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Review on Nasal Microspheres

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ABSTRACT

Microsphere is a carrier system for delivery of therapeutic candidate. Nasal microspheres offers significant advantages over other type of drug delivery system. It modulates absorption characteristics of the drug by enhancing drug residence time in the nasal cavity and subsequently may increase bioavailability profile of administered drug. Prior to formulation some physicochemical properties of drug are need to be considered like molecular weight, solubility, pKa and partition coefficient of drug etc. Till date various methods have been adopted for fabrication of nasal microspheres are like spray drying, emulsion crosslinking, Solvent Evaporation etc. Obtained microspheres can characterized for various properties like its morphology, drug content, and zeta potential etc. Present review mainly focuses on overview of microsphere technology for nasal administration of drug.

Keywords: Microspheres, Nasal, Mucociliary Clearance

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INTRODUCTION

Nasal drug delivery system has been used since ancient times to deliver drugs locally or systemically. Nasal route offers several advantages like porous endothelial membrane , highly vascularized mucosa, large surface area presence of microvilli structure of the epithelial cells, absence of first-pass hepatic metabolism, easily accessible and has good patient acceptability. This route suffers from drawbacks like mucociliary clearance, low bio-availability for large proteins, unsuitability of drug and other excipients in the formulations irritating to the mucosa, enzymatically active nature of nasal mucosa.[1] Problems like mucociliary clearance, lower membrane permeability and enzymatically active nature of nasal mucosa can be overcome by use of mucoadhesive systems, absorption enhancers like surfactants (bile salts and their derivatives) and enzyme inhibitors respectively. [2] Nasal absorption of drug is mainly affected by Physico-chemical factors like molecular weight, drug solubility and dissolution, Pka and partition coefficient of drug, Polymorphism, Prodrug, Particle size and Morphology, Design of dosage form etc. Nasal drug delivery is the promising route for drugs which show large first-pass metabolism after oral administration and poor stability in the gastrointestinal tract. The present review will mainly focuses on basic concept of microparticulate system for nasal drug delivery, methods of preparation, evaluation parameters and applications.[3-4.] Micro-particulate system was used as carrier for many drugs like Amlodipine Besylate, Atenolol, Carvedilol, Ondansetron Hydrochloride, Lamotrigine, Midazolam, Carvedilol [5-13] which are listed in table 1.

Table 1 . List of Drugs Formulated in Micro-Particulate System[5-13].

Sr.N	Drug	Polymer	Method	Comments
1	Amlodipine Besylate	Polyvinyl Alcohol (PVA)	Phase Separation Emulsification	To avoid first-pass metabolism of drug.
2	Atenolol	Hydroxy Propyl Methyl Cellulose (K4M)	Spray Drying	Better absorption , dose reduction and avoidance of first pass metabolism of drug.
3	Ondansetron HCl	Chitosan	Spray Drying	To avoid first pass metabolism and to improve therapeutic efficacy of drug.
4	Lamotrigine	Chitosan	Emulsion Solvent Evaporation	CNS Targeting.
5	Midazolam	Hydroxy Propyl Methyl Cellulose	Emulsion Cross Linking	To avoid first pass metabolism, improve the patient compliance, and to improve therapeutic efficacy of drug.
6	Midazolam	Carbopol	Emulsion Cross Linking	To avoid first pass metabolism of drug.
7	Carvedilol	Poly(D, L-Lactide-Coglycolide Acid)	Spray Drying	To avoid first pass metabolism of drug.
8	Propranolol	Bovine Serum	Emulsification-	To avoid first pass metabolism

	HCl	Albumin	Heat Stabilization	and better absorption of drug.
9	Zolmitriptan	Chitosan	Emulsion-Cross Linking	Bioavailability improvement by increasing residence time of microspheres inside nasal cavity.

Merits

1.Ease of administration[14] , amenable to self-medication[15)]and better patient compliance. [16]

2. It offers larger surface area for better drug absorption [17]

3.Drugs like propranolol , progesterone and enkephalins shows effective absorption when administered by nasal route.[18]

4.Nasal route provide some unique features like porous endothelial basement membrane, highly vascularized epithelial layer and high blood flow.[19]

5.Avoidance of first pass effect as it lacks pancreatic and gastric activity.[20]

6.Intranasal immunization can elicit both mucosal and systemic immune responses [21]

7.The olfactory region of nasal mucosa provides direct access towards brain for effective targeting CNS acting drugs like anti Alzheimer's , anti depressants, anti migraine, anti schizophrenic etc[22].

Demerits

1.The major drawback suffered by this route is presence of mucociliary clearance which reduces the contact time of applied drug delivery.[23]

2.Poor bioavailability (1% or less) for peptides due to its low membrane permeability .[24]

3.Volume that can be delivered into nasal cavity is restricted to 25–200 µl .[25]

4.It is only suitable for drug with low dose and this route is influenced by pathological conditions like rhinitis[15.]

5.Surfactants used as penetration enhancers in high concentration can damage or may dissolve nasal membrane.[26]

6.Poor ability of polar compounds to cross mucosal membranes hence leads to poor bioavailability.[27]

PHYSICOCHEMICAL PROPERTIES OF DRUG

1.Molecular Weight and Size of the Drug.

