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## Delivery of Macromolecular Drugs using Microsphere Technology

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*Introduction-* Recent years have witnessed development of protein-based therapeutic agent as the main focus of biotechnology industry. Progress made in biotechnology, genomics and combinatorial chemistry have led to the discovery of a wide variety of active moiety for specific therapeutics application. However, the common problems associated with these active moieties such as poor stability, low solubility, high potency etc, have paved way for developing a new method of drug delivery system. Considerable attention was made for the development of new drug delivery technology in recent research. The reason for developing a new drug delivery system is to apply in some clinical situations, where a constant drug delivery rate is insufficient, like delivery of insulin for patients with diabetes mellitus, gastric acid inhibitors for ulcer control, anti arrhythmic for patients with heart rhythm disorders, nitrates for patients with angina pectoris, birth control, immunization, cancer chemotherapy, selective  $\beta$ -blockade and general hormone replacement. The treatment for these clinical conditions could be optimized through the use of a novel delivery system (NDDS) (1). For delivering genetically engineered pharmaceuticals like peptides and proteins to the site of action, without incurring biological inactivation, need new system of delivery. Targeting the active moiety for better therapeutic efficacy can improve patient compliance by reducing the size and number of doses. Over the past decades major development were made in systemic delivery of protein and peptides by oral, transdermal and pulmonary routes (2-3).

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# Delivery of Macromolecular Drugs using Microsphere Technology

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## I. INTRODUCTION

Recent years have witnessed development of protein-based therapeutic agent as the main focus of biotechnology industry. Progress made in biotechnology, genomics and combinatorial chemistry have led to the discovery of a wide variety of active moiety for specific therapeutics application. However, the common problems associated with these active moieties such as poor stability, low solubility, high potency etc, have paved way for developing a new method of drug delivery system. Considerable attention was made for the development of new drug delivery technology in recent research. The reason for developing a new drug delivery system is to apply in some clinical situations, where a constant drug delivery rate is insufficient, like delivery of insulin for patients with diabetes mellitus, gastric acid inhibitors for ulcer control, anti arrhythmic for patients with heart rhythm disorders, nitrates for patients with angina pectoris, birth control, immunization, cancer chemotherapy, selective  $\beta$ -blockade and general hormone replacement. The treatment for these clinical conditions could be optimized through the use of a novel delivery system (NDDS) (1). For delivering genetically engineered pharmaceuticals like peptides and proteins to the site of action, without incurring biological inactivation, need new system of delivery. Targeting the active moiety for better therapeutic efficacy can improve patient compliance by reducing the size and number of doses. Over the past decades major development were made in systemic delivery of protein and peptides by oral, transdermal and pulmonary routes (2-3).

The controlled release technology aims to deliver the drug overcoming the difficulties associated with traditional methods of administration. Controlled delivery technology utilizes devices such as polymer based disks, microparticles or pellets, rods, which can encapsulate the drug and release in a controlled manner for a prolong period of time. Among the various devices for the controlled release, biodegradable polymer microspheres are the one most used, and can be administered by injecting into the blood stream via

intramuscular injection, subcutaneous, oral or by inhalation (4). Polymeric materials are the most diverse class of materials and offer benefits to patient health care and treatment (5). Very few of these systems have reached the market and its reason may be due to the failure in addressing the fundamental concepts of pulsatile delivery regardless of the route of administration.

Microspheres are defined as homogenous, monolithic particles with size ranging from 1-1000  $\mu\text{m}$ . They are employed as drug carriers for controlled release. Microspheres are also referred to as microparticles. Microsphere technologies have significant importance in biomedical application. Delivery of therapeutic moiety by microspheres will improve the therapeutic efficacy by providing localization of the active substance at the site of action, and thereby prolonging the release of drugs. Sensitive drugs like proteins and peptides may be protected against chemical and enzymatic degradation when the drugs are enclosed in microspheres (6-7). Microspheres are prepared from natural and synthetic materials. Different microspheres are commercially available in market like glass microspheres, polymer microspheres, and ceramic microspheres and are used for different applications.

