INCREASING THE EFFECTIVENESS OF CHITOSAN GELS TO PROTECT THE OESOPHAGEAL MUCOSA

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Abstract

The troublesome symptoms of gastro-oesophageal reflux disease are a serious health, medical, and social problem for patients. The main problem is gastro-oesophageal acid reflux. In this study, I investigated hydrogels that could prevent the destruction of the oesophageal mucosa. I investigated the effect of chitosan and poloxamer 407 on the protective properties of gels. The addition of chitosan to all the tested gels increased their pH and dynamic viscosity. Preparations containing 25% poloxamer 407 showed the highest pH. Texture tests showed the effect of the poloxamer concentration on the adhesion performance of the tested gels. The findings suggest that the gels can be used to treat advanced acid reflux.

Keywords: *acid gastro-oesophageal reflux, physiological gastro-oesophageal environment, hydrophilic gels, oesophageal mucosa, anti-inflammatory drugs, oesophageal infections*

Received: 02.05.2023
Accepted: 06.06.2023

1. Introduction

The current recommendations for patients with gastro-oesophageal reflux disease (GERD) are based on alleviating the symptoms of the disease. Hydrogels are designed to protect the oesophageal mucosa from harmful factors. An important aspect of the protective effect of gels is their ability to adhere to the mucosa for a sufficient amount of time. Studies have indicated the possibility of obtaining preparations to help solve this problem [1–9].

In this study, I investigated the influence of poloxamer 407 on the adhesive properties of gels containing chitosan. Poloxamer 407 is a thermosensitive polymer with many advantageous features. This polymer has the ability to increase viscosity, its presence in the gels may increase the adhesiveness of the preparation, and it increases the pH of the preparation. The addition of poloxamer 407 led to gels with a wide pH range. The gels were able to cover the surface of an apparatus simulating the conditions of the oesophagus. Thanks to their adhesive properties, the prepared gels should remain on the mucous membrane of the oesophagus for a prolonged time and protect it from the unfavourable effect of gastric content. These *in vitro* results should be confirmed with *in vivo* investigation, an endeavour for future studies.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: chitosan with a degree of deacetylation of 93.5% and a viscosity of 15 mPa·s, 1% in acetic acid (20°C) (Sea Fisheries Institute, Poland); methylcellulose with a viscosity of 400, 1500, 4000 mPa·s, 2% in water (20°C) (Aldrich Chemical Company Ltd., UK); poloxamer 407 (Sigma-Aldrich Chemie GmbH, Germany); and aqua purificata as required by the Farmacopoeia Poland XII.

2.2. Methods

2.2.1. Preparation of Hydrophilic Gel

Gel preparation consisted of the following stages:

1. *Preparation of gel from methylcellulose.* Gels prepared from methylcellulose (4.0 g) were combined into a homogenous excipient and the weight was adjusted to 100.0 g with distilled water (after subtracting the weight of chitosan added in step 3). To enhance gelation, the mixture was cooled to 5–10°C. The homogenous gel was weighed and enough distilled water was added to obtain the initial mass.
2. *Preparation of gel from methylcellulose and poloxamer 407.* Gels prepared from methylcellulose (4.0 g) and poloxamer 407 (20.0, 23.0, and 25.0 g) were combined into a homogenous excipient and the weight was adjusted to 100.0 g with distilled water (after subtracting the weight of chitosan added in step 3). To enhance gelation, the mixture was cooled to 5–10°C. The homogenous gel was weighed and enough distilled water was added to obtain the initial mass.
3. *Preparation of gel with chitosan.* Chitosan (1.0 g micronised powder) was added to the homogeneous gel. The solution was thoroughly mixed to a homogeneous form and cooled to 5–10°C.

2.2.2. Analytical Methods

2.2.2.1. pH

The potentiometric method was used to measure the pH of each gel. Specifically, a combined electrode integrated into a multifunctional ELMETRON CX-742 (Elmetron,

Poland) device was immersed into the investigated gel at 37°C. All gels were tested three times, and the results are reported as the average of three measurements.

