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Review Article

A Review on Microsphere – Types, Characterization and Formulation Consideration

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ABSTRACT
Microspheres are solid spherical particles ranging in size from 1-1000µm. They are spherical free flowing particles consisting of proteins or synthetic polymers, which are biodegradable in nature. There are two types of microspheres; microcapsules and micrometrics, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and in micrometrics entrapped substance is dispersing throughout the microspheres matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through particle matrix have the potential for the controlled release of drug. They are made up of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products.It is the 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. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour.

KEYWORDS: Microspheres, controlled release, micro-particulates, therapeutic efficacy, novel drug delivery

INTRODUCTION

Microspheres are homogeneous, monolithic particles which improve the treatment by providing localization of the drug at the site of action and by prolonging the drug release. [6] There are various approaches in delivering a therapeutic substance to the target site in a sustainedcontrolled release fashion.One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm. [2]

In contrast to drug delivery system, the word novel is searching something out of necessity. The drug has to be delivered for a prolonged period of time and many medicines have to be taken simultaneously in case of chronic patients. Frequent administration of drug is necessary when those have shorter half-life and all these leads to decrease in patient's compliance. [1] In order to overcome the above problems, various types of controlled release dosage forms are formulated and altered, so that patient compliance increase through prolonged effect, adverse effect decreases by lowering peak plasma concentration.The controlled release dosage form maintaining relatively constant drug level in the plasma byreleasing the drug at a predetermined rate for an extended period of time. One such in Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system.[2] Microspheres are defined as"Monolithic sphere or therapeutic agent distributedthroughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level.[4] Ithas a particle size of (1-1000nm).[3] Further, currently available slow release oral dosage forms, such as enteric coated/ double-layer tablets which release the drug for 12-24 hours still result in inefficient systemic delivery of the drug andpotential gastrointestinal irritation. Microencapsulation for oral use has been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, multi-particulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided.4 Microencapsulation is used to modify and retard drug release. Due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa.[3][4]

HISTORY

For more than a century, clinicians have worked to find a permanent treatment for scars, wrinkles, tissue deficiencies, and other contour abnormalities. Current solutions to these problems range from surgical procedures to minimally invasive injectable. Over the years, injectable fillers have evolved to include both short-term and long lasting products. The use of long-lasting, injectable augmenting agents for the correction of facial soft contour deficiencies is a rapidly expanding field fueled by an increasing public awareness of this minimally invasive alternative to surgery. The need to design new products stems from disappointment associated with many current injectable. Problems with existing products include short duration of action, migration of the injectable material, allergy to components of the filler, and other adverse events. [1] Artecoll is long-lasting dermal filler that has

been used extensively throughout the world for over a decade and has proven to be safe, effective, longlasting, and gratifying to both patients and physiciansalike. [2] The most significant complication following implantation with Artecoll, albeit rare, is granuloma formation.[5]

TYPES OF MICROSPHERES

Bio-Adhesive Microspheres:

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action. Preparation of bio-adhesive microspheres would be advantageous to have means for providing an intimates contact of the drug delivery system with the absorbing membranes. Poly-carbophil selected as polymer in the production of bio-adhesive microspheres due to its excellent bio-adhesive properties.

Magnetic Microspheres

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. [5] The different type are Therapeutic magnetic microspheres: Used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.[6]Diagnostic microspheres: Can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides. [7]

Although direct measurements of the magnetic susceptibility of magnetic microspheres can be made with a magnetic Faraday balance , as well as with MRI techniques , the results only hint at the microspheres' behavior in vivo, as for example after injection into a person's blood system. For such applications, magnetic susceptibilities only give an approximate indication of magnetic 'responsiveness' because magnetic microspheres, nano spheres and particles not only span a large range of sizes, but are also made from many different matrix materials incorporating different types and amounts of magnetic compounds. In addition, these magnetic compounds can be distributed quite differently within the microsphere. Other factors such as the solvent system, the particle's porosity, its density, surface coating and aggregation tendencies can further influence its overall magnetic responsiveness. For clinical applications in the blood circulation of a person, an all-inclusive test would be advantageous that allows choosing the most appropriate magnetic microspheres for a certain application. In addition to the direct methods, more elaborate but indirect systems have been applied to measure the magnetic susceptibility of magnetic microspheres in suspension. One of these systems is field flow fractionation (FFF) where an external magnetic field is applied perpendicular to the flow direction. The interaction of hydrodynamic and magnetic forces separates the particles. The particles' retention ratio then allows for the calculation of their magnetic susceptibility. A further improvement in analysis was reached by a 'cell tracking velocimetry' system. In this system, the movement of magnetic microspheres or magnetically labeled cells in a well-defined magnetic field is videotaped. The velocity of each particle passing the camera in laminar flow was determined by tracking its movement. By comparing the data to magnetic particles of calibrated magnetic susceptibility, information about their magnetic mobility and susceptibility was obtained.