Delivering therapeutic moiety inside the body utilizes polymeric microspheres as the carrier (8). The polymeric microspheres can be biodegradable or non biodegradable. Non biodegradable microspheres when injected inside can cause carrier toxicity when used over a long period of time however; biodegradable microspheres tend to exclude these problems and are better suited for parenteral applications. Biodegradable microspheres are prepared either from synthetic and natural polymers, and the main requirement for such products is that the degradation products should be nontoxic. The natural biodegradable polymers such as polysaccharides and proteins undergo biodegradation by enzymatic degradation in the body; synthetic polymers undergo biodegradation by utilizing the hydrolysable linkage like esters, amide, urea, and urethanes. In the past few decades more biodegradable polymers have been utilized for the development of effective delivery of drugs such as DNA, protein and incorporation of these agents can prevent the degradation in vivo and in vitro there by improve the therapeutic effect and prolong the biological activity (9-

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10). Currently biodegradable polymers like dextran, chitosan, starch, alginate, and pullulan are used for incorporation of macromolecular drugs. Although many synthetic biodegradable polymers were developed for various biomedical applications, use of natural biodegradable polymers is a better choice due to their abundance in nature, biocompatibility and modifiability (11). Among the natural biodegradable polymers, protein based polymers such as gelatin, collagen, albumin and polysaccharides- based polymers like (chitosan, hyaluronic acid, starch, and dextran) are used for drug delivery. The drug delivery based on polymer prepared from polysaccharide are gaining more important due to their cost-effectiveness, wide range of physicochemical properties, flexibility in obtaining desired release profile and ease in modification for specific applications and have wide regulatory acceptance(12). These polymers besides their application in drug delivery system, also used in various pharmaceutical formulations.

## II. MICROSPERES AS A CARRIER SYSTEM FOR DRUG MOLECULES

Several devices are used for controlled release of drug molecules, The most commonly used are biodegradable polymeric microspheres, nanoparticles, microcapsules [13-14]. These delivery devices are highly useful because they can be administered to a variety of locations in vivo via a syringe needle [13-14]. The microspheres can be incorporated with drug molecule small molecules, proteins and nucleic acid. These delivery devices have high bioavailability and show good biocompatibility, which is capable of sustaining the release of drug molecule for a longer period of time. Many drug molecules could be incorporated on biodegradable microspheres regardless of their molecular weights and water solubility using different manufacturing techniques [15-19].

## III. DIFFERENT TYPES OF MICROSPHERES USED IN DRUG DELIVERY SYSTEM

Magnetic microspheres are an important type of drug delivery system and have the ability to localize the drug concentration at the site of action. Magnetic responses are received by the magnetic carriers in response to a magnetic field from incorporated materials which are used in magnetic microspheres like chitosan, dextran etc [20]. The magnetic microspheres are the supramolecular particles, which are small (<4  $\mu\text{m}$ ) can circulate through the capillaries. The magnetic microspheres are susceptible (ferromagnetic) to get captured into micro vessels and later get dragged into the adjacent tissue with the help of magnetic fields. In magnetic microspheres some portions of the freely circulating drug molecules can be replaced by incorporating smaller amount of magnetically targeted

drug. The magnetic microspheres can be prepared by different processes continuous solvent evaporation process (CSE) by phase separation emulsion polymerization method (PSEP). Therapeutic magnetic microspheres are utilized for delivering chemotherapeutic agents to liver tumour and other drugs like proteins and peptides for drug target [21]. Magnetic microspheres can also be used for the diagnostic purpose that can be used for imaging liver metastases [22].

