Spray Dried Mucoadhesive Microspheres of Ondansetron for Nasal Administration

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ABSTRACT: Systemic delivery of drugs via nasal route has many advantages for protein and peptide drug molecules as well as conventional molecules. Mucoadhesive drug delivery systems are those that provide intimate contact of the drug with the mucosa for an extended period of time. In this current work chitosan mucoadhesive microspheres of ondansetron HCL were prepared by spray-drying method, with an aim to avoid first pass effect and to improve therapeutic efficacy of Ondansetron in treatment of nauseas and vomiting. Preformulation studies were carried out in order to find out the drug excipient interaction in order to find out the drug excipients. I.R. spectrum and TLC method was used to find out the interaction between drug and excipient. Formulations were evaluated for physical characteristics such as particle size, incorporation efficiency, swelling ability in vitro bioadhesion and in vitro drug release in pH 6.6 phosphate buffer. Average particle size of microspheres was found to be in size range 7-9 µm. All microspheres had good percentage of entrapment efficiency between 88% and 97% chitosan microspheres showed very good mucoadhesion due to electrostatic attraction between chitosan and mucin. The Fourier transformed infrared (FTIR) spectra obtained from various formulations of spray-dried microspheres showed no interaction within these formulations. The release of ondansetron hydrochloride from these microspheres was fast. These in vitro results show that spray-dried microspheres based on chitosan could be suitable nasal mucoadhesive delivery system for administration of ondansetron hydrochloride.

KEYWORDS: Ondansetron hydrochloride, Spray drying, Mucoadhesion, Microspheres.

Introduction

Although nasal administration of drug has many advantages, it is usually limited by the specific nasal morphological and physiological characteristics. One of the most important disadvantages is nasal mucociliary clearance that limits the time allowed for drug absorption to occur. Thus, mucoadhesive microspheres have been developed in order to decrease the effect of mucociliary clearance (L. Illum et al., 1987). Microspheres also exert a direct effect on the mucosa resulting in the opening of tight junctions between the epithelial cells (L. Pereswetoff, 1998). The most commonly investigated techniques to prepare mucoadhesive microspheres have been emulsion solvent evaporation techniques. (Lim S.T et al., 2000 & Morimoto. K, et al., 2003) Spray drying is an alternative process that is cheaper and faster than above techniques to prepare nasal particles from mucoadhesive polymers. (Gavini.E et al., 2007 & Gavini.E et al., 2005.)

Chitosan is a cationic biodegradable and biocompatible polysaccharide, which confers bioadhesive properties to microspheres due to its hydrophilicity. It is a polymer of choice, because it enhances the nasal absorption of low molecular weight molecules as well as peptides and proteins. (Ping H et al., 1999)

Ondansetron is a serotonin (5-Hydroxytryptamine) subtype 3 (5-HT) receptor antagonists used in management of nausea and vomiting. (Bhise S.B et al., 2007) Oral administration of ondansetron HCL is well with bioavailability of 60% with a half-life of 3.4 hrs. (David. R. et al., 1991). The aim of this work was to develop spray dried bioadhesive chitosan based microspheres for nasal delivery of ondansetron HCL and to evaluate mucoadhesive properties. The microspheres were characterized in terms of encapsulation efficiency morphology, particle size; the in vitro release behavior of the drug from the microparticles was studied.

Materials and Methods

Materials

Ondansetron hydrochloride and chitosan (degree of acetylation 79%) were gift samples obtained from
Niramaye Pharma Ltd., Nasik and Central Institute of Fisheries Technology, Cochin, India respectively. Acetic acid, Acetone, Dihydrogen potassium orthophosphate and Sodium hydroxide were purchased from S.D. Fine chemicals, Mumbai. All other chemicals were of analytical grade.

Preformulation Studies (Wells J.L, 1998)
Preformulation studies were carried out in order to find out the drug excipient compatibility. The samples of drug and excipient were intimately mixed, in equal parts and screened by TLC after storage under accelerated conditions of temperature and humidity.

Preparation of Mucoadhesive Microspheres
(Martincic A et.al, 2004 & Gavini E et.al. 2004)
Ondansetron HCL microspheres based on chitosan were prepared by spray drying of simple dispersion and oil in water (O/W) emulsion, using a LU-222 spray drier co-current (Labulima India) with a standard 0.7 mm nozzle. The liquid was fed to the nozzle with peristaltic pump, atomized by the force of the compressed air and blown together with a hot air to the chamber where the solvent in the droplets was evaporated. The dry product was then collected in collective chambers. The drying conditions were inlet air temperature 160-165 °C. Out let temp. 90-95 °C; spray pressure 2 Kg/cm² and spray rate of feed about 4-5 ml/min.

