WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 1870-1902. Research Article ISSN 2277– 7105

DESIGN, EVALUATION AND ASEPTIC REFINER TECHNIQUES FOR MICROSPHERE FORMATION

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Article Received on
10 Feb 2015,

Revised on 05 March 2015,
Accepted on 30 March 2015

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ABSTRACT

With advances in biotechnology, genomics and combinational chemistry, a wide variety of new, more potent and specific therapeutics are being created. There are various departments of medicine like cancer, pulmonary, cardiology, radiology, gynaecology, and oncology etc, numerous drugs are used and they are delivered by various types of drug delivery system. Among them microspheric drug delivery system has gained enormous attention due to its wide range of application as it covers targeting the drug to particular site to imaging and helping the diagnostic features. Because of common problems such as low solubility, high potency and/or poor stability of many of these new drugs, the means of drug delivery can impact efficacy and potential for commercialization as much as the nature of the drug itself. Thus there is a corresponding need for safer and more effective methods and devices for drug delivery. Indeed drug delivery system should be designed to provide a therapeutic agent in the needed amount, at the right time to the proper location in the body, in a manner that optimizes efficacy, increases compliance and minimizes side effects. Among a wide variety of devices that have been used for controlled drug delivery, microspheres are one of the most common types and hold several advantages. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 200µm. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particles matrix have the potential for the controlled release of drug. The objective of this article is to emphasize on the principles underlying the development and evaluation of microspheres as a controlled and

targeted drug delivery system as well as details information regarding its mechanism of drug release, evaluation techniques, advantages, applications & challenges encountered by pharma manufacturers when formulating.

KEYWORDS: Microspheres, Characterization, Mechanism of release, Evaluation, Challenges Drug Delivery.

INTRODUCTION

To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.[1] There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. The process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bioerodible polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones. For example, by taking advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behaviour. 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. Microspheres are small spherical particles, with diameters in the micrometer range(typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticles.

Biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin, the synthetic

polymer include poly lactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials chosen according to the polymer and drug solubility and stabilities, process safety and economic considerations.[2] Microspheres for oral use have been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation.

In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided.[3] Microencapsulation is used to modify and retard drug release. Due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa.[4]

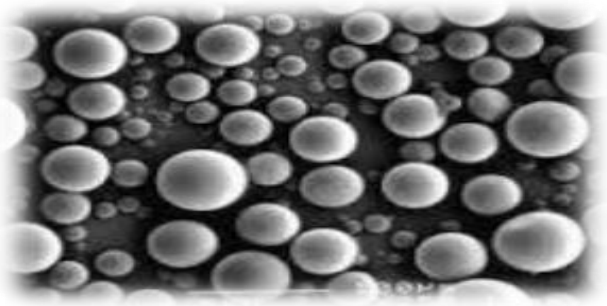


Fig.1: Microspheres.

TYPES OF MICROSPHERE[5, 6, 7]

1. Bioadhesive Microspheres[8, 9, 10]

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[11]

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field

from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different types are therapeutic magnetic microspheres and diagnostic microspheres.

i. Therapeutic Magnetic Microspheres

It is used to deliver chemotherapeutic agent to liver tumor. Drugs like proteins and peptides can also be targeted through this system.

ii. Diagnostic Microspheres

It can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.

3. Floating microspheres[12, 13]

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

4. Polymeric Microspheres[14]

The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and synthetic polymeric microspheres.

i. Biodegradable Polymeric Microspheres[15]

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer 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.

ii. Synthetic Polymeric Microspheres

The interest of synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantage of these kinds of

microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

5. Diagnostic microspheres

Diagnostic microspheres are used for imaging the liver metastases and also can be used to differentiate bowel loops from abdominal structures by formation of nano size particles supramagnetic iron oxides.[16]

6. Radioactive microspheres

Radioactive microspheres are useful for many therapy once the encapsulated diagnostic radioisotopes has been exchanged for therapeutics from the-or β-emitter group. It is used for treatment of rheumatoid arthritis, liver tumors and cystic brain tumors. However, their use remains experimental because of smaller than expected target uptake, unwanted toxicity and insufficient treatment effects that have resulted from radio chemical instability and suboptimal biodistribution of the radiopharmaceutical.[17]

Polymers used in microspheres.[18,19,20,21]

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

MICROSPHERE CHARACTERIZATION METHODS

New applications of microparticles necessitate successful technology transfer, industrial scale up, and reliable investigation methods also in preformulation and in formulation steps.

Design of experiment (DOE). Optimization with factorial based designs and analysis of the response surfaces is a powerful, efficient and systematic tool that shortens the time required for the development of dosage forms and improves research and development work.[22, 23] DOE aids the evaluation of the results of the measurements mentioned below.

Rheological measurements. It can be carried out to investigate the viscosity of the: (**i**) solvent mixture; (**ii**) aqueous and oil phases; and (**iii**) simple/multiple emulsions.

