PHARMACEUTICAL ASSESSMENT OF GYNAECOLOGICAL POWDERS WITH SODIUM ALGINATE CONTAINING A LACTIC ACID-CHITOSAN COMPLEX

Katarzyna Małolepsza-Jarmołowska

Department of Drugs Form Technology, Faculty of Pharmacy,
The "Silesian Piasts" Memorial Medical University of Wroclaw,
Borowska 211A Street, 50-556 Wrocław, Poland.
e-mail: katarzynamj@poczta.onet.pl

Abstract

The insufficient duration of the drug's contact with the vaginal mucosa does not provide adequate pH, conditioning the physiological biocenosis of the organ. This effect is hampered by numerous anatomic and physiological conditions which do not facilitate prolonged contact of the drug with the application site. This study has shown the impact of the used excipients and the ratio of lactic acid to chitosan on pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. The addition of sodium alginate and excipients to these complexes allows various formulations to be obtained over a wide range of pH. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and sodium alginate in comparison to gels without sodium alginate. Studies of the work of adhesion showed the effect of glycerol, 1,2-propylene glycol and their concentrations on the value of the work of adhesion.

Key words: *lactic acid complexed with chitosan, physiological environment of the vagina, hydrophilic powders, vaginal mucosa, anti-inflammatory drugs, vaginal infections.*

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1. Introduction

The treatment of vagina inflammatory conditions is usually long and difficult because of the frequent relapse. The condition is recognised increasingly more often due to improved diagnostic methods. Traditional therapeutic schemes recommended by global treatment centres and health organisations do not provide satisfactory results. The insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH the physiological biocenosis of the organ [1-12].

The use of a hydrophilic base for lactic acid complexed with alkaline polymers enabled the production of powders passing on natural conditions to the gels with the rheological properties of vaginal mucus. The gel remains at the site of application and provides an adequate environmental pH. Research is continuing into the optimisation of the powder composition in order to improve their adhesion properties and thus increase the efficacy of the therapy. Gynaecological powders are designed to correct the abnormal pH environment of the vagina and to achieve the physiological state.

The aim of this study was to investigate the influence of sodium alginate on the physico-chemical properties of powders for gynaecological purposes. The most important parameters influencing the properties of the tested powders, such as pH, dynamic viscosity and adhesion, have been determined. By adopting the above-mentioned assumptions, the present work tested the impact of sodium alginate on the properties of the powders. In the study, formulations were prepared with different pH and rheological properties. These powders, transforming into gels, were examined for their properties. As a result of this study, the dynamic viscosity was determined from gels obtained from powders. The test shows the adhesion of gels. By studying a range of pH in the gels, this allows the selection of the optimum formulation.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: lactic acid (P.Z.F. Cefarm (Wrocław, Poland), chitosan with a deacetylation degree of 93.5%, viscosity of 15 mPa*s, 1% in 1% acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland), methylcellulose viscosity of 4000 mPa*s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), glycerol (Sigma-Aldrich Chemie GmbH, Germany), polyoxyethylene glycol 200 [PEG-200] (Sigma-Aldrich Chemie GmbH, Germany), 1,2-propylene glycol (Sigma-Aldrich Chemie GmbH, Germany), sodium alginate (Sigma-Aldrich Chemie GmbH, Germany), and aqua purificata, acc. to FP XI.

2.2. Apparatus

1. pH meter Elmetron - CX 742 (Elmetron Poland)
2. Viscosimeter Rheotest - 2 MLW (Medingen Dresden Germany)
3. Texturometer - TA.XT.Plus Texture Analyser (Stable Micro Systems England)

2.3. Methods

2.3.1. Preparation of hydrophilic intravaginal powder

The preparation of powders containing lactic acid complexed with chitosan consisted of the following stages:

1. Preparation of the lactic acid-chitosan complex (stoichiometric weight ratio of 1:1 and 2:1).

The required amount of powdered chitosan (0.83g) was added to a known amount of lactic acid 89% (0.56g for 1:1 or 1.12g for 2:1) and was mixed. The mixture was left for 24 h until a clear, thick fluid was formed. This could be joined with methylcellulose [4].

