ejpmr, 2020,7(11), 338-344

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A REVIEW ON MICROENCAPSULATED AND NANOENCAPSULATED NASAL DRUG DELIVERY SYSTEM

Harshada A. Kadam* and Nikhil A. Gade

Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Trimbak road, Nashik- 422213, Maharashtra, India.

***Corresponding Author: Harshada A. Kadam**

Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Trimbak road, Nashik- 422213, Maharashtra, India.

Article Received on 05/09/2020 **Article Revised on 25/09/2020** **Article Accepted on 15/10/2020**

ABSTRACT

The utilization of a nasal route for drug delivery has been used in recent years within the pharmaceutical field , because it has been reveal that the many drugs have better better by nasal route than oral route. Local and systemic drug delivery are also achieved by this route of administration. But the nasal route of delivery isn't applicable to all or any drugs. The nasal delivery route has the advantages, including convenience and easy use, they have rapid onset of action, the avoidance of immediate hepatic metabolism, gut wall metabolism or destruction in the gastrointestinal tract, also the possibilities of to reduce systemic exposure, higher the bioavailability, and have a rapid brain access through the nerves. Drug delivery systems including liposomes, cyclodextrins, micro- and nanoparticles are being investigated to increase the bioavailability of medicines delivered intranasally. This criticism discusses recent progress and specific development issues regarding to colloidal drug delivery systems used in the nasal drug delivery.

KEYWORDS: Nasal delivery. Nasal bioavailability, Liposomes, Microsphere, Nanoparticles

INTRODUCTION

The nasal route is commonly used as drug delivery for the treatment of local diseases. Nasal therapy also called as 'Nasya karma' has been recognized as the style of treatment within the Ayurvedic system of Indian medicines.[1] But in recent years, this route has been received special attention as a convenient and reliable method for the systemic delivery of medicines, especially people who are ineffective by oral route due to their metabolism within the gastrointestinal tract or by first-pass effect and must be administered by injection. The nasal cavity has a large absorptive area and therefore the high vascularity of the nasal mucosa ensures that absorbed compounds are rapidly removed.[2]

Advantages of nasal drug delivery system.[3]

- Rapid onset of action.
- Absorption of drug is rapid through highly vascularised mucosa.
- Non invasive and simple for administration.
- Bypass the BBB.
- Degradation of drug that observed in GIT is avoided.
- Nasal bioavailability of small drug molecule is great.
- Bioavailability of enormous drug molecule are often increased by means of absorption enhancers.
- Alternate to parenteral route especially for proteins and peptides.
- Due to low amount of dose less side effects.
- Patient's convenience and compliance is improved.
- A self-administration is possible.

of the strategies utilized to enhance nasal drug absorption, the utilization of drug delivery systems has attracted great interest. Colloidal carriers system have demonstrated great efficacy in increasing drug bioavailability by nasal route. This text discusses recent progress in nasal drug delivery by using colloidal carriers such as micro/nanoparticles and liposomes are going to be discussed.

1. Anatomy and physiology of nasal cavity

Nasal route is employed for the systemic delivery of medication attributable to a high degree of vascularization and permeability of the nasal mucosa. In human being and other animal breeds the main functions of the nasal cavity are respire and olfaction. However, it has important protective activity once it filters, heat and humidify the inhaled air before reaching all-time low airways. Passage of the nasal cavity which runs from nasal vestibule to nasopharynx feature a depth of approximately 12-14cm. The whole extent of the nasal cavity in human adult is about 150 cm^2 and total volume is about 15 ml. By anatomically respiratory system is divided into two types:

1. Upper respiratory tract

1. Nose
2. Pharynx
3. Larynx.

2. Lower respiratory tract

1. Trachea
2. Tracheobranchial
3. Bronchioles.

Each of two nasal cavities are often subdivided into different regions: Nasal vestibule, inferior turbinate, middle turbinate, superior turbinate, olfactory region, sinus, sphenoidal sinus, and cribriform plate of ethmoid bone.

Nasal vestibule: Most anterior a part of the nasal cavity is nasal vestibule, just inside the nostrils, and presents a section about 0.6 cm². Nasal hairs are present during this area, also called vibrissae, which filtered the inhaled particles. Nasal portion is roofed by a stratified squamous and keratinized epithelium with sebaceous glands.

