Research article

Formulation of sildenafil citrate loaded nasal microspheres: An in vitro, ex vivo characterization

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Abstract
The aim of the present study was to prepare gellan gum microspheres of Sildenafil citrate, for intranasal delivery to avoid the first pass metabolism. The microspheres were prepared using spray drying method. The microspheres were evaluated for characteristics like particle size, incorporation efficiency, swelling ability, zeta potential, in-vitro mucoadhesion, ex-vivo mucoadhesion, thermal analysis, XRD study and in-vitro drug release. Treatment of in-vitro data to different kinetic equations indicated diffusion controlled drug delivery from gellan gum microspheres. The results of DSC and XRD studies revealed the molecular amorphous dispersion of Sildenafil citrate into the gellan gum microspheres. Microspheres so prepared were discrete, bulky, free flowing and showed an average encapsulation efficiency ranging from 95-98%. The formulation exhibited a good mucoadhesive strength which was determined in in vitro conditions through falling film technique and was compared with ex vivo studies. The microspheres so prepared also exhibited a good swelling index which confirmed the strong mucoadhesive property of the formulation.

Keywords: Gellan gum, Microspheres, Spray drying, Sildenafil citrate, Nasal delivery.

Introduction
Nasal drug delivery has generated interest as an alternative route for administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation and first pass metabolism. Possible pathways for a drug to permeate across the nasal mucosa are passive transport carriers mediated, transcytosis and transport through tight junctions. However nasal delivery has limitations which have restricted its use to the delivery of drug molecules is, the general rapid clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism. It has been shown that for both liquid and powder formulations that are not mucoadhesive, the half life of clearance are in the order of 15-20 min. different delivery systems based on mucoadhesive polymers have been developed which are able to increase the residence time of the formulation at the absorption site of the drugs. The use of mucoadhesive system as microspere is to provide a drug protection from enzymatic degradation and thus increase the contact time with the nasal mucosa [1, 2]. The aim of this work was the possible application of gellan gum for the preparation of mucoadhesive micro particle for the nasal administration of drugs.
Sildenafil is indicated for the treatment of male erectile dysfunction. It has been used successfully in males to remediate problems associated with impaired neural and/or hemodynamic response to sexual stimulation. Sildenafil is a cyclic guanosine-specific phosphodiesterase type 5 inhibitor that prevents the metabolism of cyclic guanosine which produces arterial smooth muscle relaxation within the corpora cavernosa of the penis and ultimately enhances penile tumescence. Inherent to its pharmacology, sildenafil produces mild decreases in systolic and diastolic blood pressure and an array of minimal side effects due to the inhibition of other types of phosphodiesterase. Its absolute bioavailability is about 35-40% after oral administration due to first pass metabolism, and its plasma half life is about 3-4 hr. Sildenafil is having a sufficient lipohillicity with its log P value reported as 1.9. All these characteristics make it a suitable candidate for nasal delivery.

Over the past decades, hydrogel polymers have attracted a great deal of attention for use as potential carriers in site specific delivery. Hydrogels are the hydrophilic, three dimensional network structures having the natural propensity to absorb large quantity of water or biological tissues. Gellan gum is an extracellular polysaccharide produced by the bacterium Pseudomonas elodea. The natural form of GG is a linear anionic hetero polysaccharide based on a terasaccharide rapid unit of glucose, glucuronic acid, and rhamnose in molar ratio of 2:1:1. The natural form of GG is partially acetylated with acetyl and L glyceryl groups located on the glucose residues. The presence of acetyl groups interferes in ion-bonding ability. On the other hand commercially available GG is deacetylated product obtained by treatment with alkali. Previous studies demonstrated the efficiency of GG in the food and pharmaceutical industry. Its pharmaceutical uses are mainly concentrated in ophthalmic drug delivery and oral sustained release preparations. Due to the characteristic property of cation induced gelation, it has been widely used in the formulation of insitu gelling ophthalmic preparations as well as an insitu gelling oral controlled release formulation. It has also been suggested that gellan gum is a promising mucoadhesive polymer for use in nasal formulations. The gellan gum formulation had a residence time of at least 4-5 hrs in the nasal cavity without any harmful effects [3, 4].

