BIOINFO Drug targets
ISSN: 2249-1759 & E-ISSN: 2249-1767, Volume 2, Issue 1, 2014, pp.-014-019.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000197

MICROSPHERES FOR INTRANASAL DELIVERY SYSTEM: AS REVIEW

KOYANI V.*, DEDAKIA A., MATHOLIYA C. AND PATEL P.
Department of Pharmaceutical Science, Saurashtra University, Rajkot- 360 001, Gujrat, India
*Corresponding Author: Email- koyanivaishali@gmail.com

Received: November 10, 2013; Accepted: May 15, 2014

Abstract- Intranasal administration is an attractive option for local and systemic delivery of many therapeutic agents. The nasal mucosa is easily accessible compared to other mucous membranes and provides a practical entrance portal for wide range of molecules. Intranasal administration is essentially painless application, provide a rapid onset of action and improve the bioavailability for brain targeted nasal drug delivery. Intranasal administration offers a variety of attractive options for local and systemic delivery of diverse therapeutic agents. The nature of the nasal mucosa provides a series of unique attributes, all of which may cosider for patient's convenience, compliance safety. Over the recent decades the interest in intranasal delivery as a non-invasive route for drugs is increased. Since the nasal mucosa is consider as a targeted drug delivery and a wide variety of therapeutic compounds may be administered intranasaly for topical, systemic and central nervous system action. In this study, a novel mucoadhesive microsphere for nasal administration was developed and investigated. The drug loaded mucoadhesive microspheres were prepared by many technique that can be proposed as an alternative to the conventional methods for preparation of microspheres. It was found that the particle size, swelling index and entrapment efficiency of microspheres increases with increasing drug to polymer ratio. Microspheres show adequate adhesion to nasal mucosa and do not have any destructive effect on nasal mucosa.

Keywords- Microspheres, nasal delivery, Mucoadhesion, Brain targeting

Introduction

Delivery of drug into brain for central nervous system (CNS) disease require for treatment, however such route of delivery is very problematic. Therapeutic effect of drug can be described by achieving a desired concentration of drug in blood or tissue for a prolonged period of time. So it is a reliable means to deliver a drug to a target site with a specificity and in a controlled manner. Microsphere used as a not controlled release but for targeted therapy also so it offer certain advantages over the conventional release dosage form for those drugs having a first pass metabolism. All these drawbacks of convensional delivery system neccesiate the development of controlled drug delivery system. Delivery of drug to CNS is problematic for a drugs having a hydrophilic in nature and having a high molecular weight because of impervious nature of Blood-Brain Barries (BBB) [1-4]. So the transport of exogenous material directly from nose-to-brain is a potential route for by passing the BBB. The nasal epithelial is highly vascularised and offers a large surface area for drug absorption and transport a drugs into systemic circulation through nasal mucosa. Microspheres are solid spherical partical having a size of 1-1000 mcm and free flowing in nature. It consists of polymer or polypeptide and they are biodegradable, biocompatible, nontoxic and well tolerated by human body. The range of techniques for the preparation of microspheres offers to control aspects of drug administration. They fascilitate the accurate delivery of small quantity of the potent drug and protectin to labile compound before and after administration [5].

Drug Selection Criteria for Nasal Delivery

It must pass through mucus layer and epithelial membrane before reaching CNS, Molecular weight, complexity and hydrophobicity/ lipophilicity of drug, pH of the solution and Pka of the drug [6], Properties of vehicles used in formulation.

The Drug Must Pass

Blood flow, enzymatic degradation, should be Stable, having a sufficient Viscosity and of required pH. Microspheres that have been used as nasal drug delivery systems are water-insoluble but absorb water into the matrix, resulting in swelling and formation of a gel. The polymeric materials in the microspheres have been albumin, starch, dextran and hyaluronic acid. The bioavailability of several peptides and proteins has been improved by using these polymers and low-molecular weight drugs have been successfully delivered in microsphere preparations [7]. The residence time in the cavity is considerably increased for microspheres compared to liquid system. There are so many other factors to increase the absorption of large hydrophilic drugs and exert a direct effect on the nasal mucosa, resulting in the opening of tight junctions between the epithelial cells described in [Fig-1].

