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Research

Preparation And Evaluation Of Microspheres Of Antidiabetic Drug By Solvent Evaporation Method

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Abstract

Microspheres, microscopic spherical particles, have garnered significant attention in recent years due to their potential applications in various fields, including pharmaceuticals, biotechnology, and medicine. This review aims to provide a comprehensive overview of the preparation and evaluation of microspheres. The study demonstrates the potential of microspheres as a controlled release delivery system for anti-diabetic therapy. The solvent evaporation method was found to be a suitable method for preparing microspheres with high entrapment efficiency and sustained release. The microspheres prepared in this study can be used for oral delivery, providing a convenient and patient-friendly treatment option for diabetes management. Various methods for preparing microspheres, such as solvent evaporation, spray drying, and emulsification, are discussed. The advantages and limitations of each method are highlighted, and the effects of formulation variables on microsphere characteristics are examined. The evaluation of microspheres, including their size, shape, surface morphology, and release kinetics, is also reviewed. Techniques such as scanning electron microscopy, transmission electron microscopy, and particle size analysis are discussed. Furthermore, the applications of microspheres in drug delivery, tissue engineering, and diagnostic imaging are explored. The challenges and future directions in microsphere research are also addressed.

Keywords: Microspheres, Anti-diabetic therapy, Preparation methods, Solvent evaporation method, Characterization, Drug delivery, Pharmaceutical applications

INTRODUCTION

MICROSPHERE

Microsphere are the carriers for control release and can be defined as solid spherical monolithic free flowing particles having a size range from 1 to 1000µm; typical size is 1 to 500µm containing dispersed drug molecules either in solution or in crystalline forms. These are made of polymeric, waxy or other protective materials, biodegradable synthetic polymers and modified natural products, such as starches, gums, proteins, fats, and polymers include poly-lactic acid and poly-glycolic acid. Microspheres are used to sustain the release of drug for localized effect[1]. Microspheres have been used for passive targeting of organs, such as liver, spleen, lung and kidney. They give protection to the drug from chemical and enzymatic degradation. In addition they reduce toxicity and improves therapeutic efficacy of drug. Microspheres are a rapidly expanding technology. Till now a numbers of drugs like anti-neoplastic, narcotic antagonists, steroid hormones, luteinizing hormone, releasing hormone analogs, elastase and other macromolecules have been incorporated into microspheres[2].

Intra-arterial injection of biodegradable microsphere can be used to produce a tumor chemoembolism. This technology is based on the chemoembolism of the drug-loaded microspheres via the tumour arterial supply. Due to their physical size, microspheres can be entrapped in capillary beds along with their load of cytotoxic drug after intra-arterial administration. As a result of this chemoembolism, the microspheres containing the cytotoxic drug can be delivered to the well-vascularized tumour tissue. The drug is then release in a sustained manner into tumour site without being dispersed into the systemic circulation. This approach has demonstrate comparable and enhanced therapeutic response with suppressed systemic toxicity of anticancer drug in animal model[2].

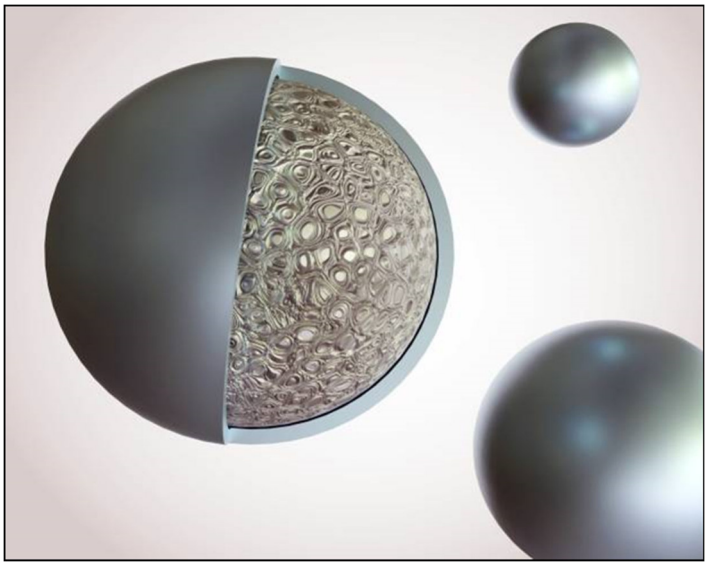


Fig 1: Microsphere

Diabetes

Diabetes mellitus taken the Greek word diabetes, meaning siphon- to pass through and the Latin word mellitus meaning sweet. Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels and also associated with developing insulin resistance, impaired insulin signaling and beta cell disfunction, abnormal glucose and lipid metabolism, subclinical inflammation increased oxidative stress. It occurs when the body is unable to produce or effectively use insulin. Insulin is a hormone produced by the pancreas that regulates blood sugar levels. Diabetes can lead to serious health complications, including heart disease, kidney damage, and blindness.

TYPES

Mostly patients with diabetes mellitus have either Type 1 diabetes (which is immune-mediated or idiopathic) Type 2 diabetes (formerly known as non-insulin dependent diabetes mellitus) is the most common form of diabetes mellitus characterized by hyperglycemia, insulin resistance and relative insulin deficiency and results from interaction

between genetic, environmental and behavioral risk factors. Diabetes also can be related to the gestational hormonal environment, genetic defects, other infections, and certain drugs.

TYPE 1 DIABETES MELLITUS

Type 1diabetes mellitus (Juvenile diabetes) is characterized by beta cell destruction caused by an autoimmune process, usually leading absolute insulin deficiency. Type 1 is usually characterized by the presence of anti-glutamic acid decarboxylase, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta cell destruction. Eventually, all type 1 diabetic patients will require insulin therapy to maintain normoglycemia.

TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus comprises 80% to 90% of all cases of diabetes mellitus. Most individuals with type 2 diabetes exhibit intra-abdominal (visceral) obesity, which is closely related to the presence of insulin resistance. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more in women, especially women with a history gestational diabetes, and in blacks, Hispanics and native Americans.

PREDIABETES

This type is the stage before type 2 diabetes. Blood glucose levels are higher than normal but not high enough to be officially diagnosed with type 2 diabetes.

GESTATIONAL DIABETES MELLITUS(GDM)

Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation. Women who develop type 1 diabetes during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy are classified with GDM. In most women who develop GDM; the disorder has its onset in the third trimester of pregnancy[32].