Atrium: Intermediate area between nasal vestibule and respiratory region is atrium. The anterior section is constituted by a stratified squamous epithelium and therefore the posterior area by psudostratified columnar cells presenting microvilli.

Respiratory region: Largest a part of the cavum is respiratory region, also called conchae, is that the cavity and it's divided in superior, middle and inferior turbinates which are projected from the lateral wall. The nasal respiratory mucosa, considered the foremost important section for delivering drugs systematically, is constituted by the epithelium, basement membrane and lamina propria. Many of the epithelial cells are covered on their apical surface with microvilli and therefore the major part of the them also has fine projections, called cilia.

Olfactory region: Location of olfactory region is at the roof of the nasal cavity and extends a brief way down the septum and lateral wall. Its neuro-epithelium is that the only part of the CNS that's directly exposed to the external environment. Olfactory receptor cells important for smell perception.

Mucus membrane of nose and its composition: The nasal mucus layer is just 5 µm thick and it is organized in two distinct layaers: an external, viscous and dense, and an indoor, fluid and serous. Overall, nasal mucus layer consists of 95% of water, 2.5-3% of mucin and 2% of electrolyte, proteins, lipids, enzymes, antibodies, sloughed epithelial cells and bacterial products.

Drug Absorption Through the Nasal Cavity

The absorption of medicine starts within the respiratory region comprising the turbinates and a part of the septum. As for all biological membrane either passively by the paracellular pathway or both passively and actively via transcellular pathway. The transport of charged drugs under basic pH conditions is also keen about the microenvironment pH effects due to the complex architecture of the nasal passages. For uncharged drugs, the nasal mucosal membrane behaves as a modified lipophillic transport barrier.[10]

The determiner on which pathway (transcellular or paracllular) is more appropriate for a particular compound is its lipophilicity. Other possible pathways for drug permeation across the nasal mucosa include carrier mediated transport, transcytosis and transport through intercellular tight junctions.[8]

Lipophilic drugs, after nasal administration, generally indicate rapid and systematic absorption. For some drugs, it is possible to obtain pharmacokinetic profiles similar to those obtained after an intravenous injection. But, the nasal absorption of hydrophilic drugs is very poor, with bioavailability less then 10% for small molecular weight drugs and less than 1% for peptides.[5,10] The main reasons for very low bioavailability of polar compounds include poor membrane permeability, rapid clearance of the drug and enzymatic degradation in the nasal cavity. For polar drugs that are not easily transported across the nasal membrane, the mucociliary clearance mechanism can quickly move the drug away from the absorption site in the nasal Cavity into the esophagus, whereby the drug is swallowed and absorption minimized.[5,10]

Using absorption promoting agents, such as surfactant,[12] phospholipids,[13] and various cyclodextrins,[14] nasal absorption of polar drugs may be enhanced. These enhancer systems work by increasing the membrane fluidity (generally by modifying the phospholipid bilayer),[5] deceasing the viscocity of mucosal layer, inhibiting proteolytic enzymes, increasing paracellular or transcellular transport, increasing the blood flow, or by combination these mechanism.[8] A more effective formulation combines an absorption enhancer with a bioadhesive polymer, which increases the residence time of formulation in the nasal cavity.[11] But care must be taken in the choice of the enhancer agent, as damage to the nasal mucosa must be avoided.

2. Methods Currently Available to study Nasal Absorption:

The better animal model used to study the nasal absorption of drugs is the rat. Studies show that for most drugs (non-peptides), the results obtained in the rat accurately forecast the absorption profiles of the drugs in the human. These are some methods that can be used to predict the nasal drug absorption in the rat, obviously each one has advantages and limitations. The *in situ*, *in vivo in situ*, and the *in vivo* method were extensively reviewed by Hussain (1998).[3]

In the *in situ* method, the perfusing solution is introduced into the nasal cavity by means of a tube that is inserted through thro esophagus to the posterior part of the nasal cavity. The surgical procedure for the in situ nasal absorption has been reported in several publications.[34] In this method, the drug solution is circulated through the nasal cavity of the rat by means of a polystatic pump. The extent of absorption is determined by analyzing periodically the amount of drug remaining in the perfusing solution.

