World Journal of Pharmaceutical research

Volume 1. Issue 4. 975-992. Review Article ISSN 2277 – 7105

MICROSPHERES –A REVIEW

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Article Received on
10 July 2012,

Revised on 05 August 2012,
Accepted on 27 August 2012

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ABSTRACT

The concept of drug delivery has been revolutionized. The current focuses on microspheres which are small, solid particles ranging in size from 1 to 1000 µm. These are made of polymeric, waxy, or other protective materials i.e. biodegradable synthetic polymers and natural products such as starches, proteins, fats and waxes. The natural polymers include albumin and gelatin; the synthetic polymers include polylactic acid and polyglycolic acid etc. Microspheres have been incorporated with antineoplastic drugs, steroid hormones, narcotic antagonists, luteinizing hrh analogs, and other macromolecules. General methods of preparation are emulsion techniques, phase separation coacervation techniques, spray drying and spray congealing and solvent extraction, in-situ polymerisation etc. Advantages of microspheres use are targeted drug delivery, optimal therapeutic effects and minimum side effects. Recent trends are floating microspheres, radio immobilisation using microspheres.

Keywords: Microspheres, floating microspheres, microencapsulation, spray coating and pan coating, phase separation coacervation.

INTRODUCTION

Morphologically, there are two general structures: microcapsules and microspheres[1]. A microcapsule is a reservoir-type system with regular or irregular shapes that contains a well defined core and envelope. The core can be solid, liquid, or gas and the envelope are made of

a continuous, porous or nonporous, polymeric phase created by one or more polymers. Alternatively, a microsphere is a homogeneous structure made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed throughout the matrix, at either the macroscopic (particulates) or molecular (dissolution) level. However, the main difference between the two systems-microcapsules and microspheres- are the nature of the microsphere matrix, in which no well-defined envelope or wall exists[2].

Drugs being incorporated in microspheres:

- Antineoplastic drugs[3-7]
- Steroids hormones[8,9]
- Narcotic antagonists[10]
- Luteinizing hormone releasing hormone analogs[11,12]
- Elastase[13]
- Other macromolecules etc[14].

Microspheres are made of polymeric, waxy, or other protective materials that may be synthetic polymers or modified natural products such as starch, waxes etc. The natural polymers include albumin and gelatin[15,16] and the synthetic polymers include polylactic acid and polyglycolic acid[17,18]. The microspheres have been used in pharmaceutical industry since 1960s[19-21], for the following applications:

- Taste and odour masking
- Protection of drugs against the environmental factors (moisture, light, heat and /or oxidation) and protection of body from adverse effects of drugs (prevention of pain on injection)[22].
- For ease of handling (e.g. conversion of oils and other liquids to solids)
- Improvement of flow properties of powders
- Safe handling of toxic substances
- Prevention or delay of volatilisation
- Separation of incompatible materials (other drugs or excipients such as buffers)
- For dispersing water-insoluble substances in aqueous media, and
- Production of sustained- release, controlled-release, and targeted medications[23-26]

Pharmaceutical application

Biologically active peptides and proteins have been delivered with the help of biodegradable microspheres. Sustained-release characteristics of microspheres reduce the need for frequent administrations and enhance patient compliance by maintaining in vivo drug levels in the therapeutic range. Poly (D, L lactide) (PLA) and poly (D, L-lactide-co-glycolide) (PLGA) are the most widely used and well characterized polymers for biodegradable micro-spheres[27-29]**.** Microspheres have also applications in injectable and inhalation products[30-32,35]. Microcapsules are also used as diagnostics, e.g. temperature sensitive microcapsules for thermo graphic detection of tumours[36]. Scratch-n- sniff (microencapsulated aromas) has been used in children's books and food and cosmetic aroma advertising[37]. Microspheres are used for isolating materials until their activity is needed. The bio technology industry employs microspheres to contain organisms and their recombinant products to aid in the isolation of these products[38].

A number of pharmaceutical microencapsulated products are available in the market, such as aspirin, theophylline and its derivatives, vitamins, pancrelipase, antihypertensive, KCL progesterone and contraceptive hormone combinations[39].

Medically microsphere has also been used for the encapsulation of live cells and vaccines. The biocompatibility of artificial cells and biomolecules such as peptides, proteins, and hormones can be improved by encapsulation which can prevent unwanted immunological reactions that would lead to inactivation or rejection[40,41].