The molecular weight and molecular size of the drug greatly influence its nasal absorption. It is believed that molecular weight of drug is having inverse relationship with its absorption.[5] Nasal absorption of drug reduces significantly when its molecular weight exceeds above 1000 daltons. Water soluble drugs move across nasal mucosa by passive diffusion through aqueous pores[28]. Fisher et al in there work studied nasal absorption of various water-soluble compounds based on there molecular weight, compounds under study were 4-oxo-4H-1-benzopyran-2-carboxylic acid, p-aminohippuric acid, insulin and dextran with molecular weight

190,194, 5200,70000 respectively. All the compounds were administered in nasal cavities of anaesthetized male wistar rat and also given by intravenous route. Nasal absorption of the administered compounds were estimated by comparison of the extent of excretion in bile and urine following intranasal and intravenous administration, it was found that 4-oxo-4H-1-benzopyran-2-carboxylic acid , p-aminohippuric acid, insulin, dextran shown 100%,75%, 15% and 2.8% absorption respectively .This data confirms an inverse relationship between molecular weight and absorption.[29] Peptides with molecular weight greater than 1000D shows poor bioavailability i:e 0.5 to 5%. Low bioavailability can be improved by co-administration of an absorption enhancers. In another study by Morten et al in 2001 , shown that the bioavailability of Peptide T was significantly increased when glycofurol or sodium glycocholate (absorption enhancer) was added to a nasal formulations. The nasal bioavailability of Peptide T in water (control formulation), 5% glycofurol, 5% glycofuro +1% sodium glycocholate and 1% sodium glycocholate was found to be 5.9, 22, 29 and 59%, respectively. This results significantly explains the outcome of study i:e improved bioavailability of peptide T by use of absorption enhancer.[30]

2. Drug Solubility and Dissolution Rate.

Drug solubility and dissolution rate affects nasal absorption of drug. Drug with poor solubility in water and high effective dose are considered to be poor candidates for nasal drug delivery system. Prior to absorption, drug should be in its solubilised form for better absorption otherwise it will be cleared off from the nasal cavity. Small volume in the nasal cavity restricts the use of drugs with large doses. The nasal secretions are aqueous in nature, hence drug should posses sufficient hydrophilicity but and at the same time drug should have sufficient lipophilicity to cross nasal mucosa.[31] Co-solvents like glycols, small quantities of alcohol, Transcutol (diethylene glycol monoethyl ether), medium chain glycerides and Labrasol are used to enhance the solubility of drugs. Drug dissolution is the prerequisite for absorption hence it should be well studied prior to formulation of nasal microsphere. [32].

3.Pka and Partition Coefficient of Drug.

Both the pH of the nasal cavity and pKa of a particular drug are important consideration in systemic absorption of drug. The pH in the range of 4.5 to 6.5 is necessary to avoid nasal irritation.[32.] pH partition theory states that , the un-ionized fraction of a drug is more permeable than ionized form.[33-34] Ohwaki et al in there study had studied the effects of dose, pH and osmolarity on the nasal absorption of secretin in rats. The maximal and minimal absorption of secretin was found at pH 3 and pH above 7 respectively. This results represent that pH of the formulation play an significant role in the absorption of drug. Authors finally concluded that the better absorption shown by the preparation is mainly due to low pH of the preparation. Low pH

of the formulation causes structural changes in epithelial cells of the nasal mucosa which serve as the key factor in nasal absorption of secretin.[35] Corbo et al studied barrier properties of mucosal membranes using progesterone and a series of its hydroxyl progesterone analogues. Membrane permeability was found to decrease as the order of hydrophilicity of the progestins increased, indicating that the lipid domain plays an important role in the barrier function of these membranes.[36]

4.Chemical State: Prodrug.

Nasal absorption of impermeable small drug molecules can be improved by using prodrug approach. Al-Ghananeem et al in there work studied the nasal route for the systemic delivery of 17-beta-estradiol using water soluble prodrug of 17beta-estradiol. . They prepared Several alkyl prodrug of 17beta-estradiol. In vivo nasal experiments were performed on rats. Radio imuno-assay using a gamma counter was used to determine the levels of 17beta-estradiol in plasma and cerebro spinal fluid (CSF) . The study revealed that rate of absorption and bioavailability was high for prodrug . Authors suggested that these prodrug are capable of producing high levels of estradiol in the CSF and as a result may have a significant value in the treatment of Alzheimer's disease[37]. In another work by Yang C et al synthesized the, L-Aspartate beta-ester, L-lysyl, and L-phenylalanyl esters of acyclovir to investigate their effectiveness in enhancing nasal absorption of plain acyclovir.[38] Prodrug approach have been used for drugs like 5-iodo-29-deoxyuridine [39-41], and L-dopa succesfuly[42].