With the in the *in vivo in situ* method, small volume of drug (50-100µL) are administered directly to the nasal cavity. The surgical procedure is similar to that described for the *in situ* recirculation studies, except that a glass tube is inserted into the posterior nasal cavity via the esophagus to keep the solution in the nasal cavity. At an appropriate time interval, the nasal cavity is rinsed with Ringer's buffer using a peristaltic pump, and the concentration of drug in the nasal cavity is determined. Furthermore, the data generated can be used directly to predict *in vivo* absorption rates.[35]

In the in vivo method, the drug is directly deposited into the nasal cavity and blood samples are periodically withdrawn and analysed. The data obtained by this method are very reproducible and reliable. This method can also be realized in large animals such as dogs, sheep, and monkeys, where the drug is administered while the animal is under anesthesia and care should be taken to minimize the physical loss of drug due to drainage.[3]

Now a days, cell culture techniques are being used for the in vitro investigation of the transport and the metabolism of drug across and in the nasal epithelium.[37,38] Fundamentally there are three different types of technique for the development of human nasal primary cell culture system: atraumatic methods, traumatic methods (surgical biopsy, surgical removal of turbinates due to sleep apnea or plastic reconstruction), and post mortem biopsy. An extensive review of cell culture condition and techniques to sample nasal tissues and cells were performed by Schimidt and co-workers (1998).[36]

Table 1. Example of Nasal Uptake of Representative Drug Models.

Sr. No.	Class	Drugs	Indication	Status	Comments
1.	Barbiturates	Barbital Phenobarbital	Ansiolitic	Human studies	Absorption increased[26]
2.	Antidiabetic	Insulin	Treatment of diabetic mellitus	Human studies	Improve patient compliance[32]
3.	Narcotics	Buprenorphine	Analgesic	Human studies	Improve drug bioavailability[24,25]
4.	Thyroid harmone	Calcitonin	Treatment of several bone diseases	Human studies	Plasmatic levels increased[33]
5.	Sex harmones	Estradiol Progesterone Testosterone	Induction of hormonal contraception, Treatment of amenorrhea	Human studies	Improve drug the bioavailability[19,20,21]

3. Drug delivery to the brain via nasal route

There has been recent interest in nasal drug delivery as a method to by-pass the blood barrier and reach the cerebral spinal fluid (CSF) in order to deliver therapeutic agents directly to the brain. The drugs absorbed via the olfactory route do not cross the blood-brain barrier (BBB), it may be possible to target substances to the CNS that would have been blocked from entering via the systemic circulation. The transport of drug across the olfactory region in the nasal cavity directly into the brain tissue or the CSF has generated much interest.[54,55]

For the drug to reach the CNS from the nasal cavity, it must cross the olfactory membrane and, depending on the pathway used, also the arachnoid membrane surrounding the arachnoid space of the CFS. The drug can use a transcellular pathway, where it is transferred by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion. This pathway is especially used for small lipophillic molecules or large molecules, but is very slow and probably not relevant in terms of drug administration. Or the drug can use the paracellular pathway by passing through the tight junctions or through open clefts in the membrane. This route enables relatively quick absorption to the CSF of hydrophilic and semi-lipophilic substances. Another pathway also used is for the drug to be transported across the olfactory neuron cells by intracellular axonal transport firstly to the olfactory bulb.[54,56]

Quantitatively determine the significance of this pathway to deliver the drugs to the brain tissue, the CSF as well the olfactory bulbs, Hussain (1998)[3] determined the levels of L-dopa after intranasal administration and compared the data with levels of L-dopa after intravenous administration. Results showed higher levels of drug in the CSF and olfactory bulbs compared to that observed after intravenous injection.

Transport directly to the brain must be done only in situations where it is therapeutically necessary, for example in the treatment of Parkinson's and Alzheimer's disease. Another situation targeting drug directly to the brain that could have potential benefit is in the treatment of human immunodeficiency syndrome (HIV), etiologic agent of AIDS and related disorders. HIV is known to

actively invade the CNS and the microglial cells in the brain are significant reservoirs of the virus. Unfortunately, little progress has been made in the search to enhance drug transport to the brain through the nasal route.

