INFLUENCE OF PECTIN AND CHITOSAN ON THE PROPERTIES OF GELS PROTECTING THE OESOPHAGEAL MUCOSA

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Abstract

Among the diseases of the digestive tract, gastro-oesophageal reflux is one of the most troublesome ailments. It is estimated that in highly developed countries, reflux symptoms occur in about 5%-10% of people every day. It has also been found that about 20% of people experience such symptoms once a week. The incidence of this disease increases with age, regardless of gender. The aim of the study was to investigate the physicochemical properties of gels intended for the protection of the oesophageal mucosa. Preparations containing 3.0% pectin showed the lowest pH. These gels can be used in the treatment of advanced alkaline reflux. The addition of chitosan to all tested gels increased their pH and dynamic viscosity. The texture tests showed the effect of pectin concentration on the adhesion work of the tested gels.

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

Received: 30.04.2021
Accepted: 23.06.2021

1. Introduction

Modern pharmacotherapy of gastro-oesophageal reflux disease is aimed at preventing symptoms and treating the disease. This condition is caused by the stomach acid flowing backwards into the oesophagus. This phenomenon can damage the oesophageal mucosa and, consequently, lead to serious health-threatening complications. Among the recommended drugs are agents that reduce the amount of acid secretion and preparations that alleviate the symptoms of the disease. Hydrogels are designed to protect the oesophageal mucosa from harmful factors. The occurrence of these ailments more than once a week is a necessary condition for the term gastro-oesophageal reflux disease. Another criterion for classification is the reaction of the content causing the symptoms of the disease. If it is caused by the acidic contents of the stomach, this form is called acid reflux. On the other hand, alkaline reflux occurs when alkaline intestinal contents enter the oesophagus. In this case, damage to the mucosa may be caused by the action of bile salts or pancreatic enzymes [1-9].

The aim of this study was to investigate the physicochemical properties of gels intended for the protection of the oesophageal mucosa. The most important parameters influencing the properties of the tested gels were investigated: pH, dynamic viscosity, adhesion and measurement of surface coverage with *in vitro* gel. The influence of pectin and chitosan on the properties of gels was investigated. Measurements were also made to illustrate the effect of the type of methylcellulose on the adhesion strength of the prepared gels. Preparations with different pH and rheological properties were prepared. On the basis of the conducted research, the dynamic viscosity of the gels was determined. The pH range of the gels allows one to choose the optimal preparation. The gels showed adhesion and the ability to cover the surface of the apparatus, simulating the conditions prevailing in the oesophagus. The gels had a specific dynamic viscosity.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: chitosan with a deacetylation degree of 93.5%, viscosity of 15 mPa*s, 1% in acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland); methylcellulose with a viscosity of 4000 mPa*s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd., Gillingham, England); citrus-apple pectin Sigma-Aldrich Chemie GmbH, Germany); and aqua purificata as required by FP XII.

2.2. Apparatuses

- pH meter (CX 742, Elmetron, Poland)
- Viscosimeter Rheotest (2 MLW, Medingen, Dresden, Germany)
- TA.XT. Plus Texture Analyser (Stable Micro Systems, England)
- Device simulating conditions in the oesophagus [10]

2.3. Methods

2.3.1. Preparation of Hydrophilic Gel

The preparation of gel consisted of two stages (1) preparation of gels from methylcellulose and citrus-apple pectin and (2) preparation of gel with chitosan.

For the first step, gels prepared from methylcellulose (4.0g) and citrus-apple pectin (1.0, 2.0 or 3.0 g) 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 the process of 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 the second step, chitosan (1.0 g) was added to the homogeneous gel. The solution was mixed thoroughly and then incubated at 5-10°C.

https://doi.org/10.15259/PCACD.26.014

2.3.2. Analytical Methods

2.3.2.1. pH Measurement

The potentiometric method was used to measure the pH of each gel. Specifically, a combined electrode integrated into an ELECTRON CX-742 multifunctional computer meter 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.

