INFLUENCE OF A CHOSEN AUXILIARY SUBSTANCE ON THE PROPERTIES OF HYDROPHILIC 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,
ul. Borowska 211A, 50-556 Wrocław, Poland.
e-mail: katarzynamj@poczta.onet.pl

Abstract
Hydrophilic powders passing gels containing lactic acid complexed with chitosan at a stoichiometric ratio of 1:1 and 8:1 revealed pH ranged from 3.92 to 4.44 and 2.36 to 2.84. Rheological studies demonstrated that the research gels obtained from powders possess the dynamic viscosity ranging from 53 to 398 mPa·s for the 1:1 stoichiometric ratio in the complex and from 19 to 242 mPa·s for the 8:1 ratio. As a result of the research, preparations with different pH values, including physiological range, were obtained. Powders show that the adhesion of the gel covering the surface of the apparatus simulates the conditions in the vagina. The gels obtained from the powders were characterised by the specific dynamic viscosity and possess the work of adhesion. The results obtained in the experimental studies proved that it is possible to produce a preparation with optimal pharmaceutical and application properties.

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

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1. Introduction

The effectiveness of anti-inflammatory drugs and drugs reconstructing the physiological environment of the vagina greatly depends on the time of contact between the therapeutic substance and the mucous membrane of the organ. The main problem in applied therapies is maintaining the continuity of treatment over 24 hours. The use of hydrophilic base for lactic acid complexed with alkaline polymers enabled the production of gels with rheological properties of vaginal discharge. The gel remains at the site of application and provides an adequate environmental pH [1 - 12].

Whilst carrying out research on the problem of the treatment of disorders of pH of the vaginal environment, powders were tested as a form of the drug carrier, with vaginal lactic acid complexed with chitosan. Application of the powders passing applications in natural conditions in the gel is designed to obtain a physiological pH of the vaginal environment. Powder passing gels were examined for their properties. The study used methylcellulose with a viscosity of 4000, 1500, 400, 25, and 15 mPa·s. In order to determine the holding capacity of a gel obtained with powders on the surface of the vaginal mucosa, studies were conducted in a device that simulates natural conditions. Rheological studies were also performed in gels formed from powders.

As a result of the research, preparations with different pH values, including the physiological range, were obtained. Powders show that adhesion of the gel covering the surface of the apparatus simulates the conditions in the vagina. The gels obtained from the powders were characterised by the specific dynamic viscosity.

2. Materials and methods

2.1. Materials

The following chemicals were used in 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 – Dorest SP 84 SL, England), and aqua purificata, acc. to FP IX.

2.2. Methods

2.2.1. Measurements of physical properties

2.2.1.1. Rheological investigations (dynamic viscosity)

Rheological investigations were performed using a rotational viscosimeter. The determinations were performed in I a and II a range 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. Viscosity and torque were calculated from appropriate formulas. The results obtained were used to plot the flow curves of the investigated gels. The results obtained in the experimental are presented in ***Table 2***.

2.2.1.2. Determination of adhesion

The determination of adhesion was performed on a biopharmaceutical model imitating the conditions in the vagina. This was a 30 cm long, 3 cm in diameter, calibrated glass

tube attached to a REMONTAR type UTU5 ultrathermostat. Water at a constant temperature of 37 °C was flowed continuously through the water jacket in the biopharmaceutical model. The measurement of adhesion determines the ability of gels obtained from powders to move. For this reason, 3 cm of gel was collected in a syringe and placed in the upper part of the model, imitating an artificial vagina. The distance of the gel flow in cm was read 5, 10, 15 and 20 minutes after application. Each gel was investigated three times and the final result was a mean of the measurements. The results obtained in the experiments are presented in ***Figure 1***.

2.2.1.3. Measurement of texture

To perform the measurements, a probe (P/1S) in the shape of a ball, built from stainless steel, with a diameter of 1 inch, was used.

The main measurement parameters were as follows: speed of downward movement of the probe during the test of 0.5 mm/s, the lifting speed of the probe was 10 mm/s, the maximum permissible force in the method of 100 g, dwell time of the probe in the gel of 10 s, and the height at which the probe was raised above the surface of the gel was 40 mm.

The assay was started by placing the gel in a cylindrical vessel with a transparent plexiglass texturometer set on the table. Then, the probe was lowered just above the surface of the gel so that there was no direct contact between them. After selecting the appropriate parameters in the program, the study was started. After contact with the surface of the gel (remains in this position for 10 seconds), the probe began to rise at a speed of 10 mm/s, to a height of 40 mm above the surface of the gel.

The study was conducted in order to illustrate the influence of the type of methylcellulose on the adhesion strength gels. All gels were tested three times and the results reported as the average of three measurements. The study sample gel is shown in ***Figure 2***.

