THE EFFECT OF CALCIUM ALGINATE ON THE NEUTRALISING PROPERTIES OF CHITOSAN GELS PROTECTING THE OESOPHAGEAL MUCOSA

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Abstract

Gastroesophageal reflux disease is one of the most frequently diagnosed conditions in the modern world. It is characterised by repeated movement of stomach acid into the oesophagus, causing irritation and symptoms such as heartburn and regurgitation. Due to their short residence time on the mucous membrane surface, the emulsions used so far do not fulfil their role. This research aimed to examine the effect of calcium alginate on the properties of gels containing chitosan. For this purpose, hydrophilic gels were prepared and tested for pH, dynamic viscosity, and their ability to move in a model simulating conditions in the oesophagus. Alginates have been shown to affect the increase of pH and give the ability to neutralise acidic stomach content. As pH exceeded the upper physiological limit, chitosan offered the potential to restore pH to the physiological range. Its addition had an additional important feature - an increase in the dynamic viscosity of gel and adhesion, which would lead to better gel adhesion to the oesophagus. In summary, the addition of chitosan to methylcellulose gels containing calcium alginate positively influenced the pH, making it more suitable for the oesophagus, and enhanced the dynamic viscosity, allowing the gel to adhere to the mucous membrane surface for protective purposes against reflux.

Keywords: *calcium alginate, physiological environment of the gastro-oesophageal, hydrophilic gels, oesophageal mucosa, anti-inflammatory drugs, oesophageal infections*

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1. Introduction

Research into preventing damage to the oesophageal mucosa caused by reflux remains fully justified, because the existing approaches do not provide satisfactory therapeutic outcomes, as proved in the available literature. Among the most commonly recommended medications are the drugs that reduce the amount of acid secreted and alleviate the symptoms of the disease, i.e., the proton-pump inhibitors. In turn, the antacids are used as supportive agents to manage gastroesophageal reflux symptoms and prevent their complications. Antacid preparations typically contain sodium bicarbonate, calcium carbonate, aluminium hydroxide, magnesium hydroxide, and magnesium carbonate. Antacids are characterised by a rapid onset of action; however, their effects are short-lived. As an alternative to antacids, which alleviate symptoms, alginates are used. The mechanism of alignates' action is based on the formation of a gel barrier on the surface of the oesophageal mucosa, which acts as a physical barrier against reflux. In contact with the gastric fluid, alginates form a sticky, cohesive gel known as a 'raft.' This raft localises in the acid pocket of the proximal stomach. Preparations based on alginates usually consist of sodium alginate, sodium bicarbonate, and sodium carbonate. In the acidic stomach environment, sodium alginate reacts with the acid to form a colloidal gel, and sodium bicarbonate releases carbon dioxide bubbles that become trapped in the gel, causing foam formation. This physical form causes the gel to float on the surface of the stomach content. Our previous studies on sodium alginate hydrophilic gels protecting the oesophageal mucosa showed a higher gel pH when adding chitosan [1–13].

The current studies broaden the previous findings on the effect of alginates and provide information on the properties of gels based on calcium alginate containing chitosan.

The results of current experimental work indicate the potential to develop preparations that prevent damage to the oesophageal mucosa caused by gastroesophageal reflux. The effect of chitosan on the properties of gels with varying pH and rheological characteristics was investigated. The tested gels demonstrated adhesive properties. Based on the initial results, the gels were further enriched with calcium alginate, which provided additional beneficial properties. The most significant outcome of this modification was the ability to produce gels with a pH suitable for neutralising reflux acid. An optimal formulation was selected based on the pH range. Dynamic viscosity measurements were also conducted, confirming that it is possible to develop a preparation with ideal pharmaceutical and application characteristics. While these *in vitro* findings are promising, they require verification through *in vivo* studies, which constitute the next phase of our research.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: chitosan of 93.5% deacetylation degree, 15 mPa·s viscosity of its 1% solution in acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland), methylcellulose of 400, 1500, 4000 mPa·s viscosity of their 2% solutions in H_2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), calcium alginate (Sigma Aldrich Chemie GmbH, Germany), aqua purificata as required by Farmacopoeia Poland XII.