Morphological study. The microparticles can be studied for appearance and the emulsions for droplet type using SEM and optical microscopy, respectively.

Particle size analysis. Microparticles could be sieved with a combined sieving system. One of the commonly used techniques for assessing the PS distribution, SSA and SPAN58 appeared to be laser diffractometry. Photon correlation spectroscopy (PCS), the Coulter® Multisizer II equipment[24] and light or electron microscopy can also be used.[25]

Drug entrapment (E) and encapsulation efficiency (EE). Very common method to measure the drug entrapment, when microparticles are dissolved with applicable solvent, then filtered and analysed with UV-spectrofotometry.[25,26] Protein and peptide content could measure with protein assay: HPLC-method,[27, 28] and Bio-Rad microassay.[29] IgG and IgA levels can be monitored by ELISA method.[30] DSC and XRD183 and EDXRF also were used to measure the actual E value.[31,32] Evaluations of the potential of EDXRF apparatus in microparticles have been performed,[**I**] its application for our purpose can be considered a novelty.

Thermoanalytical measurements (TA). TA is a useful tool in investigating e.g. the solubility of the drug in the polymer.[33] However it should be emphasized that such a solubility is determined at the melting point of the drug and not at ambient temperature. The most common techniques are TG, DSC and DMA, in which structure-dependent physical properties of polymers and drug-loaded polymeric delivery systems are measured when subjected to a controlled temperature program.[34] Interesting types are the modulated temperature DSC (MTDSC)[35], and the 'Heat-cool-reheat' technique when after the 1st heating step the sample is cooled and reheated to delete the disturbing effect of the adsorbed water, so the Tg characteristic to the polymer can be measure clearly.[36]

Raman spectroscopy (RS). Based on the measurement of Raman-scattering by a molecule, RS, FT-Raman, and surface-enhanced Raman (SERS) are used for the structural analysis of molecules, the vibrational characterization of drugs,[37,38,39] the characterization of drug stability, the quantification of complex mixtures, furthermore to confirm the possible interactions,[40] and to differentiate crystalline forms of the materials.[41]

FTIR measurement. It can also be used to characterize the parameters mentioned in connection with RS, often together with other techniques (FTIR + TGA + DSC).[42,43]

Analysis of residual organic solvents and cosolvents. Manufacturers are required to remove residual solvents completely or keep them below acceptable limits, as complete removal is often not possible. Few reports of residual solvent effects are available, such as the effect of residual CH2Cl2 on the crystallinity of the drug.[44,45]

Cumulative drug release and release profile studies. The knowledge of the BCS characteristics of a drug can also be utilized by the formulator to develop a more optimized dosage form based on fundamental mechanistic, rather than empirical information.197 The in vitro dissolution rates of the microparticles can be measured at defined rpm in 37±1 °C buffer solution/deionized water mixture of defined pH according to the USP Drug Release Test 2 criteria. Dissolution in the GI tract takes place under heterogeneous conditions, this is one of the reasons why different buffer solutions (citrate, acetate, phosphate or other) are used, although most of them do not correspond to the physiological situation in the human GI-tract. The use of surfactants in the dissolution systems has physiological significance also as natural surfactants like bile salts (wetting, micellar solubilization, and/or deflocculation). Gastric juice has a relatively low surface tension, (42.7 dyn·cm-1) compared with water (70 dyn·cm-1) which aids in the wetting of both hydrophobic and hydrophilic particles. As in vivo animal studies, generally male New Zealand white rabbits, rhesus monkeys, wild type and transgenic mice can be used[46], and the correlation of the in vitro/in vivo evaluations should be clearly established.[47,48] The types of oral biodegradable polymeric sustained release systems according to the drug release are.[49]

(i) diffusion-controlled systems (reservoir device-microcapsules; and matrix device microspheres);
(ii) dissolution-controlled systems;
(iii) erosion-controlled systems; and
(iv) swelling-controlled systems and hydrogels;
(v) chemically controlled systems;
(vi) constant or zero-order release; and
(vii) other delivery systems.

Drug diffusion can occur[50]

(i) Through polymer matrix;

(ii) Through water-filled pores/cavities; or

(iii) Through both, in parallel and/or sequence. The significance of the initial burst has not been entirely ignored, only less theories have been put forth to fully describe the phenomenon.[51]

Mathematical evaluation

The models can be selected for ideal formulation meeting the USP requirements according to the determination coefficient and the 'goodness-of-fit' test, employing the following set of equations known in the literature (Table 4). Model VI is used to describe the release from swelling-controlled systems[52], its modification were introduced by Kim-Fassihi[53], Peppas-Sahlin[54], and Colombo[55] who suggested that the distance of dissolved gel layer thickness of the polymer is the most important parameter influencing drug release The following methods are also frequently used in microparticle technology.

