BIOADHESIVE MICROSPHERES LOADED TIZANIDINE HYDROCHLORIDE - A NOVEL APPROCHE OF NASAL DRUG DELIVERY

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Abstract
In recent years growing attention has been paid to the nasal drug administration as an alternative route of administration for systemically active drugs such as proteins, peptides, hormones, and other drugs which are poorly absorbed orally and extensively metabolized in liver. Nasal route is easily accessible, convenient and reliable route with a porous endothelial membrane and a highly vascularized epithelium that provides a rapid absorption of compounds into the systemic circulation, avoiding the hepatic first pass elimination. In addition, intranasal drug delivery enables dose reduction, rapid attainment of therapeutic blood levels, quicker onset of pharmacological activity, and fewer side effects.

Introduction
The gastrointestinal tract is the major route of administration to the systemic delivery. However for some drugs this route creates problems. The gastrointestinal tract presents hostile environment, it contain enzymes, a wide range of pH conditions and varies in its composition depending upon the presence or absence of food. Those drugs which are susceptible to either acid hydrolysis or extensive metabolism in liver may exhibit poor bioavailability when given through oral route.

Parenteral route is the best route of administration to avoid this problem. However, it suffers from some problems as is associated with pain and discomfort and only can be given by medical personnel. Injectables need to be sterilized also and increases the cost. In addition certain health risks are associated with this route e.g. psychological distress, occasional allergies and hypertrophy or atrophy.

In an attempt to over these problems, alternative routes of drug administration had been investigated. Transdermal, rectal, buccal and nasal routes are other alternative routes for systemic delivery of drugs avoiding above problems to a great extent. However, transdermal route does not provide rapid blood level and is limited to controlled delivery of potent lipophilic drugs. The rectal route suffer from variable patient acceptance and depending upon the site of absorption, the drugs may be subjected to first pass effect. Buccal and sublingual routes of drug administration are of much interest, but sometime pose inconveniences during speaking, eating and drinking. Hence, the nasal administration of drug is becoming increasingly importan for systemic delivery of active drugs.

ADVANTAGES OF NASAL DRUG DELIVERY SYSTEM
- Drug degradation that is observed in the gastrointestinal tract is absent.
- Hepatic first pass metabolism is absent.
- Rapid drug absorption and quick onset of action can be achieved.
- The bioavailability of large drug molecules can be improved by means of absorption enhancer or other approach.
- The nasal bioavailability for smaller drug molecules is good.
- Drugs that are orally not absorbed can be delivered to the systemic circulation by the nasal drug delivery.
- The existence of a rich vasculature and a highly permeable structure in the nasal mucosa for systemic absorption.
- The ease and convenience of intranasal drug administration.
- Venous blood from nose passes directly into systemic circulation.
- Nasal route eliminates the intersubject variation normally associated with oral route.
Intra nasal delivery is needle free, patient friendly administration route because needles are not involved, this method of drug delivery is virtually painless. For patients who fear injections, intranasal administration offers a more acceptable alternative. Additionally, the simplicity of nasal delivery would allow for self-administration in a sitting. In general, for patients, the intranasal dosage form provides comfortable, non-threatening, less invasive therapy. This may be important in young patient populations.

Another major benefit of intranasal administration, in contrast to injectables, is that not contribute to biohazardous waste. When the drug has been delivered through intranasal administration devices may be disposed off in the normal garbage. This delivery method does not require needles, risk of accidental sticks is not a concern.

From a pharmacokinetic standpoint, absorption is rapid, which should provide fast onset of action compared to oral and intramuscular administration. Hepatic first pass metabolism is also avoided, allowing increased, reliable bioavailability. In this regard good drug candidates for nasal delivery are those that undergo extensive first pass metabolism, display erratic absorption, or require therapeutic onset.

Patent life of a particular product may be extended via development of an alternative dosage form, providing companies the opportunity to maintain their market share. So from a drug development perspective, intranasal delivery should stimulate favourable outcomes.

**LIMITATIONS OF NASAL ADMINISTRATION**

The nasal drug administration also has certain limitations.

- Rapid mucociliary clearance
- Enzymatic degradation
- Lower absorption

**Nasal metabolism**

The nasal route of administration avoids hepatic first pass metabolism, but nasal mucosa does possess enzymatic activity as a protective mechanism against exogenous chemicals. Nasal first pass metabolism may be a significant factor in the absorption of some drugs. For example, there is high content of cytochrome P450 enzymes, P450 mono-oxygenases can oxidizes many nasally administered drugs, such as nasal decongestants and anesthetic.

