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RECENT RESEARCH ON MICROSPHERES - A REVIEW

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ABSTRACT

Development of microspheres is a promising technology for controlled release and drug targeting.Various types of microspheres such as bio-adhesive, magnetic, floating, radioactive and polymeric microspheres are developed for various purposes. Microspheres occupied a central place in novel drug delivery. Literature of microspheres, their methods of preparation, evaluation, applications and recent research on microspheres are reviewed in this article.

Keywords: Microspheres, Controlled release, Targeting, Recent research, Review.

INTRODUCTION

Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems[1]. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release. Microspheres are matrix systems that contains drug throughout their structure and are potential candidates for oral controlled release. Microsphere can be defined as solid spherical particles ranging from one to 1000µm in size. The microspheres are free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature. There are two types of microspheres; microcapsules and micro-matrices. Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micro-matrices are those in which the entrapped substance is dispersed through out the microspheres matrix. 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[2].

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Advantages of Microspheres[3]:

1. They facilitate accurate delivery of small quantities of potent drug and reduce concentration of drug at site other than the target organ or tissue.
2. They provide protection for unstable drug before and after administration, prior to their availability at the site of action.
3. They provide the ability to manipulate the *in vivo* action of the drug, pharmacokinetic profile, tissue distribution and cellular interaction of the drug.
4. They enable controlled release of drug. Examples: Narcotic, Antagonist, Steroid hormones

Polymers used for microsphers[1,4]:

The following are the polymers used in the preparation of microspheres.

Synthetic polymers:

Non-biodegradable polymers: Polymethyl methacrylate (PMMA), Glycidylmethacrylate, Epoxy polymers.
Biodegradable polymers: Lactides, their glycolides and their copolymers, Polyalkyl Cyano Acrylate, Polyanhydrides.

Natural polymers:

These are obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates. Proteins: Albumin, Gelatin, And Collagen, Carbohydrates: Agarose, Carrageenan, Chitosan, Starch, Chemically Modified Carbohydrates: Poly (acryl) dextran, Poly (acryl) starch

TYPES OF MICROSPHERES:

Bioadhesive Microspheres[5,6,7] : 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.

Magnetic Microspheres[5, 8]: Magnetic microspheres are supra molecular particles that are small enough to circulate through capillaries without producing embolic occlusion (<4μm) but are sufficiently susceptible (ferromagnetic)to be captured in micro vessels and dragged into the adjacent tissues by magnetic field of 0.5-0.8 tesla.

Floating Microspheres[5, 9, 10]: Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period without affecting gastric emptying rate. The drug is released slowly at the desired rate.

Radioactive Microspheres[5, 11]: Radioactive microspheres deliver high radiation dose to the targeted are as without damaging the normal surrounding tissues. They are injected to the arteries that lead to tumour of interest. The different kinds of radioactive microspheres are α emitters, β emitters and γ emitters.

Polymeric Microspheres[5]: Biodegradable polymeric microspheres are those which contain biodegradable polymers which prolongs the residence time when contact with mucous membrane due to it's high degree of swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. Synthetic polymeric micro spheres are those which are made up of synthetic polymers and are used as used as bulking agent, fillers, embolic particles, drug delivery vehicles etc.

METHODS OF PREPARATION:

Single Emulsion Technique[1, 4, 5]:

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/dispersed inaqueous medium followed by dispersion in the non-aqueous medium e.g. oil.

In the second step of preparation, cross-linking of dispersed globule is carried out. The cross linking is achieved by two methods i.e. either by heat or by means of chemical cross linking agents including glutaraldehyde, formaldehyde, diacid chloride etc.

Double Emulsion Technique[1, 4,5,12]:

This method involves the formation of the multiple emulsion or double emulsion of type w/o/w. It is best suited to water soluble drugs, peptides, proteins and 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 poly vinyl alcohol (PVA).This results in formation of a double emulsion. Emulsion is then subjected to 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. The emulsion is then added to large quantity of water into which organic phase diffuses out.

The solid microspheres are subsequently obtained by filtration and washing with n-hexane, acetone or any organic solvent to remove traces of oil from the surface.