X-ray diffraction (XRD). Wide-angle (WAXS) and small-angle (SAXS) methods are used to get information on helical polymers, and i.e. in detecting large periodicities in structures such as lamellae, respectively. XRD can be used to quantify the crystalline drug content in microsphere.[56] The amorphous nature of the polymers can be confirmed.[57] DSC and XRD studies reveal the existence of drug-polymer interactions.[58] The first complete analysis of NSAID-loaded ethylcellulose microparticle matrix structure by TG, DSC, HPLC, and XRD was presented in 1991.[59]

NMR measurements. It can show if a rigid microsphere structure is formed due to ionic interaction between the drug and the polymer.[60] To verify that a peptide drug is not modified chemically during microencapsulation, analytical one- and two-dimensional NMR spectroscopy is used.[61]

Electron Microscopy. Freeze-Fracture Electron Microscopy shows information about the internal structure of the microparticles. Atomic force microscopy can be used to study the surface morphology and the porosity of the microspheres.[62] Confocal laser scanning microscope (CLSM) can be used to observe protein distribution within microspheres because proteins themselves show fluorescence in many cases[63] or a fluorescent marker can be added to the organic phase.[64] Confocal fluorescence microscopy (CFM) can reveal the drug

distribution in microspheres prior to and after drug release.[65] Transmission electron microscopy (TEM) analysis was used to characterize the histopathology of the ileum after oral administration of drug containing microparticles to rats.[66] CLSM and TEM were used to investigate the ability of pig ileal Peyer's patch segments to transport microspheres from GI lumen across the mucosa.[67]

Helium pycnometry is used to determine the density of the microparticles, the porosity and pore size distributions can be measured by mercury intrusion porosimetry.[68] For

surface charge measurements, the zeta potentials of suspension of microparticles and nanoparticles can be studied using a Zetasizer.[69,70,71]

Biological activity assay is used to achieve the maximum degree of retained biological activity after the microparticle preparation process. Caco-2 cell studies is used as ex vivo drug dissolution measurement, microparticles may bind to Ca2+ ions which could increase the paracellular permeability of epithelial cell monolayers by opening the tight junctions.[72]

METHODS OF PREPARATION

The choice of technique depends upon the nature of polymer as well nature of drug and the duration of therapy.[73] The most important physical chemical factors that may be controlled in microsphere manufacture are.

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

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

6. Solvent extraction

7. Solution-enhancement dispersion method

8. Wax coating Hot-melt method

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.

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

(2) 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.[74] Metformin hydrochloride microsphere are prepare by using gluteraldehyde 25% solution as a cross linking agent.[75]

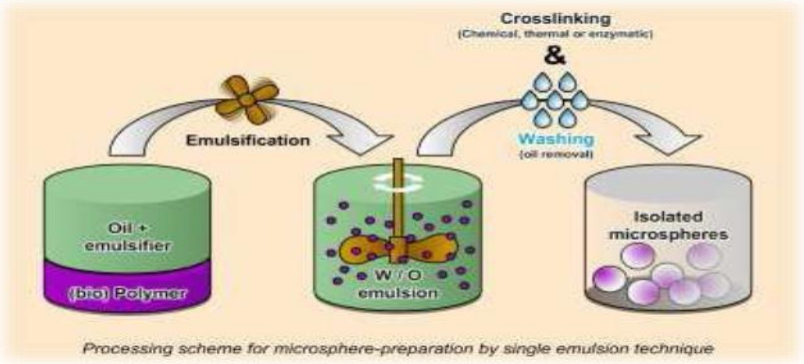


Fig.-1: Microspheres by Single Emulsion Technique

2. Double emulsion technique

It is formation of multiple emulsions i.e. W/O/W is preparing by pouring the primary w/o emulsion into aqueous solution of poly vinyl alcohol. This w/o/w emulsion put a t constant stirring for 30 min. Slowly add some water to the emulsion over a period of 30 min. collect Microcapsules by filtration and dry under vacuum.[76] 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. Disperse in oil/organic phase homogenization/vigorous i.e. formation of first emulsion then addition to aqueous solution of PVA (Poly Vinyl Alcohol) i.e. multiple emulsion formed now by addition to large aqueous phase denaturation/hardening after this separation, washings' and drying and collection of microspheres1 genistein chitosan microsphere were prepared by the o/w/o multiple emulsion method by Wu and Li (2002).[77]