Materials and methods

Materials

Gellan Gum was obtained as gift sample from Burzin and Leons, CPKelco division of the Monsento company, USA, Sildenafil Citrate was gifted by Pfizer Mumbai, India. All the other reagents used were of analytical grade.

Methods

Preparation of Microspheres

The formulation of microspheres as presented in table 1 consisted of 1:1 to 1:5 drug to polymer ratios. Gellan gum was first dispersed in water and heated upto 80°C. Sildenafil Citrate was then added in the polymer solution. The solution of each batch was spray-dried (LU222, Labultima, India) keeping the process parameters as follows: inlet temperature of 135°C, pump setting of 5ml/min, Spray pressure 2 Kg/cm². The solution was kept at a temperature about 40°C under magnetic stirring, during the spraying process. The total volume of solution used for preparation of each batch was 200ml.

Table 1. Formula for different batches of Sildenafil Citrate loaded gellan gum (GGS) microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Sildenafil :GG</th>
<th>Siuldenafil (% w/w)</th>
<th>GG (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGS1</td>
<td>1:1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GGS2</td>
<td>1:2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GGS3</td>
<td>1:3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>GGS4</td>
<td>1:4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>GGS5</td>
<td>1:5</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Characterization of Microspheres

1. Morphological examination

The morphology of microspheres was examined by scanning electron microscopy. A small amount of powder was spread on an aluminium stub, which was placed after gold sputtering in SEM chamber (JSM 6390®USA). Photographs were taken at an acceleration voltage of 20KV electron beam [5, 6].

2. Production yield, drug content and incorporation efficiency
Table 2. Characteristics of prepared Sildenafil citrate loaded microspheres.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Production Yield (%)</th>
<th>Drug Content (%)</th>
<th>Incorporation Efficiency (%±SD)*</th>
<th>Mean Particle size (μm±SD)*</th>
<th>In vitro Mucoadhesion (%±SD)*</th>
<th>Degree of swelling (α) (±SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGS1</td>
<td>52.45</td>
<td>53.16</td>
<td>96.13 ±1.25</td>
<td>9.8 ±1.39</td>
<td>86.77 ± 0.50</td>
<td>0.85 ±0.34</td>
</tr>
<tr>
<td>GGS2</td>
<td>56.70</td>
<td>59.76</td>
<td>98.95 ±2.77</td>
<td>9.71 ±1.21</td>
<td>88.25 ± 0.76</td>
<td>0.87 ±0.46</td>
</tr>
<tr>
<td>GGS3</td>
<td>65.52</td>
<td>62.25</td>
<td>98.03 ±1.05</td>
<td>9.75 ±1.88</td>
<td>89.19 ±0.36</td>
<td>0.89± 0.41</td>
</tr>
<tr>
<td>GGS4</td>
<td>68.34</td>
<td>62.45</td>
<td>95.67 ±1.59</td>
<td>10.1 ±1.15</td>
<td>89.70 ±0.90</td>
<td>1.10 ±0.50</td>
</tr>
<tr>
<td>GGS5</td>
<td>71.40</td>
<td>62.54</td>
<td>97.28 ±1.57</td>
<td>11.67 ±2.52</td>
<td>90.66 ±0.86</td>
<td>1.23±0.61</td>
</tr>
</tbody>
</table>

*n=3

The production yields of microspheres of various batches were calculated using the weight of finally dried microspheres with respect to the initial total quantity of the drug and polymer used. Percent production yields were calculated as per the formula mentioned below, and reported in Table 2 [7].