ANTIDIABETIC DRUG

Antidiabetic drugs are medications used to treat diabetes by regulating blood sugar levels.These drugs help to lower blood glucose levels, improve insulin sensitivity, and enhance glucose uptake. Antidiabetic drugs can be oral or injectable and are classified into several classes, including sulfonylureas, metformin, and DPP-4 inhibitors. They are used to manage type 2 diabetes, and in some cases, type 1 diabetes, in conjunction with insulin therapy. The goal of antidiabetic drugs is to achieve and maintain optimal blood glucose control, reducing the risk of diabetes-related complications[33].

HISTORICAL BACKGROUND OF MICROSPHERE

The concept of packaging materials within microspheres dates back to the 1930s with the work of Bungenberg de jong and coworkers on the entrapment of substances within coacervates. The first commercial applications of microencapsulation was by the National Cash Register Company for the manufacture of carbonless copying paper in 1957. The technology and applications have advanced over the last several decades. This technology is used by the medical, food, cosmetics, agricultural, graphics, and household products industries. The potential use of microspheres in the pharmaceutical industry has been considered considered since the 1960s due to some major properties of microspheres like taste and odour masking, conversion of oils and other liquids to solids for ease of handling and protection of drugs against the environment(moisture, light, heat, and oxidation),etc. Microencapsulation technology has also been used medically for the encapsulation of live cells and vaccines. Biocompatibility can be improved by the encapsulation of artificial cells and biomolecules, such as peptides, proteins, and hormones, which can prevent unwanted immunological reactions that would lead to inactivation or rejection. Microspheres are used for isolating materials until their activity is needed. The biotechnology industry employs microspheres to contain organisms and their recombinant products to aid in the isolation of these products[2,8].

IDEAL PROPERTIES OF MICROSPHERE

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradabiltity.
- Susceptibility to chemical modification.

- Controlled particle size and dispersability in aqueous vehicles for injection[3].

ADVANTAGES OF MICROSPHERE

- Microspheres have large surface areas on which ligands can be attached for drug delivery to a specific local area.
- Reliable means to deliver the drug to the target site and to maintain the desired concentration at the site of interest without untoward effects.
- Due to their macrophage uptake, microspheres can be used for targeting drugs to pathogens residing intracellularly.
- Advantages of microspheres over conventional dosage forms include biocompatibility, controllable biodegradability, absorbability and low toxicity of the degradation end products, sustained release potential and ease of administration.
- Problems associated with vaginal rings, such as erosion of the vaginal wall, ring expulsion, interference with coitus, unpleasant ring odour, difficulty with storage and sanitation can be overcome by using microspheres.
- Microspheres, injected in the form of suspension do not require surgical implantation.
- Radiolabelled microspheres can be employed for blood flow determination. Relatively large microspheres (10-15µm in diameter) are useful for regional blood flow studies in tissues and organs[2,3,4,5,20].

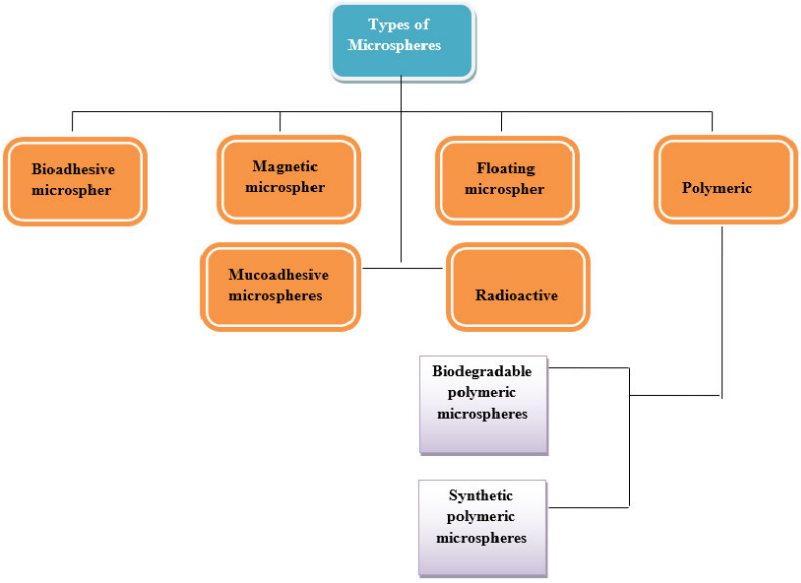
LIMITATION

Some of the disadvantages were found to be as follows:

- The modified release from the formulations.
- The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
- Differences in the release rate from one dose to another.
- Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.

Dosage forms of this kind should not be crushed or chewed[5,20].

TYPES OF MICROSPHERE[2,3,4,5,7,17,20]



Microspheres usually consist of polymers and are classified accordingly:

SYNTHETIC POLYMERS

Non-biodegradable polymers:

- ✓ Poly methyl methacrylate (PMMA),
- ✓ Acrolein,Glycidyl methacrylate,

- ✓ Epoxy polymers.

Biodegradable polymers:

- ✓ Lactides,
- ✓ Glycolides and their copolymers,
- ✓ Poly alkyl cyanoacrylates,
- ✓ Poly anhydrides.

NATURAL POLYMERS

Natural polymers are obtained from different sources like

Proteins:

- ✓ Albumin,
- ✓ Gelatin,
- ✓ Collagen.

Carbohydrates:

- ✓ Agarose,
- ✓ carrageenan,
- ✓ starch,
- ✓ chitosan.

Chemically modified carbohydrates:

- ✓ Poly dextran,
- ✓ Poly starch.

METHODS OF PREPARATION[2,3,4,7,8,18,19,25]

The microspheres can be prepared by using any of the several techniques discussed in the following sections, but the choice of technique mainly depends on the nature of polymer used, the drug, the intended use and the duration of therapy. Moreover, the method of preparation and its choice are equivocally determined by some formulation and technology related factors as mentioned below:

1. The particle size requirement
2. The drug or the protein should not be adversely affected by the process
3. Reproducibility of the release profile and the method
4. No stability problem
5. There should be no toxic product(s) associated with the final product.