2. Preparation of powder from methylcellulose and sodium alginate.

The sodium alginate (0.5g; 0.7g; 1.0g) was mixed with a known amount of methylcellulose (4.0g). Next, the mixture was added to the lactic acid complexes with chitosan and glycerol or polyoxyethylene glycol 200 or 1,2-propylene glycol (5.0g; 10.0g; 15.0g; 20.0g; 25.0g). The resulting powder was thoroughly pulverised. A homogenous powder was obtained by sieving through a mesh of size 0.16 mm.

3. Preparation of the tested gel.

A gel was obtained by mixing the powder with a known amount of distilled water (to 100.0g) and was cooled to 5–10°C to enhance the process of gelation. The homogenous gel was weighed and an additional amount of distilled water was added to obtain the initial mass of 100.0 g.

2.3.2. Analytical methods

2.3.2.1. pH-measurement

For pH measurements of the investigated gels, the potentiometric method was used, in which a combined electrode was integrated with a multifunctional computer meter ELECTRON CX-742, and immersed into the investigated gel. All gels were tested three times, and the results were reported as the average of three measurements at 37°C.

2.3.2.2. Dynamic viscosity measurement

Rheological investigations were performed using a rotational viscosimeter Rheotest 2 Medingen Dresden. The determinations were performed in I a and II a ranges on a K-1 cone with the diameter of 36 mm and a 0.917 fissure at 37°C. The shear angle was measured using 12 shear rates in an ascending direction and 11 rates in the descending direction. All gels were tested three times, and the results were reported as the average of three measurements. The values of the shear stress and viscosity were calculated from measurements at 37°C.

- shear stress for the range Ia: $\tau = c \cdot \alpha_{(1\text{-}12)} = 85 \cdot \alpha_{(1\text{-}12)}$

- viscosity for the range Ia: $\eta = \frac{\tau}{D(1-12)} \cdot 100 = \frac{85 \cdot \alpha(1-12)}{D(1-12)} \cdot 100$

- 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: $\eta = \frac{\tau}{D(1-12)} \cdot 100 = \frac{820{,}2 \cdot \alpha(1-12)}{D(1-12)} \cdot 100$

where:

τ - shear stress, [N/m^2]
η – viscosity, [mPa*s]
α - shear angle, [°]
D - shear rate, [1/s]

2.3.2.3. Measurement of adhesion

A test for texture profile analysis (TPA) was performed with Exponent Stable Micro Systems Texture Analyzer TA.XT.Plus Texture Analyser Stable Micro Systems England.

The measurements were conducted in order to illustrate the influence of the type of methylcellulose on the adhesion strength of the prepared gels.
To perform the measurements, a probe (P/1S) in the shape of a ball, built in stainless steel, with a diameter of 1 inch was used.
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 seconds). After selecting the appropriate parameters of the program, the measurements 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 were reported as the average of three measurements at 37°C.

3. Results and Discussion

3.1. pH measurement

Gels obtained from powders containing lactic acid complexed with chitosan revealed a stoichiometric weight ratio of 1:1 and 2:1 and 4.0% methylcellulose. The pH ranged from 3.92 for 1:1 gels to 3.48 for 2:1 gels [13].
The addition of 5–25% PEG-200 increased the pH range to 4.43–4.95 for 1:1 gels and 3.42–3.68 for 2:1 gels (in comparison to the previous results of 3.92 and 3.48). The further addition of 0.5%, 0.7% and 1.0% sodium alginate decreased the pH range to 4.48–4.00 for 1:1 gels (in comparison to the previous range from 4.43 to 4.95) and increased the pH to 3.78–4.37 for 2:1 gels (in comparison to the previous range from 3.42 to 3.68) in relation to the pH ranges of powders with the addition of PEG-200 (Table 1).

