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Research Article

FORMULATION AND EVALUATION OF CAPCITABINE MICROSPHERES

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Abstract: In the present work we have developed the formulation of microspheres for an anticancer drug Capcitabine 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 excepients interaction. The present study was to prepare microspheres for sustained release of using Capcitabine 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 excepients 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. FTIR and DSC results showed Capcitabine was compatible with excepients.

Key wards: Microspheres, Capcitabine, Ethyl Cellulose, HPMC, Solvent evaporation.

Introduction: 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[1-9]

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 through out 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 began 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 began as on 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[10-12].

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 powers consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 100 mm.

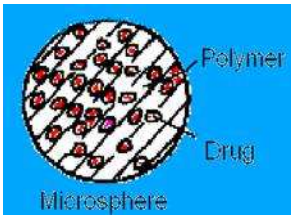


Fig: 1. Structure of microsphere.

Ideal characteristics of drug: 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**.** 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. 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. 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. 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.

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 are Control of content release , Protection of drug, Longer duration of action, Increase of therapeutic efficiency , Reduction of toxicity ,Stability , Bioavailability , Relative stability , Biocompatibility ,Water solubility and 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 intra cellularly.

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 spectrofluorometer or fluorescence microplate reader. Traditionally, this type of study has been carried out using radio labelled microspheres; however fluorescent microspheres have been shown[13-22].

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[23-25].Fig.no.2

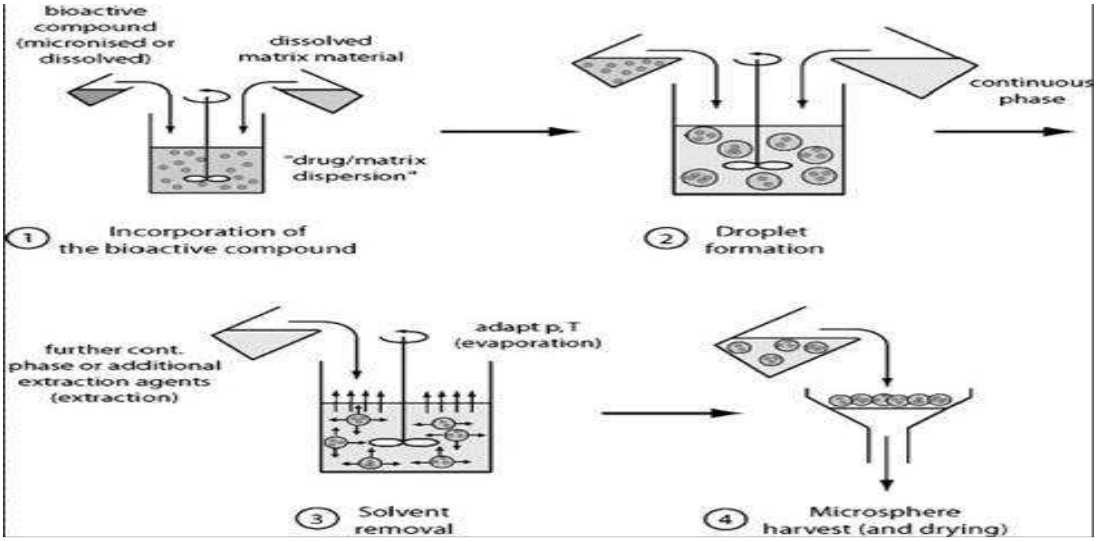


Fig: 2. Solvent evaporation.

Single emulsion technique: The micro particulate carriers of natural polymers 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. Next 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, di acid chloride etc. Heat de naturation is not suitable for thermo labile 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, separation.Fig.No.3

