THE EFFECT OF POLOXAMER 407 ON THE PROPERTIES OF HYDROPHILIC GELS CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN

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Abstract
Anatomical and physiological conditions in the vagina do not facilitate easy application of the drug neither its maintenance on the site of application. Insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH conditioning the physiological biocenosis of the organ. Gels containing lactic acid complexed with chitosan at a stechiometric ration 1:1 and 2:1 and 5 - 25% content of PEG-200 are able to move from 25 to 30 cm. Higher concentrations of the poloxamer 407: 23 and 25% result in the movability of 20 to 24 cm. The addition of 20 – 25% poloxamer 407 increases the pH to 4.55 to 5.30 for 1:1 gels and 4.00 to 4.85 for 2:1 gels. The investigations showed that the addition of a thermosensitive polymer increases the adhesive properties of the investigated gels. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 in comparison to the reference gels.

Key words: *lactic acid, chitosan, poloxamer 407, complex, vaginal infections, anti-inflammatory drugs.*

1. Introduction

Numerous reports devoted to vaginitis point to the relevance of the problem. Successful treatment of the condition is handicapped by frequent recurrences after termination of routine therapy. Anatomical and physiological conditions in the vagina do not facilitate easy application of the drug neither its maintenance on the site of application. Insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH conditioning the physiological biocenosis of the organ [1, 2].

The use of hydrophilic base for lactic acid complexed with alkaline polymers enabled production of gels with rheological properties of vaginal discharge [3 - 10]. The gel remains at the site of application and provides adequate environmental pH. Thus continuation of studies on optimization of the gels composition in order to improve their adhesion properties and thus increase the efficacy of the therapy is fully substantiated.

The aim of the study was to investigate the effect of thermosensitive polymer on pharmaceutical properties of gynecological gels containing lactic acid complexed with chitosan.

In co-operation with the Department and Clinic of Reproduction and Obstetrics Wrocław Medical University, a research has been initiated to restore the natural acidic condition of vagina environment to rebuild its physiological bacterial flora.

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). Poloxamer 407, Sigma – Aldrich Chemie GmbH, Germany. Aqua purificata, acc. to FP VI.

2.2. Methods

2.2.1. Measurements of pH and viscosity

See [10].

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 3***.

2.2.2. Determination of adhesion

The determination of adhesion was performed on a biopharmaceutical model imitating the conditions in the vagina. This is a 30 cm long and 3 cm in diameter, calibrated glass tube attached to a REMONTAR type UTU5 ultrathermostate. Water at a constant temperature of 37 °C is flowing continuously through the water jacket in the biopharmaceutical model. The measurement of adhesion determines the ability of gels to move. For this reason, 3 cm^3 of the gel was collected to a syringe and placed in the upper part of the model imitating the artificial vagina. The distance of the gel flow in cm was read 5, 10, 15, 20 and 25 minutes after application. Each gel was three times investigated and the final result was a mean of the measurements.

2.2.3. In vivo investigations

In vivo investigations were carried out at the Department and Clinic of Reproduction and Obstetrics Wrocław Medical University. The investigated gels were applied intravaginally by means of an applicator. The findings were determined on the basis of in vivo observation of the gel on the vaginal mucosa.

2.2.4. 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 and poloxamer 407. A gel was obtained from methylcellulose and poloxamer 407 by adding a known amount of this compound to the solution of polyoxyethylene glycol – 200 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 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 ration 1:1 and 2:1 and 5 - 25% content of PEG-200 are able to move from 25 to 30 cm.

Measurements performed in a biopharmaceutical model revealed that the addition of 20% poloxamer 407 to gels containing lactic acid complexed with chitosan in a stechio-

metric ration 1:1 decreases their movability to 20 to 25 cm and at 2:1 ratio, to 22 to 28 cm. Higher concentrations of the poloxamer 407: 23 and 25% result in the movability of 20 to 24 cm.

The pH of the investigated preparations was 3.42 to 4.95 for the reference gels. The addition of 20 – 25% poloxamer 407 increases the pH to 4.55 to 5.30 for 1:1 gels and 4.00 to 4.85 for 2:1 gels.

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 540.35 to 692.55 for 2:1 ratios.

All the investigations were performed at 37 °C.

Table 1. *Influence PEG-200 and poloxamer 407 on able to move investigated gels containing 4.0% methylcellulose.*

Stoichiometric ratio lactic acid to chitosan	Concentration PEG-200, %	Able to move gels with addition PEG-200	Able to move gels with PEG-200 and addition poloxamer 407, cm		
			20%	23%	25%
1:1	5	25.0	20.0	21.5	20.0
1:1	10	26.5	20.6	22.6	21.5
1:1	15	27.9	23.8	22.9	22.6
1:1	20	28.9	24.5	23.2	22.9
1:1	25	29.4	25.0	23.9	23.8
2:1	5	26.9	22.0	21.9	20.8
2:1	10	27.7	24.3	22.3	21.6
2:1	15	28.6	25.8	23.2	22.4
2:1	20	29.8	27.6	23.8	23.2
2:1	25	30.0	28.0	24.0	23.8

Table 2. *Influence PEG-200 and poloxamer 407 on pH investigated gels containing 4.0% methylcellulose.*

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
1:1	10	4.48	4.58	4.69	4.88
1:1	15	4.55	4.60	4.65	4.90
1:1	20	4.87	4.90	4.95	4.98
1:1	25	4.95	5.05	5.26	5.30
2:1	5	3.42	4.00	4.25	4.30
2:1	10	3.46	4.20	4.35	4.38
2:1	15	3.51	4.40	4.48	4.50
2:1	20	3.63	4.52	4.60	4.65
2:1	25	3.68	4.70	4.75	4.85

Table 3. *Influence PEG-200 and poloxamer 407 on reological properties investigated gels containing 4.0% methylcellulose.*

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

The investigations showed that the addition of a thermosensitive polymer increases the adhesive properties of the investigated gels.

Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 in comparison to the reference gels. The pH of the investigated preparation was in the physiological range.

4. Conclusions

1. The investigations showed that the addition of a thermosensitive polymer increases the adhesive properties of the investigated gels.
2. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing poloxamer 407 in comparison to the reference gels.
3. The pH of the investigated preparation was in the physiological range.

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