5. Physical State: Particle Size and Morphology

Particle size and morphology are important factors need to be considered for determining dissolution performance of drug. Micro particulate system suffers from drawback like feeling of grittiness when administered intra nasally, such feeling may be combated to some extent by maintaining its particle size. Particles with lesser particle size may be inhaled into the lung while the particle with greater size may be cleared off by mucociliary clearance, hence this factor should always be considered while developing intranasal micro particulate system.[43]

6. Polymorphism

Polymorphism mainly affects dissolution of drug and thereby its absorption. polymorphic form of the same drug may show different physicochemical properties and hence its determination is an important concern before developing any nasal particulate systems.[43]

MUCOCILIARY CLEARANCE

The effect evoked by cilia and mucus layer together is called as mucociliary clearance. It is generally referred as self cleaning mechanism of nose. Motile hair-like structure extending from the surface of epithelial cells called Cilia ranges from 5 to 10 mm in length and 0.1 to 0.3 mm in width respectively. The approximate number of cilia present per cell is 200, with a density of

six–eight cilia per μm^2. When measured in vitro, average beat frequency of human nasal cilia is found to be 10 Hz. Mucus exhibits Non-Newtonian flow (Viscoelastic). Normal mucociliary transit time reported is 12 to 15 min with average nasal clearance rate of 8mm/min.[44-45] Corbo et al had investigated nasal mucociliary clearance, by simple and inexpensive saccharin test. For there study school children ranging between 11-14 years of age were selected. They placed particle of diameter 1 mm on the inferior nasal turbinate 1 cm from its anterior end and the time at which initial perception of a sweet taste was sensed, recorded in minutes.[46] Rate of mucociliary clearance is primarily depends on ciliary beat frequency, hence nasal drug formulation should devoid of any effect on ciliary beat frequency of nose .[47]

METHODS FOR PREPARTION OF NASAL MICROSPHERES.

1.Spray Drying.

Spray drying is the well studied technique in the development of microsphere formulations. In this technique micro-spheres are obtained by spraying the solution mixture of drug and other excipients like polymer, permeation enhancer etc into the spray dryer. Many researcher were attempted this method for fabrication of micro-spheres. He et al in 1997, developed chitosan and ethyl cellulose microspheres by using Spray Dying technique. In his study, he used chitosan and ethyl cellulose as polymers for microsphere preparation.[48] Yeamin Huh et al in 2010, prepared spray-dried hyaluronic acid micro-spheres for intranasal delivery of fexofenadine hydrochloride, In their preparation they dissolved Fexofenadine·HCl and Hyaluronic acid in specific quantity of ethanol and water, respectively. after mixing both the solutions, sodium taurocholate and/or PEG 6000 was added into it. The prepared mixture was then spray dried to obtain nasal micro-spheres.[49] In another work, Elisabetta Gavini et al, developed nasal microsphere of metoclopramide by using 5- methylpyrrolidinone chitosan as a polymer. 5-Methylpyrrolidinone chitosan is a chitosan derivative in which the amino groups of glucosamine units of the polysaccharide backbone are partially substituted by methyl pyrrolidinone at position 5. This

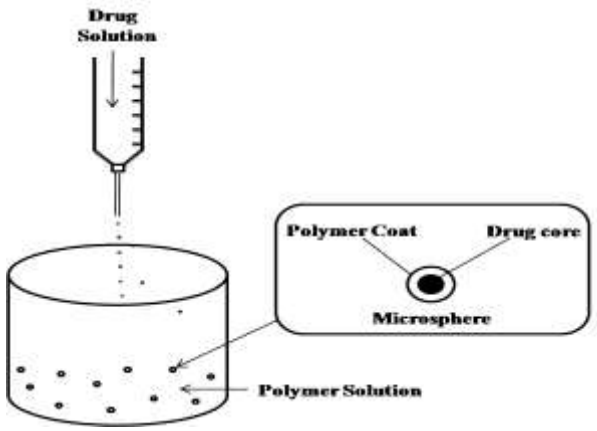

Figure 1: Phase separation and Coacervation method.

derivative of chitosan provides the biocompatibility and hydrophilic characteristics of the chitosan and pyrrolidinone moiety respectively.[50] Sadhana R. Shahi et al, prepared nasal microspheres of atenolol by using HPMC K4M polymer. Methanol and dichloromethane was used as solvent to dissolve drug and polymer. Prepared solution mixture was then spray dried to obtain microspheres. But some process parameters like size of spray nozzle, inlet temperature and spray flow rate in relation to spray dryer are required to be maintained during spray drying technique.[6]

2. Emulsion Crosslinking.

Preparation of an emulsion followed by its cross-linking using cross-linking agents like glutaraldehyde, formaldehyde, genipin etc is the main underlying principal involved in this technique. Mali et al prepared Zolmitriptan mucoadhesive microspheres of chitosan by simple w/o emulsification-crosslinking method. They used liquid paraffin (heavy and light, 1:1) as an external oil phase and chitosan solution containing drug as an internal water phase. Internal aqueous phase was then added slowly into external oily phase to obtain w/o emulsion under constant stirring. Dioctyl sodium sulfosuccinate was used as stabilizing agent and mixed with external oily phase prior to addition of aqueous phase. This emulsion was then cross-linked by using glutaraldehyde. [13].In another study, Desai et al prepared mucoadhesive microspheres of midazolam for brain targeting. HPMC was used as a mucoadhesive polymer and liquid paraffin (heavy and light 1:1 mixture) as an oil phase. Aqueous phase was prepared by dissolving HPMC in phosphate buffer followed by addition of drug. 0.5% tween 80 was used as surfactant.[9] Nasal microspheres of Levodopa [51],Simvastatin,[52] Propranolol Hydrochloride[53], Domperidone[54] were also fabricated by using this method.

