PHYSICOCHEMICAL PROPERTIES OF GELS WITH CHITOSAN THAT PROTECT THE OESOPHAGEAL MUCOSA

Katarzyna Małolepsza-Jarmołowska

Wroclaw Medical University Department of Drug Form Technology,
Faculty of Pharmacy The 'Silesian Piasts' Memorial Wroclaw Medical University,
Poland, 50-556 Wrocław, Borowska 211A Street, Poland
ORCID: 0000-0002-0127-835X

corresponding author: katarzynamj@poczta.onet.pl

Abstract

The incidence of reflux disease increases with age, regardless of gender. This disease is diagnosed more often in highly developed countries. In addition to acidic reflux, the discharge of alkaline intestinal contents into the oesophagus is a major problem. This study was undertaken to examine whether hydrogels prevent irritation of the oesophageal mucosa. The aim was to investigate the influence of chitosan and hydroxypropylmethylcellulose on the properties of chitosan-containing gels. Preparations containing 4.0% hydroxypropylmethylcellulose showed the lowest pH. These gels could be used to treat advanced alkaline reflux. The addition of chitosan to all tested gels increased their pH and dynamic viscosity. The texture tests showed the effect of the hydroxypropylmethylcellulose concentration on the adhesion work of the tested gels.

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

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1. Introduction

Correct treatment of reflux disease should be based on a reliable diagnosis and examination of the causes of the symptoms. Twenty-four hour pH measurement remains the gold standard in the detection, diagnosis, and quantification of gastro-oesophageal reflux disease. This procedure allows detecting more reflux episodes per day and over a longer duration in patients with reflux disease. Hence, the duration of the symptoms can be determined, including the total time the pH in the oesophagus is <4 or >7. Monitoring the pH enables the correct diagnosis and determination of the type of reflux: acidic or alkaline. Alkaline reflux occurs when alkaline intestinal contents enter the oesophagus. Damage to the mucous membrane may be due to the action of bile salts or pancreatic enzymes. Hydrogels have been designed to protect the mucosal membrane of the oesophagus against damaging factors [1-9].

The aim of the present work was to investigate the influence of chitosan and hydroxypropylmethylcellulose on the properties of chitosan-containing gels. To investigate the problem of alkaline reflux and eliminate unpleasant symptoms for the patient, the most important parameters influencing the properties of the prepared gels were investigated: pH, dynamic viscosity, adhesion, and measurement of the gel surface coverage *in vitro*. The influence of hydroxypropylmethylcellulose and chitosan on the properties of gels was determined. Measurements were also carried out to illustrate the effect of the type of methylcellulose on the adhesion strength of the prepared gels. The prepared gels had different pH and rheological properties. The obtained pH range and dynamic viscosity of the tested gels allow for the selection of the optimal preparation. The prepared gels showed adhesion and the ability to cover the surface of the apparatus, simulating the conditions that prevail in the oesophagus.

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 H_2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England); hydroxypropylmethylcellulose (Sigma-Aldrich Chemie GmbH, Germany); and aqua purificata as required by the Farmacopoeia Poland XII.

2.2. Methods

2.2.1. Preparation of Hydrophilic Gels

Gels were prepared from methylcellulose (4 g) and hydroxypropylmethylcellulose (1, 2, 3, or 4 g). The components were combined into a homogenous excipient and the weight adjusted to 100 g with distilled water (after subtracting the weight of chitosan added in the next stage of preparation). 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.

For gels containing chitosan, 1 g of micronised chitosan powder was added to the homogeneous gel. The whole was thoroughly mixed to a homogeneous form and cooled to 5-10°C.

2.2.2. Analytical Methods

2.2.2.1. pH Measurement

The potentiometric method to measure the pH of the prepared gels. A combined electrode integrated into a multifunctional meter (ELECTRON CX-742) was immersed

