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MICROSPHERE: A COMPLETE REVIEW

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ABSTRACT

This review gives a complete overview on how microspheres (particle size ranging from 1-1000 μm) have gained popularity for delivering a wide variety of molecules via various routes. These types of products have been prepared using various natural and synthetic polymers through suitable techniques for desired delivery of various challenging molecules. Microsphere are spherical microparticles, and are used where consistent and predictable particle surface area is important. A microsphere has a unique polymeric membrane encasing centrally located drug in it. Microspheric drug delivery system has gained enormous attention not only for prolonged release but also due to its wide range of application as it covers targeting the drug to particular site to imaging and helping the diagnostic features. Today by combining various other strategies, microspheres have found 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. This review compiles various types of microspheres, polymers used, different methods to preparation, Factors influencing, various parameters to evaluate their efficiency, its applications, and also some Recent Advancements in Microspheres.

KEYWORDS: Microspheres, microencapsulation, microencapsulation techniques, controlled drug delivery, solvent evaporation, supercritical fluid, Spray drying and spray congealing.

1) INTRODUCTION

There are several routes to deliver drugs, either locally to a specific site or systematically for distribution to the entire body, oral and pulmonary administration are the most common. The

oral route is preferred due to ease of administration to most patients, as well as the ability to achieve systematic drug distribution. However, oral delivery is not suitable for treatments in which a local drug effect is required and is not easily amenable to fragile drugs, such as proteins, that may be degraded by the digestive system. Sustained oral delivery, lasting > 24h is difficult to achieve due to the normal cycle of digestive tract. Daily or more frequent oral administration is usually necessary. To reduce dosing frequency, several strategies are being developed, such as bio-adhesive systems that adhere to the intestinal epithelium, and devices are being developed that can release some drugs in the intestine for up to 8 days.

Oral route drug administration is the most preferable route for taking medications. However, their short circulating half-life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site-specific manner.

To obtain maximum therapeutic efficacy and minimum side effects, it is necessary to deliver the agent to the target tissue in the optimal amount. In a sustained controlled release fashion, there are various approaches in delivering a therapeutic substance to the target site. Microsphere, as carrier for drug is one such approach which can be used in a sustained controlled release fashion. The range of techniques for the preparation of microspheres offers a variety of opportunities to control drug administration issue. This approach allows the accurate delivery of small quantity of the potent drugs, reduced drug concentration at the site other than the target site and the protection of the labile compound before and after the administration and prior to the site of action.

Microspheres are defined as “Monolithic sphere or therapeutic agent distributed throughout 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. It has a particle size of (1-1000nm). Microspheres are small spherical particles, with diameters 1 μm to 1000 μm. 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 micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped

substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects.

Table 1. Comparison of controlled release devices.

Controlled release vehicle	Example products/ technologies	Advantages	Disadvantages
Implantable matrix devices	GLIADEL® Wafer (Guilford Pharmaceuticals) Norplant® (Wyeth-Ayerst Laboratories)	Biodegradable (often) Localised delivery High local drug concentration	Invasive administration Device removal may be necessary
Implantable pumps	Viadur® (Duros® implant, Alza) MiniMed 2007 (Medtronic)	Long-term delivery (up to at least 1 year) Well-controlled delivery rate Programmable delivery rate	Invasive administration Complex fabrication Need for long-term drug stability Device removal usually necessary
Injectable gels and hydrogels	Alzamer® depot (Alza, Inc.) OncoGel™ (ReGel® technology, MacroMed, Inc.)	Biodegradable Injectable Relatively easy fabrication, processing Drug release can be responsive to pH, temperature etc.	Relatively large size Poor mechanical strength
Liposomes/micelles (up to 30 μm)	Doxil® (STEALTH® liposome, Alza) Abelcet® (Enzon, Inc.)	Low toxicity Low immunogenicity	Poor control of drug release rates Specific targeting Expensive to mass produce Poor stability
Microspheres (1 – 100 μm)	Nutropin Depot® (Genentech/ Alkermes) Lupron Depot® (TAP Pharmaceuticals)	Biodegradable Biocompatible Easily administered – injectable, inhalable Localised or targeted delivery possible	Difficult to control drug release rates Drug instability Difficult large-scale production
Reservoir devices	Ocusert (Dominion Pharma Ltd) Vitrasert® (Control Delivery Systems)	Localised delivery to virtually any site Controlled rate and duration of delivery	Complex fabrication Invasive administration Removal of device required

A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres. These materials include the polymers of natural and synthetic origin and also modified natural substances. Synthetic polymers employed as carrier materials are methyl methacrylate, acrolein, lactide, glycolide and their copolymers, ethylene vinyl acetate copolymer, polyanhydrides, etc. The natural polymers used for the purpose are albumin, gelatin, starch, collagen and carrageenan.

Table 2: List of marketed microsphere drug products.

Drug	Commercial Name	Company	Technology
Risperidone	RISPERDAL® CONSTA®	Janssen®/Alkermes, Inc.	Double emulsion (oil in water)
Naltrexone	Vivitrol®	Alkermes	Double emulsion (oil in water)
Leuprolide	Lupron Depot® Enantone Depot® Trenantone®	TAP Takeda Takeda	Double emulsion (water in oil in water)
Octreotide	EnantoneGyn Sandostatin® LAR	Takeda Novartis	Phase separation
Somatropin	Nutropin® Depot®	Genentech/Alkermes	AlkermesProLease® Technology (Cryogenic spray-drying)
Triptorelin	Trelstar™ depot Decapeptyl® SR	Pfizer Ferring	Phase separation
Buserelin	Suprecur® MP	Sanofi-Aventis	N/A
Lanreotide	Somatuline® LA	Ipsen-Beafour	Phase separation
Bromocriptine	Parlodel LAR ™	Novartis	Spray dry
Minocycline	Arestin®	Orapharma	N/A

Table 3: Patents of microspheres.

Sr. No.	Patent No.	Drug Used
1	CN 201110142359	Ketoprofen
2	CN 201110313846	Paclitaxel
3	CN 201210025085	5-fluorouracil
4	US 08455091	Ganciclovir
5	EP 19980924438	Cimetidine
6	EP 20070808011	Risperidone
7	CA 2217462	Cyclosporin
8	CA 2579533	Irinotecan
9	DE 1999609777	Levonorgesterel
10	DE 1994632867	Doxorubicin
11	US 3691140	Acrylate Copolymer Microspheres
12	US 5871778	Sustained Release Microsphere Preparation Containing Antipsychotic Drug
13	US 5945126	Continuous Microsphere Process
14	US 6207171	Continuous Microsphere Process
15	US 6270802	Method and Apparatus for Formulating Microspheres and Microcapsules
16	US 6998074	Method for Forming Polymer Microspheres
17	US 8440614	Polymer microspheres/Nanospheres And Encapsulating Therapeutic Proteins Therein

2) Polymers

Microspheres used usually are polymers. They are classified into two types

a. Synthetic Polymers

b. Natural polymers

a. **Synthetic polymers:** Poly alkyl cyano acrylates is a potential drug carrier for ophthalmic, oral and parenteral preparations. Poly lactic acid is a proper carrier for sustained release of anti-neoplastic agents such as cisplatin, cyclo-phosphamide, and doxorubicin and narcotic antagonist.co-polymer of poly lactic acid and poly glycolic acid are used for sustained release preparation for anti-malarial drug. Poly adipic anhydride is used to encapsulate timolol maleate for ophthalmic delivery. Poly acrolein microspheres are functional type of microspheres. They do not require any activation step since the surfacial free aldehyde groups over the poly acrolein can react with Ammonia group of protein to form Schiff's base.