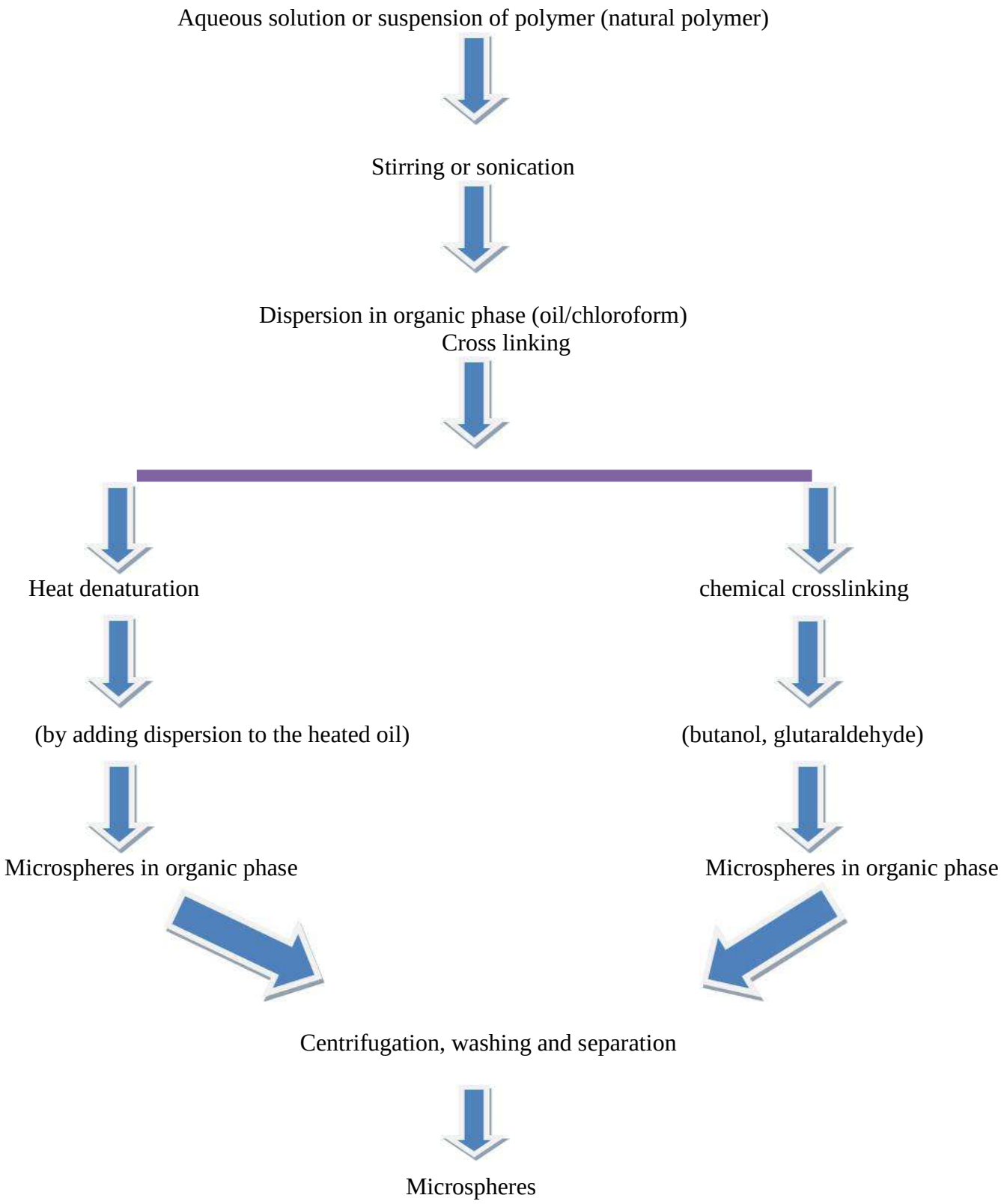
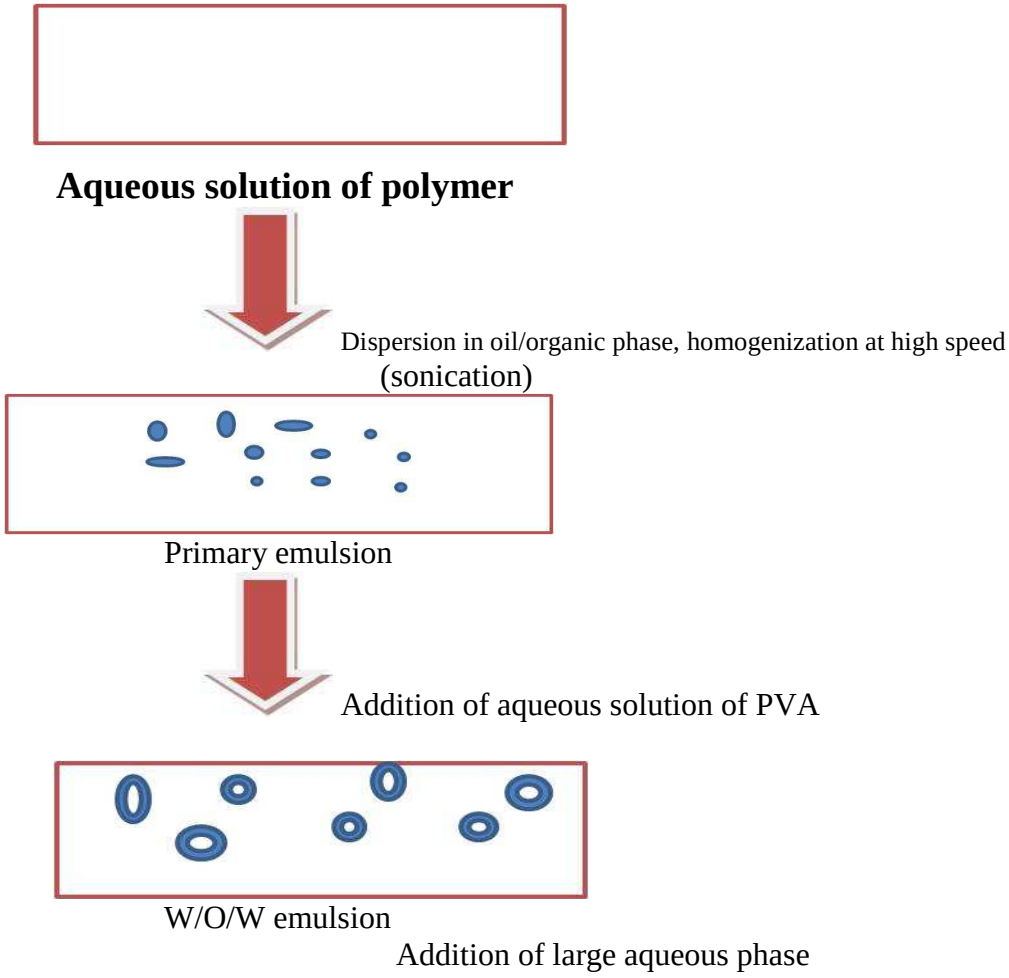
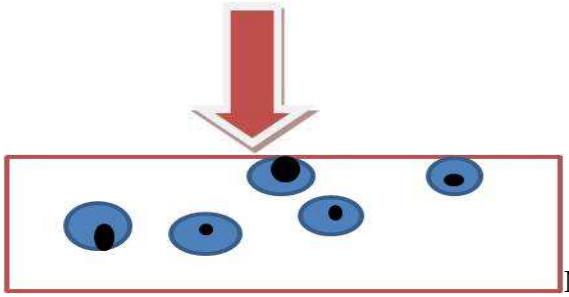


Fig: 3.Single emulsion technique.

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 to 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 leutinizing 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.No.4.




Microspheres in solution

Washing and drying

Microspheres

Fig: 4. Double emulsion technique.

Spray drying: 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 highspeed 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 sulpha ethylthiadizole 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. Microspheres are separated from hot air by cyclone separator, traces of solvent are removed by vaccum drying.Fig.No.5

Polymer in organic solvent (dichloromethane, acetone)

Drug dispersed in polymer solution under high homogenization

Atomized in a stream of hot air

Small droplets or mist form (due to evaporation of solvent)

Formation of microspheres

Microspheres are separated from hot air by cyclone separator; traces of solvent are removed by vaccum drying

Fig: 5. Spray drying.

Polymerization technique: The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as: Fig.No.6

I. Normal polymerization

II. Interfacial polymerization. Both are carried out in liquid phase.

Monomer + Bioactive material + Initiator

Heated to initiate polymerization
Initiator accelerate rate of reaction

Polymer (block)

Moulded/fragmented

Microspheres

Fig: 6. Bulk polymerization.

Normal polymerization: It is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes. In bulk, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization.Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives.Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles.Bulk polymerization has an advantage of formation of pure polymers.Fig.No.7.

