INFLUENCE OF CARBOXYMETHYLCELLULOSE SODIUM SALT ON THE PROTECTIVE PROPERTIES OF GELS WITH CHITOSAN FOR THE OESOPHAGEAL MUCOSA

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Abstract

Gastro-oesophageal reflux disease is a serious social problem that affects every sphere of life. The quality of life of people with this disease is reduced due to the presence of troublesome symptoms, which translates into reduced work efficiency and vitality. The major problem is acidic gastro-oesophageal reflux. This study was undertaken to examine whether hydrogels prevent irritation of the oesophageal mucosa. The aim was to investigate the influence of chitosan and carboxymethylcellulose sodium salt on the protective properties of prepared gels in the treatment of acid reflux. The addition of chitosan to all tested gels increased their pH and dynamic viscosity. Preparations containing 0.3% carboxymethylcellulose sodium salt showed the highest pH. The texture tests showed the effect of carboxymethylcellulose sodium salt concentration on the adhesion work of the tested gels. These gels could be used in the treatment of advanced acid reflux.

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

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1. Introduction

Symptoms of gastro-oesophageal reflux disease appear due to various factors. Thus, even in the case of quick diagnostics and properly conducted treatment, a significant portion of afflicted patients do not experience a clear improvement. Gastro-oesophageal reflux disease is associated with an abnormality in the lower oesophageal sphincter. Various factors result in the regurgitation of gastric contents into the oesophagus, resulting in unpleasant symptoms and serious complications. Gastro-oesophageal reflux disease is a serious social problem that affects every sphere of life. The quality of life of sick people is reduced due to the presence of troublesome symptoms, which translates into reduced work efficiency and vitality. Gastro-oesophageal reflux disease is a frequently diagnosed gastroenterological disease and in highly developed countries, it affects 20%-40% of the adult population. Children are also increasingly affected by this problem. Intensive research has been conducted for many years to find an effective drug for gastro-oesophageal reflux disease that provides long-term relief to patients and reduces the risk of serious complications. Monitoring the pH enables the correct diagnosis and determination of the type of reflux: acidic or alkaline. Acid reflux occurs in the case when acidic gastric contents enter the oesophagus, causing damage to the mucous membrane. Hydrogels have been designed to protect the mucosal membrane of the oesophagus against damaging factors [1-9].

The problem of acid reflux has still not been successfully addressed. One way to treat this condition is to give the patient proton pump inhibitors. In many cases, these drugs have proved to be ineffective. Research is ongoing to find an effective drug that would provide long-term relief and reduce the risk of serious complications. This study aimed to prepare gels and to test their properties, in an effort to identify preparations that could eliminate unpleasant symptoms of gastro-oesophageal reflux disease. The pH, dynamic viscosity, adhesion, and measurement of the surface coverage of the device imitating *in vivo* conditions in the oesophagus were measured for each prepared gel. The influence of carboxymethylcellulose sodium salt and chitosan on the properties of the pH and rheological properties of the preparations was determined. The obtained test results allow for the selection of the optimal preparation meeting the specific clinical condition. The pH range of the gels meets the requirements of the antacid preparation. The dynamic viscosity, adhesion, and gel displacement in the device simulating *in vivo* conditions indicate that the preparations could be maintained on the oesophageal mucosa to protect it from the irritating effect of the gastric contents.

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

2.2. Methods

2.2.1. Preparation of Hydrophilic Gels

Gels prepared from methylcellulose (4.0 g) and carboxymethylcellulose sodium salt (0.3, 0.5, 0.7, or 1.0 g) were combined into a homogenous excipient and the weight was 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, 1.0 g of micronised chitosan powder was added to the homogeneous gel. The mixture was mixed thoroughly to a homogeneous form and cooled to 5-10°C.

2.2.2. Analytical Methods

2.2.2.1. pH Measurement

The potentiometric method was used to measure the pH of the gels. Specifically, a combined electrode integrated into a multifunctional ELECTRON CX-742 computer meter was immersed in 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 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 values 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 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

A texture profile analysis (TPA) test was performed with an Exponent Stable Micro Systems Texture Analyzer TA.XT. Plus Texture Analyser Stable Micro Systems (England). A stainless-steel probe (P/1S) in the shape of a ball, with a diameter of 2.54 cm, was used to perform the measurements. The measurement parameters were as follows: the speed of the 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 program, 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 surface of the gel. 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. 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

The pH of gels containing 4.0% methylcellulose (400 cp, 1500 cp, or 4000 cp) 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 (Table 1).