4. Colloidal carriers as drug delivery systems

The purpose of any drug delivery system is to supply a specific amount of drug to the proper site in the body to attain and maintain the desired therapeutic drug concentrations. The drug delivery system should deliver drug at a rate by the needs of the body over a specified period of treatment.

The two main barriers for drugs reaching biological compartments in adequate quantities: poor stability and finite transport across the epithelia. Therefore, appropriate delivery systems for these compounds would protect the drugs form the biological environment and facilitate transport though biological barriers.

Particulate carriers in colloidal dimensions, like liposomes, nanoparticles and microparticles, have attracted considerable interest as drug carriers for achieving controlled delivery of medicine at specific sites within the body. The usefulness of liposomes and micro/nanoparticles in topical administration and especially nasal administration has been studied for only 10 years.[54] The use of liposomes, microparticles and nanoparticles for targeting drug delivery as well as their potential applications in nasal drug delivery will be discussed.

4.1. Liposomes

Liposomes **(Fig.1)** are small artificial bilayer vesicles which is spherical in shape that can be prepared from natural non-toxic phospholipids and cholesterol. Liposomes are good systems for drug delivery due to their size, hydrophobic and hydrophilic character, also biocompatibility. Their unique amphiphilic nature makes them suitable for many drug delivery strategies. Liposomes are classified into three classes based on their size and number of bilayers. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50 nm in diameter, containing an aqueous solution in the core. Large unilamellar vesicles (LUV) that have a diameter between 100-3000 nm are a heteregenous group of vesicles similar to SUVs and are enclosed by a single lipid bilayer. Liposomes were first reported by Bangham in the 1960s. Since then, they have been analysed as a possible delivery system for drugs administered as a possible delivery system by various routes.

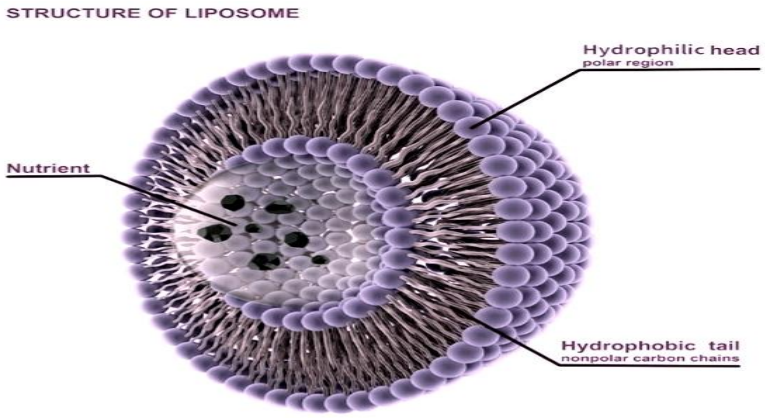

Fig. (1). Liposomes structure formed by phospholipids.

The choice of bilayer components determines the fluidity and the charge of the bilayer. For example, unsaturated phosphotidyl (PC) species from natural sources (egg or soybean PC) form a fluid, much more permeable and less stable bilayer, whereas saturated phospholipids with long acyl chains such as dipalmitoyl-PC form a rigid, rather impermeable bilayer structure.

The ability to trap solute varies among different types of liposomes. SUVs offer the benefit of homogeneity and reproducibility in size distribution. However, a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are three to four times more efficient in terms of trapping water-soluble drug but seem to be somewhat less stable than other types of vesicles. In liposomes characteristics the important determinant in drug entrapment is the physicochemical charactercteristics of the drug itself. Polar drugs are trapped in the aqueous spaces, and non-polar drugs bind to the lipid bilayers of the vesicle. Polar drugs are released when the bilayer is broken, while non-polar drugs remain attached with the bilayer unless it is bursted by temperature or exposure to lipoproteins.