Floating Microspheres

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric contentand increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of striking and dose dumping. One another way it produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug (ketoprofen) given through this form. [8]

Radioactive Microspheres

Radio mobilization therapy microspheres sized 10-30 nm are of larger than capillaries and get stepped in first capillary bed when they come across. They are injected to the arteries that lead to tumor of interest. Radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. [9] It differs from drug delivery system, as radio activity is not released from microspheres but acts from within radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, βemitters, γ emitters. [10] Radioactive microspheres used in diagnostic application are given in table 1.

Application	Type of radioactive microspheres used
Blood flow measurements	Polystyrene-microsphere labeled with the □-emitters ^{141}Ce, ^{114}mIn, ^{85}Sr, ^{57}CO, ^{51}Cr, etc.
Liver and spleen imaging	^{99m}Tc-macro-aggregated albumin
Bone marrow imaging	^{99m}Tc-sulfur colloid
Bone marrow imaging	^{99m}Tc-sulfur colloid
Gated blood pool study	^{111}In- or ^{51}Cr-labeled red blood cells
Infection localization	^{111}In-labeled leukocytes ^{111}In-labeled liposomes ^{99m}Tc-labeled liposomes
Investigation of the biodistributionand fate of (drug-loaded) microspheres	^{3}H, ^{14}C-labeled microspheres

Future developments with radioactive microspheres include the preparation of more homogenous, mono-sized microspheres that will allow for better and more reliable bio distribution results. Materials are being tested which are more biocompatible and ideally even biodegrade after delivering the radioisotopes. The labeling methods are being improved such that highly stable radioactive microspheres can be produced in a single, short step using a simple radiolabeling kit. No purification of the radioactive microspheres is needed, and the radioisotope should be

chosen for radio biologic and diametric reasons. [9] Various radioactive microspheres used in therapeutic application are given in table 2.

Polymeric Microspheres

The different types of polymeric microspheres can be classified as Biodegradable polymeric microspheres and Synthetic polymeric microspheres.Different types of polymers and their application are given in table 3.

Biodegradable polymeric microspheres

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bio adhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymer [10] and the release pattern in a sustained manner. The main drawback is, in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release. However they provide wide range of application in microsphere based treatment.

Table 2 Radioactive microspheres for therapeutic applications [16]

Application	Type of radioactive microspheres used
Radioembolization of liver and spleen tumors	^{90}Y-glass microsphere ^{186}Re/^{188}Re-glass microspheres ^{188}Re-Aminex A27 microspheres 35S-colloid
Radiosynoviorthesis of arthritic joints	90Y-resin microspheres 186Re-sulfur-colloid 188Re-macro-aggregated albumin 90Y-labeled poly(lactic acid) microsphere
Local radiotherapy	165Dy-acetylacetone poly(lactic acid) microspheres 166Ho-acetylacetone poly(lactic acid) microspheres 186Re/188Re-labeledpoly(lactic acid) microspheres
Peritoneal ovarian tumor metastases treatment, cystic brain tumors Local restenosis prevention in coronary arteries	32P-chromate 141Ce microspheres

Synthetic polymeric microspheres

The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc. and proved to be safe and biocompatible. [11]But the main disadvantage of these kind of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage. [12]

CHARACTERIZATION OF MICROSPHERES

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

Percent yield

The percent yield of each batch of microsphere was obtained on weight basis of microspheres with respect to the total expected weight of drug and polymer

$$\%\text{Yield} = \frac{\text{weight of microspheres}}{\text{Total weight of drug}} \times 100$$

Table 3 Various types of polymers and their application [10]

POLYMER	MECHANISM
Modified starch, HPMC, Carbopol 974P	Slower release of drug.
Ethyl Cellulose	Controlled release for longer period of time.
PLGA, Chitosan	Vaccine delivery.
PLA, PLGA, Starch cyano acrylateetc (PEG-) liposomes.	Drug delivery without toxic side effects.
Magnetic polystyrene microspheres	Specific cell labeling.
Polymer resins such as Agarosepolyacroline, sephadex	Affinity chromatography.
Chitosan coated PlGA microspheres	Targeted drug delivery
Polyvinyl alcohol, polyacrylamide	Adsorption of harmful substances in blood.