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

2.2.2.2. Dynamic Viscosity Measurement

Rheological investigations were performed using a Rheotest 2 rotational viscosimeter (Dresden, Germany). The determinations were performed in the Ia and IIa ranges on a K-1 cone with a diameter of 36 mm and a 0.917 fissure at 37°C. 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 values of the shear stress and viscosity were calculated from measurements at 37°C. The following equations were used:

i. shear stress for range Ia: $\tau = c \times \alpha_{(1\text{-}12)} = 85.0 \times \alpha_{(1\text{-}12)}$

ii. viscosity for range Ia: $\eta = \frac{\tau}{D(1-12)} \times 100 = \frac{85.0 \times \alpha(1-12)}{D(1-12)} \times 100$

iii. shear stress for range IIa: $\tau = c \times \alpha_{(1\text{-}12)} = 820.2 \times \alpha_{(1\text{-}12)}$

iv. viscosity for range IIa: $\eta = \frac{\tau}{D(1-12)} \times 100 = \frac{820.2 \times \alpha(1-12)}{D(1-12)} \times 100$

Meaning of the symbols: τ – shear stress, [N/m^2]; η – viscosity, [mPa*s]; α – shear angle, [°]; D – shear rate, [1/s].

2.2.2.3. Adhesion Measurement

Texture profile analysis (TPA) was performed with an Exponent Stable Micro Systems Texture Analyzer TA.XT. Plus Texture Analyser Stable Micro Systems (England). A probe (P/1S) in the shape of a ball, built of stainless steel and with a diameter of 2.54 cm, was used to perform the measurements. The measurement parameters were as follows: 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 them (the probe remained in this position for 10 s). After selecting the appropriate parameters of the programme, the measurement started. The probe began to rise at a speed of 10 mm/s to the height of 40 mm above the surface of the gel after contact with the gel surface. All gels were tested three times, and the results are reported as the average of three measurements at 37°C.

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

Due to the lack of a suitable measuring device, a model simulating conditions in the oesophagus was constructed [10]. This model comprises a glass tube 25 cm long, modelled on a water cooler, with a double wall, finished on both sides with a wide opening. The model is connected to a thermostat so that water, previously 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 is provided with a measuring scale in millimetres. The model is placed in a vertical position using a tripod so that the measurement resembles physiological conditions the most. A plastic medical syringe with a scale in millimetres is also mounted vertically under the glass tube. It has no piston and the tip is closed, making it possible to collect hydrogel that flows down the walls. With a medical syringe, 5 ml of hydrogel is applied to the top of the tube

in a uniform motion. The times it takes the hydrogel to flow 5, 10, 15, 20, and 25 cm and to the bottom of the tube are recorded. Hydrogel that travels the entire length of the apparatus is collected into a syringe placed under the glass tube. The total measurement time is 10 min. Next, the volume of hydrogel that has drained into the syringe is read or the height on the scale of the glass tube at which the preparation has stopped has been recorded. The results are presented as the average of three measurements.

3. Results and Discussion

3.1. pH Measurement

For the gels containing 4.0% methylcellulose (400 cp, 1500 cp, or 4000 cp), the pH was from 5.96 to 5.73. The addition of 1.0% chitosan reduced the pH, with a range from 6.60 to 5.82 (Table 1).

The addition of 1.0%, 2.0%, 3.0%, or 4.0% hydroxypropylmethylcellulose decreased the pH, with a range from 4.45 to 3.77 (compared with previous range of 5.96-5.73). A modification of the tested gels with of 1.0% chitosan decreased the pH, with a range from 4.68 to 4.40 (compared with the previous range of 6.60-5.82) (Table 1).

Table 1. Influence of chitosan on the pH of gels containing 4.0% methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC).

Gel components	pH of gels with 4.0% MC and HPMC	pH of gels with 4.0% MC, HPMC, and 1.0% chitosan
MC 400cp	5.96	6.60
MC 1500cp	5.77	5.98
MC 4000cp	5.73	5.82
MC 400 cp + 1.0% HPMC	4.45	4.68
MC 1500 cp + 1.0% HPMC	4.32	4.64
MC 4000 cp + 1.0% HPMC	3.38	4.55
MC 400 cp + 2.0% HPMC	4.23	4.58
MC 1500cp + 2.0% HPMC	4.12	4.53
MC 4000cp + 2.0% HPMC	4.10	4.45
MC 400cp + 3.0% HPMC	3.92	4.56
MC 1500cp + 3.0% HPMC	3.86	4.51
MC 4000cp + 3.0% HPMC	3.80	4.49
MC 400cp + 4.0% HPMC	3.87	4.52
MC 1500cp + 4.0% HPMC	3.83	4.50
MC 4000cp + 4.0% HPMC	3.77	4.40

The use of methylcellulose and hydroxypropylmethylcellulose produced formulations with a wide pH range. The pH decreased as the hydroxypropylmethylcellulose concentration increased. The addition of chitosan also produced formulations with a wide pH range, although it was always in the physiological range of 4.0-7.0 at 37°C. Formulations containing 4.0% hydroxypropylmethylcellulose showed the lowest pH, which is an important feature and can be used in the treatment of advanced alkaline reflux. Gels containing 1.0%-3.0% hydroxypropylmethylcellulose and chitosan could be used in the event there is a risk of alkaline reflux.