Monomer + Bioactive material + Initiator

Dispersion in water and stabilizer

Droplet

Vigorous agiration Polymerization by heat

Hardened microspheres

Sepearation and drying

Microspheres

Fig: 7.Suspension polymerization.

Interfacial polymerization: It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.Fig.No.8.

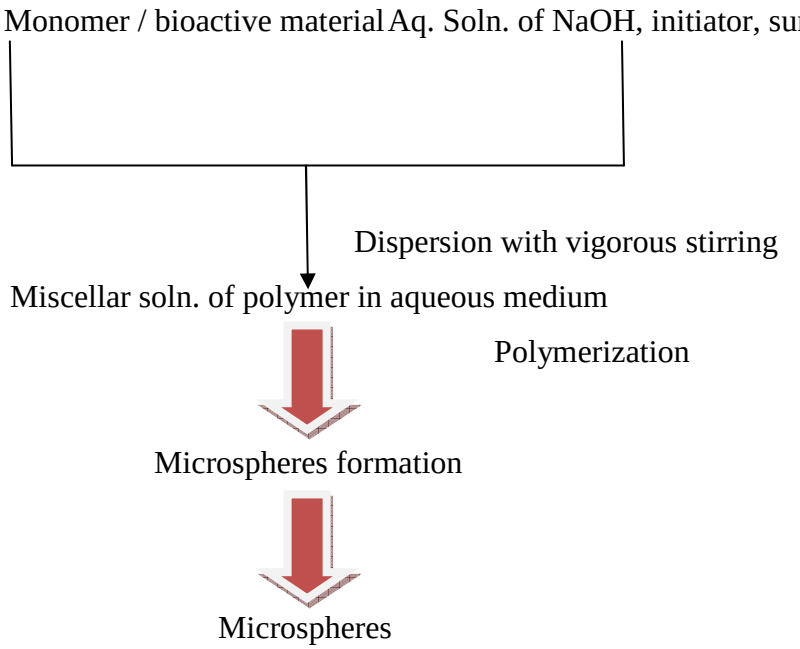


Fig: 8.Emulsion polymerization.

Phase separation technique: 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 coacervation. 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 coacervation 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.Fig.No.9.

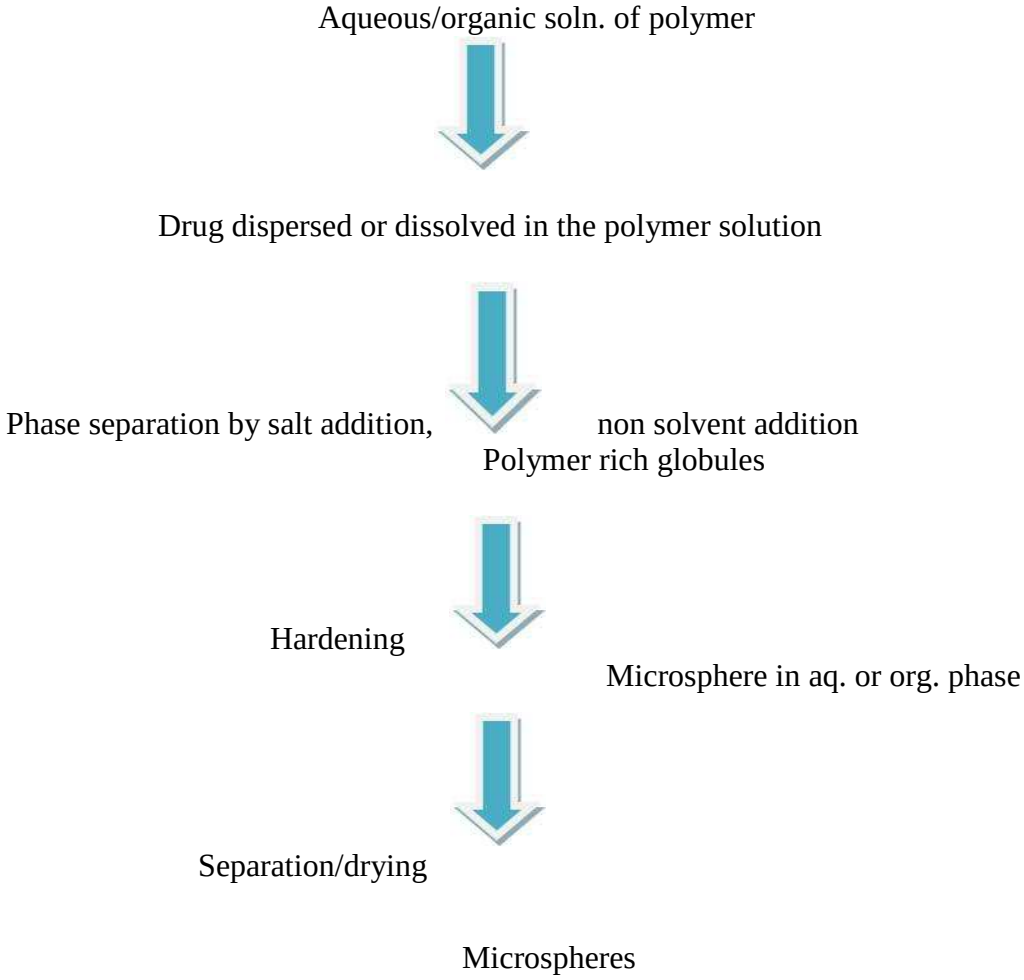


Fig: 9. Phase separation technique.

Drug loading and Drug release kinetics: Principally the active components are loaded over the microsphere by using two methods: 1) During the preparation of microsphere. 2) After the formation of microsphere.

The active component can be loaded by means of the physical entrapment, chemical linkage and surface adsorption. The entrapment largely depends on method of preparation and nature of the drug or polymer

Maximum loading can be achieved by incorporating the drug during the time of preparation but it may get affected by many other process variables such as method of preparation, presence of additives (e.g. cross linking agent, surfactant stabilizers, etc.) heat of polymerization, agitation intensity, etc. Release of the active constituent is an important consideration in case of microspheres. The release profile from the microspheres depends on the nature of the polymer used in the preparation as well as on the nature of the active drug. The release of drug from

both biodegradable as well as non-biodegradable microspheres is influenced by structure or micro-morphology of the carrier and the properties of the polymer itself.