Particle size and shape

The most widely used procedures to visualize micro-particles are conventional light microscopy (LM) and scanning electron microscopy (SEM). Both can be used to determine the shape and outer structure of micro-particles. 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. SEM provides higher resolution in contrast to the LM. 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. Conflocal fluorescence microscopy1 is used for the structure characterization of multiple walled microspheres. Laser light scattering and multi size coulter counter other than instrumental methods, which can be used for the characterization of size, shape and morphology of the microspheres.

Electron spectroscopy for chemical analysis:

The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA). ESCA provides a means for the determination of the atomic composition of the surface. The spectra obtained using ECSA can be used to determine the surfacial degradation of the biodegradable microspheres.

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.

Density determination:

The density of the microspheres can be measured by using a multi volume pychnometer. Accurately weighed sample in a cup is placed into the multi volume pychnometer. 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 volume and hence the density of the microsphere carrier is determined.

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. The electrophoretic mobility can be related to surface contained charge, ionisable behavior or ion absorption nature of the microspheres.

Surface carboxylic acid residue

The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugates is prepared by the reaction of c14-glycine ethyl ester hydro chloride with the microspheres. The glycine residue is linked using the water soluble condensing 1-ethyl-3 (3-dimethyl amino propyl) carbidiimide (EDAC). The radioactivity of the conjugate is then measured using liquid scintillation counter. Thus the carboxylic acid residue can be compared and correlated. The free carboxylic acid residue can be measured for hydrophobic or hydrophilic or any other derivatized type of the microspheres.

Surface amino acid residue

Surface associated amino acid residue is determined by the radioactive C14-acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly. EDAC is used to condense the amino group and the C14 –acetic acid carboxylic acid residue. The method used for determining the free amino or the free carboxylic acid residues are based on indirect estimation, by measuring the radioactivity of the C14 having acetic acid or the glycine conjugate. The accuracy of the method however, depends on the time allowed for conjugation of the radioactive moiety and the reactivity of free functional group.

Capture efficiency

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

% Entrapment = Actual content/Theoretical content x 100

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. The advancing and receding angle of contact are 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.

***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 [13].

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 overhead 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 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.

Dissolution apparatus

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

Other methods

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

***In vivo* methods**

Methods for studying the permeability of intact mucosa comprise of techniques that exploit the biological response of the organism locally or systemically and those that involve direct local measurement of uptake or accumulation of penetrants 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.

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, cat, hamster, pigs, and sheep have been reported. In general, the procedure involves anesthetizing the animal followed by administration of the dosage form. In case of rats, the esophagus is ligated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed.

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 multicomponent 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* correlations**

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.

Percent of Drug Dissolved *In Vitro* Vs Peak Plasma Concentration

One of the ways of checking the in vitro and in vivo correlation is to measure the percent of the drugreleased from different dosage forms and also to estimate the peak plasma concentrations achieved by them and then to check the correlation between them. It is expected that a poorly formulated dosage form releases amount of drug than a well formulated dosage form, and, hence the amount of drug available for absorption is less for poorly formulated dosage form than from a well formulateddosage form.

Percent of Drug Dissolved Vs Percent of Drug Absorbed

If the dissolution rate is the limiting step in the absorption of the drug, and is absorbed completely after dissolution, a linear correlation may be obtained by comparing the percent of the drug absorbed to the percent of the drug dissolved. If the rate limiting step in the bioavailability of the drug is the rate of absorption of the drug, a change in the dissolution rate may not be reflected in a change in the rate and the extent of drug absorption from the dosage form.

Dissolution Rate Vs Absorption Rate

The absorption rate is usually more difficult to determine than the absorption time. Since the absorption rate and absorption time of a drug are inversely correlated, the absorption time may be used in correlating the dissolution data to the absorption data. In the analysis of in vitro and in vivo drug correlation, rapid drug absorption may be distinguished from the slower drug absorption by observation of the absorption time for the dosage form. The quicker the absorption of the drug theless is the absorption time required for the absorption of the certain amount of the drug. The time required for the absorption of the same amount of drug from the dosage form is correlated.

