EFFECT OF A POLYVINYL ALCOHOL AND SELECTED AUXILIARY SUBSTANCES ON THE PROPERTIES OF THERMOSENSITIVE HYDROPHILIC GELS CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN

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Abstract
Gels containing lactic acid complexed with chitosan at a stechiometric ratio 1:1 and 2:1 and 5-25% content of PEG-200 reveal pH from 3.42 to 4.95. The addition of 20 – 25% poloxamer 407 increases the pH from 4.55 to 5.30 for 1:1 gels and from 4.00 to 4.85 for 2:1 gels. The addition of 0.5% polyvinyl alcohol decreases the pH of the investigated gels from 4.00 to 4.55 for 1:1 gels and from 3.85 to 4.40 for 2:1 gels. The addition of poloxamer 407 at concentrations of 20 to 25% increases the dynamic viscosity from 506.14 to 641.20 for 1:1 and from 540.35 to 692.55 for 2:1 ratios. The addition of 0.5% polyvinyl alcohol increases the dynamic viscosity from 590.20 to 720.63 for 1:1 and from 560.90 to 698.82 for 2:1 ratios. The addition of polyvinyl alcohol and auxiliary substances reduces the pH and maintains high adhesion.

Key words: *lactic acid complexed with chitosan, thermosensitive polymer - poloxamer 407, glycerol, 1,2-propylene glycol, polyvinyl alcohol, vaginal infections.*

1. Introduction

Commonly applied drug forms tend to leave the vagina when the patient assumes an upright position. The effectiveness of anti-inflammatory drugs and drugs reconstructing physiological environment of vagina greatly depends on the time of contact between therapeutic substance and the mucous membrane of the organ. The main problem in applied therapies is to maintain the continuity of treatment during 24 hours. The use of hydrophilic base for lactic acid complexed with alkaline polymers enabled production of gels with rheological properties of vaginal discharge. The gel remains at the site of application and provides adequate environmental pH [**1 - 11**].

The use of a thermosensitive polymer (poloxamer 407) affects further adhesion of the investigated preparations [**12**].

The aim of the study was to investigate the effect of adjuvant substances on optimization properties of the vaginal gels containing lactic acid complexed with chitosan.

2. Materials and methods

2.1. Materials

The following chemicals were used in experiments: lactic acid (P.Z.F. Cefarm Wrocław, Poland), chitosan with deacetylation degree of 93.5% (Sea Fisheries Institute, Gdynia, Poland), polyoxyethylene glycol 200 (LOBA–Chemie, Wien–Fishamend Austria), methylcellulose (Aldrich Chemical), glycerol (Sigma – Aldrich Chemie GmbH), 1,2-propylene glycol (Sigma – Aldrich Chemie GmbH), poloxamer 407 (Sigma – Aldrich Chemie GmbH, Germany), polyvinyl alcohol (Sigma – Aldrich Chemie GmbH), aqua purificata, acc. to FP VIII.

2.2. Methods

2.2.1. Measurements of rheology

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 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 obtained results were used to plot the flow curves of the investigated gels. The results obtained in the experimental are presented in ***Table 5 - 8***.

2.2.2. Technology of manufacture of hydrophilic intravaginal gel

The production of gel 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 gel from methylcellulose, polyvinyl alcohol and poloxamer 407.

A gel was obtained from methylcellulose, polyvinyl alcohol and poloxamer 407 by adding a known amount of this compound to the solution of polyoxyethylene glycol 200 or glycerol or 1,2-propylene glycol 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.

Lactic acid complexes with chitosan was added to methylcellulose, polyvinyl alcohol and poloxamer 407 gel and stirred until an homogenous gel was obtained. Distilled water was added to obtain the initial mass.

3. Results and discussion

Gels containing lactic acid complexed with chitosan at a stechiometric ratio 1:1 and 2:1 and 5 - 25% content of PEG-200 reveal pH from 3.42 to 4.95. The addition of 20 - 25% poloxamer 407 increases the pH from 4.55 to 5.30 for 1:1 gels and from 4.00 to 4.85 for 2:1 gels (***Table 1***).

The addition of 0.5% polyvinyl alcohol decreases the pH of the investigated gels from 4.00 to 4.55 for 1:1 gels and from 3.85 to 4.40 for 2:1 gels (***Table 2***).

The addition of 5-25% glycerol decreases the pH of investigated gels from 3.50 to 4.20 (1:1) and from 3.30 to 4.00 (2:1) (***Table 3***).