Synthetic polymers are divided into two types

(A) Non-biodegradable polymers

For examples: Poly methyl methacrylate acrolein (PMMA), Glycidyl methacrylate, Epoxy polymers.

(B) Biodegradable polymers

For example: Lactides and Glycolides and their copolymers, Poly alkyl cyano acrylates, Polyanhydrides and Poly-ε-caprolactone (PCL)

b. **Natural polymers:** Albumin is widely distributed natural protein. This is considered as a potential carrier of drugs or proteins (for their site-specific localization). It is widely used for the targeted drug delivery to the tumour cells in cancer. Gelatin microspheres can be used as carrier system capable of delivering the drugs or biological response modifiers as interferon to phagocytes. Starch, polysaccharide belongs to carbohydrate class. It consists of glucopyranose as principle unit, which on hydrolysis yields D-glucose. Starch, being a poly saccharide consists of a large number of free hydroxyl groups. By means of these free hydroxyl groups a large number of active substances can be incorporated within as well as active on surface of microspheres. Chitosan is a deacylated product of chitin. The chitosan effect has been considered because of its Charge. It is insoluble at neutral and alkaline PH values, but it forms salts with inorganic and organic salts. Upon dissolution, the amino groups of chitosan get hydrogenated, and the resultant polymer gets positively charged.

They 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 dextran, Poly starch. (microsphere-comparative study).

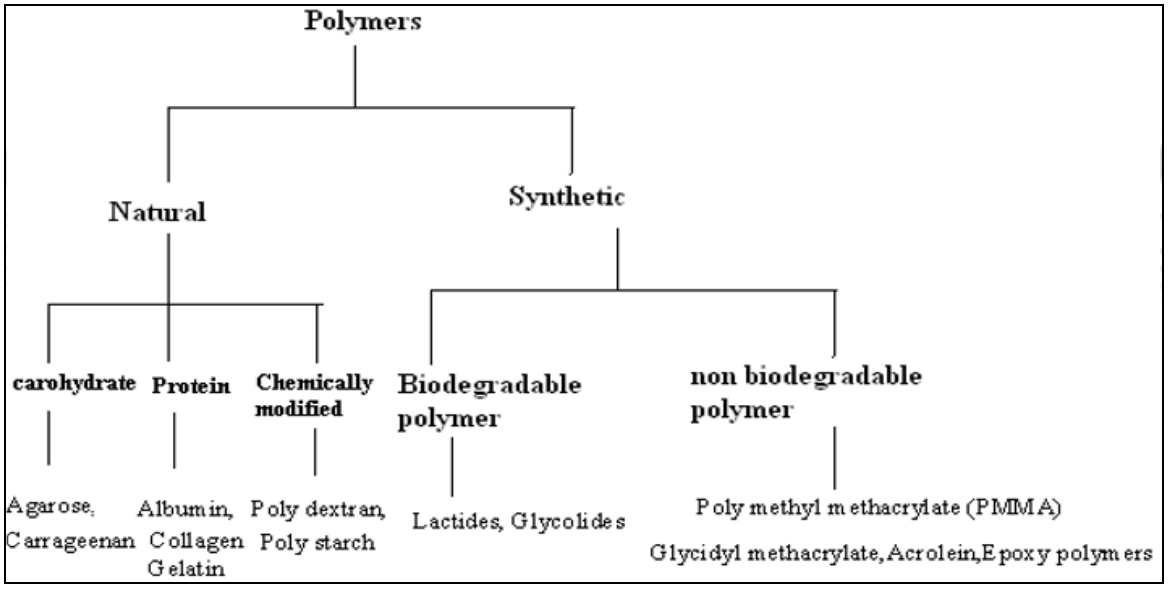

Figure 1: Classification of polymers.

The microsphere in pharmaceutical industry has been considered since the 1960s for their following applications

- Masking of taste and odour.
- Delay of volatilization
- Safe in case of toxic substances.
- Flow of powder is improved
- Sustained-release, controlled-release, targeted medication can produce
- Reduced dose dumping
- Best for incompatible materials
- Provide protection to drug against the environment etc.

3) Ideal characteristics of microspheres

- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersability in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.

- Biocompatibility with a controllable biodegradability.
- Susceptibility to chemical modification.
- Longer duration of action
- Provide protection of drug
- Sterilizability
- Water solubility
- Relative stability
- Bio-resorbability
- Control of content release
- Increase of therapeutic efficiency
- Reduction of toxicity
- Target ability
- Polyvalent

4) Advantages: of microspheres over single unit dosage forms

- Microspheres provide constant and prolonged therapeutic effect.
- Convert liquid to solid form & to mask the bitter taste.
- Reduces the dosing frequency and thereby improve the patient compliance.
- They could be injected into the body due to the spherical shape and smaller size.
- Protect the drug from enzymatic and photolytic cleavage hence found to be best for drug delivery of protein.
- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere morphology allows a controllable variability in degradation and drug release
- Microspheres spread out more uniformly in the GIT, thus avoiding exposure of the mucosa locally to high concentration of drug. Protects the GIT from irritant effects of the drug.
- Microspheres ensure more reproducible drug absorption.
- The risk of dose dumping also seems to be considerably lower than with single unit dosage form.
- Microspheres allow the administration of much smaller doses than are normally required. This reduces local irritation when compared to single unit dosage forms.

- Drug discharge in the stomach may be hindered and local unwanted effects may be reduced or eliminated.
- Microspheres possess many other advantages such as high bioavailability, rapid kinetic of absorption and improvement of patient compliance.
- Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour.

5) Limitations: Some of the disadvantages were found to be as follows:

- The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
- The fate of polymer matrix and its effect on the environment.
- The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
- Reproducibility is less.
- Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to be encapsulated.
- The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.
- The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
- Differences in the release rate from one dose to another.
- Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
- Dosage forms of this kind should not be crushed or chewed.

6) Types of microspheres

1. Bio-adhesive microspheres
2. Magnetic microspheres
3. Floating microspheres
4. Radioactive microspheres
5. Polymeric microspheres

a) Biodegradable polymeric microspheres

b) Synthetic polymeric microspheres

1. **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.

2. **Magnetic microspheres:** This kind of delivery system is very much important which localises 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. The different types are

a. Therapeutic magnetic microspheres used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.

b. Diagnostic microspheres, used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano-size particles supra-magnetic iron oxides.