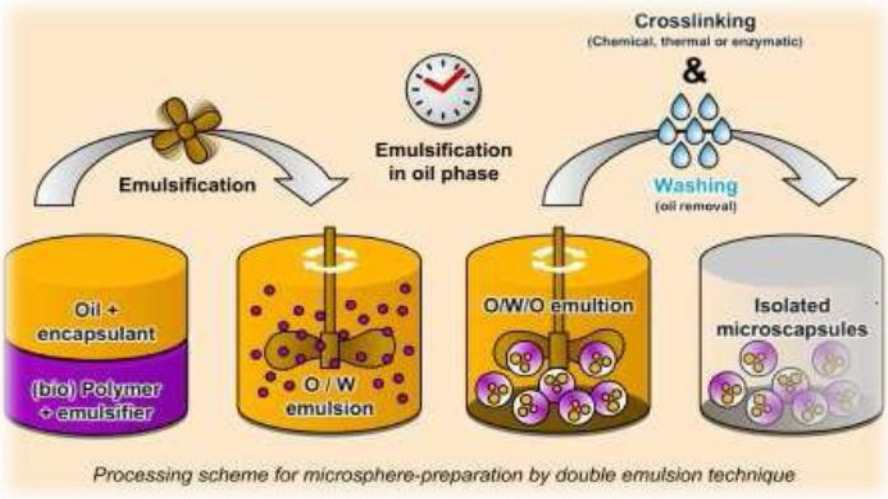


Fig.-2: Microspheres by Double Emulsion Technique

3. Polymerization techniques

Mainly two techniques are using for the preparation of microsphere are classified as.

(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.[78]

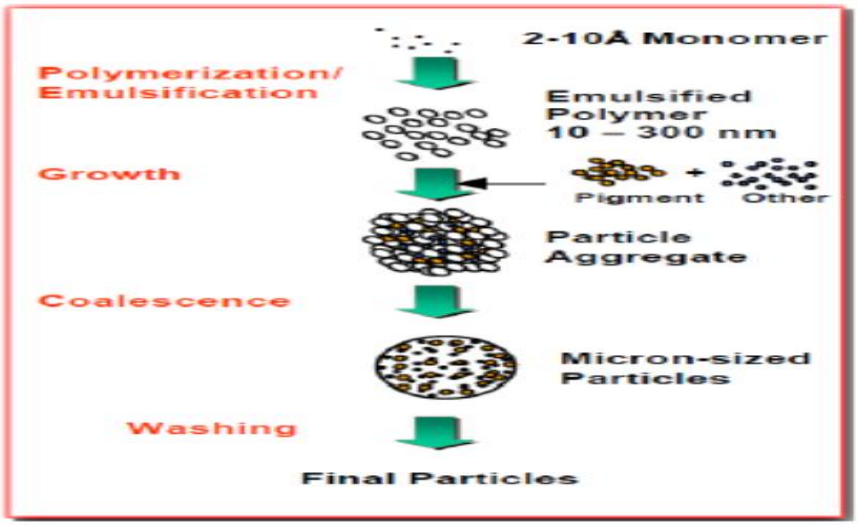


Fig.-3: Microspheres by Polymerization techniques

(b) Interfacial polymerization[79,80]

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. Spray drying and spray congealing

Concept of spray drying technique (fig 1) 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.[81] 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 mononitrate10 and sulpha ethylthiadizole11 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.[82]

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.[83] Spray drying technique is also useful for preparing chitosan microsphere[84], 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.[85,86] In 1994 Giunchedi et al. was used spray drying used for the preparation of PCL microspheres of ketoprofen.[87] (c2) He used 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.[88]

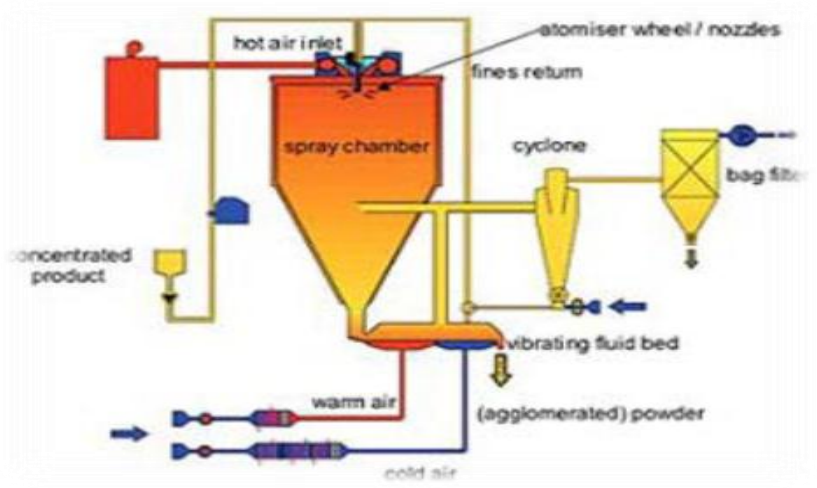


Fig.-4: Spray drying method for preparation of microspheres.

Advantages and disadvantages[89, 90]

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.