Production yield = \[ \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \]

**Actual drug content and incorporation efficiency**

Actual drug content was determined using UV spectrophotometer at a wavelength of 292(UV-spectrophotometer-1700, Shimadzu, Kyoto, Japan). The percent incorporation efficiency was calculated from actual drug content. The weighed weighed quantity of microspheres (M_{actual}) and theoretical amount of drug and polymers in microspheres calculated from the quantity added in the fabrication process (M_{theoretical}) was substituted in the following equation to get the incorporation efficiency [8].

%Incorporation efficiency = \[ \frac{M_{actual}}{M_{theoretical}} \times 100 \quad (1) \]

**3. Particle size Measurement**

A microscopic image analysis technique for determination of particle size was applied. The morphology and particle sizes were determined in a Motic DMW2-223 digital microscope (Motic Instruments Inc, Canada) equipped with a 1/3” CCD camera imaging accessory and computer controlled image analysis software (Motic images 2000, 1.3 version). The microspheres were dispersed on a microscope slide. A microscopical field was scanned by video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined.

**4. Zeta Potential Study**

The microparticles were dispersed in distilled water. This dispersion was filled in zeta cell and placed in the Zeta Sizer (Nano ZS, Malvern Instruments, and UK). The zeta potential was determined with the help of software.

**5. In Vitro mucoadhesive strength determination**

A freshly cut 2cm² piece of sheep nasal mucosa was obtained and cleaned by washing with isotonic saline solution. One hundred milligram of microparticles was placed on mucosal surface which was fixed over poly ethylene support. About 100μl of simulated nasal electrolytes (SNES :aqueous solution containing 8.77 mg/ml NaCl, 2.98mg/ml KCl and 0.59 mg/ml CaCl₂) was placed on microspheres and this plate was incubated for 15min in desiccators at 90% relative humidity to allow the polymer to interact with the membrane. The support was then fixed at an angle of 45° relative to the horizontal plane. The nasal mucosa...
was thoroughly washed with phosphate buffer (pH 6.6) at the rate of 5ml/min using a peristaltic pump. Sixty min after administration of microspheres, the concentration of drug in collected perfusate was spectrophotometrically determined. The microsphere amount corresponding to the drug amount in perfusate was determined. The adhered microspheres amount was estimated from the difference between the applied microparticles amount and the flowed microparticles amount. The ratio of adhered microparticles was computed percent mucoadhesion using following equation [9].

\[
\% \text{ Mucoadhesion} = \frac{\text{Amount of drug in washout liquid}}{\text{Actual amount of drug in applied microparticles}} \times 100
\]

6. In vitro swelling studies [10]
The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium. Accurately weighed amounts of microspheres (10mg) were placed as Millipore filter NY 11, 0.22 μm using a Franz diffusion cell (16ml) filled with phosphate buffer pH 6.6 and kept for 3.5 min. The following formula was then 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, \( W_s \) = weight of microspheres after swelling

7. Thermal Analysis
Differential scanning calorimetry (DSC) was performed on drug loaded and blank microspheres. DSC measurement was done on a mettler Toledo DSC 822c. The thermograms were obtained at a scanning rate of 10ºC/min over a temperature range of 20ml/min.

8. X-Ray Diffraction (XRD) Studies
The crystallinities of Sildenafil citrate and Sildenafil citrate loaded microspheres was determined using an x-ray diffractometer (Brrucker Axs, 08 Advance).


In vitro drug release test of the microspheres was performed using Franz diffusion cell with Dialysis membrane (cut off Mol. Weight.12000). The membrane was equilibrated before carefully dispersing the sample equivalent to 15 mg of drug onto the donor compartment. The donor compartment contained 3ml of SNES and receiver compartment was filled with phosphate buffer solution pH 6.6 that was within the pH range in nasal cavity and maintained at 37ºC±0.5ºC. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer at 292nm.

**Figure 1.** Scanning electron micrograph of Sildenafil Citrate loaded microspheres.

10. Histopathological examination of Nasal Mucosa
The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.6) for 6hrs after collection was compared with tissue incubated in diffusion chamber with formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope to detect damage to the tissue [11].