Estimation of regional blood flow can be done with hematogenously delivered microspheres. The criterion for determination is as follows:

(1). When appropriately sized microspheres are used, then the regional blood flow is proportional to the number of microspheres trapped in the region of interest.

(2). A number of excellent review articles describe and validate the use of microspheres for the measurement of regional organ perfusion. A classic review by Heyman, *et al.* contains many details for radioactive microsphere use that apply to stable-isotope labelled microspheres - the only difference being that the assay of the microspheres is performed by Bio PAL, which uses neutron activation technology for measuring microsphere content[42].

The number of microspheres injected must be calculated to assure a sufficient number to accurately determine blood flow to the organ of interest. Per tissue segment, a minimum of 400 to 600 microspheres is needed for a 95% confidence interval on a blood flow measurement. As a result, the following equation is used to estimate the minimum total number of microspheres needed per injection[43,44],

$$\mathbf{Y} = 1.2x106 + 1.9x105\mathbf{X},$$

where Y is the minimum number of microspheres needed for injection and X is the mass of the subject in kg (i.e., 1.5 million for a rat, 2.5 million for a rabbit, 5 million for a small canine, 7 million for a large canine, 9 million for a swine). This equation is designed for an average myocardial study.

Manufacturing of microsphere- There are many methods for the manufacturing of microsphere, some of these are as follows:-

1. Spray coating and pan coating
2. Phase separation coacervation
3. Solvent evaporation
4. Spray congealing
5. In-situ polymerisation etc.

Spray coating and pan coating: In this method, the solid core particles are rotated in coating pans which are then sprayed with liquid coating material. The size range of core particle is usually µm to few mm. The process of coating is continued until a uniform coat is obtained. Coating a large number of small particles may provide a safer and more consistent release pattern than coated tablets. In addition to this several batches of microspheres can be prepared with different coating thickness and different release patterns. The Wurster process uses the principle of fluidisation for coating the core particles. The fluidised bed technique provides more uniform coating than pan coating method. Explosion proof units have to be designed to prevent any explosion in the enclosed fluidised chamber. This process is not used as much for the microsphere manufacture[45,46].

Phase separation coacervation: There are various methods which are effectively employed for the coacervates phase separation. The choice of method is largely dependent on polymer used and set of conditions under which the process is carried out. The method is based on salt addition, addition of the incompatible polymer, non solvent addition, and change in pH. This

method is designed for preparing the reservoirs type of the system, i.e. to encapsulate water soluble drugs e.g. proteins and peptides. However when the drug is hydrophobic in nature e.g. steroids some of the preparation are of matrix type. In matrix type device, the drug or the protein is soluble in the polymer phase[47].

→ The principle of decreasing the solubility of the polymer in the organic phase affects the formation of polymer rich phase called the coacervates.
→ The coacervation can also be brought about by addition of the third component in the system which results in the formation of two phases, one rich in the polymer and the other one, i.e. supernatant, depleted of the polymer.
→ In this technique, the polymer is first dissolved in a suitable solvent and then dispersion of drug is done by making its aqueous solution, if hydrophilic or dissolved in the polymer solution itself, if hydrophobic. Changing the solution conditions causes the phase separation.
→ Continuous stirring should be done to control the size of the microparticles. The agglomeration can be avoided by stirring the suspension using a suitable speed stirrer.
→ The process variables are very important since the rate of achieving the coacervate determines the distribution of the polymer film, the particle size and the agglomeration of the formed particles. Therefore, the process variables are critical as they control the kinetic of the formed particles since there is no defined state for attaining the equilibrium.

Solvent extraction or evaporation method

Emulsion solvent removal method [48] has been used extensively in pharmaceutical industries for various purposes such as controlled drug delivery, masking the taste and odour of drugs, protection of the drugs from degradation, and protection of the body from the toxic effects of the drugs. Among the various microencapsulation methods, the most widely used technique to prepare microcapsules of water insoluble drugs (within the water insoluble polymer) is the emulsion solvent evaporation technique. This method is used for the preparation of microparticles, and the method involves the removal of the organic phase by extraction of the organic solvent. Microspheres are formed by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and drug[48-50].