The drugs could be released through the microspheres by any of the three methods, first is the osmotically driven burst mechanism, second by pore diffusion mechanism, and third by erosion or the degradation of the polymer. In osmotically driven burst mechanism, water diffuse into the core through biodegradable or non-biodegradable coating, creating sufficient pressure that ruptures the membrane. The burst effect is mainly controlled by three factors the macromolecule/polymer ratio, particle size of the dispersed macromolecule and the particle size of the microspheres. The pore diffusion method is named so because as penetrating water front continue to diffuse towards the core. The polymer erosion, i.e. loss of polymer is accompanied by accumulation of the monomer in the release medium. The erosion of the polymer begins with the changes in the microstructure of the carrier as water penetrates within it leading to the plasticization of the matrix[26-31].

Drug release from the non-biodegradable type of polymers can be understood by considering the geometry of the carrier. The geometry of the carrier, i.e. whether it is reservoir type where the drug is present as core, or matrix type in which drug is dispersed throughout the carrier, governs overall release profile of the drug or active ingredients.

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[32-37.]

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.

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

Density determination: Density measured by using a multivolume psychometer.

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

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

$$\% \text{ Entrapment} = \text{Actual content/Theoretical content} \times 100$$

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.

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 form 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 both 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 of penetrate at the surface. Some of the earliest and simple studies of mucosal permeability utilized the 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[38-41].

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 legated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed.

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.

Applications:

1. Microspheres in vaccine delivery: The prerequisite of a vaccine is protection against the micro organism or its toxic product. An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. The aspect of safety and minimization of adverse reaction is a complex issue The aspect of safety and the degree of the production of antibody responses are closely related to mode of application. Biodegradable delivery systems for vaccines that are given by parenteral route may overcome the shortcoming of the conventional vaccines.

The interest in parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages including: 1. Improved antigenicity by adjuvant action. 2. Modulation of antigen release. 3. Stabilization of antigen.

2. Targeting using micro particulate carriers: The concept of targeting, i.e. site specific drug delivery is a well established dogma, which is gaining full attention. The therapeutic efficacy of the drug relies on its access and specific interaction with its candidate receptors. The ability to leave the pool in reproducible, efficient and specific manner is center to drug action mediated by use of a carrier system. Placement of the particles indiscrete anatomical compartment leads to their retention either because of the physical properties of the environment or biophysical interaction of the particles with the cellular content of the target tissue.

3. Monoclonal antibodies mediated microspheres targeting: Monoclonal antibodies targeting microspheres are immune microspheres. This targeting is a method used to achieve selective targeting to the specific sites. Monoclonal antibodies are extremely specific molecules. This extreme specificity of monoclonal antibodies (Mabs) can be utilized to target microspheres loaded bioactive molecules to selected sites. Mabs can be directly attached to the microspheres by means of covalent coupling. The free aldehyde groups, amino groups or hydroxyl

groups on the surface of the microspheres can be linked to the antibodies. The Mabs can be attached to microspheres by any of the following methods are 1. Non specific adsorption. 2. Specific adsorption. 3. Direct coupling. 4. Coupling via reagents.

4. Chemo embolisation: Chemo embolisation is an endovascular therapy, which involves the selective arterial embolisation of a tumour together with simultaneous or subsequent local delivery the chemotherapeutic agent. The theoretical advantage is that such embolisations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of the tumour. Chemo embolisation is an extension of traditional percutaneous embolisation techniques.

5. Imaging: The microspheres have been extensively studied and used for the targeting purposes. Various cells, cell lines, tissues and organs can be imaged using radio labelled microspheres. The particle size range of microspheres is an important factor in determining the imaging of particular sites. The particles injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is exploited for the scintiographic imaging of the tumour masses in lungs using labeled human serum albumin microspheres.

6. Topical porous microspheres: Microsponges are porous microspheres having myriad of interconnected voids of particle size range 5-300 µm. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils etc., are used as the topical carries system further, these porous microspheres with active ingredients can be incorporated into formulations such as creams, lotions and powders. Microsponges consist of non collapsible structures with porous surface through which active ingredients are released in a controlled manner.

7. Surface modified microspheres: Different approaches have been utilized to change the surface properties of carriers to protect them against phagocytic clearance and to alter their body distribution patterns .The adsorption of the poloxamer on the surface of the polystyrene, polyester or poly methyl methacrylate microspheres renders them more hydrophilic and hence decrease their MPS uptake. Protein microspheres covalently modified by PEG

derivatives show decreased immunogenicity and clearance. The most studied surface modifiers are 1. Antibodies and their fragments. 2. Proteins. 3. Mono-, oligo- and polysaccharides.4. Chelating compounds (EDTA, DTPA or Desferroxamine). 5. Synthetic soluble polymers.

Such modifications are provided surface of microspheres inorder to achieve the targeting to the discrete organs and to avoid rapid clearance from the body.

Floating microspheres: Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These are in strict sense, spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs[20]. Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.

Advantages: 1.Improves patient compliance by decreasing dosing frequency.2.Gastric retention time is increased because of buoyancy.3. Enhanced absorption of drugs which solubilise only in stomach.4.Drug releases in controlled manner for prolonged period.5.Site-specific drug delivery to stomach can be achieved.6.Avoidance of gastric irritation, because of sustained release effect.

7. Better therapeutic effect of short half-life drugs can be achieved.

Mechanism: When microspheres come in contact with gastric fluid the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However a minimal gastric content needed to allow proper achievement of

buoyancy. Hollow microspheres of acrylic resins, eudragit, polyethylene oxide, and cellulose acetate; polystyrene floatable shells; polycarbonate floating balloons and gelucire floating granules are the recent developments.

Applications:

1. The floating microspheres can be used as carriers for drugs with so-called absorption windows, these substances, for example antiviral, antifungal and antibiotic agents (Sulphonamides, Quinolones, Penicillins, Cephalosporins, Aminoglycosides and Tetracyclines) are taken up only from very specific sites of the GI mucosa.

2. Hollow microspheres of non-steroidal anti inflammatory drugs are very effective for controlled release as well as it reduces the major side effect of gastric irritation; for example floating microspheres of Indomethacin are quiet beneficial for rheumatic patients.