2.2. Methods

2.2.1. Preparation of Hydrophilic Gel

First, methylcellulose (4.0 g) (MC) and calcium alginate (0.5, 0.7, or 1.0 g) were combined into a homogeneous mixture. Then, distilled water was added in the amount providing

100.0 g of the final mass mixture (considering the chitosan mass, which will be added in the next stage of gel preparation). The mixture was cooled to ca. 5 - 10°C. The resulting homogeneous gel was weighed, and the distilled water was refilled. In the next step, the homogeneous gel was supplemented with chitosan (1.0 g of micronised powder). The gel was mixed thoroughly. When a homogeneous consistency had been reached, the preparation was cooled to 5 - 10°C.

2.2.2. pH Measurements

The preparations were first subjected to pH measurement. A potentiometric method was used. To measure the pH of the gels, a combined electrode integrated with the ELECTRON CX-742 multifunctional multimeter was immersed in each of them. All pH measurements of the gels were performed at 37°C. The study was repeated three times. The final result is the average of these multiple measurements.

2.2.3. Dynamic Viscosity

To determine the rheological properties of the tested preparations, measurements were made with a Rheotest 2 rotational viscometer (Medingen Dresden). The measurements were carried out at 37°C in the range Ia and IIa. A K-1 cone with a diameter of 36 mm was used. The measurement gap applied equals 0.917 cm. The shear angle was measured using 12 shear rates in the increasing direction and 11 shear rates in the decreasing direction. Three replicates were analysed for each formulation and the final results were averaged. The values of subsequent parameters: shear stress for the range Ia (Equation 1), dynamic viscosity for the range Ia (Equation 2), shear stress for the range IIa (Equation 3), and dynamic viscosity for the range IIa (Equation 24), were calculated.

$$\tau = c \cdot \alpha_{(1\text{-}12)} = 85.0 \cdot \alpha_{(1\text{-}12)}, \tag{1}$$

$$\eta = \frac{\tau}{D_{(1\text{-}12)}} \cdot 100 = \frac{85.0 \cdot \alpha_{(1\text{-}12)}}{D_{(1\text{-}12)}} \cdot 100, \tag{2}$$

$$\tau = c \cdot \alpha_{(1\text{-}12)} = 820.2 \cdot \alpha_{(1\text{-}12)}, \tag{3}$$

$$\eta = \frac{\tau}{D_{(1\text{-}12)}} \cdot 100 = \frac{820.2 \cdot \alpha_{(1\text{-}12)}}{D_{(1\text{-}12)}} \cdot 100. \tag{4}$$

where:

τ – shear stress [N/m^2];
η – viscosity [mPa·s];
α – shear angle [°];
D – shear rate [1/s].

2.2.4. Evaluation of Adhesion Properties

The texture of the prepared gels was examined using the Exponent Stable Micro Systems TA.XT texture analyser (Plus Stable Micro Systems England Texture Analyzer). A texture profile analysis (TPA) test was performed at 37°C. The basic measurement tool was a ball-shaped probe (P/1S) made of stainless steel, 2.54 cm in diameter. During the measurement, the probe's downward movement speed was 0.5 mm/s while the probe's lifting speed was 10 mm/s. The height at which the probe was raised was 40 mm, and the maximum allowable force equalled 100 g. During the measurement, the probe stayed in the gel for 10 s. Placing the gel in a transparent cylindrical Plexiglas vessel was the first research

stage. To start the measurement, the probe was placed directly on the gel surface (the probe touched the gel surface for 10 s). After selecting the appropriate parameters of the program, the probe was detached from the gel surface and began to rise to a height of 40 mm above its surface. The speed of the moving probe was 10 mm/s. All formulations were tested three times and the final results represent the average of these three measurements.

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

Due to the lack of an automatic device, a model was constructed to simulate the conditions in the oesophagus [14].

