EFFECT OF A HYDROPHILISING SUBSTANCE ON THE VISCOSITY OF GYNAECOLOGICAL GELS 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. Szewska 38 Street, 50-139 Wrocław, Poland
E-mail: katarzynamj@poczta.onet.pl

Abstract
Thus ensuring a continuous action of a drug also during daily activity time of a patient is a vital question in gynaecological treatment. The main problem in applied therapies is to maintain the continuity of treatment during 24 hours. The addition of 5-25% glycerol or 1,2-propylene glycol decreases the pH of investigated gels with calcium alginate to 4.20 to 4.88 (1:1) and 3.69 to 4.19 (2:1); 4.29 to 4.88 (1:1) and 3.62 to 4.40 (2:1). The addition of 5-25% glycerol or 1,2-propylene glycol increases the dynamic viscosity gels with calcium alginate from 595.14 to 820.25 for 1:1 and 640.15 to 769.12 for 2:1; 585.40 to 815.56 for 1:1 and 648.52 to 780.10 for 2:1 ratios. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of calcium alginate and glycerol or 1,2-propylene glycol in comparison to the reference gels.

Key words: *lactic acid complexed with chitosan, calcium alginate, thermosensitive polymer-poloxamer 407, hydrophilic gels, vaginal infections, anti-inflammatory drugs.*

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.

Many drugs conform to this requirement only when a patient is in horizontal position.

Permanent contact of the drug form with the vaginal mucosa during daily activities of the patient is an indispensable condition for 24-hour therapy.

This condition may be fulfilled by drug forms with high ability to adhere to vaginal mucosa. Available literature does not offer any significant progress in the efficacy of vaginitis therapy [1 - 3].

The use of hydrophilic gels with high adhesion properties and ability to spread over the vaginal mucosa enable prolonged action of the drug [4 - 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 affects further adhesion of the investigated preparations [12].

The aim of the study was to investigate optimization of pharmaceutical properties of hydrophilic gels containing lactic acid complexed with chitosan.

2. Materials and methods

2.1. Materials

Lactic acid – P.Z.F. Cefarm (Wrocław, Poland). Chitosan - deacetylation degree of 93.5% – Sea Fisheries Institute (Gdynia, Poland). Polyoxyethylene glycol 200 - LOBA – Chemie, Wien – Fishamend (Austria). Methylcellulose, Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL – (England). Glycerol, Sigma – Aldrich Chemie GmbH, Germany. 1,2-Propylene glycol, Sigma – Aldrich Chemie GmbH, Germany. Calcium alginate, SIGMA – Aldrich Chemical Company Ltd. (Gillingham, England). Poloxamer 407, Sigma – Aldrich Chemie GmbH, Germany. Aqua purificata, acc. To FP VIII.

2.2. Methods

2.2.1. Measurements of pH and viscosity

(see [11])

2.2.1.1. Determination of pH

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.

2.2.1.2. 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 4 and 5***.

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 with poloxamer 407, calcium alginate and glycerol or 1,2-propylene glycol.

A gel was obtained from methylcellulose with poloxamer 407 and calcium alginate by adding a known amount of this compound to the solution of 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 with poloxamer 407 and calcium alginate 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 stoichiometric ratio 1:1 and 2:1 and 5 - 25% content of polyoxyethylene glycol 200 reveal pH from 3.42 to 4.95. The addition of 20 - 25% poloxamer 407 increases the pH 4.55 to 5.30 for 1:1 gels and 4.00 to 4.85 for 2:1 gels (***Table 1***).

The addition of 0.5; 0.7; 1.0% calcium alginate decreases the pH of the investigated gels 4.54 to 4.89; 4.29 to 4.85; 4.19 to 4.51 for 1:1 gels and 3.82 to 4.26; 3.92 to 4.39; 3.64 to 3.99 for 2:1 gels.

The addition of 5 - 25% glycerol or 1,2-propylene glycol decreases the pH of investigated gels with calcium alginate 4.20 to 4.88 (1:1) and 3.69 to 4.19 (2:1); 4.29 to 4.88 (1:1) and 3.62 to 4.40 (2:1) (***Table 2*** and ***3***).