Table 1. Influence of PEG-200 and sodium alginate on the pH of gels obtained from investigated powders containing 4% methylcellulose

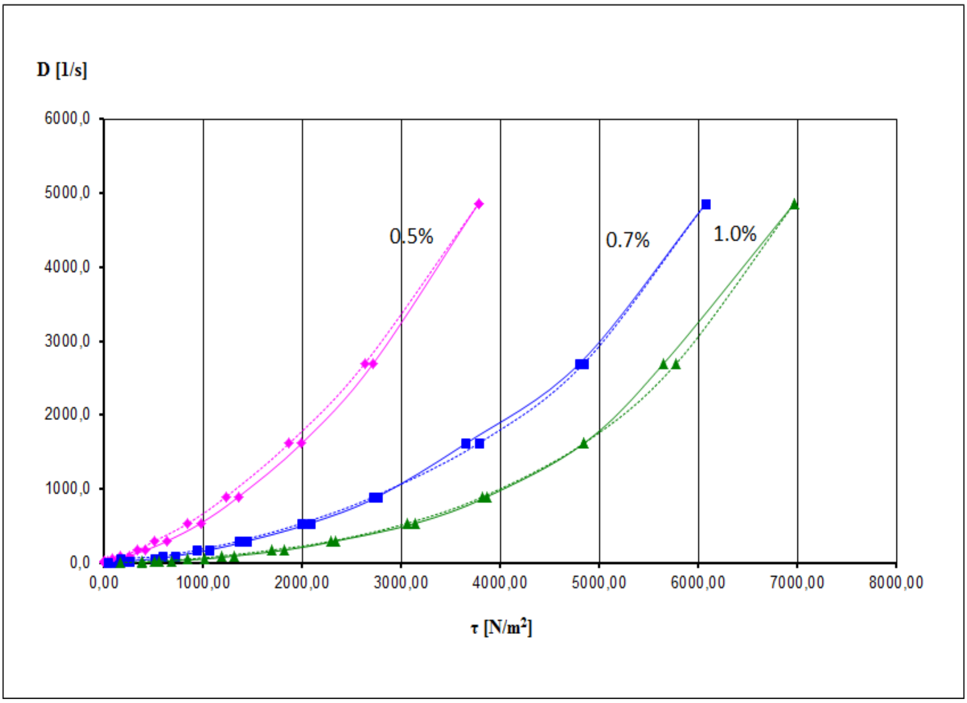
Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration [%] PEG-200	pH gels with addition PEG-200	pH gels with PEG-200 and addition 0.5% sodium alginate	pH gels with PEG-200 and addition 0.7% sodium alginate	pH gels with PEG-200 and addition 1.0% sodium alginate
1:1	5	4.43	4.10	4.08	4.00
1:1	10	4.48	4.22	4.10	4.05
1:1	15	4.55	4.35	4.12	4.10
1:1	20	4.87	4.40	4.25	4.15
1:1	25	4.95	4.48	4.30	4.20
2:1	5	3.42	4.04	3.81	3.78
2:1	10	3.46	4.10	3.85	3.80
2:1	15	3.51	4.13	3.87	3.83
2:1	20	3.63	4.25	3.90	3.90
2:1	25	3.68	4.37	3.99	3.93

The addition of 5–25% of 1,2-propylene glycol increased the pH range to 4.49–4.97 for 1:1 gels and 3.90–4.50 for 2:1 gels [14]. The further addition of 0.5%, 0.7% and 1.0% sodium alginate decreased the pH range to 4.42–4.88 for 1:1 gels (in comparison to the previous range from 4.49 to 4.97) and 3.72–4.30 for 2:1 gels (in comparison to the previous range from 3.90 to 4.50) in relation to the pH range of powders with the addition of 1,2-propylene glycol. All gels with the weight ratios of 1:1 and 2:1 showed a pH in the physiological range (3.5–5.0) at 37°C. The addition of sodium alginate and excipients allowed various formulations to be obtained with a wide range of pH. Formulations containing the complex at a ratio of 2:1 showed the lowest pH, which is an important feature that can be used in the treatment of advanced bacterial vaginosis. The use of methylcellulose with sodium alginate allows different formulations to be obtained with a wide range of pH.

3.2. Rheological tests

The rheological analyses demonstrated that the researched gels obtained from powders possessed a dynamic viscosity of 398 mPa*s for the 1:1 stoichiometric weight ratio in the complex and 356 mPa*s for the 2:1 ratio [13].