Results and discussion
The spray drying technique described here appears to be a suitable method for the preparation of gellan gum microspheres loaded with Sildenafil Citrate. It is the one step process, easy and rapid; also it combines drying of the feed and embedding of drug into a one step operation. Aqueous solution of gellan gum is
known to undergo gelation up to warming to body temperature as well as in presence of cations; hence the spray drying solution kept at about 40ºC to avoid temperature induced gelation during the spray drying process. The spray dried microspheres were obtained as off white powder.

Table 3. In vitro release kinetic parameter of Sildenafil citrate loaded gellan gum microspheres.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Coefficient of Determination (R²)</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Highuchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGS1</td>
<td>0.9445</td>
<td>0.9567</td>
<td>0.9883</td>
<td></td>
</tr>
<tr>
<td>GGS2</td>
<td>0.7785</td>
<td>0.9125</td>
<td>0.9567</td>
<td></td>
</tr>
<tr>
<td>GGS3</td>
<td>0.8234</td>
<td>0.9654</td>
<td>0.9854</td>
<td></td>
</tr>
<tr>
<td>GGS4</td>
<td>0.8678</td>
<td>0.9549</td>
<td>0.9834</td>
<td></td>
</tr>
<tr>
<td>GGS5</td>
<td>0.9345</td>
<td>0.9375</td>
<td>0.9971</td>
<td></td>
</tr>
</tbody>
</table>

Characterization of Microspheres
1. Morphological Examination
All gellan gum microspheres were spherical with smooth surfaces (Fig.1). These microspheres had no hole or rupture on the surface, such morphology would result in slow clearance and good deposition pattern in nasal cavity [12, 13].

Figure 2. Zeta potential distribution curve of Sildenafil loaded gellan gum microspheres.

2. Production yield, Drug content and Incorporation efficiency
The production yield of spray dried microspheres was between 50 to 71%. Increasing the concentration of polymer slightly increased the yield. 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 [14]. The determination of drug content shows good uniformity. In addition, they were close to their percentages of theoretical content which were 7.5% to 2.5% for drug to polymer ratio 1:1 to 1:5. All microspheres had good incorporation efficiency between 95% and 98% (Table 2). These results indicate very good reproducibility of the spray drying method.

3. Particle size measurement
Particle size of microspheres is one of the most important characteristic as a nasal drug delivery. The mean particle size of microsperes ranged from 9.5 to 11.5μm (Table 2). It has been suggested that 10μm particle size is most suitable for nasal administration [15].

Figure 3. DSC spectra of Sildenafil, gellan gum blank microspheres (GGB) and Sildenafil loaded microspheres (GGS).

4. Zeta potential study
All the microspheres prepared were negatively charged, indicating the presence of gellan gum at the surface of all microspheres formed (Fig. 2). Studies have shown that polymers with charge density can serve as good mucoadhesive agents. It has also been reported that anion polymers are more effective bioadhesive than polycations or non-ionic polymers.

5. In vitro mucoadhesion
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. Percent mucoadhesion was increased with increase in polymer concentration (Table 2). This could be because of more availability of hydroxyl function groups to interaction with mucin. The functional groups available on the surface of the polymer favoring mucoadhesion include hydroxyl, carboxyl, and amine. Being carbohydrate, gellan gum is rich in these functional group contents hence showed higher percentage of mucoadhesion [16].

6. In-vitro swelling studies
In vitro swelling properties of the spray dried microspheres were expressed as degree of swelling estimated by use of equation (3). Swelling capacity of the microspheres was determined by gellan gum in the preparation. The maximum swelling (degree of swelling) was observed with microspheres containing highest concentration of gellan gum (Table 2).

![Figure 4](image)

**Figure 4.** X-ray Diffractogram of Sildenafil, gellan gum blank microspheres (GGB) and Sildenafil loaded microspheres (GGS).