3. Solvent Evaporation Method.

This method involves the formation of either water in oil (w/o) or oil in water (o/w) type of emulsion followed by controlled heating to evaporate solvent present in it. Kellaway et al prepared nasal microsphere of Fluorescein isothiocyanate–dextran by using water-in-oil (w/o) emulsion solvent evaporation technique. In this study, water-in-oil (w/o) emulsion was prepared by using Mannide Monooleate as an emulsifying agent and mineral oil as an oil phase. Obtained emulsion was then subjected to controlled heating for removal of aqueous phase. Mineral oil was then decanted from the aqueous free volume to obtain microspheres. [55] Nasal microsphere of drugs like Lamotrigine [8], Neostigmine Bromide[56], Somatostatin Acetate [57] are also prepared by using this method. Some authors utilized the same approach for preparation of nasal microspheres by using single and double emulsion solvent evaporation technique.

Single Emulsion Solvent Evaporation.

It involves solubilization of given polymer in suitable solvent (aqueous or oily) followed by

addition of drug. This mixture was then added into aqueous or oily solvent to prepare oil in water (o/w) or water in oil (w/o) emulsion respectively. Resultant emulsion was then left for evaporation of volatile solvent either by stirring or controlled heating for specific period of time. After complete evaporation of solvent , prepared microspheres are collected ,washed and stored in suitable container.[58]

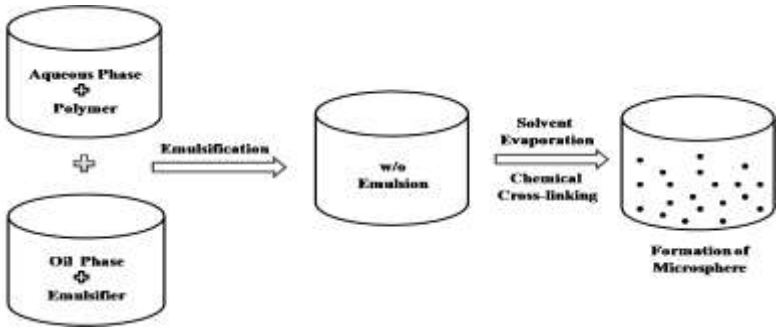

Figure 2: Single Emulsion Solvent Evaporation Technique

Double Emulsion Solvent Evaporation.

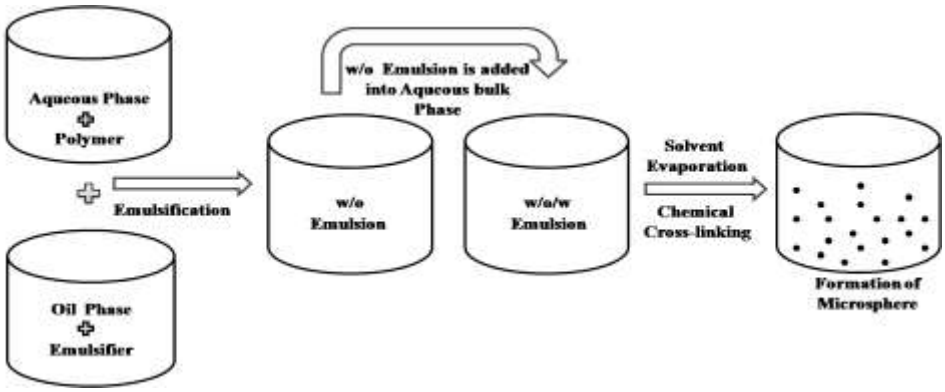

Figure 3: Double Emulsion Solvent Evaporation Technique.

It involves preparation of primary emulsion (w/o or o/w) which is further added into aqueous or oily solvent to form double emulsion (w/o/w or o/w/o). resultant double emulsion was then left for evaporation of volatile solvent either by stirring or controlled heating for specific period of time. after complete evaporation of solvent , prepared microspheres are collected ,washed and stored in suitable container.[58]

4. Ionotropic Gelation Method.

Prasanth et al formulated Salbutamol sulphate alginate (**SSA**) microspheres by Ionotropic Gelation Method. They dissolved sodium alginate in distilled water then Salbutamol sulphate was added into it. This mixture was then homogenized to obtain Salbutamol Sulphate-Sodium Alginate (**SS-SA**)dispersion. This dispersion of drug was then added dropwise into solution of gelling agent to get microspheres.[59] Counter ions play an important role in formation of microspheres. Counter ions can be categorized into 3 sections like high molecular weight counterions (e.g. octyl sulphate, lauryl sulphate, hexadecyl sulphate, cetylstearyl sulphate), low

molecular weight counter ions (e.g. pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolyphosphate, hexametaphosphate), and hydrophobic counter ions (e.g. alginate, -carragenan, poly-1-hydroxy-1-sulphonate-propene-2, polyaldehydro-carbonic acid) Polymer dissolved in suitable solvent is allowed to drop in the solution of counter ions which leads to formation of microsphere. [60]

5. Phase separation and Coacervation method

Nagamani et al formulated irbesartan loaded microspheres by Phase separation and Coacervation method. Weighed amount of gelatin was dissolved in distilled water by heating the solution and then drug is dispersed in above gelatin solution .This dispersion was then added gradually to sunflower oil, and finally cross linked by using formaldehyde. Obtained microspheres was then subjected for drying at 37°C and stored in colored glass containers.[61]

Characterization of Microspheres

Microspheres are unique carrier system for delivery of many drug molecules and can be characterized by using various techniques which are summarized in table 2

Table 2. Characterization of Micro-spheres[62-66].