5. 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.[91] For coating and coring of particle wax is use

mostly. In which encapsulate the drug by dispersion in the molted 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.[92]

6. Solvent evaporation method[93]

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) (fig 2). Bogatajet al. (2000) prepared microsphere by using liquid paraffin/ acetone as the solvents by evaporation method. The drug solution (in acetone) was dispersed in chitosan solution and this mixture was emulsified in liquid paraffin and stirred. The suspension of microspheres was filtered, washed and dried. Magnesium stearate was also added for preventing agglomeration as a Agglomerationpreventing agent. The results showed that average particle size decreased with increasing amount of magnesium stearate used for microsphere preparation.[94] Lim et al. (2000) investigated the comparison of mucoadhesive microspheres of hyaluronic acid, chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelatin prepared by complex coacervation.[95]

7. 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 etc1. 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.

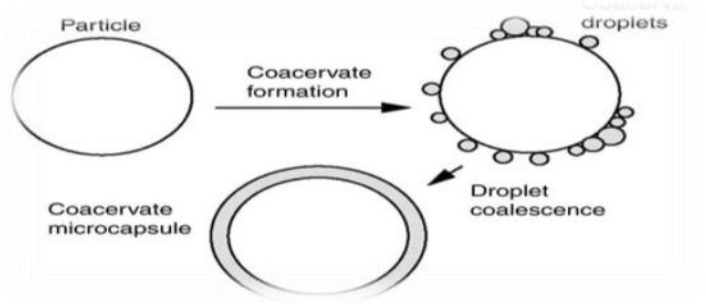


Fig.- 5: Schematic diagram of the formation of a coacervation around a core material

But this method is not suitable for organic solvents and glutaraldehyde which are toxic in nature. Berthold et al. (1996a) prepared prednisolone sodium phosphate loaded chitosan microspheres using sodium sulphate as a precipitant. 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.[96]

8. Solvent extraction,[97]

In this method preparation of microparticles, involves removal of the organic phase by extraction of the organic solvent. Isopropanol can be use as water miscible organic solvents. By extraction with water, Organic phase is removed. Hardening time of microsphere can be decrease by this method. 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**.**

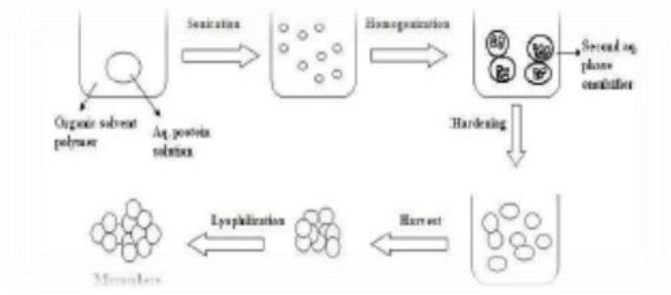


Fig.6: Microsphere preparation by solvent extraction

9. Emulsification method

Multiple emulsions may also be formed[98] 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

The mechanism of drug release from microspheres can occur in the following ways[99]

- **Diffusion:** On contact with aqueous fluids in the gastrointestinal tract (GIT), water diffuses into the interior of the particle. Drug dissolution occurs and the drug solutions diffuse across the release coat to the exterior.
- **Erosion:** Some coatings can be designed to erode gradually with time, thereby releasing the drug contained within the particle.
- **Osmosis:** In allowing water to enter under the right circumstances, an osmotic pressure can be built up within the interior of the particle. The drug is forced out of the particle into the exterior through the coating.

FACTORS THAT DETERMINE THE PROPERTIES OF MICROPARTICLES

A range of production parameters influence the physicochemical parameters of the resulting microspheres.[100] Critical formulation parameters for the W1/O/W2 preparation process are.

Mechanical stirring. When W1/O emulsion is prepared by vortex-mixing, the obtained microspheres are large,[101] however, when by sonication is applied, a microfine and homogeneous emulsion is formed.[102] The EE was reported to increase with increasing mixing rate,[103] whereas other authors found no relationship between these parameters.[104]

Viscosity. The more viscous the polymer solution is, the more difficult it is to break it down into smaller droplets, which leads to larger microparticles. A highly viscous phase and low mixing intensity can be useful in the preparation of microparticles containing sensitive drugs. Increase in the W1/O viscosity is related to an increase in the EE,[105] but W1-phase with higher viscosity will permit the water pass into this phase resulting in swelling and releasing their content into the W2-phase.[106]

Osmotic gradient. The W1 phase usually contains stabilizers (protein, surfactant). The semipermeable surfactant membrane allows some concentration difference, but once the maximum limit is reached (around 10% w/w), transfer of the water droplets through the oil phase will occur. When the W2-concentration is nil, water can penetrate into the W1-droplets, resulting increased PS and viscosity of W2 phase. When the W2-concentration is

twice the W1- concentration, internal water will migrate (W1_W2) resulting smaller droplets.[107]

Volume of the phases. The volume of the W1-phase affects the solidification time, as it decreases, an increase in E[108] and a small decrease in PS[109] can be observed. Low oil phase volume yields a viscous and concentrated polymer solution, so it is more difficult for the oil phase to be broken into smaller droplets, which results in increased PS[110] and porous matrix. The increase in the W2-phase volume leads to an increase in both the PS and E,[111] which is related to the reduced mixing or dispersion efficiency during the 2nd emulsification step due to the larger volume. Generally there is a practical limit of increasing the W1- (_1) and W2-phase (_2) fractions (0.60<_1<0.75 and 0.60<_2<0.80), because either the W1/O emulsion will become far too viscous to be dispersed, or it might invert.