The addition of 0.3%, 0.5%, 0.7%, or 1.0% carboxymethylcellulose sodium salt decreased the pH, with a range from 6.66 to 6.25. The addition of 1.0% chitosan decreased the pH, with a range from 7.53 to 6.45 (Table 1).

Table 1. Influence of chitosan on the pH of gels containing 4.0% methylcellulose (MC) and carboxymethylcellulose sodium salt (CMC-Na).

Gel components	pH of gels with MC and CMC-Na	pH of gels with MC, CMC-Na, 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 + 0.3% CMC-Na	6.66	7.53
MC 1500 cp + 0.3% CMC-Na	6.60	7.41
MC 4000 cp + 0.3% CMC-Na	6.59	7.32
MC 400 cp + 0.5% CMC-Na	6.48	7.28
MC 1500cp + 0.5% CMC-Na	6.47	7.15
MC 4000cp + 0.5% CMC-Na	6.39	7.05
MC 400cp + 0.7% CMC-Na	6.35	6.90
MC 1500cp + 0.7% CMC-Na	6.32	6.86
MC 4000cp + 0.7% CMC-Na	6.28	6.71
MC 400cp + 1.0% CMC-Na	6.33	6.63
MC 1500cp + 1.0% CMC-Na	6.28	6.54
MC 4000cp + 1.0% CMC-Na	6.25	6.45

The use of methylcellulose and carboxymethylcellulose sodium salt produced formulations with a wide pH range. The pH decreased as the carboxymethylcellulose sodium salt concentration increased. The addition of chitosan to the formulations produced gels with a wide pH range, all within the physiological range of 4.0-7.0 at 37°C.

Formulations containing 0.3% carboxymethylcellulose sodium salt showed the highest pH, which is an important feature and can be used in the treatment of advanced acid reflux. Gels containing 0.7%-1.0% carboxymethylcellulose sodium salt and chitosan could be used for acid 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).

The addition of carboxymethylcellulose sodium salt to methylcellulose increased the dynamic viscosity, with a range from 257 to 496 mPa*s the gels (Table 2). Enrichment with 1.0% chitosan further increased the dynamic viscosity, with a range from 366 to 573 mPa*s (Table 2).

Table 2. Influence of chitosan on the dynamic viscosity of gels containing 4.0% methylcellulose (MC) and carboxymethylcellulose sodium salt (CMC-Na)

Gel components	Dynamic viscosity of gels with MC and CMC-Na [mPa*s]	Dynamic viscosity of gels with MC, CMC-Na, and 1.0% chitosan [mPa*s]
MC 400cp	142	246
MC 1500cp	254	328
MC 4000cp	365	457
MC 400 cp + 0.3% CMC-Na	257	366
MC 1500 cp + 0.3% CMC-Na	348	393
MC 4000 cp + 0.3% CMC-Na	393	470
MC 400 cp + 0.5% CMC-Na	276	428
MC 1500cp + 0.5% CMC-Na	362	443
MC 4000cp + 0.5% CMC-Na	447	497
MC 400cp + 0.7% CMC-Na	310	456
MC 1500cp + 0.7% CMC-Na	398	469
MC 4000cp + 0.7% CMC-Na	465	537
MC 400cp + 1.0% CMC-Na	336	474
MC 1500cp + 1.0% CMC-Na	420	542
MC 4000cp + 1.0% CMC-Na	496	573

The dynamic viscosity of the gels increased as the carboxymethylcellulose sodium salt concentration increased. The addition of chitosan further increased the dynamic viscosity of the tested gels. The observed process of increasing dynamic viscosity in gels due to the added polymers may significantly impact the ability of these preparations to adhere to the oesophageal mucosa.

The thixotropic properties of the tested gels were also analysed. This is clearly illustrated in Figures 1-3. The obtained results prove the possibility of using the tested preparations in medicine due to their promising features.