Sr.No	Technique Used	Parameter Assessed
1	Scanning Electron Microscopy (SEM)	Morphology of Microspheres (Shape and Surface Characteristics) In vitro degradation of the microspheres by its morphology
2	Laser Diffraction Spectroscopy (LDS)	Particle Size Analysis
3	FT-IR Spectroscopy	Compatibility Studies
4	Powder X-Ray Diffraction (PXRD)	Particle Behaviour
5	UV-Spectroscopy	Drug Content/ Drug Loading
6	Optical Microscopy	Particle Size Analysis
7	Photon-Correlation Spectroscopy	Zeta-Potential
8	Laser Doppler Anemometry	Zeta-Potential
9	Thermal Analysis (Differential Scanning Calorimetry)	physical state of the drug inside the polymer
10	Confocal Laser Scanning Microscopy (CLSM)	Localization of drug, i.e., on the surface and/or inside the microsphere,

Morphology of Microspheres.

The morphology (Shape and Surface Characteristics) of the micro-particles can be studied by using Scanning Electron Microscopy (SEM) with the help of scanning electron microscope. operations involved in this technique are carried out at an inert atmosphere with the help of inert gases like argon and nitrogen. Photographic images produced by scanning electron microscope of respective micro-spheres can be helpful to predict its morphology.[62]

Particle Size Analysis.

The size distribution of the micro-particles can be measure by Laser Diffraction (LD)(62) or by

Microscopical Image Analysis [63] or by Optical Microscopy[66]. Martinac et al. in there work had examined 3000 micro-particles by Microscopical image Analysis technique. Then obtained data is subjected for analysis by software to get particle size distribution.

Compatibility Studies.

Compatibility studies can be predict out with the help of Fourier transform infra-red spectroscopic (FT-IR) technique. Velaga et al in there work did compatibility studies between zolmitriptan(drug) and chitosan(polymer) by using Fourier transform infra-red spectroscopic (FT-IR) analysis. They used the same technique for determining the extent of cross-linking performed on chitosan micro-particles. Presence of an extra peak or absence of peak in the IR spectra of standard drug when formulated in micro-particles represents the interaction between drug and non drug components (polymer).[62]

Drug Content.

UV-spectroscopic technique are now a days widely used to find out drug concentration present in micro-particles. Martinac and co-researchers were used UV-spectroscopic technique for the determination of Loratadine in the prepared micro-spheres. UV absorbance of extracted solution of drug from micro-particles at its λmax can be used to find out its concentration present in micro-particles. Similarly samples withdrawn during dissolution test at respective time intervals can be analyzed by UV-spectroscopic technique to find out its drug content. [62-63]

Zeta-potential of the microspheres.

Zeta-potential of the micro-particles can be measured by Photon-Correlation Spectroscopy[63] or by Laser Doppler Anemometry[65]

Physical State of Drug Inside The Polymer

Thermal analytical technique like Differential Scanning Calorimetry(DSC) can be used to determine physical state of drug inside the polymer whether it is available in crystalline or amorphous form. Presence of an endothermic or exothermic peak in DSC curve will reflect the physical status of the drug inside the polymer. along with DSC ,the X-Ray Diffraction (XRD) Studies are also carried out to know the physical status of drug.[66-67]

Drug Location .

Confocal Laser Scanning Microscopic technique can be used to locate the drug present on the surface and/or inside the micro-spheres.[66]

In-Vitro Swelling Studies.

Coulter Laser Diffraction technique can be use to determine in-vitro swelling properties of the prepared microspheres. Gavini et al in there work allowed to suspend micro-spheres in the phosphate buffer (pH 7.0) for 5 min under magnetic stirring. Particle size distributions, the mean diameters (dvs) are then determine and placed in following formula to find out in vitro swelling

index of micro-spheres,

Swelling Index = (d2- d1)/d1

with d1 is the dvs measured in silicon oil and d2 is the dvs measured after 5 min on contact with the buffer solution[68].

In-Vitro Mucoadhesion Studies

Determination of mucoadhesive strength is an important concern in case of mucoadhesive micro-spheres. Gavini et al in their work were studied the in-vitro mucoadhesion of micro-particles, by pouring sample of Ten milligrams of microparticles on filter paper which was saturated with mucin solution for 10 minutes in a chamber with controlled humidity and at room temperature. Microparticles sticking on the surface were then recovered by washing the filter paper with water and the amount of the drug was determined by an UV spectrophotometric analysis.[68]

In-vitro Diffusion Studies

Franz diffusion cell is widely used for in-vitro diffusion studies. Jose et al carried out in-vitro diffusion studies of lorazepam microspheres by using dialysis membrane (Spectra/Por® dialysis tubing). Donor compartment was filled with simulated nasal fluid and receptor compartment with phosphate buffer maintained at pH 6.2. amount of drug diffuse was calculated by using UV-spectroscopic analysis.[69]

Histopathological Evaluation of Mucosa.

