THE EFFECT OF A CARBOXYMETHYLCELLULOSE SODIUM SALT ON THE PROPERTIES OF HYDROPHILIC POWDERS CONTAINING A LACTIC ACID-CHITOSAN COMPLEX

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Abstract

The investigated powders on a methylcellulose base revealed good adhesion to the vaginal mucosa and were present on it throughout the daily activities of the patient. This property enabled the continuity of the 24-hour action of the drug. The treatment of vagina inflammatory conditions is usually long and difficult because of the frequent relapse.
Powders were transformed into gels and were tested for their properties. Formulations were prepared with varying pH and rheological properties. The test showed the work of adhesion of gels. All gels with ratios of lactic acid to chitosan of 1:1 and 2:1 showed a pH in the physiological range at 37°C. Additionally, carboxymethylcellulose sodium salt and excipients allow various formulations with a wide range of pH to be obtained. Rheological investigation revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and carboxymethylcellulose sodium salt in comparison to gels without carboxymethylcellulose sodium salt.

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

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1. Introduction

Current methods for the treatment of inflammatory conditions in the vagina are based on antibiotic and chemical therapy, which destroys the physiological conditions in the vaginal environment. Commonly applied drug forms tend to leave the vagina when the patient assumes a vertical position.

The use of a hydrophilic base as a vehicle for the complexes enabled powders passing under natural conditions in gels with rheological properties of the vaginal mucus to be produced. Thus formed gels do not flow out. The gels uniformly cover the mucous membrane and remain there during the daily activities of the patient. In effect, the normal physiological pH is restored, which enables the reconstruction of natural bacterial flora. The effect was achieved thanks to high adhesive properties and the ability of the gel to spread, which provides the prolonged action of the preparation [1-12].

The aim of the study was to investigate the optimisation of the pharmaceutical properties of hydrophilic powders containing lactic acid complexed with chitosan. These powders must form a gel that expresses properties close to those of the natural conditions of the vagina to cover the vaginal mucosa. Therefore, the present study investigated the most important parameters influencing the properties of the tested powders: pH, dynamic viscosity and adhesion.

This work also investigated the effects of carboxymethylcellulose sodium salt additives on the properties of the powders. These powders, forming gels, were examined for their properties. The formulations were prepared with varying pH and rheological properties. The test shows the adhesion of gels. The results allowed the dynamic viscosity of the gels obtained from powders to be defined. A range of pH values in the gels allowed 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 acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland), methylcellulose with a viscosity of 4000 mPa*s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), polyoxyethylene glycol 200 [PEG-200] (Sigma-Aldrich Chemie GmbH, Germany), 1,2-propylene glycol (Sigma-Aldrich Chemie GmbH, Germany), glycerol (Sigma-Aldrich Chemie GmbH, Germany), carboxymethylcellulose sodium salt **[**CMC-Na] (Aldrich Chemical Company Ltd. Gillingham, England), 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 a hydrophilic intravaginal powder

The preparation of powder 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 combined with methylcellulose [4].

2. Preparation of a powder from methylcellulose and carboxymethylcellulose sodium salt.

The carboxymethylcellulose sodium salt (CMC-Na) (0.3g; 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 polyoxyethylene glycol 200 (PEG-200) or 1,2-propylene glycol or glycerol (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 100.0g.

2.3.2. Analytical methods

2.3.2.1. pH-measurement

For pH measurement of the investigated gels, the potentiometric method was used, in which a combined electrode integrated into a multifunctional computer meter ELECTRON CX-742 was immersed in 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 range 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 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]

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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: speed of downward movement of the probe during the test was 0.5 mm/s, and 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 measurements were 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 were 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 lactic acid to chitosan 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 from 4.43 to 4.95 for 1:1 gels and from 3.42 to 3.68 for the 2:1 ratio gels (in comparison to the previous range of 3.92 and 3.48, respectively). The further addition of 0.3%, 0.5%, 0.7% and 1.0% carboxymethylcellulose sodium salt decreased the pH from 4.20 to 4.75 for 1:1 gels (in comparison to the previous range of 4.43 to 4.95) and increased the pH from 3.80 to 4.48 for the 2:1 ratio gels (in comparison to the previous range of 3.42 to 3.68) in relation to the pH range of powders with the addition of PEG-200 (Table 1).

