THE USE OF DEXTRAN IN GYNECOLOGICAL POWDERS CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN

Katarzyna Małolepsza-Jarmołowska

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

Abstract

Powders for gynecological use pass under natural conditions in gels covering the vaginal mucosa. This study has shown the impact of used excipients and the ratio of lactic acid to chitosan on pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. All gels with complexes in the ratios of 1:1 and 2:1 (lactic acid:chitosan) showed a pH in the physiological range 3.5-5.0 at 37°C. The addition of dextran and excipients to these complexes allows various formulations over a wide range of pH to be obtained. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and dextran in comparison to gels without dextran. 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. The presented work shows that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane.

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

Received: 28.05.2017
Accepted: 19.07.2017

1. Introduction

The effectiveness of anti-inflammatory drugs and drugs that reconstruct the physiological environment of the vagina greatly depend on the time of contact between the therapeutic substance and the mucous membrane of the organ. This condition may be fulfilled by drug forms with a high ability to adhere to the vaginal mucosa. The available literature does not offer any significant progress in the efficacy of vaginitis therapy.

The use of hydrophilic powders with high adhesion properties, dynamic viscosity above 100 mPa*s and the ability to spread over the vaginal mucosa enable prolonged action of the drug. Preparations that remain at the site of application produce adequate pH 3.5-5.0 in the environment due to the content of lactic acid complexed with chitosan. Powders for gynecological use pass under natural conditions in the gel covering the vaginal mucosa [1-12].

The aim of this study was to investigate the influence of dextran on the physico-chemical properties of powders for gynecological purposes. The most important parameters influencing the properties of the tested powders, such as pH, dynamic viscosity, adhesion, have been determined.

By adopting the above-mentioned assumptions, the present work tested the impact of dextran on the properties of the powders. In the study, formulations were prepared with different pH and rheological properties.

These powders, passing in 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 work of adhesion of gels. By studying a range of pH of 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% (Sea Fisheries Institute, Gdynia, Poland), methylcellulose (Aldrich Chemical Company Ltd. Gillingham, England), glycerol (Sigma-Aldrich Chemie GmbH, Germany), 1,2-propylene glycol (Sigma – Aldrich Chemie GmbH, Germany), dextran (Sigma – Aldrich Chemie GmbH, Germany), aqua purificata, acc. to FP X.

2.2. Methods

2.2.1. 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.

The required amount of powdered chitosan was added to a known amount of lactic acid 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 dextran.

The dextran was mixed with a known amount of methylcellulose. Next, the mixture was added to the lactic acid complexes with chitosan and glycerol or 1,2-propylene glycol. The resulting powder was thoroughly pulverized.. 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 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.

2.2.2. Analytical methods

2.2.2.1. 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. Prior to the measurement, the computer meter was calibrated by two buffer solutions with pH 7.00 and pH 4.00. All gels were tested three times, and the results were reported as the average of three measurements at 37°C.

2.2.2.2. 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 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.

2.2.2.3. Measurement of adhesion

A test for texture profile analysis (TPA) was performed with Exponent Stable Micro Systems Texture Analyzer TA-XT 2 plus.

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 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

Gels obtained from powders containing lactic acid complexed with chitosan revealed stoichiometric ratios of 1:1 and 2:1 and 4% methylcellulose (4000 mPa*s). Their pH ranged from 3.92 for 1:1gels to 3.48 for 2:1gels [13].

The addition of 5-25% glycerol increased the pH range to 4.40 - 4.94 for 1:1gels and 3.86 - 4.41 for 2:1 gels. The further addition of 1.0% dextran decreased the pH range to 4.37 - 4.90 for 1:1gels and 3.82 - 4.00 for 2:1 gels in relation to the pH ranges of powders with the addition of glycerol (Table 1).