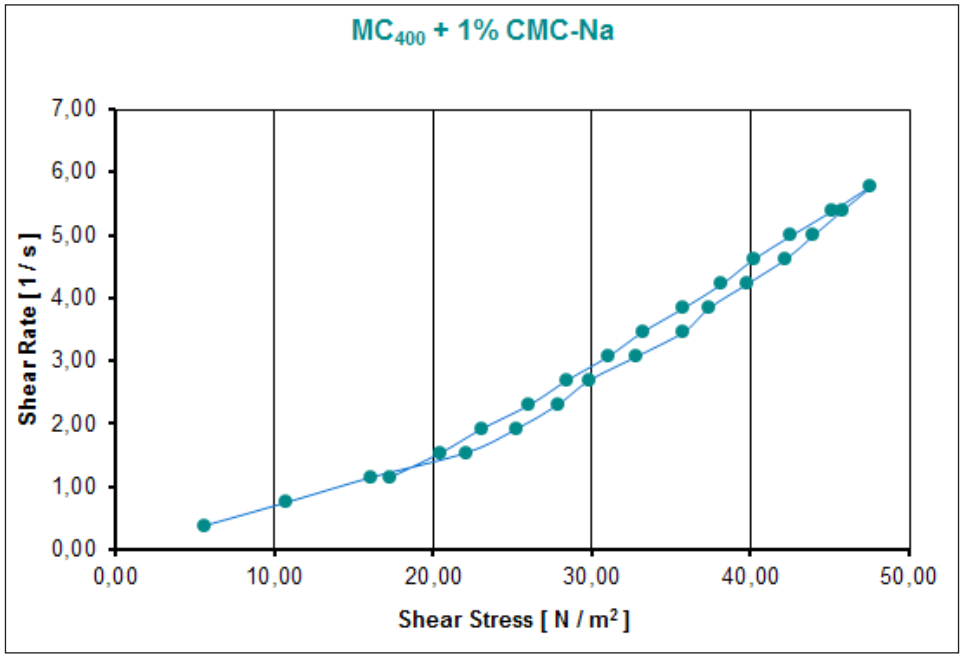

Figure 1. Measurement of the rheological properties of 4% methylcellulose 400 cp gel with 1.0% carboxymethylcellulose sodium salt.

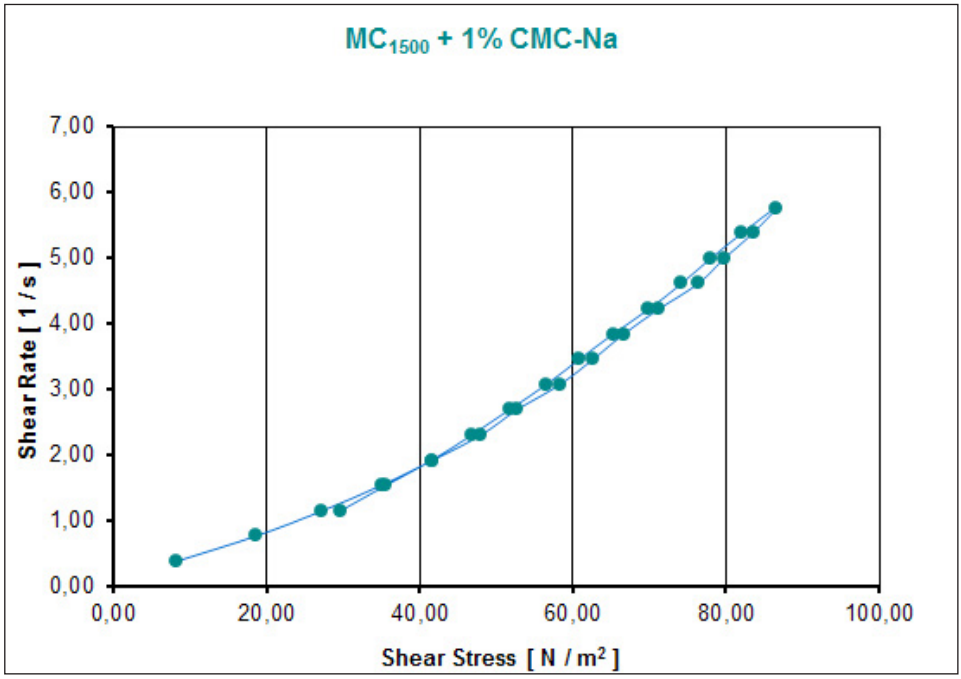

Figure 2. Measurement of the rheological properties of 4% methylcellulose 1500 cp gel with 1.0% carboxymethylcellulose sodium salt.