Vyas and co-workers Observed that nifedipine bearing multilamellar liposomes administered via nasal route could be employed successfully to attain a constant plasma profile of the same. Mucoadhesive agents, carbopol and chitosan were also incorporated to keep the formulation at the administered site and to enhance the drug bioavailability through the nasal route. The results revealed that extend the contact time of the drug within absorptive surfaces by means of suitable mucoadhesive agents delayed clearance and increased bioavailability of the intranasally administered drug. Using liposomes as colloidal carriers allowed a reduction in the drug dose

while maintaining therapeutic blood concentrations for at least 48h.

Efficient drug delivery systems based on liposomal approaches need to posses special features. First, good chemical, colloidal, and biological stability are required. Second, specific targeting requires structural and/or chemical changes related to functional groups incorporated into the bilayer, like carbohydrates, antibodies, ligands, growth factors, or magnetic nanoparticles, optimizing drug effects. Other disadvantages of liposomes include interactions with lipoproteins, increased free radicals production, and complete saturation of the immune system. In addition, liposomes have reveal less encapsulation efficiency, poor storage stability, problems in sterilization and fast leakage of water soluble drugs in the blood. As such, their ability to control drug release may not be adequate.

4.2 Microspheres

Microsperes are solid particles be made up of macromolecular substances that range in size from 1 to 1000 µm. Microspheres are matrix systems, where the drug is dispersed throughout the polymeric matrix. Polymers used to prepare microspheres must be biocompatible and biodegradable, and considering nasal administration, the mucoadhesive polymers offer more prolonged contact time with the mucosa. Microspheres has advantage of protection of the incorporated drug from enzymes and, due to their sustained drug release, may also result in the desirable blood concentration profiles.

The mechanism by which microspheres increases the bioavailability of drugs are due to properties of the polymer. Mucoadhesive polymers provide a short-term adhesion between the microspheres and mucus and/or the epithelial cells surface. Mucoadhesive polymers when used to prepare drug carrries for the nasal route promote an increase in the in the residence time within the nasal cavity, intensify the contact between nasal mucosa and drug. Chitosan microspheres are an example; this polymer has good mucoadhesive properties and microspheres produced with chitosan show an increased residence time of drug formulations in the nasal cavity providing improved systemic delivery.

Microspheres have been explored as nasal dosage forms, with the aim to decrease the nasal clearance, and thereby, increase nasal drug absorption. They observed that albumin, starch and dextran microspheres, with diameter of about 45µm, have clearance half-values of 3h, compared with 15 min for solutions and powder formulations, These microspheres were absorbed water and formed a gel-like consistancy that was cleared slowly from the nasal cavity.

In the market, there are microspheres systems for nasal drug delivery. The system based on the degradable starch microsphere is known as Spherex®. Another microshere system, Sephadex®, based on dextran cross linked with epichlorohydrine has also been used for nasal drug delivery. For both starch and dextran systems, the drugs are loaded to the microspheres by a lyophilization process.

4.3 Nanoparticles

Nanoparticles are solid, colloidal particles be made up of macromolecular substances varying in size from 10 to 1000 nm. The drug can be, entrapped, adsorbed, encapsulated into a nanoparticle. Depending on the method of preparation, nanocapsule (Fig. 2-a) **or** nanosphere (Fig. 2-b) can be acquire with different properties and release characteristics for the encapsulated therapeutic agent. Nanocapsules those are vesicular systems in which the drug is limited to a cavity surrounded by a unique polymer membrane, whereas nanospheres are matrix type systems in which the drug is physically and uniformally dispersed. The design of transmucosal drug carriers has led to the conclusion that nanoparticles can, indeed, cross epithelium, and besides, the surface distribution of the nanoparticles influence the intensity of this transport.[59]

The polymers used for the formation of nanoparticles consist of synthetic polymers such as polylactide-polyglycolide copolymers, polyacrylates and polycaprolactones or natural polymers such as albumin, gelatin, alginate, collagen and chitosan.[60]

