

THE PROPERTIES OF INTRAVAGINAL GLOBULES CONTAINING A LACTIC ACID-CHITOSAN COMPLEX

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Abstract

Continuing the implementation of research on the problem of treatment of vaginal pH disorders, intravaginal globules were examined as vaginal drug form which carries the lactic acid complexes with chitosan. The intravaginal application of globules that transform into gel under natural conditions is aimed at achieving the physiological pH of the vaginal environment. Formulations were prepared with varying pH and rheological properties. The test showed the work of adhesion of gels. All gels with ratios of 1:1 and 2:1 of lactic acid to chitosan showed a pH in the physiological range at 37°C. Additional hydroxypropylmethylcellulose and excipients allows 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 hydroxypropylmethylcellulose in comparison to the gels without hydroxypropylmethylcellulose. The study of the work of adhesion showed the effect of hydroxypropylmethylcellulose and their concentration on the value of the work of adhesion.

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

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1. Introduction

Constant contact between the drug form and the vaginal mucosa during the patient's daily activities is the basic condition for successful therapy. This condition can be met by forms of the drug with high adhesion to the vaginal mucosa. Available literature does not inform about a significant progress in the effectiveness of treatment of bacterial vaginosis [1-3].

Continuing the implementation of research on the problem of treatment of vaginal pH disorders, intravaginal globules containing methylcellulose and gelatin were examined as vaginal drug form which carries the lactic acid complexes with chitosan. The intravaginal application of globules that transform into gel under natural conditions and is aimed at achieving the physiological pH of the vaginal environment [4-14].

The aim of the study was to examine the pharmaceutical properties of gynecological globules that transform into a gel in natural conditions and are intended to cover the mucous membrane with a gel.

In an effort to solve the problem, the effect of hydroxypropylmethylcellulose and hydrophilizing substances such as polyoxyethylene glycol 200 on the properties of beads was investigated. Formulations were prepared with varying pH and rheological properties. As a result of the research was obtained preparations with different pH values including the physiological range. Globules show the adhesion of the gel covering the surface of the apparatus simulates the conditions in the vagina. The gels obtained from the globules were characterized by the thixotropy and specific dynamic viscosity.

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 viscosity of 4000 mPa*s, 2% in H₂O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), gelatin (LOBA – Chemie, Wien – Fishamend), polyoxyethylene glycol 200 [PEG-200] (Sigma-Aldrich Chemie GmbH, Germany), hydroxypropylmethylcellulose, Sigma – Aldrich Chemie GmbH, Germany), aqua purificata as required by you FP XI.

2.2. Apparatus

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

2.3. Methods

2.3.1. Preparation of hydrophilic intravaginal globules

The preparation of globules 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 [4].

2. Obtaining the excipient:

a) Preparation of gel from methylcellulose and hydroxypropylmethylcellulose

A gel was obtained from methylcellulose and hydroxypropylmethylcellulose by adding a known amount of this compound to the solution of hydrophilizing substance in water. In order to enhance the process of gelation, the mixture was cooled to 5 - 10 °C. The homogenous gel was weighed and enough distilled water was added to obtain the initial mass.

b) Preparation of gel from gelatin

Gelatin was left with water until swelling was completed and then dissolved by heating. The lactic acid - chitosan complex was added to liquid gelatinous gel and heated until an homogenous gel was obtained. Distilled water was added to obtain the initial mass.

c) Preparation of the excipient and pouring into the form

Gels prepared from methylcellulose (4.0g), hydroxypropylmethylcellulose (1.0g; 2.0g; 3.0g; 4.0g) and gelatin (16.0g) with the lactic acid - chitosan complex were combined into a homogenous excipient and supplemented with distilled water. The excipient was poured into a form that had been previously covered with a thin layer of polyoxyethylene glycol – 200.

3. Preparation of the tested gel.

A gel was obtained by dissolved by heating the globules in water bath.

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 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 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: $\tau = c \cdot \alpha_{(1-12)} = 85.0 \cdot \alpha_{(1-12)}$

• viscosity for the range Ia: $\eta = \frac{\tau}{D(1-12)} \cdot 100 = \frac{85.0 \cdot \alpha(1-12)}{D(1-12)} \cdot 100$

• shear stress for the range IIa: $\tau = c \cdot \alpha_{(1-12)} = 820.2 \cdot \alpha_{(1-12)}$

• 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/m²]; 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.

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 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 measurement 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 globules containing lactic acid complexed with chitosan revealed stoichiometric weight ratio of 1:1 and 2:1 lactic acid to chitosan and 4.0% methylcellulose. Their 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 ranged from 4.43 to 4.95 for 1:1 gels and from 3.42 to 3.68 for the 2:1 ratio gels (in compare to previous range from 3.92 and 3.48). Further addition of 1.0%, 2.0%, 3.0% and 4.0% of hydroxypropylmethylcellulose decreased the pH from 4.35 to 4.00 for 1:1 gels (in compare to previous range from 4.95 to 4.43) and increased the pH from 3.60 to 4.33 for the 2:1 ratio gels (in compare to previous range from 3.42 to 3.68) in relation to the pH range of gels with the addition of PEG-200 (Table 1).

