PROTECTION OF THE OESOPHAGEAL MUCOSA WITH CHITOSAN GELS

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Abstract

In developed countries, gastro-oesophageal reflux is one of the most frequently diagnosed diseases. Although acid reflux is more well known, alkaline reflux is equally troublesome. In either of the two cases, the oesophageal mucosa is destroyed. The aim of this study was to analyse the possibility of eliminating the problem by using hydrogels containing dextran and chitosan to prevent irritation of the oesophageal mucosa. The addition of chitosan to all tested gels increased their dynamic viscosity, enabling better adhesion and, consequently, better protection of the mucous membrane. The addition of dextran reduced the pH of the tested gels, which allowed for the neutralisation of alkaline reflux. Based on the texture tests, chitosan and dextran increased work of adhesion.

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

Received: 01.04.2024
Accepted: 05.07.2024

1. Introduction

Gastro-oesophageal reflux disease continues to be a common disease. Indeed, based on the available literature, acid and alkaline reflux have yet to be solved effectively. It is estimated that up to half of adults complain about symptoms of this disease. A potential treatment for this disease is the use of protective gels that can adhere to the mucous membrane for a long time. The tested hydrogels are designed to protect the oesophageal mucosa against harmful factors [1–9].

The aim of the study was to investigate the effect of dextran on the properties of chitosan-containing hydrogels. First, the effect of chitosan on the physicochemical properties of the tested gels was examined. The prepared hydrogels showed a range of pH and rheological properties. Based on the *in vitro* tests, the gels can be assumed to remain at the site of application to protect the oesophageal mucosa against the irritating effects of alkaline reflux. The texture tests showed the influence of dextran concentration on the adhesion of the hydrogels. Moreover, the wide pH range of the hydrogels would allow one to select an optimal hydrogel for each patient. These *in vitro* findings require clinical confirmation.

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%, viscosity of 15 mPa·s, 1% in acetic acid (20°C) (Sea Fisheries Institute, Poland); methylcellulose with a viscosity of 400, 1500 and 4000 mPa·s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd., England); dextran with a molecular weight of 40000 (Sigma-Aldrich Chemie GmbH, Germany); and aqua purificata as required by Farmacopoeia Poland XII.

2.2. Methods

2.2.1. Preparation of Hydrogels

The gel preparation steps were:

1. Methylcellulose (4.0 g) and dextran (0.25, 0.5 or 1.0 g) were combined into a homogeneous mixture. Then, distilled water was added to reach a final mass of 100.0 g (accounting for the mass of chitosan that will be added in step 2). The mixture was cooled to 5–10°C. The resulting homogeneous gel was weighed. The amount of distilled water necessary to obtain the starting mass was added.
2. Chitosan was added to the homogenous gel (1.0 g of micronised powder). The gel was mixed thoroughly. After obtaining a homogeneous mixture, the preparation was cooled to 5–10°C.

2.2.2. Analytical Methods

2.2.2.1. pH Measurement

A potentiometric method was used to measure the pH of the prepared hydrogels at 37°C by immersing an electrode integrated with the CX-742 multifunctional multimeter (ELMETRON, Poland). pH was measured three times; the final result is the average of the three measurements.

2.2.2.2. Dynamic Viscosity

A Rheotest 2 rotational viscometer (Medingen, Germany) was used to determine the rheological properties of the prepared hydrogels. The measurements were carried out in the range Ia and IIa. A K-1 cone with a diameter of 36 mm was also used.

The measurement gap was 0.917 cm at 37°C. The shear angle was measured using 12 shear rates in the increasing direction and 11 shear rates in the decreasing direction. The measurements were carried out three times at 37°C. The result is the average of the three measurements. The dynamic viscosity and shear stress were calculated from the results of the measurements using equations (1)–(4):

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

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

2.2.2.3. Measurement of Adhesion

The texture of the prepared hydrogels was examined using the Exponent TA.XT Texture Analyzer. Plus System (Stable Micro Systems, UK). The measurement tool was a ball-shaped probe (P/1S) made of stainless steel with a diameter of 2.54 cm. The following parameters were used: the speed of the probe was 0.5 mm/s; the probe lifting speed was 10 mm/s; and the height at which the probe was raised was 40 mm. During the test, the maximum allowable force was 100 g, and the probe stayed in the gel for 10 s. The measurement was started by placing the gel in a transparent cylindrical plexiglass vessel. Then, the probe was lowered above the gel surface until it contacted it directly; this contact lasted for 10 s. After selecting the appropriate program parameters, the probe detached from the gel surface and began to rise to a height of 40 mm at 10 mm/s. The preparations were tested three times at 37°C, and the results are presented as the average of three measurements.

