Development and evaluation of ion-dependent in-situ nasal gelling systems of metoclopramide hydrochloride as an antimigraine model drug

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Abstract: Oral Metoclopramide hydrochloride undergoes first-pass metabolism. Nasal delivery could protect drugs from this effect. Mucoadhesive in-situ gels for Metoclopramide hydrochloride with gellan gum were formulated and evaluated. Metoclopramide Hydrochloride in-situ nasal gels (10% w/w) were prepared at concentration of gellan gum 0.2%, 0.4%, 0.6% and 0.8% w/v with Xanthan gum 0.1%, 0.15% and 0.2% w/v as bioadhesive polymer and benzalkonium chloride (0.01%) as preservative. The formulations were evaluated for hydrogel formation, viscosity, in vitro release, in-vitro mucoadhesive force and histopathology of nasal tissues. The formulation of 0.6% gellan gum with 0.15% Xanthan gum was tested for accelerated stability studies. Increasing gum concentration was accompanied by slower release rate during the first hr. Release kinetics followed diffusion model. Microscopic results did not show any mucosal changes after diffusion study of the optimized formulation. In-vitro mucoadhesion showed that in-situ gels can be retained in nasal mucosal tissue for prolonged time in the order: 0.8% w/v > 0.6% > 0.4% > 0.2% gellan gum. The 0.6% in-situ gel remained liquid for 6 months without any turbidity or gelation.

The in-situ gel is a promising approach for the intranasal delivery of Metoclopramide hydrochloride. This gel combines the advantage of a solution and its administration convenience with favorable residence time and sustained release and hence expected improved drug absorption.

Keywords- Metoclopramide hydrochloride, nasal in situ gel, gellan gum, Xanthan gum

Introduction

Migraine is a physiological condition, in which a person suffers from tremendous headache. Generally, this headache affects only one side of the head and body. Migraine attacks are more common to those persons who take too much of stress or are work alcoholic. In such people, the blood flow in the brain muscles drops, as a result of too much load, squeezing the arteries. When the person suddenly relaxes, these tight brain muscles expand, stretching the blood vessel walls. The blood pumped with each heartbeat, then, pushes the vessels further, causing immense pain\(^1\). Though the exact cause of migraine has not been identified, there are a number of factors that can trigger the severe headache. The typical migraine headache is unilateral and pulsating, lasting from 4 to 72 hours. Migraine is a neurological syndrome characterized by altered bodily perceptions, headaches, and nausea. Physiologically, the migraine headache is a neurological condition more common to women than to men\(^1\). In other words Migraine is a familial disorder characterized by recurrent attacks of headache widely variable in intensity, frequency and duration. Attacks are commonly unilateral and are usually associated with anorexia, nausea and vomiting\(^2\). So, there is much attention has been paid to the use of the nasal route for the systemic delivery of drugs that are conventionally administered by injection. The nasal cavity has many advantages as a potential site for drug delivery; being readily accessible facilitates self-medication, which may improve patient compliance compared to parenteral routes. The nasal mucosa is having a relatively large absorptive surface area and is highly vascularized. Furthermore, the blood is drained directly from the nose into the systemic circulation, thus, avoiding first pass metabolism predominantly by the liver. Delivery of biologics and a variety of other substances from the nasal passages to the brain has now been documented in numerous animal and clinical studies.\(^2\)

In situ nasal gels have many advantages over conventional modes of drug administration: extended nasal retention time, reduction in frequency of drug administration, and improvement of patient compliance. Gelrite-based in situ gelling nasal formulations have been developed for many drugs.
such as Fluorescein dextran [3], mometasone furoate [4] and gastrodin [5].