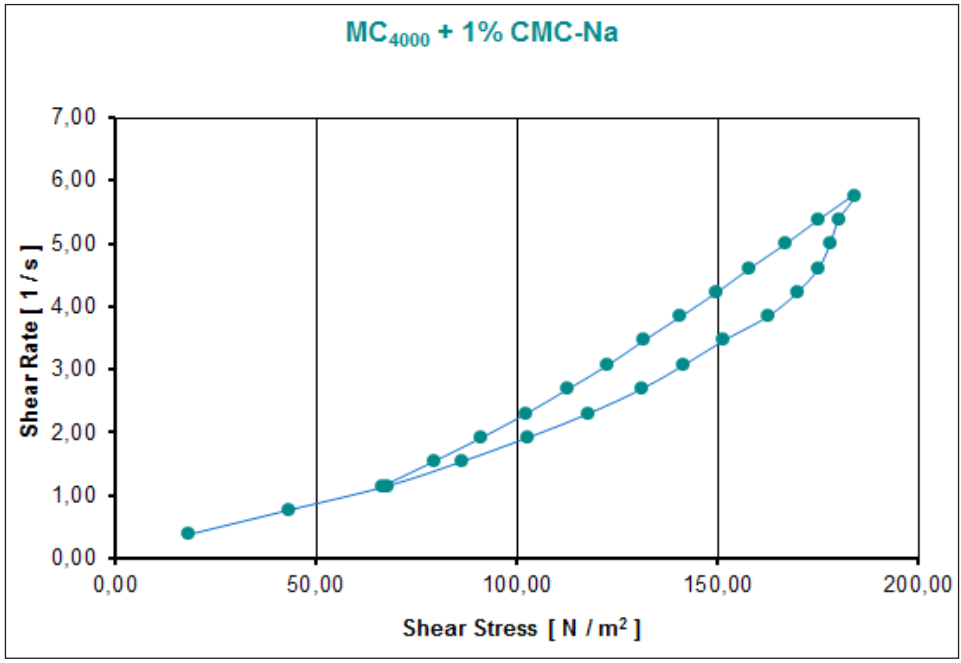

Figure 3. Measurement of the rheological properties of 4% methylcellulose 4000 cp gel with 1.0% carboxymethylcellulose sodium salt.

3.3. Adhesion

The work of adhesion of the gels containing methylcellulose 400 cp, 1500 cp, or 4000 cp was 39.2-51.9 g/s. The addition of 1.0% chitosan to these gels increased the work of adhesion to 74.1-78.0 g/s.

When adding 0.3%, 0.5%, 0.7%, or 1.0% carboxymethylcellulose sodium salt to the gels containing methylcellulose, the work of adhesion increased to 58.2-67.2 g/s. Enrichment of these gels with 1.0% further increased the work of adhesion to 77.1-97.8 g/s. The graphs presented in Figures 4-6 allow calculating the area under the curve and above the x-axis (time). These values represent the force needed to separate the probe from the hydrogel over time, a measure of adhesion. The adhesiveness of the gel containing 4.0% methylcellulose 400 cp and 1.0% carboxymethylcellulose sodium salt was 55.5 g/s (Figure 4), the adhesiveness of the gel containing 4.0% methylcellulose 1500 cp and 1.0% carboxymethylcellulose sodium salt was 88.8 g/s (Figure 5), and the adhesiveness of the gel containing 4.0% methylcellulose 4000 cp and 1.0% carboxymethylcellulose sodium salt was 97.8 g/s (Figure 6). A value >5.0 g/s indicates good adhesion. Hence, all preparations showed high adhesion to the oesophageal mucous membrane model. Based on the present study, it is possible to obtain gels with high adhesiveness to an oesophageal mucous membrane with dynamic viscosity above 100 mPa*s.