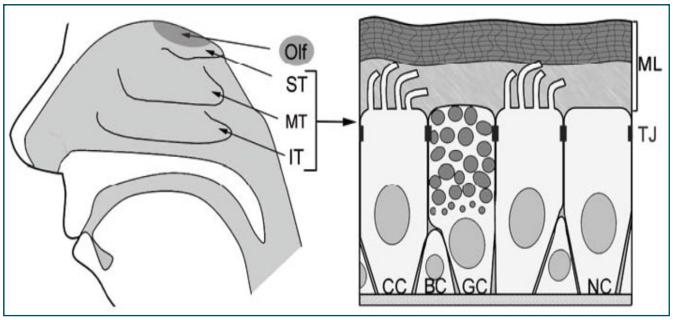

Fig. 1- The approximate place of the olfactory epithelium (Olf), the superior turbinate (ST), middle turbinate (MT) and inferior turbinate (IT) and to the right a ciliated columnar cell (CC), basal cell (BC), goblet cell (GC), nonciliated columnar cell (NC), tight junctions (TJ) and mucus layer (ML). A comparison can be made with the frontal section of the porcine snout.

Advantages and Limitation

Advantages

- Easily injected into body due to the smaller size and spherical shape.
- Hepatic firstpass metabolism is absent.
- Drug degradation can not occur.
- It provide constant relese and prolonged therapeutic effect.
- Maximum drug utilization will improve drug bioavailability.
- Drug absorption is rapid and onset of action is quick.
- Conventional route when compared with parenteral route.

Limitation

- Drug cannot be removed after administration.
- Differ in a release rate from one dose to another.
- Surface area for absorption is less when compared to GIT
- The release rate may be vary from a variety of factors like food and others.
- It contains high drug loading and thus any loss of integrity of dosage form may lead to potential toxicity [8].

Brief Application of Nasal Drug Delivery System

Brain Targeted Drug Delivery through Nasal Cavity

This delivery system is beneficial in major diseases like Parkinson's disease, Alzheimer's disease or pain because it requires rapid and/or specific targeting of drugs to the brain. The nasal delivery system to brain will increase the fraction of drug that reach the CNS after nasal delivery because the olfactory region located at the upper remote parts of the nasal passages offers the potential for certain compounds to circumvent the blood-brain barrier and enter into the brain through the nasal route. Certain neurotrophic factors such as NGF, IGF-I, FGF and ADNF have been delivered intranasally to the CNS shows increase the bioavailability of drug in the brain as shown in [Fig-2]. The human studies, with proteins such as AVP, CCK analogue, MSH/ACTH and insulin have suggested that they are delivered directly to the brain from the nasal route.

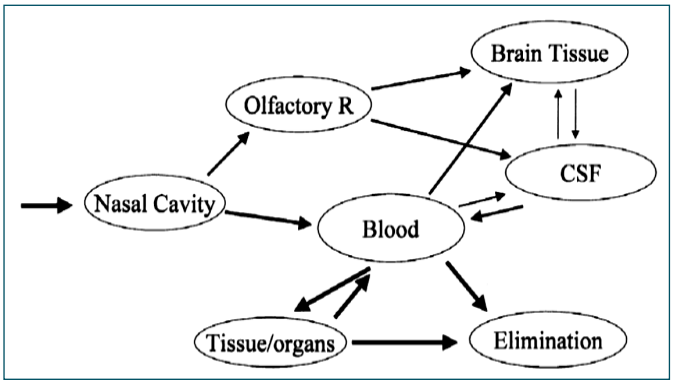

Fig. 2- Possible routes of transport between the nasal cavity and the brain and CSF [9].

Protein-Peptide Drug Delivery

Because of their physico-chemical instability, peptides & proteins have a generally low oral bioavailability. They are also susceptible to hepato-gastrointestinal first-pass metabolism. Examples are insulin, calcitonin, pituitary hormones etc. [31]. These peptides and proteins are relatively high molecular weight hydrophilic polar molecules, are poorly absorbed across biological membranes with low bioavailability when administered as a liquid solution. We are using the absorption enhancers to overcome this problem like surfactants, glycosides, cyclodextrin and glycols to increase the bioavailability. Nasal route is providing the best route for delivering such biotechnological products.