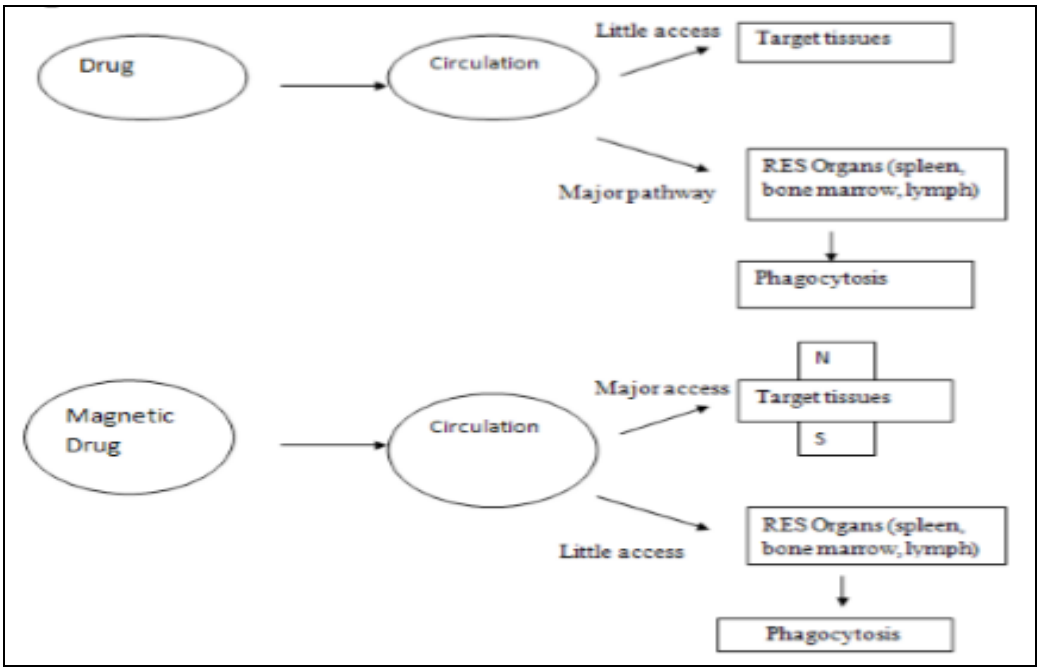

Figure 2: Drug targeting via magnetic and non-magnetic systems.

3. **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 floating

microspheres are gastro-retentive drug delivery systems based on effervescent and non-effervescent approach. The size of microsphere is less than 200 mm and is available in free-flowing powders. Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. The drug is released slowly at the desired rate, and the system is found to be floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover, it also reduces chances of dose dumping. It produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug (ketoprofen) is given in the form of floating microspheres.

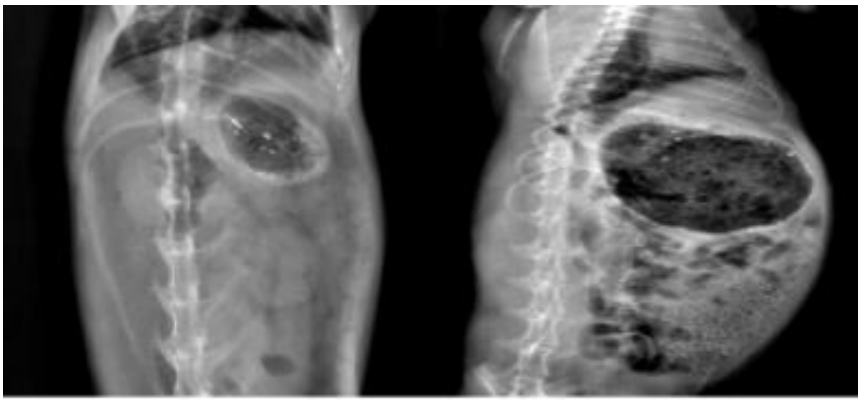

Figure 3: Floating microspheres observed in upper part of GIT at 12h and 24h.

The gastro retentive dosage forms (GRDFs) has been designed in large part based on the following approaches: (a) low density form of the DF that causes buoyancy above gastric fluid; (b) high density DF that is retained in the bottom of the stomach; (c) bio-adhesion to the stomach mucosa; (d) slowed motility of the GIT by concomitant administration of the drugs or p'ceutical excipients; (e) expansion by swelling or unfolding to a large size which limits emptying of the DF through the pyloric sphincter.

It is useful for drugs which act locally in the stomach, poorly soluble in alkaline pH, having narrow window of absorption, unstable in intestine or colonic environment, primarily absorbed in the stomach and higher absorption in upper part of intestine can be used to deliver through floating system.

4. **Radioactive microspheres:** Radio embolization therapy microspheres sized 10-30 nm are of larger than the diameter of the capillaries and gets tapped in first capillary bed when they come across. They are injected in the arteries that leads them to tumour of

interest so all these conditions radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery system, as radio activity is not released from microspheres but acts from within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

5. **Polymeric microspheres:** The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and Synthetic polymeric microspheres.

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 prolong 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 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. E.g. As a drug carrier, chitosan has been investigated for the sustained delivery of many oral formulations and parenteral formulations. One of the widely used polymer for a variety of pharmaceutical applications to be blended with CS is poly(vinyl alcohol) (PVA), because of its permeability, biocompatibility, biodegradability, excellent chemical resistance and physical properties. It exhibits minimal cell adhesion and protein adsorption.

The chief advantage of using biodegradable polymers is that after performing their tasks they breakdown in a biologically friendly manner.