Table 1. *Influence PEG-200 and poloxamer 407 on pH investigated gels*

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200, %	pH gels with addition PEG-200	pH gels with PEG-200 and addition poloxamer 407		
			20%	23%	25%
1:1	5	4.43	4.55	4.62	4.84
	10	4.48	4.58	4.69	4.88
	15	4.55	4.60	4.65	4.90
	20	4.87	4.90	4.95	4.98
	25	4.95	5.05	5.26	5.30
2:1	5	3.42	4.00	4.25	4.30
	10	3.46	4.20	4.35	4.38
	15	3.51	4.40	4.48	4.50
	20	3.63	4.52	4.60	4.65
	25	3.68	4.70	4.75	4.85

Table 2. *Influence polyvinyl alcohol, PEG-200 and poloxamer 407 on pH investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200, %	pH gels with addition PEG-200	pH gels with PEG-200 and addition poloxamer 407		
			20%	23%	25%
1:1	5	4.43	4.00	4.07	4.29
	10	4.48	4.03	4.14	4.33
	15	4.55	4.05	4.10	4.35
	20	4.87	4.35	4.40	4.43
	25	4.95	4.45	4.50	4.55
2:1	5	3.42	3.85	4.10	4.15
	10	3.46	4.05	4.15	4.20
	15	3.51	4.15	4.20	4.33
	20	3.63	4.25	4.28	4.38
	25	3.68	4.30	4.36	4.40

Table 3. *Influence polyvinyl alcohol, glycerol and poloxamer 407 on pH investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration glycerol, %	pH gels with addition PEG-200	pH gels with glycerol and addition poloxamer 407		
			20%	23%	25%
1:1	5	4.43	3.50	3.64	3.74
	10	4.48	3.54	3.72	3.83
	15	4.55	3.62	3.88	3.92
	20	4.87	3.74	3.95	4.17
	25	4.95	3.86	4.15	4.20
2:1	5	3.42	3.30	3.54	3.90
	10	3.46	3.37	3.65	4.10
	15	3.51	3.48	3.68	4.20
	20	3.63	3.54	3.74	4.35
	25	3.68	3.65	3.83	4.00

Table 4. *Influence polyvinyl alcohol, 1,2- propylene glycol and poloxamer 407 on pH investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration 1,2-propylene glycol, %	pH gels with addition PEG-200	pH gels with 1,2- propylene glycol and addition poloxamer 407		
			20%	23%	25%
1:1	5	4.43	3.90	4.15	4.20
1:1	10	4.48	4.05	4.19	4.26
1:1	15	4.55	4.11	4.22	4.34
1:1	20	4.87	4.23	4.28	4.38
1:1	25	4.95	4.29	4.36	4.40
2:1	5	3.42	3.70	3.82	4.00
2:1	10	3.46	3.79	3.94	4.20
2:1	15	3.51	3.88	4.04	4.31
2:1	20	3.63	3.97	4.14	4.40
2:1	25	3.68	4.08	4.17	4.20

The addition of 5 - 25% 1,2-propylene glycol decreases the pH of investigated gels from 3.90 to 4.40 (1:1) and from 3.70 to 4.20 (2:1) (***Table 4***).

Rheological studies demonstrated that the reference gels possess the dynamic viscosity from 159.16 to 354.41 for the 1:1 stechiometric ratio in the complex and from 236.27 to 388.16 for 2:1 ratio. The addition of poloxamer 407 at concentrations of 20 to 25% increases the dynamic viscosity from 506.14 to 641.20 for 1:1 and from 540.35 to 692.55 for 2:1 ratios (***Table 5***).

The addition of 0.5% polyvinyl alcohol increases the dynamic viscosity from 590.20 to 720.63 for 1:1 and from 560.90 to 698.82 for 2:1 ratios (***Table 6***).

The addition of 5 - 25% glycerol increases the dynamic viscosity from 670.82 to 770.45 for 1:1 and from 650.00 to 750.66 for 2:1 ratios (***Table 7***).