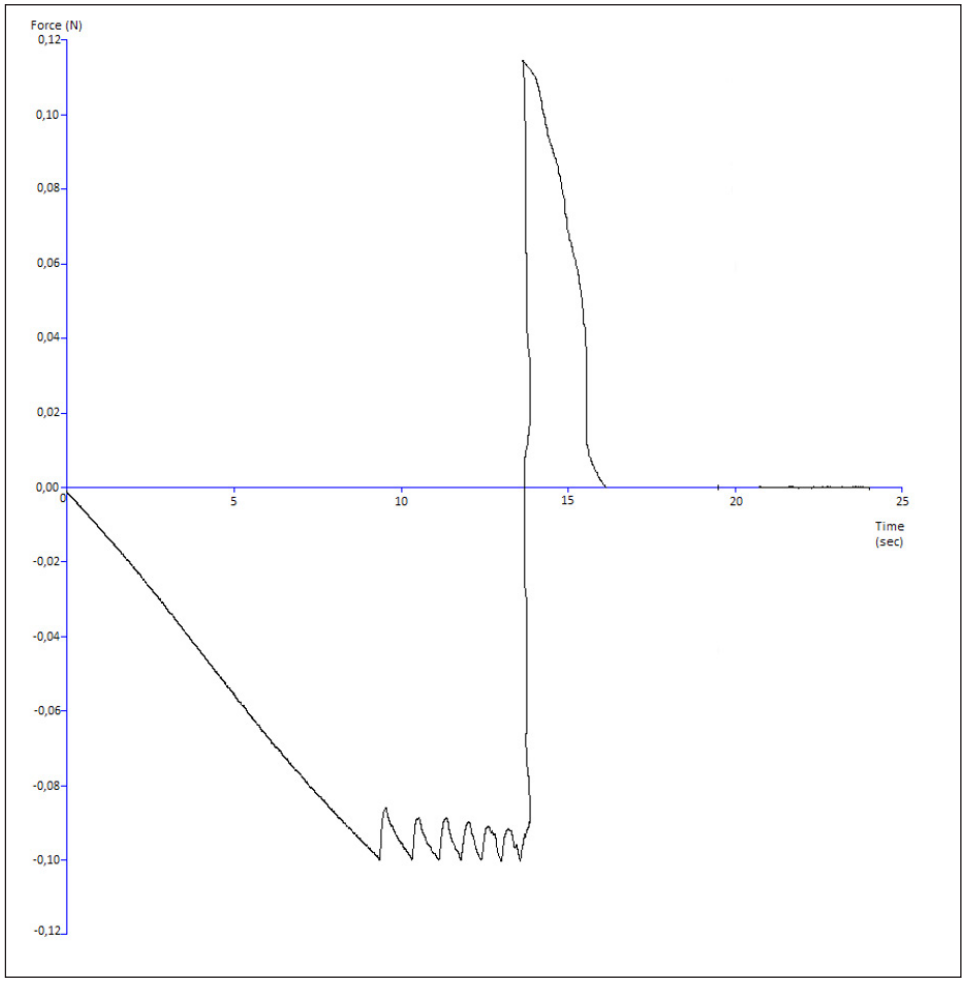

Figure 4. Measurement of the texture of 4% methylcellulose 400 cp gel with 1.0% carboxymethylcellulose sodium salt.

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

The prepared gels could coat the model surface, although the coating capacity depended on the initial methylcellulose viscosity: as the viscosity increased, less gel flowed into the syringe (Table 3). The addition of 1.0% chitosan further decreased the amount of gel collected in the syringe.

The addition of 0.3%, 0.5%, 0.7%, or 1.0% carboxymethylcellulose to gels with methylcellulose decreased the gel outflow (to 3.9-1.4 ml). Enrichment with 1.0% chitosan further reduced the gel outflow to 1.9-0.1 ml (Table 3).

The results have shown that it is possible to obtain gels with high adhesion to the oesophageal mucous membrane. The carboxymethylcellulose sodium salt concentration affects the ability of the gel to adhere to the surface. Gels with 1.0% chitosan showed higher adhesion compared with gels without chitosan. Overall, gels containing methylcellulose 4000 cp and 0.3%, 0.5%, 0.7%, or 1.0% carboxymethylcellulose sodium salt and gels