Polymerization[1, 4, 5, 13]:

The polymerization techniques conventionally used for the preparation of themicro spheres, are mainly classified as: Normal polymerization and Interfacial polymerization

Normal Polymerization[14]

Normal polymerization proceeds and is carried out using different techniques as bulk, suspension, Precipitation, emulsion and micellar polymerisation processes. In bulk polymerization, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization. Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain aninitiator and other additives. Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles. Bulk polymerizationhas an advantage of formation of pure polymers.

Interfacial Polymerization[1]:

It involves the reaction of various monomers at the interface between the two immiscible liquid phasesto form a film of polymer that essentially envelops the dispersed phase. In this technique two reacting monomers are employed, one of which is dissolved in the continuous phase while the other being dispersed in the continuous phase.

Phase Separation/ Coacervation[1, 4, 15]:

Phase separation method is mainly designed for preparing the reservoir type of the system. This method is used to encapsulate water soluble drugs e.g. peptides, proteins and some of preparations having matrix type particular, when the drug is hydrophobic in nature e.g. steroids. In this technique the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophobic or dissolved in polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution conditions by the salt addition, on-solvent addition, addition of the incompatible polymer or change in pH.

Spray Drying [1,4,16]:

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 instantaneously leading the formation of microspheres.

Solvent Extraction[1]:

Solvent extraction method is used for the preparation of the micro particles, involves removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as isopropanol. Organic phase is removed by extraction with water. This process decreases the hardening time for the microspheres. 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.

Emulsion Solvent Evaporation[1, 4,5,17]:

In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0.2 %sodium of PVP as emulsifying agent. The above mixture was agitated at 500 rpm then the drug and polymer (eudragit) was transformed into fine droplet which solidified into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralised water and desiccated at room temperature for 24 hrs 4.8 Emulsion solvent diffusion technique[1, 4]. The colon floating microspheres were prepared using emulsion solvent diffusion technique in order to improve the residence time. The drug polymer mixture was dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture was added drop wise to sodium lauryl sulphate (SLS) solution. The solution was

stirred with propeller type agitator at room temperature at 150rpm for 1 hr. Thus the formed floating microspheres were washed and dried in desiccators at room temperature.

EVALUATION OF MICROSPHERES[1, 4, 5]:

Microspheres are evaluated for the following:

1. **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.

2. **Electron Spectroscopy for Chemical Analysis**: Determines surface chemistry

3. **Attenuated Total Reflectance Fourier Transform-Infrared Spectroscopy**: Determines degradation of polymeric matrix

4. **Capture Efficiency**: Entrapment (%) = Actual content/Theoretical content x 100

5**. Thermal Analysis**: Differential Scanning Calorimetry (DSC)

6. **Swelling Index**: Characterization of microspheres is performed with swelling index technique. Different solutions (100mL) such as distilled water and buffer solutions of pHs (1.2, 4.5, 7.4) are taken and alginate microspheres (100mg) are placed in a wire basket and kept on the above solutions and swelling is allowed at 37^0C and changes in weight difference between initial weight of microspheres and weight due to swelling was measured by taking weight periodically and soaking with filter paper.

Swelling Index = (Initial weight – Final weight) / Initial weight × 100

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

Buoyancy (%) = $W_f/(W_f + Ws)$

Where, W_f and Ws are the weights of the floating and settled microparticles.

8. **Micromeritic Properties:** Micromeritic properties such as tapped density, bulk density, compressibility index, angle of repose are to be studied.

9. **Release Studies:** Drug release from different type of microspheres are carried out by using different suitable dissolution media, mostly by rotating paddle apparatus (USP / BP).The speed of rotation varies from 50-100 rpm. The samples are taken at a specific time interval and replaced by same amount of dissolution medium. The active ingredient withdrawn in the sample is analysed as per monograph requirement and release profile is determined by using the plot amount released as a function of time. Dialysis and Franz diffusion cell technique is also used.

APPLICATIONS OF MICROSPHERES:

Microspheres in Vaccine Delivery

An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. Biodegradable delivery systems for vaccines that are given by parenteral route may overcome the shortcoming of the conventional vaccines. The interest in parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages including:

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

Microspheres in Chemotherapy:

The most promising application of microspheres is possible to used as carriers for anti- tumor agents. Enhanced endocytic activity and leaky vasculature administrated microspheres. Stealth microspheres are prepared by coating with soluble polyoxy ethylene. The accumulation of non-stealth microspheres in Reticulo Endothelial System (RES) may also be exploited for cancer chemotherapy[18-.19]

Microspheres for DNA Delivery:

Microspheres have been recently used as a delivery vehicle for the transfer of plasmid DNA which leads to improve the transfer of plasmid DNA and their stability in the bioenvironment[20]. A novel system for gene delivery based on the use of DNA-gelatin microspheres/ nanoparticles formed by salt induced complex coacervation of gelatin and plasmid DNA.