Non-Peptide Drug Delivery

small non- peptide lipophilic drugs having a low molecular weight (below 1000 Daltons) are well absorbed through the nasal mucosa even though absence of permeation enhancer. Nasal membrane containing highly vascularised epithelium and it contains large surface area it is readily accessible for drug absorption. Drugs with extensive first pass metabolism, such as propranolol, progesterone, estradiol, nitroglycerin, sodium chromoglyate can be rapidly absorbed through the nasal mucosa with a systemic bioavailability of approximately 100% [30]. These drugs can reach widespread circulation within few minutes after dosing directly into the systemic circulation. In fact, many orally administered drugs are poorly absorbed and less efficiently than those from intranasal administration.

Examples of non-peptide drugs that being studied for nasal delivery and shown good bioavailability includes:

1. Adrenal corticosteroids
2. Sex hormones: estrogene, progesterone, no-rethindrone, and testosterone.
3. Narcotics and antagonists: bupenorphine, nalorphine.
4. Histamine and antihistamines: disodium cromoglycate, meclizine.
5. Antimigrane drugs: dihydro erogotamine, ergotamine.
6. Phenicillin, cephalosporins, gentamycin
7. Antivirals: Phenyl-p-guanidine benzoate, envirox-ime.
8. Inorganic compounds: Inorganic salts, colloidal gold, colloidal carbon, colloidal silver.
9. Vitamins: vitamin B
10. Cardiovascular drugs: Nitroglycerine, isosobide dinitrate, propanolol, and colifilium tosylate.
11. Autonomic nervous system:
    - Sympathomimetics: phenylephrine, ephedrine, epinephrine.
    - sympatholytics: Xylometazoline, dopamine and dobutamine.
    - Parasympathomimetics: nicotine, metacholine
    - Parasympatholytics: scopolamine, atropine, ipatropium
    - Prostaglandins
12. CNS stimulants: Nicotine, cocaine, lidocaine.

Vaccine Drug Delivery

Nasal mucosal sites gives first line of defense against the microorganisms entered into the body, it act by filtering the pathogens from the inspired air by mucociliary clearance and compaction. Nose-associated lymphoid tissue (NALT) is called Waldeyer´s Ring in human beings and acts as an effective site for targeting immune system. Nasal mucus mainly contains protective proteins such as complement as well as neutrophils, immunoglobulins (IgA, IgG, IgM, IgE) and lymphocytes [32]. Main reasons for delivering vac-

cines through the nasal route are 1) the nasal mucosa is the first site of contacts with inhaled pathogens, 2) The nasal passages are rich in lymphoidal tissue, 3) Creation of immune responses, 4) cost effective, non-injectable, safe, patient convenience. Nasal delivery of vaccines has been reported to produce systemic immune response as well as local immune response and providing additional protection to nasal lining [32]. Delivering the vaccine to the nasal cavity itself stimulates the production of local secretory IgA antibodies as well as IgG, providing an additional first line of de-fense, which helps to eliminate the pathogen before it becomes established [33]. The common diseases like meningitis, measles, pertussis, and influenza causing pathogens are mainly enter into the body through the nasal mucosal surfaces and hence good candidates for preparing nasal vaccines. Nasally administered vaccines, especially if based on attenuated live cells or adjuvanted by means of an immunostimulator or a delivery system, can induce both local and systemic (i.e. humoral and cell-mediated) immune responses. Recently, for the diseases like anthrax and influenza are treated by using the nasal vaccines prepared by using the recombinant Bacillus anthracis protective antigen (rPA) and chitosan respectively [34].

Polymers Used in Microsphere

Drugs and Polymers

- **Drug-** wide variety of drugs like NSAIDS, small molecular weight therapeutic agent to high molecular weight peptides like LHRH agonist and steroidal hormones, antibiotics and chemotherapeutic agent.
- **Polymers-** water soluble polymers are mucoadhesive in nature and swellable network joined with cross-linking agent. We can use both natural and synthetic polymers which are biodegradable and biocompatible in nature. Natural polymers like chitosan, sodium alginate, guar gum and in synthetic polymers we can use ethyl cellulose, polyvinyl alcohol and HPMC.
- **Cross-Linking Agents-** generally citric acid, gluteraldehyde, and tripolyphosphate.