METHODS

- Solvent evaporation
- Hot melt
- Solvent removal
- Single emulsion
- Double emulsion
- Spray drying and spray congealing
- Freeze drying
- Wax coating
- Chemical and thermal cross linking
- Co-acervation phase separation
- Multiple emulsion
- Ionic gelation
- Polymerization method

SOLVENT EVAPORATION METHOD

One of the oldest and widely used methods of microspheres preparation is the solvent evaporation technique. In this method, drug/polymer/solvent mixture (i.e. the oil phase) is emulsified in water in order to form an oil-in-water (o/w) emulsion. To assist emulsification, a surfactant is normally dissolved in the water phase before the o/w emulsion is formed. A good example is partially hydrolyzed (88%) poly (vinyl alcohol) (PVA). Once the desired oil phase droplet size and emulsion stability have been obtained, the system is stirred at a constant rate and the solvent

evaporates. It is known as solvent evaporation method because most of the solvent disappears on evaporation. The organic solvent can also be removed by heating, vacuum or by stirring. Once solvent evaporation appears to be completed, the capsules are separated from the suspending medium by filtration, washed and then dried[2].

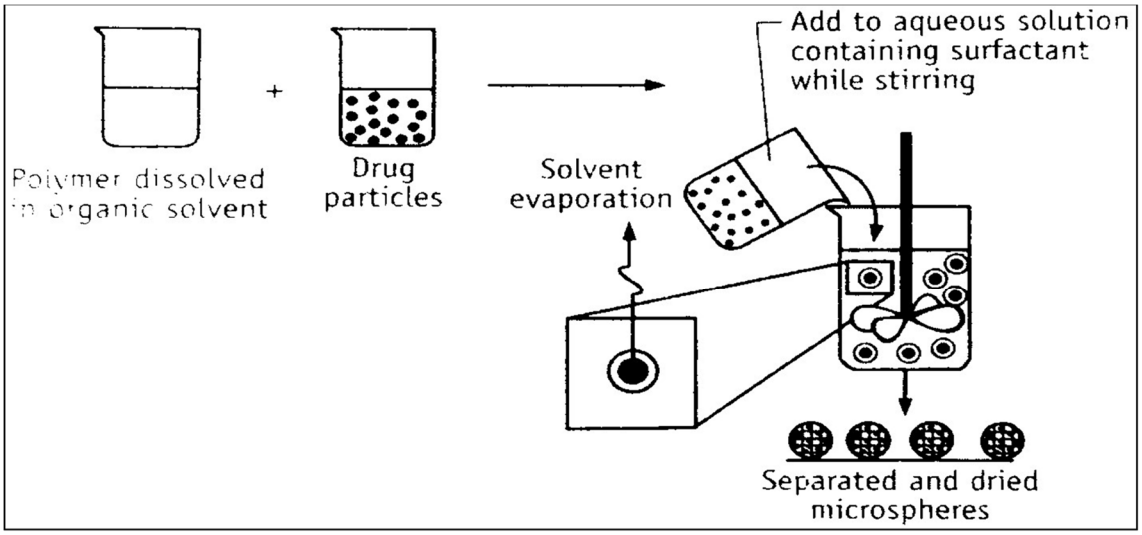


Fig 2: Solvent Evaporation Method

HOT MELT METHOD

The polymer is first melted and then mixed with solid particles of the drug that have been sieved to less than 50 µm. The mixture is suspended in a non-miscible solvent (like silicone oil), continuously stirred, and heated to 5°C above the melting point of the polymer. Once the emulsion is stabilized, it is cooled until the polymer particles solidify. The resulting microspheres are washed by decantation with petroleum ether. The primary objective for developing this method is to develop a microencapsulation process suitable for the water labile polymers, e.g. poly anhydrides. Microspheres with diameter of 1- 1000 µm can be obtained and the size distribution can be easily controlled by altering the stirring rate. The only disadvantage of this method is moderate temperature to which is a drug is exposed.

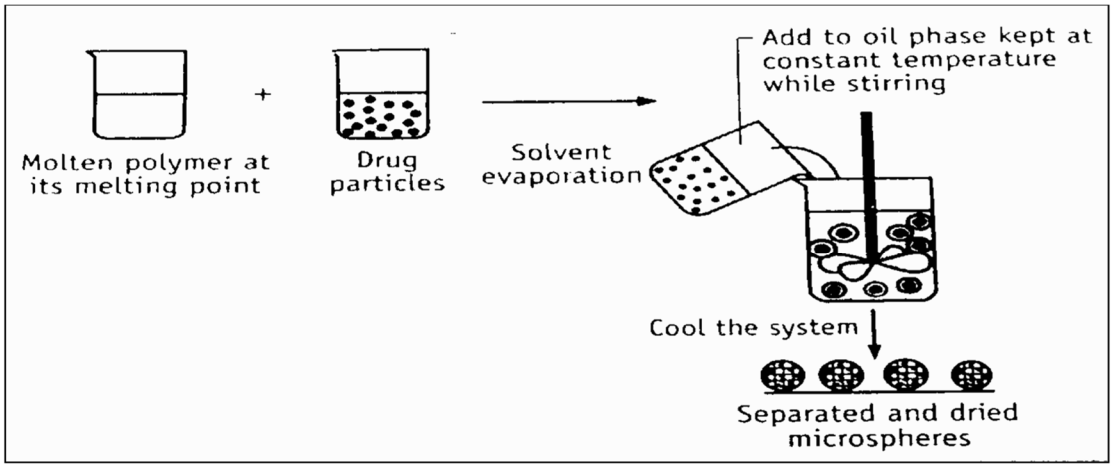


Fig 3: Hot Melt Method

SOLVENT REMOVAL METHOD

It is a non-aqueous method of microencapsulation, particularly suitable for water labile polymers such as the polyanhydrides. In this method, drug is dispersed or dissolved in a solution of the selected polymer in a volatile organic solvent like methylene chloride. This mixture is then suspended in silicone oil containing span 85 and methylene

chloride. After pouring the polymer solution into silicone oil, petroleum ether is added and stirred until solvent is extracted into the oil solution. The resulting microspheres can then be dried in vacuum.