3. Floating microspheres are especially effective in delivery of sparingly soluble and insoluble drugs. It is known that as the solubility of a drug decreases, the time available for drug dissolution becomes less adequate and thus the transit time becomes a significant factor affecting drug absorption. For weakly basic drugs that are poorly soluble at an alkaline pH, hollow microspheres may avoid chance for solubility to become the rate-limiting step in release by restricting such drugs to the stomach. The positioned gastric release is useful for drugs efficiently absorbed through stomach such as Verapamil hydrochloride. The gastro-retentive floating microspheres will alter beneficially the absorption profile of the active agent, thus enhancing its bioavailability.

4. Hollow microspheres can greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentrations at the gastric mucosa, thus eradicating *Helicobacter pylori*from the sub-mucosal tissue of the stomach and making it possible to treat stomach and duodenal ulcers, gastritis and oesophagitis.

5. The drugs recently reported to be entrapped in hollow microspheres include Prednisolone, Lansoprazole, Celecoxib, Piroxicam, Theophylline.

Characterization:

Floating microspheres are characterized by their micromeritic properties such as particle size, tapped density, compressibility index, true density and flow properties. Particle size is measured using an optical microscopy and mean particle size was calculated by measuring 200 to 300 particles with the help of calibrated ocular micrometer. True density is determined by liquid displacement method; tapped density and compressibility index are calculated by measuring the change in volume using a bulk density apparatus; angle of repose is determined by fixed funnel method. The hollow nature of microspheres is confirmed by scanning electron microscopy.

The compressibility index was calculated using following formula:

$$I = V_b - V_t / V_b \times 100$$

Where, V_b is the bulk volume and V_t is the tapped volume. The value given below 15% indicates a powder with usually give rise to good flow characteristics, whereas above 25% indicate poor flow ability.

Floating Behavior:

Fifty milligrams of the floating microspheres were placed in 100 ml of the simulated gastric fluid (SGF, pH 2.0) containing 0.02% w/v Tween 20. The mixture was stirred at 100 rpm with a magnetic stirrer. After 8 hours, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight was achieved. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

$$\text{Buoyancy (\%)} = W_f / W_f + W_s$$

Where, W_f and W_s are the weights of the floating and settled microparticles

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[38-41].

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).

Preparation of microspheres: The microspheres were prepared by (o/w) solvent evaporation method, since Capecitabine 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 an 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(Table no.1). 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 10.

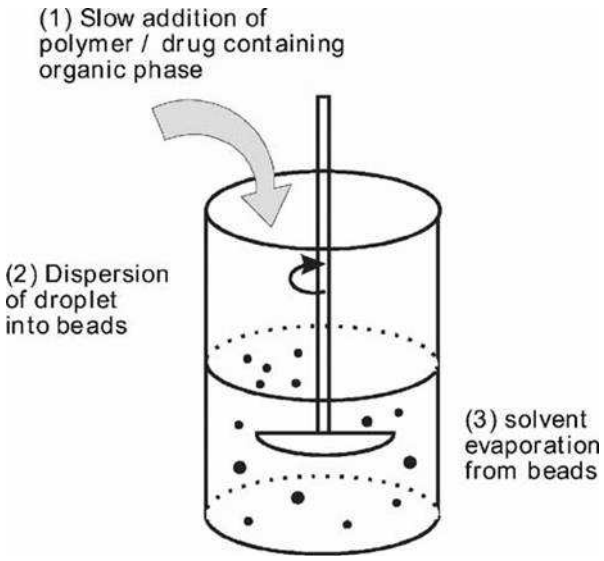


Fig: 10. Process of microsphere preparation.

Table No-1: Formulation table for Capecitabine microspheres.

Formulation	Drug (g)	Ethyl Cellulose (g)	HPMC (g)	DCM (ml)	PVA (0.46% w/v)	SPEED (rpm)
F1	0.5	2	1	20	160 ml	800
F2	0.5	1	2	20	160 ml	800

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

Results and Discussion:

Micromeritic properties:

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 ($\rho 0$) $=M/V_0$

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: Hausnerís 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).

Fourier transform infrared (FTIR) spectral studies: FTIR spectra of the Ethyl Cellulose, HPMC, Capecitabine and Capecitabine-loaded microspheres were obtained. In order to investigate the possible reaction between Ethyl cellulose and Capecitabine, Capecitabine was treated with Ethyl Cellulose. The ratio (mL/mg) and concentration of Ethyl Cellulose as well as capecitabine 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, capecitabine 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.11-14

