THE PROPERTIES OF THERMOSENSITIVE GYNECOLOGICAL POWDERS CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN

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Abstract

The investigations showed that the addition of a 20%, 23% and 25% poloxamer 407 increases the adhesive properties of the investigated powders, but at the same time it increases their pH. The enrichment of the composition of the tested powders containing 5% glycerol and 5% 1,2-propylene glycol resulted in increased pH of the formulations. A modification of the composition of the tested powders containing 5% glycerol and 5% 1,2-propylene glycol has increased the range of the dynamic viscosity of formulations. The enrichment of the composition of the tested powders containing 5% hydroxypropylmethylcellulose resulted in increased the range of the dynamic viscosity and decreased pH of the formulations. Laboratory tests have shown that it is possible to obtain thermosensitive gels with high adhesion properties to vaginal mucous membrane.

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

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1. Introduction

Recurrent vaginitis is a clinical problem that still lacks successful solution. The use of hydrophilic gels with high adhesion properties and ability to spread over the vaginal mucosa enable prolonged action of the drug. The preparations, remaining at the site of application, produce adequate pH in the environment thanks to the content of lactic acid complexed with chitosan [1 - 12].

Thermosensitive gynecological formulations are designed to correct abnormal pH environment of the vagina and the supply to the physiological state. Aim of this work was to study the pharmaceutical properties of powders for gynecology which under natural conditions turn into the thermosensitive gels covering the vaginal mucosa. The influence of hydroxypropylmethylcellulose and hydrophilizing substances such as glycerol, propylene glycol-1,2 on the properties of the powders containing poloxamer 407 was tested. Formulations were prepared with varying pH and rheological properties. Powders becomes thermosensitive gels were examined for their properties. The adhesion test of the thermosensitive gel were performed in the texturometer. The thermosensitive gels obtained from thermosensitive powders possess the work of adhesion with different values. On the basis of studies, the dynamic viscosity of the gels obtained from powders was defined. A wide range of pH of the gels allows the selection of the optimum formulation.

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 with deacetylation degree of 93.5% (Sea Fisheries Institute, Gdynia, Poland), methylcellulose (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), hydroxypropylmethylcellulose (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), poloxamer 407 (Sigma – Aldrich Chemie GmbH, Germany), glycerol (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), 1,2-propylene glycol (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), aqua purificata, acc. to FP IX.

2.2. Methods

2.2.1. 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 24h until a clear, thick fluid was formed that could be joined with methylcellulose [4].

2. Obtaining the excipient - preparation of powder from methylcellulose and poloxamer 407

A powder was obtained from methylcellulose, hydroxypropylmethylcellulose and poloxamer 407, by adding a known amount of this compound to the lactic acid complexes with chitosan and glycerol or 1,2-propylene glycol. The resulting powder was thoroughly pulverized. 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 supplemented with distilled water to the initial weight.

2.2.2. Measurements of physical properties

2.2.2.1. Rheological investigations (dynamic viscosity)

Rheological investigations were performed using a rotational viscosimeter Rheotest-2 Medingen Dresden. The determinations of viscosity 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.

2.2.2.2. Measurement of texture

The measurements was performed using a probe (P/1S) in the shape of a ball, built in stainless steel, with a diameter of 1 inch.

Main measurement parameters were as follows: speed of downward movement of the probe during the test, and was 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 10 s, the height at which probe raised above the surface of the gel 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 is no direct contact between them. After selecting the appropriate parameters in the program, started to study. The probe after contact with the surface of the gel (remains in this position for 10 seconds) began to rise at a speed of 10 mm /s at 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.

3. Results and Discussion

Gels obtained from powders, containing lactic acid complexed with chitosan reveal a stoichiometric ratio 1:1, 2;1, 3:1, 4:1 and 8:1. Their pH ranged from 3.92 to 4.44 for gels 1:1 and from 2.36 to 2.84 for 8:1 ratio [12]. The

addition of 20%, 23% or 25% poloxamer 407 increases the pH ranged from 4.00 to 4.91 for gels 1:1 and from 2.56 to 3.42 for 8:1 ratio. Further addition of the 25% poloxamer 407 and 5% glycerol increases the pH ranged from 4.30 to 5.15 for gels 1:1 and from 2.89 to 3.57 for 8:1 ratio (Table 1).