Fluorescent Microspheres:

These are made up of polystyrene or poly vinyl toluene, mono disperse system ranging in size from20nm to 4µm. Preparation of fluorescent microspheres comprising, swelling the polymeric microsphere so that fluorescent dyes may enter the microsphere pores.Unswelling the polymeric microspheres so that the fluorescent dyes become physically entrapped in the pores[21].

Adjuvant Effect for Vaccines:

An adjuvant effect of the microspheres/nano particles with either matrix entrapped or surface adsorbed vaccines have been demonstrated in several studies on substances or oral administration. Poly methyl methacrylate microspheres containing the influenza antigen induced significant antibody response. Oral delivery of antigens with microspheres may be an elegant means of producing an increase Immunoglobin A (Ig A) antibody response.

Microspheres for Ocular Delivery:

The most applications of drug loaded ophthalmic delivery systems are for glaucoma therapy, especially cholinergic agonists like pilocarpine. The short elimination half life of aqueous eye drops can be extended from a very short time (1-3 min) to prolonged time (15-20 min) using microspheres which have biodegradable properties e.g: Poly alkyl cyanoacrylate.

Microspheres for Lymph targeting:

The major purpose of lymph targeting is to provide an effective anticancer chemotherapy to prevent the metastasis of tumor cells by accumulating the drug in the regional lymph node. Example:

• Poly alkyl cyanoacrylate microspheres bearing anticancer drugs for tumor of peritoneal cavity.
• Poly (lactide-co-glycolide) microspheres for the lymphatic of diagnostic agents.

RECENT RESEARCH ON MICROSPHERES:

Several studies are reported on microspheres, their preparation and evaluation for various purposes employing a variety of polymers. A summary of recent research on microspheres is given in Table 1.

CONCLUSION

Microspheres form an important drug delivery strategy for controlled release and targeting. Microspheres containing anti neo-plastic drugs, steroid hormones, vaccines, proteins and peptides, antiviral, antifungal and antibiotic drugs, anti-diabetic drugs and anti inflammatory drugs are investigated extensively for controlled release by various routes and for targeting. In recent years studies on microspheres have been increased as it has become a promising technology in the areas of drug delivery, proteomics and genomics and also for studying bio molecular interactions.

