International Journal of Advances in Pharmaceutics
E-ISSN: 2320-4923; P-ISSN: 2320-4931
Journal DOI: https://doi.org/10.7439/ijap
Journal home page: http://ssjournals.com/index.php/ijap
Review Article

Review on microspheres as a drug delivery carrier

A. Krishna Sailaja* and Anusha K

RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Hyderabad, India

QR Code

***Correspondence Info:**
Dr. A. Krishna Sailaja
Associate Professor
RBVRR Women's College of Pharmacy,
Affiliated to Osmania University, Hyderabad, India

***Article History:**
Received: 09/05/2017
Revised: 23/05/2017
Accepted: 23/05/2017
DOI: https://doi.org/10.7439/ijap.v6i5.4152

Abstract

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. One such approach is using microspheres as carriers for drugs. In this article importance of microsphere as a novel drug delivery carrier to attain site specific drug delivery was discussed.

Keywords: polymer, SEM, Entrapment efficiency.

1. Introduction

Microspheres are characteristically free flowing powders consisting of protein or synthetic polymers which are biodegradable in nature and ideally having a particle size less than 200 µm[1,2]. In contrast to drug delivery system, the word novel is searching something out of necessity. The drug has to be delivered for a prolonged period of time and many medicines have to be taken simultaneously in case of chronic patients. Frequent administration of drug is necessary when those have shorter half-life and all these leads to decrease in patient's compliance [3]. In order to overcome the above problems, various types of controlled release dosage forms are formulated and altered, so that patient compliance increase through prolonged effect, adverse effect decreases by lowering peak plasma concentration[4]. The controlled release dosage form maintaining relatively constant drug level in the plasma by releasing the drug at a predetermined rate for an extended period of time.

One such in Microspheres as carriers of drug become an approach of controlled release dosage form in novel drug delivery system. 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)[5].

2. Advantages of microspheres [6]

- Increase bioavailability
- Alter the drug release & separation of reactive core from other materials.
- Improve the patient's compliance
- Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.
- Reduce the reactivity of the core in relation to the outside environment.
- The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles *in vivo*.
- Decrease evaporation rate of the volatile core material.
- Convert liquid to solid form & to mask the bitter taste.

- Protects the GIT from irritant effects of the drug.
- Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.
- Controlled release delivery biodegradable microspheres are used to control drug release rates thereby decreasing toxic side effects, and eliminating the inconvenience of repeated injections [7,8].

3. Classification of polymers

Microspheres used usually are polymers. They are classified into two types [9]-

3.1 Synthetic polymers:

It is divided into two types

a. Non-biodegradable polymers

e.g. Poly methyl methacrylate (PMMA), Acrolein, Glycidyl methacrylate, Epoxy polymers.

b. Biodegradable polymers

e.g. Lactides, Glycolides & their co polymers, Poly alkyl cyano acrylates, Poly anhydrides.

3.2 Natural polymers:

It is 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.

4. Types of Microspheres

4.1. Bio-adhesive microspheres [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.

4.2. 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 like chitosan, dextran etc receive magnetic responses to a magnetic field from incorporated materials.

A. Therapeutic magnetic microspheres

They are used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.

B. 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 supra magnetic iron oxides.

4.3. Floating microspheres [12]

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 increases gastric residence and causes 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. Drug (ketoprofen) given through this form.

4.4. Radioactive microspheres [13]

Radio emobilisation therapy microspheres sized 10-30 nm are of larger than capillaries and gets tapped in first capillary bed when they come across. They are injected to the arteries that lead 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, ß emitters, γ emitters.

4.5. Polymeric microspheres

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

A. Biodegradable polymeric microspheres [14]

The natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bio adhesive 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. However they provide wide range of application in microsphere based treatment.

B. Synthetic polymeric microspheres [15]

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. Techniques used in the preparation of microspheres

The choice of the technique mainly depends on the nature of the polymer used, the drug, the equivocally determined by some formulation and technology related factors as mentioned below

The particle size requirement:

a) The drug or the protein should not be adversely affected by the process.
b) Reproducibility of the release profile and the method.
c) No stability problem.
d) There should be no toxic product(s) associated with the final product[16]

Different types of techniques are employed for the preparation of the microspheres using hydrophobic and hydrophilic polymers as matrix materials are,

5.1. Single emulsion technique [17,18]

The micro particulate carriers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. Next cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, di acid chloride etc. Heat denaturation is not suitable for thermolabile substances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation.