Table 1. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% glycerol on pH gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose 4000 mPa*s	pH gels with addition methyl-cellulose 1500 mPa*s	pH gels with addition methyl-cellulose 400 mPa*s	pH gels with addition methyl-cellulose 25 mPa*s	pH gels with addition methyl-cellulose 15 mPa*s
1:1	4.30	4.48	4.64	4.89	5.15
2:1	3.99	4.38	4.58	4.75	4.98
3:1	3.68	3.82	4.25	4.28	4.67
4:1	3.28	3.42	3.67	3.76	4.29
8:1	2.89	3.00	3.34	3.38	3.57

The enrichment of the composition 5% 1,2-propylene glycol of the tested powders containing 25% poloxamer 407 and 5% resulted in increased pH ranged of the formulation to between 4.35 to 5.29 for gels 1:1 and from 2.99 to 3.66 for 8:1 ratio (Table 2).

Table 2. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% 1,2-propylene glycol on pH gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose 4000 mPa*s	pH gels with addition methyl-cellulose 1500 mPa*s	pH gels with addition methyl-cellulose 400 mPa*s	pH gels with addition methyl-cellulose 25 mPa*s	pH gels with addition methyl-cellulose 15 mPa*s
1:1	4.35	4.56	4.78	4.95	5.29
2:1	4.12	4.49	4.66	4.82	5.11
3:1	3.79	3.91	4.32	4.37	4.78
4:1	3.35	3.55	3.77	3.81	4.31
8:1	2.99	3.12	3.48	3.43	3.66

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The enrichment of the composition of the tested powders 5% glycerol and 5% hydroxypropylmethylcellulose containing 25% poloxamer 407 resulted in increased pH ranged of the formulation to between 4.04 to 4.80 for gels 1:1 and from 2.50 to 3.24 for 8:1 ratio (Table 3).

Table 3. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% glycerol on pH gels obtained from investigated powders with 5% hydroxypropylmethylcellulose

Stoichiometric ratio lactic acid to chitosan	**pH gels with addition methyl-cellulose 4000 mPa*s**	**pH gels with addition methyl-cellulose 1500 mPa*s**	**pH gels with addition methyl-cellulose 400 mPa*s**	**pH gels with addition methyl-cellulose 25 mPa*s**	**pH gels with addition methyl-cellulose 15 mPa*s**
1:1	4.04	4.12	4.30	4.55	4.80
2:1	3.65	4.00	4.25	4.46	4.62
3:1	3.39	3.42	4.09	4.03	4.34
4:1	3.00	3.08	3.50	3.44	4.01
8:1	2.50	2.82	3.06	3.10	3.24

Further addition 5% 1,2-propylene glycol and 5% hydroxypropylmethylcellulose of the composition of the tested powders containing 25% poloxamer 407 and resulted in increased pH ranged of the formulation to between 4.06 to 4.89 for gels 1:1 and from 2.70 to 3.80 for 8:1 ratio (Table 4).

Table 4. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% 1,2-propylene glycol on pH gels obtained from investigated powders with 5% hydroxypropylmethylcellulose

Stoichiometric ratio lactic acid to chitosan	**pH gels with addition methyl-cellulose 4000 mPa*s**	**pH gels with addition methyl-cellulose 1500 mPa*s**	**pH gels with addition methyl-cellulose 400 mPa*s**	**pH gels with addition methyl-cellulose 25 mPa*s**	**pH gels with addition methyl-cellulose 15 mPa*s**
1:1	4.06	4.29	4.50	4.66	4.89
2:1	3.85	4.17	4.40	4.52	4.80
3:1	3.47	3.60	4.05	4.00	4.46
4:1	3.00	3.23	3.50	3.55	4.03
8:1	2.70	2.86	3.35	3.46	3.80

Rheological studies demonstrated that the researches gels obtained from powders possess the dynamic viscosity from 53 mPa*s to 398 mPa*s for the 1:1 stoichiometric ratio in the complex and from 19 mPa*s to 242 mPa*s for 8:1 ratio. A modification of the composition of the tested powders containing 20% poloxamer 407 has increased the range of the dynamic viscosity of formulations suitably from 269 mPa*s to 556 mPa*s for gels 1:1 and from 220 mPa*s to 508 mPa*s for 8:1 ratio (Table 5).