The addition of 5–25% of 1,2-propylene glycol increased the pH from 4.49 to 4.97 for 1:1 gels and from 3.90 to 4.50 for 2:1 gels. The further addition of 0.3%, 0.5%, 0.7% and 1.0% of carboxymethylcellulose sodium salt decreased the pH from 4.32 to 4.80 for 1:1 gels (in comparison to the previous range of 4.49 to 4.97) and from 3.79 to 4.38 for 2:1 gels (in comparison to the previous range of 3.90 to 4.50) in relation to the pH range of powders with the addition of 1,2-propylene glycol (Table 2).

Table 1. Influence of PEG-200 and CMC-Na on the pH of gels obtained from investigated powders containing 4.0% 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 0.3% CMC-Na	pH gels with PEG-200 and addition 0.5% CMC-Na	pH gels with PEG-200 and addition 0.7% CMC-Na	pH gels with PEG-200 and addition 1.0% CMC-Na
1:1	5	4.43	4.30	4.26	4.23	4.20
1:1	10	4.48	4.33	4.20	4.19	4.24
1:1	15	4.55	4.40	4.25	4.20	4.29
1:1	20	4.87	4.48	4.30	4.25	4.32
1:1	25	4.95	4.75	4.45	4.42	4.38
2:1	5	3.42	4.19	4.19	3.95	3.80
2:1	10	3.46	4.23	4.20	4.10	3.86
2:1	15	3.51	4.38	4.23	4.14	3.96
2:1	20	3.63	4.45	4.28	4.22	4.00
2:1	25	3.68	4.48	4.30	4.25	4.15

PEG-200 = polyoxyethylene glycol 200
CMC-Na = carboxymethylcellulose sodium salt

Table 2. Influence of 1,2-propylene glycol and CMC-Na on the pH of gels obtained from investigated powders containing 4.0% methylcellulose

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration [%] 1,2-propylene glycol	pH gels with addition 1,2-propylene glycol	pH gels with 1,2-propylene glycol and addition 0.3% CMC-Na	pH gels with 1,2-propylene glycol and addition 0.5% CMC-Na	pH gels with 1,2-propylene glycol and addition 0.7% CMC-Na	pH gels with1,2-propylene glycol and addition 1.0% CMC-Na
1:1	5	4.49	4.19	4.21	4.29	4.32
1:1	10	4.52	4.29	4.20	4.19	4.17
1:1	15	4.60	4.40	4.25	4.20	4.20
1:1	20	4.88	4.48	4.30	4.25	4.25
1:1	25	4.97	4.80	4.35	4.30	4.26
2:1	5	3.90	4.15	4.12	3.77	3.79
2:1	10	4.11	4.20	4.15	3.80	3.90
2:1	15	4.22	4.33	4.20	3.83	3.97
2:1	20	4.39	4.40	4.26	3.85	4.00
2:1	25	4.50	4.38	4.30	4.26	4.20

The addition of 5–25% of glycerol increased the pH from 4.40 to 4.94 for 1:1 gels and from 3.86 to 4.41 for 2:1 gels. The further addition of 0.3%, 0.5%, 0.7% and 1.0% of carboxymethylcellulose sodium salt decreased the pH from 4.17 to 4.60 for 1:1 gels (in comparison to the previous range of 4.40 to 4.94) and from 3.75 to 4.32 for 2:1 gels (in comparison to the previous range of 3.86 to 4.41) in relation to the pH range of powders with the addition of glycerol (Table 3).

Table 3. Influence of glycerol and CMC-Na on the pH of gels obtained from the investigated powders containing 4.0% methylcellulose

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration [%] glycerol	pH gels with addition glycerol	pH gels with glycerol and addition 0.3% CMC-Na	pH gels with glycerol and addition 0.5% CMC-Na	pH gels with glycerol and addition 0.7% CMC-Na	pH gels with glycerol and addition 1.0% CMC-Na
1:1	5	4.40	4.38	4.30	4.25	4.17
1:1	10	4.44	4.26	4.20	4.23	4.19
1:1	15	4.54	4.38	4.25	4.20	4.20
1:1	20	4.84	4.45	4.30	4.25	4.25
1:1	25	4.94	4.60	4.35	4.30	4.26
2:1	5	3.86	4.15	4.12	3.77	3.75
2:1	10	4.06	4.20	4.15	3.80	3.77
2:1	15	4.16	4.27	4.20	3.83	3.79
2:1	20	4.31	4.30	4.26	3.85	3.82
2:1	25	4.41	4.32	4.29	3.90	3.87