5.2. Double emulsion technique (Multiple emulsions)

Double emulsion method of microsphere preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w .and is best suited to the water-soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as the synthetic polymers. A number of hydrophilic drugs like leutinizing hormone releasing hormone (LH-RH) agonist, vaccines, protein/peptides and conventional molecules are successfully incorporated in to the microspheres using the method of double emulsion solvent evaporation/extraction.

5.3. Polymerization techniques

The polymerization techniques conventionally used for the preparation of the microspheres are mainly classified as: I. Normal polymerization

II. Interfacial polymerization.

Both are carried out in liquid phase.

Normal polymerization is carried out using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization processes.

In **Bulk polymerization**, a monomer or a mixture of monomers along with the initiator or catalyst is usually heated to initiate polymerization. Polymer so obtained may be moulded as microspheres. Drug loading may be done during the process of polymerization.

Suspension polymerization also referred as bead or pearl polymerization. Here it is carried out by heating the monomer or mixture of monomers as droplets dispersion in a continuous aqueous phase. The droplets may also contain an initiator and other additives.

Emulsion polymerization differs from suspension polymerization as due to the presence initiator in the aqueous phase, which later on diffuses to the surface of micelles.

Bulk polymerization has an advantage of formation of pure polymers, but it is very difficult to dissipate the heat of reaction, which can adversely affect the thermo labile active ingredients. On the other hand the suspension and emulsion polymerization can be carried out at lower temperature.

Interfacial polymerization

Interfacial polymerization essentially precedes involving 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. The monomers present in either phase diffuse rapidly and polymerize rapidly at the interface. Monomer droplet, the formed carrier is of capsular (reservoir) type. The interfacial polymerization is not widely used in the preparation of the microspheres because of toxicity associated with the unreacted monomer, high permeability of the film, high degradation of the drug during the polymerization, fragility of microcapsules, non-biodegradability of the microspheres.

5.4. Phase separation coacervation technique

The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called coacervates. The coacervation can be brought about by addition of the third component to the system which results on the formation of the two phases, one rich in the polymer while the other one, i.e. supernatant, depleted of the polymer. The methods are based on salt addition, non-solvent additi0on, addition of the incompatible polymer or change in pH.

5.5. Spray drying

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the

polymer solution under high- speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 µm. Microspheres are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying. One of the major advantages of the process is feasibility of operation under aseptic conditions. The spray drying process is used to encapsulate various penicillin's. 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 microspheres.

5.6. Non-aqueous solvent evaporation method

In these methods the polymer is dissolved in an organic solvent such as dichloromethane, chloroform, alcohol or ethyl acetate, either alone or in combination. The drug is either dissolved or dispersed into the polymer solution and this solution containing the drug is emulsified in to an aqueous phase to make oil in water emulsion by using a surfactant or an emulsifying agent. After the formation of a stable emulsion, the organic solvent is evaporated either by increasing the temperature or by continuous stirring. Solvent evaporation for preparation of embryonic microspheres under pressure or by continuous stirring, determines the size and morphology of the microspheres. It had been reported that the rapid removal of the solvent from the embryonic microspheres leads to the precipitation at the o/w interface. This leads to the formulation of cavity in the microspheres, making them hollow

5.7. Ionic gelation method:

Ionotropic gelation is based on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogels. These anions forms meshwork structure by combining with the polyvalent cations and induce gelation by binding mainly to the anion blocks. The hydrogel beads are produced by dropping a drug-loaded polymeric solution into the aqueous solution of polyvalent cations. The cations diffuses into the drug-loaded polymeric drops, forming a three dimensional lattice of ionically crossed linked moiety.