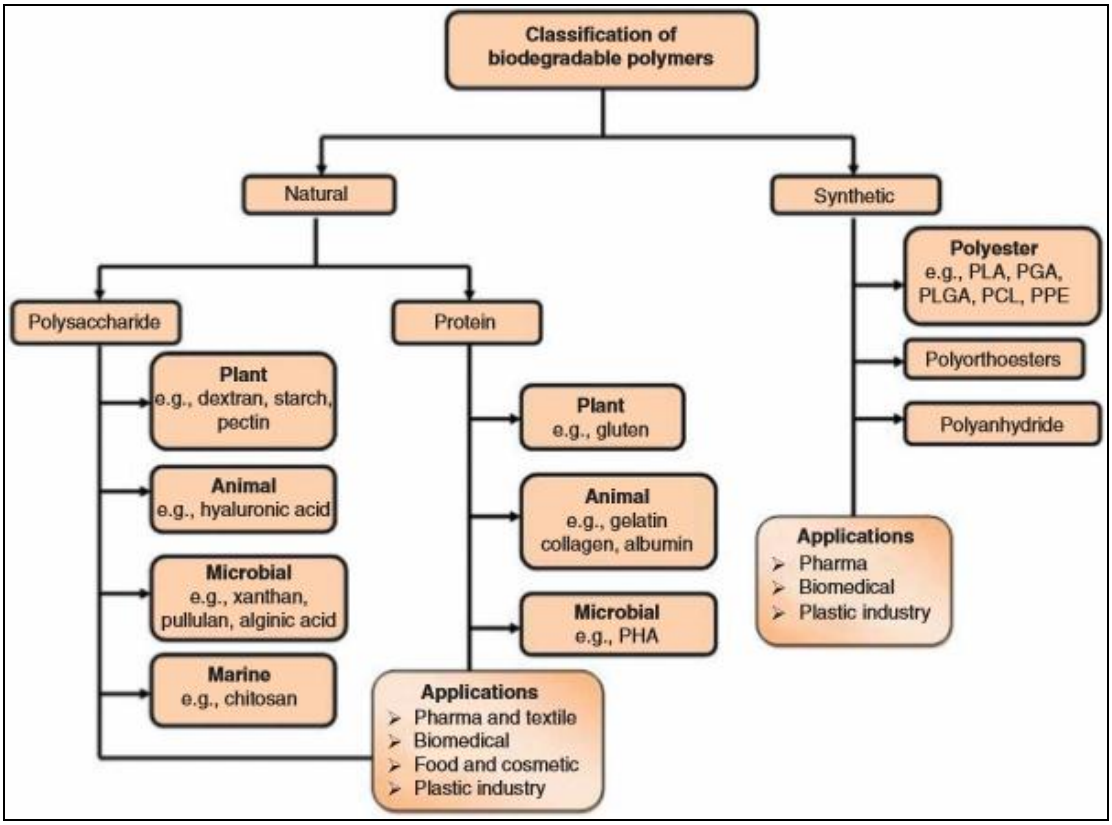

Figure 4: Classification of biodegradable polymers.

Synthetic polymeric microspheres: 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 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.

The various types of polymers are used for preparation of microspheres e.g.

- Albumin microspheres
- Gelatin microspheres
- Starch microspheres
- Dextran microspheres
- Poly lactide and poly glycolide microspheres
- Polyanhydride microspheres and poly-phosphazene microspheres
- Chitosan microspheres
- Polysaccharides or lipid cross linked chitosan microspheres
- Carrageenan microspheres
- Alginate microspheres

- Poly (alkyl cyanoacrylate) microspheres.

7) Methods of preparation

The choice of technique depends upon the nature of polymer as well nature of drug and the duration of therapy. The most important physical chemical factors that may be controlled in microsphere manufacture are

- The particle size requirement
- Molecular weight of polymer
- Polymer to drug ratio
- No stability problem
- Final product should be non-toxic.
- Total mass of drug and polymer
- Reproducibility
- Controlled particle size and dispersability in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale

8) Microencapsulation

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment. The products obtained by this process are called microparticles, microcapsules and microspheres which differentiate in morphology and internal structure. When the particle size is below 1mm they are known as nanoparticles, nano-capsules, nano-spheres, respectively, and particles having diameter between 3–800mm are known as microparticles, microcapsules or microspheres. Particles larger than 1000mm are known as macroparticles. Microencapsulation can be done

1. To protect the sensitive substances from the external environment,
2. To mask the organoleptic properties like colour, taste, odour of the substance,
3. To obtain controlled release of the drug substance,
4. For safe handling of the toxic materials,
5. To get targeted release of the drug and
6. To avoid adverse effects like gastric irritation of the drug, e.g. aspirin is the first drug which is used to avoid gastric irritation.

9) Microencapsulation techniques

Various techniques are available for the encapsulation of core materials. Broadly the methods are divided into three types. Different types of microencapsulation techniques are:

1. Chemical methods;
2. Physico-chemical methods; and
3. Physico-mechanical methods.

Table 4: Different techniques used for microencapsulation.

Chemical processes	Physico-chemical processes	Physico-mechanical process
Interfacial polymerization	Coacervation and phase separation	Spray drying and congealing
In situ polymerization	Sol-gel encapsulation	Fluid bed coating
Poly condensation	Supercritical CO_2 assisted microencapsulation	Pan coating
		Solvent evaporation

The above-mentioned techniques are widely used for microencapsulation of several pharmaceuticals. Among these techniques, fluidized bed or air suspension method, coacervation and phase separation, spray drying and spray-congealing, pan coating and solvent evaporation methods are widely used. Depending on the physical nature of the core substance to be encapsulated the technique used will be varied.

Table 5: Microencapsulation processes and their applicability.

Microencapsulation process	Nature of the core material	Approximate particle size (μm)
Air suspension	Solids	5–5000*
Coacervation and phase separation	Solids and liquids	2–5000*
Multi-orifice centrifugation pan coating	Solids and liquids	1–5000*
Spray drying and congealing	Solids	600–5000*
Solvent evaporation	Solids and liquids	600
	Solids and liquids	5–5000*

*The 5000 μm size is not a particle size limitation. The methods are also applicable for macrocoating

Techniques for microsphere preparation

1. Single emulsion techniques
2. Double emulsion techniques
3. Polymerization

a. Normal polymerization

- Bulk
- Suspension
- Emulsion

b. Inter-facial polymerization

4. Phase separation coacervation technique
5. Spray drying and spray congealing
6. Solvent extraction
7. Emulsification method
8. Wax coating and Hot-melt method
9. Quasi emulsion solvent diffusion
10. Supercritical fluid (SCF) technique

1. **Single emulsion technique:** There are several Proteins and carbohydrates, which are prepared by this technique. In which the natural polymers are dissolved in aqueous medium and the followed by dispersion in oil phase i.e. non-aqueous medium. That is the first step in Next step cross linking is carried out by two methods:

a) **Cross linking by heat:** by adding the dispersion into heated oil, but it is unsuitable for the Thermolabile drugs.

b) **Chemical cross-linking agents:** By using agents i.e. formaldehyde, di acid chloride, glutaraldehyde etc. but it is having a disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing and separation. Chitosan solution (in acetic acid) by adding to Liquid paraffin containing a surfactant resulting formation of w/o emulsion. Metformin hydrochloride microsphere are prepared by using gluteraldehyde 25% solution as a cross linking agent.

2. **Double emulsion technique:** Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited for water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. It is best suited to water soluble drugs, peptides, proteins and the vaccines. Natural as well as synthetic polymer can use for this

method. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like luteinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/ extraction.

3. **Polymerization:** Existing marketed microsphere products are composed of homo and copolymers of lactides and glycolides (PLGA). These polymers have a long history of human safety and regulatory approval. The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as:

a. Normal polymerization
b. Interfacial polymerization.

Both are carried out in liquid phase.

a. Normal polymerization: In bulk polymerization, a monomer or a mixture of number 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 by adding the drug during the process of polymerization. It is a pure polymer formation technique but it is very difficult to dissipate the heat of reaction which affects the thermo labile active ingredients. Suspension polymerization is carried out of lower temperature and also refer to as pearl polymerization in which heating the monomer mixture with active drug as droplets dispersion in continuous aqueous phase. Microsphere size obtained by suspension techniques is less the 100 µm. Emulsion polymerization is differ from the suspension as due presence of initiator in aqueous phase but is also carried out at low temperature as suspension external phase normally water in last two techniques so through which heat can easily dissipate formation of higher polymer at faster rate is possible by these techniques but association of polymer with the un reacted monomer and other additives can occur.
b. Interfacial polymerization: It involves the reaction of various monomers at the interface between the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase. In this technique two reacting monomers are employed; one is dissolve in continuous phase while other is disperse in continuous phase (aqueous in

nature) throughout which the second monomer is emulsified. Two conditions arise because of solubility of formed polymer in the emulsion droplet. That is formation is monolithic type of carrier if the polymer is soluble in droplet. Capsular type formed if the polymer is insoluble in droplet.