The use of methylcellulose and carboxymethylcellulose sodium salt allows various formulations with a wide range of pH to be obtained. The pH decreased with an increasing concentration of carboxymethylcellulose sodium salt in gels obtained from powders. All gels with the lactic acid–chitosan complex at 1:1 and 2:1 weight ratios showed a pH in the physiological range of 3.5–5.0 at 37°C. The addition of carboxymethylcellulose sodium salt and excipients allowed various formulations with a wide range of pH to be obtained. Formulations containing the complex at the weight ratio of 2:1 showed the lowest pH, which is an important feature and can be used in the treatment of advanced bacterial vaginosis.

3.2. Rheological tests

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

The enrichment of the composition of the tested powders with 5–25% glycerol resulted in an increase in the dynamic viscosity of the formulation from 591 to 681 mPa*s for 1:1 gels and from 615 to 699 mPa*s for 2:1 gels (Table 4).

A modification of the composition of the tested powders with 0.3%, 0.5%, 0.7% and 1.0% of carboxymethylcellulose sodium salt increased the dynamic viscosity of formulations from 635 to 816 mPa*s for 1:1 gels and from 660 to 780 mPa*s for 2:1 gels (Table 4).

Table 4. Influence of glycerol and CMC-Na on the viscosity of gels obtained from investigated powders containing 4.0% methylcellulose

Stoichiometric weight ratio of lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration of glycerol [%]	Dynamic viscosity of gels with glycerol [mPa*s]	Dynamic viscosity of gels with glycerol and 0.3% CMC-Na [mPa*s]	Dynamic viscosity of gels with glycerol and 0.5% CMC-Na [mPa*s]	Dynamic viscosity of gels with glycerol and 0.7% CMC-Na [mPa*s]	Dynamic viscosity of gels with glycerol and 1.0% CMC-Na [mPa*s]
1:1	5	681	689	699	752	816
1:1	10	654	678	689	775	778
1:1	15	623	669	685	740	756
1:1	20	599	646	659	723	732
1:1	25	591	635	642	663	686
2:1	5	699	729	741	772	780
2:1	10	663	712	736	758	762
2:1	15	645	679	695	753	759
2:1	20	629	672	689	740	743
2:1	25	615	660	681	735	739

The addition of 5–25% of 1,2-propylene glycol increased the dynamic viscosity from 609 to 699 mPa*s for 1:1gels and from 629 to 726 mPa*s for 2:1 gels [14]. The further addition of 0.3%, 0.5%, 0.7% and 1.0% of carboxymethylcellulose sodium salt increased the dynamic viscosity from 645 to 861 mPa*s for 1:1 gels and from 672 to 846 mPa*s for 2:1 gels.

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and carboxymethylcellulose sodium salt in comparison to the gels without carboxymethylcellulose sodium salt. The dynamic viscosity increased with increasing concentrations of carboxymethylcellulose sodium salt in gels obtained from powders.

3.3. Adhesion tests

The 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 66.30 g/s for gels obtained with 5.0% glycerol and 1.0% carboxymethylcellulose sodium salt (Figure 1), and 74.40 g/s for gels obtained with 5.0% 1,2-propylene glycol and 1.0% carboxymethylcellulose sodium salt (Figure 2). 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 over time. The value of the work of adhesion tests and the obtained graphs (Figures 1 and 2) indicate good adherence of the obtained gels. Values of adhesiveness above 5.0g/s indicate 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, 1,2-propylene glycol and its concentration 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 the vaginal mucous membrane, with a dynamic viscosity above 100 mPa*s.