6. Applications

6.1 Microspheres in vaccine delivery

The prerequisite of a vaccine is protection against the microorganism or its toxic product. An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. The aspect of safety and minimization of adverse reaction is a complex issue [19]. The aspect of safety and the degree of the production of antibody responses are closely related to mode of application. Biodegradable delivery systems for vaccines that are given by parenteral route may overcome the shortcoming of the conventional vaccines [20]. The interest in parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages including:

- Improved antigenicity by adjuvant action
- Modulation of antigen release
- Stabilization of antigen.
- Targeting using micro particulate carriers

The concept of targeting, i.e. site specific drug delivery is a well-established dogma, which is gaining full attention. The therapeutic efficacy of the drug relies on its access and specific interaction with its candidate receptors. The ability to leave the pool in reproducible, efficient and specific manner is center to drug action mediated by use of a carrier system. Placement of the particles indiscrete anatomical compartment leads to their retention either because of the physical properties of the environment or biophysical interaction of the particles with the cellular content of the target tissue.

6.2. Chemoembolisation

Chemoembolisation is an endovascular therapy, which involves the selective arterial embolisation of a tumour together with simultaneous or subsequent local delivery of the chemotherapeutic agent. The theoretical advantage is that such embolisations will not only provide vascular occlusion but will bring about sustained therapeutic levels of chemotherapeutics in the areas of the tumour. Chemoembolisation is an extension of traditional percutaneous embolisation techniques.

6.3 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 .The adsorption of the poloxamer on the surface of the polystyrene, polyester or poly methyl methacrylate microspheres renders them more hydrophilic and hence decrease their MPS uptake. 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 on surface of microspheres in order to achieve the targeting to the discrete organs and to avoid rapid clearance from the body.

6.4 Monoclonal antibodies mediated microspheres

Monoclonal antibodies are extremely specific molecules. This extreme specificity of monoclonal antibodies (Mabs) can be utilized to target microspheres loaded bioactive molecules to selected sites. Mabs can be directly attached to the microspheres by means of covalent coupling. The free aldehyde groups, amino groups or hydroxyl groups on the surface of the microspheres can be linked to the antibodies. The Mabs can be attached to microspheres by any of the following methods.

1. Nonspecific adsorption 2. Specific adsorption 3. Direct coupling 4. Coupling via reagents

6.5 Imaging

The microspheres have been extensively studied and used for the targeting purposes. Various cells, cell lines, tissues and organs can be imaged using radio labelled microsphere. The particle size range of microspheres is an important factor 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 labeled human serum albumin microspheres.

6.6 Topical porous microspheres

Microsponges 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. These porous microspheres with active ingredients can be incorporated into formulations such as creams, lotions and powders. Microsponges consist of non-collapsible structures with porous surface through which active ingredients are released in a controlled manner [21].

6.7 Mcrospheres in Gene delivery [22]**:**

Gene delivery systems include viral vectors, cationic liposomes, polycation complexes, and microencapsulated systems. Viral vectors are advantageous for gene delivery because they are highly efficient and have a wide range of cell targets. However, when used in vivo they cause immune responses and oncogenic effects. To overcome the limitations of viral vectors, non-viral delivery systems are considered for gene therapy. Non-viral delivery system has advantages such as ease of preparation, cell/tissue targeting, low immune response, unrestricted plasmid size, and large-scale reproducible production. Polymer has been used as a carrier of DNA for gene delivery applications.

6.8. Microspheres for Oral drug delivery [23]

The potential of polymer films containing diazepam as an oral drug delivery was investigated in rabbits. The results indicated that a film composed of a 1:0.5 drug-polymer mixture might be an effective dosage form that is equivalent to the commercial tablet dosage forms. The ability of polymer to form films may 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 polymer a unique polymer for oral drug delivery applications.

6.9. Microspheres for DNA delivery [24]**:**

Microspheres have been recently used as a delivery vehicle for the transfer of plasmid DNA which leads to improve the transfer of plasmid DNA and their stability in the bio- environment. Truong-Le & Coworkers (1998) developed a novel system for gene delivery based on the use of DNA-gelatin microspheres/ nanoparticles formed by salt induced complex coacervation of gelatin & plasmid DNA.