Table 1- Effect of polymers on bioadhesive microspheres [27,28,29]

Drug	Route of Administration	Bioadhesive polymers use	Application of polymers
Clonazepam	Nasal	Gelatin-Chitosan	Higher concentration of drug is achieved in brain
Gentamicin	Nasal	DSM+LPC	Combination of these polymers improves nasal absorption
Insulin	Nasal	DSM+LPC	Helps to deliver insulin via nasal route
Human growth Hormone (hGH)	Nasal	DSM+LPC	Improves absorption
Propanalol Hcl	Nasal	Gelatin-Chitosan	Controlled blood level profile as well as increased bioavailability of drug

Methodology

Spray Drying

In this method, polymer is first dissolved in a suitable volatile organic solvent such as acetone, dichloromethane etc. The drug in the solid form is dispersed in the polymer solution under high-speed homogenization [10]. This dispersion is then atomized in a stream of hot air. The atomization leads to the formulation of the small droplets or the fine mist from which the solvent evaporate instaneously leading the formation of the microspheres in a size range 1-100 mm. Microparticles are separated while the trace of solvent is removed by vaccum drying. One of the major advantages of process is feasibility of operation under aseptic conditions [Fig-3].

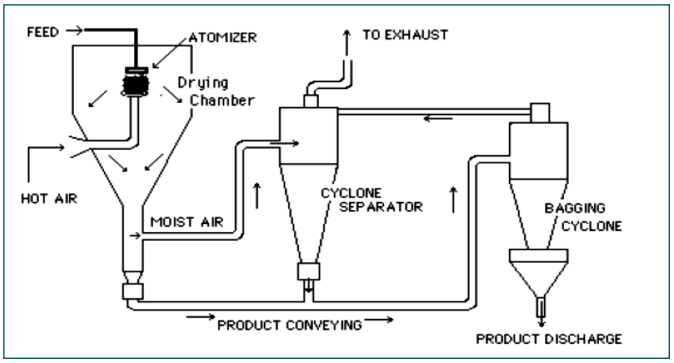

Fig. 3- Spray Dryer

Solvent Evaporation

The processes is carried out by using liquid manufacturing vehicle. The polymers used for coating the microcapsule are dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microcapsulated is dissolved or dispersed in a coating polymer volatile solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size of microcapsule. It is heated if necessary to evaporate the solvent from this mixture and polymer shrinks around the core. If the coating material is dissolved in a core polymer solution, reservoir-type microcapsule are formed. The solvent Evaporation technique is shown in [Fig-4].

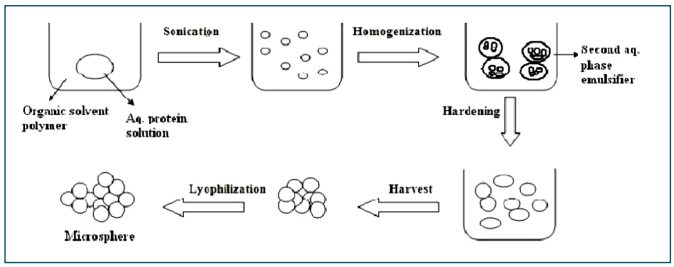

Fig. 4- Solvent evaporation method

Co-Acervation Method

Co-acervation thermal change: Performed by weighed amount of ethyl cellulose was dissolved in cyclohexane with vigorous stirring at 80°C by heating. Then the drug was finely pulverised and added with vigorous stirring on the above solution and phase separation was done by reducing temperature and using external cooling like ice bath. Then above product was washed twice with cyclohexane and air dried then passed through sieve (sieve no. 40) to obtain individual microcapsule [11]. Co-acervation non solvent addition: Developed by weighed amount of ethyl cellulose was dissolved in toluene containing propylisobutylene in closed beaker with magnetic stirring for 6 hr. at 500 rpm and the drug is dispersed in it and stirring is continued for 10-15 minutes. Then phase separation is done with continuous stirring. After that the microcapsules were washed with n-hexane and air dried for 2 hr. and then in oven at 50°C for 4 hr. [11].

