EFFECT OF A DEXTRANE AND HYDROPHILIZING 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 1.0% dextrane decreases the pH of investigated gels from 4.40 to 4.90 (1:1) and from 4.00 to 4.48 (2:1). 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 1.0% dextrane increases the dynamic viscosity from 665.23 to 750.28 for 1:1 and from 645.50 to 740.16 for 2:1 ratios. The addition of dextrane and glycerol or 1,2-propylene glycol reduces the pH and maintains high adhesion. The use of dextrane and hydrophilizing substances allowed to obtain physiological range pH.

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

1. Introduction

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. Commonly applied drug forms tend to leave the vagina when the patient assumes an upright position.

The use of hydrophilic gels with high adhesion properties and ability to spread over the vaginal mucosa enable prolonged action of the drug [**1 - 11**]. The preparations, remaining at the site of application, produce adequate pH in the environment thanks to the content of lactic acid complexed with chitosan. 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

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

2.2. Methods

2.2.1. Rheological investigations

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. Method 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, dextrane and poloxamer 407.

A gel was obtained from methylcellulose, dextrane 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, dextrane 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 1.0% dextrane decreases the pH of investigated gels from 4.40 to 4.90 (1:1) and from 4.00 to 4.48 (2:1) (***Table 2***).

The addition of 5-25% glycerol decreases the pH of investigated gels from 4.00 to 4.50 (1:1) and from 3.50 to 4.35 (2:1) (***Table 3***).

The addition of 5-25% 1,2-propylene glycol decreases the pH of investigated gels from 4.25 to 4.68 (1:1) and from 3.90 to 4.38 (2:1) (***Table 4***).

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 dextrane, 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	4.40	4.48	4.56
	10	4.48	4.45	4.52	4.64
	15	4.55	4.53	4.68	4.76
	20	4.87	4.67	4.75	4.85
	25	4.95	4.72	4.86	4.90
2:1	5	3.42	4.00	4.28	4.33
	10	3.46	4.08	4.31	4.37
	15	3.51	4.14	4.23	4.40
	20	3.63	4.21	4.34	4.45
	25	3.68	4.30	4.40	4.48

Table 3. *Influence dextrane, 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	4.00	4.14	4.20
	10	4.48	4.10	4.15	4.28
	15	4.55	4.16	4.21	4.34
	20	4.87	4.22	4.30	4.45
	25	4.95	4.31	4.44	4.50
2:1	5	3.42	3.50	3.81	4.06
	10	3.46	3.59	3.89	4.19
	15	3.51	3.63	3.99	4.27
	20	3.63	3.72	4.08	4.31
	25	3.68	3.88	4.23	4.35

Table 4. *Influence dextrane, 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	4.25	4.32	4.44
	10	4.48	4.34	4.42	4.50
	15	4.55	4.41	4.49	4.56
	20	4.87	4.52	4.57	4.60
	25	4.95	4.61	4.65	4.68
2:1	5	3.42	3.90	4.08	4.12
	10	3.46	3.95	4.14	4.22
	15	3.51	4.10	4.20	4.29
	20	3.63	4.16	4.24	4.32
	25	3.68	4.23	4.30	4.38

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 1.0% dextrane increases the dynamic viscosity from 665.23 to 750.28 for 1:1 and from 645.50 to 740.16 for 2:1 ratios (***Table 6***).

The addition of 5 - 25% glycerol increases the dynamic viscosity from 685.99 to 798.66 for 1:1 and from 667.34 to 780.00 for 2:1 ratios (***Table 7***).

The addition of 5 - 25% 1,2-propylene glycol increases the dynamic viscosity from 720.44 to 898.99 for 1:1 and from 700.89 to 825.55 for 2:1 ratios (***Table 8***).

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 dextrane, 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	665.23	686.32	750.28
	10	253.15	678.42	692.42	746.22
	15	270.02	683.00	699.11	738.87
	20	354.41	690.12	712.98	730.89
	25	159.16	698.34	720.87	724.65
2:1	5	253.15	645.50	686.44	740.16
	10	236.27	652.31	692.13	732.68
	15	270.02	660.22	712.77	728.55
	20	236.27	678.09	715.00	719.99
	25	388.16	689.66	700.25	716.43

Table 7. *Influence dextrane, 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 20% poloxamer		
			20%	23%	25%
1:1	5	354.41	685.99	734.87	798.66
	10	253.15	699.13	745.99	787.76
	15	270.02	703.54	752.65	776.41
	20	354.41	706.88	748.13	769.32
	25	159.16	723.58	750.00	754.39
2:1	5	253.15	667.34	690.86	780.00
	10	236.27	672.08	707.32	773.88
	15	270.02	680.65	730.54	765.31
	20	236.27	692.76	700.61	754.57
	25	388.16	734.99	740.00	744.12

Table 8. *Influence dextrane, 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	720.44	780.85	898.99
1:1	10	253.15	731.80	785.99	886.21
1:1	15	270.02	742.76	764.32	872.55
1:1	20	354.41	754.09	784.44	860.44
1:1	25	159.16	760.00	788.11	850.99
2:1	5	253.15	700.89	730.84	825.55
2:1	10	236.27	726.21	745.07	820.00
2:1	15	270.02	739.11	755.88	810.78
2:1	20	236.27	746.52	767.44	790.52
2:1	25	388.16	758.79	779.32	788.88

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 dextrane and glycerole or 1,2-propylene glycol reduces the pH and maintains high adhesion. The use of dextrane and hydrophilizing substances allowed to obtain physiological range pH.

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of dextrane and auxiliary 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 investigations showed that the addition of dextrane and glycerol or 1,2-propylene glycol reduces the pH and maintains high adhesion.
3. The use of dextrane and hydrophilizing substances 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 dextrane and auxiliary 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.

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