Table 1: Summary of Recent Research on Microspheres

S. No	Drug/Category	Polymers Used	Method of Preparation	Purpose / Result	Reference
1	Cephalexin (Antibiotic)	Sodium alginate, Guargum	Ion gelation technique	Mucoadhesive polymers Guar gum in combination with sodiumalginate provides extended gastric retention and has ability to coat gastric mucosa Uniformly.	22
2	Propanolol Hydro chloride (Anti Hypertensive)	Sodium carboxy methyl cellulose, carbopol 934p, HPMCK4M	Solvent evaporation	Enhancing bioavailability	23
3	Sitagliptin phosphate (Anti Diabetic)	Eudragit RS100,HPMC	Non aqueous solvent evaporation technique	Prolonging gastric retention of the dosage form	24
4	Aceclofenac (NSAID)	EUDRAGIT S 100, EUDRAGIT L 100	Response surface methodology	Increase gastric residence of the drug	25
5	Cephalexin (Antibiotic)	Ethyl cellulose	Emulsion solvent evaporation	Prolonged drug release in the stomach at least 12 hrs	26
6	Salbutamol sulphate (Anti Asthmatic), theophylline (Anti Asthmatic)	Ethyl cellulose	Emulsion solvent evaporation	Matrix microspheres have a potential for the prolongation and simultaneous delivery of drugs	27
7	Mefenamic acid (NSAID)	Chitosan	Thermal and Glutaraldehyde cross linking	Use of chitosan to control the release of poorly water soluble drugs	28
8	Salbutamol sulphate (Broncho Dilator)	Chitosan	Spray drying method	Spray drying is useful for preparation of salbutamol sulphate loaded chitosan microspheres in presence of and absence of crosslinking agent	29
9	Aceclofenac (NSAID)	Sodium alginate	Ionic gelation Method	Decrease the dosing frequency and also prevent gastric hemarrahage	30
10	Perindopril erbumine (Anti Hypertensive)	Ethyl cellulose, HPMC, PVP K30, EUDRAGIT S 100, PVPK90	Double emulsion solvent diffusion method	Prolong the drug release in a stomach for up to 12 hrs	31
11	Losartan (Anti Hypertensive)	Ethyl cellulose, Sodium alginate, Acycoat L30D, Acycoat E 30 D	Solvent evaporation, w/o emulsion, solvent evaporation method	Solvent evaporation method gaves Maximum yield.	32
12	Losartan potassium (Anti Hypertensive)	Chitosan, Sodium alginate	Emulsification solvent evaporation method	Smoothness of the losartan potassium microspheres was increased by increasing polymer concentration	33
13	Glipizide (Anti Diabetic)	Acrycoat S100 USP, Eudragit RS100	Emulsion solvent diffusion technique	Maintain a constant drug concentration in the serum for a longer period of time	34
14	Diltiazem hydrochloride (Anti Hypertensive)	Ethyl cellulose	Emulsion solvent evaporation method	Enhance the uptake of hydrophilic substance across epithelial layer	35
15	Metformin (Anti Diabetic)	Ethylcellulose, HPMC, Carbopol 934 P, Chitosan	Non aqueous solvent evaporation method	Chitosan as a polymer exhibited maximum prolonged drug release at GIT P^H or at least 15hrs	36
16	Glimepiride (Anti Diabetic)	Ethyl Cellulose, Eudragit RS 100, Eudragit RL100	Emulsification solvent evaporation method	Size of the microspheres was increased with increasing concentration of polymer	37
17	Carvedilol (Anti Hypertensive)	Ethyl cellulose, PEG 6000	Spray drying	The characterization of microspheres revealed the poor flow ability of the spray dried	38

18	Nifedipine (Anti Hypertensive)	Eudragit RL 100	Solvent evaporation	Enhancing bioavailability	39
19	Nifedipine (Anti Hypertensive)	Sodium Alginate, HPMC, Carbopol	Ionic gelation method	Drug release from microspheres was found slow followed by first order kinetics with non Fickian release mechanism	40
20	Amoxicillin trihydrate (Antibiotic)	Eudragit RS 100	Solvent evaporation	Delivery of protein and peptide drugs	41
21	Ritonovir (Anti Viral)	Mixture of sodium alginate and HPMC	Ionic gelation method	Increase the gastric retention time of drug	42
22	Indomethacin (NSAID)	Ethyl cellulose N 10, Ethyl cellulose N 100	Emulsion solvent evaporation	Ethylcellulose N10 and N100 membrane materials indicated differences in release patterns of microspheres. Microspheres exhibited lower burst effect with ethyl cellulose N100	43
23	Metformin hydrochloride (Anti Diabetic)	Sodium Carboxy Methyl Cellulose, Carbopol 934P, HPMC K 4M	Emulsion solvent evaporation	Reduce the dosing frequency and improving patient compliance by designing sustained release Mucoadhesive microspheres	44
24	Ascorbic acid (Anti Oxidant)	Ethyl cellulose	Phase separation coacervation technique	Derived properties of ascorbic acid loaded microspheres, the prepared product has uniform size and shape, spherical in particle size and passable flow property.	45
25	Metformin Hcl (Anti Diabetic)	Eudragit RL 100, cellulose acetate butyrate	o/o emulsion solvent evaporation method	Drug loaded floating microspheres will overcome the drawbacks associated with drug in conventional tablet form reducing plasma drug conc. fluctuations.	46
26	Lamivudine (Anti Viral)	Acryl coat L 30D and S 100	Solvent evaporation method	Plasma concentration was maintained above the minimum effective concentration for longer time after administration of microspheres	47
27	Melaxicam (Anti Inflammatory)	Polyvinyl Alcohol, PEG 6000, Gelatin	Emulsion solvent evaporation method	Emulsion solvent evaporation method is suitable for encapsulating lipophilic drugs.	48
28	Glipizide (Anti Diabetic)	Ethyl Cellulose, HPMC, Methocel K 100 M CR	Solvent evaporation method	Use of this approach has the potential not only to improve the Therapeutic effectiveness of the drug but also to allow a reduction in the total drug needed.	49

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