2.3.2.2. Dynamic Viscosity Measurement

Rheological investigations were performed using a Rheotest 2 rotational viscosimeter. 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 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 using the following equations:

- shear stress for the range Ia:

$$\tau = c \times \alpha_{(1\text{-}12)} = 85.0 \times \alpha_{(1\text{-}12)}$$

- viscosity for the range Ia:

$$\eta = \frac{\tau \qquad \tau}{D(1-12)D(1-12)} \cdot 100 = \frac{85.0 \cdot \alpha(1-12)85.0 \cdot \alpha(1-12)}{D(1-12) \qquad D(1-12)} \times 100$$

- shear stress for the range IIa:

$$\tau = c \cdot \alpha_{(1\text{-}12)} = 820.2 \cdot \alpha_{(1\text{-}12)}$$

- viscosity for the range IIa:

$$\eta = \frac{\tau \qquad \tau}{D(1-12)D(1-12)} \cdot 100 = \frac{820.2 \cdot \alpha(1-12)820.2 \cdot \alpha(1-12)}{D(1-12) \qquad D(1-12)} \times 100.$$

For the above equations, τ is the shear stress (N/m^2), η is the viscosity (mPa*s), α is the shear angle and D is the shear rate (1/s).

2.3.2.3. Measurement of Adhesion

Texture profile analysis was performed with the TA.XT. Plus Texture Analyser. To perform the measurements, a probe (P/1S) in the shape of a ball, built of stainless steel and with a diameter of 2.54 cm was used. The measurement parameters were as follows: 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 at 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, and the results are reported as the average of three measurements at 37°C.

https://doi.org/10.15259/PCACD.26.014

2.3.2.4. Measurement of the Ability to Coat a Surface With a Gel

Due to the lack of a suitable measuring device, a model simulating conditions in the oesophagus was constructed [10]. The model simulating the *in vivo* conditions of the oesophagus is 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 most resembles physiological conditions. 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 in it a hydrogel tube that flows down the walls. To measure the ability of the preparation to move, 5 ml of hydrogel was applied in a uniform motion to the inner wall of the tube with a separate medical syringe. The experiment lasted 10 minutes; the times the hydrogel took to reach 5, 10, 15, 20 and 25 cm as well as the bottom of the apparatus were recorded. The hydrogel was collected into a syringe placed under the glass tube. The volume of hydrogel that had drained into the syringe was read or the height on the scale of the glass tube at which the preparation stopped was been recorded. The results of measurements are the average of three measurements.

3. Results and Discussion

3.1. pH Measurement

The pH of the gels containing 4.0% methylcellulose (400, 1500 and 4000 cp) ranged from 5.96 to 5.73. The addition of 1.0% chitosan increased the pH from 6.60 to 5.82 (Table 1). The addition of 1.0%, 2.0% or 3.0% citrus-apple pectin decreased the pH of the gels from 4.17 to 4.06 (compared with the previous range of 5.96 to 5.73). A modification of the composition of the tested gels with 1.0% chitosan decreased the pH from 4.67 to 4.50 (compared with the previous range of 6.60 to 5.82; Table 1).

Table 1. Influence of chitosan on the pH of gels containing 4.0% methylcellulose and citrus-apple pectin

Gels with 4.0% MC and pectin	pH of gels with 4.0% MC and pectin	pH of gels with 4.0% MC, pectin and 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 + 1.0% pectin	4.17	4.67
MC 1500 cp + 1.0% pectin	4.14	4.60
MC 4000 cp + 1.0% pectin	4.08	4.50
MC 400 cp + 2.0% pectin	4.10	4.52
MC 1500 cp + 2.0% pectin	4.11	4.60
MC 4000 cp + 2.0% pectin	4.15	4.66
MC 400 cp + 3.0% pectin	4.00	4.58
MC 1500 cp + 3.0% pectin	4.04	4.60
MC 4000 cp + 3.0% pectin	4.06	4.64

Abbreviations: MC, methylcellulose; pectin, citrus-apple pectin

The use of methylcellulose and citrus-apple pectin produced various formulations with a wide pH range. The pH decreased as the concentration of citrus-apple pectin increased in the gels (compared with the previous range of 5.96 to 5.73). All gels with chitosan showed a pH in the physiological range of 4.0-7.0 at 37°C. The addition of chitosan produced various formulations with a wide pH range. Formulations containing 3.0% citrus-apple pectin showed the lowest pH, which is an important feature and could be used in the treatment of advanced alkaline reflux. Gels containing 1.0%-2.0% citrus-apple pectin and chitosan could be used in the treatment of acid reflux.

