In-Situ Gelling System based on Thiolated Gellan Gum as New Carrier for Nasal Administration of Dimenhydrinate

Hitendra Mahajan*, Hannan Shaikh, Surendra Gattani, Pankaj Nerkar
R.C. Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, 425 405, India.

ABSTRACT: The purpose of the present study was to develop intranasal delivery system of dimenhydrinate using thiolated gellan gum and formulations were modulated so as to have gelation at physiological ion content after intranasal administration. Gelation was determined by physical appearance. The mucoadhesive force in terms of detachment stress, determined using sheep nasal mucosa, increased with increasing concentration of thiolated polymer. The results of in vitro drug permeation studies across sheep nasal mucosa indicate that effective permeation could be significantly increased by using in situ gelling formulation with thiolated polymer concentration. Finally, histopathological examination did not detect any changes during in vitro permeation studies. In conclusion the gel formulation of dimenhydrinate with in situ gelling and mucoadhesive properties with increased permeation rate is promising for prolonging nasal residence time and thereby nasal absorption.

KEYWORDS: Thiolated gellan gum; in situ gel; Dimenhydrinate; Mucoadhesion; Nasal

Introduction
Nasal administration is a non-invasive method of administration when oral administration of drug gives an undesirably show effect, or when drug is highly metabolized or incompletely absorbed in the gastrointestinal tract. Possible pathways for a drug to permeate across the nasal mucosa are passive transportation, carrier mediated, transcytosis and transport through intercellular tight junctions. However nasal delivery system has limitations which have restricted its use to delivery of few drug molecules in general rapid clearance from nasal cavity (Illum et al., 2003).

Different delivery systems based on polymers have been developed which are able to increase the residence time of the formulation at absorption site of drugs (Illum et al., 2003). In recent years there has been an increasing interest in water soluble polymer that are able to form gels after application to delivery site. These so called in-situ gelling polymers are highly advantageous compared with other polymer because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption they swell to form a strong gel that is capable of prolonging the residence time of the active substance. Gellan gum is a novel ion sensitive polysaccharide polymer; it is native from extra cellular microbial polysaccharides produced by bacterium Pseudomonas elodea that are capable of forming gels within seconds of contact with liquid of high cation concentrations. Further improvement concerning in situ gelling of already well established polymer could possibly be achieved by immobilization of thiol group. These so called thiomers are capable for increase in viscosity, residence time (Alexander et al., 2002, Andreas et al., 2003).

Dimenhydrinate is one of the OTC drugs used to prevent motion sickness which has been used by oral and injectable administration. It is an antihistaminic used for the prevention and treatment of nausea, vomiting, dizziness, and vertigo associated with motion sickness. Dimenhydrinate is rapidly absorbed orally, but extensively metabolized by the liver. It should be administered 30 min before travelling, but the orally administered antiemetic drug tends to be discharged by vomiting (Drug Facts and comparisons).

On the contrary, intravenous (IV) administration renders rapid effects to a patient, but the onset of effects is too rapid to cause undesirable effects, additionally, it results in a local pain. Practicing the IV route in journey is highly impossible. In the current scenario of globalization; travelling has become one of the most essential needs of
human beings. Travelling is mostly associated with a variety of discomforts one of them is motion sickness, which is observed in about 38% individuals.

The aim of this work was to study the possible application of thiolated gellan gum for preparation of mucoadhesive in-situ gel system for nasal administration of drugs. A untreated gellan gum has been used as a comparison.

**Experimental**

**Materials and Methods**

The products used in the formulations were gellan gum (Deacetylated): CPKelco division of the Monsanto Company (USA), dimenhydrinate: RPG Life sciences Ltd. (India), 2-imminothiolane hydrochloride (Merck, Switzerland) All other materials and solvents obtained from commercial sources were of analytical grade. Ultra-pure water was used throughout the studies.

**Modification of gellan gum with 2-iminothiolane.**

Gellan gum were dissolved in 50ml of water after adjusting the pH-6.5 with 1m NaoH. 200mg of 2-iminothiolane was added. Reaction was allowed to for 14 hrs at room temperature under continuous stirring. For purification, resulting polymer was dialyzed against 5mM HC\( \text{Cl} \), twice to obtain final pH 3. Polymer was frozen at -30 °C for further use (Alexander et al., 2006).

**Formulation of in situ gel** (Balasubramanyam et al., 2003).

Gellan gum was weighed and dispersed in ultra-pure water. The dispersions were then stirred for 20 minutes at 100°C in water bath and then cooled to room temperature. Dimenhydrinate (2.5 % w/v) was added during the cooling process. The solutions were allowed to equilibrate for at least 16 hours at 4-8°C temperature. Appropriate quantities of mannitol and benzalkonium chloride were added simultaneously. The final pH of formulations was adjusted in between 4.5 to 5.5 using 0.1N HC\( \text{Cl} \).

The formulations were filled in 10-mL amber colored glass vials, capped with rubber closures and sealed with aluminum caps. In their final pack, the formulations were terminally sterilized by autoclaving at 121°C and 15 Pa for 20 minutes. Sterilized formulations were stored in a refrigerator (4-8 °C) until further use. Prepared formulations were evaluated for following physical parameters.