Type of organic solvent-cosolvent. Ever since microparticles have been formulated, the problem of the organic solvent as an important parameter has been present. The integrity of the forming microsphere wall is controlled by the rate of extraction of the organic solvent to the W2 phase and also by the rate of its evaporation from the W2 phase. The rate of solvent extraction is limited by the water-solubility of the organic solvent used, while the evaporation rate depends on its boiling point.

When polar cosolvent is used in the organic polymer solution and is emulsified into the aqueous medium, at the water-organic interface, cosolvents with low affinity for the polymer are the first to diffuse out from the W1/O emulsion droplet (depending on their physicochemical properties) until it attains equilibrium with the W2-phase.[112] Addition of a polar cosolvent and therefore fast partitioning and extraction can decrease the interfacial tension between the organic and aqueous phases, and form a dense wall, which can prevent the confluence of the aqueous phases, and ensure a low PS and a dense microsphere structure with high EE.[113,114] Addition of a cosolvent can increase the porosity, leading to drug loss and therefore a lower EE.[115,116]

Polar cosolvents may act in two opposite ways: (**i**) increasing the polymer precipitation rate and (**ii**) at the same time decreasing E, due to the confluence of the aqueous phases; thus, there can be a sensitive balance between these effects.

Temperature. Below RT the diffusion and evaporation rate of solvents become slow.[117] Above 30 °C, it is easier for the droplets to collide with each other and they may coalesce together at the same time with solidification, since the viscosity of the oil medium is lower at higher temperature.[118] When the solidifying microspheres are exposed to T > Tg of polymer, it will change to its rubbery state which is more flexible and fluent, so the polymer can move through the matrix and fill gaps and coat the existing drug crystals, as in situ micro-coating.[119]

Stabilizers. Addition of buffers (TRIS or PBS82) to the W1-phase could promote an influx of water from the W2-phase due to a difference in osmotic pressure. The addition of salts to the W2-phase results in formation of a dense and homogenous polymer matrix, although they could reduce the solubility of organic solvents in water, resulting the precipitation of polymer.[120]

EVALUATION OF MICROSPHERES

Particle size analyser

Microsphere (50 mg) was suspended in distilled water (5mL) containing 2%w/v of tween 80, To prevent microsphere aggregation, the above suspension is sonicated in water bath and the particle size was expressed as volume mean diameter in micrometer.[121]

Optical microscopy

This method was used to determine particle size by using optical microscope (Meizer OPTIK) The measurement was done under 450x (10x eye piece and 45x objective) and100 particles were calculated.[121]

Scanning electron microscopy (SEM)

Surface morphology was determined by the method SEM. In this microcapsule were mounted directly on the SEM sample slub with the help of double sided sticking tape and coated with gold film under reduced pressure.[121]

Swelling index

This technique was used for Characterization of sodium alginate microspheres were performed with swelling index technique Different solution (100mL) were taken such as (distilled water, buffer solution of pH(1.2, 4.5, 7.4) were taken and alginate microspheres (100mg) were placed in a wire basket and kept on the above solution and swelling was

allowed at 37^0C and changes in weight variation between initial weight of microspheres and weight due to swelling was measured by taking weight periodically and soaking with filter paper.[124]

Entrapment efficiency

Microspheres containing of drug (5mg) were crushed and then dissolved in distilled water with the help of ultrasonic stirrer for 3 hr, and was filtered then assayed by uv-vis spectroscopy. Entrapment efficiency is equal to ratio of actual drug content to theoretical drug content.[125]

X-ray diffraction

Change in crystalinity of drug can be determined by this technique. Microperticles and its individual components were analysed by the help of D & discover (Bruker, Germony).Scanning range angle between 8 0C - 70 0C. Scan speed - 4o/min Scintillation detector Primary silt=1mm Secondary silt=0.6 mm.

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 was weighed and heated on alumina pan at constant rate of 10oc/min under nitrogen flow of 40 ml/min.

UV-FTTR (Fourier transform infra red)

The drug polymer interaction and also degredation of drug while processing for microencapsulation can be determined by FTIR.

Stability studies

By placing the microspheres in screw capped glass container and stored them at following conditions.