In this method water miscible organic solvent such as isopropanol is used and the organic phase is removed by extraction with water. Hardening time for the microspheres is decreased

by this process. One variation of the process involves direct addition of the drug or protein to polymer organic solution. The rate of solvent removal depends on the temperature of water, ratio of emulsion volume to the water and the solubility profile of the polymer. For large scale production of emulsions, colloidal mills, and high pressure homogenizers are most often used [54]. Stirring is the simplest and most straight forward method that is exclusively used in laboratories to generate droplets of the emulsion. Increasing the mixing or stirring speed generally results in decreased microsphere mean size[55].

Spray drying and congealing: Spray drying and spray congealing methods are based on the drying of the mist of the drug and polymer used in the air. In spray drying the solvent is removed by applying high temperature and in spray congealing the solvent is removed by decreasing the temperature or by cooling. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. Then the drug in the solid form is dispersed in the polymer solution. The process is carried out under high speed homogenization. This dispersion is then subjected to hot air atomisation which 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. Separation of microparticles from the hot air is done by means of the cyclone separator while vacuum drying removes the traces of solvent[56-58].

In-situ polymerisation: There are two types of polymerization techniques:

1. Normal polymerization
2. Interfacial polymerization

Normal polymerization: normal polymerisation is carried out using different techniques such as:

1) Bulk polymerization: A monomer or a mixture of monomers is usually heated along with the initiator to initiate the polymerization. The rate of the reaction is facilitated or increased by adding catalyst or the initiator to the reaction mixture. The polymer thus obtained may be moulded or fragmented as microspheres. Drug loading can be achieved by the process of adsorption of drug or it may be added during the process of polymerization.
2) 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.

3) Emulsion polymerization: However, differs from the suspension polymerization as due to presence of the initiator in the aqueous phase, which eventually diffuses to the surface of the micelles or the emulsion globules.

Interfacial polymerization

In Interfacial polymerization technique two reacting monomers are used; one of which is dissolved in the continuous phase while the other being dispersed in the continuous phase. Generally the continuous phase is aqueous in nature through which the second monomer is emulsified. The monomers present in either phase diffuse rapidly and polymerize rapidly at the interface. Two conditions arise depending upon the solubility of formed polymer in the emulsion droplet. If the polymer is soluble in the droplet it will lead to the formation of the monolithic type of the carrier on the hand if the polymer is insoluble in the monomer droplet, the formed carrier is of capsular (reservoir) type. The degree of polymerization can be controlled by the temperature of the system, by the reactivity of the monomer chosen, their concentration, and the composition of the vehicle of either phase. Controlling the droplets or globules size of the dispersed phase can control the particle size. The polymerization reaction can be controlled by maintaining the concentration of the monomers, which can be achieved by addition of an excess of the continuous phase. The interfacial polymerization is not widely used in the preparation of the microparticles because of certain drawbacks, which are associated with the process, such as[59]:

• Toxicity associated with the un-reacted monomer
• High permeability of the film
• High degradation of the drug during the polymerization
• Fragility of microcapsules
• Non-biodegradability of the microparticles.

Loading of the drug

The active components are loaded over the microspheres principally at two intervals, i.e. during the preparation of the microsphere or after the formation of the microspheres by incubating them with the drug/protein. The active component can be loaded by means of physical entrapment, chemical linkage and surface adsorption. The entrapment largely depends on the nature of drug and the polymer used and the method of preparation. Maximum loading can be achieved by incorporating the drug during the time of preparation

but it may get affected by many other process variables such as method of preparation, process additives, heat of polymerisation, agitation intensity, etc. The adsorption of the drugs/proteins depends on some factors such as the nature of the polymers (Kiplings 1965). The Frendluich equation is

$$X / m = kc^{P}_{eq}$$

where k is a constant related to capacity of the adsorbent for the adsorbate.

Drug release kinetics: In case of microspheres, the release of active constituents is a very important factor. Many theoretically possible mechanisms may be followed for the release of drug from the microparticles –

1. Liberation due to degradation or polymer erosion.
2. Self diffusion through the pore.
3. Release from the surface of the polymer.