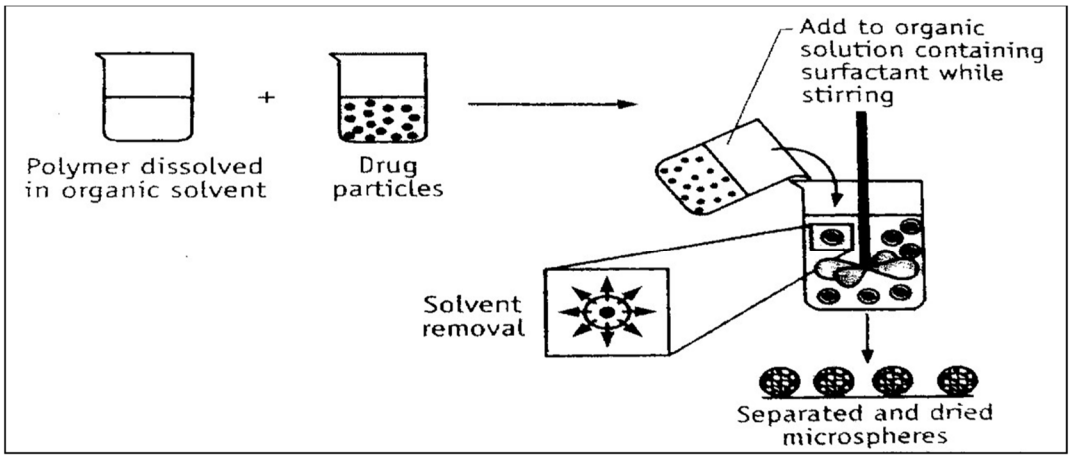


Fig 4: Solvent Removal Method

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, 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, separation. 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.

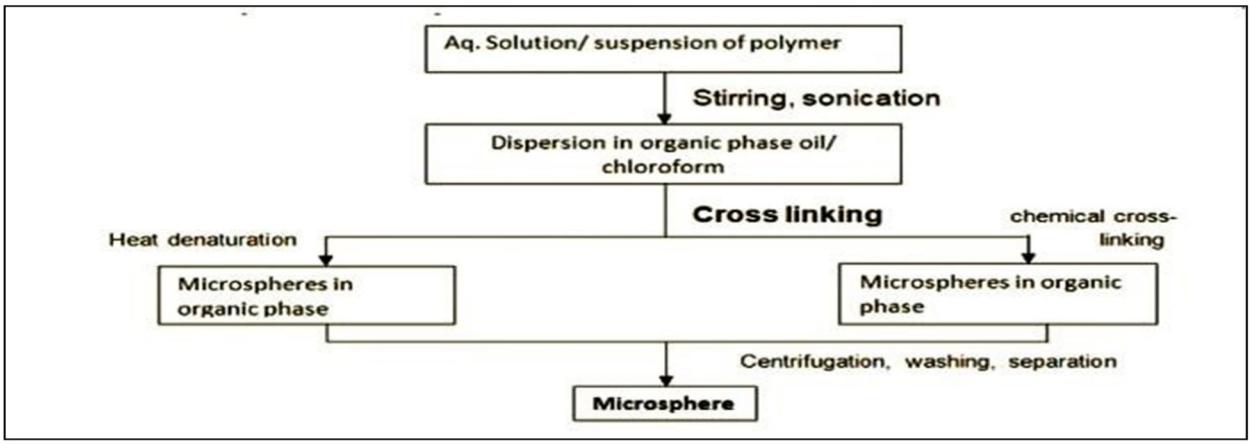


Fig 5: Single Emulsion Method

DOUBLE EMULSION METHOD

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.

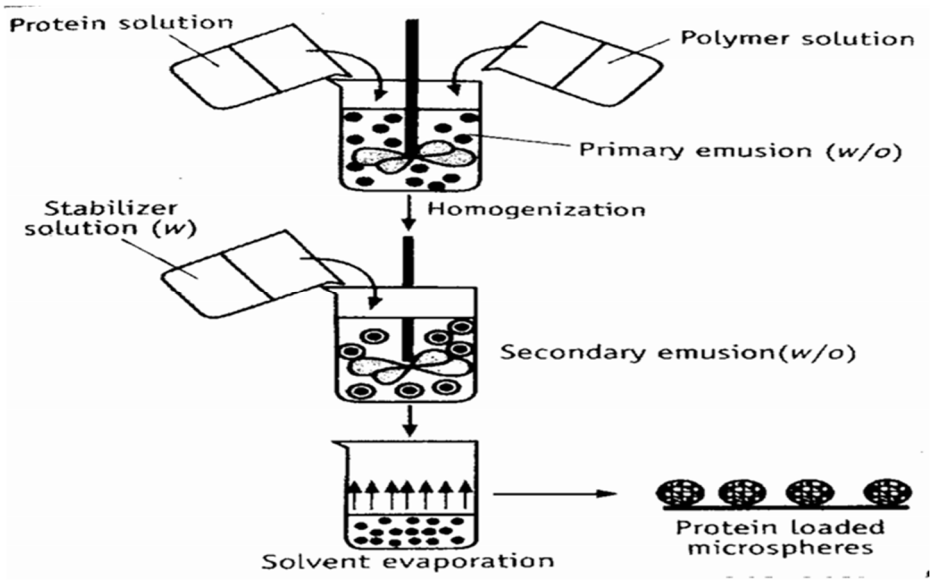


Fig 6: Double Emulsion Method

MULTIPLE EMULSION METHOD

In this method, primary emulsion of polymers like ethyl cellulose or methyl cellulose is prepared. The primary emulsion is then re-emulsified in aqueous medium. During this phase, discrete microspheres are formed under optimized conditions.

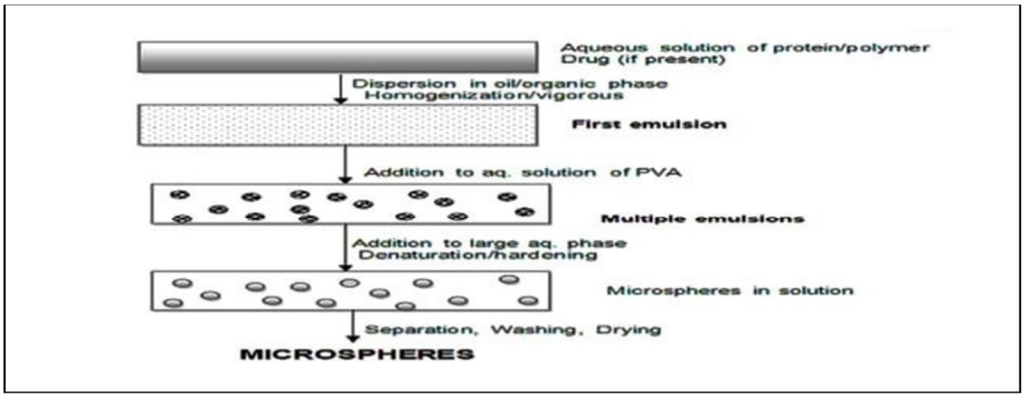


Fig 7: Multiple Emulsion Method

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.