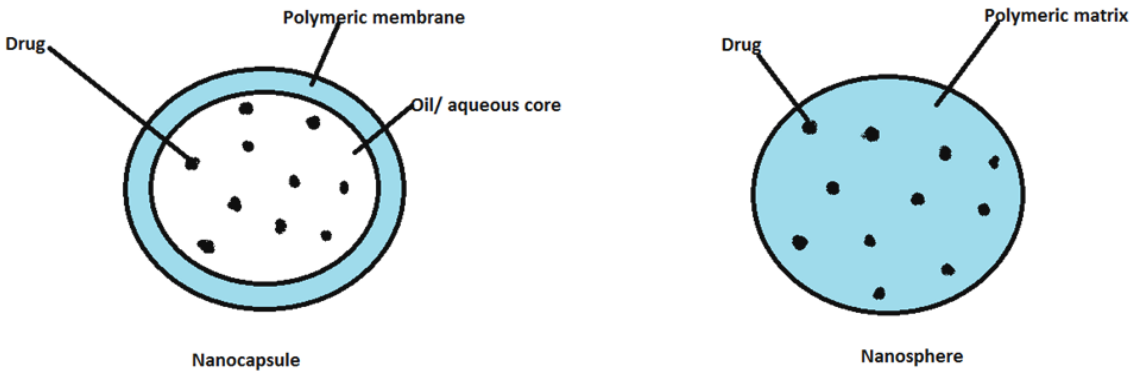

Fig. (2). Nanoparticle structure: a) Nanocapsule b) Nanosphere.

The potential of polyethylene glycol (PEG)-corona nanoparticles for nasal protein administration has been explored due the positive effect of the PEG in preserving the stability of nanosystem in contact with mucosal components.[59] While nanoparticles without PEG-corona led to a significant absorption of the protein and showed a circulation in the blood stream for prolonged periods of time. Chitosan nanoparticles also have been explored as carrier for delivering vaccines intranasally. Efficiently, these nanoparticles are able to deliver the antigen to the immune system, eliciting high and long-lasting humoral as well as mucosal immune responses. The nanoparticles cross the nasal mucosa and reach the antigen presenting cells. In this environment, the particles might deliver the associated antigen for extended periods of time.[59]

The potential of chitosan nanoparticles as a nasal delivery vehicle for peptides has been investigated. A possible mechanism for this behavior is that the nanoparticles that adhere to the nasal mucosa and transiently open the tight junctions, thereby facilitating the transport of he associated insulin at the mucosal surface.[63] The nanoparticles were able to increase significantly the hypoglycemic response to nasally absorbed insulin.[64]

CONCLUSION

The nasal passage is an interesting route for drug administration and has potential for both systemic and local treatment. Although simple solutions represent the majority of all nasal dosage forms, significant effort directed towards new drug delivery systems for nasal administration. The potential of colloidal carriers for transmucosal drug delivery system has fascinate great interest. These systems can maintain their drug activity within site of action and are suitable for poorly water-soluble drugs. Solid, biodegradable micro/nanoparticles have reveal their advantage over liposomes by increasing their stability and ability to control the drug release.

The main approaches, nowadays, are in the use of nasal cavity for vaccination, especially against respiratory infection.

ACKNOWLEDGMENTS

All the author's are equally contributed for preparation of this review article.