2.2.2.2. Dynamic Viscosity

A Rheotest 2 rotational viscosimeter (Medingen, Germany) was used to determine the rheological properties of the gels. The measurements were made at 37°C in the Ia and IIa range on a K-1 cone with a diameter of 36 mm and a 0.917 fissure. The shear angle was measured using 12 shear rates in the ascending direction and 11 shear rates in the descending direction. All gels were tested three times, and the results are reported as the average of three measurements. The shear stress and viscosity were calculated with the following equations:

- Shear stress for the Ia range: $\tau = c \cdot \alpha_{(1-12)} = 85.0 \cdot \alpha_{(1-12)}$; (1)
- Viscosity for the Ia range: $\eta = \frac{\tau}{D_{(1-12)}} \cdot 100 = \frac{85.0 \cdot \alpha_{(1-12)}}{D_{(1-12)}} \cdot 100$; (2)
- Shear stress for the IIa range: $\tau = c \cdot \alpha_{(1-12)} = 820.2 \cdot \alpha_{(1-12)}$ (3)
- Viscosity for the IIa range: $\eta = \frac{\tau}{D_{(1-12)}} \cdot 100 = \frac{820.2 \cdot \alpha_{(1-12)}}{D_{(1-12)}} \cdot 100.$ (4)

The symbols in the above equations mean the following:
τ [N/m^2] – shear stress;
η [mPa·s] – viscosity;
α [°] – shear angle;
D [1/s] – shear rate.

2.2.2.3. Measurement of Adhesion

A texture profile analysis was performed with a TA.XTPlus Texture Analyser (Stable Micro Systems, UK). A stainless steel probe (P/1S) in the shape of a ball and with a diameter of 2.54 cm was used for the measurements. The following parameters were used: the speed of downward movement of the probe during the test was 0.5 mm/s, the lifting speed of the probe was 10 mm/s, the maximum permissible force was 100 g, the dwell time of the probe in the gel was 10 s, and the height at which the probe was raised above the surface of the gel was 40 mm. The measurement was started by placing the gel in a cylindrical vessel with a transparent plexiglass texturometer. Then, the probe was lowered just above the surface of the gel so that there was direct contact between the gel and the probe (the probe remained in this position for 10 s). After selecting the appropriate parameters of the program, the measurement started. The probe began to rise at a speed of 10 mm/s to a height of 40 mm above the surface of the gel after contact with the surface of the gel. All gels were tested three times at 37°C, and the results are reported as the average of three measurements.

2.2.2.4. Measurement of the Gel's Ability to Coat a Surface

Due to the lack of a suitable measuring device, a previously developed model simulating the conditions in the oesophagus was used [10]. The model is a glass tube 25 cm long, modelled on a water cooler, with a double wall, finished on both sides, and with a wide opening. The model is connected to a thermostat so that water, heated to 37°C (body temperature), can constantly flow through the space between the inner and outer walls. The outer wall of the glass tube has a measuring scale in millimetres. The model is placed in a vertical position using a tripod so that the measurement resembles

physiological conditions. A plastic medical syringe with a scale in millimetres is mounted vertically under the glass tube. It has no piston and the tip is closed, and thus it can collect hydrogel that flows down the tube walls. With a medical syringe, 5 ml of hydrogel was applied to the top of the tube in a uniform motion. The times it took the hydrogel to flow 5, 10, 15, 20, and 25 cm and to the bottom of the tube were recorded. Hydrogel that travelled the entire length of the apparatus was collected in a syringe placed under the glass tube. The total measurement time is 10 min. Next, the volume of hydrogel that drained into the syringe was read or the height on the scale of the glass tube at which the preparation stopped was recorded. The results are presented as the average of three measurements.

3. Results and Discussion

3.1. pH Measurement

Gels containing 4.0% methylcellulose (400, 1500, and 4000 cp) had a pH from 5.96 to 5.73. The addition of 1.0% chitosan increased the pH, with a range from 6.60 to 5.82. The addition of 20%, 23%, 25% poloxamer 407 increased the pH, with a range from 6.23 to 8.68 the gels (compared with the range from 5.96 to 5.73 for gels with just methylcellulose). Finally, the addition of 1.0% chitosan to the gels with methylcellulose and poloxamer 407 increased the pH, with a range from 6.72 to 8.98 (compared with the range of 6.60 to 5.82 for gels with methylcellulose and poloxamer 407) (Table 1).

Table 1. The influence of chitosan on the pH of gels containing 4.0% methylcellulose (MC) and poloxamer 407 (P407).