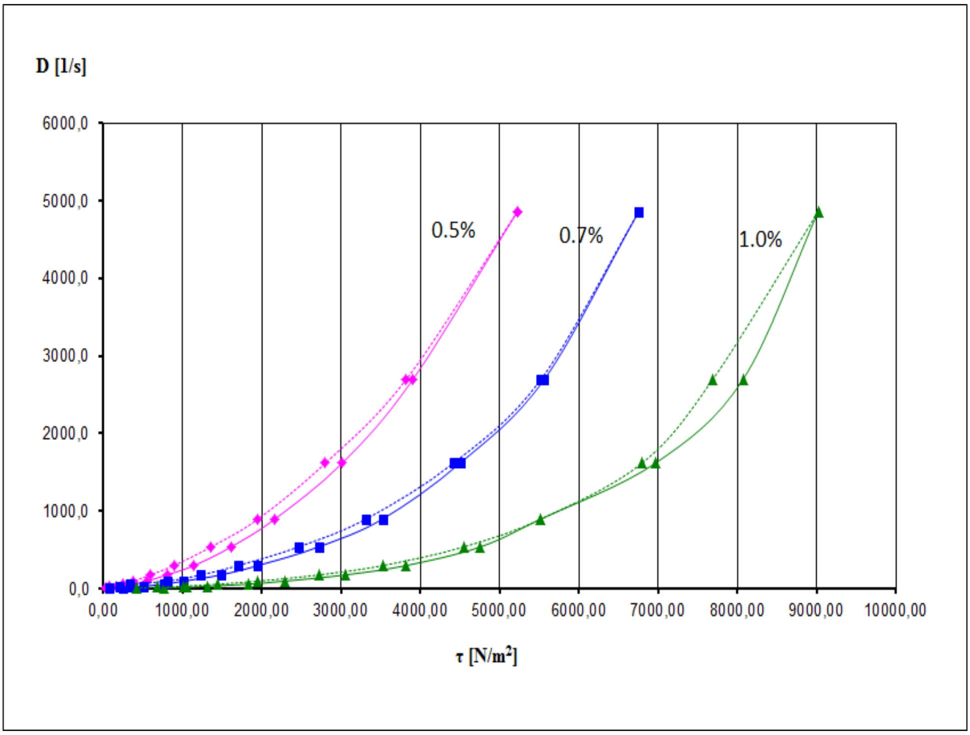
The addition of 5–25% of PEG-200 increased the dynamic viscosity range to 564–679 mPa*s for 1:1 gels and 602–681 mPa*s for 2:1 gels. The addition of 0.5%, 0.7% and 1.0% sodium alginate increased the dynamic viscosity range to 589–746 mPa*s for 1:1 gels and 642–789 mPa*s for 2:1 gels (Figure 1).



D - shear rate, τ - shear stress

Figure 1. Influence of 0.5%, 0.7%, 1.0% sodium alginate on the viscosity of gels obtained from investigated powders containing 4.0% methylcellulose and 5.0% PEG-200

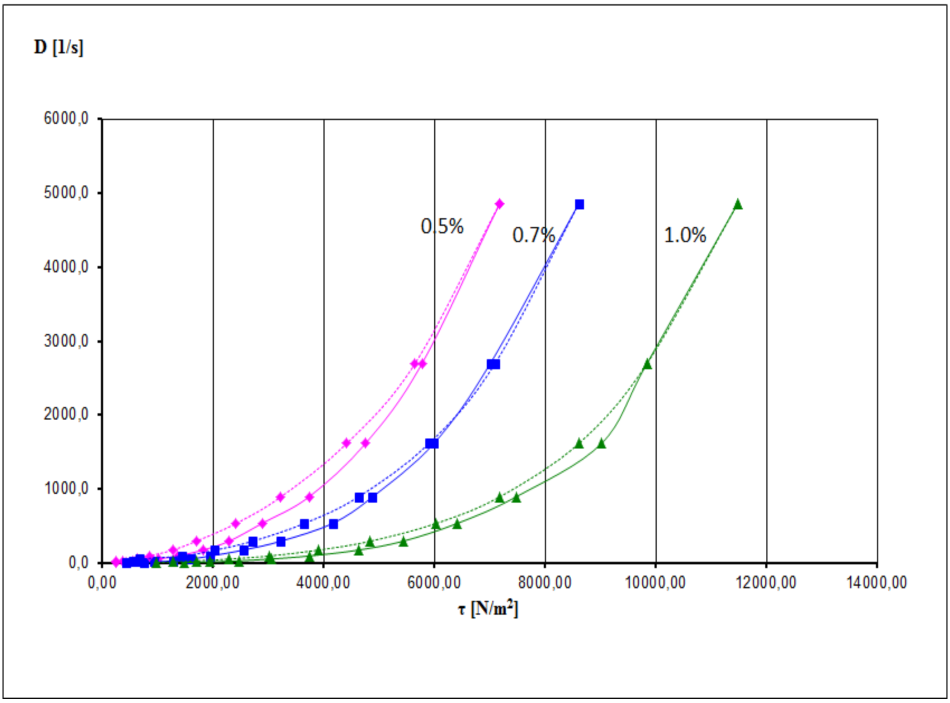
The enrichment of the composition of the tested powders with 5–25% glycerol resulted in an increased dynamic viscosity range of the formulation to between 591 and 681 mPa*s for 1:1 gels and from 615–699 mPa*s for 2:1 gels [14].
A modification of the composition of the tested powders with of 0.5%, 0.7% and 1.0% sodium alginate increased the range of the dynamic viscosity of the formulations to 611–762 mPa*s for 1:1 gels and 645–799 mPa*s for 2:1 gels (Figure 2).