There are many other types of enzymes in the nasal mucosa, which can act on conventional drugs. Example includes dehydrogenases, hydroxylases, carboxylesterases, carbonic anhydrases, and various phases II conjugative enzymes. The development of new nasal dosage forms should therefore include some considerations of the nature, extent and location of the drug's metabolism in the nose. Not all metabolism is undesirable, however, and certain enzymes, such as esterases, open the possibility of using prodrugs as a mean of improving nasal delivery.

**Mucociliary clearance**

Mucociliary clearance is a non-specific defensive function which also presents a barrier to the drug absorption. The mucus layer is normally 5-20µm thick, consisting of mainly water containing glycoproteins, ions and various other proteins such as enzymes and immunoglobulins. Glycoprotein gives the mucus its viscous character, which causes foreign particles to become trapped, cleared to the GI tract, and ultimately eliminated from the body. The mucus is actually divided into two layers, the one closest to the cell surface being a less viscous, the watery substance. This aids clearance by the lubricants the passage of the mucus over the cell surface and easing the action of cilia. The cilia work in ratchet like way by engaging the viscous

**Enzyme inhibition**

Substances like bile salts (e.g. sodium glycocholate) and surfactants (e.g. polyoxy-9-lauryl ether) in combination with drug modify the properties of nasal mucosa, inhibiting the enzyme activity in the nasal membrane and reduce the viscosity of mucous thereby allow for an easier diffusion of the drug through this layer, thereby enhancing absorption of drugs.

**Enhancing nasal absorption:**

The mechanism of action of absorption enhancer is increasing the rate at which drug passes through the nasal mucosa. Many enhancers act by altering the structure of epithelial cells in some way, but they should accomplish this while causing no damage or permanent change to nasal mucosa.

General requirement of an ideal penetration enhancer are as follows.
1. It should lead to an effective increase in the absorption of the drug.
2. It should not cause permanent damage or alteration to the tissue.
3. It should be non irritant and nontoxic.
4. It should be effective in small quantity.
5. The enhancing effect should occur when absorption is required.
6. The effect should be temporary and reversible.
7. It should be compatible with other excipients.

**BIOADHESIVE CONTROLLED DRUG DELIVERY**

Bio-adhesion is defined as a state in which two bodies, one or both of which are of biological nature, are held together for extended period of time by interfacial force, or bio-adhesion is defined as the ability of the material (synthetic or biological) to adhere to a biological tissue for a prolonged period of time. For drug delivery purposes, the polymer/drug carrier is usually a non biological macromolecular or hydrocolloid material that adheres primarily to mucus layer or alternatively may attach to the underlying epithelium. The delivery system which utilizes property of bioadhesion of certain water soluble polymers, which become adhesive on hydration, and hence can be used for targeting a drug to a particular region of the body for extended period of time.

Technology has improved to such a level that dosage forms could deliver drugs for days to years. Despite these advances, long-term drug delivery via many routes, especially the oral route, has been limited. Regardless of the time period for drug release from the device, the extent of drug absorption is determined in many instances by the residence time of the device at the absorption site. Thus, the advantages of controlled-release drug delivery have not been fully appreciated.

Extensive efforts have recently been focused on targeting a drug or drug delivery system in a particular region of the body for extended period of time, not only for local targeting of drugs but also for the better control of systemic drug delivery.

**ADVANTAGES OF BIOADHESIVE SYSTEMS**

Bioadhesive systems have three distinct advantages when compared to conventional dosage forms:

- The bioadhesive systems are readily localized in the region applied to improve and enhance the bioavailability of drugs.
- These dosage forms facilitate intimate contact of the formulation with underlying absorption surface.
- The bioadhesive dosage forms also prolong residence time of the dosage form at the site of application and absorption to permit once or twice a day dosing.
- A polymeric device also allows for slow, controlled and predictable drug release overtime and reduces the initial drug loading concentration needed. This reduction also decreases the toxicity and waste of expensive drugs as well as improves patient compliance.