Metoclopramide hydrochloride is used as Dopamine receptor antagonist; antiemetic. Chemically it is 4-amino-5-chloro-N-[2-[diethyl amino] ethyl]-2-methoxybenzamide hydrochloride. It is available as white or almost white, crystalline powder or crystals, which is very soluble in water, freely soluble in alcohol, sparingly soluble in methylene chloride. This antiemetic, chemically related to the procainamide, acts predominantly as a dopamine antagonist. It also has 5-HT4 agonist properties [6], [7]. However, the oral bioavailability of Metoclopramide hydrochloride is highly variable showing values between 32 and 98% due to extensive pre-systemic metabolism. Oral forms of Metoclopramide hydrochloride often get vomited out before systemic absorption compelling parenteral or rectal administration where both methods result in low patient compliance. In this regard, the intranasal delivery seems to be an attractive alternative.

In situ gel, or in vivo gel, environment sensitive gel, is a new dosage form which has been applied as nasal drug delivery recently. Compared with liquid nasal formulations, nasal in situ gels are instilled as low viscosity solutions into the nasal cavity and upon contact with the nasal mucosa, the polymer changes conformation producing a gel, so it cannot only prolong the contact time between the drug and the absorptive sites in the nasal cavity, but also release drug slowly and continuously. Hence, it is especially useful for those drugs used chronically. The phase transition can be induced by a shift in pH as for cellulose acetate phthalate, a shift in temperature as for the thermo gelling Poloxamer 407 or by the presence of cations as for gellan gum [8].

Gellan gum is an anionic deacetylated, exocellular polysaccharide secreted by Pseudomonas elodea with a tetrasaccharide repeating unit of 1β-L-rhamnose, 1 β-d-glucuronic acid and 2β d-glucose. The mechanism of gelation involves the formation of double-helical junction zones followed by aggregation of the double-helical segments to form a 3-D network by complexation with cations and hydrogen bonding with water [8]. Since human nasal mucosa is covered with approximately 0.1 ml mucus, which consists of sodium, potassium, and calcium ions, a solution-gel phase transition can be expected.

In the present study, a nasal delivery system of ion-activated in situ gel for Metoclopramide hydrochloride with gellan gum combined with xanthan gum was developed, and its rheological characteristics, drug content, stability and hydrogel formation in vitro were investigated.

Materials and methods

Materials

Metoclopramide Hydrochloride and Xanthan Gum were gifted by the Sun Pharmaceuticals Ltd. (Baroda, India). Gellan gum was obtained from Gujarat Pharmalab Pvt Ltd. (Ahmadabad, India) and all other reagents were of commercially analytical-grade.

A. Preformulation Studies:

Determination of λmax of Metoclopramide Hydrochloride

A stock solution of 100 μg/ml of Metoclopramide hydrochloride was prepared by dissolving 10 mg in 100 ml of deionized distilled water. The resulting solution was scanned between 200 nm to 400 nm using double beam UV-visible spectrophotometer (2201-systronics, India.).

Fourier transform infrared spectral studies

Fourier transform infrared (FTIR) spectra were taken on FTIR (model-8400, shmadzu) to investigate any possible chemical reactions between the drug and the polymer. FTIR spectra of the pure drug and physical mixture of drug with polymers were obtained. Pure drug (Metoclopramide hydrochloride) and polymers were subjected to FTIR studies alone and in combinations. 3 mg of pure drug / combination of drug - polymer were triturated with 97 mg of potassium bromide in a smooth mortar. The mixtures were placed in the sample holder and were analyzed by FTIR to study the interference of polymers with the drug.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to evaluate the thermal behavior of pure drug and physical mixture of the drug and excipients using a DSC-60 (shimadzu corporation, Japan.). Samples, 5–10 mg, were weighed and sealed in standard aluminum pans and then scanned over a temperature range from 50 to 300°C at a heating rate of 10.00°C / min.