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

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200 in %	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 glycerol and calcium alginate on pH investigated gels containing 4.0% methylcellulose and 25% poloxamer 407.*

Stoichiometric ratio lactic acid to chitosan	Concentration glycerol, %	pH gels with addition glycerol	pH gels with glycerol and addition calcium alginate		
			0.5%	0.7%	1.0%
1:1	5	4.44	4.42	4.25	4.20
	10	4.48	4.50	4.32	4.21
	15	4.58	4.62	4.48	4.23
	20	4.88	4.75	4.59	4.40
	25	4.98	4.88	4.76	4.44
2:1	5	3.90	3.80	3.74	3.69
	10	4.10	3.84	3.80	3.74
	15	4.20	3.89	3.85	3.81
	20	4.35	4.00	3.90	3.88
	25	4.45	4.19	4.00	3.92

Table 3. *Influence 1,2-propylene glycol and calcium alginate on pH investigated gels containing 4.0% methylcellulose and 25% poloxamer 407.*

Stoichiometric ratio lactic acid to chitosan	Concentration 1,2-propylene glycol, %	pH gels with addition 1,2-propylene glycol	pH gels with 1,2-propylene glycol and addition calcium alginate		
			0.5%	0.7%	1.0%
1:1	5	4.52	4.50	4.32	4.29
	10	4.60	4.57	4.40	4.21
	15	4.61	4.63	4.52	4.25
	20	4.90	4.76	4.58	4.40
	25	5.10	4.88	4.66	4.45
2:1	5	4.00	3.78	3.69	3.62
	10	4.20	3.85	3.74	3.65
	15	4.31	3.93	3.86	3.70
	20	4.40	4.26	3.94	3.79
	25	4.55	4.40	4.20	3.85

Table 4. *Influence glycerol and calcium alginate on rheological properties investigated gels containing 4.0% methylcellulose and 25% poloxamer 407.*

Stoichiometric ratio lactic acid to chitosan	Concentration glycerol, %	Dynamic viscosity gels with addition glycerol, mPa·s	Dynamic viscosity gels with glycerol and addition calcium alginate, mPa·s		
			0.5%	0.7%	1.0%
1:1	5	680.30	768.99	782.45	820.25
	10	600.11	740.22	768.53	812.34
	15	599.43	698.31	753.66	800.10
	20	555.12	648.15	660.23	795.00
	25	590.22	595.14	611.28	786.22
2:1	5	698.35	710.43	722.41	769.12
	10	610.45	686.00	699.23	760.00
	15	579.14	660.88	750.11	755.34
	20	587.26	659.14	680.24	732.88
	25	598.31	640.15	670.33	700.10

Table 5. *Influence 1,2- propylene glycol and calcium alginate on rheological properties investigated gels containing 4.0% methylcellulose and 25% poloxamer 407.*

Stoichiometric ratio lactic acid to chitosan	Concentration 1,2-propylene glycol, %	Dynamic viscosity gels with addition 1,2-propylene glycol, mPa·s	Dynamic viscosity gels with 1,2-propylene glycol and addition calcium alginate, mPa·s		
			0.5%	0.7%	1.0%
1:1	5	690.28	760.30	780.11	815.56
	10	650.33	720.44	754.29	798.22
	15	600.44	682.00	743.00	780.00
	20	580.21	590.00	704.44	779.45
	25	599.58	585.40	652.11	730.29
2:1	5	700.34	720.69	755.00	780.10
	10	630.11	687.00	699.88	767.99
	15	582.28	666.87	690.31	755.51
	20	590.42	654.66	681.22	702.22
	25	600.54	648.52	669.00	698.75

Rheological studies demonstrated that the reference gels possess the dynamic viscosity from 159.16 to 354.41 for the 1:1 stoichiometric 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 540.35 to 692.55 for 2:1 ratios.

The addition of 0.5; 0.7; 1.0 calcium alginate increases the dynamic viscosity from 580.25 to 780.40 for 1:1 and 610.56 to 745.50 for 2:1 ratios.

The addition of 5 - 25% glycerol or 1,2-propylene glycol increases the dynamic viscosity gels with calcium alginate from 595.14 to 820.25 for 1:1 and 640.15 to 769.12 for 2:1; 585.40 to 815.56 for 1:1 and 648.52 to 780.10 for 2:1 ratios (***Table 4*** and ***5***).

All the investigations were performed at 37 °C.

The investigations demonstrated that the thermosensitive polymer - poloxamer 407 increases significantly the dynamic viscosity of hydrophilic gels, but at the same time it increases their pH.

The use of calcium alginate resulted in a decrease of the pH. The addition of glycerol or 1,2-propylene glycol caused further decrease of the pH.

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of calcium alginate and glycerol or 1,2-propylene glycol in comparison to the reference gels.

The analysis of results obtained in the experimental studies revealed differentiated values of dynamic viscosity of gels depending on their composition.

The broad and differentiated range of dynamic viscosity, thixotropy and the liquefaction border for the investigated preparations obtained in own studies enables the selection of an adequate preparation. The obtained gels behaved as non-newtonian fluids, plotting a hysteresis loop on the graph illustrating the shearing stress – shearing rate relationship. The obtained parameters gels should provide positive application effects.

4. Conclusions

1. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 with the addition of calcium alginate and glycerol or 1,2-propylene glycol in comparison to the reference gels.
2. The investigations revealed that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane.
3. Revealed differentiated values of dynamic viscosity of gels depending on their composition.

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