Percent of Drug Dissolved Vs Serum Drug Concentration

For drugs whose absorption from GIT is dissolution rate limited, a linear correlation may be established between the percent of drug dissolved at specified times and the serum drug concentrations at corresponding times.

Percent of Drug Dissolved Vs Percent of the Dose Excreted in urine

The percent of a drug dissolved and the percent of drug absorbed are linearly correlated. There exists a correlation between the amount of drug in body and the amount of drug excreted in the urine. Therefore, a linear relation may be established between the percent of the drug dissolved and the percent of the dose excreted in the urine.[11]

Advantages

1. 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.
2. Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.
3. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour [4, 7].
4. The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles *in vivo.*
5. Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intracellular.
6. Blood flow determination. [11]
7. Albumin Microspheres provide constant and prolonged therapeutic effect.
8. Reduces the dosing frequency and thereby improve the patient compliance.

9. They could be injected into the body due to the spherical shape and smaller size.
10. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
11. Albumin microsphere morphology allows a controllable variability in degradation and drug release.
12. Reduces GI toxic effects.
13. Albumin has non-antigenic property and ability to control the physicochemical characteristics of the microspheres produced, depending on the cross-linking methods and characteristics of cross-linking agent.

Disadvantages

1. Dosage forms of this kind should not be crushed or chewed
2. Larger size of extended release products may cause difficulties in ingestion or transit through the gut.
3. Possibility of distal intestinal toxicological manifestations because of sustained release and enteric coated NSAID formulations.

Applications

Microspheres in vaccine delivery

The prerequisite of a vaccine is protection against the microorganism 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 reactionis a complex issue [4, 8]. 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 advantagesincluding:

1. Improved antigenicity by adjuvant action
2. Modulation of antigen release
3. Stabilization of antigen.

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.

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

1. Nonspecific adsorption
2. Specific adsorption
3. Direct coupling
4. Coupling via reagents

Chemoembolisation

Chemoembolisation 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. Chemoembolisation is an extension of traditional percutaneous embolisation techniques.

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 labeled 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.

Topical porous microspheres

Micro-sponges are porous microspheres having myriad of interconnected voids of particle size range 5-300 μm. These micro-sponges 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. Micro-sponges consist of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner.

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.

Medical applications

1. Release of proteins, hormones and peptides over extended period of time.

2. Gene therapy with DNA plasmids and also delivery of insulin.
3. Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid,diphtheria, birth control.
4. Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra-arterial/ intravenous application.
5. Tumour targeting with doxorubicin and also treatments of leishmaniasis.
6. Magnetic microspheres can be used for stem cell extraction and bone marrow purging.
7. Used in isolation of antibodies, cell separation and toxin extraction by affinity chromatography.
8. Used for various diagnostic tests for infectious diseases like bacterial, viral, and fungal.

Radioactive applications

1. Can be used for radioembolisation of liver and spleen tumours.
2. Used for radiosynvectomy of arthritis joint, local radiotherapy, interactivity treatment.
3. Imaging of liver, spleen, bone marrow, lung etc and even imaging of thrombus in deep vein thrombosis can be done.

Other applications

1. Fluorescent microspheres can be used formembrane based technologies for flowcytometer, cell biology, microbiology,Fluorescent Linked Immuno-Sorbent Assay.
2. Yttrium 90 can be used for primary treatment of hepatocellular carcinoma and also used for pretransplant management of HCC with promising results.[9]

Preparation of Microspheres

The microspheres can be prepared by using any of the several techniques given below but choice of the technique mainly depends on the nature of the polymer used, the drug, the intended use and the duration of therapy.

Single Emulsion Technique

The micro particulate carriers of natural polymers i.e., those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium e.g., oil. In the second step of preparation 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 agent used include gluteraldehyde, formaldehyde, terephthaloyl chloride, di-acid chloride, etc.Cross linking by heat is carried out by adding the dispersion, to previously heated oil. Heat denaturation is however, not suitable for the thermo labile drugs while the chemical cross-linking suffers disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation.