**FACTORS AFFECTING BIOADHESION**

The bioadhesive power of a polymer is affected by the nature of the polymer and also by the nature of the surrounding media. The factors influencing the bioadhesion are summarized below:

1. **Polymer related factors**
   - Molecular weight
   - Concentration of active polymer
   - Flexibility of polymer chains
   - Spatial confirmation
   - Swelling

2. **Environment related factors**
   - pH of polymer-substrate interface
   - Applied strength
   - Initial contact time

3. **Physiological factors**
   - Mucin turnover
   - Disease state
MECHANISM OF BIOADHESION

For bioadhesion to occur, a succession of phenomena, whose role depends on the nature of the bioadhesive, is required. The first stage involves an intimate contact between a bioadhesive and a membrane, either from a good wetting of the bioadhesive surface, or from the swelling of the bioadhesive. In the second stage, after contact is established, penetration of the bioadhesive into the crevices of the tissue surface or interpenetration of the chains of the bioadhesive with those of the mucus takes place. Low chemical bonds can then settle.

To explain the fundamental mechanisms of adhesion. In a particular system one or more theories contribute to the formation of bioadhesive bonds.

Proposed theories of bioadhesion include wetting, diffusion, electronic, adsorption and fracture.

Proposed Method

Bioadhesive microspheres of chitosan are prepared by simple “Emulsification Phase Separation Technique.”

- Polymer - Chitosan
- Drug – Tizanidine HCL
- Cross linking agent- Glutaraldehyde
- Type of external phase- Heavy liquid paraffin and light liquid paraffin oil
- Surfactant- DOSS(dioctyl sulfosuccinate)

MATERIAL AND METHOD:

Microspheres were prepared by Emulsification phase separation technique. Sixteen batches were prepared with following process variables:

- Polymer concentration- Chitosan
- Drug concentration- Tizanidine HCL
- Amount of cross linking agent- Glutaraldehyde
- Type of external phase- Heavy liquid paraffin and light liquid paraffin oil
- Stirring speed- 1400 to 2000 rpm
- Concentration of surfactant- DOSS(dioctyl sulfosuccinate)

FORMULATION OF BIOADHESIVE MICROSPHERES

Mucoadhesive microspheres of chitosan were prepared by simple emulsification phase separation technique. 100mL of paraffin oil mixture of 50mL heavy liquid paraffin and 50mL light liquid paraffin oil was placed in 500mL plastic beaker. Chitosan 200mg was dissolved in 2% acetic acid solution. The drug 100mg was added in it and the suspension was extruded through syringe in 100ml of liquid paraffin containing 0.2% DOSS(dioctyl sulfosuccinate). This addition was accompanied with stirring of paraffin oil with the help of high-speed stirrer (Remi stirrer) After 20 minutes of stirring, 1ml of glutaraldehyde (25%, solution as cross linking agent) was added and stirring was continued for 3 hours after the complete addition of chitosan solution into oil.

Suspension of chitosan microspheres in paraffin oil thus obtained were allowed to stand to let the microspheres settle down under gravity. Clear supernatant liquid was decanted and microspheres obtained as residue were washed 3-4 times with the solvent cyclohexane to remove oil and finally washed with water to remove excess of glutaraldehyde. After the final wash, microspheres were allowed to dry in air. Dry powder thus obtained was collected and stored in desiccator at room temperature.
### TABLE: Formulation optimization

<table>
<thead>
<tr>
<th>BATCH CODE</th>
<th>DRUG: POLYMER</th>
<th>STIRRING SPEED (rpm)</th>
<th>QTY OF GLUT (ml)</th>
<th>EXTERNAL PHASE</th>
<th>DOSS</th>
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<td>A1</td>
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<td>-</td>
</tr>
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<td>2000</td>
<td>1</td>
<td>-</td>
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</tr>
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### TABLE: Drug incorporation efficiency

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<td>A3EP3</td>
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<tr>
<td>A3DOSS1</td>
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<tr>
<td>A3DOSS2</td>
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TABLE: Degree of swelling of prepared microspheres

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<th>BATCH CODE</th>
<th>DEGREE OF SWELLING</th>
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<td>A1</td>
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<tr>
<td>A2</td>
<td>1.95</td>
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<tr>
<td>A3</td>
<td>1.08</td>
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<tr>
<td>A4</td>
<td>1.03</td>
</tr>
<tr>
<td>P1</td>
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<tr>
<td>P2</td>
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<tr>
<td>P3</td>
<td>1.45</td>
</tr>
<tr>
<td>A3R1</td>
<td>1.1</td>
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<td>A3R2</td>
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<tr>
<td>A3G1</td>
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<tr>
<td>A3G2</td>
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<td>A3EP1</td>
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<tr>
<td>A3DOSS2</td>
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RESULTS AND DISCUSSION
In the present study Tizanidine HCl microspheres were prepared by emulsification followed by cross linking technique and were studied for their shape, size, drug incorporation efficiencies, drug release rates and surface characteristics. Chitosan was selected as a polymer for the preparation of mucoadhesive microspheres due to its biodegradable and mucoadhesive properties. The effect of formulation variables such as polymer concentration, drug concentration, amount of cross linking agent, stirring rate, type of external phase was studied.