Table 1. Influence of glycerol and dextran on the pH of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of glycerol [%]	pH of gels with glycerol	pH of gels with glycerol and 1.0% dextran
1:1	5	4.40	4.37
1:1	10	4.44	4.41
1:1	15	4.54	4.52
1:1	20	4.84	4.81
1:1	25	4.94	4.90
2:1	5	3.86	3.82
2:1	10	4.06	3.88
2:1	15	4.16	3.92
2:1	20	4.31	3.99
2:1	25	4.41	4.00

The addition of 5-25% of 1,2-propylene glycol increased the pH range to 4.49 - 4.97 for 1:1gels and 3.90 - 4.50 for 2:1 gels. The further addition of the 1.0% dextran decreased the pH range to 4.47 - 4.92 for 1:1gels and 3.88 - 4.45 for 2:1 gels in relation to the pH range of powders with the addition of 1,2-propylene glycol (Table 2).

Table 2. Influence of 1,2-propylene glycol and dextran on the pH of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of 1,2-propylene glycol [%]	pH of gels with 1,2-propylene glycol	pH of gels with 1,2-propylene glycol and 1.0% dextran
1:1	5	4.49	4.47
1:1	10	4.52	4.51
1:1	15	4.60	4.58
1:1	20	4.88	4.85
1:1	25	4.97	4.92
2:1	5	3.90	3.88
2:1	10	4.11	3.98
2:1	15	4.22	4.20
2:1	20	4.39	4.30
2:1	25	4.50	4.45

The rheological analyses demonstrated that the researched gels obtained from powders possessed 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 increased dynamic viscosity range of the formulation to between 591 and 681 mPa*s for 1:1gels and from 615 to 699 mPa*s for 2:1 gels (Table 3).
A modification of the composition of the tested powders with 1.0% dextran increased the range of the dynamic viscosity of the formulations to 683 - 753 mPa*s for 1:1gels and 710 - 782 mPa*s for 2:1 gels (Table 3).

Table 3. Influence of glycerol and dextran on the viscosity of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	**Concentration of glycerol [%]**	**Dynamic viscosity of gels with glycerol [mPa*s]**	**Dynamic viscosity of gels with glycerol and 1.0% dextran [mPa*s]**
1:1	5	681	753
1:1	10	654	734
1:1	15	623	708
1:1	20	599	690
1:1	25	591	683
2:1	5	699	782
2:1	10	663	761
2:1	15	645	739
2:1	20	629	725
2:1	25	615	710

The addition of 5-25% of 1,2-propylene glycol increased the dynamic viscosity range to 609 - 699 mPa*s for 1:1gels and 629 - 726 mPa*s for 2:1 gels. Addition of 1.0% dextran increased the dynamic viscosity range to 663 - 746 mPa*s for 1:1gels and 662 - 789 mPa*s for 2:1 gels (Table 4).

Table 4. Influence of 1,2-propylene glycol and dextran on the viscosity of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of 1,2-propylene glycol [%]	Dynamic viscosity of gels with 1,2-propylene glycol [mPa*s]	Dynamic viscosity of gels with 1,2-propylene glycol and 1.0% dextran [mPa*s]
1:1	5	699	746
1:1	10	667	723
1:1	15	634	701
1:1	20	615	686
1:1	25	609	663
2:1	5	726	789
2:1	10	678	767
2:1	15	655	714
2:1	20	636	706
2:1	25	629	662

The investigation of gels obtained from powders demonstrated the work of adhesion - the adhesiveness of the gel to the probe was 35.86 g/s for gels obtained with 5.0% glycerol and 1.0% dextran (Figure 1), and it was 49.62 g/s for gels obtained with 5.0% 1,2-propylene glycol and 1.0% dextran (Figure 2).