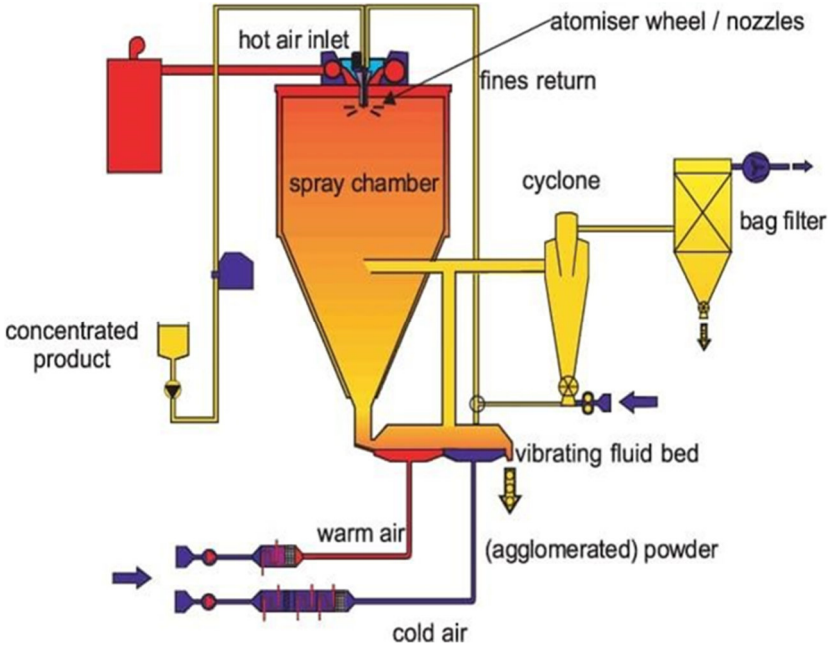


Fig 08: Spray Drying Method

FREEZE DRYING METHOD

Freeze-drying is effectively used in protein API microspheres praparation. The method is freezing, sublimation, main drying, and secondary drying. At the freezing step, account is taken of the eutectic point of the components. During the process, lyoprotectants or cryoprotectants will stabilise API molecules by removing water, creating a glass matrix, lowering intermolecular interaction by forming hydrogen bonds between the molecules or dipole - dipole interactions. It's a beneficial cycle for heat tolerant molecules, given its high expense. Freeze-drying produces solidification and then enables the reconstitution of particles in an aqueous media.

WAX COATING METHOD

Wax used to encapsulate the main components, by dissolving or dispersing the product in melted wax. The waxy paste or mixture, such as frozen liquid paraffin, is released by high intensity blending with cold water. The water is heated up for at least an hour. The substance is stirred up for at least 1 hour. Then the external layer (liquid paraffin) is decanted and the microspheres are immersed in a non-miscible solvent and dry air is required to dry. For the surface ingredients, carnauba wax and beeswax can be used and both should be combined to obtain desirable characteristics.

CHEMICAL AND THERMAL CROSS LINKING

Citric acid, as a cross-linking agent was added to 30 mL of an aqueous acetic acid solution of chitosan (2.5% wt/vol) maintaining a constant molar ratio between chitosan and citric acid (6.90 × 10−3 mol chitosan: 1 mol citric acid). The chitosan cross-linker solution was cooled to 0°C and then added to 25 mL of corn oil previously maintained at 0°C, with stirring for 2 minutes. This emulsion was then added to 175 mL of corn oil maintained at 120°C, and cross-linking was performed in a glass beaker under vigorous stirring (1000 rpm) for 40 minutes. The microspheres obtained were filtered and then washed with diethyl ether, dried, and sieved.

Glutaraldehyde cross linking:

A 2.5% (w/v) chitosan solution in aqueous acetic acid was prepared. This dispersed phase was added to continuous phase (125 mL) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (wt/vol) Span 85 to form a water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a 3- blade propeller stirrer). A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% v/v)

was added at 15, 30, 45, and 60 minutes. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed, first with petroleum ether (60°C80°C) and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde, respectively. The microspheres were then finally dried in vacuum desiccators.

PHASE SEPARATION COACERVATION TECHNIQUE

Phase separation method is specially designed for preparing the reservoir type of the system, i.e. to encapsulate water soluble drugs e.g. peptides, proteins, however, some of the preparations are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of polymer rich phase called the coacervates. In this technique, the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophilic or dissolved in polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution conditions by using methods such as salt addition, non solvent addition, addition of the incompatible polymer or change in pH. The process is carried out under continuous stirring to control the size of the microparticles.

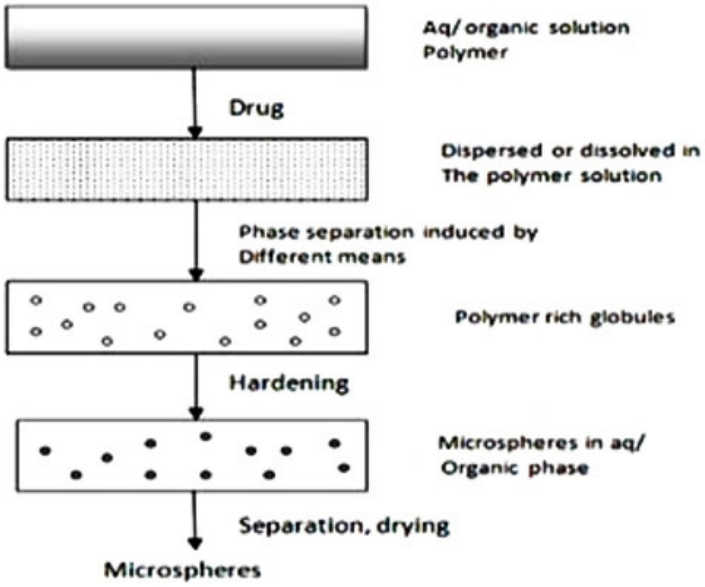


Fig 9: Phase Separation Method

IONIC GELATION METHOD

Ionotropic gelation is depend on the tendency of polyelectrolytes to cross connect to develop hydrogel beads often called gelispheres in the existence of counter ions. Gelispheres are Circular cross linked polymeric hydrophilic agent capable of substantial gelation and thickening in model biological fluids and drug release regulated by polymer relaxation via it. The hydrogel beads are formed by dumping a drug-laden polymeric solution into the polyvalent cations aqueous solution. The cations migrate through the drug-laden hydrophilic compounds, creating a three-dimensional lattice the moiety is ionically crosslinked. Biomolecules may also be placed into these gelispheres to maintain their three-dimensional form under moderate conditions.