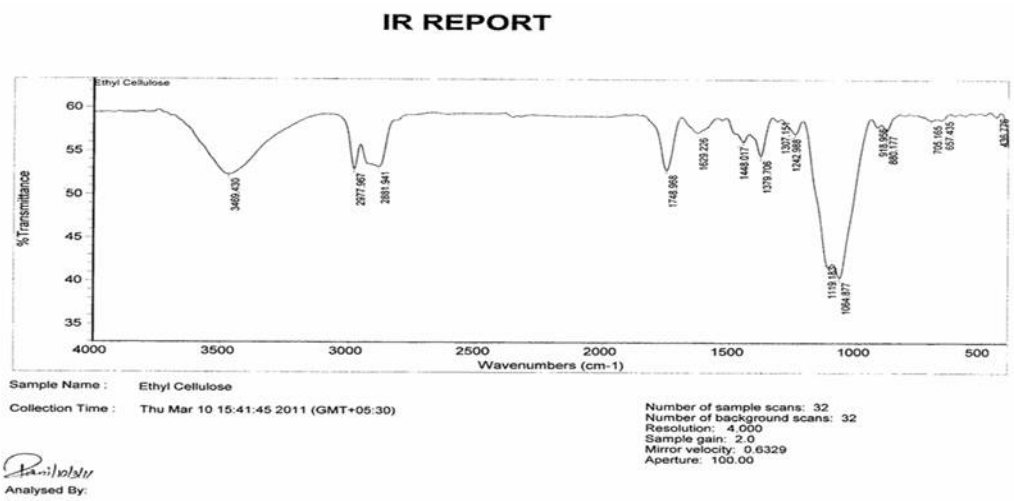


Fig. No.11. FTIR OF PURE CAPECITABINE

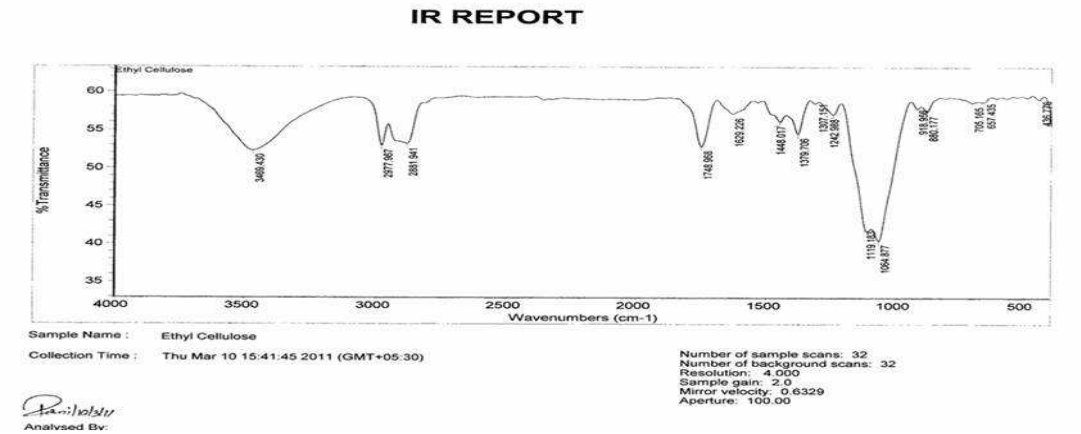


Fig. No.12. FTIR OF ETHYL CELLULOSE

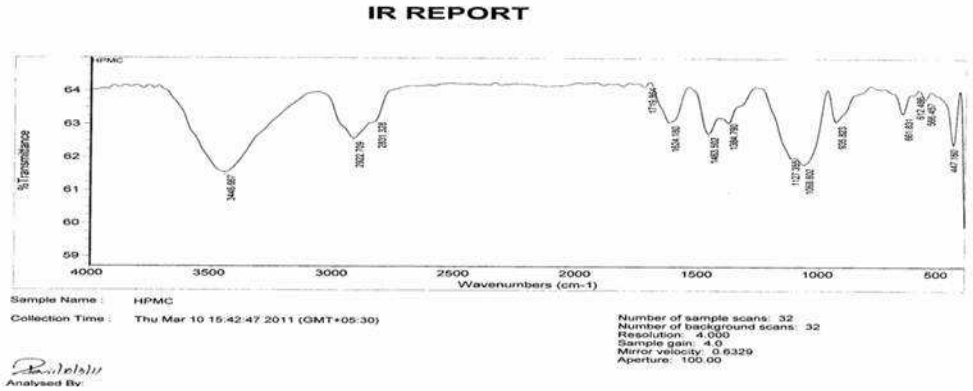


Fig. No.13. FTIR OF HPMC

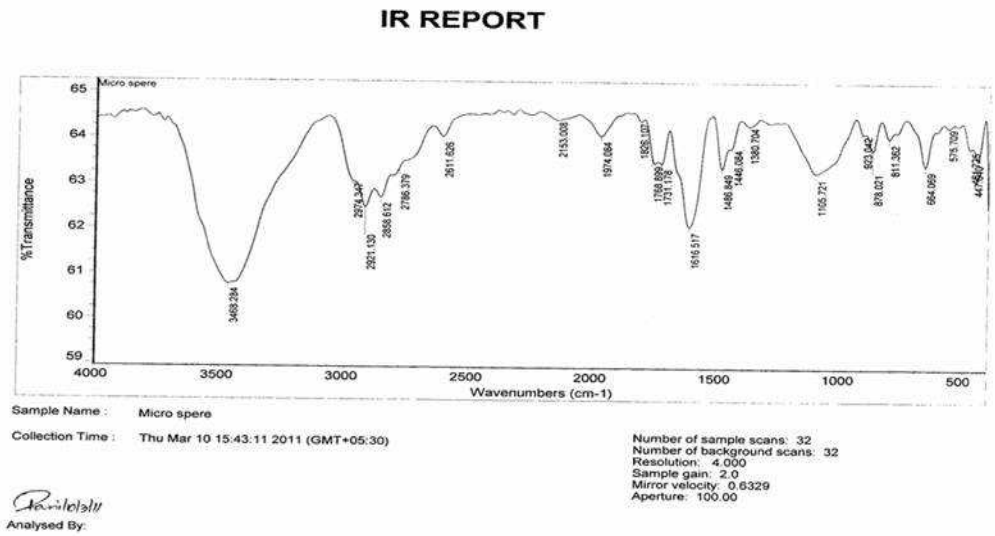


Fig. No.14. FTIR OF MICROSPEARS

Differential scanning calorimetry (DSC) studies: Differential scanning calorimetry (DSC) was performed on Ethyl Cellulose, HPMC, Capecitabine and Capecitabine-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).fig no. 15-18.