D - shear rate, τ - shear stress

Figure 2. Influence of 0.5%, 0.7%, 1.0% sodium alginate on the viscosity of gels obtained from investigated powders containing 4.0% methylcellulose and 5.0% glycerol

The addition of 5-25% of 1,2-propylene glycol increased the dynamic viscosity range to 609–699 mPa*s for 1:1 gels and 629–726 mPa*s for 2:1 gels [14]. The addition of 0.5%, 0.7% and 1.0% sodium alginate increased the dynamic viscosity range to 663–780 mPa*s for 1:1 gels and 662–807 mPa*s for 2:1 gels (Figure 3).



D - shear rate, τ - shear stress

Figure 3. Influence of 0.5%, 0.7%, 1.0% sodium alginate on the viscosity of gels obtained from investigated powders containing 4.0% methylcellulose and 5.0% 1,2-propylene glycol

Each of the tested hydrogels showed thixotropic properties, creating hysteresis loops in the graphs, depending on the shear rate and shear stress. With the increase in shear rate, the value of the dynamic viscosity of the tested formulations decreased. This testifies to the pseudoplastic nature of the studied gels (Figure 1-3). The rheological assessments revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and sodium alginate in comparison to the gels without sodium alginate.

3.3. Adhesion tests

Tested gels obtained from the powders possessed the work of adhesion - the adhesiveness at 37°C. The existence of a field under the curve above the axis of time shows adhesion. For example, the adhesiveness of gel to the probe was 32.54 g/s for gels obtained with 5.0% glycerol and 1.0% sodium alginate (Figure 4), and was 71.25 g/s for gels obtained with 5.0% 1,2-propylene glycol and 1.0% sodium alginate (Figure 5). These values correspond to the area under the curve - area under the curve of dependence of force needed to separate the probe from the hydrogel from time. The value of the work of adhesion tests and the obtained graphs (Figure 4, 5) indicate good adherence of the obtained gels. A value of adhesiveness above 5.0g/s shows good adhesion. The presented studies have shown that it is possible to obtain gels with high adhesion to the vaginal mucous membrane. The study of the work of adhesion showed the effect of glycerol and 1,2-propylene glycol, and their concentrations, on the value of the work of adhesion. The gels obtained from powders showed good adhesion. The present study has shown that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane, with a dynamic viscosity above 100 mPa*s.