The model simulating the natural, physiological conditions in the oesophagus *in vivo* [14] consists of a 25 cm long glass tube, modelled on a water cooler, with a double wall, ending with a wide opening on both sides. The entire device was continuously water-thermostated to maintain a human body temperature (37°C). The outer wall of the glass tube is equipped with a millimetre scale. The whole model was suspended on a tripod in a vertical position. A plastic medical syringe with a millimetre scale was placed vertically under the opening of the glass tube. The plunger was removed from the syringe, and its tip was closed with a cap to provide suitable conditions for collecting the hydrogel flowing down on the model walls. The measurement of the preparation's ability to move and its run-off time begins by applying 5 mL of hydrogel to the inner wall of the model's glass tube. Then, measurements of the time the hydrogel flowed towards the bottom of the glass model were performed within a maximum of 10 minutes. The time needed for the formulations to reach 5, 10, 15, 20, and 25 cm of the tube length was recorded. If the formulations reached the final 25 cm tube length, the excess was collected in a syringe placed under the glass tube, and after completing the 10-minute measurement, the volume of hydrogel that had flowed into the syringe was noted. For the preparations that did not flow through the whole tube length, the distance the gel reached after 10 minutes was taken. The average of the three independent measurements is considered the final result.

3. Results and Discussion

3.1. pH Measurements

Gel preparations containing 4.0% methylcellulose with initial static viscosity: 400, 1500, and 4000 mPa·s showed a pH ranging from 5.96 to 5.73 (Table 1). Modifying the composition of the neat methylcellulose gels with the chitosan resulted in the pH increase to 6.60 - 5.82. The addition of calcium alginate to methylcellulose gels, providing 0.5, 0.7, or 1.0% concentration of the alginate, causes an increase in the gels' pH to 7.03 - 7.57. Simultaneously, for the corresponding formulations containing chitosan (at a 1% concentration), the addition of calcium alginate resulted in an increase in pH, from the initial 5.82 – 6.60 values to 6.35 - 6.99 (Table 1).

The studies have shown that the obtained gels can effectively neutralise acidic pH (lower than 4.0) and reach physiological pH in the oesophagus, 4.0 - 7.0. The gels containing calcium alginate have a pH within the 7.03 - 7.57 range that is significantly higher than the pH of the reference MC gels. The addition of chitosan allows the gels to reach the physiological pH range from 6.35 to 6.99.

Table 1. Influence of chitosan on the pH of gels containing methylcellulose (MC) and calcium alginate.

Gel	pH of gel without chitosan	pH of gel with chitosan
MC 400	5.96	6.60
MC 1500	5.77	5.98
MC 4000	5.73	5.82
MC 400 + 0.5% calcium alginate	7.03	6.35
MC 1500 + 0.5% calcium alginate	7.19	6.44
MC 4000 + 0.5% calcium alginate	7.43	6.54
MC 400 + 0.7% calcium alginate	7.10	6.65
MC 1500 + 0.7% calcium alginate	7.14	6.70
MC 4000 + 0.7% calcium alginate	7.41	6.82
MC 400 + 1.0% calcium alginate	7.33	6.86
MC 1500 + 1.0% calcium alginate	7.50	6.92
MC 4000 + 1.0% calcium alginate	7.57	6.99

Research on preparations based on methylcellulose has shown the possibility of obtaining a series of preparations with various properties. Methylcellulose gels enriched with chitosan simulate the physiological conditions ranging from 4.0 to 7.0 at 37°C. The pH range of these gels was wide, which allows for a more accurate selection of the gels according to specific application and physicochemical conditions. The calcium alginate introduced into the formulations had a very beneficial effect on pH regulation. Comparing the initial pH range of MC gels, i.e., 5.96 to 5.73, calcium alginate increased the pH values. The highest pH was reached for the formulations with the highest concentration of calcium alginate, i.e., 1.0%. The observed changes are of great application significance, making it possible to neutralise the acid content in a gentle way, bringing the pH to the physiological level.