Preparation of nasal Formulations

Gellan gum solutions of various concentrations were prepared by adding the gum to deionized water and heating up to 90°C while stirring. After cooling to below 40°C, Xanthan gum, Metoclopramide hydrochloride (10% w/v), mannitol (5%, w/v), and Benzalkonium chloride (0.01%, w/v) were added and mixed well. Three kinds of Metoclopramide hydrochloride in situ gels were prepared at the concentrations of Gellan gum which were 0.2%, 0.4%, 0.6% and 0.8% (w/v) with combination of Xanthan gum concentrations 0.1%, 0.15% and 0.2% (w/v) respectively.
Table 1: Formulas for developed Ion dependent nasal in-situ gel for Metoclopramide HCl

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>I-1</th>
<th>I-2</th>
<th>I-3</th>
<th>I-4</th>
<th>I-5</th>
<th>I-6</th>
<th>I-7</th>
<th>I-8</th>
<th>I-9</th>
<th>I-10</th>
<th>I-11</th>
<th>I-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Metoclopramide</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>10</td>
</tr>
<tr>
<td>hydrochloride(% w/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Gellan gum(% w/v)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Xanthan gum(% w/v)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Mannitol(% w/v)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Benzalkonium chloride(% w/v)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Each formulation contain 10mg/ml of Metoclopramide HCl

B. Characterization of the Prepared Formulations

Gelation study

It is the temperature at which the liquid phase makes a transition to gel. A gelation temperature range suitable for thermoreversible nasal gel would be 30-36°C. Gelation point was considered as the temperature where formulations would not flow when test tubes were tilted to 90° angle, as the temperature was gradually increased.

pH of the gels

The pH of each batch was measured using digital pH meter which was calibrated using buffers of pH 4 and pH 7 before the measurements.

Content uniformity[^9]

Formulations were tested for content uniformity. Bottles (n=3) containing the formulation were properly shaken for 2–3 min. The formulation, 1.0 ml was transferred into a 100-ml volumetric flask and 50 ml of simulated nasal fluid was added. The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a 0.45-mm filter membrane and the drug concentration was determined with a UV-Visible spectrophotometer at 272.4 nm.

Rheological studies[^9]

Viscosity of the prepared formulations was measured by using Brookfield LVDV-E Viscometer. The suitable spindle was lowered perpendicularly into the fixed volume of gel which was to be measured. The spindle was rotated at varying speeds and the suitable speed was selected. The temperature was increased initially above 40°C and then the viscosity was measured as the system was allowed to cool gradually.

Gel strength determination[^9]

A sample of 50g of the nasal gel was put in a 100 ml graduated cylinder and gelled in a thermostatically controlled water bath at 37°C. A weight of 35 g was placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.

Determination of Mucoadhesive Strength[^10]

The mucoadhesive strength was determined by using the modified method reported by Choi et al[^10]. The mucoadhesive potential of each formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. 50mg of gel was placed on first slide
and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm² was determined from the minimal weight that detached the mucosal tissue from surface of each formulation.

\[
\text{Mucoadhesive Strength (dynes/cm}^2\) = \frac{mg}{A},
\]

Where, \(m\) = weight required for detachment in gram,
\(g\) = Acceleration due to gravity (980cm/s²),
\(A\) = Area of mucosa exposed.

The nasal mucosa was changed for each measurement.

**In-vitro Release Studies**

Drug release from gel was tested with nasal diffusion cell, using dialysis membrane (mol.wt.12000 to 4,000) with permeation area of 2 cm². 20ml of simulated nasal fluid pH 6.6 was added to the acceptor chamber. Gel containing drug equivalent to 10mg was placed in donor compartment. At predetermined time points (1 hr), Samples (1 ml) were withdrawn from the acceptor compartment, replacing the sampled volume with SNF after each sampling for a period of 8 hrs. The samples were suitably diluted and measured spectro photometrically at 272.4nm. The concentration of drug was determined from a previously constructed calibration curve. (\(y = 0.039x + 0.003, R^2 = 0.9988\))