1. Ambient humid condition
2. Room temperature (27+/-2 0C)
3. Oven temperature (40+/-2 0C)
4. Refrigerator (5 0C -80C).

It was carried out of a 60 days and the drug content of the microsphere was analysed.[120]

Zeta potential

The polyelectrolyte shell was prepared by incorporating chitosan of different molecular weight into the W2 phase and the resulting particles were determined by zeta potential measurement.[121]

Advantages of Microspheres[121]

- It protects the unstable and sensitive material from prior environment.
- It improves solubility, dispersibility and flow ability.
- It is helpful for safety handling of toxic material.
- To improve the bioavailability
- To improve the therapeutic efficiency of drug.
- To improve the stability of drug.
- Helpful for masking of odor and taste of drug.

Disadvantage of Microspheres

1. The modified release from the formulations.
2. 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.
3. Differences in the release rate from one dose to another.
4. 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.
5. Dosage forms of this kind should not be crushed or chewed.[123]

Application of microspheres

- Microspheres provide constant and prolonged therapeutic effect.
- 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.
- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere is most useful for the preparation of tablets, capsules or parenteral dosage forms.
- Microsphere can be used to prepare enteric-coated dosage forms, so that the medicament will be selectively absorbed in the intestine rather than the stomach.
- It can be used to mask the taste of bitter drugs.

- It has been used to protect drugs from environmental hazards such as humidity, light, oxygen or heat.
- The separations of incompatible substances, for example, pharmaceutical eutectics have been achieved by encapsulation.
- Microsphere can be use to reduce the volatility. An encapsulated volatile substance can be stored for longer times without substantial evaporation.
- The hygroscopic properties of many interior materials may be reduced by microsphere.
- Many drugs have been microsphere to reduce gastric irritation.
- Microsphere technique has also been proposed to prepare intrauterine contraceptive device.

Table: List of drugs and vaccines which are given as microspheres

DRUG	REASON	FORMULATION
Chlorpromazine (Anti psychotic).	Long term administration has side effects and extra pyramidal effects and so low dose would overcome both this disadvantage in microcapsule form the occurrence Of degree of catatonia reduces &hence better therapeutic profile can be achieved.	Microcapsule
Glipizide (antidiabetic).	It has short biological half life of 3.4+/- 0.7hr and is rapidly eliminated because of its short half life and chronic use attempts are made to control release parental in the form of microspheres.	Microspheres
Salbutamol sulfate (β2-bronchodilator).	It has short biological half life i.e. 4-6 hr. In case of drugs having short half life. Lot of medicines has to be taken simultaneously for longer period of time so to overcome this problem microencapsulation has been done which is capable of releasing the drug gradually.	Microencapsulation
Pentoxifylline (xanthine derrivative).	It has short half live and low bioavailability so to overcome the above problems microspheres were prepared for pre oral	Microspheres
Tramadol .Hcl(synthetic opoid).	Half life of the drug is 5 hr and the usual dose is 50-100mg every 4-6 hr with maximum dosage of 400mg/day.when given in the form of immediate release formulation adverse effect like headache, nausea, were more which decreased when given in the form of sustained release formulation and hence patient compliance increased, as frequency of dosing decreased and this was achieved by microsphere formulation.	

ASEPTIC MICROSPHERE REFINER TECHNIQUES

A. Microsphere Refiner from PSL for sterile microsphere formulation using contained discharge and unique filter plate design

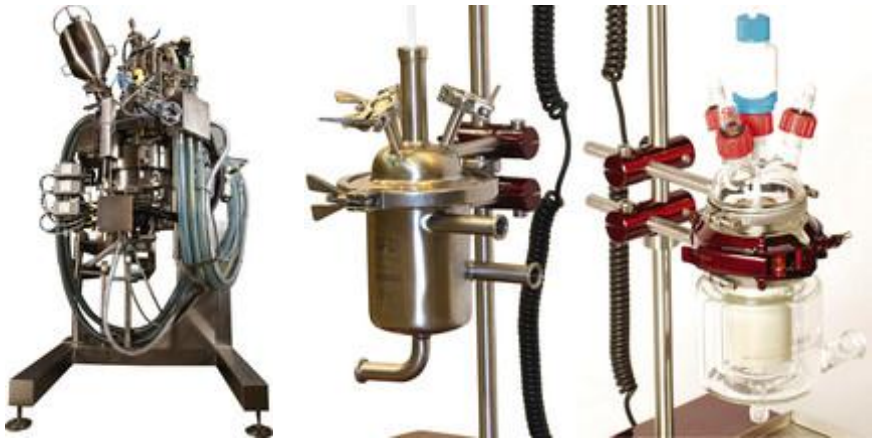
B. Installed large scale sterile filter dryer for efficient filtration, washing and drying of polymeric microsphere with SIP and process skid.

C. Small scale production of microsphere and other sterile products with the agitated nutsche filter dryer technology

(A) **(B)** **(C)**

D. Unique 150° tiltable aseptic discharge with PSL large scale pressured ANFD for sterile microspheres recovery after filtration, washing and drying.