Gel composition	pH	pH of the gel containing 1.0% chitosan
MC 400 cp	5.96	6.60
MC 1500 cp	5.77	5.98
MC 4000 cp	5.73	5.82
MC 400 cp + 20% P407	6.23	6.72
MC 1500 cp + 20% P407	6.65	6.88
MC 4000 cp + 20% P407	6.89	7.20
MC 400 cp + 23% P407	6.92	7.45
MC 1500 cp + 23% P407	7.69	7.91
MC 4000 cp + 23% P407	7.87	8.61
MC 400 cp + 25% P407	7.93	8.70
MC 1500 cp + 25% P407	8.25	8.65
MC 4000 cp + 25% P407	8.68	8.98

The gels containing methylcellulose and poloxamer 407 have a wide pH range. The pH of the gel increases as the poloxamer 407 concentration increases. The addition of 1.0% chitosan further increases the pH. Some of the preparations, with poloxamer 407 alone as well as with the addition of 1.0% chitosan, show a pH that is higher than the physiological range in the oesophagus (4.0–7.0). Specifically, the gels containing 23% poloxamer 407 with methylcellulose 1500 or 4000 cp and the gels containing 25%

poloxamer 407 and methylcellulose 400, 1500, or 4000 cp have a pH of 7.69 to 8.68. The gel containing 1.0% chitosan, 20% poloxamer 407, and methylcellulose 400 cp, and the gels containing 1.0% chitosan, 23% or 25% poloxamer 407, and methylcelluloses 400, 1500, or 4000 cp show a pH of 7.20–8.98. These preparations could be useful to neutralise high acid reflux.

3.2. Rheological Tests

The gels containing 4.0% methylcellulose 400, 1500, and 4000 cp had a dynamic viscosity of 142–365 mPa·s. The addition of 1.0% chitosan the methylcellulose gels increased the dynamic viscosity to 246–457 mPa·s. The addition of 20%, 23%, and 25% poloxamer 407 to the methylcellulose gels increased the viscosity to 708–843 mPa·s. Finally, the addition of 1.0% chitosan to the gels containing methylcellulose and poloxamer 407 increased the dynamic viscosity to 806–880 mPa·s (Table 2).

Table 2. The influence of chitosan on the dynamic viscosity of gels containing 4.0% methylcellulose (MC) and poloxamer 407 (P407).

Gel composition	Dynamic viscosity [mPa·s]	Dynamic viscosity of the gel containing 1.0% chitosan [mPa·s]
MC 400 cp	142	246
MC 1500 cp	254	328
MC 4000 cp	365	457
MC 400 cp + 20% P407	708	806
MC 1500 cp + 20% P407	720	831
MC 4000 cp + 20% P407	743	840
MC 400 cp + 23% P407	764	833
MC 1500 cp + 23% P407	782	842
MC 4000 cp + 23% P407	804	865
MC 400 cp + 25% P407	815	809
MC 1500 cp + 25% P407	821	827
MC 4000 cp + 25% P407	843	880

Experimental studies have shown that 400 cp, 1500 cp and 4000 cp methylcellulose gels have a specific value of dynamic viscosity. The thermosensitive polymer poloxamer 407 markedly and dose-dependently increases the dynamic viscosity of the gel preparations. The addition of 1% chitosan further increases the dynamic viscosity. This increased dynamic viscosity may help to increase the adhesion of these preparations to the oesophageal mucosa. This feature is very important in protecting the mucosa from the harmful effects of acidic gastric contents to which the oesophageal mucosa could be exposed.

3.3. Adhesion

At 37°C, the work of adhesion of the gels containing 4.0% methylcellulose 400, 1500, and 4000 cp was 39.2–51.9 g/s. The addition of 1.0% chitosan the methylcellulose gels increased the viscosity to 74.1–78.0 g/s. The addition of 20%, 23%, and 25% poloxamer 407 to the methylcellulose gels increased the work of adhesion to 78.7–114.2 g/s. Finally,

the addition of 1.0% chitosan to the gels containing methylcellulose and poloxamer 407 increased the work of adhesion to 86.3–120.3 g/s (Table 3).

A work of adhesion above 5.0 g/s indicates good adhesion. Hence, the work of adhesion of all gel preparations indicate the potential to adhere to the oesophageal mucous membrane. Poloxamer 407 dose-dependently increases the work of adhesion. Moreover, 1.0% chitosan also increases the work of adhesion. Overall, it is possible to obtain gels with high adhesiveness to the oesophageal mucous membrane and with a dynamic viscosity above 100 mPa·s.

Table 3. The influence of chitosan on the work of adhesion of gels containing 4.0% methylcellulose (MC) and poloxamer 407 (P407).