**Mechanism of drug Release**

In order to elucidate kinetics of the drug release, data were analyzed using: zero-order equation, \(Q = Q_0 + Kt\); first-order equation, \(Q = Q_0 e^{-Kt}\) and Higuchi’s square root model, \(Q = K\sqrt{t}\). Where, \(Q\) is the amount of drug released in time “\(t\)”. \(Q_0\) is the initial dose, \(K\) is the release constant of the respective equations, and \(t\) is the release time. In order to find out the drug release mechanism from the mucoadhesive systems, the data were fitted to Korsmeyer–Peppas’ power equation. \(M_t/M_{\infty} = K_t^n\) where, \(M_t/M_{\infty}\) is the fraction of drug released in time “\(t\)” \(K\) is a constant incorporating structural and geometrical characteristics of the drug/polymer system, and \(n\) is the release exponent, which is indicative of the drug-release mechanism. When \(n\) is equal to 0.5, the drug is released from the polymer with Fickian diffusion mechanism. If 0.5<\(n<1\), it indicates anomalous or non-Fickian release, whereas if \(n = 1\), it indicates zero-order release.

**In –vìtro Permeation Study**

Fresh nasal tissue was removed from nasal cavity of sheep obtained from local slaughter house. Tissue was inserted in the nasal diffusion cell (Franz diffusion cell) with permeation area of 0.785 cm². Similar way as in drug release study gel containing drug equivalent to 10mg was kept in donor compartment. At predetermined time point sampling was done. Blank samples (without drug) were run simultaneously throughout the experiment. Amount of drug permeated was determined by UV- spectrophotometry. Cumulative percentage drug release after1 h \((t_1)\) and 8 h \((t_8)\) were calculated using the Beer-Lambert calibration curve in the linearity range of 0–20 µg/mL⁻¹.

**Histopathological Evaluation of Mucosa**

The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.8) for 6 h 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 and damage to the tissue.

**Accelerated stability studies**

Stability studies were carried out on gel formulation according to ICH (International Conference on Harmonization) guidelines. A sufficient quantity of in situ gel in glass bottles was stored in desiccator containing saturated solution of sodium chloride, which gave a relative humidity of 75±5%. The desiccator was placed in a hot air oven maintained at 40±2 °C, and samples were withdrawn at 0, 30, 60 and 90 days intervals. Selected formulation I-7 was stored at (4±2) °C, room temperature (25±2) °C, and (40±2) °C for a period of 3 months. The formulations were evaluated at periodic for drug content, pH, gelling capacity, viscosity and in vitro drug release. The drug content remaining and the viscosity of formulation were measured at predetermined time interval.

**Results and Discussion**

Metoclopramide hydrochloride exhibited \(\lambda_{max}\) at 272.4 nm. Linearity was observed in the range of 2 to 20µg/ml with the \(r^2\) value of 0.998. FTIR and DSC studies were carried out on pure drug as well as its combination with selected polymers. At the outset, as a preformulation study, IR characteristics of Metoclopramide hydrochloride with the polymer resemble almost the IR structural characteristics of pure drug indicated the compatibility between the drug and polymers. The infrared spectra of Metoclopramide hydrochloride and physical mixture
of formulation containing drug, gellan gum and Xanthan gum shows in figure 1 and Figure 2 respectively. Drug spectrum shows prominent peaks at 3379.05 cm$^{-1}$, 3396.41 cm$^{-1}$, 1595.02 cm$^{-1}$, 703.97 cm$^{-1}$ corresponding to -NH stretching, -OH stretching, C=O and C-Cl stretching respectively (Figure 1). Drug: polymer mixture spectrum (Figure 2) shows absence of characteristic drug peaks at 3379.05 cm$^{-1}$. Subtraction spectrum did not show the characteristic peak of drug at 3379.05 cm$^{-1}$ corresponding to –NH stretching. In both cases it was observed that the characteristic bands did not shift appreciably, suggesting the lack of any interaction between the drug and excipients.

DSC thermograms were recorded for pure Metoclopramide hydrochloride and physical mixture of drug and polymers (Figure 3 and 4). In both cases it was observed that the characteristic endotherm (corresponding to melt of the drugs) did not shift appreciably, suggesting the lack of any interaction between the drug and excipients.