The use of methylcellulose and hydroxypropylmethylcellulose allows various formulations with a wide range of pH to be obtained. The pH decreased with increasing concentration of hydroxypropylmethylcellulose 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 hydroxypropylmethylcellulose 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.

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Table 1. Influence of PEG-200 and HPMC on the pH of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

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 1.0% HPMC	pH gels with PEG-200 and addition 2.0% HPMC	pH gels with PEG-200 and addition 3.0% HPMC	pH gels with PEG-200 and addition 4.0% HPMC
1:1	5	4.43	4.05	4.04	4.02	4.00
1:1	10	4.48	4.11	4.05	4.04	4.02
1:1	15	4.55	4.23	4.10	4.06	4.05
1:1	20	4.87	4.30	4.15	4.12	4.10
1:1	25	4.95	4.35	4.20	4.15	4.11
2:1	5	3.42	4.00	3.97	3.62	3.60
2:1	10	3.46	4.05	4.00	3.65	3.62
2:1	15	3.51	4.17	4.05	3.68	3.64
2:1	20	3.63	4.25	4.11	3.70	3.67
2:1	25	3.68	4.33	4.15	3.75	3.72

HPMC - Hydroxypropylmethylcellulose

Table 2. Influence of PEG-200 and HPMC on the viscosity of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Conc. PEG-200 [%]	Dynamic viscosity of gels with addition PEG-200 [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 1.0% HPMC [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 2.0% HPMC [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 3.0% HPMC [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 4.0% HPMC [mPa*s]
1:1	5	354.41	783.57	785.27	786.54	790.64
1:1	10	334.17	780.96	784.99	785.33	789.76
1:1	15	280.02	778.78	782.68	784.44	788.96
1:1	20	234.41	775.87	780.35	782.85	787.54
1:1	25	139.16	773.24	779.43	780.22	785.98
2:1	5	368.14	844.94	850.11	860.68	866.85
2:1	10	250.02	830.78	817.92	855.32	855.35
2:1	15	233.15	828.96	838.48	845.24	850.67
2:1	20	224.56	826.59	835.33	840.31	834.23
2:1	25	216.27	823.15	824.23	820.87	824.51

HPMC - Hydroxypropylmethylcellulose

3.2. Rheological tests

Rheological studies demonstrated that the gels obtained from globules possessed a dynamic viscosity of the formulation from 139.16 to 354.41 mPa*s for the 1:1 stoichiometric ratio in the complex and from 216.27 to 368.14 mPa*s for the 2:1 ratio. A modification of the composition of the tested globules with 1.0%, 2.0%, 3.0% and 4.0% of hydroxypropylmethylcellulose increased the dynamic viscosity of formulations from 773.24 to 790.64 mPa*s for 1:1gels and from 817.92 to 866.85 mPa*s for 2:1 gels (Table 2).

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and hydroxypropylmethylcellulose in comparison to the gels without hydroxypropylmethylcellulose. The dynamic viscosity increased with increasing concentration of hydroxypropylmethylcellulose in gels obtained from globules.

3.3. Adhesion tests

Tested gels possessed the work of adhesion - the adhesiveness at 37 °C . Work of adhesion demonstrated that the gels obtained from globules possessed a value of the formulation from 76.5 to 35.7 g/s for the 1:1 stoichiometric ratio in the complex and from 47.6 to 35.7 g/s for the 2:1 ratio. A modification of the composition of the tested globules with 1.0%, 2.0%, 3.0% and 4.0% of hydroxypropylmethylcellulose increased the work of adhesion of formulations from 140.0 to 176.1 g/s for 1:1gels and from 167.1 to 182.1 g/s for 2:1 gels (Table 3). The study of the work of adhesion showed the effect of hydroxypropylmethylcellulose and their concentration on the value of the work of adhesion. The gels showed good adhesion.

Table 3. Influence of PEG-200 and HPMC on the work of adhesion of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Conc. PEG-200 [%]	Work of adhesion of gels with addition PEG-200 [g/s]	Work of adhesion of gels with PEG-200 and addition 1.0% HPMC [g/s]	Work of adhesion of gels with PEG-200 and addition 2.0% HPMC [g/s]	Work of adhesion of gels with PEG-200 and addition 3.0% HPMC [g/s]	Work of adhesion of gels with PEG-200 and addition 4.0% HPMC [g/s]
1:1	5	76.5	160.5	166.2	169.8	176.1
1:1	10	74.3	156.7	164.1	168.0	175.8
1:1	15	56.4	149.2	159.8	167.1	175.0
1:1	20	48.3	145.8	157.5	164.1	166.5
1:1	25	35.7	140.0	146.9	150.6	154.3
2:1	5	47.6	178.2	179.8	180.8	182.1
2:1	10	45.8	175.8	177.0	179.8	180.9
2:1	15	40.5	170.5	171.8	175.2	176.9
2:1	20	38.3	168.9	169.9	171.6	173.6
2:1	25	35.7	167.1	168.9	170.3	171.8

HPMC - Hydroxypropylmethylcellulose

4. Conclusions

The researches demonstrated that the impact of hydroxypropylmethylcellulose, used excipients and the ratio of lactic acid to chitosan affected the pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from globules. The obtained formulations have a pH in the desired physiological range and have high viscosity and adhesiveness. The gels showed good adhesion.