Single Emulsion Technique

The micro particulate carriers of natural polymers 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 crosslinking of the dispersed globule is occur. 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 acid chloride, glutaraldehyde and formaldehyde etc. Heat denaturation is not suitable for thermolabile substances. Chemical crosslinking 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. The nature of the surfactants used to stabilize the emulsion phases can greatly influence the size, size distribution, surface morphology, loading, drug release, and bio-performance of the final multiparticulate product [12,13] [Fig-5].

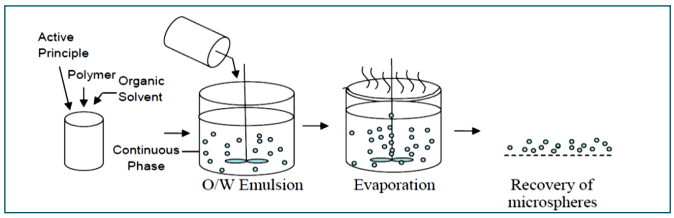

Fig. 5- Single Emulsion Method

Multiple Emulsion Method

Oral controlled release drug delivery of indomethacin was prepared by multiple emulsion technique. In this technique, powder drug was dispersed in solution (methyl cellulose) followed by emulsification in ethyl cellulose solution in ethyl acetate. The primary emulsion was then added into aqueous medium. The discrete microspheres were formed during this phase under optimised condition [14].

Polymerization Techniques

The polymerization techniques conventionally used for the preparation of the microspheresare mainly classified as [13,15].

- Normal Polymerization
- Interfacial Polymerization.

Both are carried out in liquid phase.

Normal Polymerization

It is carried out using different techniques as suspension, precipitation, emulsion, bulk and micellar polymerization processes. In bulk polymerization, a monomer or different monomers along with the initiator or catalyst is usually heated to initiate polymerization and microspheres are formed. Drug loading may be done between polymerization process. Suspension polymerization also referred as bead or pearl polymerization. Here it is also carried out by heating the monomer or mixture of monomers as droplets dispersion with initiator and other additives in a continuous aqueous phase. In Emulsion polymerization, initiator present in the aqueous phase, which later on diffuses to the surface of micelles.

Interfacial Polymerization

It involves the reaction of different monomers at the interface of the two immiscible liquid phases to form a film of polymer that essentially envelops the dispersed phase.

Solvent Extraction

Solvent evaporation method is used for the preparation of microparticles. It involves removal of the organic phase by extraction with the organic solvent. The method involves water miscible organic solvents such as isopropanol and is removed by extracting it with water. That leads to decreases the hardening time for microspheres. One variation of the process involve direct addition of the drug or protein to polymer organic solution. The rate of solvent removal depends on the temperature of water, ratio of emulsion to the water and the solubility of polymer [Fig-6].

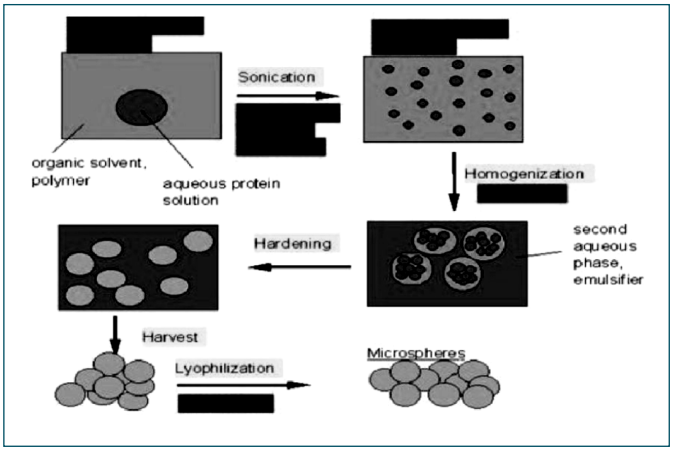

Fig. 6- Solvent Extraction Method

Ionic Gelation

Alginate/chitosan particulate system for diclofenac sodium release was prepared using this technique. 25 % (w/v) of diclofenac sodium was added to 1.2 % (w/v) sodium alginate solution. In order to get the complete solution stirring is continued and after that it was added dropwise to a solution containing Ca2+ /Al3+ and chitosan solution in acetic acid. Microspheres were kept for 24 hrs. in original solution. So they are internally gellify and separated by filtration. The complete release was obtained at pH 6.4-7.2 [14] [Fig-7].