For the simple dispersion system chitosan was solubilized in 0.5% acetic acid solution at 1 % (W/V) concentration. Ondansetron HCL was dissolved at different concentration in above solution and subjected to spray drying under process condition described above.

In that way, different polymer drug ratios (2:1 to 4:1) were obtained for the preparation of ondansetron HCL loaded chitosan microspheres (Table 1)

For the O/W emulsion system, ondansetron HCL was dissolved in acetone and chitosan in 0.5% acetic acid. The drug solution was added drop wise to the polymer solution within 5 min under stirring at 3000 rev / min. During the spraying process the emulsion was kept at a room temperature, under magnetic stirring. No phase separation in the emulsion during the spray drying process was observed, thus rendering unnecessary the use of surfactants. The emulsion was subjected to spray drying under conditions described above. The amount of drug and polymer were different depending on the composition of the microspheres to be prepared. (Table 1)

The volume of feed sprayed for the preparation of each batch was always 200ml. The type and composition of spray dried systems in the preparation of ondansetron HCL- loaded microspheres.

Characterization of Microspheres
Particle Size analysis (Gavini E et.al. 2006)
All the microspheres were evaluated with respect to their particle size and shape by optical microscopy using Motique optical microscope. Average of 100 microspheres was used for study. The average particle size was express as the volume surface diameter (Fig. 1)

Table 1. The type and composition of spray dried systems in the preparation of microspheres.

<table>
<thead>
<tr>
<th>Type of Spray Dried System Formulations</th>
<th>Dispersion</th>
<th>Emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OND° 1</td>
<td>OND° 2</td>
</tr>
<tr>
<td>Concentration of Ondansetron (%w/v)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Concentration of Chitosan (% w/v)</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Chitosan: Ondansetron</td>
<td>2:1</td>
<td>3:1</td>
</tr>
</tbody>
</table>

a- Ondansetron spray dried microspheres based on simple dispersion system.

b- Ondansetron spray dried microspheres based on emulsion system.
Determination of production yield
(Martin. A.et.al.1996)

The yields of production were calculated as the weight percentage of the final product after drying, with respect to the total amount of ondansetron HCL and chitosan used for the preparation.

Drug content and Entrapment efficiency
(Martin. A.et.al.1996)

Ondansetron HCL in microspheres of each formulation was extracted in phosphate buffer pH 6.6. The concentration of ondansetron HCL was determined using a UV spectrophotometer at a wavelength 248nm (UV-spectrophotometer 1700) Shimadzu, preliminary UV scan showed that the presence of the polymers did not interfere with the absorbance of ondansetron hydrochloride at 248 nm. The actual amount of drug loaded relative to the theoretical amount in the microspheres was calculated in a percentage and expressed as entrapment efficiency.

Swelling Property
(Jain S.K et al., 2004 & Garcia A.A et al., 2001)

The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium. Accurately weighed amount of microspheres (10mg) were placed on Millipore filter (NY 11 0.22 μm) using a Franz diffusion cell (12.5ml) with phosphate buffer (pH 6.6) and kept for 3.5 min.

The following formula was used for calculation of degree of swelling.

\[ \alpha = \frac{W_s - W_o}{W_s} \]

Where

\( \alpha \) = degree of swelling, \( W_o \) = initial weight of microspheres and
\( W_s \) = of microspheres after swelling

Adhesion Property

A freshly cut piece, of intestine (5 cm long) of sheep obtained from local abattoir within 1 hour of killing the animal was cleaned by washing with isotonic saline solution. An accurate weight of microspheres was placed on mucosal surface which was attached over a poly ethylene plate. This plate was incubated for 15 min. in desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally fixed at an angle of 45° relative to the horizontal plane. Phosphate buffer pH 6.6 warmed at 37° C was peristaltically pumped at a rate of 5 ml/min over the tissue. The duration for complete washing of microspheres from sheep intestine was recorded and averaged from 5 determinations (Table 2).
Table 2. Characteristics of the Ondansetron HCL Microspheres.