The compositions of the various batches of the Metoclopramide hydrochloride mucoadhesive in situ nasal gelling system are shown in table 1. Xanthan gum was used as mucoadhesive agents combined with gellan at the concentration of 0.2, 0.4, 0.6, 0.8% w/v and 0.1, 0.15, and 0.2% w/v, respectively provided the defined fluidity of the liquid formulation.

The physico-chemical properties of the prepared Metoclopramide hydrochloride formulations are shown in table 2 and 3. The drug content, clarity and pH of the formulations were found to be satisfactory. The two main pre-requisites of mucoadhesive systems are viscosity and gelling capacity (speed and extent of gelation). Moreover, a mucoadhesive system should preserve its integrity without dissolving or eroding for a prolonged period of time to facilitate sustained release of the drug to the nasal tissues$^{11}$. All the formulations showed instantaneous gelation when contacted with the simulated nasal fluids (SNF). However, the nature of the gel which was formed depended to the polymer concentration. In the case of Metoclopramide hydrochloride formulation batch I-1, I-5 and I-9 showed the weakest gelation, due to the presence of minimal amount of gellan (0.2%).

### Appearance and gelation

All the formulation developed has a clear appearance in the sol form when stored at room temperature not exceeding 32 $^\circ$C.

**pH**

An acidic or alkaline formulation is bound to cause irritation on the mucosal membrane and hence this parameter assumes significance while developing a Mucoadhesive formulation. Surface pH of the formulation I-1 to I-12 varies from 5.7 $\pm$ 0.252 to 6.5 $\pm$ 0.224. Each sample was analyzed in triplicate (n=3) within acceptable range. The results reveal that all the formulations provide an acceptable pH in the range of 5.5 to 7.0 (salivary pH). Hence, they may not produce any local irritation to the mucosal.

### Drug content:

Drug content of the developed formulations I-1 to I-12 varied from 98.60 $\pm$ 0.06 % to 100.34 $\pm$ 0.26 % which was within the required limits.

<table>
<thead>
<tr>
<th>Form. code</th>
<th>Appearance</th>
<th>Gelling capacity</th>
<th>pH $\pm$ S.D.</th>
<th>Drug content $\pm$ S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>Transparent Solution</td>
<td>---</td>
<td>5.7 $\pm$ 0.265</td>
<td>99.62$\pm$ 0.31</td>
</tr>
<tr>
<td>I-2</td>
<td>Transparent and less viscous solution</td>
<td>+</td>
<td>6.1 $\pm$ 0.153</td>
<td>100.17 $\pm$ 0.51</td>
</tr>
<tr>
<td>I-3</td>
<td>Transparent Solution</td>
<td>++</td>
<td>6.2 $\pm$ 0.265</td>
<td>99.56 $\pm$ 1.13</td>
</tr>
<tr>
<td>I-4</td>
<td>Transparent Solution</td>
<td>+++</td>
<td>6.5 $\pm$ 0.200</td>
<td>98.80 $\pm$ 0.99</td>
</tr>
<tr>
<td>I-5</td>
<td>Transparent and less viscous solution</td>
<td>+</td>
<td>5.8 $\pm$ 0.210</td>
<td>100.27 $\pm$ 0.16</td>
</tr>
<tr>
<td>I-6</td>
<td>Transparent and viscous solution</td>
<td>+++</td>
<td>6.1 $\pm$ 0.251</td>
<td>100.00 $\pm$ 0.10</td>
</tr>
<tr>
<td>I-7</td>
<td>Transparent Solution</td>
<td>+++</td>
<td>6.2 $\pm$ 0.265</td>
<td>99.69 $\pm$ 0.10</td>
</tr>
<tr>
<td>I-8</td>
<td>Transparent and viscous solution</td>
<td>++++</td>
<td>5.7 $\pm$ 0.252</td>
<td>99.08 $\pm$ 0.27</td>
</tr>
<tr>
<td>I-9</td>
<td>Transparent Solution</td>
<td>+++</td>
<td>6.2 $\pm$ 0.132</td>
<td>100.34 $\pm$ 0.26</td>
</tr>
<tr>
<td>I-10</td>
<td>Transparent and viscous solution</td>
<td>++++</td>
<td>6.5 $\pm$ 0.224</td>
<td>99.93 $\pm$ 0.16</td>
</tr>
<tr>
<td>I-11</td>
<td>Transparent Solution</td>
<td>+++</td>
<td>6.3 $\pm$ 0.288</td>
<td>99.15 $\pm$ 0.16</td>
</tr>
<tr>
<td>I-12</td>
<td>Transparent Solution</td>
<td>++</td>
<td>6.4 $\pm$ 0.098</td>
<td>98.60 $\pm$ 0.06</td>
</tr>
</tbody>
</table>