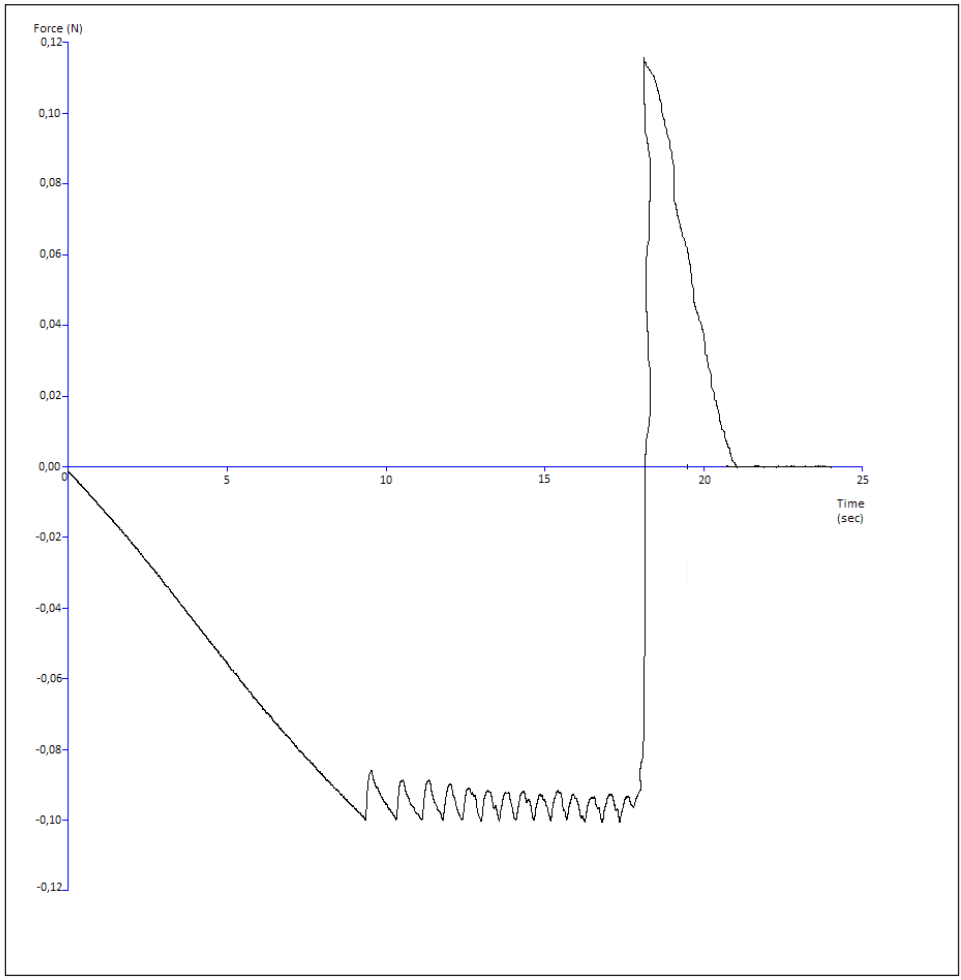

Figure 5. Measurement of the texture of 4% methylcellulose 1500 cp gel with 1.0% carboxymethylcellulose sodium salt.

containing methylcellulose 1500 cp and 0.7% carboxymethylcellulose sodium salt are maintained on the test surface and do not flow out (Table 3).

Table 3. Influence of chitosan on the ability of the gel to coat a surface with a gel containing 4.0% methylcellulose (MC) and carboxymethylcellulose sodium salt (CMC-Na)

Gel components	Surface coating of gels with MC and CMC-Na [cm] after 10 min	Surface coating of gels with MC, CMC-Na, 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 + 0.3% CMC-Na	25.0 + 3.9 ml S	25.0 + 1.9 ml S