PREFORMULATION STUDIES[9,10,11,28,29,30,31]

1.ORGANOLEPTIC PROPERTIES

Organoleptic properties of the drug were characterized on the basis of appearance, color, odor and taste by visual inspection.

2.MELTING POINT DETERMINATION

The melting point of a drug was determined by using digital melting point apparatus .In this method, a tiny amount of drug was introduced into a small capillary tube, attaching this to the stem of a thermometer centered in a

heating bath, heating the bath slowly, and observing the temperatures at which melting of drug begins and is completed. The melting point was recorded and compared with literature value.

3.DETERMINATION OF SOLUBILITY

Qualitative solubility

Qualitative solubility of drug in different solvent was determined according to (USP NF, 2007). Drug (10 mg) was accurately weighed and transferred into a 10 ml test tube then, it was dissolved in the respective solvents (1 ml each) such as distilled water, PBS (pH 7.4), methanol, ethanol, dimethylsulphoxide, isopropyl acetate, ether, acetone and hexane. The solubility (mg/ml) was observed by visual inspection and compared with that available in literature.

Quantitative solubility

Quantitative solubility analysis of drug was done by taking 10ml of each solvent and drug in 10 mg(s) into the solvent till saturation of solvent. Solutions were filtered and absorbance was recorded using UV spectrophotometer and the concentration of drug dissolved in respective solvents was calculated. Different solvents like distilled water and PBS (pH 7.4) were used for the solubility determination. This is done to determine the capacity of the solvent for dissolving the drug in it.

4.LIPOPHILICITY (PARTITION COEFFICIENT)

The partition coefficient of a chemical compound provides a thermodynamic measure of its hydrophilicity-lipophilicity balance. The partition coefficient of a substance between noctanol and water is referred to as log Po/w,

$$\textbf{Log } P_{Oct/wat} = \textbf{log } ([\textbf{solute}]_{octanol}/[\textbf{solute}]_{water}^{un\text{-}ionized})$$

5.FTIR SPECTROSCOPY

FTIR spectra of the pure drug were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). Sample were ground thoroughly with KBr powder in mortar and pestle, in a weight ratio of 1:100 and then pressed the mixture in dies set in pellet press under a hydraulic pressure of 15 tons for a minute. Release the pressure by rotating the side valve in anticlockwise direction to take of the pellet from the dies set. Then, the pellet was placed in the sample holder and spectral scanning was taken in the wavelength region between 4000 and 400 cm-1 at a resolution of 4 cm-1 and scan speed of 2 mm/sec13 .

6.DRUG EXCIPIENT COMPATIBILITY SCREENING BY FTIR

FTIR spectra of gum ghatti (Gg), polyvinyl alcohol (PVA), and a physical mixture of Gg: PVA: miltefosine in a weight ratio of 1:1:1 were obtained using FTIR spectrometer (FTIR-8400S spectrophotometer, Shimadzu, Japan). Each sample were ground thoroughly with KBr powder in a weight ratio of 1:100 and then pellets were prepared using a hydraulic pellet press under a hydraulic pressure of 15 tons for a minute. Then, the pellet was placed in the sample holder and spectral scanning were taken in the wavelength region between 4000 and 400 cm-1 at a resolution of 4 cm-1 and scan speed of 2 mm/sec. IR spectra of the physical mixture was then compared with the IR spectra of pure drug and polymer to find out the evidence of any compatibility.

7.DIFFERENTIAL SCANNING CALORIMETRIC (DSC) STUDY

DSC analysis was performed on the pure drug by using Perkin-Elmer instrument. Initially, the moisture was removed by heating the samples and then, each sample (about 3-7 mg) was accurately weighed into platinum crucible 40 µl aluminium pan in hermetically sealed condition, where alpha alumina powder used as a reference. Thermograms were recorded from 50°C to 300°C at the heating rate of 20°C/min under a constant flow of an inert nitrogen gas atmosphere with the flow rate of 20 ml/min15 . The DSC spectra used to find out the exotherm peak position or any change in their position compared with the standard spectra.

EVALUATION OF MICROSPHERE[1,24,26,27,28.]

PHYSIOCHEMICAL EVALUATION

CHARACTERIZATION PARTICLE SIZE AND SHAPE

Light microscopy (LM) provides a control over coating parameters in case of double walled microspheres. The microspheres structures can be visualized before and after coating and the change can be measured microscopically.Scanning electron microscopy (SEM) allows investigations of the microspheres surfaces and after particles are cross-sectioned, it can also be used for the investigation of double walled systems.

1. **Attenuated total reflectance FT-IR 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 ATRFT-IR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions.

2. **Density determination:**

The density of the microspheres can be measured by using a multi volume pycnometer. Accurately weighed sample in a cup is placed into the multi volume pycnometer. Helium is introduced at a constant pressure in the chamber and allowed to expand. This expansion results in a decrease in pressure within the chamber. Two consecutive readings of reduction in pressure at different initial pressure are noted. From two pressure readings the density of the microsphere carrier is determined.

3. **Isoelectric point:**

The micro electrophoresis is an apparatus used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined. The mean velocity at different PH values ranging from 3-10 is calculated by measuring the time of particle movement over a distance of 1 mm. By using this data the electrical mobility of the particle can be determined.

4. **Entrapment efficiency:**

Microspheres containing of drug (5mg) were crushed and then dissolved in distilled water with the help of ultrasonic stirrer for 3 hr., and was filtered then assayed by UV-VIS spectroscopy. Entrapment efficiency is equal to ratio of actual drug content to theoretical drug content.

% Entrapment = Actual content/Theoretical content x 100

5. **Swelling index:**

This technique was used for Characterization of microspheres were performed with swelling index technique Different solution (100mL) were taken such as (distilled water, buffer solution of pH(1.2, 4.5, 7.4) were taken and microspheres (100mg) were placed in a wire basket and kept on the above solution and swelling was allowed at 37°C changes in weight variation between initial weight of microspheres and weight due to swelling was measured by taking weight periodically and soaking with filter paper.

6. **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. The angle of contact is measured at the solid/air/water interface. The angle of contact is measured by placing a droplet in a circular cell mounted above objective of inverted microscope. Contact angle is measured at 200C within a minute of deposition of microspheres.

7. **Scanning electron microscopy (SEM) study:**

The Samples were analyzed through SEM and it was well qualified from a back scattered electron sensor for image analysis and conducting the x - Ray diffraction analysis (EDXA) for elemental structure determination where particular elements have been identified. In this method the sample was scanned in parallel lines using a centered electron beam. Microspheres were then placed on a sample holder for SEM characterization preceded by coating with a conductive metal like platinum or zirconium using a sputter coater. The sample was then scanned with a guided, fine electron beam. The surface properties of the sample were derived from the secondary electrons leaked from the sample surface.