In order to obtain maximum therapeutic efficacy and for minimising the toxicity and side effects [60] it becomes necessary to deliver the drug in the optimal amount to the target tissue for the required period of time[60]. Desired drug release can be achieved by rate-controlling membranes or by implanted biodegradable polymers containing dispersed medication. To maintain the desired concentration at the site of interest without untoward effects micro particulate drug delivery systems are considered and accepted as a reliable means[61]. For improving patient compliance and for prolonging the duration of drug effect microencapsulation is a useful method. Eventually the total dose may be reduced since a steady plasma concentration is maintained and the adverse reactions can be minimised[62]. In recent years much research in drug delivery has been focused on degradable polymer microspheres. Administration of medication via such systems is advantageous because it can provide organ-targeted release and can be ingested or injected, can be used for desired release profiles[48-50,63,64].

In most of the cases, a combination of more than one mechanism for drug release may operate. The drug could be released through the microspheres by any of the following mechanisms, osmotically driven burst mechanism, pore diffusion mechanism or by erosion or biodegradation of the polymer.

Applications of microspheres: Applications are explained as follows –

(A) Magnetic microspheres

These microspheres are used for delivering the drug at localised disease site. Magnetic drug delivery by particulate carriers is used for this very purpose. In magnetic targeting, a drug or therapeutic radioisotope is bound to a magnetic compound, injected into a patient's blood stream, and then stopped in the target area with a powerful magnetic field[65].

(B) Radioactive microspheres

Therapeutic radioactive microspheres (radio labelled microspheres) are appropriate for therapy when the encapsulated diagnostic radioisotope has been exchanged for a therapeutic one from the α- or β-emitter group. Typical uses include local application for the treatment of rheumatoid arthritis, liver tumours and cystic brain tumour. However, their use remains experimental because of unwanted toxicity, smaller than expected target uptake and insufficient treatment effects that have resulted from radio chemical instability and suboptimal bio-distribution of the radiopharmaceutical moiety. In spite of proven superior results of many radiation therapies there exists a general negative attitude towards the use of radioactive substances[66-68]. Few therapeutic applications of radioactive microspheres are tabulated in Table: 1

Table: 1 Therapeutic application of microspheres[69]

Type of radioactive microspheres	Applications
90Y-glass microspheres, 186Re/188Re-glass Microspheres	Radio immobilisation of liver and spleen tumours
35S-colloid, 90Y-resin microspheres, 169Er.citrate	Radiosynovectomy of arthritis joints 90Y-labeled poly (lactic acid) microspheres, 211At-microspheres
212Pb-sulfur colloid	Local radiotherapy
Chromium 32P-phosphate, 90Y-silicate	Intracavity treatment

Table: 2 Diagnostic applications of radioactive microspheres

Type of radioactive microspheres	Applications
111In or 51Cr-labelled red blood cells	Gated blood pool study
111In-labeled platelets 99mTc-sulfur colloid	Thrombus imaging in deep vein thrombosis Polystyrene microspheres labelled with γ-emitters
141Ce, 57Co, 114mIn, 85Sr, 51Cr	Blood flow measurements
3H, 14C-labelled microspheres 141Ce polystyrene microspheres	Investigation of biodistribution and fate of drug loaded microspheres
99mTc-impregnated carbon particles 99mTc-macro aggregated human serum albumin	Lung scintigraphy
99mTc-macro aggregated human serum albumin	Radio immobilisation
99mTc-macro aggregated human serum albumin	Liver and spleen imaging
99mTc-sulfur colloid 99mTc-antimony sulphide colloid	Bone marrow imaging

(C) Perfect count microspheres

These microspheres are meant for invitro diagnostic use. These microspheres are meant for determination of absolute counts of cells in peripheral blood, bone marrow, leukophoresis and culture medium samples using flow cytometry. These are micro-bead-based single platform system, which can be used in combination with monoclonal antibodies conjugated with different flurochromes for absolute counts, which helps to identify the cell subpopulations for which the absolute count is intended[70,71].

(E) Microsphere sensors

Optical microspheres are proving to be best candidates for label-free biochemical sensors. Light of resonant frequencies circulates on the surface of the microsphere in the form of whispering-gallery modes (WGMs). High-*Q* factor of microspheres allow the interaction between the WGM and the surrounding medium. The WGM's resonant wavelength is extremely sensitive to slight changes in refractive index near the sphere's surface when

molecules bind to or are removed from the surface. Microsphere sensors are used for heavy-metal detection, detection of protein and DNA molecules and refractometric sensing. Microsphere sensors have also been used for small molecule detection. Detection of small molecules is challenging because the transduction signal in label free sensors is generally proportional to the mass of the target molecule[72].