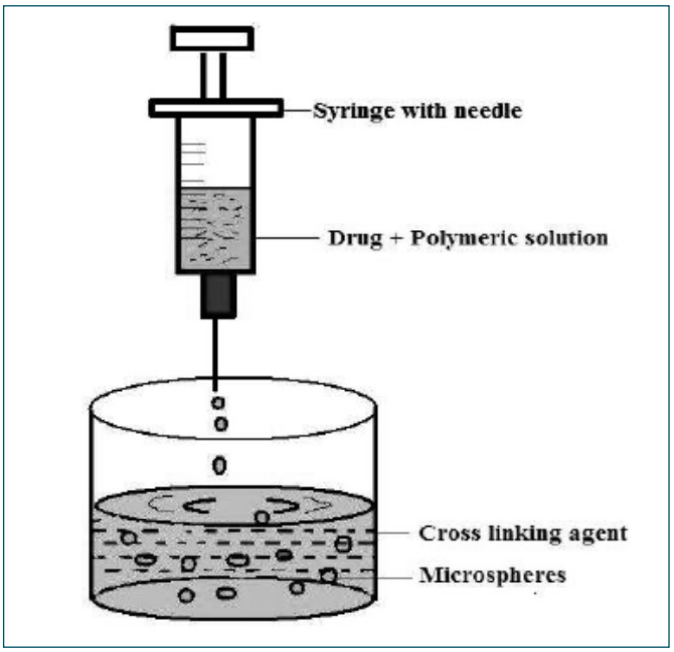

Fig. 7- Ionic Gelation Method

Preparation of Microspheres by Using Cross Linking Agent

A 2.5% (wt/vol) chitosan solution dissolved in aqueous acetic acid. This dispersed phase was added to continuous phase (125 mL) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (wt/vol) Span 85 to form a water in oil (w/o) emulsion. Stirring was continued at 2000rpm using a 3- blade propeller stirrer. A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% vol/vol) was added. Stirring was continued for 2.5 hours and separated by filtration under vacuum and washed first with petroleum ether (60°C- 80°C) and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde. The microspheres were then finally dried in a vacuum desiccators [16-17].

Preparation of Cellulose Microspheres

A solution of ethylcellulose in acetone was added to liquid paraffin containing emulgent (Span 85) while stirring at 1500 rpm for 5 to 6 hours at 25-30°C. Subsequently suitable amount of petroleum ether was added to the dispersion, filtered, and dried at ambient temperature. The resultant microspheres were washed with water followed by petroleum ether to remove traces of liquid paraffin. The microspheres were desiccated under vacuum [8].

Preparation of Tripolyphosphate Microspheres

Microspheres were formed by dropping the bubble-free dispersion of chitosan (2.5% wt/vol) through a disposable syringe (10 mL) onto a gently agitated (magnetic stirrer) 5 or 10% (wt/vol) TPP solution. Chitosan microspheres were separated after 2 hrs. by filtration and rinsed with distilled water, then they were air dried [8].

Charecterization and Evaluation of Microspheres

Particle Size and Shape

The microspheres were evaluated for particle size and shape. A stereomicroscope was used for this purpose, which was calibrated using calibration micrometers. The microscope was equipped with the software, Image Manager through a camera. A suspension of the microspheres was prepared using Nujol on a slide and covered with the cover slip. For preparing a suspension, 50 mg microsphere was suspended in 5mL distilled water containing 2% w/v of tween 80 to prevent aggregation. Then solution is sonicated in water bath and the particle size was expressed as volume mean diameter (mm). This slide was observed under the microscope. An image was clicked and this image was used for the particle size and shape analysis. The magnification of the microscope used for observations was 115X. Around 250 particles size was measured for each batch on the different portions of the slide. From this size distribution, the average particle size and standard deviation were calculated for each batch of microspheres [18,19,22,23].