7. Thermal analysis
In an effort to assess the physical state of the drug, the gellan gum microspheres, we have analyzed Sildenafil Citrate (SC), gellan gum blank microspheres (GGB), and drug loaded microspheres (GGS) using DSC. The results are displayed in Fig.3. The DSC thermogram showed a short endothermic peak at 188°C due to the melting of the drug. In blank microspheres thermal transition at 251°C can be see, which is attributed to the melting of the gellan gum polymer (Fig.3). In the DSC thermogram of the drug loaded peak for drug loaded microspheres was not appeared (Fig.3). DSC studies revealed that Sildenafil were molecularly dispersed inside of the microspheres.

**Table 4.** Coefficient and exponent of release according to Mt/M∞=Ktn for prepared microspheres.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Kinetic Constant (k)</th>
<th>Release exponent (n)</th>
<th>Coefficient of Determination (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGS1</td>
<td>-1.532</td>
<td>0.6754</td>
<td>0.9689</td>
</tr>
<tr>
<td>GGS2</td>
<td>-1.239</td>
<td>0.4299</td>
<td>0.9858</td>
</tr>
<tr>
<td>GGS3</td>
<td>-0.937</td>
<td>0.4375</td>
<td>0.9460</td>
</tr>
<tr>
<td>GGS4</td>
<td>-1.543</td>
<td>0.5198</td>
<td>0.9790</td>
</tr>
<tr>
<td>GGS5</td>
<td>-1.542</td>
<td>0.4510</td>
<td>0.9947</td>
</tr>
</tbody>
</table>

8. XRD studies
The X-ray diffraction spectra recorded for pure Sildenafil Citrate (SC) gellan gum blank microspheres (GGB) and drug loaded microspheres (GGS) are presented in Fig. 4. These studies are useful to investigate the crystallinity of drug in the polymeric microspheres. Ondansetron has shown characteristic intense peaks between 2θ of 5 and 25 but in case of blank microspheres and drug loaded microspheres no intense peaks were observed between 2θ of 5 and 25, indicating amorphous nature of drug after entrapment into the gellan gum microspheres by spray drying.

9. In vitro drug Release Study
The drug release profiles from various formulations of microspheres shown in Fig.5. Microspheres prepared with gellan gum, moderately sustained the drug release to five hrs without any lag time. The rate and extent of Sildenafil release from microspheres significantly decreased with an increase in gellan concentration. The drug release from the microspheres was at slower rate due to ionic gelation (cross linking of gellan with cations in SNE).
Sildenafil release from the multiparticulate system was almost complete, regulated and extended until 6 hours in F5 batch so the F5 batch was optimized. In order to investigate the release mechanism, the release data were fitted to models representation zero order, first order and Highuchi’s square root of time [17]. The examination of coefficient of determination values indicated that drug release from the microspheres formulation followed the diffusion control mechanism (Highuchi model). A more stringent test was used to distinguish between the mechanisms of drug release.

The release data were fitted to the Peppas exponential model [18, 19]. Mt/M∞ = Kt^n, where Mt/M∞ is the fraction of drug release after time t, k is the kinetic constant and n is the release exponent which characterizes the drug transport mechanism. The values for kinetic constant and release exponent are listed. The n values as shown in table 3 and Table 4 were in the range of 0.4510 to 0.6754 indicating that all the prepared formulations followed the fickion diffusion controlled mechanism of drug release.

10. Histopathological evaluation of nasal mucosa
The microphotographs were taken of nasal mucosa following incubation with microsphere formulations for more than six hrs (Fig 6). Examination of tissue showed ciliated respiratory epithelium and normal goblet cell appearance. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells was detected.

Conclusion
The result of our present study clearly indicated promising potentials of gellan gum microspheres for delivering drug intranasally and could be viewed as attention to conventional dosage form. Gellan gum microspheres size of about 10μm and a negative zeta potential could be prepared by a conventional spray drying method.
before establishing Sildenafil nasal delivery as an alternative.

References