CHITOSAN GELS TO PREVENT REFLUX DISEASE

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Abstract

Reflux disease is one of the most frequently diagnosed conditions in developed countries. The problem is both the acidic content of the stomach and the alkaline content of the intestines. In each case, the oesophageal mucosa is destroyed. I analysed hydrogels that prevent irritation of the oesophageal mucosa. Specifically, I investigated the effect of chitosan and sodium alginate on the properties of gels protecting the oesophagus. Gels containing chitosan and 1.0% sodium alginate can be used to treat advanced alkaline reflux. The addition of chitosan to all the tested gels increased their pH and dynamic viscosity, making it possible to neutralise acid reflux. Texture studies showed the influence of chitosan and sodium alginate on the adhesiveness of the tested gels.

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

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1. Introduction

Gastro-oesophageal reflux describes the reverse flow of acidic gastric contents into the oesophagus. Based on the literature, the problem of acid reflux has not been effectively resolved. Among the recommended drugs are agents that reduce the amount of secreted acid and preparations to alleviate the symptoms of the disease. The results of experimental studies show the possibility of obtaining preparations that prevent damage to the oesophageal mucosa as a result of gastro-oesophageal reflux [1–9].

The aim on the work was to investigate the influence sodium alginate on the properties of chitosan-containing gels. I examined the effect of chitosan on the properties of each gel, which had various pH and rheological properties, to determine the optimal preparation. I also determined the dynamic viscosity of the gels. The results showed that it is possible to produce a preparation with optimal pharmaceutical and application properties. The gels could adhere to and cover the surface of an apparatus simulating the conditions of the oesophagus. Due to their adhesive properties, the tested gels should stay on the oesophageal mucosa for a long time and protect it from the adverse effects of gastric or bile contents. The wide range of pH of the investigated gels enables preparing a gel with an optimal pH for the oesophagus. These *in vitro* findings require verification *in vivo*, an endeavour of subsequent 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, Gdynia, Poland); methylcellulose with a viscosity of 400, 1500, and 4000 mPa·s, 2% in water (20°C) (Aldrich Chemical Company Ltd., UK); sodium alginate (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

The 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 sodium alginate.* Gels prepared from methylcellulose (4.0 g) and sodium alginate (0.5, 0.7, and 1.0 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 the 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 was 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 that ranged 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 0.5%, 0.7%, and 1.0% sodium alginate decreased the pH, with a range from 5.95 to 5.41 (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 containing methylcellulose and sodium alginate decreased the pH, with a range from 6.27 to 5.63 (compared with the range from 6.60 to 5.82 for gels that just contained methylcellulose and chitosan) (Table 1).

Table 1. The influence of chitosan on the pH of gels containing 4.0% methylcellulose (MC) and sodium alginate (NaAlg).

Gel composition	pH	pH of the gels 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 + 0.5% NaAlg	5.95	6.27
MC 1500 cp + 0.5% NaAlg	5.79	6.15
MC 4000 cp + 0.5% NaAlg	5.77	6.00
MC 400 cp + 0.7% NaAlg	5.79	5.97
MC 1500 cp + 0.7% NaAlg	5.64	5.88
MC 4000 cp + 0.7% NaAlg	5.60	5.83
MC 400 cp + 1.0% NaAlg	5.59	5.80
MC 1500 cp + 1.0% NaAlg	5.51	5.72
MC 4000 cp + 1.0% NaAlg	5.41	5.63

The results show that it is possible to obtain a variety of preparations with a wide pH range in the case of methycellulose and sodium alginate. The pH of the gels decreases as the sodium alginate concentration increases. All gels with chitosan show a pH in the physiological range of 4.0–7.0 at 37°C. The addition of chitosan produces various

formulations with a wide pH range. Formulations containing 1.0% sodium alginate have the lowest pH, which is an important feature and can be used in the treatment of advanced alkaline reflux. Gels containing 1.0% sodium alginate and chitosan can be used to treat alkaline reflux by mildly neutralising the physiological reaction.

3.2. Rheological Tests

Rheological analysis demonstrated that the gels prepared from 4.0% methylcellulose (400, 1500, and 4000 cp) possessed 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 0.5%, 0.7%, and 1.0% sodium alginate to the methylcellulose gels increased the dynamic viscosity to 250–468 mPa·s. Finally, enrichment of methylcellulose and sodium alginate gels with 1.0% chitosan increased the dynamic viscosity to 289–562 mPa·s (Table 2).

Table 2. The influence of chitosan on the dynamic viscosity of gels containing 4.0% methylcellulose (MC) and sodium alginate (NaAlg).