Drug Entrapment Efficiency

Microspheres containing of drug (5mg) were crushed and then these microspheres dissolved in distilled water with the help of ultrasonic stirrer for 3 hr. and then filtered and assayed by UV spectroscopy and then entrapment efficiency is calculated. Entrapment efficiency is equal to ratio of actual drug content to theoretical drug content [20].

$$\%\,Entrapment = \frac{Actual\ Content}{Theoretical\ Content} \times 100$$

Swelling Index

Swelling index was determined by measuring the swelling efficiency of microspheres in the given buffer. Exactly weighed amount of microspheres were allowed to swell in given buffer. The excess liquid drops were removed by blotting and then the swollen microspheres were reweighed. The hydrogel microspheres then dried in an oven at 60° for 5 h until there was no change in the dried mass of sample [21]. The swelling index of the microsphere was calculated by using the formula:

$$\text{Swelling index} = \frac{(\text{mass of swollen microspheres - mass of dry microspheres})}{(\text{mass of dry microspheres})} \times 100$$

Attenuated Total Reflectance FT-IR Spectroscopy

FT-IR is mainly used to determine the degradation of the polymeric matrix of the carrier system. The surface of the microspheres is investigated and measuring alternated total reflectance (ATR). The IR beam passing through the ATR cell reflected many times through the sample to provide surface material IR spectra. The ATR-FTIR provides information about the surface composition of the microspheres depending upon manufacturing conditions and procedures.

Stability Studies

Stability studies were performed by placing the microspheres in screw capped glass container and stored them at following conditions:

1. Ambient humid condition
2. Room temperature (27+/-2°C)
3. Oven temperature (40+/-2°C)
4. Refrigerator (5°C -8°C).

It was carried out for 60 days and the drug content of the microsphere was analysed [24].

X-ray Diffraction

X-ray diffraction is mainly used to determine the Change in crystalline of drug. Microperticles and its individual components were analysed by the help of an x-ray diffractometer. Scanning range angle between 8°C - 70°C.

Scan speed - 40/min

Scintillation detector

Primary silt = 1 mm

Secondary silt = 0.6 mm [11].

Isoelectric Point

The micro electrophoresis is an apparatus used to determine the electrophoretic mobility of microspheres from which the isoelectric point can be determined. The mean velocity was determined at different Ph values ranging from 3-10 calculated by measuring the time of particle movement over a distanace of 1 mm. By using this data, electrical mobility of the particle can be determined. The electrophoretic mobility can be related to surface charge, ionisable behaviour of the microspheres.

UV-FTTR (Fourier Transform Infrared)

The drug polymer interaction and degradation of drug while processing for microencapsulation can be determined by FTIR [25].

Measurement of the *in vitro* Release

A modification of the USP-III (reciprocating cylinder type) apparatus

was used. The media used in this method was phosphate buffer of pH 6.4 and the volume of media taken was 25 ml that will just touch the surface of the reciprocating cylinder's mesh # 400. The temperature of the media was maintained at 37 ± 0.5°C. The media was kept covered throughout the experiment and the dipping mechanism was stopped in static conditions. Periodical samples of 3 ml were withdrawn and replaced it after uplifting the assembly. The samples were taken after stirring the media. Samples were filtered through the 0.45 µm nylon filter and, after suitable dilution; absorbances were taken at suitable wavelength. The experiment was performed in triplicate and average values reported [26].

Conclusion

It has been observed that the 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, greater bioavailability and better patient compliance. Its applications are enormous because they are not only used for drug delivering but also for imaging tumours, detecting biomolecular interaction etc. So microspheres will have an important role to play in the advancement of medicinal field in future.

Conflicts of Interest: None declared.

References

[1] Stoner C.L., Cleton A., Johnson K., Oh D.M., Hallak H., Brodfuehrer J., Surendran N. and Han H.K. (2004) *Int. J. Pharm*, 269 (1), 241-249.

[2] Hou T., Wang J., Zhang W. and Xu X. (2007) *ADME evaluation in Drug Discovery*, 208-218.

[3] Prajapati R.K., Mahajan H.S. and Surana S.J. (2011) *Indian Journal of Novel Drug Delivery* 3(1), 9-16.

[4] Romeo V.D., Meireles J., Sileno A.P., Pimplaskar H.K. and Behl C.R. (1998) *Adv. Drug Delivery*, 29, 89-116.

[5] Woo B.H., Jiang G., Jo Y.W. and DeLuca P.P. (2001) *Pharmaceutical Research*, 18(11), 1600-1606.