3.2. Rheological Tests

The gels containing methylcellulose 400 cp, 1500 cp, or 4000 cp possessed a dynamic viscosity from 142 to 365 mPa*s. The addition of 1.0% chitosan increased the viscosity, with a range from 246 to 457 mPa*s (Table 2).

Modifying the gel composition with 1.0%, 2.0%, 3.0%, or 4.0% hydroxypropylmethylcellulose increased the viscosity from 190 to 464 mPa*s (Table 2). Enrichment of these gels with 1.0% chitosan increased the dynamic viscosity, with a range from 210 to 534 mPa*s (Table 2).

Table 2. Influence of chitosan on the viscosity of gels containing 4.0% methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC).

Gel components	Dynamic viscosity of gels with 4.0% MC and HPMC [mPa*s]	Dynamic viscosity of gels with 4.0% MC, HPMC, and 1.0% chitosan [mPa*s]
MC 400cp	142	246
MC 1500cp	254	328
MC 4000cp	365	457
MC 400 cp + 1.0% HPMC	190	210
MC 1500 cp + 1.0% HPMC	290	323
MC 4000 cp + 1.0% HPMC	390	428
MC 400 cp + 2.0% HPMC	215	293
MC 1500cp + 2.0% HPMC	285	356
MC 4000cp + 2.0% HPMC	412	462
MC 400cp + 3.0% HPMC	246	375
MC 1500cp + 3.0% HPMC	320	449
MC 4000cp + 3.0% HPMC	449	515
MC 400cp + 4.0% HPMC	257	394
MC 1500cp + 4.0% HPMC	354	461
MC 4000cp + 4.0% HPMC	464	534

The experiments showed that 400 cp, 1500 cp, and 4000 cp methylcellulose gels have specific dynamic viscosities. The addition of hydroxypropylmethylcellulose increased the dynamic viscosities: as the hydroxypropylmethylcellulose concentration increased, the dynamic viscosity increased. The addition of chitosan further increased the dynamic viscosity. The ability to increase dynamic viscosity based on adding polymers may increase the adhesion of these preparations to the oesophageal mucosa. This feature is crucial in protecting the mucosa from the harmful effects of the alkaline content that enters the oesophagus.

3.3. Adhesion

The adhesion at 37°C of the gels containing methylcellulose 400 cp, 1500 cp, or 4000 cp ranged from 39.2 to 51.9 g/s. The addition of 1.0% chitosan increased the viscosity, with a range from 74.1-78.0 g/s (Table 3).

The addition of 1.0%, 2.0%, 3.0%, or 4.0% hydroxypropylmethylcellulose increased the viscosity to 41.5-49.8 g/s (Table 3). Enrichment with 1.0% chitosan increased dynamic viscosity to 75.6-81.6 g/s (Table 3).

An adhesiveness >5.0 g/s indicates good adhesion. Hence, all the prepared gels showed high adhesion to the oesophageal mucous membrane. The addition of 1.0% chitosan markedly increased adhesion. These results have demonstrated that it is possible to obtain gels with high adhesion to the oesophageal mucous membrane with dynamic viscosity above 100 mPa*s.

Table 3. Influence of chitosan on the work of adhesion of gels containing 4.0% methylcellulose (MC) and hydroxypropylmethylcellulose (HPMC).