Gel composition	Work of adhesion [g/s]	Work of adhesion of the gel containing 1.0% chitosan [g/s]
MC 400 cp	39.2	74.1
MC 1500 cp	48.3	76.0
MC 4000 cp	51.9	78.0
MC 400 cp + 20% P407	78.7	86.3
MC 1500 cp + 20% P407	82.5	89.5
MC 4000 cp + 20% P407	90.4	95.2
MC 400 cp + 23% P407	95.8	98.5
MC 1500 cp + 23% P407	99.8	102.5
MC 4000 cp + 23% P407	100.3	108.1
MC 400 cp + 25% P407	104.9	111.2
MC 1500 cp + 25% P407	108.6	114.6
MC 4000 cp + 25% P407	114.2	120.3

3.4. Measurement of the Gel's Ability to Coat a Surface

The coating capacity of the gel preparations at 37°C depended on the initial methylcellulose viscosity (400, 1500, and 4000 cp). At 400 cp, 4.5 ml of the gel flowed into the syringe and at 4000 cp, 4.0 ml flowed into the syringe. After the addition of 1.0% chitosan, 2.0 ml of the methylcellulose 400 cp gel and 1.0 ml of the methylcellulose 4000 cp gel flowed into the syringe. The addition of 20%, 23%, and 25% poloxamer 407 reduced the gel outflow to 2.7 to 0.5 ml. Finally, the addition of 1.0% chitosan to the gels containing methylcellulose and poloxamer 407 reduced the gel outflow to 0.0–1.0 ml (Table 4).

These results demonstrate that it is possible to obtain gels with high adhesiveness to the oesophageal mucous membrane. The addition of 1.0% chitosan increases the adhesiveness of the gels. Overall, the addition of chitosan to methylcellulose and poloxamer 407 produces gels that coat the surface of the apparatus without flowing out.

Table 4. The influence of chitosan on the ability of gels containing 4.0% methylcellulose (MC) and poloxamer 407 (P407) to coat a surface.

Gel compositionnents	Surface coating of the gel [cm] after 10 min	Surface coating of the gel containing 1.0% chitosan [cm] after 10 min
MC 400 cp	25.0 + 4.5 ml S	25.0 + 3.0 ml S
MC 1500 cp	25.0 + 4.1ml S	25.0 + 2.5 ml S
MC 4000 cp	25.0 + 4.0 ml S	25.0 + 1.7 ml S
MC 400 cp + 20% P407	25.0 + 2.7 ml S	25.0 + 1.0 ml S
MC 1500 cp + 20% P407	25.0 + 2.0 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 20% P407	25.0 + 1.5 ml S	25.0 + **0.0 ml** S
MC 400 cp + 23% P407	25.0 + 1.9 ml S	25.0 + **0.0 ml** S
MC 1500 cp + 23% P407	25.0 + 1.7 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 23% P407	25.0 + 1.0 ml S	25.0 + **0.0 ml** S
MC 400 cp + 25% P407	25.0 + 1.7 ml S	25.0 + **0.0 ml** S
MC 1500 cp + 25% P407	25.0 + 1.0 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 25% P407	25.0 + 0.5 ml S	25.0 + **0.0 ml** S

Note. 25.0 + 1.0 ml S means the gel coated the entire 25.0 cm length of the apparatus and 1.0 ml of gel was collected in the syringe. S = syringe.

4. Conclusions

This study demonstrated the influence of chitosan and poloxamer 407 on pH, dynamic viscosity, adhesion, and *in vitro* coverage of the tested surface with methylcellulose gel. The gel preparations have a wide pH range suitable to neutralise acidic and very acidic contents that enter the oesophagus. Due to their adhesive properties, the tested gels should remain on the oesophageal mucosa for a long time and protect it from the adverse effects of acidic contents regurgitated from the stomach. In addition, the high dynamic viscosity of the gels containing 1.0% chitosan and poloxamer 407 could provide better adhesion of gels in patients with a damaged oesophageal mucosa. The results show that it is possible to produce a preparation with optimal pharmaceutical properties. These preparations may be of great importance in individualised therapy depending on the severity of acid and very acid reflux. The *in vitro* results need to confirmed with *in vivo* experiments, an endeavour for future research.

5. Acknowledgements

This research was financially supported by the Ministry of Health subvention according to number of SIMPLE SUBZ.D190.23.032 from the IT Simple system of Wroclaw Medical University.

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