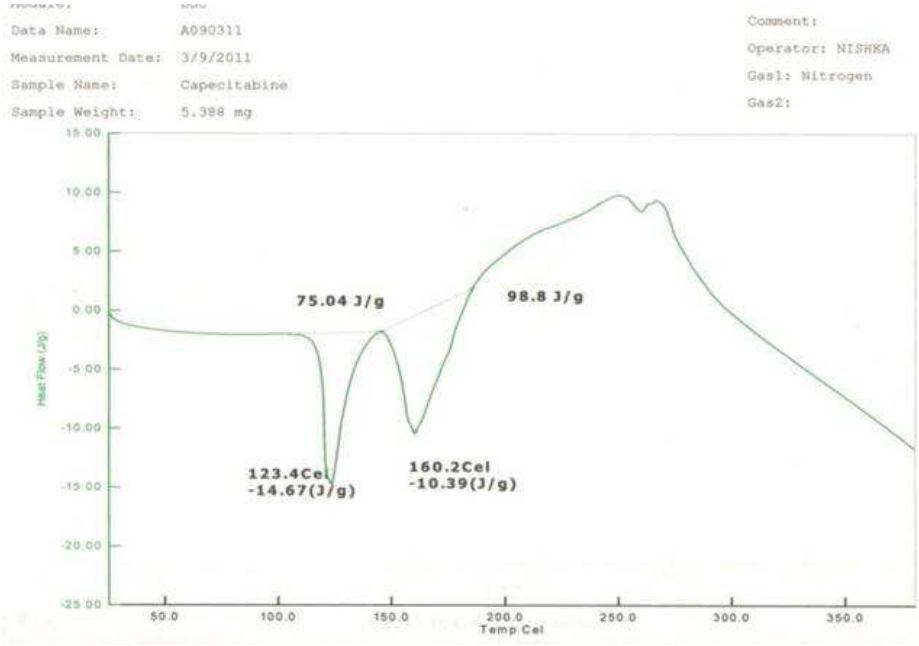


Fig. No.15. DSC OF CAPECITABINE

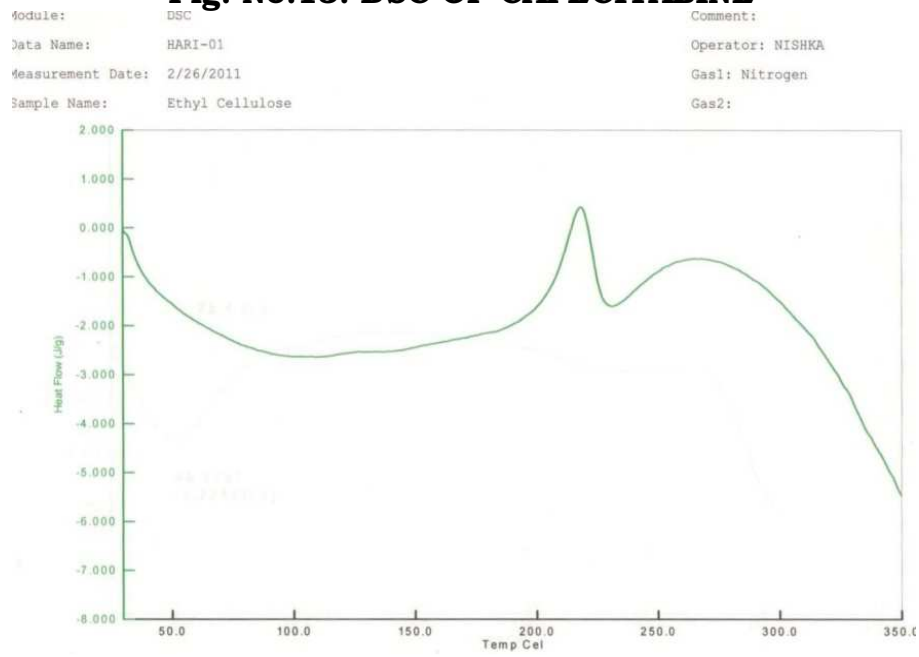


Fig. No.16. DSC OF ETHYL CELLULOSE

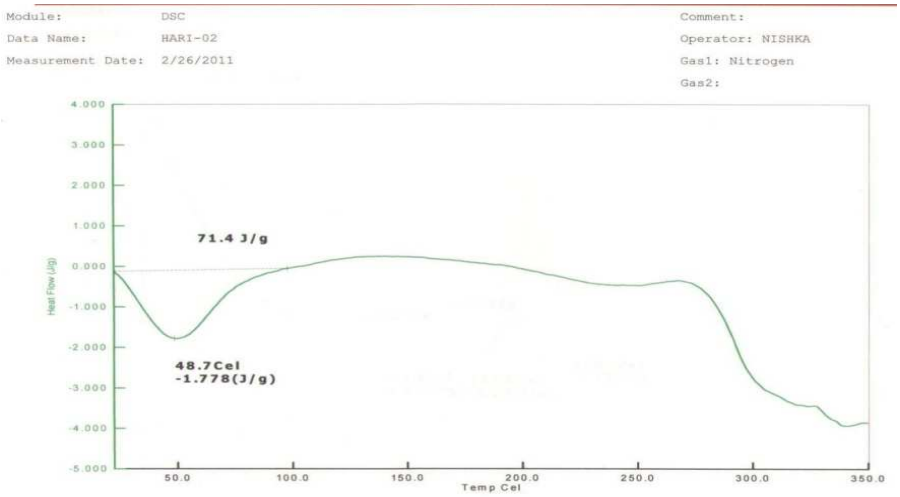


Fig. No.17. DSC OF HPMC

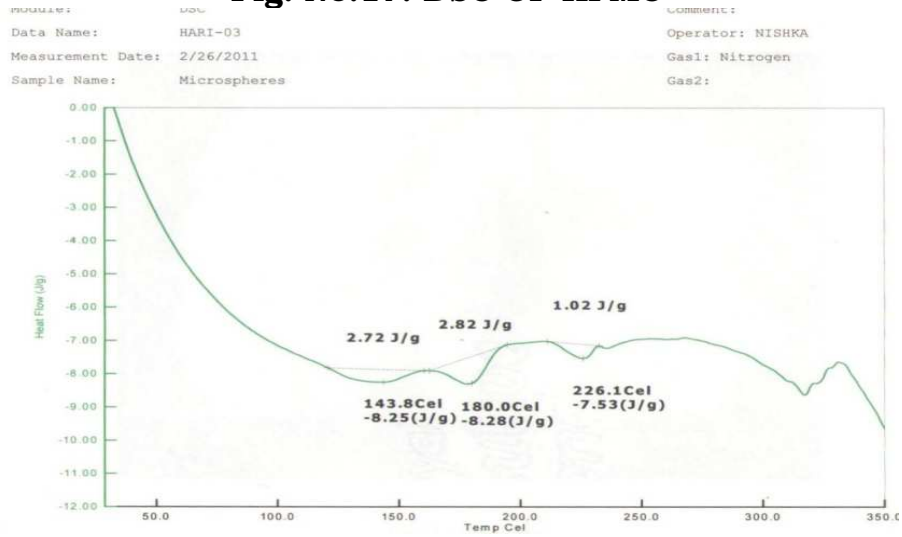


Fig. No.18. DSC OF PHYSICAL MIXTURE OF CAPECITABINE, EC & HPMC

Drug entrapment: Drug loaded microspheres (50mg) were powdered and suspended in 5oml of methanol.the resultant dispersion was kept for half hour in sonicator and filtered. Fom this above solution 1ml was diluted to 25ml with distilled water and analyzed spectrophotometricaly at 240nm .(Table no.4)

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

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

Surface morphology: The shape and surface topography of the microspheres were studied by scanning electron microscopy (SEM).fig no.19.