The oral controlled drug delivery system is one of the most promising route because of the low cost and high level of patient compliance. The effective oral drug delivery may depend on several factors like, gastrointestinal transit time of the dosage form, gastric emptying process, release of drug from the dosage form and finally the site of absorption. The gastric emptying process is one of the variables, which depends on the type of dosage form and the fed and the fasted state of stomach. The normal gastric residence time is between 5 minutes to 2 hours. In empty stomach the transit of dosage forms takes place by the electrical activity in the stomach and the interdigestive myoelectric cycle or migrating myoelectric complex. The retentive systems can remain in the gastric region for several hours and there by prolong the gastric residence time of drugs [23]. Prolongation of the gastric retention can improve the bioavailability of the drug, improves solubility for those drugs which show poor solubility at high pH and also reduce drug wastage. Floating type microspheres are one such group having bulk density lower than the gastric fluid and remain buoyant in stomach. The floating microsphere does not affect the gastric emptying time and the release of drug is initiated at the desired rate. Floating drug delivery system can be divided into effervescent systems and non-effervescent systems. The mechanism by which the floating microspheres release the drug is when the microspheres come in contact with gastric fluid the polymer forms a colloidal gel barrier, which controls the rate of fluid penetration into the device resulting in drug release. When exterior surface of the delivery system dissolves, a gel layer will be maintained by the hydration of the adjacent hydrocolloid layer. The buoyancy of the microspheres is initiated by the entrapment of air by the swollen polymer [25-26]. The floating type microspheres prepared from acrylic resins, eudragit, cellulose acetate, polyethylene oxide, polystyrene floating balloons and gelucire floating granules are the recent developments in this category.

Microspheres with bioadhesion property are another group, which can be utilized for drug targeting to a particular region of the body for a prolonged period of time. The term mucoadhesion was coined for the adhesion of polymers with the surface of mucosal layer. The adhesion takes place via interfacial forces between drugs to the membrane by using the sticking property of

water soluble polymers. The adhesion between polymers attached to the mucin layer of mucosal tissue is an example of bioadhesion[27]. The mucoadhesion is used when mucosal layer, which is present in number of regions which include urogenital tract, airways, GIT, ear, nose and eye. Hence these sites can be utilized as potential sites for bioadhesive system. The mucoadhesive drug delivery system can be used for oral, nasal, buccal, vaginal, rectal and ocular route of administration. These microspheres exhibit prolonged resident time and offers intimate contact with the absorption site and offer better medicinal action [21].

Radioactive microspheres are the groups which can emit beta or gamma radiation either alone or in combination. These microspheres are available in wide range of biocompatible glass compositions such as aluminosilicate, borate, silicate and borosilicate. The ideal characteristic required for radioactive microspheres are bioabsorbability or bio-inertness and they can be in the size range of 10-30 nm. These microspheres are injected to the arteries and get localized to the tumor of interest. The radioactive microspheres can deliver high radiation dose to the target area without causing any side effect to the normal surrounding tissues.

The floating microspheres also called hollow microspheres and are referred to as the gastro-retentive drug delivery system by non effervescent approach. The floating microspheres have low bulk density than the gastric fluid and helps to remain buoyant in stomach without affecting the gastric emptying rate. Floating microspheres are spherical empty particles without a core and are free flowing powders consisting of proteins or synthetic polymers with a size range of 1-1000  $\mu\text{m}$ . The release of drug from the floating microspheres takes place slowly resulting in increase gastric retention with reduced fluctuations in plasma drug concentration (28-29). These types of microspheres are prepared by solvent diffusion and evaporation methods to create a hollow inner core. The characterization of floating microspheres can be done based on micromeritic properties such as particle size distribution, tapped density, true density, flow properties, compressibility index, scanning electron microscopy, in vitro floatability studies, in vitro drug release studies and stability studies etc.