<table>
<thead>
<tr>
<th>Type of Spray Dried System</th>
<th>Formulation</th>
<th>% Production Yield (± S.D)</th>
<th>Average Particle Size µm (± S.D)</th>
<th>% Entrapment Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dispersion</td>
<td>OND1</td>
<td>16.6 ± 0.16</td>
<td>8.21 ± 1.0</td>
<td>88.6</td>
</tr>
<tr>
<td></td>
<td>OND2</td>
<td>17.93 ± 0.24</td>
<td>8.94 ± 1.13</td>
<td>92.03</td>
</tr>
<tr>
<td></td>
<td>OND3</td>
<td>17.93 ± 0.24</td>
<td>8.94 ± 1.13</td>
<td>95.33</td>
</tr>
<tr>
<td></td>
<td>OND4</td>
<td>18.18 ± 0.13</td>
<td>8.53 ± 1.17</td>
<td>96.62</td>
</tr>
<tr>
<td></td>
<td>ONE1</td>
<td>18.20 ± 0.33</td>
<td>8.02 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ONE2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ONE3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ONE4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emulsion</td>
<td>ONE1</td>
<td>18.39 ± 0.21</td>
<td>7.51 ± 1.89</td>
<td>90.36</td>
</tr>
<tr>
<td></td>
<td>ONE2</td>
<td>17.50 ± 0.35</td>
<td>7.60 ± 1.21</td>
<td>94.91</td>
</tr>
<tr>
<td></td>
<td>ONE3</td>
<td>18.56 ± 0.38</td>
<td>7.77 ± 1.67</td>
<td>95.27</td>
</tr>
<tr>
<td></td>
<td>ONE4</td>
<td>18.46 ± 0.28</td>
<td>7.93 ± 2.10</td>
<td>97.59</td>
</tr>
</tbody>
</table>

Fig. 2 (a) I.R. Spectrum Ondansetron HCL, (b) Ondansetron Loaded chitosan microspheres based on dispersion system and (c) emulsion system.
In vitro drug release

The in vitro drug release test of the microspheres was performed on Franz diffusion cell with dialysis membrane (cut off M.W. 12000). The receptor compartment contained phosphate buffer solution pH 6.6 that was within the pH range in nasal cavity and maintained at 37°C ± 0.5°C. The membrane was equilibrated before carefully dispersing the sample equivalent to 15mg of drug onto the donor compartment containing 3ml of SNES (SNES: aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml CaCl2). Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer at 450 nm.

Results

Spray drying is good technique for the preparation of chitosan microparticles. It is one step process, easy and rapid, as it combines drying of the feed and embedding of the drug into a one step operation.

Preformulation Studies

Preformulation studies revealed that chitosan is the suitable mucoadhesive polymer for ondansetron HCL by spray drying technique.

Characterization of microspheres

Eight samples of ondansetron HCL loaded microspheres were prepared by spray-drying method (Table 1). Microspheres differed in the type of spray dried system (Simple dispersion and Emulsion) in concentration of polymer solutions used and in drug polymer ratio (1:2 to 1:5). The main characteristics of spray dried systems and microspheres prepared are summarized in Table 2.

The chitosan microspheres were spherical. The average particle size of microspheres ranged from 7 to 9 µm. The size distribution of the microspheres was narrow. (Table 2). Such particle sizes were considered to be suitable for nasal administration. It was also noted that increasing the drug to polymer ratio slightly increased the size of microspheres.

Production yields were always relatively low (16 to 18 %). As previously observed these values can be justified by the low quantity of feed used for the preparation of each batch and by the structure of the spray drier, which lacked a trap to capture the smallest and lightest particles.

The reproducibility of the spray-drying method is good, as indicated by drug content and encapsulation efficiency. Drug contents are always close to the theoretical values and good encapsulation efficiencies are obtained. Encapsulation efficiencies always high, increases with increasing drug to polymer weight ratios. An almost total encapsulation was obtained between 89 and 98%.

Swelling Property

Swelling capacity of the microspheres was mostly determined by chitosan content in the preparation, since chitosan was the only component in the spray dried system with swelling abilities. The maximum swelling degree of swelling 1.25 was observed with microspheres (OND.5). Microspheres with lower chitosan content formulations OND 2 and ONE 2 were characterized by lower swelling ability than the microspheres with higher chitosan content OND 4 & ONE 4. (Table 3). The type of spray drying system (i.e. simple dispersion and emulsion) has no significant effect on swelling ability of microspheres.