The results obtained in the experimental studies demonstrated that it is possible to produce a preparation with optimal pharmaceutical and application properties.

5. References

- [1] Donders GGG, Larson PG, Platz – Christensen JJ, Hallen A, Meijden W, Wölnner – Hanssen P; (2009) Variability in diagnosis of clue cells, lactobacillary grading and white blood cells in vaginal wet smears with conventional bright light and phase contrast microscopy. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 145, 109 – 112. Doi.org/10.1016/j.ejogrb.2009.04.012
- [2] Schwebke JR; (2009) New Concepts in the Etiology of Bacterial Vaginosis. *Curr. Infect. Dis. Rep.* 11, 143 – 147. Doi: 10.1007/s11908-009-0021-7
- [3] Brandt M, Abels C, May T, Lohmann K, Schmidts – Winkler I, Hoyme UB; (2008) Intravaginally applied metronidazole is as effective as orally applied in the treatment of bacterial vaginosis, but exhibits significantly less side effects. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 141, 158 – 162. Doi.org/10.1016/j.ejogrb.2008.07.022
- [4] Kubis AA, Małolepsza-Jarmołowska K; (1996) Studies on gynecological hydrophilic preparations comprising lactic acid. Part 1: Effects of lactic acid and hydrophilic agents on physical and chemical properties of methylcellulose gels. *Pharmazie* 51, 989 – 990.
- [5] Małolepsza-Jarmołowska K, Kubis AA; (1999) Studies on gynecological hydrophilic lactic acid preparations. Part 2: Effects of Eudragit® E-100 on properties of methylcellulose gels. *Pharmazie* 54, 441 – 443.
- [6] Małolepsza-Jarmołowska K, Kubis AA; (2000) Studies on gynaecological hydrophilic lactic acid preparations. Part 3: Effects of chitosan on the properties of methylcellulose gels. *Pharmazie* 55, 610 – 611.
- [7] Małolepsza-Jarmołowska K, Kubis AA; (2001) Studies on gynaecological hydrophilic lactic acid preparations. Part 4: Effects of polyvinyl pyrrolidone K-90 on properties of methylcellulose gels. *Pharmazie* 56, 160 – 162.
- [8] Małolepsza-Jarmołowska K, Kubis AA, Hirnle L; (2003) Studies on gynaecological hydrophilic lactic acid preparations. Part 5: The use of Eudragit® E-100 as lactic acid carrier in intravaginal tablets. *Pharmazie* 58, 260 – 262.
- [9] Małolepsza-Jarmołowska K, Kubis AA, Hirnle L; (2003) Studies on gynaecological hydrophilic lactic acid preparations. Part 6: Use of Eudragit® E-100 as lactic acid carrier in intravaginal tablets. *Pharmazie* 58, 334-336.
- [10] Małolepsza-Jarmołowska K; (2006) Studies on gynaecological hydrophilic lactic acid preparations. Part 7: Use of chitosan as lactic acid carrier in intravaginal tablets (globuli vaginales). *Pharmazie* 61, 780 - 782.
- [11] Małolepsza-Jarmołowska K; (2007) Studies on gynaecological hydrophilic lactic acid preparations. Part 8: Use of chitosan as lactic acid carrier in intravaginal tablets. *Acta Pol. Pharm.* 64, 69 - 72.
- [12] Małolepsza-Jarmołowska K; (2010) The effect of poloxamer 407 on the properties of hydrophilic gels containing lactic acid complexed with chitosan. Monograph vol.

- XV ed. by M. Jaworska „Progress on Chemistry and Application of Chitin and Its Derivatives“ 15, 143-148.
- [13] Małolepsza-Jaromłowska K; (2014) Research of pharmaceutical properties of hydrophilic powders containing lactic acid complexed with chitosan „Progress on Chemistry and Application of Chitin and Its Derivatives“ 14, 79-86 vol. XIX, ed. by M. Jaworska Doi:10.15259/PCACD.19.09
- [14] Małolepsza-Jaromłowska K; (2017) Influence of a pectin on the properties of hydrophilic powders containing a lactic acid-chitosan complex „Progress on Chemistry and Application of Chitin and Its Derivatives“ 17, 135-142 vol. XXII, ed. by M. Jaworska Doi:10.15259/PCACD.22.13