4. **Phase separation coacervation technique:** It is the simple separation of a micromolecular solution into two immiscible liquid-phase. In this process, the polymer is solubilized to for a solution. This process is designed for preparing the reservoir type system e.g. encapsulate water soluble drugs i.e. peptides, proteins etc. The principle of coacervation is decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, formation of dispersion of drug particles in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Matrix types preparations can also be prepared by this process for hydrophilic drug e.g. steroids, Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer.

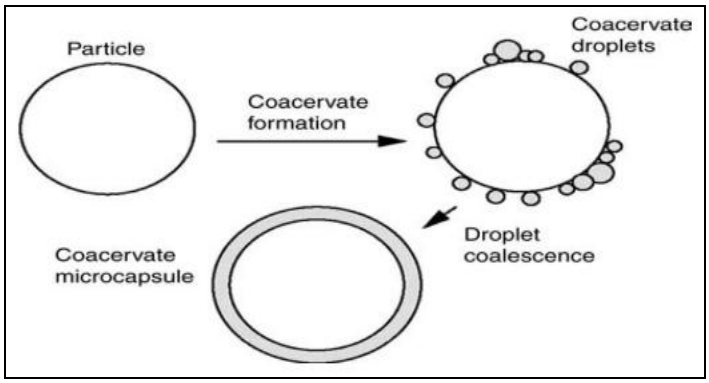

Figure 5: Schematic diagram of the formation of a coacervate around a core material.

But this method is not suitable for organic solvents and glutaraldehyde which are toxic in nature. Prednisolone sodium phosphate loaded chitosan microspheres using sodium sulphate as a precipitant were prepared. Addition of sodium sulphate to the solution of chitosan in acetic acid resulted in decreased solubility of chitosan, leading to precipitation of chitosan as a poorly soluble derivative.

5. **Spray drying and spray congealing:** Concept of spray drying technique depending upon the removal of solvent or the cooling of solution the two processes are spray drying &

spray congealing. Evaporation is the basic mechanism in spray drying, whereas in spray congealing it is that of a phase inversion from a liquid to a solid. Both processes are similar, except for energy flow. Spray drying is the most widely used industrial process involving particle formation and drying. Therefore, spray drying is an ideal process where the end product must comply with precise quality standards regarding particle size distribution, residual moisture content, bulk density, and particle shape.

Principle: Three steps involved in spray drying

a. **Atomization:** of a liquid feed change into fine droplets.
b. **Mixing:** it involves the passing of hot gas stream through spray droplets which result in evaporation of liquids and leaving behind dried particles.
c. **Dry:** Dried powder is separated from the gas stream and collected.

In this technique 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, this form small droplets or the fine mist, from which the solvent evaporates instantaneously leading the formation of the microspheres. The size range is 1-100 µm. By using hot air separate of Microparticle by means of the cyclone separator while the traces of solvent are removed by vacuum drying. Advantages of the process are feasibility of operation. This technique is very useful to encapsulate various penicillins. Thiamine mononitrate and sulpha ethylthiadizole are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous microparticles.

The sprays are produces by either rotary (wheel) or nozzle atomizers. Evaporation of moisture from the droplets and formation of dry particles proceed under controlled temperature and airflow conditions.

The microsphere size is controlled by the rate of spraying, nozzle size, temperature (in drying and collecting chambers.) and the feed rate of polymer drug solution. The quality of product is improved by addition plasticizer spray flow rate should kept constant around 6ml/min.

Spray drying technique is also useful for preparing chitosan microsphere5, In 1999 He et.al. Used formaldehyde as a crosslinking and also reported a novel method in which cimetidine

and famotidine were entrapped in microspheres prepared by spray drying of multiple emulsion (o/w/o or w/o/w). They found that the release of the drugs from microspheres by this novel method was significantly sustained as compared to those prepared by conventional spray drying or o/w emulsion method. Spray drying was used for the preparation of PCL microspheres of ketoprofen. The organic solution of the drug and two polymers, cellulose acetate butyrate and PCL was made in a mixture of dichloromethane and chloroform (1:1). The prepared solution was sprayed through a nozzle in a spray-drier under different experimental conditions. Solid microspheres were collected into final bottom vessel spray-drier.

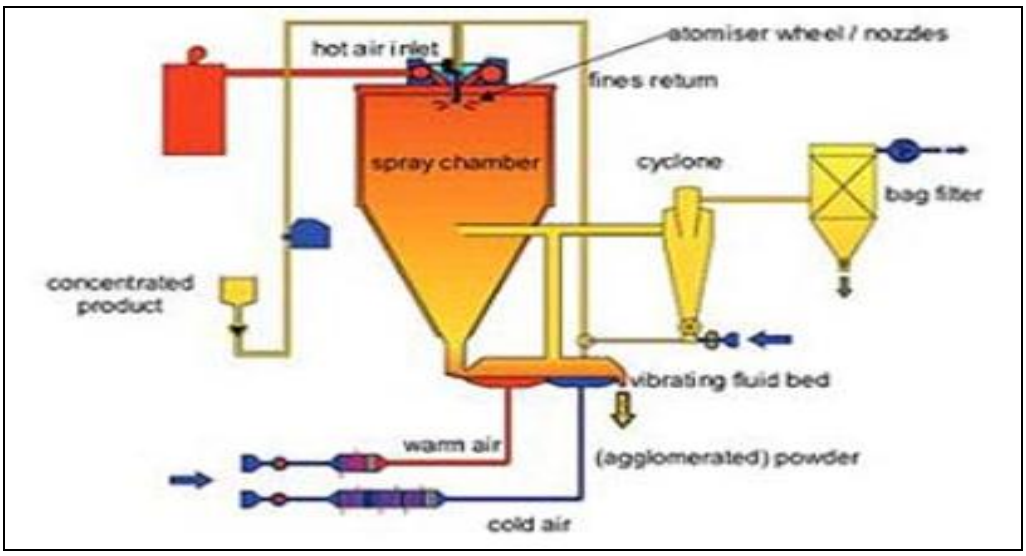

Figure 6: Spray drying method.

Advantages and disadvantages

Spray drying is very useful for pulmonary drug delivery as well as for oral dosages form and it is remarkable versatility of the technology, and a wide range of product can be obtained by this technique. It is very flexible and reproducible method that, why number of industries use this technique for drying operation. It can be designed to virtually any capacity required easily. Can be used with both heat-resistant and heat sensitive products. Powder quality remains constant during the dryer. Particles which produced uniform in size and frequently hollow thus reduce the bulk density of the product. But there are some drawbacks in technique; the equipment is very bulky and expensive. The overall thermal efficiency is low, as the large volumes of heated air pass through the chamber without contacting a particle.