- No gelation, + Gelation occurred in few min and remained for few h, ++ Gelation immediate, remained for few h, +++ Gelation immediate, and for extended period, ++++ Very stiff gel

**Table 2: Appearance pH gelling capacity and drug content estimation of various formulations**

ISSN: 2278-5299

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Table 3: Evaluation parameters of formulations.

<table>
<thead>
<tr>
<th>Form. Code</th>
<th>Mucoadhesive force (dynes/cm²) ± S.D.</th>
<th>Gel strength (sec.) ± S.D.</th>
<th>Viscosity (cPs) ± S.D.</th>
<th>Viscosity (cPs) ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>2401.00 ± 120.05</td>
<td>8.93 ± 0.28</td>
<td>109.33 ± 8.08</td>
<td>--</td>
</tr>
<tr>
<td>I-2</td>
<td>2681.12 ± 124.95</td>
<td>12.83 ± 0.35</td>
<td>266.67 ± 23.25</td>
<td>1279 ± 19.51</td>
</tr>
<tr>
<td>I-3</td>
<td>3301.38 ± 180.07</td>
<td>15.00 ± 1.20</td>
<td>314.17 ± 1.89</td>
<td>1346.67 ± 25.17</td>
</tr>
<tr>
<td>I-4</td>
<td>3921.63 ± 151.06</td>
<td>21.47± 0.57</td>
<td>369.17± 5.75</td>
<td>1919.00± 28.83</td>
</tr>
<tr>
<td>I-5</td>
<td>5202.17 ± 242.59</td>
<td>31.13 ± 1.41</td>
<td>259.67 ± 5.03</td>
<td>1855.00± 35.00</td>
</tr>
<tr>
<td>I-6</td>
<td>5022.09 ± 91.69</td>
<td>40.70 ± 3.61</td>
<td>281.00± 3.61</td>
<td>3779.67± 35.70</td>
</tr>
<tr>
<td>I-7</td>
<td>5222.18 ± 180.08</td>
<td>47.17 ± 1.34</td>
<td>462.67 ± 11.68</td>
<td>4369.00± 16.82</td>
</tr>
<tr>
<td>I-8</td>
<td>5822.43 ± 681.75</td>
<td>51.53 ± 1.41</td>
<td>541.67 ± 27.54</td>
<td>349.00 ± 31.19</td>
</tr>
<tr>
<td>I-9</td>
<td>7403.08 ± 916.90</td>
<td>50.97 ± 1.56</td>
<td>638.00 ± 3.00</td>
<td>4246.00± 32.08</td>
</tr>
<tr>
<td>I-10</td>
<td>8203.42 ± 1510.60</td>
<td>53.43 ± 1.34</td>
<td>785.00 ± 5.57</td>
<td>5297.33 ± 876.07</td>
</tr>
<tr>
<td>I-11</td>
<td>10404.33± 346.55</td>
<td>55.83 ± 2.76</td>
<td>841.67 ± 10.41</td>
<td>5653.33± 412.48</td>
</tr>
<tr>
<td>I-12</td>
<td>11004.58± 346.55</td>
<td>62.80 ± 0.14</td>
<td>1109.33± 70.68</td>
<td>6149.67± 26.65</td>
</tr>
</tbody>
</table>