3.2. Rheological Tests

Rheological studies demonstrated that the gels obtained from methylcellulose 400, 1500 and 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). A modification of the composition of the tested gels with 1.0%, 2.0% or 3.0% pectin increased the viscosity of the gels, with a range from 150 to 450 mPa*s (Table 2). The enrichment of the composition of the tested gels with 1.0% chitosan increased the dynamic viscosity of the pectin gels, with a range from 237 to 549 mPa*s (Table 2).

The rheological investigations revealed an increase in the dynamic viscosity of preparations with citrus-apple pectin compared with the gels without citrus-apple pectin. The dynamic viscosity increased as the citrus-apple pectin concentration increased. The addition of chitosan notably increased the dynamic viscosity of the tested gels.

3.3. Adhesion Tests

The adhesiveness of the gels at 37°C was examined. The gels obtained from methylcellulose (400, 1500 and 4000 cp) possessed a work of adhesion from 39.2 to 51.9 g/s.

Table 2. Influence of chitosan on the viscosity of gels containing 4.0% methylcellulose and citrus-apple pectin

Gels with 4.0% MC and pectin	**Dynamic viscosity of gels with 4.0% MC and pectin (mPa*s)**	**Dynamic viscosity of gels with 4.0% MC, pectin and 1.0% chitosan (mPa*s)**
MC 400 cp	142	246
MC 1500 cp	254	328
MC 4000 cp	365	457
MC 400 cp + 1.0% pectin	150	237
MC 1500 cp + 1.0% pectin	261	370
MC 4000 cp + 1.0% pectin	359	454
MC 400 cp + 2.0% pectin	189	300
MC 1500 cp + 2.0% pectin	256	354
MC 4000 cp + 2.0% pectin	380	472
MC 400 cp + 3.0% pectin	275	389
MC 1500 cp + 3.0% pectin	341	450
MC 4000 cp + 3.0% pectin	450	549

Abbreviations: MC, methylcellulose; pectin, citrus-apple pectin

The addition of 1.0% chitosan increased the viscosity, with a range from 74.1 to 78.0 g/s (Table 3). A modification of the composition of the tested gels with 1.0%, 2.0% or 3.0% citrus-apple pectin increased the viscosity of the gels, with a range from 40.0 to 53.7 g/s (Table 3). The enrichment of the composition of the tested citrus-apple pectin gels with 1.0% chitosan resulted in increased dynamic viscosity, with a range from 76.7 to 90.6 g/s (Table 3).

An adhesiveness above 5.0g/s indicates good adhesion. The results showed that it is possible to obtain gels with high adhesion to the oesophageal mucous membrane. The gels with 1.0%, 2.0% or 3.0% citrus-apple pectin showed good adhesion. The gels with 1.0% chitosan showed markedly greater adhesion compared with gels without chitosan. The present study has shown that it is possible to obtain gels with high adhesiveness to oesophageal mucous membrane, with a dynamic viscosity above 100 mPa*s.

3.4. Measurement of the Ability to Coat a Surface With a Gel

The gels were tested for their ability to coat a surface at 37°C. The coating capacity depended on the initial methylcellulose viscosity. At a viscosity of 400 cp, 4.5 ml of the gel flowed out of the apparatus and at 4000 cp, 4.0 ml flowed out of the apparatus. After the addition of 1.0% chitosan, 2.0 ml flowed out of the apparatus for the 400 cp methylcellulose gel and 1.0 ml flowed out of the apparatus for the 4000 cp methylcellulose gel (Table 4). Modification of the composition of the tested gels with the addition of 1.0%, 2.0% or 3.0% citrus-apple pectin reduced the gel outflow, with a range from 4.2 to 1.9 ml. The addition of 1.0% chitosan reduced the gel outflow, with a range from 1.6 to 0.1 ml (Table 4).