Double Emulsion Technique

Involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to the water-soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as the 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 then subjected to the homogenization or the sonication before addition to the aqueous solution of the polyvinyl alcohol (PVA). This results in the formation of the double emulsion. The emulsion is then subjected to the solvent removal either by solvent evaporation or by solvent extraction process. The solvent evaporation is carried out by maintaining emulsion at reduced pressure or by stirring the emulsion so that the organic phase evaporates out. In the latter case, the emulsion is add to the large quantity of water (with or without surfactant) into which organic phase diffuses out. The solid microspheres are subsequently obtained by filtration and washing. A number of hydrophilic drugs like luteinizing hormone releasing hormone (LH-RH) agonist; vaccines.

POLYMERIZATION TECHNIQUES

The polymerization techniques used for the preparation of the microspheres are mainly classified as:

Normal polymerization

Bulk polymerization: A monomer or a mixture of monomer along with the initiator is usually heated to initiate the polymerization and carry out the process. The catalyst or the initiator is added to their action mixture to facilitate or accelerate the rate of the reaction. The polymer so obtained may be molded or fragmented as microspheres. For loading of drug, adsorptive drug loading or adding drug during the process of polymerization may be adopted.

The suspension polymerization: It is carried out by heating the monomer or mixture of monomers with active principles (drugs) as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives.

The emulsion polymerization: However, differs from the suspension polymerization as due to presence of the initiator in the aqueous phase, which later on diffuses to the surface of the micelles or the emulsion globules.

Interfacial polymerization

In Interfacial polymerization technique two reacting monomers are employed; one of which is dissolved in thecontinuous phase while the other being dispersed inthe continuous phase. The continuous phase is generallyaqueous in nature through which the second monomer isemulsified. The monomers present in either phase diffuserapidly and polymerize rapidly at the interface. Twoconditions arise depending upon the solubility of formedpolymer in the emulsion droplet. If the polymer is solublein the droplet it will lead to the formation of the monolithic type of the carrier on the hand if the polymeris insoluble in the monomer droplet, the formed carrier isof capsular (reservoir) type.The degree of polymerization can be controlled by thereactivity of the monomer chosen, their concentration,and the composition of the vehicle of either phases and bythe temperature of the system. Controlling the droplets orglobules size of the dispersed phase can control theparticle size. The

polymerization reactions can becontrolled by maintaining the concentration of themonomers, which can be achieved by addition of anexcess of the continuous phase. The interfacialpolymerization is not widely used in the preparation ofthe micro-particles because of certain drawbacks, which areassociated with the process such as:

1. Toxicity associated with the unreacted monomer
2. High permeability of the film
3. High degradation of the drug during the polymerization
4. Fragility of microcapsules
5. Non-biodegradability of the micro-particles

PHASE SEPERATION AND COACERVATION

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. In matrix type device, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the coacervates. The coacervation can be brought about by addition of the third component to the system which results in the formation of the two phases, one rich in the polymer, while the other one. In this technique, the polymer is first dissolved in a suitable solvent and then making its aqueous solution disperses drug. Phase separation is then accomplished by changing the solution conditions by using any of the method mentioned above. The process is carried out under continuous stirring to control the size of the micro-particles. The process variables are very important since the rate of achieving the coacervate 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 as the process of microsphere formation begins the polymerize globules start to stick and form the agglomerates. Thus the process variable critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

SPRAY DRYING AND CONGEALING

Spray drying and spray congealing 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 the 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 small droplets or the fine mist from which the solvent evaporates leading to the formation of microspheres in a size range 1-100μm. Micro particles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying.

SOLVENT EXTRACTION

This method is used for the preparation of micro-particles, involves the removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvent such as isopropanol; organic phase is removed by extraction with water. The process decreases the hardening time for the microspheres. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer.

QUASSI EMULSION SOLVENT DIFFUSION

A novel quasi-emulsion solvent diffusion method to prepare the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Micro-sponges can be prepared by a quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol (PVA). The internal phase is consisting of drug; ethyl alcohol and polymer is added at an amount of 20% of the polymer in order to facilitate the plasticity. At first, the internal phase is prepared at 60ºC and added to the external phase at room temperature. After emulsification, the mixture is continuously stirred for 2 hours. Then the mixture can be filtered to separate the micro-sponges. The product is then washed and dried by vacuum oven at 40ºC for 24hours.

CONCLUSION

In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective in vivo delivery and supplements as miniature versions of diseased organ and tissues in the body. New applications for microspheres are discovered every day such as Assay, Buoyancy, Ceramics, Cosmetics, Drug delivery, Electronic paper, Insulation, Personal Care, Spacers, Standards, Retro-reflective, Thickening Agent etc.

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