6.10 Recent Applications of Controlled Release Microspheres

Controlled-release microspheres are in development for a number of interesting and important applications, especially for delivery of large, fragile drugs like proteins and nucleic acids. Several recent examples are described below. Controlled-Release Vaccines Vaccination has been highly successful for controlling or even eradicating many important types of infectious diseases, and new or improved vaccines are being heavily investigated for AIDS, hepatitis B, anthrax, and SARS.A frequent problem is the need for repeated administrations. Single-shot Vaccine delivery systems should provide the antigen(s) and adjuvant on a prescribed schedule and maintain the bioactivity of the antigen, both during fabrication of the delivery device and during the often prolonged residence time of the device in the body. To enhance vaccine stability, researchers have been focusing on several approaches, including the use of adjuvants to protect the protein antigens or by choosing different microsphere materials. A major advantage of microspheres for vaccination is that they can be passively targeted to antigen-presenting cells (APCs) such as macrophages and dendritic cells. The ability of APCs to phagocytose particulates is dependent on the particle size. In particular, 1- to 10-µm diameter microspheres are optimally taken up by APCs in a number of tissues and have been shown to enhance antigen-specific T-helper lymphocyte (Th) responses thus leading to an enhancement in antigen-specific antibody responses) and elicit a cytotoxic T lymphocyte (CTL) response T-cell activation in response to antigen encapsulating microspheres has been shown to be 100-1000 fold better than antigen alone.

6.11 Stabilization of Encapsulated Protein Therapeutics

A major problem with protein encapsulation in polymer particles is loss of protein bioactivity. Damage to

proteins can occur during fabrication of the particles via shear stresses or other physical forces, through contact with organic solvents, and by loss of water (e.g., upon lyophilization) as well as during incubation and release in the warm, moist, in vivo environment.

Two types of damage occur most often:

(i) Covalent or non-covalent intermolecular aggregation and (ii) Denaturation. Several studies have investigated the mechanisms of damage. Protein stability can be enhanced by the addition of excipients to prevent aggregation and stabilize the folded protein structure or through judicious choice of polymer employed for fabrication of the devices.

7. Future Challenges

Future challenges of microspheres look bright particularly in the area of medicinal field because of its wide spectrum of application in molecular biology, e.g.: microsphere based genotyping platform is used to detect six single nucleotide polymorphism, yittrium-90 microspheres are used to prevent tumour after liver transplantation and it's advanced way in delivery of vaccines and proteins.

7.1 Microspheres in cancer therapy

Cancer microsphere technology is the latest trend in cancer therapy. It helps the pharmacist to formulate the product with maximum therapeutic value and minimum or negligible range side effects. Cancer is a disease in which the abnormal cells are quite similar to the normal cells, with just minute genetic or functional change. A major disadvantage of anticancer drugs is their lack of selectivity for tumor tissue alone, which causes severe side effects and results in low cure rates. Thus, it is very difficult to target abnormal cells by the conventional method of the drug delivery system. Microsphere technology is probably the only method that can be used for site-specific action, without causing significant side effects on normal cells.

Table 1: Marketed products of microspheres

Drug	Commercial Name	Company	Technology	Indication
Risperidone	RESPERDA^{R},CONSTA^{R}	Jansenn/Alkermes,inc.	Double emulsion(oil in water)	Schizophrenia; Bipolar Disorder
Naltrexone	VIVITROL^{R}	Alkermes	Double emulsion(oil in water)	Alcohol dependence
Octreotide	Sandostatin LAR	Novartis	Phase separation	Acromegaly
Somatropin	Nutropin^{R} Depot^{-a}	Genentech /alkermes	Alkermes prolease^{R} Technology (cryogenic spray drying method)	Growth deficiencies
Bromocriptine	Parlodel LAR	Novartis	Spray drying	Parkinsonism
Minocycline	Arestin	Orapharma		Periodontitis

References

[1]. Jain N.K., Controlled and Novel drug delivery, 04 Edition, 236-237, 21.

[2]. Vyas S.P. and Khar R.K., Targeted and Controlled drug delivery, 07 Edition, 418.

[3]. Ghulam M., Mahmood A., Naveed A., Fatima R.A., Comparative study of various microencapsulation techniques. Effect of polymer viscosity on microcapsule characteristics, *Pak. J. Sci.* 2009; 22 (3): 291-300.

[4]. Mathew Sam T., Devi Gayathri S., Prasanth V. V., Vinod B., Suitability of factorial design in determining the processing factors affecting entrapment efficiency of albumin microspheres, *Journal of Pharmacy Research*. 2010; 3(5): 1172-1177.