E. Feasibility studies and trials can be carried out in the GFD lab nutsche filter dryers of PSL, for direct process scale-up and filtration mesh porosities assessment



(D) **(E)**

Microsphere production is experiencing a fast growing demand due to its revolutionary characteristics and applications. Pharmaceutical drug manufacturers are facing new

challenges during process scale-up from R&D stage to commercial formulation of microsphere drugs.

Microspheres are small spherical particles, having a required size range (typically 5µm up to 250µm). These particles can be manufactured from various natural and synthetic materials such as polymer microspheres. New applications for microspheres are discovered every day from coating to cosmetics and cancer research. PSL has developed process solutions for this type of drug delivery, as biodegradable polymer microspheres can work as miniature time release capsules for parenteral drugs, within pharmaceutical and biotechnology industries.

Suspended microspheres obtained from various micro encapsulation processes require unique aseptic handling that differs from typical challenging sieving and nitrogen drying operations. Following the synthesis stage, microspheres require being washed, classified by size, filtered then dried under appropriate conditions to gain the final free flowing injectable or inhalation microsphere product. Microspheres are random in size and need to be filtered and classified into the micron size range desired before drying. PSL sterile refiners have been designed to meet these criteria from washing to classifying and drying.

PSL microsphere technology actually consists of two process units that allow the classification of the microspheres: the microsphere scalper and the microsphere refiner: The microsphere scalper retains the oversized particles and the microsphere refiner retains the required particles and removes the undersized ones. It works as an agitated filter dryer to maintain the homogeneity of the solution. The mesh complies with the requirements of the particle size distribution for the filtration. They have multiple valves for the washing and a heating/cooling system for the drying of the cake.

With PSL's unique design, size classification is readily achieved, which is the most difficult task associated with microsphere formulation. Most microsphere processes require sterility, PSL solutions have been designed with Steam-In-Place and drainability capabilities. The discharge being part of the sterility envelope, the Microsphere Refiner is tiltable.

The Microsphere Refiner benefits

- Suspended microspheres side & bottom filtration to avoid mesh blocking
- Accurate size distribution and filtration process
- Wide range of porosities & types of mesh

- SIP, CIP and efficient drainability for ensured aseptic process
- High yield batch homogeneity
- Direct scale up from R&D to commercial production
- Total product recovery
- Process experts in filtration and drying solutions manufactured in-house

Feasibility studies with GFD

The GFD is a true miniature version of a Microsphere Refiner allowing direct lab scale filtration, classification, washing and drying of microspheres. Providing continuity for feasibility studies now with both bottom and side filtration. The removable filtration basket guarantees maximum product recovery.

The GFD is suitable for any laboratories' needs and is available for trials in 2 sizes: MINI LAB (0.002sq.m) and LAB (0.01sq.m). PSL's Glass Filter Dryer works under vacuum and has a heated jacket for drying. Simply email the GFD Sales direct for more information on this innovative piece of lab equipment.

With the new side filtration technology small scale feasibility tests can be carried out with the GFD product range to filtrate and dry up to 50 grams of cake prior to scale up to the R&D Microsphere Scalping and Refiner technologies classifying and drying up to 2kgs with sterile discharge.

CHALLENGES IN MICROSPHERE FORMULATION

Pharmaceutical drug manufacturers are facing new challenges during process scale-up from R&D stage to commercial formulation of microsphere drugs. Traditionally sieving operation is achieved on non-sterile laboratory scale for microsphere drugs. But when sterile large scale production is needed, manufacturers are facing issues with blockage, sterility and poor product recovery.

Suspended microspheres obtained from various micro encapsulation processes require unique aseptic handling that differs from typical challenging sieving and nitrogen drying operations. The manufacturing processes for microsphere formulation from the upstream stage of the microsphere creation (emulsification, droplet formation...) to the downstream operations of harvesting the microsphere in a sterile manner with control size distribution need to develop

new technologies. Microsphere Refiner technology development for small to pilot scale classification, washing, filtration and drying of micro particles is the current challenges.

CONCLUSION

It has been observed that microspheres are better choice of drug delivery system than many other types of drug delivery system because it is having the advantage of target specificity and better patient compliance. Microsphere production is experiencing a fast growing demand due to its revolutionary characteristics and applications. Pharmaceutical drug manufacturers are facing new challenges during process scale-up from R&D stage to commercial formulation of microsphere drugs. Microsphere Refiner technology development for sterile large scale production of microsphere formulation from the upstream stage of the microsphere creation (emulsification, droplet formation...) to the downstream operations of harvesting the microsphere is the current challenges to satisfy regulatory complaisance.

ACKNOWLEDGEMENTS

The authors are grateful to Principal and Management, Channabasweshwar Pharmacy College, Latur, Maharashtra to encourage scientific publication.

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