Table 5. Influence of the composition of powders containing 4% methylcellulose, 20% poloxamer 407 on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	**η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 400 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 25 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 15 mPa*s**
1:1	556	504	370	321	269
2:1	537	498	365	310	253
3:1	520	487	351	291	240
4:1	515	470	347	283	231
8:1	508	386	339	277	220

A modification of the composition of the tested powders containing 23% poloxamer 407 has increased the range of the dynamic viscosity of formulations suitably from 371 mPa*s to 568 mPa*s for gels 1:1 and from 338 mPa*s to 517 mPa*s for 8:1 ratio (Table 6).

Further addition of the 25% poloxamer 407 resulted in a further increase in dynamic viscosity of the formulations from 402 mPa*s to 579 mPa*s for gels 1:1 and from 368 mPa*s to 530 mPa*s for 8:1 ratio (Table 7) .

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Table 6. Influence of the composition of powders containing 4% methylcellulose, 23% poloxamer 407 on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	**η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 400 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 25 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 15 mPa*s**
1:1	568	510	460	411	371
2:1	545	502	452	403	365
3:1	533	499	447	396	354
4:1	529	486	432	387	341
8:1	517	475	428	376	338

Table 7. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	**η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 400 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 25 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 15 mPa*s**
1:1	579	526	479	439	402
2:1	560	519	471	430	398
3:1	552	501	468	422	387
4:1	541	499	459	414	379
8:1	530	487	447	409	368

A modification 5% glycerol of the composition of the tested powders containing 25% poloxamer 407 has increased the range of the dynamic viscosity of formulations suitably from 408 mPa*s to 590 mPa*s for gels 1:1 and from 361 mPa*s to 560 mPa*s for 8:1 ratio (Table 8).

Table 8. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% glycerol on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s	η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s	η[mPa*s] gels with addition methyl-cellulose 400 mPa*s	η[mPa*s] gels with addition methyl-cellulose 25 mPa*s	η[mPa*s] gels with addition methyl-cellulose 15 mPa*s
1:1	590	554	501	452	408
2:1	586	546	498	449	397
3:1	573	531	487	437	383
4:1	569	528	474	426	377
8:1	560	512	460	417	361

Further addition 5% 1,2-propylene glycol of the 25% poloxamer 407 resulted in a further increase in dynamic viscosity of the formulations from 502 mPa*s to 624 mPa*s for gels 1:1 and from 468 mPa*s to 587 mPa*s for 8:1 ratio (Table 9).

Table 9. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 1,2-propylene glycol on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders

Stoichiometric ratio lactic acid to chitosan	η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s	η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s	η[mPa*s] gels with addition methyl-cellulose 400 mPa*s	η[mPa*s] gels with addition methyl-cellulose 25 mPa*s	η[mPa*s] gels with addition methyl-cellulose 15 mPa*s
1:1	624	579	529	539	502
2:1	617	568	516	530	498
3:1	609	559	505	522	487
4:1	598	546	559	514	479
8:1	587	535	547	509	468

A modification 5% glycerol and 5% hydroxypropylmethylcellulose of the composition of the tested powders containing 25% poloxamer 407 has increased the range of the dynamic viscosity of formulations suitably from 719 mPa*s to

845 mPa*s for gels 1:1 and from 678 mPa*s to 813 mPa*s for 8:1 ratio (Table 10).

Table 10. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 5% glycerol on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders with 5% hydroxypropylmethylcellulose

Stoichiometric ratio lactic acid to chitosan	**η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 400 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 25 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 15 mPa*s**
1:1	845	806	772	750	719
2:1	840	798	768	746	710
3:1	834	788	762	739	705
4:1	822	780	759	732	695
8:1	813	776	752	729	678

Further addition 5% 1,2-propylene glycol and 5% hydroxypropylmethylcellulose of the 25% poloxamer 407 resulted in a further increase in dynamic viscosity of the formulations from 725 mPa*s to 886 mPa*s for gels 1:1 and from 680 mPa*s to 849 mPa*s for 8:1 ratio (Table 11).