#### IV. PREPARATION OF MICROSPHERES

Varieties of techniques have been developed for the preparation of microspheres to be used as drug delivery system-combinations of phase separation or precipitation, emulsion/solvent evaporation (30-36), and spraying methods (37-41). The control of the particle size and size distribution can be initiated by varying the fabrication parameters. The incorporation of the drug on to microspheres can be done by several different ways

depending on the properties of the drug molecule. A self assembled polydisperse porous microspheres preparation was studied and was prepared by self assemble of three peptides containing various conformationally flexible and rigid  $\gamma$ -amino acids. The three peptides used for the self assembly are peptide 1, which adopts an extended backbone confirmation. The peptide 2 will form a turn like structure and peptide 3 can adopt a kink-like conformation. These peptides are consistent with increase rigidity of the N-terminally  $\gamma$ -amino acids. These peptides in solid state in methanol-water system (19:1) solution they assemble and form polydisperse porous microspheres. The SEM images have revealed that microspheres have a diameter range from 500 nm-1 $\mu\text{m}$  (42). The most common method especially at the lab scale is emulsion-solvent extraction/evaporation methods. In this process a polymeric solution containing the drug to be encapsulated are emulsified in a non-solvent phase (continuous phase) containing the stabilizer. The emulsions are then prepared by any of the physical methods adopted such as homogenization and sonication(43). The components are chosen in such a way that the solvent is slightly soluble in the non-solvent. Once the emulsification is affected the solvent is extracted into the continuous phase and allowed to evaporate, while the non-solvent may penetrate into the polymeric droplets. The loss of solvent will help the disperse phase to get enriched in polymer until the droplets get harden to become particle. The microspheres so formed are then filtered, washed and lyophilized. Microspheres containing Aceclofenac are prepared by emulsion solvent evaporation techniques by using sodium PVP as emulsifying agent and eudragit as polymer (44).

Emulsion cross linking method is also another method for fabricating the microspheres. A typical example for microspheres prepared by this method include chitosan microspheres, in which chitosan gel in dispersed in toluene containing span 80 as emulsifying agent and resulting w/o emulsion are crosslinked by glutaraldehyde. The microspheres obtained were then washed with acetone and toluene, later collected as free flowing powder (45). The microspheres can also be prepared by Co-acervation phase separation method. The first step involved in Co-acervation method is formation of three immiscible phases namely- liquid manufacturing vehicle, core material and coating material. The core material (drug) were added on a coating polymer solution and is made immiscible in the liquid state by using one of the techniques like changing temperature, addition of salt, addition of non solvent, inducing polymer-polymer interaction and finally addition of incompatible polymer to the polymer solution. The polymeric coating material in liquid form is then deposited on to core material and finally the microspheres were rigidized by cross linking,



desolvation or thermal treatments (46-47). The spray drying techniques for preparing microspheres, involves the dispersion of the core material on a liquefied coating material and later the mixture are sprayed in an environment for effecting solidification of coating followed by rapid evaporation of solvent. The spray drying is a rapid process, but sometimes may loose crystallinity due to the fast drying process (48). A novel quasi-emulsion solvent diffusion method was developed for preparing a controlled-release microsphere made of acrylic polymer containing ibuprofen. The procedure involves an ethanol solution of ibuprofen and acrylic resin was mixed in an aqueous medium with stirring. The fine dispersion of ethanolic droplet such as coacervates formed in the aqueous media is later solidified gradually to form microspheres during agitation (49). The microspheres prepared by multiple emulsion method, involves the dispersion of the powdered drug in polymeric solution. The dispersion was then emulsified by using ethyl cellulose solution in ethyl acetate. The primary emulsion so formed was then re-emulsified in aqueous medium to get discrete microspheres (48).

Another technique adopted for the preparation of microsphere is ionic gelation, which involves the addition of drug in an aqueous polymeric solution with continous stirring. The resulted mixture was added drop wise to solution containing calcium/aluminum compounds and chitosan solution in acetic acid. The microspheres formed were kept in original solution for 24 hrs for internal gellification and later separated by filtration (48).

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