In this type of study, generally bovine nasal mucosa is exposed to the nasal formulation which is then subjected observed under light microscope. Microscopic images obtained under light microscope can reveal extent of damage or injury to the tissues of nasal mucosa if any.[69]

CONCLUSION

Microspheres are considered as better choice of drug delivery system because it offers many options like better target specificity and patient compliance. Nasal microspheres is the unique carrier system for delivery of many drugs unsuitable by other routes. Use of mucoadhesive polymer in such type of system may be used to improve bioavailability of given therapeutic moiety by increasing its residence time in nasal cavity. Though such type of carrier system offers many merits but further comprehensive study is needed, to overcome many drawbacks associated with it.

REFERENCES

1. Peter E, Erik B. Routes of Delivery: Case Studies Nasal delivery of peptide drugs. Adv Drug Deliv Rev 1992; 8: 165-77.
2. Chandler S, Lisbeth I, Thomas N. Nasal absorption in the rat. I: A method to demonstrate the histological effects of nasal formulations. Int J Pharm 1991. 7019-27.

3. Pujara PC, Shao Z, Duncan MR, Mitra AK. Effects of formulation variables on nasal epithelial cell integrity: Biochemical evaluations. Int J Pharm 1995; 114: 197-03.
4. Merkus FWHM, Schipper NGM, Hermens WAJJ, Romeijn SG, Verhoef JC. Absorption enhancers in nasal drug delivery: efficacy and safety. J Control Release 1993; 24: 201-08.
5. Swamy NGN, Abbas Z. Preparation and In Vitro Characterization of Mucoadhesive Polyvinyl Alcohol Microspheres Containing Amlodipine Besylate for Nasal Administration. Ind J Pharm Edu Res 2012; 46: 52-58.
6. Shahi SR, Tribhuwan SD, Tadwee IK, Gupta SK, Zadbuke NS, Shivanikar SS. Formulation of Atenolol Mucoadhesive Microspheres for Nasal Delivery by Spray Drying Technique: In vitro/ Ex vivo Evaluation. Der Pharm Sini 2011; 2 (5): 54-63.
7. Mahajan H, Gattani S, Surana S. Spray dried mucoadhesive microspheres of ondansetron for nasal administration. Int J Pharm Sci Nano 2008; 1(3): 267-74.
8. Dave K, Purohit S. Formulation And In Vitro Characterization Of Intranasal Mucoadhesive Microspheres Of Lamotrigine Using Chitosan By Glutaraldehyde Cross Linking. Int J Pharm Bio Sci 2013; 4(3): 402 – 15.
9. Desai S, Vidyasagar G, Shah V, Desai D. Preparation And In Vitro Characterization of Mucoadhesive Microspheres of Midazolam: Nose To Brain Administration. Asian J Pharm Clin Res 2011; 4(1): 100-02.
10. Desai S, Vidyasagar G, Desai D. Brain targeted nasal midazolam microspheres. Int J Pharm Biomed Sci 2010; 1(2): 27-30.
11. Prajapati RK, Mahajan HS, Surana SJ. PLGA Based Mucoadhesive Microspheres for Nasal Delivery: In Vitro / Ex Vivo Studies. Indian J Nov Drug Deliv 2011; 3(1): 9-16.
12. Yadav A, Jain DK. Preparation And Characterization Of Micro particulate System Of Propranolol Hydrochloride. J Drud Del Ther 2011; 1(2): 15-20.
13. Mali S, Akarte A, Sapate M, Baviskar D, Jain D. Formulation And Evaluation of Zolmitriptan-Chitosan Nasal Mucoadhesive Microspheres. IJAPR 2013; 4(08): 2109 – 2117.
14. Al-Ghananeem AM, Sandefer EP, Doll WJ, Page RC, Yunik C, Digenis GA. Gamma scintigraphy for testing bioequivalence: A case study on two cromolyn sodium nasal spray preparations. Int J Pharm 2008; 357: 70–76.
15. Ugwoke MI, Agu RU, Vanbilloen H, Baetens J, Augustijnsa P, Verbekea N, et al. Scintigraphic evaluation in rabbits of nasal drug delivery systems based on carbopol 971p and carboxymethylcellulose. J Control Release 2000; 68: 207–214.
16. Wang j, Tabata Y, Morimoto K. Aminated gelatin microspheres as a nasal delivery system for peptide drugs: Evaluation of in vitro release and in vivo insulin absorption in

rats. J Control Release 2006; 113: 31–37.