[6] Illum L. (2003) *J. Control Release*, 87, 187-198.

[7] Yamaya M., Finkbeiner W.E., Chun S.Y. and Widdicombe J.H. (1992) *Am. J. Physiol.*, 262, L713-L724.

[8] Sharma A. (2012) *Journal of Pharmacy Research*, 5(2), 773-777.

[9] Chaudhari M.A., Jadhav M.K. and Kadam M.V. (2010) *International Journal of Pharmaceutical Sciences Review and Research*, 5(1), 8-17.

[10] Andersen I. and Proctor D.F. (1983) *European Journal of Respiratory Diseases*, 127, 37-40.

[11] Ghulam M., Mahmood A., Naveed A. and Fatima R.A. (2009) *Pak. J. Sci*., 22 (3), 291-300.

[12] Sworbrik J.C. and Boylen J.C. and Dekker M. (1988) *Encyclopedia of Pharmaceutical Technology*, 10, 1-29.

[13] Ratcliff J.H., Hunnyball I.M., Smith A. and Wilson C.G. (1984) *J. Pharmacol*., 4, 431-436.

[14] Sam M.T., Gayathri D.S., Prasanth V. and Vinod B. (2008) *The Internet Journal of Pharmacology*, 6(1) 262-269.

[15] Fujimoto S., Miyazaki M., Endoh F., Takahashi O., Okui K. and Morimoto Y. (1985) *Cancer*, 56(10), 2404-2410.

[16] Keith S. (2006) *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 30(6), 996-1008.

[17] Illum L. (2003) *Journal of Controlled Release*, 87(1), 187-198.

[18] Kannan K., Karar K.P. and Manavalan R. (2009) *J. Pharm. Sci. & Res*., 1(1), 36-39.

[19] Shaji J., Poddar A. and Iyer S. (2009) *Indian J. Pharm. Sci*., 71 (6), 715-718.

[20] Soni L.M., Kumar M. and Namdeo P.K. (2012) *International Journal of Drug Delivery*, 2(1), 64-68.

[21] Luppi B., Bigucci F., Mercolini L., Musenga A., Sorrenti M., Catenacci L., Zecchi V. (2009) *J. Pharm. Pharmacol*, 61(2), 151-157.

[22] King M. (1980) *Biorheology*, 17, 249-254.

[23] Nagai T. and Machida Y. (1990) *Bioadhesive Drug Delivery Systems, CRC Press: Boca Raton, Florida*, 169-178.

[24] Tamizharasi S., Rathi J.C. and Rathi V. (2008) *Indian Journal of Pharmaceutical Sciences*, 70(3), 333-337.

[25] Surini S., Anggriani V. and Anwar E. (2009) *J. Med. Sci*., 9(6), 249-256.

[26] Rout P.K. and Nayak B.S. (2009) *Asian Journal of Pharmaceutical and Clinical Research*, .2(4).

[27] Shaji J., Poddar A. and Iyer S. (2009) *Indian Journal of pharmaceutical Sciences*, 71(6), 715-718.

[28] Patel J.K. (2010) *Pharmaceutical Reviews*, 4(6).

[29] Dandagi M.P., Masthiolimath S.V., Gadad P.A. and Iliger R.S. (2007) *Indian Journal of Pharmaceutical Sciences*, 69(3), 402-407.

[30] Ramaprasad Y.V. (1996) *Indian J. Pharm. Sci.*, 58, 1-8.

[31] O'Hagan D.T. and Illum L. (1990) *Crit. Rev. Ther. Drug Carrier Syst*., 7(1), 35-97.

[32] Mestecky J., Moldoveanu Z., Michalek S.M., Morrow C.D., Compans R.W., Schafer D.P. and Russell M.W. (1997) *Journal of Controlled Release*, 48(2-3), 243-257.

[33] Durrani Z., McInerney T.L., McLain L., Jones T., Bellaby T., Brennan F.R. and Dimmock N.J. (1998) *Journal of Immunological Methods*, 220(1), 93-103.

[34] Read R.C., Naylor S.C., Potter C.W., Bond J., Jabbal-Gill I., Fisher A. and Jennings R. (2005) *Vaccine*, 23(35), 4367-4374.