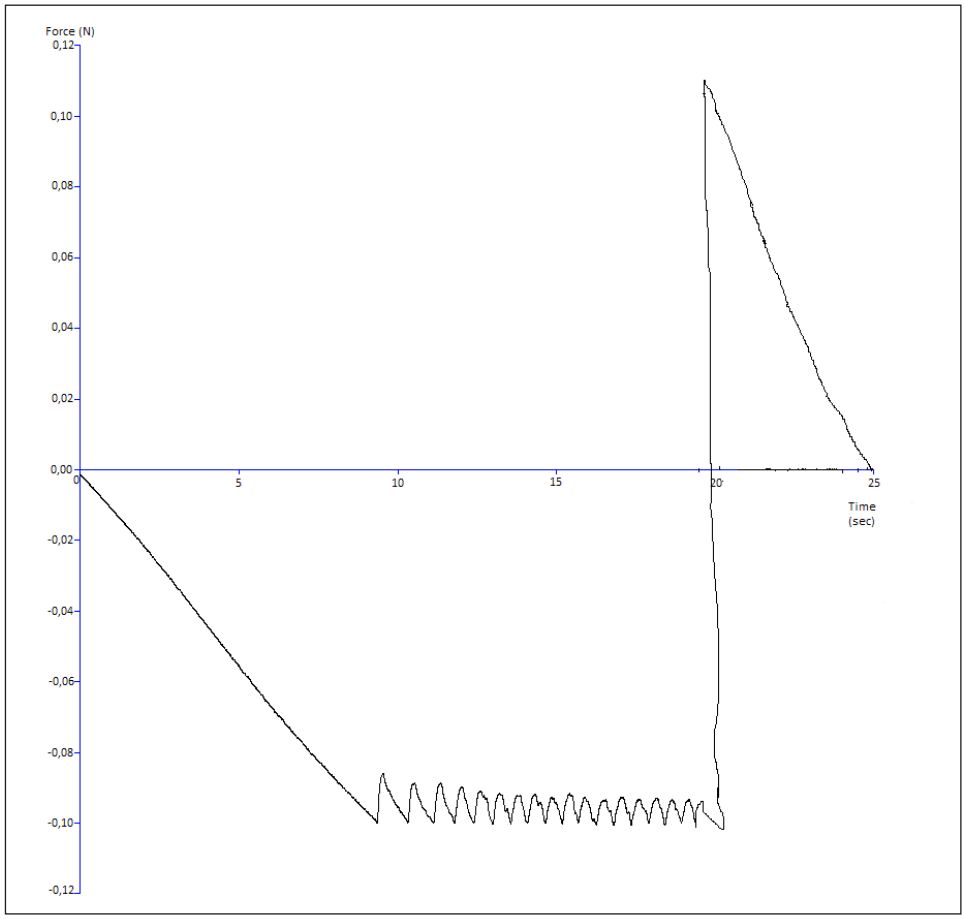

Figure 6. Measurement of the texture of 4% methylcellulose 4000 cp gel with 1.0% carboxymethylcellulose sodium salt.

Gel components	Surface coating of gels with MC and CMC-Na [cm] after 10 min	Surface coating of gels with MC, CMC-Na, and 1.0% chitosan [cm] after 10 min
MC 1500 cp + 0.3% CMC-Na	25.0 + 3.5 ml S	25.0 + 0.6 ml S
MC 4000 cp + 0.3% CMC-Na	25.0 + 3.1 ml S	25.0 + 0.0 ml S
MC 400 cp + 0.5% CMC-Na	25.0 + 3.6 ml S	25.0 + 1.5 ml S
MC 1500cp + 0.5% CMC-Na	25.0 + 3.2 ml S	25.0 + 0.3 ml S
MC 4000cp + 0.5% CMC-Na	25.0 + 2.9 ml S	25.0 + 0.0 ml S
MC 400cp + 0.7% CMC-Na	25.0 + 3.3 ml S	25.0 + 0.4 ml S
MC 1500cp + 0.7% CMC-Na	25.0 + 3.0 ml S	25.0 + 0.0 ml S
MC 4000cp + 0.7% CMC-Na	25.0 + 2.6 ml S	25.0 + 0.0 ml S
MC 400cp + 1.0% CMC-Na	25.0 + 3.0 ml S	25.0 + 0.1 ml S
MC 1500cp + 1.0% CMC-Na	25.0 + 2.3 ml S	25.0 + 0.0 ml S
MC 4000cp +1.0% CMC-Na	25.0 + 1.4 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 research has shown the possibility of obtaining preparations with specific properties conducive to protect the oesophageal mucosa. The obtained gels have a suitable pH to neutralise acidic content entering the oesophagus. The addition of chitosan and carboxymethylcellulose sodium salt increases the dynamic viscosity. Such features of the prepared gels, combined with the established adhesion and the ability to move in the device simulating *in vivo* conditions, guarantee the adherence of the preparations to the oesophageal mucosa. Coating the mucosa with an antacid gel preparation could protect it from damage. The findings indicate that preparations could be tailored to each patient to reduce acid reflux and to protect the oesophageal mucosa. The presented *in vitro* results require *in vivo* verification, which is the subject of further research.

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