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 + 0.5% NaAlg	250	289
MC 1500 cp + 0.5% NaAlg	295	345
MC 4000 cp + 0.5% NaAlg	398	467
MC 400 cp + 0.7% NaAlg	278	347
MC 1500 cp + 0.7% NaAlg	343	423
MC 4000 cp + 0.7% NaAlg	452	497
MC 400 cp + 1.0% NaAlg	290	390
MC 1500 cp + 1.0% NaAlg	369	479
MC 4000 cp + 1.0% NaAlg	468	562

The rheological properties of the prepared gels indicate that the addition of sodium alginate dose-dependently increases the dynamic viscosity. The addition of chitosan to the gels containing methylcellulose and sodium alginate preparations further increases the dynamic viscosity. The gels containing 400, 1500, and 4000 cp methylcellulose have different specific dynamic viscosity values. The observed increase in the dynamic viscosity of gels due to the added polymers may have a large impact on the increase in the adhesion of these preparations to the oesophageal mucosa. This feature is very important in protecting the mucosa from the harmful effects of the alkaline content retracted into the oesophagus.

3.3. Adhesion

At 37°C, the gels obtained from 4.0% methylcellulose (400, 1500, and 4000 cp) possessed a work of adhesion of 39.2–51.9 g/s. The addition of 1.0% chitosan the methylcellulose gels increased the work of adhesion to 74.1–78.0 g/s. The addition of 0.5%, 0.7%, and 1.0% sodium alginate to the methylcellulose gels increased the work of adhesion to 52.3–65.8 g/s. Finally, the addition of 1.0% chitosan to the gels containing methylcellulose and sodium alginate increased the work of adhesion to 79.2–92.5 g/s (Table 3).

A work of adhesion above 5.0 g/s indicates good adhesion. The data show that it is possible to obtain gels with high adhesion to the oesophageal mucous membrane. The addition of sodium alginate and chitosan markedly increase the adhesiveness of the gels. Overall, these results show that 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 sodium alginate (NaAlg).

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 + 0.5% NaAlg	52.3	79.2
MC 1500 cp + 0.5% NaAlg	56.9	82.3
MC 4000 cp + 0.5% NaAlg	58.7	88.4
MC 400 cp + 0.7% NaAlg	54.4	81.6
MC 1500 cp + 0.7% NaAlg	57.3	86.3
MC 4000 cp + 0.7% NaAlg	60.4	89.1
MC 400 cp + 1.0% NaAlg	58.6	86.7
MC 1500 cp + 1.0% NaAlg	62.7	89.9
MC 4000 cp + 1.0% NaAlg	65.8	92.5

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

The coating capacity of the gels at 37°C varied depending 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 of the gel flowed into the syringe. After the addition of 1.0% chitosan, 3.0 ml of the methylcellulose 400 cp gel and 1.7 ml of the methylcellulose 4000 cp gel flowed into the syringe (Table 4). The addition of 0.5%, 0.7%, and 1.0% sodium alginate reduced the gel outflow to 3.6 to 2.0 ml. The addition of 1.0% chitosan to the gels containing methylcellulose and sodium alginate further reduced the gel outflow to 2.0 to 0.0 ml.

Taken together, the findings demonstrate that the viscosity of methylcellulose, the sodium alginate concentration, and the presence of chitosan all affect the adhesiveness of the gel to a simulated oesophageal surface. The higher the viscosity of methylcellulose and the sodium alginate concentration, the more gel stays on the surface.

Table 4. The influence of chitosan on the ability of gels containing 4.0% methylcellulose (MC) and sodium alginate (NaAlg) to coat a surface.

Gel composition	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 + 4.1 ml 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 + 0.5% NaAlg	25.0 + 3.6 ml S	25.0 + 2.0 ml S
MC 1500 cp + 0.5% NaAlg	25.0 + 3.1 ml S	25.0 + 1.7 ml S
MC 4000 cp + 0.5% NaAlg	25.0 + 2.9 ml S	25.0 + **0.0 ml** S
MC 400 cp + 0.7% NaAlg	25.0 + 3.3 ml S	25.0 + 1.5 ml S
MC 1500 cp + 0.7% NaAlg	25.0 + 3.0 ml S	25.0 + 0.5 ml S
MC 4000 cp + 0.7% NaAlg	25.0 + 2.4 ml S	25.0 + **0.0 ml** S
MC 400 cp + 1.0% NaAlg	25.0 + 2.6 ml S	25.0 + 0.1 ml S
MC 1500 cp + 1.0% NaAlg	25.0 + 2.1 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 1.0% NaAlg	25.0 + 2.0 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

Chitosan and sodium alginate affect the pH, dynamic viscosity, adhesion, and *in vitro* coverage of methylcellulose gels. The preparations show a wide range of pH, allowing the selection of the optimal gel to neutralise the alkaline contents entering 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 alkaline contents. The results show that it is possible to produce a preparation with optimal pharmaceutical and application properties. These gels can be adapted to the individual needs of patients with alkaline reflux. These *in vitro* findings require *in vivo* verification, which is the endeavour of future research.

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