Values expressed as Mean±S.D, n =3

Mucoadhesive force measurement
The mucoadhesive force significantly increased as the concentration of mucoadhesive polymers increased over the concentration range of 0.1-0.2 %. 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 mucoadhesive polymer concentration (Table 3). In mucoadhesion process, both weak and strong interactions (i.e. van der Waals interaction, hydrogen bonding and ionic bonding) can develop between certain types of functional groups on the polymer (e.g. hydroxyl or carboxyl groups) and glycoprotein network of the mucus layer or the glycoprotein chains attached to the epithelial cells for example in the nose. In order to develop strong adhesive bonds, the establishment of strong intimate molecular contact between the polymer and glycoprotein chain is essential [9]. Gellan gum form gels in the presence of mono and divalent cations, here with physiological cations from nasal electrolytes have a key role in Mucoadhesion strength [13]. The rapid fluid uptake from mucus layer enabling the polymer chain to penetrate mucin network and establish adhesive bond has a key role in mucoadhesion.

Viscosity
The formulations exhibited pseudoplastic rheology, as evidenced by shear thinning and an increase in the shear stress with increased angular velocity. The viscosity was directly dependent on the polymeric content of the formulations. When the in situ gels were mixed with SNF, a gel immediately formed and the elastic and viscous gel. In the selection of the concentration of the gelling polymer, a compromise is sought between satisfactory gel strength for use as a delivery vehicle and an acceptable viscosity for ease of spraying. All gellan gum formulations, either in solution or in gel, showed pseudo plastic behavior (Fig. 6 and 7). The viscosity of the test gels increased with increasing concentrations of gellan gum, and a large viscosity change was found when gellan gum underwent sol–gel transition at lower concentrations (0.2%, 0.4% and 0.6%). Due to a very viscous solution obtained with 0.8% gellan gum, a slight viscosity increase was observed after gel formation. The observed increase in viscosity with increase in concentration has been noted previously for gellan gum and is attributed to a consequence of increasing chain interaction with polymer concentration rising [3]. Formulation I-7 (containing gellan gum 0.6% w/v with mucoadhesive polymer xanthan gum 0.15% w/v) exhibited optimum mucoadhesive strength. The results also showed that the presence of combination of polymers significantly increased the viscosity as well as the mucoadhesive property. There was increased in gel strength might be the result of an increase of polysaccharide chain flexibility causing a theroreversible conformational change and making the gellan helices more accessible to the cations and at increased temperature, in salt excess solution, the hydration of gellan gum helices decreases and the charges on the helices may be drastically reduced because of the presence of cations. This can result in hydrophobic interactions between adjacent helices, inducing an increase in gel structure [19].

Drug release study
Metoclopramide hydrochloride release from nasal preparations was moderate under sink condition (Figure 8, 9...
Formulation I-1 containing 0.2% w/v of gellan gum with 0.1% w/v xanthan gum showed 98.01 ± 2.75% cumulative drug released. At fixed drug concentrations, the release rate depended on gellan gum concentration; the higher the gellan gum concentration, the lower the rate of drug release. The release of the drug from the formulation depends upon the type of polymer used, its concentration used and the viscosity of the formulation. The results clearly showed that the mucoadhesive nasal in-situ gelling systems have the ability to retain Metoclopramide hydrochloride in its matrix network and that the premature drug release will not occur. Drug release from the I-1 containing less amount of gellan gum was completely depleted within 2-3 hrs. Gellan gum, an anionic polysaccharide, when incorporated as a part (0.2% to 0.8% w/v), controlled the release rate of the drug significantly (p<0.001) (Figure 8, 9 and 10). Gellan gum undergoes gelation in the presence of cations, via a chemical bonding between the divalent cations and two COO$^-$ groups of guluronic acid molecules in gellan chains. It is evident from the figure 3 that even at lower concentrations of gellan gum; the drug release was sustained for an extended period. Drug release seemed to slow down with an increase in gellan concentration.