17. Luppi B, Bigucci F, Abruzzo A, Corace G, Cerchiara T, Zecchi V. Freeze-dried chitosan/pectin nasal inserts for antipsychotic drug delivery. Eur J Pharm Biopharm 2010; 75: 381–87.
18. Illum L, Jorgensen H, Bisgaard H, Krogsgaard O, Rossing N. Bioadhesive microspheres as a potential nasal drug delivery system. Int J Pharm 1987; 39: 189-99.
19. Hascicek C, Gonul N, Erk N. Mucoadhesive microspheres containing Gentamycin sulfate for nasal administration: preparation and in vitro characterization. Farmaco Sci 2003 58; 11-16.
20. Galgatte UC, Kirange RH, Pote CW, Thanvi DA, Chaudhari PD. Preparation and evaluation of spray dried mucoadhesive microspheres of metaclopramide hydrochloride for nasal delivery. Int J Pharm Sci Nano 2013; 6(1): 1994-07.
21. Davis SS. Nasal vaccines. Adv Drug Deliv Rev 2001; 51: 21–42.
22. Kumara M, Misra A, Babbar AK, Mishra AK, Mishra P, Pathak K. Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. Int J Pharm 2008; 358: 285–91.
23. Vyas SP, Goswami SK, Singh R. Liposomes based nasal delivery system of nifedipine: Development and characterization. Int J Pharm 1995; 118: 23-30.
24. Shahnaza G, Vettera A, Barthelmesa J, Rahmata D, Laffleura F, Iqbala J. Thiolated chitosan nanoparticles for the nasal administration of leuprolide: Bioavailability and pharmacokinetic characterization. Int J Pharm 2012; 428: 164– 70.
25. Rao JV, Gangadi JR, Kumar SM, Venu K, Jayaveera KN. Formulation And Evaluation Of Mucoadhesive Microspheres For Intra -Nasal Delivery. IJPT 2010; 2(4): 1158-98.
26. Alagusundaram M, Chengaiah B, Gnanaprakash K, Ramkanth S, Chetty MS, Dhachinamoorthi D. Nasal drug delivery system - an overview. Int. J. Res. Pharm. Sci. 2010; 1(4): 454-465.
27. .Soane RJ, Hinchcliffe M, Davis SS, Illum L. Clearance characteristics of chitosan based formulations in the sheep nasal cavity. Int J Pharm 2001; 217: 183–91.
28. Agrawal V, Mishra B. Recent Trends In Drug Delivery System: Intranasal Drug Delivery. Indian J Exp Biol 1999; 37: 6-16.
29. Fischer AN, Brown K, David SS, Par GD, Smith DA. The effect of molecular size on the nasal absorption of water-soluble compounds in the albino rat. J Pharm Pharmacol. 1987; 39(5): 357-62.
30. Baggera MA, Nielsena HW, Bechgaard E. Nasal Bioavailability of Peptide T In Rabbits: Absorption Enhancement By Sodium Glycocholate And Glycofurol. Eur J Pharm Sci

2001; 14: 69–74

31. Ali A, Prajapati SK, Singh D, Kumar B, Shafat K. Enhanced Bioavailability Of Drug Via Intranasal Drug Delivery. System. Int Res J Pharm 2012; 3(7): 68-74.
32. Kushwaha S, Keshari RK, Rai AK. Advances In Nasal Trans-Mucosal Drug Delivery. Journal of Applied Pharmaceutical Science 2011; 01 (07): 21-28.
33. Dey S, Mahanti B, Mazumder B, Malgope, Dasgupta S. Nasal drug delivery: An approach of drug delivery through nasal route. Der Pharm Sini 2011; 2 (3): 94-106.
34. .Pratapwar AS, Agrawal VA, Chiddarwar AP. Formulation And Biological Factors Influencing The Absorption Of Drugs Through Nasal Epithelium And Current Nasal Formulations - An Overview. Pharmacophore 2012; 3 (1): 37-43.
35. Ohwaki K, Ando H, Watanabe S, Miyake Y. Effects of dose, pH, and osmolarity on nasal absorption of secretin in rats. J. Pharm. Sci 1985; 74: 550–52.
36. Corbo DC, Liu JC, Chien YW. Characterization of the barrier properties of mucosal membranes. J Pharm Sci 1990; 79(3):202-6.
37. Al-Ghananeem AM, Traboulsi AA, Dittert LW, Hussain AA. Targeted Brain Delivery Of 17 Beta-Estradiol Via Nasally Administered Water Soluble Prodrugs. AAPS PharmSciTech. 2002;3(1): E5.
38. Yang C, Gao H, Mitra AK. Chemical stability, enzymatic hydrolysis, and nasal uptake of amino acid ester prodrug of acyclovir. J Pharm Sci. 2001; 90(5): 617-24.
39. Ghosh MK, Mitra AK. Effects of 59-ester modification on the physicochemical properties and plasma protein binding of 5-iodo-29-deoxyuridine. Pharm. Res 1991; 6: 771–75.
40. Ghosh MK, Mitra AK. Enhanced delivery of 5-iodo-29-deoxyuridine to the brain parenchyma. Pharm. Res. 1992; 9: 1173–76.
41. Narukar MN, Mitra MK. Synthesis, physicochemical properties, and cytotoxicity of a series of 59-ester prodrugs of 5-iodo-29-deoxyuridine. Pharm. Res 1988; 5: 734–37.
42. Kao HD. Enhancement of Delivery of L-Dopa by the Administration of Its Prodrugs Via the Nasal Route(Ph.D. Thesis). University of Kentucky, Lexington, Kentucky; 1995.
43. Behl CR, Pimplaskar HK, Sileno AP, DeMeireles J, Romeo VD. Effects of Physicochemical Properties And Other Factors On Systemic Nasal Drug Delivery. Adv Drug Deliv Rev 1998; 29: 89–116.
44. Romeijn SG, Verhoef JC, Marttin E, Merkus FWHM. The effect of nasal drug formulations on ciliary beating in vitro. Int J Pharm 1996; 135: 137-45.
45. Marttin E, Schipper NGM, Verhoef JC, Merkus FWHM. Nasal mucociliary clearance as a factor in nasal drug delivery. Adv Drug Deliv Rev; 29: 13–38.