**Gelation studies** (Yong et al., 2001, Zaki etal., 2007)

A 10-mL transparent vial containing a magnetic bar and 2 mL of each formulation was placed on a magnetic stirrer. The Simulated Nasal Fluid (SNF- aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml CaCl\( \text{2} \)) per litre (Illium et al.,) which has the cationic composition of nasal secretions was added slowly while stirring (Murthy et al., 2006). The gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked and graded as indicated in Table 2. Each preparation was tested thrice to check the repeatability of the measurement.

**Viscosity measurements** (Balasubramanyam et al., 2003)

Viscosities of formulations before and after gelation were measured by Brookfield DV-E viscometer using Spindle number-3 at 100 rpm shear rate. Fig. 1 displays viscosities of all formulations.

**Gel strength determination** (Yong et al., 2001)

It is expressed in terms of time (in seconds) required by a 35g piston for penetration of 5 cm distance, through the 50g gel formulation. Test was performed using ‘Gel strength apparatus modified at laboratory as mentioned by Yong, et al. Polymer solution (50 g) was placed in a 100 ml-measuring cylinder and gelation was induced by SNF. The apparatus for measuring gel strength (weight: 35 g) was then placed on to the gel. The gel strength was measured as the time (in seconds) required for moving the apparatus 5 cm down through the gel. In those cases that took more than 5 min to drop the apparatus into the gel, suitable weights were placed on top of the apparatus and gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel.

**Table 1**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation composition (%W/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimenhydrinate</td>
<td>GG1 2.5 2.5 2.5 2.5</td>
</tr>
<tr>
<td>Gellan gum</td>
<td>0.15 0.3 0.15 0.3</td>
</tr>
<tr>
<td>Mannitol</td>
<td>4 4 4 4</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>0.001 0.001 0.001 0.001</td>
</tr>
<tr>
<td>Ultra pure Water</td>
<td>q.s q.s q.s q.s</td>
</tr>
</tbody>
</table>
Evaluation of the mucoadhesive strength (Murthy et al., 2006)

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method described by Murthy et al. In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughter house. Tissues were immediately used after separation. At the time of testing, a section of nasal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The upper probe was attached to pre calibrated force displacement transducer SS12LA, (BIOPAC Systems Inc.) connected to the Student’s Physiograph apparatus (Medicaid systems, India). The surface area of each exposed mucosal membrane was 4.2 cm². At room temperature, fixed amount of samples of each formulation were placed on the lower probe. The probes were equilibrated and maintained at 37°C. Probe with nasal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.2 gm was applied for 2 minutes to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The mucoadhesive force, expressed as the detachment stress in dyne/cm², was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation (1).

\[
\text{Detachment Stress} = \frac{m \times g}{A}
\]

Where, \(m\) is the weight added to the balance in gram; \(g\) is the acceleration due to gravity taken as 980 cm/s²; and \(A\) is surface area of mucosa tissue in cm²

Before each measurement a fresh

Drug content determination (Balasubramanyam et al., 2003)

The vials containing the preparation were shaken for 2–3 minutes manually and 100 μL of the preparation was transferred to 25-mL volumetric flasks with a micropipette and the final volume was made up with phosphate buffer pH 6.6. Dimenhydrinate concentration was determined at 278 nm (Shimadzu, UV-1700).

In vitro diffusion (Zaki et al., 2007)

In-vitro diffusion study of formulated in situ gels was carried out on Franz diffusion cell having 2.0 cm diameter and 16 mL capacity. Dialysis membrane having molecular weight 12000 – 14000 kDa (HiMedia) was used as diffusion membrane. Dialysis membrane were soaked in phosphate buffer pH 6.6 for 24 hrs prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.6; dialysis membrane was mounted on cell. The temperature was maintained at 34°C. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 2.5 mg of Dimenhydrinate was placed in the donor chamber. Gelation was induced using SNF. At predetermined time intervals, 0.5 mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with pH 6.6 after each sampling, for a period of 300 minutes. The samples withdrawn were filtered and used for analysis. Blank samples (without Dimenhydrinate) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV spectrophotometer at 278 nm.

Results and Discussion

Gelation study

Formulations containing gellan gum were clear while thiolated gellan preparation showed slight turbidity. Gelation studies were carried out using SNF. In these studies the gelling capacity (speed and extent of gelation) for all formulations were determined. After easy instillation in nasal cavity the liquid polymeric solutions should undergo rapid sol to gel transition by means of ionic gelation. Thus the in situ formed gel should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration. Gelation characteristics was assessed on an ordinal scale ranging between ++ and +++ as below (Table2). All formulations (both untreated and thiolated polymer) showed instantaneous gelation, depending upon the polymer concentration.

| Table 2 Physical parameters of gellan gum series formulations. |
|-------------------------|----------------|----------------|----------------|----------------|----------------|
| Formulation | Degree of Gelation | Gel Strength(Seconds) | Drug Content (%) | Mucoadhesion Force(dyne/cm²) | |
| GG1 | ++ | 15 | 95.31 | 172.67 | |
| GG2 | +++ | 25 | 99.48 | 203.71 | |
| GGT1 | ++ | 16 | 95.48 | 317.11 | |
| GGT2 | +++ | 29 | 99.96 | 720.31 | |
Viscosity

The apparent viscosity values were measured for liquid formulations and gels, using Brookfield viscometer DV-E with spindle 3 at 100 rpm. Figure 1 shows the marked increase in viscosity as observed in the formulations of thiolated gellan gum.