3.2. Rheological Properties

Gels prepared based on methylcellulose with static viscosities of 400, 1500, and 4000 mPa·s showed dynamic viscosity values ranging from 142 to 365 mPa·s (Table 2). The addition of chitosan (to the final 1% chitosan concentration) causes an increase in the dynamic viscosity to 246 - 457 mPa·s. Modifying the neat MC gels' composition with calcium alginate (0.5, 0.7, and 1.0% concentration in the final formulation) resulted in an increase in the dynamic viscosity to 245 - 356 mPa·s. Further enrichment of the composition containing methylcellulose and calcium alginate with a chitosan of 1.0% concentration resulted in an increase in the dynamic viscosity of the preparations. Three-component gels exhibited dynamic viscosity from 279 to 565 mPa·s.

To increase the effectiveness of gels protecting the oesophageal mucosa, an attempt has been made to increase the viscosity by using calcium alginate. The addition of this substance to the tested preparations significantly increased the dynamic viscosity of individual gels. It was also found that the dynamic viscosity of the gels increased with increasing calcium alginate concentration. Enriching the tested preparations with chitosan resulted in a further significant increase in the dynamic viscosity value. Methylcellulose gels with static viscosities of 400, 1500, and 4000 mPa·s have a specific dynamic viscosity

value. The effect of calcium alginate on the dynamic viscosity can significantly impact the tested preparations' adhesion to the oesophageal mucosa. This feature is essential in protecting the mucous membrane against the harmful effects of acidic food content.

Table 2. Influence of chitosan on the viscosity of gels containing methylcellulose (MC) and calcium alginate.

Gel	The dynamic viscosity of the gel without chitosan [mPa·s]	The dynamic viscosity of the gel with chitosan [mPa·s]
MC 400	142	246
MC 1500	254	328
MC 4000	365	457
MC 400 + 0.5% calcium alginate	245	279
MC 1500 + 0.5% calcium alginate	262	368
MC 4000 + 0.5% calcium alginate	380	480
MC 400 + 0.7% calcium alginate	246	290
MC 1500 + 0.7% calcium alginate	275	386
MC 4000 + 0.7% calcium alginate	297	499
MC 400 + 1.0% calcium alginate	268	340
MC 1500 + 1.0% calcium alginate	320	436
MC 4000 + 1.0% calcium alginate	356	565

3.3. Adhesive Properties

Gel preparations obtained from methylcellulose 400, 1500, and 4000 mPa·s were tested to determine their adhesion work value. All tests were carried out at 37°C. The values of the work of adhesion for neat MC-based gels ranged from 39.2 to 51.9 g/s. The addition of chitosan increased the adhesion to 74.1 - 78.0 g/s (Table 3). When calcium alginate was added to the neat MC formulations, with the amount providing 0.5, 0.7, and 1.0% concentration of alginate, an increase in adhesion to 52.8 - 68.5 g/s was observed. Further enriching the MC/calcium alginate compositions with chitosan caused an increase in adhesion of the preparations to 86.6 to 92.4 g/s. The gels designed for the oesophageal mucosa protection should be characterised by a work of adhesion higher than 5.0 g/s, which is recognised as good adhesion. Experimental research has shown that it is possible to obtain preparations with high enough adhesion to the oesophageal mucosa. The study showed the possibility of obtaining gels with high oesophageal mucosa adhesiveness with a dynamic viscosity above 100 mPa·s.

3.4. Surface-Coating Properties

The evaluation of the ability of the preparation to cover the tested surface showed that the results were highly dependent on the initial static viscosity of methylcellulose: 400, 1500, and 4000 mPa·s. Preparations made of methylcellulose with a viscosity of 400 mPa·s flow relatively fast through the test tube and were collected in the syringe in an amount of 4.5 mL (Table 4). When methylcellulose of 4000 mPa·s was used, the gel collected in the syringe reached 4.0 mL. Adding chitosan reduced the collected amount to 3.0 mL for the

gels formed with 400 mPa·s methylcellulose and 1.7 mL for the gels created with MC of 4000 mPa·s (Table 4).