Surface morphology and particle size
Shape of the microspheres were studied with the help of optical micrographs. The microspheres were spherical with smooth surface, glossy in nature and were not aggregated.

- The mean particle diameter of the prepared microspheres varied from 55.3 to 89.5 µm.
- As the concentration of chitosan was increased, the size of microspheres also increased proportionally. The increase in mean particle diameter may be due to increase in viscosity with increasing polymer concentration. This viscosity affect the droplet formation during the emulsification process.
- Average particle size increases with increasing drug concentration and particles were more widely distributed.
- Particle size was further increased with increasing amount of cross linking agent. This results in formation of more cross linked structure which further increases the viscosity of the formulation medium thereby leading to formation of larger microspheres.
- The microspheres were prepared under the stirring condition of 2000, 1700, 1400 rpm, on decreasing the rpm particle size increased. It may be due to the increased mechanical shear force, which resulted in the decrease in droplet size during the emulsification process.

Swelling ability
The swelling ability of microspheres in phosphate buffer pH6.6 were determined.

- The swelling of microspheres increases the particle size and dissolution of microspheres.
- Chitosan microspheres swell quickly within 30mins.
Maximum swelling was observed with the microspheres with less cross linking agent and swellability decreases with increase in cross linking density and amount of polymer. This could be due to increasing cross linking of hydroxyl group of the polymer with the cross linking agent.

**Drug Incorporation efficiency**

- The Drug Incorporation efficiency were found to be good with all batches and was minimum in batch A3EP3 and maximum in batch A3.
- The results show that an increase in concentration of polymer caused slight increase in the Drug Incorporation efficiency. This is evident by the comparison of batch P1, P2, P3. This slight increase in Drug Incorporation efficiency may be due to formation of larger microspheres with increasing polymer concentration, entrapping greater amount of drug.
- The Drug Incorporation efficiency were increased by increasing drug: polymer ratio from 1:4 to 1:3 and 1:3 to 1:2, but DIE were increased more by increasing drug: polymer ratio from 1:4 to 1:2.

**Release studies**

- Batch no. A3,(drug :polymer ratio 1:3,2000 rpm,Glu 1ml,DOSS 0.1%) show the highest release rate and Batch no.A3D0SS2( drug :polymer ratio 1:3,2000 rpm,Glu 1ml,DOSS 0.3%) show the lowest release rate.
- It was observed that drug polymer ratio has marked influence on drug release profile. An increase in drug polymer ratio from 1:4 to 1:3 and 1:3 to 1:2, increased the drug release rate, therefore t50% (time taken for 50% drug release) decreased significantly.
- The release rate of drug is also affected by stirring rate during preparation of microspheres. The microspheres prepared at 2000 rpm have faster release rates than microspheres prepared at 1700 and 1400 rpm. The comparison of the batch sets A3,A3R1 and A3R2 for 2000,1700 and 1400 rpm show the difference in t50% value. This may be due to increase in mechanical shear force which resulted in increased surface area. The release rate of drug from microspheres is directly proportional to surface area.

**CONCLUSION**

The use of microspheres is of interest for bioadhesion purpose because these pharmaceutical dosage form have a large specific surface, which is indicative of a high interactive potential with biological surfaces. Chitosan appears to be a suitable polymer for the preparation of mucoadhesive microspheres capable of adhering to the mucus layer. It has been used as a microspheres material owing to its versatile biodegradability, biocompatibility and natural origin.

Chitosan being a biodegradable and biocompatible polymer, one would expect that it will not cause any deleterious effects or toxic response in the nasal mucosal cavity even if used for prolonged periods.

Formulating bioadhesive microspheres of TIZANIDINE HYDROCHLORIDE increases the contact time with nasal mucosa, its plasma half life which in turn increases the bioavailability. It has advantage of delivering the effective systemic concentration of the drug by enhancing its uptake for prolonged periods, so there will be no need of frequent administration. Drug can be administered at lower dose with fewer side effects.

The results of present study clearly indicated promising potential of chitosan microspheres for delivering TIZANIDINE HYDROCHLORIDE intranasally. This could be viewed as a potential alternative to conventional dosage forms of TIZANIDINE HYDROCHLORIDE.

**REFERENCES**