6. **Solvent extraction/evaporation:** For the formation of the emulsion between polymer solution and an immiscible continuous phase in aqueous (o/w) as well as non-aqueous phase (w/o). This procedure requires both the drug and the polymer to be insoluble in water, while the polymer should also be soluble in the water-immiscible solvent. Solvent evaporation method is used for the preparation of micro particles, involving removal of the organic phase by extraction of the organic solvent. The method involves water miscible organic solvents such as is o-propanol. Organic phase is removed by extraction with water. This 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.

The solvent evaporation method has been used extensively to prepare PLA and PLGA microspheres containing many different drugs.

An alcohol-in-oil emulsion can be used to prepare microspheres of highly water- soluble compounds. Microspheres ranging in size between 200 and 5OO μm in diameter can be manufactured and the release rates can be modified by the incorporation of plasticizers and/or surfactants.

E.g. eudragit based microspheres offer a high degree of protection from premature drug release in simulated GIT conditions and deliver most of the drug load in the colon and allow drug release to occur at the desired site by emulsion solvent evaporation system.

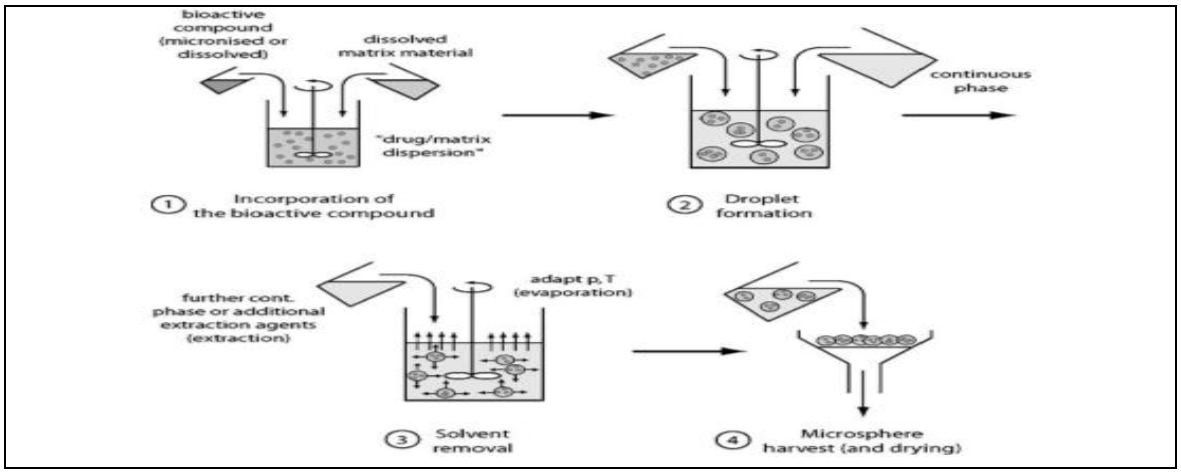

Figure 7: Schematic overview over the four principal process steps in microsphere preparation by solvent extraction/evaporation.

Table 6: Examples of hydrophobic drugs encapsulated using solvent evaporation technique.

Name of drug	Polymer
Cisplatin, 5-fluorouracil (anticancer agents)	PLGA PLGA
Lidocaine (local anesthetics)	PLGA PLA
Naltrexone, cyclazocine (narcotic antagonists)	PLA PLA PELA (PLA + PEG)
Progesterone (hydrophobic steroids)	PLGA PLA PLA

Table 7: Examples of hydrophilic drugs encapsulated using solvent evaporation technique

Name of drug	Method
Insulin	o/o w/o/w o/w dispersion
Proteins	o/o w/o/w
Peptides	w/o/w co-solvent o/w dispersion o/o
Vaccine	w/o/w

7. **Emulsification method:** Multiple emulsions may also be formed for example; a heated aqueous drug solution can be dispersed in molten wax to form a water-in-oil emulsion, which is emulsified in a heated external aqueous phase to form a water-in-oil-in-water emulsion. The system is cooled and the microcapsules collected. For highly aqueous soluble drugs, a non-aqueous phase can be used to prevent loss of drug to the external phase. Another alternative is to rapidly reduce the temperature when the primary emulsion is placed in the external aqueous phase.

8. **Wax Coating and Hot Melt:** In this technique polymer is disperse in suitable dispersion medium and slowly cooled to form the microspheres. The polymers which having low melting point fabricated into microspheres by this technique easily. For coating and coring of particle wax is use mostly. In which encapsulate the drug by dispersion in the melted wax. The wax suspension is dispersed by high speed mixing into cold solution for

example liquid paraffin. Agitate the mixture for one hour. Then decanted the external phase and suspended microspheres collect from solvent. And allow drying it in air. It is inexpensive method as comparison to others and drug release is more rapid. Mostly Carnauba wax and beeswax can be used as the coating materials and these can be mixed in order to achieve desired characteristics.

9. **Quasi emulsion solvent diffusion:** A novel quasi-emulsion solvent diffusion method to manufacture the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Micro-sponges can be manufactured by a quasi-emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol. The internal phase consists of drug, ethanol and polymer. The concentration of polymer is in order to enhance plasticity. At first, the internal phase is manufactured at 60ºC and then added to the external phase at room temperature. After emulsification process, 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 a day.

10. **Supercritical fluid (SCF) technique:** A new supercritical fluid (SCF) technique was developed for the preparation of microspheres for pulmonary drug delivery (PDD). This technique, based on the anti-solvent process, has incorporated advanced engineering design features to enable improved control of the particle formation process. SCF processes have been used for fine particles of proteins and peptides. However, the main difficulty arising for protein processing with these techniques is related to the exposure of the protein to organic solvents, which often results in a significant bioactivity decrease and re-agglomeration during storage.

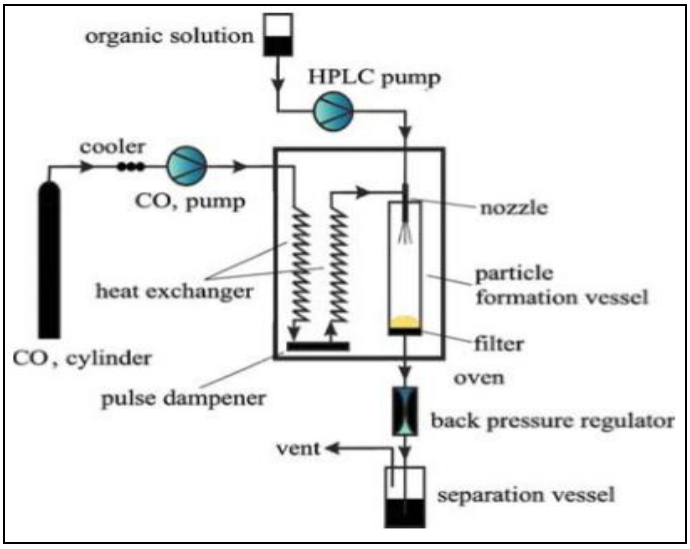

Figure 8: Crystec SCF apparatus.

10) Factors influencing encapsulation efficiency

The efficacy of this microencapsulation process is dependent on many factors, including organic solvent, rate of solvent removal, and amount of organic solvent or drug solubility, drug to polymer ratio, partition coefficient, polymer composition and molecular weight, and method of method of manufacture etc.