Table 3. Influence of chitosan on the adhesion of gels containing methylcellulose (MC) and calcium alginate.

Gel	Work of adhesion of gel without chitosan [g/s]	Work of adhesion of gel with chitosan [g/s]
MC 400	39.2	74.1
MC 1500	48.3	76.0
MC 4000	51.9	78.0
MC 400 + 0.5% calcium alginate	52.8	86.6
MC 1500 + 0.5% calcium alginate	60.6	88.3
MC 4000 + 0.5% calcium alginate	62.3	89.8
MC 400 + 0.7% calcium alginate	50.9	86.3
MC 1500 + 0.7% calcium alginate	62.0	88.9
MC 4000 + 0.7% calcium alginate	65.2	89.5
MC 400 + 1.0% calcium alginate	60.8	87.5
MC 1500 + 1.0% calcium alginate	65.4	89.8
MC 4000 + 1.0% calcium alginate	68.5	92.4

Table 4. Influence of chitosan on surface-coating ability of gels containing methylcellulose (MC) and calcium alginate.

Gel	Surface coated with gel without chitosan [cm] after 10 min	Surface coated with gel with chitosan [cm] after 10 min
MC 400	25.0 + 4.5 mL S	25.0 + 3.0 mL S
MC 1500	25.0 + 4.1mL S	25.0 + 2.5 mL S
MC 4000	25.0 + 4.0 mL S	25.0 + 1.7 mL S
MC 400 + 0.5% calcium alginate	25.0 + 2.0 mL S	25.0 + 1.5 mL S
MC 1500 + 0.5% calcium alginate	25.0 + 1.5 mL S	25.0 + 1.0 mL S
MC 4000 + 0.5% calcium alginate	25.0 + 1.0 mL S	25.0 + **0.0 mL** S
MC 400 + 0.7% calcium alginate	25.0 + 2.5 mL S	25.0 + **0.0 mL** S
MC 1500 + 0.7% calcium alginate	25.0 + 2.0 mL S	25.0 + **0.0 mL** S
MC 4000 + 0.7% calcium alginate	25.0 + 1.5 mL S	25.0 + **0.0 mL** S
MC 400 + 1.0% calcium alginate	25.0 + 2.0 mL S	25.0 + **0.0 mL** S
MC 1500 + 1.0% calcium alginate	25.0 + 1.5 mL S	25.0 + **0.0 mL** S
MC 4000 + 1.0% calcium alginate	25.0 + 1.0 mL S	25.0 + **0.0 mL** S

Note. 25.0 + 1.0 mL S means 25.0 cm coated with gel + 1.0 mL collected in syringe (S)

Modification of the neat MC-based composition with calcium alginate resulted in a reduction in the amount of gel collected in the syringe to 1.0 - 2.0 mL. Further enrichment of the composition of the tested gels with chitosan reduced the collected gel by 1.5 mL. No gel flowing into the syringe was observed for several samples, described as 0.0 mL S (Table 4). The presented research, conducted at 37°C, showed that it is possible to prepare gels with high adhesion to the oesophageal mucosa. Testing the effectiveness of these gels to cover the surface, it was found that calcium alginate and its concentration affect the ability of the gel to adhere to the surface. Gels with 1.0% of chitosan showed higher adhesion when compared to gels without this polymer.

4. Conclusions

The presented studies have shown that it is possible to obtain preparations that neutralise acidic content that irritates the oesophagus. Acidic gastric content regurgitated into the oesophagus can be neutralised using prepared gels, the pH of which exceeds the upper limit of physiological pH. The noted wide range of dynamic viscosity values allows the selection of an appropriate one for specific application conditions. The adhesive properties of the gels tested in the *in vitro* model indicate that they will remain on the oesophageal mucosa for a long time. The advantage of these preparations lies in the possibility of personalising the therapy thanks to the variety of their properties, which can be easily adjusted by changing the gel composition. The studies have shown an apparent effect of calcium alginate on the evaluated gel's parameters and the impact of adding chitosan. The experiments conducted require confirmation in *in vivo* conditions.

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