2.2.2.4. Measurement of the Ability of the Hydrogel to Coat a Surface

Due to the lack of availability of an appropriate measuring device, a model was constructed to simulate the physiological conditions in the oesophagus [10]. It is a 25-cm-long glass tube, modelled on a water cooler, with double walls and ending with a wide opening on both sides. The entire device is thermostated. Water is maintained at 37°C, the temperature of the human body, through continuous heating and flows constantly between the inner and outer walls of the model. The outer wall of the glass tube is equipped with a measurement scale in millimetres. A plastic medical syringe is placed vertically under the mouth of the glass tube. The syringe has a scale in millimetres on its surface. The plunger is removed from the syringe and its tip is closed with a cap. The hydrogel flowing down the glass walls of the model can be collected in a syringe. With a medical syringe, 5 ml of the prepared hydrogel was applied to the top of the tube in a uniform motion. The time it took the hydrogel to flow 5, 10, 15, 20, and 25 cm and to the bottom of the tube was 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. 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

Table 1 presents the pH of the prepared hydrogels. The hydrogels containing 4.0% methylcellulose (400, 1500 or 4000 cp) showed an initial pH range of 5.96 to 5.73. After adding 1.0% chitosan, the pH increased, with a range of 6.60 to 5.82. The addition of dextran (0.25%, 0.5% or 1.0%) decreased the pH, with a range from 5.09 to 4.46 (compared to the previous range of 5.96 to 5.73). Finally, adding both 1.0% chitosan and dextran reduced the pH, with a range of 5.36 to 4.68 (compared with the previous range of 6.60 to 5.82).

Table 1. The influence of chitosan on the pH of hydrogels containing 4.0% methylcellulose (MC) and dextran.

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 + 0.25% dextran	5.09	5.36
MC 1500 cp + 0.25% dextran	4.79	5.28
MC 4000 cp + 0.25% dextran	4.69	5.15
MC 400 cp + 0.5% dextran	4.82	5.25
MC 1500 cp + 0.5% dextran	4.78	4.99
MC 4000 cp + 0.5% dextran	4.64	4.85
MC 400 cp + 1.0% dextran	4.65	4.82
MC 1500 cp + 1.0% dextran	4.53	4.74
MC 4000 cp + 1.0% dextran	4.46	4.68

The methylcellulose gels enriched with chitosan had a wide pH range (from 4.0 to 7.0) at 37°C, which allows for the selection of hydrogels with physicochemical properties for specific applications. The introduction of dextran reduced the pH. There was a relationship between the dextran concentration and pH: the higher the dextran concentration, the lower the pH. Consistently, gels containing the highest dextran concentration (1.0%) showed the lowest pH. These gels could be used to neutralise the alkaline content in a gentle way, bringing the pH to the physiological level.

3.2. Rheological Tests

Table 2 shows the rheological test results. The hydrogels prepared with methylcellulose (400, 1500 or 4000 cp) showed a dynamic viscosity of 142–365 mPa·s. The addition of 1.0% chitosan increased the dynamic viscosity to 246–457 mPa·s. The addition of dextran (0.25%, 0.5% or 1.0%) also increased the dynamic viscosity to 202–348 mPa·s. Finally, the addition of 1.0% chitosan and dextran (0.25%, 0.5% or 1.0%) increased the dynamic viscosity to 268–540 mPa·s.

Table 2. The influence of chitosan on the dynamic viscosity of hydrogels containing 4.0% methylcellulose (MC) and dextran.

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 + 0.25% dextran	202	268
MC 1500 cp + 0.25% dextran	259	354
MC 4000 cp + 0.25% dextran	378	469
MC 400 cp + 0.5% dextran	239	279
MC 1500 cp + 0.5% dextran	265	378
MC 4000 cp + 0.5% dextran	284	480
MC 400 cp + 1.0% dextran	256	325
MC 1500 cp + 1.0% dextran	299	420
MC 4000 cp + 1.0% dextran	348	540

Dextran was added to the gels in an attempt to increase the dynamic viscosity so that the preparations could be used to protect the oesophageal mucosa. The dynamic viscosity of the gels increased as the dextran concentration increased. The addition of chitosan further increased the dynamic viscosity. The increased dynamic viscosity could enhance the ability of the preparation to adhere to the oesophageal mucosa and thus protect it against the harmful effects of alkaline food content.