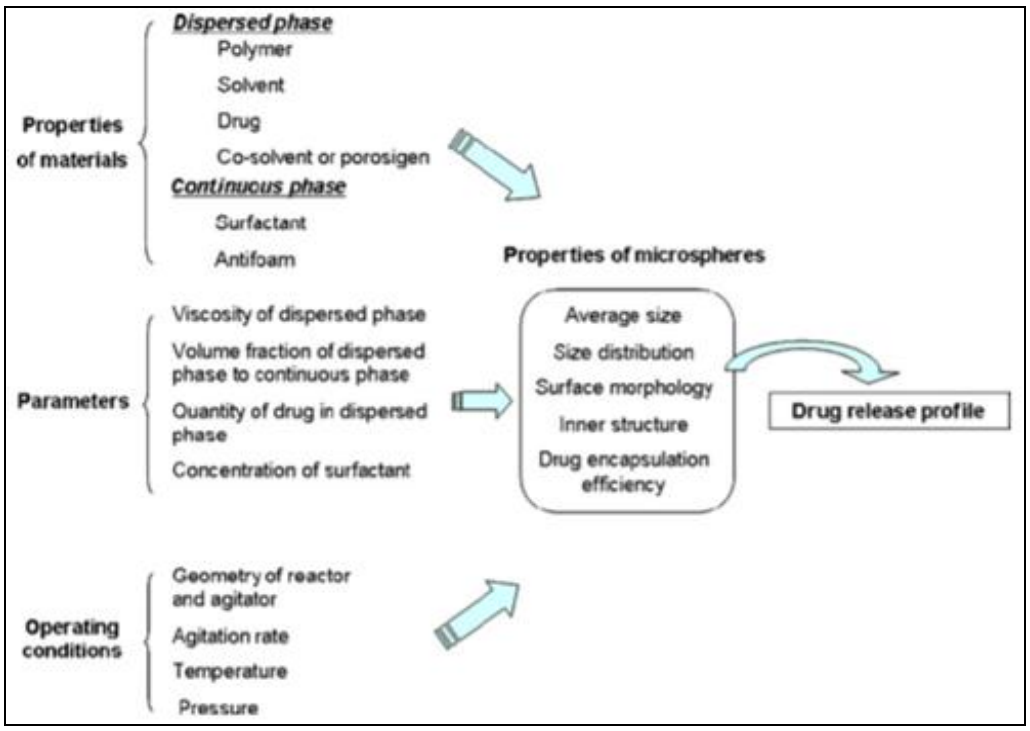

Figure 9: Scheme of the factors influencing the properties of microspheres.

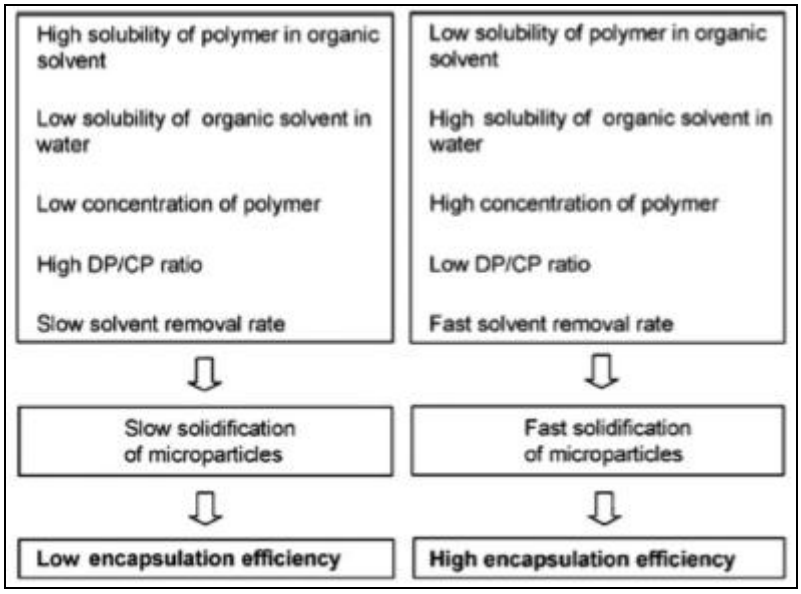

Figure 10: Factors influencing encapsulation efficiency.

11) Evaluation of microspheres

1. **Particle size and shape:** The most widely used procedures to visualize microparticles are conventional light microscopy (LM) and scanning electron microscopy (SEM).

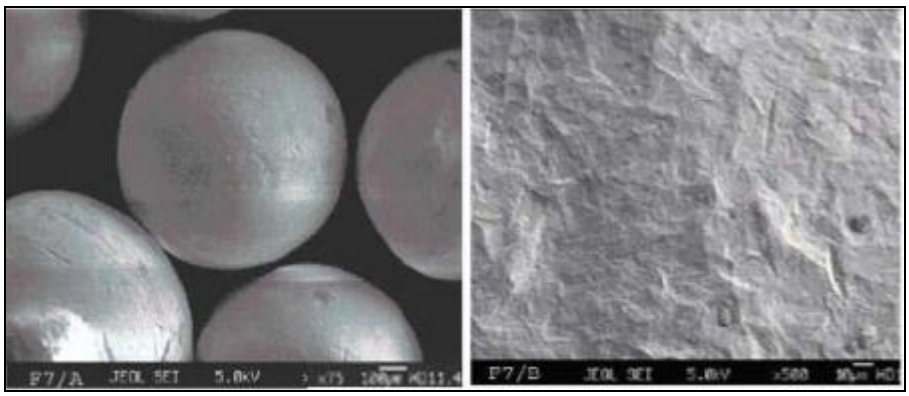

Figure 11: SEM of microspheres.

2. **Electron spectroscopy for chemical analysis:** The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA).
3. **FTIR:** The drug polymer interaction and also degradation of drug while processing for microencapsulation can be determined by FTIR.
4. **X-ray diffraction:** Change in crystallinity of drug can be determined by this technique. Micro particles and its individual components are analysed by the help of XRD Instrument. Scanning range angle between 80°C - 70°C.

5. **Thermal analysis:** Thermal analysis of microcapsule and its component can be done by using

   Differential scanning calorimetry (DSC)

   Thermo gravimetric analysis (TGA)

   Differential thermometric analysis (DTA)

   Accurately the sample is weighed and heated on alumina pan at constant rate of 10°c/min under nitrogen flow of 40 ml/min.
6. **Density determination:** The density of the microspheres can be measured by using a multi volume pycnometer.
7. **Isoelectric point:** The micro electrophoresis is used to measure the electrophoretic mobility of microspheres from which the isoelectric point can be determined.
8. **Angle of contact:** The angle of contact is measured to determine the wetting property of a micro particulate carrier.
9. **In vitro methods:** Release studies for different type of microspheres are carried out by using different suitable dissolution media, mostly by rotating paddle apparatus (USP / BP).
10. **Drug entrapment efficiency:** Microspheres containing of drug (5mg) are crushed and then dissolved in distilled water with the help of ultrasonic stirrer for 3 hr, filtered then assayed by uv-vis spectroscopy. Drug entrapment efficiency can be calculated using following equation,

    % Entrapment = Actual content/Theoretical content x 100.
11. **Swelling index:** The swelling index of the microsphere was calculated by using the formula,

    Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 100.
12. **Contact angle:** Contact angle is used to define the hydrophilicity/ hydrophobicity of the microspheres based on their wetting properties.
13. **Flow properties:** As microspheres are powder dosage forms, it is essential to measure flow properties in order to understand the type of flow, and therefore avoid segregation/ dosage nonuniformity. Understanding the flow properties is also important for packaging the final drug product as well as product administration.
14. **Stability evaluation of microspheres:** Storage stability studies are conducted under various environmental conditions such as temperature and humidity to assess product quality, and define storage conditions and ultimately the shelf life of the drug product.