2.2.2. Technology of manufacture of hydrophilic intravaginal powder

The production of powder containing lactic acid complexes with chitosan consisted of the following stages:

1. Obtaining the lactic acid-chitosan complex.

Chitosan combines with organic acids by means of I-order amine groups. This property was used in the preparation of the complex. The required amount of powdered chitosan was poured onto a weighed amount of lactic acid. The mass was stirred until a homogenous suspension was obtained. The mixture was left for 24 h until a clear, thick fluid was formed that could be joined with methylcellulose [4].

2. Obtaining the excipient - preparation of powder from methylcellulose.

A powder was obtained from methylcellulose by adding a known amount of this compound to the lactic acid complexes with chitosan. The resulting powder was thoroughly pulverised. Homogenous powder was obtained sieved through a sieve having a mesh size of 0.16 mm.

3. Obtaining the gel from powder for researches.

A gel was obtained from powder by adding a known amount distilled 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.

3. Results and discussion

Gels obtained from powders, containing lactic acid complexed with chitosan, reveal a stoichiometric ratio of 1:1, 2;1, 3:1, 4:1 and 8:1 and 4% methylcellulose. Their pH ranged from 3.92 to 4.44 for 1:1 gels and from 2.36 to 2.84 for the 8:1 ratio (***Table 1***).

Rheological studies demonstrated that the research gels obtained from powders possess dynamic viscosity from 53 to 398 mPa·s for the 1:1 stoichiometric ratio in the complex and from 19 to 242 mPa·s for the 8:1 ratio (***Table 2***).

Measurements performed in a biopharmaceutical model revealed that the gels obtained from powders containing lactic acid complexed with chitosan are able to move from 25 to 30 cm.

Statistical evaluation was performed. The analysis was carried out on the basis of multivariate analysis of variance (ANOVA). The level of significance was $\alpha = 0.05$. Differentiating factors were used in the study of different types of methylcellulose viscosity: 4000, 1500, 400, 25, 15 mPa·s. In these studies, it has been shown that the composition of each formulation affects their runoff and was considered statistically significant ($p < 0.0001$). The longest path flowing, and thus the greatest ability to move the gel under conditions

Table 1. *Influence viscosity methylcellulose on pH gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	3.92	3.96	4.17	4.25	4.44
2:1	3.48	3.82	4.04	4.19	4.25
3:1	3.17	3.25	3.44	3.65	3.90
4:1	2.75	2.87	2.90	2.95	3.09
8:1	2.36	2.58	2.65	2.78	2.84

Table 2. *Influence viscosity methylcellulose on rheological properties (dynamic viscosity η in mPa·s gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	η in mPa·s gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	398	254	165	110	53
2:1	356	232	159	98	44
3:1	305	221	143	87	38
4:1	286	204	136	71	24
8:1	242	198	129	62	19

simulating natural gels obtained were characterised with methylcellulose -based powders, with a viscosity of 15 mPa·s. The shortest route runoff showed gels formed from powders containing methylcellulose with a viscosity of 4000 mPa·s (***Figure 1***).

The researches of gels obtained from powders possess the work of adhesion - the energy needed to separate the gel from the probe 36.50 g/s (***Figure 2***, see page 80) for the 1:1 stoichiometric ratio in the complex and 8.74 g/s for 8:1 ratio.

All of the researches were performed at 37 °C.

The studies have shown that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane. The use of methylcellulose with different values of the viscosity allows different formulations to be obtained with a wide range of pH. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan at a stoichiometric ratio 1:1 in comparison to the gels with ratio 8:1.