Based on these findings, it is possible to obtain gels with high adhesion to the oesophageal mucous membrane. Examination of the ability to coat the surface with gel

Table 3. Influence of chitosan on the adhesiveness of gels containing 4.0% methylcellulose and citrus-apple pectin

Gels with 4.0% MC and pectin	Work of adhesion of gels with 4.0% MC and addition pectin (g/s)	Work of adhesion of gels with 4.0% MC, pectin and 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 + 1.0% pectin	40.0	76.7
MC 1500 cp + 1.0% pectin	41.4	78.9
MC 4000 cp + 1.0% pectin	42.6	80.3
MC 400 cp + 2.0% pectin	43.4	79.1
MC 1500 cp + 2.0% pectin	45.9	82.9
MC 4000 cp + 2.0% pectin	48.3	84.3
MC 400 cp + 3.0% pectin	46.5	82.8
MC 1500 cp + 3.0% pectin	49.8	86.5
MC 4000 cp + 3.0% pectin	53.7	90.6

Abbreviations: MC, methylcellulose; pectin, citrus-apple pectin

Table 4. Influence of chitosan on ability of gel to coat a surface with a gel containing 4.0% methylcellulose and citrus-apple pectin

Gels with 4.0% MC and pectin	Surface coating of gels with 4.0% MC and pectin after 10 min (cm)	Surface coating of gels with 4.0% MC, pectin and 1.0% chitosan after 10 min (cm)
MC 400 cp	25.0 + 4.5 ml S	25.0 + 2.0 ml S
MC 1500 cp	25.0 + 4.1ml S	25.0 + 1.5 ml S
MC 4000 cp	25.0 + 4.0 ml S	25.0 + 1.0 ml S
MC 400 cp + 1.0% PVP K-30 pectin	25.0 + 4.2 ml S	25.0 + 1.6 ml S
MC 1500 cp + 1.0% PVP K-30 pectin	25.0 + 3.3 ml S	25.0 + 1.4 ml S
MC 4000 cp + 1.0% PVP K-30 pectin	25.0 + 3.0 ml S	25.0 + 0.0 ml S
MC 400 cp + 3.0% PVP K-30 pectin	25.0 + 3.6 ml S	25.0 + 0.9 ml S
MC 1500 cp + 3.0% PVP K-30 pectin	25.0 + 3.4 ml S	25.0 + 0.2 ml S
MC 4000 cp + 3.0% PVP K-30 pectin	25.0 + 2.8 ml S	25.0 + 0.0 ml S
MC 400 cp + 5.0% PVP K-30 pectin	25.0 + 2.4 ml S	25.0 + 0.2 ml S
MC 1500 cp + 5.0% PVP K-30 pectin	25.0 + 2.1 ml S	25.0 + 0.1 ml S
MC 4000 cp + 5.0% PVP K-30 pectin	25.0 + 1.9 ml S	25.0 + 0.0 ml S

Abbreviations: MC, methylcellulose; pectin, citrus-apple pectin; S, syringe; 25.0 + 1.0 ml S means the gel coated the entire 25.0-cm length of the syringe and 1.0 ml flowed out and was collected in the syringe

showed that citrus-apple pectin and their concentration affect the ability of the gel to adhere to the surface. Gels with 1.0% chitosan showed high adhesion compared with gels without chitosan (Table 4).

Taken together, this study showed the effect of pectin and chitosan on pH, dynamic viscosity and adhesiveness of methylcellulose gels. These gels have the ability to coat an *in vitro* surface. It is possible to produce a formulation with optimal pharmaceutical and application properties. Due to the wide pH range, high dynamic viscosity, adhesion and the ability to cover the test surface, these gels can be adjusted to the individual needs of patients.

4. Conclusions

The research showed the effect of citrus-apple pectin and chitosan on the pH, dynamic viscosity, adhesiveness and *in vitro* coverage of the tested surface with methylcellulose gel. The obtained preparations had a pH in the desired physiological range, high dynamic viscosity, adhesion and the ability to cover the tested surface. The results have shown that it is possible to produce a preparation with optimal pharmaceutical and application properties. Due to their adhesive properties, the tested gels should stay on the oesophageal mucosa for a long time and protect it against the adverse effects of gastric or bile contents. The wide pH range of the tested gels enables the selection with the optimal pH for the oesophagus depending on the type of reflux. The presented data and *in vitro* tests require *in vivo* verification, which is the purpose of further research.

5. Acknowledgements

This research was financially supported by the Ministry of Health according to the grant SIMPLE SUB.D190.21.098 from the IT Simple system of Wroclaw Medical University.

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