REFERENCES

1. Hicke A. J., Pharmaceutical Inhalation Aerosol Technology, 2nd ed Marcel Dekker, Inc: NewYork, 2004.
2. Hinchcliffe, M.; Illum, L. *Adv.* Drug Del. Rev., 1999; *35*(2-3): 199-234.
3. Singh kumar Arun.Nasal cavity: A promising transmucosal platform for drug delivery and research approach from nasalto brain targeting. Journal of Drug Delivery and Therapeutics, 2012; 23: 22-33.
4. Jones, N. Adv. Drug Del. Rev., 2001; *51*(1): 5-19.
5. Illum, L. J. Control. Release, 2003; *87*(1-3): 187-198.
6. Marttin, E.; Schipper, N.G.M.; Verhoef, J.C.; Merkus, F.W.H.M. Adv. Drug Del. Rev., 1998; *29*(1-2): 13-38.
7. Zhou, M.; Donovan, M.D. Int. J. Pharm., 1996; 135(1-2): 115-125.
8. Arora, P.; Sharma, S.; Garg, S. Drug Discovery Today, 2002; 7(18): 967-975.
9. Behl, C.R.; Pimplaskar, H.K.; Sileno, A.P.; Meireles, J.; Romeo, V.D. Adv. Drug Del. Rev., 1998; 29(1-2): 89-116.
10. Gibson, R.E.; Olanoff, L.S. J. Control. Release, 1987; 6(1): 361-366.
11. Illum, L. Drug Discovery Today, 2002; 7(23): 1184-1189.
12. Hirai, S.; Yahiki, T.; Mima, H. Int. J. Pharm., 1981; 9(2): 173-184.
13. Illum, L.; Farraj, N.F.; Critchley, H.; Johansen, B.R.; Davis, S.S.Int. J. Pharm., 1989; 57(1): 49-54.
14. Verhoef, J.C.; Schipper, N.G.M.; Romeijn, S.G.; Merkus, F.W.H.M. J. Control. Release, 1994; 29(3): 351-360.
15. Aggarwal, R.; Cardoso, A.; Homer, J.J. Clin.Otolaryngol., 2004; 29: 201 –205.
16. Salib, R.J.; Howarth, P.H. Drug Saf., 2003; 26(12): 863-893.
17. Szefler, S.J. J. Allergy Clin. Immunol., 2002; 109(6); 1037-1038.
18. Brannon, M.D.; Herron, J.M.; Reidenberg, P.; Affrime, M.B. Clin. Ther., 1995; 17: 637-647.
19. Dalton, M; Bromham, D.; Osbome, J.; Dalton, K. Br. J. Obstet. Gynecol., 1987; 94: 84-88.
20. Rigg, L.A.; Milanes, B.; Villanueva, B.; Yen, S.S.C. J. Clin. Endocrinol. Metab., 1977; 45: 1261-1264.
21. Brittebo, E.B.; Raffer, J.J. J. Steroid Biochem., 1984; 20: 1147-1151.
22. Hussain, A.A.; Hirai, S.; Bawarshi, R. J. Pharm. Sci., 1979; 68: 1196-1199.
23. Hussain, A.A.; Foster, T.; Hirai, S.; Kashihara, T.; Batenhorst, R.; Jone, M. J. Pharm. Sci., 1980; 69: 1240-1243.
24. Hussain, A.A.; Kimura, R.; Huang, C.H.; Kashihara, T. Int. J. Pharm., 1984; 21: 233-237.
25. Eriksen, J.; Jensen, N.; Jensesn, M.; Biamo, H.; Frills, P.; Brewster, D. J. Pharm. Pharmacol., 1989; 41: 803-805.
26. Huang, C.; Kimura, R.; Nassar, R.; Hussain, A.A. J. Pharm. Sci.,1985; 74: 608-611.
27. Kaneo, Y. Acta Pharm. Suec., 1983; 20: 379-388.
28. Hill, A.B.; Bowley, C.J.; Nahwold, M.L.; Knigh, P.R.; Kirsh, M.M.; Denlinger, J.K. Anesthesiol., 1981; 54: 346-348.
29. Rubinstein, A. Antimicrob. Agents Chemother., 1983; 23: 778-779.
30. Dyke, C.V.; Barash, P.G.; Jatlow, P.I.; Byck, R. Science, 1977; 195: 859.
31. Rapoport, A.M.; Bigal, M.E.; tepper, S.J. Sheftell, F.D. CNS Drugs, 2004; 18(10): 671-685.