Mucoadhesion Studies

Mucoadhesion studies were carried out to ensure the adhesion of the formulation to the mucosa for a prolonged period of time at the site of absorption. All the microspheres has mucoadhesion time more than 120 min. (Table 3) such excellent mucoadhesion of chitosan microspheres was from the electrostatic attraction between chitosan and mucin. Moreover, the linear molecule of chitosan expressed sufficient chain flexibility for interpenetration and entanglement.

Infrared absorption study

Infrared absorption study

In an effort to investigate the possible chemical interaction of drug with polymer matrixes, we have analyzed (a) pure ondansetron HCL, (b) blank chitosan microspheres and (c) ondansetron HCL loaded microspheres using FTIR. Ondansetron HCL has shown a characteristic band at 756 cm⁻¹ due to 0- disubstituted benzene, the band at 1458 and 1479 cm⁻¹ are due to methyl group. The C-N stretching vibrations are observed at 1259 cm⁻¹. The band at 1531 cm⁻¹ due to aromatic C=C and the band at 1638 was due to C=N, C=O in six member ring. Peaks at 756, 1259, 1458, 1479, 1531, 1638 for ondansetron HCL have also been observed in the drug loaded microspheres; indicating the chemical stability of ondansetron after entrapment.

Swelling Property

In vitro drug release tests of microspheres were carried out in phosphate buffer pH 6.6 with 6 hrs. The result shows that the drug release was moderately sustained up to 5 hrs, Fig. 3 and 4 shows the drug release profiles from various formulations of microspheres. No relevant differences are found between the microspheres prepared by different spray dried systems.
Table 3. Degree of Swelling and Mucoadhesion of Formulations.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Formulations</th>
<th>Degree of Swelling (α)</th>
<th>Mucoadhesion Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OND1</td>
<td>0.68</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>2</td>
<td>OND2</td>
<td>0.74</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>3</td>
<td>OND3</td>
<td>0.85</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>4</td>
<td>OND4</td>
<td>1.25</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>5</td>
<td>ONE1</td>
<td>0.69</td>
<td>&gt; 120</td>
</tr>
<tr>
<td>6</td>
<td>ONE2</td>
<td>0.72</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>7</td>
<td>ONE3</td>
<td>0.84</td>
<td>&gt; 180</td>
</tr>
<tr>
<td>8</td>
<td>ONE4</td>
<td>1.22</td>
<td>&gt; 180</td>
</tr>
</tbody>
</table>

Fig. 3 *in vitro* Drug Release Profile of Microspheres Prepared from Dispersion System.

Fig. 4 *in vitro* Drug Release Profile of Microspheres Prepared from Emulsion System.
Discussion

Microspheres containing ondansetron HCl based on chitosan can be easily produced by spray-drying of simple dispersion and emulsion systems. They show similar properties with respect to particle size, production yield, entrapment efficiency, degree of swelling, and in vitro drug release. The particle size is suitable for nasal administration. It has been suggested that 4 µm is a sufficient particle size for intranasal administration. The swelling studies was an important attribute of studying clearance of the microspheres system can be probably due to the fact that the microspheres undergo a process of taking up water and swelling which results in polymer/mucus interaction and increased viscosity leading to mucociliary clearance. However microspheres based on chitosan are characterized by better mucoadhesiveness due to electrostatic attraction. These properties make microspheres based on chitosan suitable for the nasal administration; in fact the mucoadhesiveness might prolong the residence time of the formulation inside the nasal cavity. Varying the proportion of polymer could alter the drug release and mucoadhesive property of the microspheres. The infrared absorption study indicating the chemical stability of ondansetron HCl after entrapment on the basis of these results, ondansetron microspheres based on chitosan may be considered a promising nasal delivery system.

Conclusion

The results of our present study clearly indicated promising potential of chitosan microspheres for delivery ondansetron intranasally and could be viewed as a potential alternative to conventional dosage forms. However extensive pharmacokinetic and pharmacodynamic studies are required to establish a correlation if any, before establishing intranasal ondansetron delivery as an alternative. Because chitosan is biocompatible polymer, we would expect it not to cause any deleterious effects or toxic response in the nasal mucosal cavity even if used for prolonged periods. To evaluate the biocompatibility of the polymer with nasal mucosa, appropriate histopathological investigations are required.

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References