Fig. No. 19. SEM ANALYSIS

Particle size measurements: Particle size was measured by using light scattering technique (Microtrack). Size 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 .20)(Table no.2-4).

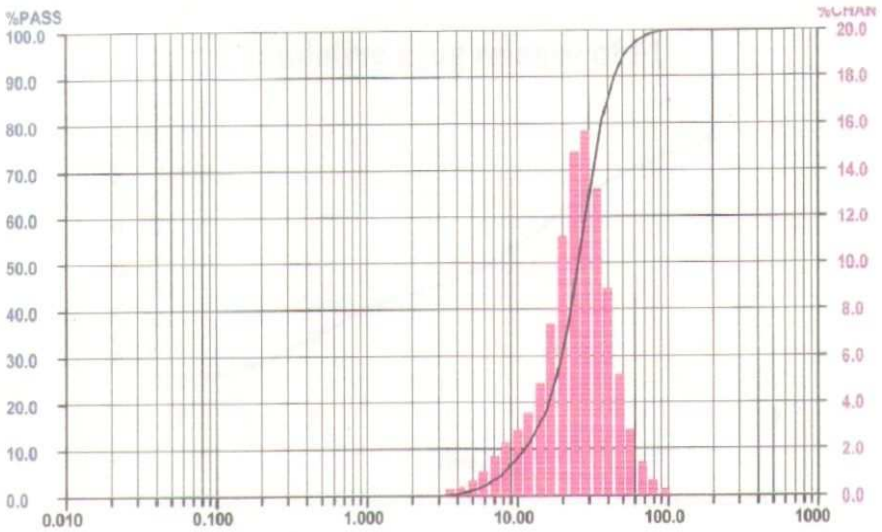


Fig. No.20. PARTICAL SIZE ANALYSIS

In vitro drug release: 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 37^0 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. Then the Capecitabine concentration was determined spectrophotometrically at 240nm.(Fig no .21-23)(Table no.5&6).

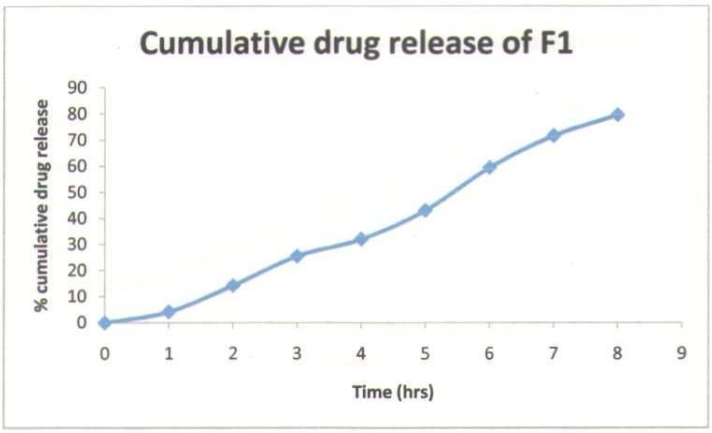


Fig. No.21. CUMULATIVE DRUG RELEASE OF F1

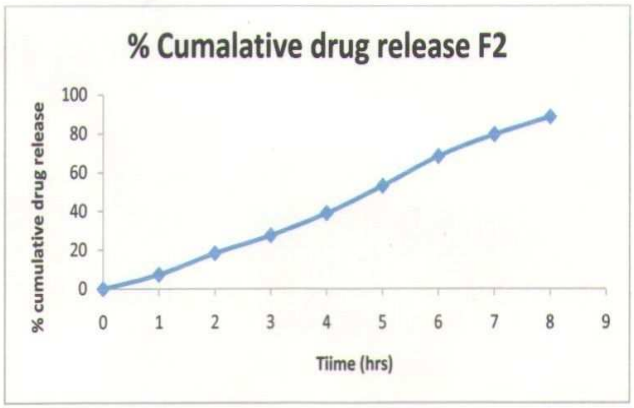


Fig. No.22. CUMULATIVE DRUG RELEASE OF F2

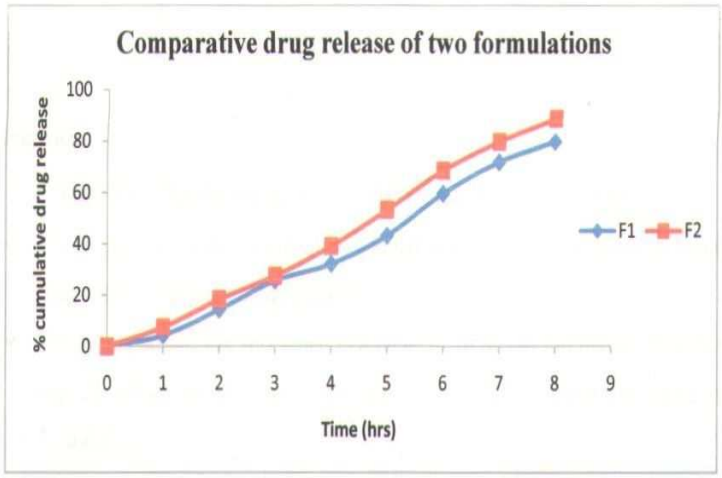


Fig. No.23. COMPARATIVE DRUG RELEASE OF F1 AND F2

Table No-2: Micromeritic properties of microspheres.

Formulation code	DERIVED PROPERTIES		FLOW PROPERTIES		
	Bulk density	Tapped density	Angle Of repose	Carr's index	Hausners 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

Table No-3: Yield of microspheres.

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

Table No-4: Entrapment efficiency.

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

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

Discussion:

With the obtained results the following points are noticed, The microspheres thus obtained was found to be spherical as given in Figure 20 (Page no 57) 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 22 and Fig. 23 (Page no 59). 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.24. 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 Capecitabine 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 upto 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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