32. Shaikh, I.M.; Jadhav, K.R.; Ganga, S.; Kadam, V.J.; Pisal, S.S.Curr. Pharm. Biotechnol., 2005; 6(5): 387-395.
33. Reginster, J.Y. Osteoporos Int., 1993; 3(2): 3-7.
34. Huang, C.H.; Kimura, R.; Bawarshi-Nassar, R.; Hussain, A. J. Pharm. Sci., 1985; 74: 608–611.
35. Faraj, J.A.; Hussain, A. Aramaki, Y.; Iseki, K. J. Pharm. Sci.,1990; 79: 698–702.
36. Schmidt, M. C.; Peter, H.; Lang , S. R.; Ditzinger, G.; Merkle, H. P. Adv. Drug Del. Rev., 1998; 29: 51-79.
37. Werner, U.; Kissel, T. Pharm. Res., 1995; 12: 565–571.
38. Werner, U.; Kissel, T. Pharm. Res., 1996; 13: 978–988.
39. Buchanan,J.A.; Yeger, H.; Tabcharani,J.A.; Jensen, T.J.; Auerbach, W.; Hanrahan, J.W.; Riodan, J.R.; Buchwald,M. J. Cell. Sci., 1990; 95: 109–123.
40. Cremaschi, D.; Porta, C.; Ghirardelli, R.; Manzoni, C.; Caremi, I. Biochim. Biophys. Acta, 1996; 1280: 27–33.
41. Hosoya, K.I.; Kubo, H.; Natsume,H.; Sugibayashi,;K.; Morimoto, Y.; Yamashita, S. Biopharm. Drug Dispos., 1993; 14: 685–695.
42. Kubo, H.; Hosoya, K.I.; Natsume, H.; Sugibayashi, K.; Morimoto,Y. Int. J. Pharm., 1994; 103: 27–36.
43. Reardon, P.M.; Gochoco, C.H.; Audus, K.L.; Wilson, G.; P Smith, L. Pharm. Res., 1993; 10: 553–561.
44. Hersey, S.J.; Jackson, R.T. J. Pharm. Sci. 1987; 76: 876–879.
45. De Fraissinette, A.; Kolopp, M.; Schiller, I.; Fricker, G.; Gammert, A.; Vonderscher, J.; Richter, F. Cell Biol. Toxicol., 1995; 11: 295–301.
46. Soane, R.J.; Frier, M.; Perkins, A.C.; Jones, N.S.; Davis, S.S.; Illum, L. Int. J. Pharm., 1999; 178(1): 55–65.
47. Mygind, N.; Dahl, R. Adv. Drug Del. Rev., 1998; 29(1-2): 3-12.
48. Mortazavi, S.A.; Smart, J.D. J. Pharm. Pharmacol., 1994: 46(2): 86–90.
49. Passali, D.; Bellussi, L. Acta Otolaryngol., 1988; 106(3-4): 281–285.
50. Dowley, A.C.; Homer, J.J. Clin. Otolaryngol., 2001; 26: 488-490.
51. Jones, M.S.; Quraishi, S.; Mason, J.D. Int. J. Clin. Pract., 1997; 51(5): 308-311.
52. Revington, M.; Lacroix, J.S.; Potter, E.K. J. Physiol., 1997; 505(3): 823–831.
53. Machida, M.; Sano, K.; Arakawa, M.; Hayashi, M.; Awazu, S. Biol. Pharm. Bull., 1994; 17(10): 1375–1378.
54. Illum, L. J. Control Release, 2003; 87(1-3): 187-198.
55. Graff, C.L.; Pollack, G.M. J. Pharm. Sci., 2005; 94(6): 1187-1195.
56. Bagger, M.A.; Bechgaard, E. Eur. J. Pharm. Sci., 2004; 21(2-3): 235-242.
57. Mathison, S.; Nagilla, R.; Kompella, U.B. J. Drug Target, 1998; 5: 415–441.
58. Krishnamoorthy, R.; Mitra, A.K. Adv. Drug Del. Rev., 1998; 29(12): 135-146.
59. Alonso, M.J. Biomed. Pharmacot., 2004; 58(3): 168-172.
60. Panyam, J.; Labhasetwar, V. Adv. Drug Del. Rev., 2003; 55(3): 329-347.
61. Hans, M.L.; Lowman, A.M. Cur. Opnion. Sci., 2002; 6: 319-327.
62. Vila A, Sánchez A, Tobío M, Calvo P, Alonso MJ. J. Control. Release, 2002; 78(1-3): 15–24.
63. Janes, K.A.; Calvo, P.; Alonso, M.J. Adv. Drug Del. Rev., 2001; 47(1): 83-97.
64. Fernandez-Urrusuno, R.; Calvo, C.; Remuñan-López, C.; Vila-Jato J.L.; Alonso, M.J. Pharm. Res., 1999; 16: 1576–81.