Table 5. *Influence PEG-200 and poloxamer 407 on rheological properties investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200, %	Dynamic viscosity gels in mPa*s with addition PEG-200	Dynamic viscosity gels in mPa*s with PEG-200 and addition poloxamer		
			20%	23%	25%
1:1	5	354.41	506.14	520.65	641.20
	10	253.15	537.53	554.41	595.67
	15	270.02	571.28	523.17	565.67
	20	354.41	520.65	571.28	537.53
	25	159.16	565.12	502.52	583.15
2:1	5	253.15	540.35	590.70	692.55
	10	236.27	553.78	586.90	588.16
	15	270.02	575.04	557.53	555.67
	20	236.27	553.15	547.53	554.41
	25	388.16	650.12	648.53	580.43

Table 6. *Influence polyvinyl alcohol, PEG-200 and poloxamer 407 on rheological properties investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200, %	Dynamic viscosity gels in mPa*s with addition PEG-200	Dynamic viscosity gels in mPa*s with PEG-200 and addition poloxamer		
			20%	23%	25%
1:1	5	354.41	590.20	658.45	720.63
	10	253.15	623.00	646.41	705.88
	15	270.02	634.22	650.50	690.00
	20	354.41	644.73	688.30	682.11
	25	159.16	658.99	662.09	680.43
2:1	5	253.15	560.90	640.55	698.82
	10	236.27	563.78	586.90	688.16
	15	270.02	575.04	567.13	570.60
	20	236.27	566.15	647.00	589.41
	25	388.16	588.11	648.56	580.43

Table 7. *Influence polyvinyl alcohol, glycerol and poloxamer 407 on rheological properties investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration glycerol, %	Dynamic viscosity gels in mPa*s with addition PEG-200	Dynamic viscosity gels in mPa*s with glycerol and addition poloxamer		
			20%	23%	25%
1:1	5	354.41	670.82	688.46	770.45
	10	253.15	682.56	698.23	768.00
	15	270.02	688.11	699.00	763.77
	20	354.41	690.33	688.30	689.52
	25	159.16	695.75	690.09	750.00
2:1	5	253.15	650.00	660.00	750.66
	10	236.27	661.56	656.10	688.16
	15	270.02	675.09	667.73	670.00
	20	236.27	676.11	687.08	689.46
	25	388.16	688.00	688.51	680.00

Table 8. *Influence polyvinyl alcohol, 1,2-propylene glycol and poloxamer 407 on rheological properties investigated gels.*

Stoichiometric ratio lactic acid to chitosan	Concentration 1,2-propylene glycol, %	Dynamic viscosity gels in mPa*s with addition PEG-200	Dynamic viscosity gels in mPa*s with 1,2-propylene glycol and addition poloxamer		
			20%	23%	25%
1:1	5	354.41	690.55	798.44	860.88
1:1	10	253.15	699.12	828.09	848.00
1:1	15	270.02	720.00	799.97	850.76
1:1	20	354.41	760.88	699.00	789.58
1:1	25	159.16	755.44	691.09	750.00
2:1	5	253.15	680.11	723.98	790.20
2:1	10	236.27	688.55	700.19	688.16
2:1	15	270.02	686.07	690.93	690.80
2:1	20	236.27	689.13	687.08	689.46
2:1	25	388.16	688.00	688.51	682.00

The addition of 5 - 25% 1,2-propylene glycol increases the dynamic viscosity from 690.55 to 860.88 for 1:1 and from 680.11 to 790.20 for 2:1 ratios (***Table 8***).

All the investigations were performed at 37 °C.

The investigations revealed that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane.

The addition of polyvinyl alcohol and auxiliary substances reduces the pH and maintains high adhesion. The use of polyvinyl alcohol and glycerole or 1,2 propylene glycol allowed to obtain physiological range pH.

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of polyvinyl alcohol and hydrophilizing substances in comparison to the reference gels.

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 investigations demonstrated that the thermosensitive polymer - poloxamer 407 increases significantly the adhesive properties of hydrophilic gels, but at the same time it increases their pH.
2. The addition of polyvinyl alcohol and auxiliary substances reduces the pH and maintains high adhesion.
3. The use of polyvinyl alcohol and glycerol or 1,2 propylene glycol allowed to obtain physiological range pH.
4. The rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of polyvinyl alcohol and hydrophilizing substances in comparison to the reference gels.
5. The results obtained in the experimental studies proved that it is possible to produce a preparation with optimal pharmaceutical and application properties.

5. References

1. Schwebke JR; (2009) New Concepts in the Etiology of Bacterial Vaginosis. Curr. Infect. Dis. Rep. 11, 143 – 147.
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.
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.
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.