12) Applications

- Parenteral applications of polymeric microspheres for delivery of biological molecules such as proteins, DNA and vaccines have been extensively studied in preclinical and early clinical phases with promising results
- Drug loaded microspheres can be formulated in to tablets offering the advantages of biocompatibility and biodegradability.
- Monoclonal antibodies mediated microspheres targeting: Monoclonal antibodies (Mabs) targeting microspheres are immune-microspheres. This targeting is a method used to achieve selective targeting at specific sites.

Attachment of microspheres to Mabs by any of the following methods:

1. Non-specific adsorption
2. Specific adsorption
3. Direct coupling
4. Coupling with reagents.

- **Microspheres in vaccine delivery:** The prerequisite of a vaccine is protection against the micro-organism or its toxic product. 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.
4. Targeting using microparticulate carriers.

- **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. 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 in order to achieve the targeting to the discrete organs and to avoid rapid clearance from the body.

- **Imaging:** The particle size plays an important role 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 labelled 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.
- **Ophthalmic drug delivery:** Microspheres developed using polymer exhibits favorable biological behaviour such as bio-adhesion, permeability-enhancing properties, and interesting physico-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles. Eg. Chitosan, Alginate, Gelatin.
- **Oral drug delivery:** The ability of microspheres containing polymer to form films permit its use in the formulation of film dosage forms, as an alternative to pharmaceutical tablets. The pH sensitivity, coupled with the reactivity of the primary amine groups, make microspheres more suitable for oral drug delivery applications. Eg. Chitosan, Gelatin.
- **Gene delivery:** Microspheres could be a useful oral gene carrier because of its adhesive and transport properties in the GI tract. Eg. Chitosan, Gelatin, viral vectors, cationic liposomes, polycation complexes.
- **Nasal drug delivery:** Polymer based drug delivery systems, such as microspheres, liposomes and gels have been demonstrated to have good bio-adhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailability and residence time of the drugs to the nasal route. Eg. Starch, Dextran, Albumin, Chitosan+Gelatin.
- **Intratumoral and local drug delivery:** In order to deliver paclitaxel at the tumor site in therapeutically relevant concentration, polymer films are fabricated. Mixture of drug has

promising potential for use in controlled delivery in the oral cavity. Eg. Gelatin, PLGA, Chitosan and PCL.

- **Buccal drug delivery:** Polymer is an excellent polymer to be used for buccal delivery because it has muco/bio-adhesive properties and can act as an absorption enhancer. Chitosan, Sodium alginate.
- **Gastrointestinal drug delivery:** Polymer granules having internal cavities prepared by de acidification when added to acidic and neutral media are found buoyant and provided a controlled release of the drug. eg. Eudragit, Ethyl cellulose+Carbopol BSA, Gelatin.
- **Transdermal drug delivery:** Polymer has good film-forming properties. The drug release from the devices is affected by the membrane thickness and cross-linking of the film. Eg. Chitosan, Alginate, PLGA.
- **Colonic drug delivery:** Polymer has been used for the specific delivery of insulin to the colon. Eg. Chitosan.
- **Vaginal drug delivery:** Polymer, modified by the introduction of thioglycolic acid to the primary amino groups of the polymer is widely used for the treatment of mycotic infections of the genitourinary tract. Eg. Chitosan, Gelatin, PLGA.
- **Targeting by using microparticulate carriers:** Pellets are prepared with polymer by using the extrusion/spheronization technology. Eg. Chitosan, Microcrystalline cellulose.

13) Recent Advancements in Microspheres

- **Important utilizations of chitosan polymer Cholesterol lowering effects:** Chitosan and cellulose were used as examples of fibres with high, intermediate and low bile acid-binding capacities, respectively. The serum cholesterol levels in a control group of mice fed a high fat/high cholesterol diet for 3 weeks increased about 2-fold to 4·3mM and inclusion of any of these fibres at 7·5% of the diet prevented this increase from occurring. In addition, the amount of cholesterol accumulated in hepatic stores due to the HFHC diet was reduced by treatment with these fibres.
- **Increase Stability of Drug:** Chitosan polymer is used to increase the stability of the drug in which the drug is complexed with chitosan.
- **Orthopaedic Patients:** Chitosan is a biopolymer that exhibits osteo conductive, enhanced wound healing and antimicrobial properties which make it attractive for use as a bioactive coating to improve Osseo integration of orthopaedic and craniofacial implant devices. It has been proven to be useful in promoting tissue growth in tissue repair and accelerating wound-healing and bone regeneration.

- **Cosmetics industry:** Cosmetic compositions are disclosed for the treatment of hair or skin, characterized by a content of new quaternary chitosan derivatives of the formula. The chitosan derivatives have a good substantial, particularly to hair keratin, and prove to have hair strengthening and hair conditioning characteristics. e.g.; Hair setting lotion, Oxidation Hair-coloring Composition, Hair toning Composition, Skin Cream, Hair treatment Composition, Gel-form.
- **Dental Medicine:** Chitosan have been recognized to accelerate wound healing to attain an aesthetically valid skin surface, and to prevent excess scar formation. In dental medicine, chitosan is also applied as a dressing for oral mucous wound and a tampon following radical treatment of maxillary sinusitis. Furthermore, it is being investigated as an absorbing membrane for periodontal surgery. Chitosan has a variety of biological activities and advertised as a healthy food that is effective for improvement and/or care of various disorders, arthritis, cancer, diabetes, hepatitis, etc.
- **Chitosan as Permeation Enhancer:** It has been reported that chitosan, due to its cationic nature is capable of opening tight junctions in a cell membrane. This property has led to a number of studies to investigate the use of chitosan as a permeation enhancer for hydrophilic drugs that may otherwise have poor oral bioavailability, such as peptides. Because the absorption enhancement is caused by interactions between the cell membrane and positive charges on the polymer, the phenomenon is pH and concentration dependant. Furthermore, increasing the charge density on the polymer would lead to higher permeability.
- **Chitosan as Mucoadhesive Excipient:** Bio-adhesivity is often used as an approach to enhance the residence time of a drug in the GI tract, hereby increasing the oral bioavailability. A comparison between chitosan and other commonly used polymeric excipients indicates that the cationic polymer has higher bio-adhesivity compared to other natural polymers, such as cellulose, Xantham gum, and starch.

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