3.3. Adhesion

Table 3 presents the work of adhesion results. At 37°C, the hydrogels prepared with methylcellulose (400, 1500 or 4000 cp) had a work of adhesion of 39.2–51.9 g/s. The addition of 1.0% chitosan increased the work of adhesion to 74.1–78.0 g/s. Moreover, the addition of dextran (0.25%, 0.5% or 1.0%) increased the work of adhesion to 48.6–66.2 g/s. Finally, the hydrogels containing 1.0% chitosan and 1% dextran had the highest work of adhesion: 82.5–90.0 g/s (Table 3).

Table 3. The influence of chitosan on the work of adhesion of hydrogels containing 4.0% methylcellulose (MC) and dextran.

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 + 0.25% dextran	48.6	82.5
MC 1500 cp + 0.25% dextran	59.1	84.2
MC 4000 cp + 0.25% dextran	59.9	86.9
MC 400 cp + 0.5% dextran	50.6	83.8
MC 1500 cp + 0.5% dextran	61.9	87.5
MC 4000 cp + 0.5% dextran	64.9	88.6
MC 400 cp + 1.0% dextran	59.4	85.2
MC 1500 cp + 1.0% dextran	63.2	88.0
MC 4000 cp + 1.0% dextran	66.2	90.0

A work of adhesion value above 5.0 g/s is indicative of good adhesion. All hydrogels showed good work of adhesion, especially the gels containing chitosan and dextran, indicating the ability to adhere to the oesophageal mucosa. The results showed that it is possible to obtain gels with high adhesive properties to the oesophageal mucosa, with a dynamic viscosity above 100 mPa·s.

3.4. Measurement of the Ability of the Hydrogel to Coat a Surface

At 37°C, the ability of the prepared hydrogels to coat a surface depended on the initial methylcellulose viscosity (400, 1500 or 4000 cp). At 400 cp, 4.5 ml flowed into the syringe; however, at 4000 cp, 4.0 ml flowed into the syringe. After adding 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. The addition of dextran (0.25%, 0.5% or 1.0%) further reduced the amount of gel that flowed into the syringe, with a range of 3.0 to 1.1 ml. Finally, the addition of 1.0% chitosan reduced the amount of gel that flowed into the syringe to 2.2 to 0.0 ml. The hydrogels containing 1% chitosan, methylcellulose 4000 cp and 0.5% or 1.0% dextran; 1% chitosan, methylcellulose 1500 cp and 0.5% or 1.0% dextran; or 1% chitosan, methylcellulose 400 cp and 1.0% dextran remained entirely on the test surface (Table 4).

Table 4. The influence of chitosan on the ability of hydrogels containing 4.0% methylcellulose (MC) and dextran to coat a surface.

Gel composition	Surface coating of the gel [cm] after 10 min	Surface coating of the gels 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 + 0.25% dextran	25.0 + 3.0 ml S	25.0 + 2.2 ml S
MC 1500 cp + 0.25% dextran	25.0 + 2.7 ml S	25.0 + 1.5 ml S
MC 4000 cp + 0.25% dextran	25.0 + 2.0 ml S	25.0 + 0.9 ml S
MC 400 cp + 0.5% dextran	25.0 + 2.6 ml S	25.0 + 0.5 ml S
MC 1500 cp + 0.5% dextran	25.0 + 2.1 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 0.5% dextran	25.0 + 1.3 ml S	25.0 + **0.0 ml** S
MC 400 cp + 1.0% dextran	25.0 + 2.0 ml S	25.0 + **0.0 ml** S
MC 1500 cp + 1.0% dextran	25.0 + 1.7 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 1.0% dextran	25.0 + 1.1 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. Abbreviation: S, syringe.

4. Conclusions

This study produced hydrogels that could neutralise the alkaline content that irritates the oesophagus. Based on an *in vitro* model, these gels have an excellent adhesion ability and could remain on the oesophageal mucosa for a long time. There was a wide range of dynamic viscosity, allowing for the selection of the appropriate preparation. These results may lead to significant improvements in the treatment of alkaline reflux, including the ability to personalise treatment. The next goal will be to validate the *in vitro* with *in vivo* experiments.

5. Acknowledgements

This research was financially supported by the Ministry of Health subvention from the IT Simple System of Wroclaw Medical University (SIMPLE SUBZ.D190.24.070).

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