DETERMINATION OF DRUG CONTENT

An accurately weighed quantity of the microspheres equivalent to 20mg of drug was taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of phosphate buffer (pH 6.8) repeatedly. The extract was transferred to a 100ml volumetric flask and the volume was made up using phosphate buffer (pH 6.8). The solution was filtered and the absorbance was measured after suitable dilution at 233 nm by using UV-visible spectrophotometer. The drug content was estimated in triplicate using a calibration curve constructed in the same solvent.

DETERMINATION OF PERCENTAGE YIELD

The prepared microspheres were collected and weighed. The measured weight was divided by the total amount of all non- volatile components which were used for the preparation of the microspheres. The yield percentage was determined with the following formula:

Yield (%) = Weight of microspheres/Total expected weight of drug and polymer X 100

MICROMERITIC PROPERTIES

Microspheres were characterized for their micromeritic properties such as bulk density, tapped density, compressibility index, Hausner`s ratio, and angle of repose.

1. **Bulk density**

An exact quantity „M‟ of microsphere was taken and was placed into a measuring cylinder. Volume „V‟ occupied by the microspheres was noted without disturbing the cylinder and bulk density was calculated using the following equation;

Bulk density (Pb) = M/V.

2. **Tapped density**

To calculate the tapped density, a cylinder containing a specified amount (M) of microspheres was exposed to a fixed number of taps (about 100) until the bed of microspheres reached the minimum. The following equation was used to obtain the tap density and final volume after tapping.

Tapped Density (Pp) = M/Vo

3. **Angle of repose**

This characteristic was discovered to predict flowability. The angle of repose of the microspheres was calculated using the fixed funnel method and the formula,

Angle of repose (ϕ) = tan-1 [2h/d] - h

Where, h is height and d is the diameter of the microsphere pile that is on a paper after making the microspheres flow from the glass funnel.

4. **Carr's index or % compressibility**

A high Carr's index is indicative of the tendency to form bridges can be calculated by using following formula:

Carr's index or %compressibility Index or C= Vt – Vb x 100 Vt

Where, Vt = Tapped density, Vb = Bulk density

5. **Hausner's ratio**

Hausner's ratio is measures of the propensity of a powder to be compressed and the flow ability of granule. A higher Hausner's ratio indicates greater cohesion between particles.

Hausner's Ratio = Vt / Vb

Where, Vt = Tapped density, Vb = Bulk density

IN VITRO METHODS

1. **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.

2. **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 abuffer. 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.

3. **Modified keshary chien cell**

A specialized apparatus was designed in the laboratory. It comprised of a KesharyChien cell containing distilled water (50ml) at 370 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.

4. **Dissolution apparatus**

Standard USP or BP dissolution apparatus have been used to study in vitro release profiles using rotating elements, paddle 26 and basket 27 Dissolution medium used for the study varied from 100-500 ml and speed of rotation from 50-100 rpm.

5. **Stability studies**

By placing the microspheres in screw capped glass container and stored them at following conditions: ™

- Ambient humid condition ™
- Room temperature (27+/-2 0C) ™
- Oven temperature (40+/-2 0C) ™
- Refrigerator (5 0C -80C).

It was carried out of a 60 days and the drug content of the microsphere was analysed.

IN VIVO METHODS

1. 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 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.

2. Buccal absorption test

The buccal absorption test was developed by Beckett & Triggs 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.

APPLICATION OF MICROSPHERES IN PHARMACEUTICAL INDUSTRY[3,4,7,8]

Microspheres developed using polymer exhibits favourable biological behaviour such as bioadhesion, permeability-enhancing properties, and interesting physicochemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles. e.g. Chitosan, Alginate, Gelatin .

1. ORAL DRUG DELIVERY:

The ability of microspheres containing polymer to form films permit its use in the formulation of film dosage forms, as an alternative to pharmaceutical tablets. The pH sensitivity, coupled with the reactivity of the primary amine groups, make microspheres more suitable for oral drug delivery applications. e.g. Chitosan, Gelatin.

2. GENE DELIVERY:

Microspheres could be a useful oral gene carrier be-cause of its adhesive and transport properties in the GI tract. e.g. Chitosan, Gelatin, viral vectors, cationic liposome, polycation complexes and Gene therapy with DNA plasmids and also delivery of insulin. It is also beneficial in vaccine delivery also as the prerequisite of a vaccine is protection against the microorganism or its toxic product. Biodegradable delivery system for vaccines that are given by Parenteral route may overcome the shortcoming of conventional vaccines. Several parenteral vaccines have been encapsulated in biodegradable polymeric microspheres, including the tetanus and diphtheria vaccine.

3. NASAL DRUG DELIVERY:

Polymer based drug delivery systems, such as micro-spheres, liposomes and gels have been demonstrated to have good bioadhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailability and residence time of the drugs to the nasal route. e.g. Starch, Dextran, Albumin, Chitosan + Gelatin .

4. INTRATUMORAL AND LOCAL DRUG DELIVERY:

In order to deliver paclitaxel at the tumor site in therapeutically relevant concentration, polymer films are fabricated. Mixture of drug has promising potential for use in controlled delivery in the oral cavity e.g. Gelatin, PLGA, Chitosan.

5. BUCCAL DRUG DELIVERY:

Polymer is an excellent polymer to be used for buccal delivery because it has muco / bioadhesive properties and can act as an absorption enhancer. Chitosan, Sodium alginate.

6. GASTROINTESTINAL DRUG DELIVERY:

Polymer granules having internal cavities prepared by de acidification when added to acidic and neutral media are found buoyant and provided a controlled release of the drug e.g. Eudragit, Ethyl cellulose + Carbopol BSA, Gelatin.

7. TRANSDERMAL DRUG DELIVERY:

Polymer has good film-forming characteristics. The release profile from of the devices is impacted by the membrane thickness as well as crosslinking of a film. Chitosan-alginate polyelectrolyte structure has also been prepared in-situ in beads and microspheres for potential uses in packaging, controlled release systems and surgical instruments. Polymer gel beads are an impressive highly biocompatible vehicle for chemotherapy of inflammatory cytokines for medications like prednisolone that also showed extended release action enhancing treatment effectiveness. The amount of drug discharge was found to also be depend on the characteristics of cell wall used. A mixture of chitosan membrane and chitosan hydrogel known to contain lidocaine hydrochloride, a local anaesthetic is a great comprehensive process for controlled drug release and release kinetics.

