STUDIES ON CHITOSAN GELS WITH POLYVINYL ALCOHOL TO PROTECT THE OESOPHAGEAL MUCOSA

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Abstract
Young children have an incompletely developed digestive tract and they are susceptible to numerous disorders due to the return of acid and alkaline content to the oesophagus. The aim of the study was to analyse the ability of hydrogels containing chitosan and polyvinyl alcohol to prevent irritation of the oesophageal mucosa in children. The addition of chitosan to the tested gels increased their pH, which could be useful to neutralise gastric reflux, and dynamic viscosity. The texture tests revealed that chitosan and polyvinyl alcohol significantly increased the work of adhesion work.

Keywords: *mild treatment of reflux in children, physiological gastro-oesophageal environment, hydrophilic gels, oesophageal mucosa, anti-inflammatory drugs, oesophageal infections*

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1. Introduction

Health problems related to the digestive tract affect people of all ages, including children. Diagnosing gastro-oesophageal reflux in children is a difficult task. For a long time, the so-called gold standard in this diagnosis was 24-h oesophageal pH-metry. A more modern method has recently been introduced; it uses the phenomenon of resistance, that is, oesophageal impedance. This method is much more effective than pH-metry because it allows detecting not only acid gastroesophageal reflux, but also episodes of weak acid or alkaline reflux. Research into ways to treat gastro-oesophageal reflux have suggested the possibility of using hydrogels that protect the oesophageal mucosa against harmful factors [1–9].

The aim of the study was to investigate the effect of polyvinyl alcohol on the properties of hydrogels containing chitosan that could be used to treat gastro-oesophageal reflux in children. These gels were designed so that they could be swallowed easily while thoroughly cover the oesophageal mucosa to protect it from the irritating effects of refluxed content. The prepared hydrogels had a range of pH and rheological properties and good adherence to the surface of a device imitating the physiological conditions of the oesophagus. The prepared hydrogels should remain on the oesophageal mucosa for a long time and protect it against the adverse effects of refluxed content. The *in vitro* results require confirmation with *in vivo* tests, which will be performed in subsequent research.

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 or 4000 mPa·s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd., UK), polyvinyl alcohol 87%–90% hydrolysed, average molecular weight 30000–70000, viscosity 4–6 cP, 4% in H_2O (20°C) (Sigma-Aldrich Chemie GmbH, Germany); and aqua purificata as required Farmacopoeia Poland XII.

2.2. Methods

2.2.1. Preparation of Hydrophilic Gel

The gel preparation steps were:

1. Methylcellulose (4.0 g) and polyvinyl alcohol (0.1, 0.3 or 0.5 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

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.

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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: $\eta = \frac{\tau}{D(1-12)} \times 100 = \frac{85.0 \times \alpha(1-12)}{D(1-12)} \times 100$ (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: $\eta = \frac{\tau}{D(1-12)} \times 100 = \frac{820.2 \times \alpha(1-12)}{D(1-12)} \times 100$ (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 each prepared hydrogel. The hydrogels containing 4.0% methylcellulose (400, 1500 or 4000 cp) presented a pH that ranged from 5.96 to 5.73. The addition of 1% chitosan increased the pH, with a range from 6.60 to 5.82. The addition of polyvinyl alcohol (0.1%, 0.3% or 0.5%) to the gels containing 4.0% methylcellulose decreased pH, with a range from 5.93 to 5.34 (compared with the previous range from 5.96 to 5.73). Finally, the addition of 1.0% chitosan and polyvinyl alcohol reduced the pH of the gels, with a range from 6.24 to 5.55 (compared with the previous range from 6.60 to 5.82).

The use of methylcellulose along with polyvinyl alcohol provided gels with a wide pH range from 5.0 to 7.0. Of note, these gels fall within the physiological pH range of the oesophagus (4.0–7.0). Hence, gels containing methylcellulose, chitosan and polyvinyl alcohol could be of great importance to neutralise the oesophageal pH and thus treat alkaline reflux in children.

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

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 + 0.1% polyvinyl alcohol	5.93	6.24
MC 1500 cp + 0.1% polyvinyl alcohol	5.77	6.12
MC 4000 cp + 0.1% polyvinyl alcohol	5.75	5.99
MC 400 cp + 0.3% polyvinyl alcohol	5.70	5.94
MC 1500 cp + 0.3% polyvinyl alcohol	5.63	5.87
MC 4000 cp + 0.3% polyvinyl alcohol	5.58	5.81
MC 400 cp + 0.5% polyvinyl alcohol	5.57	5.78
MC 1500 cp + 0.5% polyvinyl alcohol	5.50	5.70
MC 4000 cp + 0.5% polyvinyl alcohol	5.34	5.55

3.2. Rheological Tests

Table 2 presents the rheological test results. The hydrogels containing methylcellulose (400, 1500 or 4000 cp) had 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 polyvinyl alcohol (0.1%, 0.3% or 0.5%) to the gels increased the dynamic viscosity to 266–483 mPa·s. Finally, adding 1% chitosan and polyvinyl alcohol increased the dynamic viscosity to 294–579 mPa·s.

The dynamic viscosity of the gels increased as the polyvinyl alcohol concentration increased. Enrichment with chitosan further increased the dynamic viscosity. These data indicate that the gels could potentially adhere to the oesophageal mucosa and thus protect against the harmful effects of alkaline content refluxed into the oesophagus.

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

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 + 0.1% polyvinyl alcohol	266	294
MC 1500 cp + 0.1% polyvinyl alcohol	298	356
MC 4000 cp + 0.1% polyvinyl alcohol	400	478
MC 400 cp + 0.3% polyvinyl alcohol	280	359
MC 1500 cp + 0.3% polyvinyl alcohol	348	445
MC 4000 cp + 0.3% polyvinyl alcohol	462	512
MC 400 cp + 0.5% polyvinyl alcohol	305	423
MC 1500 cp + 0.5% polyvinyl alcohol	379	487
MC 4000 cp + 0.5% polyvinyl alcohol	483	579

3.3. Adhesion

Table 3 shows the work of adhesion of the prepared hydrogels at 37°C. The hydrogels containing methylcellulose (400, 1500 cp or 4000 cp) had a work of adhesion of 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. The addition of polyvinyl alcohol (0.1%, 0.3% or 0.5%) also increased the work of adhesion to 55.4–68.1 g/s. Finally, the addition of 1.0% chitosan and polyvinyl alcohol (0.1%, 0.3% or 0.5%) increased the work of adhesion to 82.5–95.1 g/s.

A work of adhesion value above 5.0 g/s indicates good adhesion. Hence, the obtained gels are characterised by high adhesion to the oesophageal mucosa. The addition of 0.1%, 0.3% or 0.5% polyvinyl alcohol had a positive influence on the work of adhesion. Overall, it was possible to obtain hydrogels with high adhesive properties to the oesophageal mucosa.

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

Gel composition	Work of adhesion [g/s]	Work of adhesion of gels 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 + 0.1% polyvinyl alcohol	55.4	82.5
MC 1500 cp + 0.1% polyvinyl alcohol	58.6	84.2
MC 4000 cp + 0.1% polyvinyl alcohol	62.6	90.6
MC 400 cp + 0.3% polyvinyl alcohol	58.3	85.1
MC 1500 cp + 0.3% polyvinyl alcohol	60.4	87.5
MC 4000 cp + 0.3% polyvinyl alcohol	64.1	90.3
MC 400 cp + 0.5% polyvinyl alcohol	60.8	88.6
MC 1500 cp + 0.5% polyvinyl alcohol	64.5	92.2
MC 4000 cp + 0.5% polyvinyl alcohol	68.1	95.1

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

The ability of each hydrogel to coat the surface of the *in vitro* model was evaluated at 37°C. The coating properties of the gels depended on the initial static viscosity of methylcellulose (400, 1500 or 4000 cp). At 400 cp, 4.5 ml of the gel flowed into the syringe, while at 4000 cp, 4.0 ml of the gel 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 0.1%, 0.3% or 0.5% polyvinyl alcohol notable reduced the amount of gel that flowed into the syringe (from 3.0 to 0.2 ml). Finally, of the addition of 1.0% chitosan and polyvinyl alcohol had the greatest impact: the amount of gel that flowed into the syringe ranged from 1.5 to 0.0 ml (Table 4).

Table 4. The influence of chitosan on ability of hydrogels containing 4.0% methylcellulose and polyvinyl alcohol to coat the model surface.

Gel composition	Surface coating with the gel [cm] after 10 min	Surface coating with the gel 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 + 0.1% polyvinyl alcohol	25.0 + 3.0 ml S	25.0 + 1.5 ml S
MC 1500 cp + 0.1% polyvinyl alcohol	25.0 + 2.7 ml S	25.0 + 0.6 ml S
MC 4000 cp + 0.1% polyvinyl alcohol	25.0 + 2.2 ml S	25.0 + **0.0 ml** S
MC 400 cp + 0.3% polyvinyl alcohol	25.0 + 2.9 ml S	25.0 + 0.5 ml S
MC 1500 cp + 0.3% polyvinyl alcohol	25.0 + 2.0 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 0.3% polyvinyl alcohol	25.0 + 1.6 ml S	25.0 + **0.0 ml** S
MC 400 cp + 0.5% polyvinyl alcohol	25.0 + 2.5 ml S	25.0 + **0.0 ml** S
MC 1500 cp + 0.5% polyvinyl alcohol	25.0 + 0.8 ml S	25.0 + **0.0 ml** S
MC 4000 cp + 0.5% polyvinyl alcohol	25.0 + 0.2 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

The results of this work showed the significant impact of chitosan and polyvinyl alcohol on several parameters of hydrogels, including the pH, dynamic viscosity, adhesion and *in vitro* coverage of the model surface. The wide pH range of the gels allows for the selection of the optimal gel for each juvenile patient depending on the content that enters the oesophagus. Due to their adhesive properties, the presented hydrogels should remain on the oesophageal mucosa for a long time and protect it against the adverse effects of regurgitation from the stomach. Although these *in vitro* results are very promising, they require verification with *in vivo* experiments. This is the focus of future research.

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