Gel components	Work of adhesion of gels with 4.0% MC and HPMC [g/s]	Work of adhesion of gels with 4.0% MC, HPMC, and 1.0% chitosan [g/s]
MC 400cp	39.2	74.1
MC 1500cp	48.3	76.0
MC 4000cp	51.9	78.0
MC 400 cp + 1.0% HPMC	41.5	75.6
MC 1500 cp + 1.0% HPMC	42.8	76.2
MC 4000 cp + 1.0% HPMC	43.2	77.1
MC 400 cp + 2.0% HPMC	42.6	76.2
MC 1500cp + 2.0% HPMC	44.7	78.3
MC 4000cp + 2.0% HPMC	46.9	79.5
MC 400cp + 3.0% HPMC	44.8	78.6
MC 1500cp + 3.0% HPMC	46.5	79.9
MC 4000cp + 3.0% HPMC	48.6	80.5
MC 400cp + 4.0% HPMC	46.8	79.2
MC 1500cp + 4.0% HPMC	48.4	80.8
MC 4000cp + 4.0% HPMC	49.8	81.6

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

The coating capacity depends on the initial methylcellulose viscosity (400 cp, 1500 cp, or 4000 cp). At a viscosity of 400 cp, 4.5 ml of gel flowed into the syringe, while at 4000 cp, 4.0 ml of gel 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 (Table 4).

The addition of 1.0%, 2.0%, 3.0%, or 4.0% hydroxypropylmethylcellulose reduced the gel outflow to 4.3-1.7 ml. The addition of 1.0% chitosan reduced the gel outflow to 2.5-0.1 ml (Table 4).

These results have shown that it is possible to obtain gels with high adhesion to the oesophageal mucous membrane. The addition of hydroxypropylmethylcellulose improved the ability of the gel to adhere to the surface. The addition of 1.0% chitosan showed higher adhesion compared with gels without chitosan. Gels containing methylcellulose 4000 cp along with 3.0% or 4.0% hydroxypropylmethylcellulose are maintained on the surface to be tested and do not flow out (Table 4).

Table 4. Influence of chitosan on the ability of gels containing 4.0% methylcellulose and hydroxypropylmethylcellulose (HPMC) to coat a surface

Gel components	Surface coating of gels with 4.0% MC and HPMC [cm] after 10 min	Surface coating of gels with 4.0% MC, HPMC, and 1.0% chitosan [cm] after 10 min
MC 400cp	25.0 + 4.5 ml S	25.0 + 3.0 ml S
MC 1500cp	25.0 + 4.1ml S	25.0 + 2.5 ml S
MC 4000cp	25.0 + 4.0 ml S	25.0 + 1.7 ml S
MC 400 cp + 1.0% HPMC	25.0 + 4.3 ml S	25.0 + 2.5 ml S
MC 1500 cp + 1.0% HPMC	25.0 + 4.1 ml S	25.0 + 1.9 ml S
MC 4000 cp + 1.0% HPMC	25.0 + 3.9 ml S	25.0 + 1.2 ml S
MC 400 cp + 2.0% HPMC	25.0 + 4.2 ml S	25.0 + 2.1 ml S
MC 1500cp + 2.0% HPMC	25.0 + 4.0 ml S	25.0 + 1.5 ml S
MC 4000cp + 2.0% HPMC	25.0 + 3.6 ml S	25.0 + 0.9 ml S
MC 400cp + 3.0% HPMC	25.0 + 3.8 ml S	25.0 + 0.5 ml S
MC 1500cp + 3.0% HPMC	25.0 + 3.3 ml S	25.0 + 0.1 ml S
MC 4000cp + 3.0% HPMC	25.0 + 3.0 ml S	25.0 + **0.0 ml** S
MC 400cp + 4.0% HPMC	25.0 + 2.7 ml S	25.0 + 0.2 ml S
MC 1500cp + 4.0% HPMC	25.0 + 2.2 ml S	25.0 + 0.1 ml S
MC 4000cp + 4.0% HPMC	25.0 + 1.7 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

Gel preparations with specific pH, dynamic viscosity, adhesion, and the ability to cover the surface imitating the oesophagus *in vitro* conditions were obtained. The research showed the effect of chitosan and hydroxypropylmethylcellulose on pH, dynamic viscosity, adhesion, and *in vitro* coverage of the tested surface with methylcellulose gel. The pH range of the preparations enables the selection of the optimal gel for the neutralisation of 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 against the adverse effects of alkaline contents. This study has shown that it is possible to produce a preparation with optimal pharmaceutical and application properties. Thanks to the obtained pH range, high dynamic viscosity, adhesion, and the ability to cover the tested surface, these gels could be adapted to the individual needs of patients with alkaline reflux. The presented assumptions and *in vitro* tests require *in vivo* verification, which is the subject of future research.

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