8. MONOCLONAL ANTIBODIES:

Monoclonal antibodies or targeting microspheres are biologically immune microspheres. This type of targeting is used to achieve selective targeting to specific sites of the body organ. Monoclonal Antibodies are extremely specific molecules which bind to the specific part of the body system through which absorption takes place via

- Non specific adsorption and specific adsorption
- Direct coupling
- Coupling via reagents

9. IMAGING:

Diameter of microspheres plays an important role in determining the imaging of targeted sites using already labelled microspheres having radio activity. The microspheres injected via IV route apart from the portal vein will usually become entrapped in the area of lungs. This phenomenon is specifically used for scintigraphic imaging of tumour masses in lungs using human serum albumin microspheres.

10. TOPICAL POROUS MICROSPHERES:

Microsponges are porous microspheres having myriad of interconnected voids of size range 5 to 300µm. these sponges having capacity to engulf the various active ingredients such as emollients, fragrances, essential oils which is used for the topical application.

11. MEDICAL APPLICATION:

- Release of proteins, peptides and hormones over the extended period of time.
- Passive targeting of leaky tumor vessels, active targeting of tumor cells, antigens, by parenteral route.
- Magnetic Microspheres can be used for used for stem cell extraction and bone marrow purging.
- Used for Various diagnostic test for infectious disease like bacterial, viral and fungal.

12. RADIOACTIVE APPLICATION:

It can be beneficial for the embolisation of various liver and spleen tumors which is used for radio synvectomy of local radiotherapy, arthritis, imaging of liver, bone marrow, local radiotherapy and even imaging of thrombus in deep vein thrombosis can be done.

13. VACCINE DELIVERY

The precondition of a vaccine is safety toward the microbes and its harmful component. An ideal vaccine should satisfy this same necessity of effectiveness, protection, affordability in application and charge. The aspect of protection and avoidance of severe effects is a complicated.The aspect of safeness and the extent of the manufacturing of antibody responses are intently linked to mode of application. Biodegradable delivery technology for vaccines which are provided by intravenous path may resolve the shortcoming of this same conventional vaccines. The involvement in parenteral (subcutaneous, intramuscular, intradermal) carrier exists even though those who offer significant benefits.

14. COLONIC DRUG DELIVERY:

Polymer has been used for the specific delivery of insulin to the colon e.g. Chitosan.

15. VAGINAL DRUG DELIVERY:

Polymer, modified by the introduction of thioglycolic acid to the primary amino groups of the polymer is widely used for the treatment of mycotic infections of the genitourinary tract e.g. Chitosan, Gelatin, PLGA.

16. TARGETING BY USING MICRO PARTICULATE CARRIERS:

The concept of targeting is a well established dogma, which is gaining full attention now a days. The response produced by the drug depends on its access and interaction with receptor usually pellets method is reported which can be prepared by using extrusion / Spheronization technology e.g. microcrystalline cellulose (MCC) and chitosan.

17. OTHER APPLICATIONS

Microspheres are used for membrane technology developed for mass spectrometry, cell biology, cell biology; Fluorescent connected Immuno-Sorbent Assay. Yttrium could be used for standard treatment of hepatocellular carcinoma and even used besides pre transplant management of HCC with promising results. Applications of microencapsulation in other industry sectors are various. Carbonless copying paper, photosensitive paper, microencapsulated fragrances such as "scent-strips" (also known as "snap-n-burst") and microencapsulated aromas ("scratch-n-sniff"') are the best known microencapsulated products. These other products are usually prepared by the use of gelatin – acacia coacervation complex. Scratch-n-sniff has been used in children's literature and in the development of nutrition and cosmetics fragrance advertising. Microcapsules also are heavily included as diagnostic tests, for example, temperature-sensitive microcapsules for temperature dependent visual detection of cancer. In the biotech industry microcapsules microbial cells are used for the production of recombinant and proteins.

BENEFITS OF MICROSPHERES IN ANTIDIABETIC THERAPY[3,7,20,21]

IMPROVED BIOAVAILABILITY

Microspheres can enhance the bioavailability of antidiabetic drugs by protecting them from degradation and ensuring controlled release.

CONTROLLED DRUG RELEASE

Microspheres can provide sustained release of antidiabetic drugs over a prolonged period, reducing the need for frequent dosing and improving patient compliance.

REDUCED TOXICITY

Microspheres can reduce the toxicity associated with antidiabetic drugs by minimizing peak plasma concentrations and preventing drug accumulation.

TARGETED DELIVERY

Microspheres can be designed to target specific sites in the body, such as the liver or pancreas, to enhance the efficacy of antidiabetic therapy.

ENHANCED THERAPEUTIC EFFICACY

Microspheres can improve the therapeutic efficacy of antidiabetic drugs by providing a consistent and sustained release of the active ingredient.

IMPROVED PATIENT COMPLIANCE

Microspheres can improve patient compliance by reducing the frequency of dosing and minimizing side effects.

PROTECTION FROM ENZYMATIC DEGRADATION

Microspheres can protect antidiabetic drugs from enzymatic degradation, ensuring that the active ingredient remains effective for a longer period.

ABILITY TO DELIVER MULTIPLE DRUGS

Microspheres can be designed to deliver multiple antidiabetic drugs simultaneously, providing a more comprehensive treatment approach.

BIOCOMPATIBILITY AND BIODEGRADABILITY

Microspheres can be prepared from biocompatible and biodegradable materials, ensuring safe and non-toxic delivery of antidiabetic drugs.

CONCLUSION

Microspheres have emerged as a versatile tool in drug delivery system for antidiabetic therapy and various fields, including pharmaceuticals, biotechnology, cosmetics, and materials science. Additionally, microspheres have shown great promise in tissue engineering, regenerative medicine, and diagnostic applications. Their ability to provide controlled and sustained release of antidiabetic drugs, improve bioavailability, reduce toxicity, and enhance therapeutic efficacy makes them an attractive option for managing diabetes. It may include oral, targeted, sustained, topical, naso-pulmonary and various biotechnology applications such as gene therapy etc. Overall, the use of microspheres in antidiabetic therapy has the potential to revolutionize the management of diabetes by providing a more effective, safe, and patient-friendly treatment approach. Further research and development are needed to fully explore the potential of microspheres in antidiabetic therapy and to translate these findings into clinical practice.

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