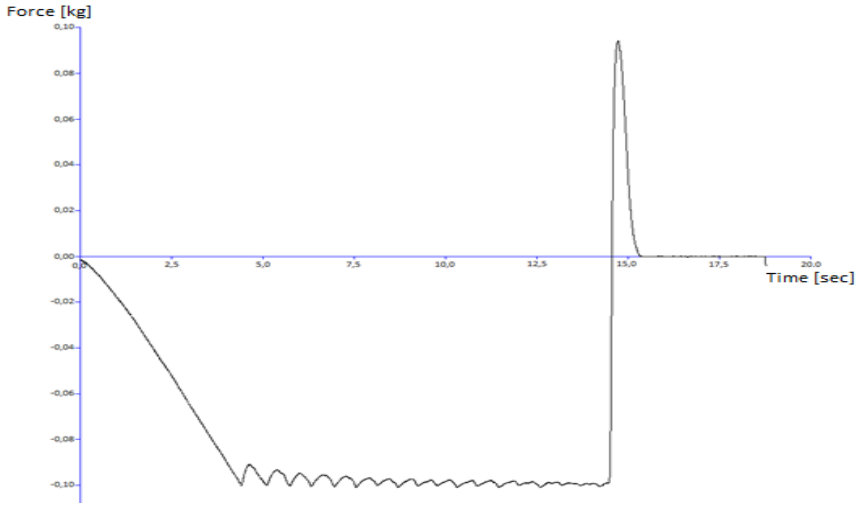

Figure 1. Measurement of texture of gels with 5.0% glycerol and 1.0% dextran with the addition of 4000 mPa*s methylcellulose and a stoichiometric ratio of lactic acid to chitosan of 1:1

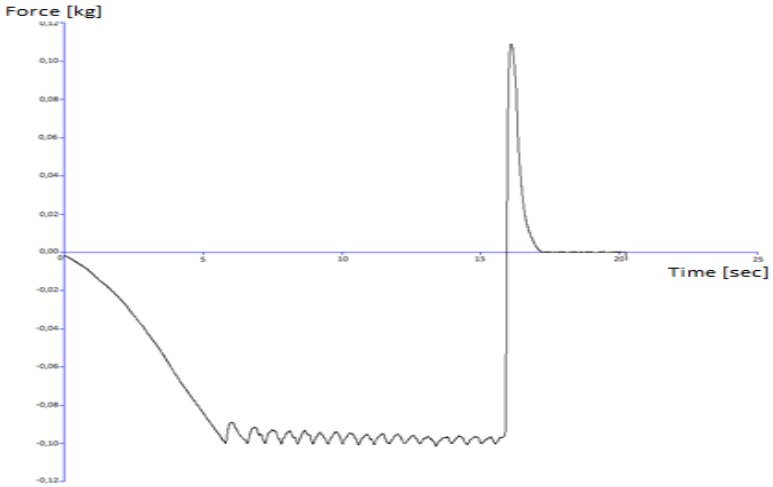

Figure 2. Measurement of texture of gels with 5.0% 1,2-propylene glycol and 1.0% dextran with the addition of 4000 mPa*s methylcellulose and a stoichiometric ratio of lactic acid to chitosan of 1:1

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. The use of methylcellulose with dextran allows different formulations to be obtained with a wide range of pH. The rheological assessments revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and dextran in comparison to the gels without dextran. The results of the work of adhesion showed the effect of glycerol, 1,2-propylene glycol and their concentration on the value of the work of adhesion.

This study has also 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.

All gels with the ratios of 1:1 and 2:1 showed a pH in the physiological range (3.5–5.0) at 37°C. The addition of dextran 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. 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 can be adapted to the individual needs of the patients.

4. Conclusions

The results have shown the impact of dextran, used excipients and the ratio of lactic acid and chitosan on 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.

5. References

[**1**] 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

[**2**] 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

[**3**] Donders GGG, Larson PG, Platz – Christensen JJ, Hallen A, Meijden W, Wölner – 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

[**4**] Kubis AA, Małolepsza-Jarmołowska K; (1996) Studies on gynecological hydrophiling preparations comprising lactic acid. Part 1: Effects of lactic acid and hydrophiling 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-Jarmołowska 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“ XIX, 79-86. **DOI**:10.15259/PCACD.19.09