(G) Fluorescent microspheres

These are made of polystyrene or poly vinyl toluene, mono disperse system. There size ranges from 20nm to 4µm. Preparation of fluorescent microspheres involves swelling the polymeric microsphere followed by incorporation of fluorescent dyes in the microspheres pores. The main applications for Estapor® Fluorescent Microspheres (commercial fluorescent microspheres) are the following: Membrane-based technologies Flow Cytometry, Embolization, Confocal Microscopy FLISA (Fluorescent Linked Immunosorbent Assay), and Toxicology, Cell Biology, Microbiology, Biosensors, Biochips and Micro fluidics[73].

(H) Microspheres in molecular biology

During the study of the underlying genetic causes of disease a need for multiplexed analytical genotyping methods have been increased. Although microarray platforms have attempted to fulfil this need, their acceptance in the clinical diagnostic setting has been limited. Microspheres have been used for the detection of six single nucleotide polymorphisms (SNPs) believed to be associated with venous thromboembolism, a classic example of a complex, multifactorial disorder involving multiple genetic abnormalities[74-78]. Pyrosequencing is real-time DNA sequencing by synthesis[79,80]. Pyrosequencing currently has many applications, including determination of single nucleotide polymorphisms, resequencing of PCR products, microbial typing, and analysis of secondary DNA structures such as hairpins. The pyrosequencing technique utilizes DNA templates which are attached to magnetic microspheres, which can easily be put on an electrowetting chip in solution. On a digital micro fluidic platform, pyrosequencing could be accomplished by merging droplet containing the magnetic microspheres with the wash droplets and then resolving the double-volume droplet through droplet splitting.

Some products in market

Tretinoin Gel Microsphere: Tretinoin gel microsphere, 0.1%, is a formulation containing 0.1% by weight tretinoin for the topical treatment of acne vulgaris.

Dexamethasone microspheres: These microspheres are used for its anti inflammatory action. With previous investigations on dexamethasone loaded microspheres and composites, the suppression of acute inflammation by dexamethasone containing composites is consistent[81-83].

Azithromycin microspheres: Azithromycin extended release (Zmax®, Pfizer Inc) is a novel single-dose administration formulation of azithromycin approved by FDA in June 2005. It is currently being used for the treatment of community acquired pneumonia and acute bacterial sinusitis (Zmax package insert). The immediate-release formulation azithromycin has been available in the United States since 1992 under the trade name Zithromax®, and is approved and widely used for community-acquired pneumonia, acute bacterial sinusitis, otitis media, acute bacterial exacerbations of chronic obstructive pulmonary disease, pharyngitis/tonsillitis, uncomplicated skin and skin structure infections, urethritis and cervicitis, and genital ulcer disease in men (Zithromax package insert). Azithromycin revolutionized antibiotic care as it shortened treatment time for infections from 7–14 days to 1–5 days with comparable efficacy[84-87].

Acetazolamide microspheres: Acetazolamide is a carbonic anhydrase inhibitor and it is widely used in the treatment of glaucoma and also used as diuretic. The half life of this drug is relatively short i.e. 3-4 hrs and usually administered 3 – 4 times daily in the form of an immediate release formulation [88].These microspheres were prepared by solvent evaporation technique. Encapsulation matrix consists of water insoluble polymers like Eudragit RS and Eudragit RL using this technique[89].

Degradable starch microspheres: These are the most frequently used microsphere system for nasal drug delivery. Degradable starch microspheres (DSM) are also known as Spherex. These micro- spheres are prepared by an emulsion polymerization technique where starch is cross-linked with epi-chlorohydrine[90].

CONCLUSION

Microsphere offer vast advances in the pharmaceutical field. The recent use allows targeting the delivery of such drugs which offers difficulties in their normal delivery. Now higher dose can be administered as microspheres thus limiting gastrointestinal side-effects and allowing a full course of antibiotics to be given in a single dose. In recent years, studies of microspheres

have been increased so that it may be used in more diverse applications and it is evident that the range of its applications is vast and enormous. For biologists, microspheres have emerged as an exciting new platform in the investigation of cellular processes and bimolecular interactions. The future certainly looks bright for microspheres, particularly in the areas of proteomics, genomics and drug discovery.

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