46. Corbo GM, Foresi A, Bonfitio P, Mugnano A, Agabiti N, Colet PJ. Measurement of nasal mucociliary clearance. Arch Dis Child 1989; 64: 546-50.
47. Vetter A, Augustijns P, Bernkop-Schnürch A. Solubilizing agents in nasal formulations and their effect on ciliary beat frequency. Toxicol In Vitro 2012; 26: 150–56.
48. Ping H, Davis SS, Illum L. In vitro evaluation of the mucoadhesive properties of chitosan. Microspheres. Int J Pharm 1998; 166: 75–68.
49. Huha Y, Choa HJ, Yoona IS, Choia MK, Kim JS, Oh E et al. Preparation and evaluation of spray-dried hyaluronic acid microspheres for intranasal delivery of fexofenadine hydrochloride. Eur J Pharm Sci 2010; 40: 9–15.
50. Gavini E, Rassu G, Muzzarelli C, Cossu M, Giunchedi P. Spray-Dried Microspheres Based On Methylpyrrolidinone Chitosan As New Carrier For Nasal Administration of Metoclopramide. Eur J Pharm Biopharm 2008; 68: 245–52.
51. Chandrakala V, Saral M. Optimization and Characterization of Nasal Microparticles for Levodopa Delivery to CNS. RJPBCS 2013; 4(2): 882.
52. Tatiya GD, Basarkar GD. Formulation Development and In Vitro Evaluation of Mucoadhesive Microspheres of Simvastatin for Nasal Delivery. Int. J. Pharm. Sci. Rev. Res 2013; 21(2): 334-41.
53. .Dandagi PM, Mastiholimath VS, Gadad AP, Iliger SR. Mucoadhesive Microspheres of Propranolol Hydrochloride for Nasal Delivery. Indian J Pharm Sci 2007; 402-07.
54. Yadav AV, Mote HH. Development of Biodegradable Starch Microspheres for Intranasal Delivery. Indian J Pharm Sci 2008; 70(2): 170–74.
55. Abd El-Hameed MD, Kellaway IW. Preparation and in vitro characterization of mucoadhesive polymeric microspheres as intra-nasal delivery systems. Eur J Pharm Biopharm 1997; 44: 53-60.
56. Nanjwade BK, Parikh KA, Deshmukh RV, Nanjwade VK, Gaikwad KR, Thakare SA,et al. Development and Evaluation of Intranasal Mucoadhesiv Microspheres of Neostigmine Bromide. Pharm Anal Acta 2011; 2:2: 2153-35.
57. Herrmann J, Bodmeier R. Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on W/O/W-multiple emulsions. Int J Pharm 1995; 126(1-2): 129–38.
58. Yıldız A, Okyar A, Baktır G, Araman A, Ozsoy Y. Nasal administration of heparin-loaded microspheres based on poly(lactic acid). Farmaco Sci 2005; 60: 919–24.
59. Prasanth VV, Chakraborty A, Mathew ST, Parthasarathy G, Mathappan R, Thoppil CS. Formulation And Evaluation Of Salbutamol Sulphate -Alginate Microspheres By Ionotropic Gelation Method. IJCP 2011; 7 (07): 1-4.

60. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, Dhawan S. Chitosan microspheres as a potential carrier for drugs. Int J Pharm 2004; 274: 1–33.
61. Nagamani B, Rajanraju CH, Saratchandra. K, Koteswararao GSN., Uma devi P. Effect Of Natural Polymers On Controlled Release Of Irbesartan From Acacia And Kernel Loaded Microspheres. Int. J. Inv. Pharm.Sci 2013; 1(4): 282-87.
62. Alhalaweh A, Andersson S, Velaga SP. Preparation of zolmitriptan–chitosan microparticles by spray drying for nasal delivery. Eur J Pharm Sci 2009; 38: 206–14.
63. Martinaca A, Filipovic-Grcica J, Voinovich D, Perissuttib B, Franceschinis E. Development and bioadhesive properties of chitosan-ethyl cellulose microspheres for nasal delivery. Int J Pharm 2005; 291: 69–77.
64. Li Y, Jiang HL, Zhu KJ, Liu JH, Hao YL. Preparation, characterization and nasal delivery of a-cobrotoxin-loaded poly(lactide-co-glycolide)/ polyanhydride microspheres. J Control Release 2005; 108: 10– 20.
65. He P , Davis SS, Illum L. Chitosan microspheres prepared by spray drying. Int J Pharm 1999; 187: 53–65.
66. Pereswetoff-Morath L, Edman P. Dextran microspheres as a potential nasal drug delivery system for insulin - in vitro and in vivo properties. Int J Pharm 1995; 124: 37-44.
67. Goud K, Desai H, Jin P. Preparation and Characterization of Drug-Loaded Chitosan–Tripolyphosphate Microspheres by Spray Drying. Drug Dev Res 2005; 64:114–128.
68. Gavini E, Rassu G, Muzzarelli C, Cossu M, Giunchedi P. Spray-dried microspheres based on methylpyrrolidinone chitosan as new carrier for nasal administration of metoclopramide. Eur J Pharm Biopharm 2008; 68: 245–52.
69. Jose S, Ansa CR, Cinu TA, Chacko AJ, Aleykutty NA, Ferreira SV, Souto EB, Thermo-sensitive gels containing lorazepam microspheres for intranasal brain targeting. Int J Pharm 2013; 441: 516– 26.