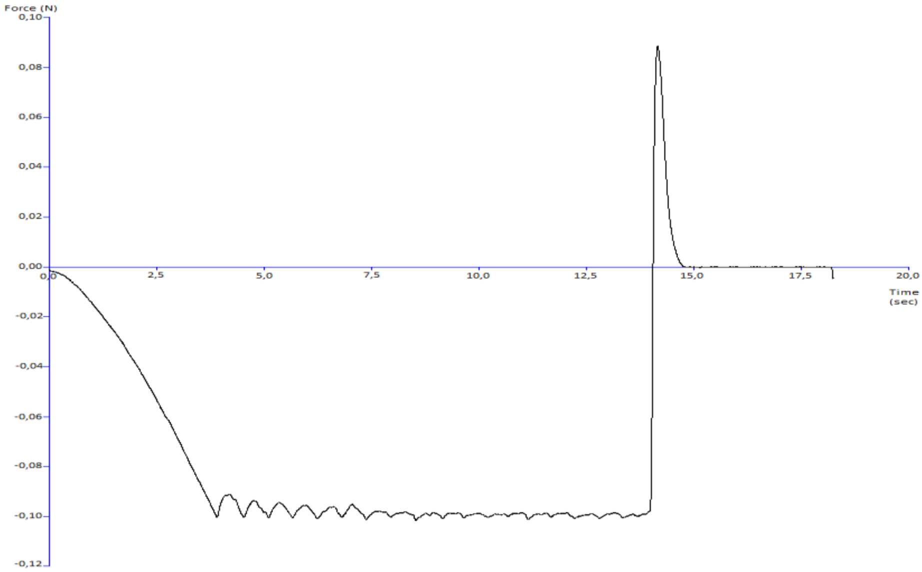


Figure 4. Measurement of texture of 4.0% gels of methylcellulose with a stoichiometric ratio of lactic acid to chitosan of 1:1 and the addition of 1.0% sodium alginate and 5.0% glycerol

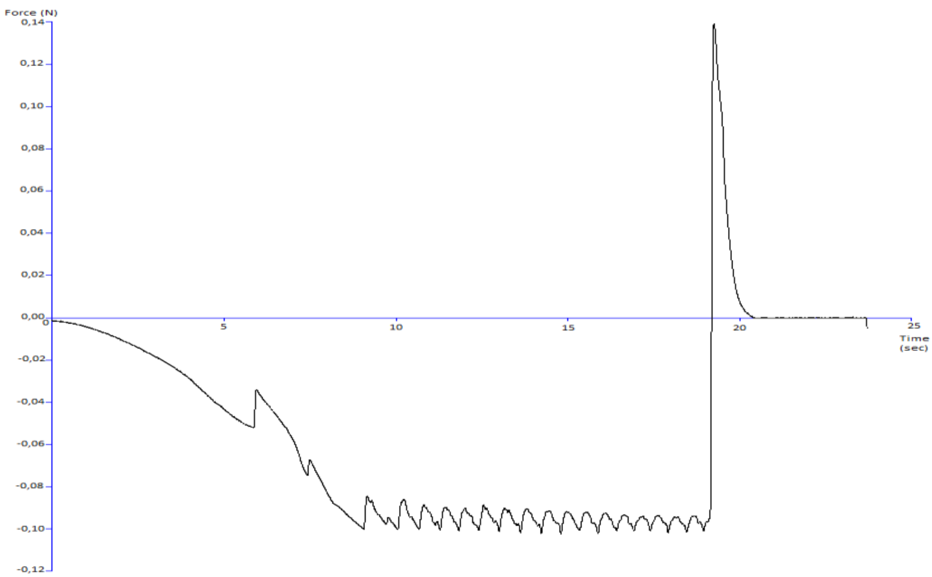


Figure 5. Measurement of the texture of 4.0% gels of methylcellulose with a stoichiometric ratio of lactic acid to chitosan of 1:1 and the addition of 1.0% sodium alginate and 5.0% 1,2-propylene glycol

This study has shown the impact of the used excipients and the ratio of lactic acid to chitosan on pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. The results obtained in the experimental studies demonstrated that it is possible to produce a preparation with optimal pharmaceutical and application properties. Due to the wide pH range, high dynamic viscosity and adhesiveness of the gels obtained from the powders, the powders can be adapted to the individual needs of the patients.

4. Conclusions

The results have shown the impact of sodium alginate, the used excipients and the ratio of lactic acid and chitosan on the pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. The obtained formulations have a pH in the physiological range, along with high viscosity and adhesiveness. Thixotropic properties indicate the possibility of covering the mucosa with the formed gel. The results of adhesion tests and the obtained graphs indicate good adherence of the obtained gels.

5. References

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