[5]. Karmakar U., Faysal M.M., Diclofenac as microspheres, *The Internet Journal of Third World Medicine*. 2009; 8(1).

[6]. Alagusundaram M, Madhu Sudana Chetty C, Umashankari K, Badarinath AV, Lavanya C and Ramkanth S "Microspheres As a Novel Drug Delivery System : A Review" *IJCTR*. 2009; 1:526-534.

[7]. Luna B, Mark N. Feinglos "Oral Agents In The Management Of Type 2 Diabetes Mellitus" *Am Fam Physician*, 2001 May , 1747-1757.

[8]. Targeted drug delivery http://en.wikipedia.org/wiki/Targeted_drug_delivery, assessed on [Last cited on 2012 June 21].

[9]. Alagusundaram. M, Microspheres as a novel drug delivery system – A Review, *International Journal of ChemTech Research*, 2009; 1: 526.

[10]. Sipai Altaf Bhai. M. Vandana yadav, Mamatha. Y, Prasanth V. V., Mucoadhesive Microsphere An overview. *American Journal of Pharmtech Research*, 2012; 2(1): 237-258.

[11]. Guojun Liu, Husheng Yang, Jiayun Zhou, Preparation of magnetic microsphere from water-in-oil emulsion stabilized by block copolymer dispersant,. Biomacromolecules, 2005; 6:1280-1288.

[12]. Lachman LA, Liberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy. 3rd edition 1991; Varghese Publishing House, Mumbai, India: P-414-415.

[13]. Amsden BG and Goosen M. An examination of the factors affecting the size, distribution, and release

characteristics of polymer microbeads made using electrostatics. *J. Control. Rel.*1997; 43:183– 196.

[14]. Cleland JL, Duenas ET, Park A, Daugherty A, Kahn J, Kowalski J, and Cuthbertson A. Development of poly-(D,L-lactide-co-glycolide) microsphere formulations containing recombinant human vascular endothelial growth factor to promote local angiogenesis. *J. Control. Rel.* 2001; 72(1–3): 13– 24. 14.

[15]. Jain NK. Controlled and Novel drug delivery, CBS Publishers New Delhi, India; 4th Edition, 236- 237.

[16]. Khar, R.K., Vyas, S.P. Targeted and Controlled Drug Delivery- Novel Carrier Systems., 1st edition, CBS Publications and Distributors, New Delhi, 2002; 417-418.

[17]. P.B. O'Donnell and J.W. Mc Ginity. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Del.1997; *Rev*, 28:25-42.

[18]. Piush Khare and Sanjay K. Jain, Influence of Rheology of Dispersion Media in the Preparation of Polymeric Microspheres through Emulsification Method. *AAPS PharmSciTech*., 2009; 10(4):1295-1300).

[19]. Funden Berg H.H., Stites D.P., Caldwel J. L .and Wells J.V. In: Basic and clinical immunology, 2nd ed., Lange Medical, Los Altosca, 1978.

[20]. Capron A.C., Locht C. and Fracchia G.N, Vaccine.12, 667; Edelman R. (1993) vaccine 11,1361; Drews J. (1984) Immunostimulantien, Klin. Wochenscher. 62, 254; Spier K.E. (1993) vaccine 11:1450.

[21]. Nachts S. and Martin K., In: The microsponges a novel topical programmable delivery formulation, Marcel Dekker Inc., Newyork., 1990; 299

[22]. Yun YH, Goetz DJ, Yellen P, Chen W., Hyaluronan microspheres for sustained gene delivery and site-specific targeting. *Biomaterials*, 2004; 25(1): 147-57.

[23]. Lamprecht, Hiromitsu Yamamoto, Hirofumi Takeuchi, Yoshiaki Kawashima, pH-sensitive microsphere delivery increases oral bioavailability of calcitonin. *Journal of Controlled Release*, 2004; 98: 1-9.

[24]. Liu J, Meisner D, Kwong E, Wu XY, Johnston MR., A novel trans-lymphatic drug delivery system: implantable gelatin sponge impregnated with PLGA-paclitaxel microspheres. *Biomaterials*, 2007; 28(21): 3236-3244.