Drug release mechanism

The correlation coefficient ($r^2$) values for various release models viz., zero-order, first-order, and Higuchi models, were found. The $r^2$ values suggest that the drug release from the mucoadhesive system predominately followed Higuchi’s square root of time kinetics, as the values for $r^2 Q$ vs. $t^{1/2}$ (0.912-0.987) were found. First order rate kinetic coefficient was varied from 0.838 to 0.998 and zero order kinetic coefficients were found to be 0.970 to 0.999. Whereas Release exponent, $n$, was >0.5 but <1, for I-1, 2, 3, 4, 9, 10, 11 and I-12, indicating that release mechanism was followed an anomalous or non-Fickian release and suggesting a coupled erosion-diffusion mechanism for the tested Metoclopramide hydrochloride mucoadhesive system. Whereas selected optimized formulation I-7 and I-5, 6, 8 were followed zero order kinetics. The light micrograph was taken of nasal mucosa following diffusion study of 8 h. Examination of tissue showed None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells was detected (Figure 5).

Stability studies

All the formulation showed good stability at 27 °C/60% RH. There were no significant changes in visual appearance and clarity; pH remained constant for entire stability period; drug content did not deviate by than 2% indicating that the drug is stable in the in situ gel formulations and also there was no significant variation in the in vitro release studies at the end of 30 day period. A formulation intended for a nasal

Conclusions

Metoclopramide Hydrochloride was successfully formulated as an in situ gelling system using Gellan gum. The formulated systems provided sustained release of the drug over an 8-hour period in vitro and the developed formulations were devoid of any deleterious effect on the nasal tissues. Hence, this can be viewed as a viable alternative to conventional nasal drops by virtue of its ability to enhance nasal residence time and thereby intranasal bioavailability. The ease of administration coupled with its ability to provide sustained release could probably result in less frequent administration, thus enhancing patient compliance.

Acknowledgements

The authors are grateful to Samanvay trust (Botad, Gujarat) for providing facilities for this research work. The author thanks to Dr. A.N. Lumbhani, Principal, Shree Samanvay Institute of Pharmaceutical education and Research, Botad (Gujarat) for helping and providing necessary facilities to carry out this work. Also thanks to Dr. Sanjay Gandhi, Histopathologist, Green Cross Laboratory, Ahmedabad for writing the report about the nasal mucosal integrity.
Figure 2. FTIR spectra of physical mixture of drug and polymers

Figure 3. DSC thermogram of pure Metoclopramide hydrochloride drug

Figure 4. DSC thermogram of physical mixture of drug and polymers
Figure 5. Light Photomicrograph of the Nasal Mucosa Normal Mucosa (A) and Metoclopramide hydrochloride ion-dependent in situ nasal gel treated Mucosa (D).

Figure 6. Rheological profile of ion dependent in-situ gelling system before gelation

Figure 7. Rheological profile of ion dependent in-situ gelling system after gelation

Figure 8. Drug diffusion profile of in-situ gelling system containing 0.1% w/v of xanthan gum

Figure 9. Drug diffusion profile of in-situ gelling system containing 0.15% w/v of xanthan gum

Figure 10. Drug diffusion profile of in-situ gelling system containing 0.2% w/v of xanthan gum

Table 4: Kinetic values obtained from different plots of the Ion dependent nasal in situ gel formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order plots</th>
<th>First order plots</th>
<th>Higuchi’s plots</th>
<th>Korsmeyer’s peppas equation</th>
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<tr>
<td></td>
<td>$R^2(a)$</td>
<td>$R^2(a)$</td>
<td>$R^2(a)$</td>
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<td>0.912</td>
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<td>0.649</td>
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<td>0.635</td>
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<tr>
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<td>0.981</td>
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<tr>
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<td>0.838</td>
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</tbody>
</table>

ISSN: 2278-5299
Correlation coefficient \( \text{Mt}/\text{M}_\infty = k_t n \).

References: