THE INFLUENCE OF HYDROPHILISING SUBSTANCES ON THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN GELS WITH A PROTECTIVE EFFECT ON THE OESOPHAGEAL MUCOSA

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Abstract

This study aimed to analyse hydrogels intended to prevent irritation of the oesophageal mucosa in individuals sensitive or allergic to commonly used substances that neutralise acidic gastric contents. Such sensitivities can hinder effective management of gastrointestinal disorders involving the stomach and oesophagus. The research focused on assessing the influence of hydrophilizing agents: glycerol, polyethylene glycol 200, and propylene glycol-1,2 on the properties of chitosan-containing gels. In vitro experiments demonstrated that hydrogels formulated with chitosan and the selected hydrophilizing agents can potentially alleviate reflux symptoms. The incorporation of chitosan resulted in an increase in gel pH and dynamic viscosity, both of which support the neutralisation of acidic gastric contents. Texture analysis further revealed that both chitosan and the hydrophilizing substances significantly enhanced the adhesive properties of the gels, as indicated by increased adhesion work. These findings suggest that the developed formulations offer an effective alternative for individuals with sensitivities to standard antacid therapies.

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

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1. Introduction

Different agents that cover the oesophageal mucosa and neutralise irritating contents are used to relieve the effects of gastroesophageal reflux. However, these substances are washed out too quickly from the mucosal surface. Generally, anatomical and physiological conditions make it difficult to effectively protect the mucosa from acid and alkaline reflux destruction.

When methylcellulose gels protect the mucosa, increased wettability of methylcellulose is needed. Glycerol, as a hydrophilizing substance, actively binds water. When added to hydrogels, it gives them moisturising properties. Moreover, the presence of glycerol affects the stability and rheological properties of the preparations through its ability to form hydrogen bonds with water molecules, affecting the structure of the polymer network. These properties affect the preparations' elasticity and coating capabilities. Similar properties are demonstrated by polyoxyethylene glycol 200 and propylene glycol-1,2, positively impacting the structure and physicochemical properties of the tested hydrogels [1–13].

The aim of the work was to investigate the influence of hydrophilizing substances on the adhesive properties of chitosan-containing gels.

Experimental results proved the possibility of increasing adhesion by incorporating hydrophilizing substances into the composition of methycellulose-based gels. Increasing the adhesion of gels and, consequently, extending the time the preparation remains on the mucous membrane could significantly increase the effectiveness of the therapy. Prepared formulations exhibited various rheological properties and values of adhesion work. An additional positive aspect is the expected pH of the preparations. It was found that it is possible to produce a preparation with optimal pharmaceutical and application properties. The current studies can help to accomplish the difficult task of long-term coverage of the oesophageal mucosa and its adequate protection against irritating factors that lead to chronic complications. Laboratory tests require clinical confirmation.

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 H2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), glycerol (Aldrich Chemical Company Ltd. Gillingham, England), polyoxyethylene glycol 200 (Aldrich Chemical Company Ltd. Gillingham, England), propylene glycol-1,2 (Aldrich Chemical Company Ltd. Gillingham, England), 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 glycerol or polyoxyethylene glycol 200 or propylene glycol-1,2 (5.0 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. Evaporated 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 was 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

The pH of gels containing 4.0% methylcellulose with static viscosities of 400, 1500, and 4000 mPa·s ranged from 5.96 to 5.73 (Table 1). The addition of chitosan, providing a polymer concentration of 1.0%, increased the pH of the preparations to 6.60 - 5.82. The addition of glycerol to neat MC-based formulations, resulting in 5% glycerol concentration, caused an increase in the pH value of the gels to 6.08 to 6.02. Analogous gels doped with chitosan of 1.0% concentration in the formulation resulted in an increase in the pH to 6.78 - 5.91. In turn, the addition of polyoxyethylene glycol 200 at 5.0% concentration to neat MC-based gels resulted in a pH decrease to 5.82 - 5.70, while further modification with chitosan caused further changes in the pH to 5.90 to 5.78. Next, the propylene glycol-1,2 was added to the MC formulations, increasing the gels' pH value to 6.53 - 6.45. The modification of MC/propylene glycol-1,2 formulations with chitosan resulted in an increase in the pH of the gels to ca. 6.90 - 6.63 (compared to the previous range, i.e. from 6.60 to 5.82) (Table 1).

All gel preparations were formed using methylcellulose. The modification of the gel's composition with glycerol, polyoxyethylene glycol 200 or propylene glycol-1,2 allows the formation of preparations over a wide pH range. During the tests, a significant increase in the pH of the gels with the addition of glycerol was observed. Adding chitosan to the tested preparations causes a considerable increase in the pH of the gels. Incorporation of polyoxyethylene glycol 200 into the tested gels resulted in a decrease in the pH of the gels, which was observed (5.82 to 5.70) in relation to both the reference gels and the gels containing glycerol. A similar relationship occurred in the case of gels enriched with chitosan (5.90 to 5.78). The gels containing propylene glycol-1,2 as a hydrophilizing substance presented the highest pH values. This range, initially found as 6.45 - 6.53, increased to 6.63 - 6.90 after chitosan addition.

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

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 + glycerol	6.08	6.78
MC 1500 + glycerol	6.04	6.23
MC 4000 + glycerol	6.02	5.91
MC 400 + polyoxyethylene glycol 200	5.82	5.90
MC 1500 + polyoxyethylene glycol 200	5.72	5.83
MC 4000 + polyoxyethylene glycol 200	5.70	5.78
MC 400 + propylene glycol-1,2	6.53	6.90
MC 1500 + propylene glycol-1,2	6.50	6.85
MC 4000 + propylene glycol-1,2	6.45	6.63

3.2. Rheological Properties

The rheological measurements showed that gels obtained from methylcellulose with 400, 1500 and 4000 mPa·s static viscosity had dynamic viscosity 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 values to 246 to 457 mPa·s. Modifying the gels' composition with glycerol increased the dynamic viscosity of the preparations to 243 to 389 mPa·s. Further enriching the composition with chitosan resulted in an increase in the dynamic viscosity values of the preparations to 255 to 474 mPa·s. The addition of polyoxyethylene glycol 200 increases the dynamic viscosity of the preparations, ranging from 348 to 412 mPa·s. Modifying these compositions with chitosan increased the dynamic viscosity of the preparations to 369 to 495 mPa·s. In turn, the addition of propylene glycol-1,2 increases the dynamic viscosity of the preparations, ranging from 257 to 397 mPa·s, after modification. Adding chitosan to these preparations resulted in an increase in the dynamic viscosity to 268 to 482 mPa·s (Table 2).

Rheological tests of the prepared gels showed that the addition of hydrophilizing substances increased the dynamic viscosity values of individual preparations. The dynamic viscosity value of the gels was varied. The highest dynamic viscosity values were obtained by gels containing polyoxyethylene glycol 200. The addition of chitosan to these gels further increases the dynamic viscosity value. Lower dynamic viscosity values are presented by gels containing propylene glycol-1.2. Enriching these gels' composition with chitosan addition increases the dynamic viscosity value. Gels containing glycerol as a hydrophilizing substance show the lowest dynamic viscosity values. The addition of chitosan also increases the dynamic viscosity value of these gels. Experimental tests have demonstrated that methylcellulose gels with static viscosities of 400, 1500, and 4000 mPa·s have a specific dynamic viscosity value. Increasing the dynamic viscosity value in preparations due to adding hydrophilizing substances and chitosan can significantly impact their adhesion to the oesophageal mucosa. This is an essential feature in protecting the mucosa from the harmful effects of acidic contents entering the oesophagus.

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

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 + glycerol	243	255
MC 1500 + glycerol	268	346
MC 4000 + glycerol	389	474
MC 400 + polyoxyethylene glycol 200	348	369
MC 1500 + polyoxyethylene glycol 200	365	389
MC 4000 + polyoxyethylene glycol 200	412	495
MC 400 + propylene glycol-1,2	257	268
MC 1500 + propylene glycol-1,2	272	359
MC 4000 + propylene glycol-1,2	397	482

3.3. Adhesive Properties

Adhesion tests conducted at 37°C showed that the gels obtained from methylcellulose with static viscosities of 400, 1500, and 4000 mPa·s exhibited adhesion work values ranging from 39.2 to 51.9 g/s (Table 3). The addition of chitosan increased the adhesion to 74.1 - 78.0 g/s. Modifying the composition of the tested gels with glycerol increased the work of adhesion value to 42.6 - 59.8 g/s. Further enriching the composition with chitosan, at a concentration of 1.0%, resulted in an increase in the adhesion value to 78.2 - 81.8 g/s. Modifying the composition of the tested gels with polyoxyethylene glycol 200 caused an increase in the work of adhesion to 64.5 - 69.4 g/s. Enriching the composition of the tested gels with chitosan at a concentration of 1.0% resulted in an increase in the adhesion value of the preparation, ranging after modification from 84.3 to 95.1 g/s (Table 3). Modifying the composition of the tested gels with propylene glycol-1,2 increased the value of the adhesion work of the gels to 51.3 - 61.2 g/s, and the value rises to 85.8 - 80.3 g/s when chitosan is added.

An adhesion work value above 5.0 g/s indicates good adhesion. The presented research has shown that it is possible to obtain gels characterised by high adhesion to the oesophageal mucosa. The study indicated the influence of glycerol, polyoxyethylene glycol 200, and propylene glycol-1,2 at 5% concentration on the work of adhesion. Gels enriched with chitosan at 1% concentration showed better adhesion properties than those without this polymer. 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

Tests of the gels' ability to cover the surface of the *in vitro* model were carried out at 37°C. It was found that the coating properties depended on the initial static viscosity of methylcellulose (Table 4).

Table 3. Influence of chitosan on surface-coating ability of gels containing methylcellulose (MC) and hydrophilizing substances.

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 + glycerol	42.6	78.2
MC 1500 + glycerol	53.8	79.1
MC 4000 + glycerol	59.8	81.8
MC 400 + polyoxyethylene glycol 200	64.5	84.3
MC 1500 + polyoxyethylene glycol 200	67.9	89.2
MC 4000 + polyoxyethylene glycol 200	69.4	95.1
MC 400 + propylene glycol-1,2	51.3	80.3
MC 1500 + propylene glycol-1,2	55.8	82.7
MC 4000 + propylene glycol-1,2	61.2	85.8

Table 4. Influence of chitosan on the surface-coating ability of gels containing methylcellulose (MC) and hydrophilizing substances.

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 + glycerol	25.0 + 2.6 mL S	25.0 + 1.2 mL S
MC 1500 + glycerol	25.0 + 2.3 mL S	25.0 + 0.4 mL S
MC 4000 + glycerol	25.0 + 2.0 mL S	25.0 + **0.0 mL** S
MC 400 + polyoxyethylene glycol 200	25.0 + 1.6 mL S	25.0 + 0.3 mL S
MC 1500 + polyoxyethylene glycol 200	25.0 + 1.3 mL S	25.0 + **0.0 mL** S
MC 4000 + polyoxyethylene glycol 200	25.0 + 1.0 mL S	25.0 + **0.0 mL** S
MC 400 + propylene glycol-1,2	25.0 + 2.3 mL S	25.0 + 0.6 mL S
MC 1500 + propylene glycol-1,2	25.0 + 2.1 mL S	25.0 + 0.4 mL S
MC 4000 + propylene glycol-1,2	25.0 + 1.5 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)

When gel formed with methylcellulose of 400 mPa·s was tested, 4.5 mL of the gel was collected in the syringe, while for the preparations made of 4000 mPa·s MC, 4.0 mL was collected. Adding chitosan reduced these values to 3.0 and 1.7 mL, respectively (Table 4).

Modification of the composition with glycerol showed a close relationship with a decrease in the gel flow, resulting in the gels collected in the syringe equaling 2.6 - 2.0 mL. Enrichment of these compositions with chitosan caused a reduction in the amount that flowed out of the test tube to 1.2 or even no gel was collected (Table 4). Adding polyoxyethylene glycol to the gels reduced the mobility of the gels, observed as 1.6 - 1.0 mL gels collected. Adding chitosan to these preparations limited the gel flow capacity. As a result, a maximum of 0.3 mL of gel was collected. Finally, the addition of propylene glycol-1,2 caused a decrease in the distance covered by the gel in 10 minutes, observed as 2.3 - 1.5 mL of collected gel. The addition of chitosan reduced the gel flow to the limits of 0.6 - 0.0 mL collected.

The studies proved that it is possible to obtain preparations characterised by high adhesion to the oesophageal mucosa using the proposed compounds within a given concentration. Hydrophilic substances used, i.e. glycerol, polyoxyethylene glycol 200 and propylene glycol-1,2, significantly affect the ability of the gel to adhere to the surface. Preparations containing 1.0% chitosan are characterised by high adhesion compared to gels free of this polymer. The preparations composed of 4000 mPa·s static viscosity methylcellulose with glycerol, polyoxyethylene glycol 200, and propylene glycol-1,2, as well as gel formed with 1500 mPa·s methylcellulose with polyoxyethylene glycol 200, remain on the tested surface and do not flow out (Table 4).

4. Conclusions

Gastroesophageal reflux occurs in varying degrees of severity. An additional problem is the sensitivity or allergy of patients to commonly used substances that combat reflux symptoms. The studies aimed to verify the possibility of obtaining preparations effective in the reflux treatment using methylcellulose and methylcellulose/chitosan gels with the addition of hydrophilizing substances. The results showed a significant effect of chitosan and hydrophilizing substances on many important gel parameters, including their pH, dynamic viscosity, adhesion and *in vitro* coating of the tested surface. The pH range of the obtained preparations allows the selection of the optimal gel neutralising acidic and very acidic content that enters the patients' oesophagus. Due to their adhesive properties, the presented gels should remain on the oesophageal mucosa for a long time and protect it from the adverse effects of acidic reflux of stomach contents. An essential aspect of these studies is that gels containing chitosan and hydrophilizing substances exhibited high values of dynamic viscosity, which enables sufficient adhesion of preparations in patients with damaged oesophageal mucosa.

The results of experimental studies have shown that it is possible to produce a gel with optimal pharmaceutical and application properties. These preparations can be of great importance in individualised therapy. Thanks to the favourable pH range, high dynamic viscosity, adhesion, and ability to cover the tested surface, these gels can be adapted to the individual needs of patients with gastric reflux and those who are sensitive or allergic to traditional antacids. Preparations with specific pH values, dynamic viscosity, adhesion and ability to cover the surface imitating the oesophagus *in vitro* were obtained. *In vitro* studies and their assumptions require verification *in vivo*.

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6. References

[1] Mousa HM, Rosen R, Woodley FW, Orsi M, Armas D, Faure C, Fortunato J, O'connor J, Skaggs B, Nurko S; (2011) Esophageal impedance monitoring for gastroesophageal reflux. J Pediatr Gastroenterol Nutr 52(2), 129–139. **DOI:** 10.1097/MPG.0b013e3181ffde67

[2] Maret-Ouda J, Markar SR, Lagergren J; (2020) Gastroesophageal reflux disease: A review. JAMA 324(24), 2536–2547. **DOI:** 10.1001/jama.2020.21360

[3] Katz PO, Gerson LB, Vela MF; (2013) Guidelines for the diagnosis and management of gastroesophageal reflux disease. Am J Gastroenterol 108(3), 308–328. **DOI:** 10.1038/ajg.2012.444

[4] Raban M, Żak A, Litak J, Turska M, Grochowski C; (2017) Gastroesophageal reflux disease - unit description, diagnosis and treatment. J Educ Health Sport 7(7), 215–225. **DOI:** 10.5281/zenodo.825357

[5] Vakil N, Van Zanten SV, Kahrilas P, Dent J, Jones R, the Global Consensus Group; (2006) The Montreal definition and classification of gastroesophageal reflux disease: A global evidence-based consensus. Am J Gastroenterol 101(8), 1900–1920. **DOI:** 10.1111/j.1572-0241.2006.00630.x

[6] Singh M, Lee J, Gupta N, Gaddam S, Smith BK, Wani SB, Sullivan DK, Rastogi A, Bansal A, Donnelly JE, Sharma P; (2013) Weight loss can lead to resolution of gastroesophageal reflux disease symptoms: A prospective intervention trial. Obesity 21(2), 284–290. **DOI:** 10.1002/oby.20279

[7] Sasankan P, Thota PN; (2022) Evaluation and management of Gastroesophageal reflux disease: A brief look at the updated guidelines. Cleve Clin J Med 89(12), 700–703. **DOI:** 10.3949/ccjm.89a.22059

[8] Ze EY, Kim BJ, Kang H, Kim JG; (2017) Abdominal visceral to subcutaneous adipose tissue ratio is associated with increased risk of erosive esophagitis. Dig Dis Sci 62(5), 1265–1271. **DOI:** 10.1007/s10620-017-4467-4

[9] Gao F, Gao Y, Chen X, Qian J, Zhang J; (2017) Comparison of Oesophageal function tests between Chinese non-erosive reflux disease and reflux hypersensitivity patients. BMC Gastroenterol 17(1), 67. **DOI:** 10.1186/s12876-017-0624-7

[10] Nathan KG, Genasan K, Kamarul T; (2023) Polyvinyl Alcohol-Chitosan Scaffold for Tissue Engineering and Regenerative Medicine Application: A Review. Mar Drugs 21(5), 304. **DOI:** 10.3390/md21050304

[11] Azelee NI, Ramli AN, Manas NS, Salamun N, Man RC, Enshasy HA; (2019) Glycerol in food, cosmetics and pharmaceutical industries: basics and new applications. IJSTR 8(12), 553–558.

[12] Ibrahim M, Ramadan E, Elsadek NE, Emam SE, Shimizu T, Ando H, Ishima Y, Elgarhy OH, Sarhan HA, Hussein AK, Ishida T; (2022) Polyethylene glycol (PEG): The nature, immunogenicity, and role in the hypersensitivity of PEGylated products. J Control Release 351, 215–230. **DOI:** 10.1016/j.jconrel.2022.09.031

[13] Małolepsza-Jarmołowska K, Bazan H; (2024) Studies on chitosan gels with polyvinyl alcohol to protect the oesophageal mucosa. Prog Chem Appl Chitin Deriv 29, 186–193. **DOI:** 10.15259/PCACD.29.014

[14] Małolepsza-Jarmołowska K; (2019) Influence of chitosan on the properties of gels protecting of the esophageal mucosa. Prog Chem Appl Chitin Deriv 24, 135–144. **DOI:** 10.15259/PCACD.24.012