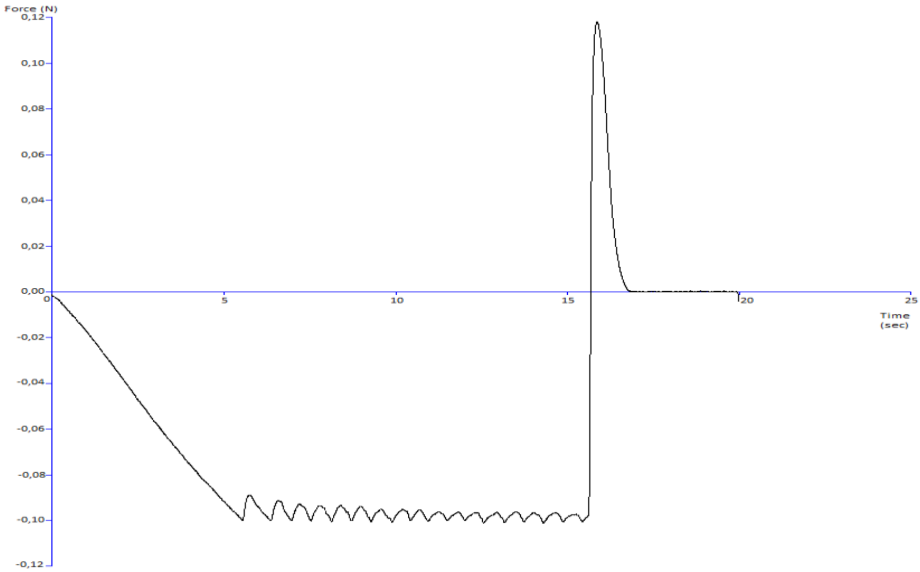

Figure 1. Measurement of the texture of 4.0% methylcellulose gels with a stoichiometric ratio of lactic acid to chitosan of 1:1 and the addition of 1.0% carboxymethylcellulose sodium salt and 5.0% glycerol

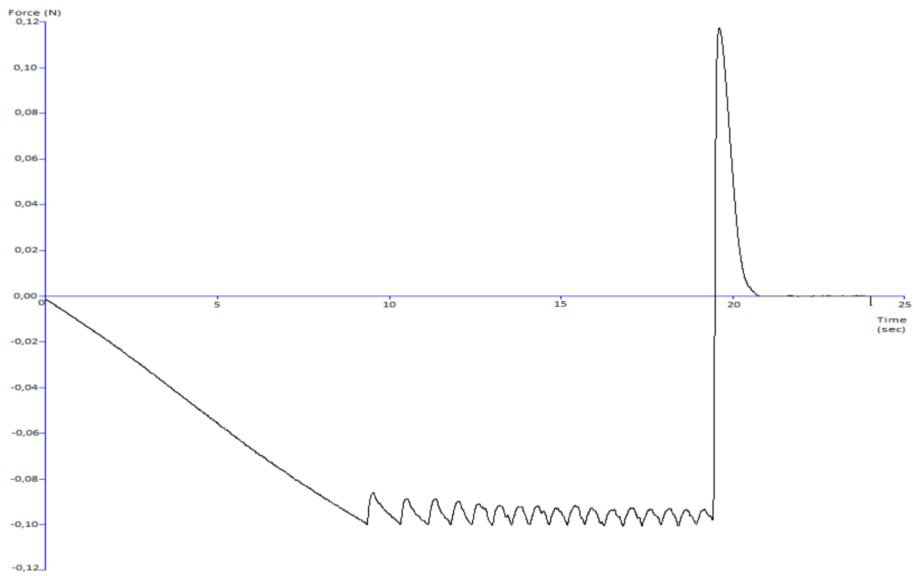

Figure 2. Measurement of the texture of 4.0% methylcellulose gels with a stoichiometric ratio of lactic acid to chitosan of 1:1 and the addition of 1.0% carboxymethylcellulose sodium salt and 5.0% 1,2-propylene glycol

The present study demonstrates the impact of the used excipients and the ratio of lactic acid to chitosan on pH, dynamic viscosity and the adhesiveness of methylcellulose gels obtained from powders.
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, powders may be adapted to the individual needs of the patients.

4. Conclusions

The research demonstrated that the impact of carboxymethylcellulose sodium salt, excipients used and the ratio of lactic acid to chitosan affected the pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. The obtained formulations have a pH in the desired physiological range and high viscosity and adhesiveness. The work of adhesion tests and the obtained graphs indicate good adherence of the obtained gels.
The results obtained in the experimental studies demonstrated that it is possible to produce a preparation with optimal pharmaceutical and application properties.

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