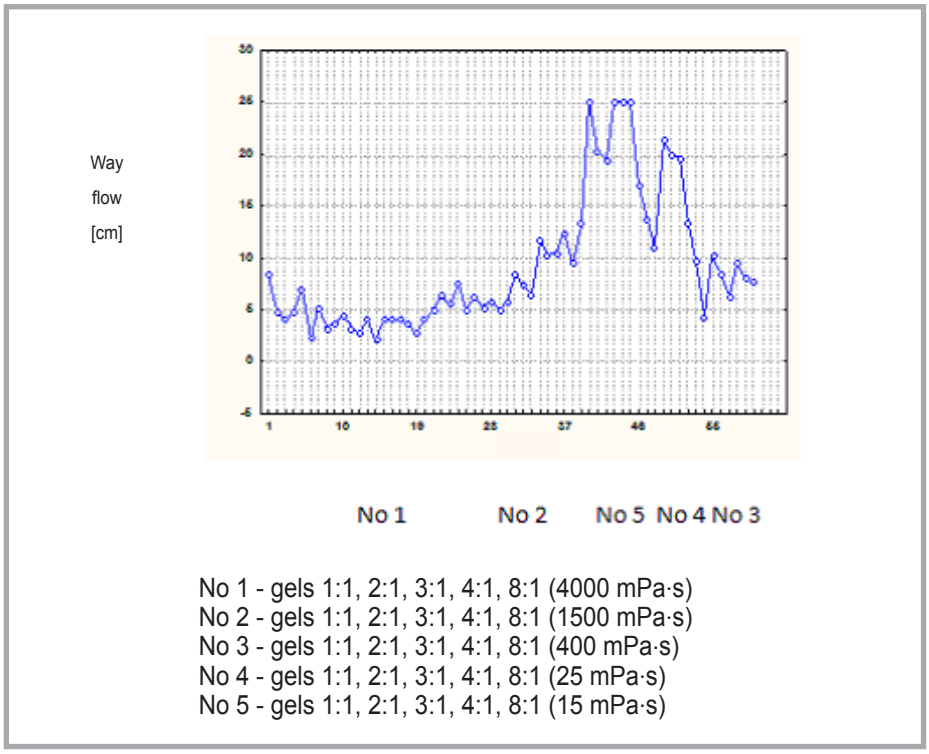

Figure 1. *Way flow research gels with addition methylcellulose ranged from 4000 to 15 mPa·s and stoichiometric ratio lactic acid to chitosan ranged from 1:1 to 8:1.*

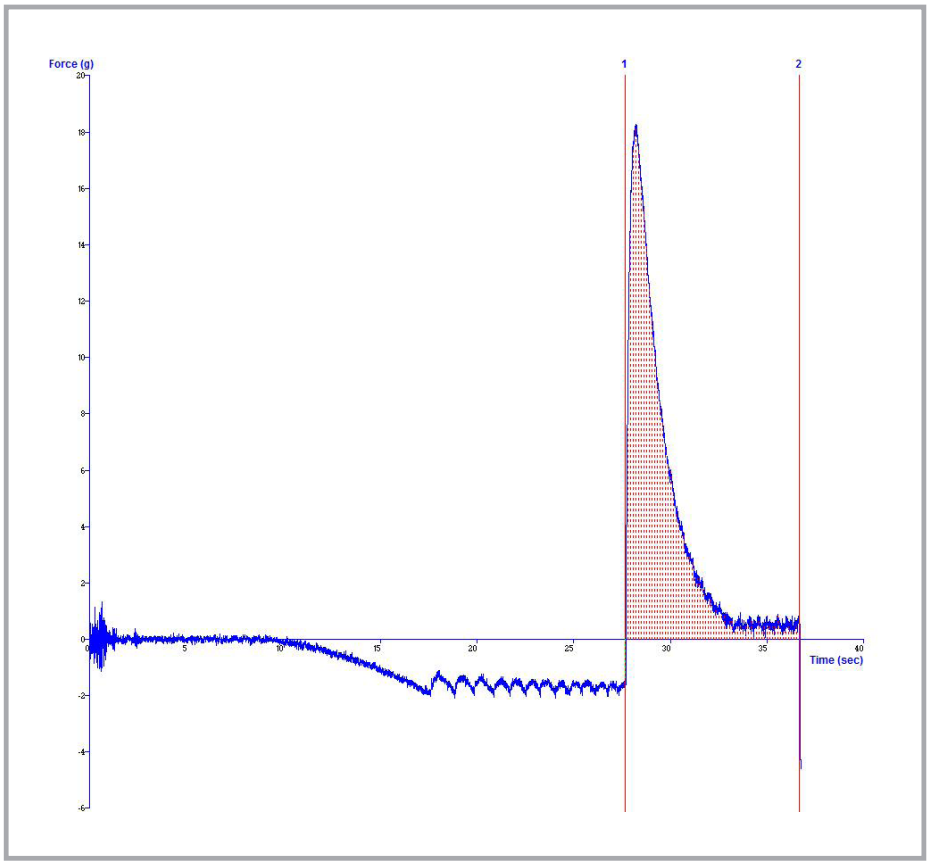

Figure 2. *Measurement of texture of gel with addition methylcellulose 4000 mPa·s and stoichiometric ratio lactic acid to chitosan 1:1.*

Measurements performed in a biopharmaceutical model revealed that the gels obtained from powders containing lactic acid complexed with chitosan are able to move from 25 cm to 30 cm. The gels obtained from powders possess the work of adhesion - the energy needed to separate the gel from the probe with different values.

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

4. Conclusions

1. The researches demonstrated that methylcellulose with different values of viscosity significantly affect the adhesive properties of hydrophilic gels obtained from powders, but at the same time, a wide range of pH.

2. Powders show that the adhesion of the gel covering the surface of the apparatus simulates the conditions in the vagina.

3. The gels obtained from the powders were characterised by the specific dynamic viscosity.

4. The gels obtained from powders possess the work of adhesion.

5. References

1. Schwebke JR; (2009) New concepts in the aetiology 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: 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: 10.1016/j.ejogrb.2009.04.012.
4. Kubis AA, Małolepsza-Jarmołowska K; (1996) Studies on gynaecological 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 gynaecological 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.