Gel strength

In the development of nasal in situ gelling system, the gel strength is important in finding the condition, which allow easy administration as droplets and delay the post nasal drip or anterior leakage. The gel strength was found to be affected by concentrations of gelling polymers (Table 2). Optimal in situ gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosa without leakage after administration. The thiolated gellan gum gel was found to increase the gel strength. The gel strength values between 25 to 50 seconds were considered sufficient. The gel strength duration of less than 25 seconds may not retain its integrity and may erode rapidly while gels having strength greater than 50 seconds are too stiff and may cause discomfort to the mucosal surfaces. GGT and GG formulation series showed the gel strength values in range 23 to 31 seconds which are in acceptable for nasal delivery.

Mucoadhesion

All the formulations were subjected to mucoadhesion studies by the method reported by Murthy et al. Fig. 2 displays the comparison of mucoadhesion of GG1, GG2, GGT1 and GGT2 formulation series. The mucoadhesion force is an important parameter for in situ gelling nasal formulations since it prolongs the nasal clearance of gels and increases its residence time in nasal cavity. The enforcement of the mucoadhesive forces in the nasal in situ gels is believed to be based on formation of disulfide bonds between thiol groups of the thiomers and cysteine rich subdomains of mucus glycoproteins (Leitner et al., 2003). The stronger the bioadhesive force more is the nasal residence time. But if the mucoadhesion is too strong the gel can damage the mucosal membrane. Figure 2 shows comparison of mucoadhesion of all the gellan gum series formulations. GGT2 shows the significant increases in mucoadhesion property as compared to untreated gellan gum formulation.
Drug content determination

Results of drug content are mentioned in Table 2. The drug content of the formulations was ranging from 95.31 to 99.96.

*In vitro* diffusion (Yong et al., 2001)

*In vitro* release studies of formulations were performed using the Franz diffusion cell with dialysis membrane phosphate buffer of pH 6.6 used as diffusion media. Release profiles of GGT and GG formulation series are elaborated in Figure 3.

The release profiles exhibited an inflection point, which indicated the gel formation in the donor compartment of diffusion cell. During gel formation, a portion of drug might be loaded in to the gel matrix, thus the cross linking of polymer reduces the drug release rate. The initial rapid release of dimenhydrinate was may be due to formation of pre hydrated matrix containing water filled pores due to presence of aqueous vehicle. The results showed that the formed gels had the ability to extend the release of dimenhydrinate for the duration of about 180 to 300 min. Formulations of untreated gellan gum, gellan gum (thiolated) showed the complete drug release up to duration of 180 min. Release profiles of formulation GGT2 were found to be slightly rapid than untreated polymer preparation.

*Ex vivo* permeation

Formulation GGT2 was further subjected to *ex vivo* permeation studies on basis of their physical parameter and drug release study using the sheep nasal mucosa. Permeation profiles of GGT2 formulation is shown in Figure 4. The percent drug permeated after 5 hours was found to be 99.96 % as compared to aqueous solution of drug which was found 84.42%. GGT2 shows good permeation.

Histopathological study (Rita et al., 2006).

Photomicrographs of sheep nasal mucosa after the permeation studies were observed for histopathological changes in comparison with the phosphate buffer treated mucosa (Figure 5). The section of mucosa treated with formulation GGT2 showed very slight degeneration of nasal epithelium along with slight erosion. There was increased vascularity in basal membrane and superficial part of sub mucosa as compared with PBS-treated mucosa.

This might be the result of mucoadhesive and permeability enhancing property of thiolated gellan gum in the formulation.

There was no sign of remarkable destructive effect of formulations on the treated nasal mucosa.
Fig. 4 Permeation study of gellan gum series formulation.

Fig. 5 Photomicrograph of sheep nasal mucosa used in mucosal toxicity study of in situ gel formulation (GGT2).

Conclusion
The potential of gel formulation to prolong the residence time at the site of absorption has been attributed to its mucoadhesive property. In the present study attempt was made to enhance mucoadhesiveness of gellan gum by addition of thiol functional group. In situ containing dimenhydrinate based on gellan gum and thiolated gellan gum were developed. They show similar property with respective to viscosity and in-vitro drug diffusion. However, formulations based on thiolated gellan gum are characterized by better mucoadhesiveness and improved permeation profile than gellan gum formulation. On the basis of these preliminary results in situ gel may be considered a promising nasal delivery system based on thiolated gellan gum.

References
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