Table 11. Influence of the composition of powders containing 4% methylcellulose, 25% poloxamer 407 and 1,2-propylene glycol on rheological properties (dynamic viscosity η[mPa*s]) gels obtained from investigated powders with 5% hydroxypropylmethylcellulose

Stoichiometric ratio lactic acid to chitosan	**η[mPa*s] gels with addition methyl-cellulose 4000 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 1500 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 400 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 25 mPa*s**	**η[mPa*s] gels with addition methyl-cellulose 15 mPa*s**
1:1	886	836	791	760	725
2:1	875	823	788	752	716
3:1	869	819	779	749	706
4:1	858	808	768	733	698
8:1	849	798	764	725	680

The gels obtained from powders possess the work of adhesion - the energy needed to separate the gel from the probe 5.64 g/s without poloxamer 407 (Fig. 1) for the 1:1 stoichiometric ratio in the complex and 12.45 g/s with poloxamer 407 (Fig. 2) for 1:1 ratio.

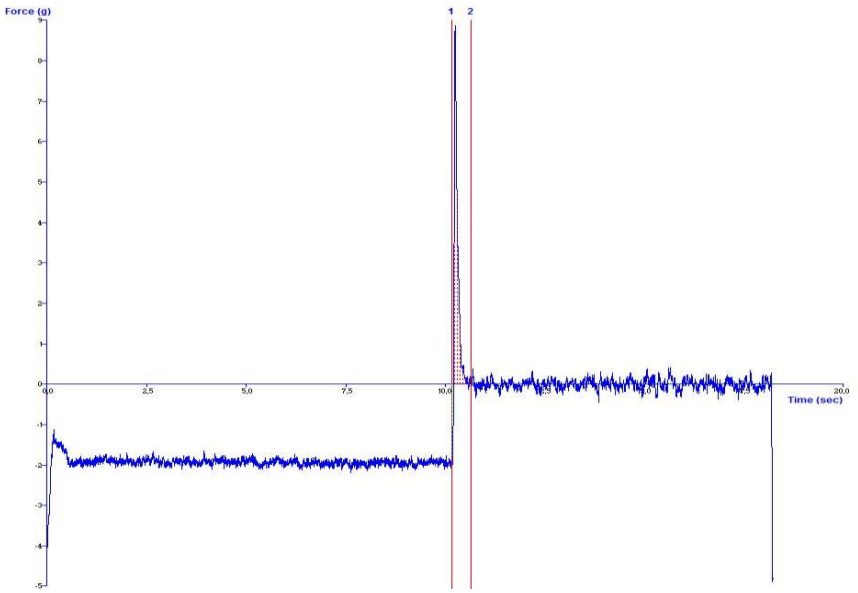

Figure 1. Measurement of texture of gel with addition methylcellulose 4000 mPa*s and stoichiometric ratio lactic acid to chitosan 1:1 without poloxamer 407

Laboratory tests have shown that it is possible to obtain thermosensitive gels with high adhesion properties to vaginal mucous membrane. The investigations showed that the addition of a 20%, 23% and 25% poloxamer 407 increases the adhesive properties of the investigated gels, but at the same time it increases their pH. The enrichment of the composition of the tested powders containing 5% glycerol and 5% 1,2-propylene glycol resulted in increased pH of the formulations. A modification of the composition of the tested powders containing 5% glycerol and 5% 1,2-propylene glycol has increased the range of the dynamic viscosity of formulations. The enrichment of the composition of the tested powders containing 5% hydroxypropylmethylcellulose resulted in increased values the range of the dynamic viscosity and decreased pH of the formulations. The thermosensitive gels obtained from thermosensitive powders possess the work of adhesion with different values. Results obtained in the experimental studies proved that it is possible to produce a thermosensitive preparation with optimal pharmaceutical and application properties.

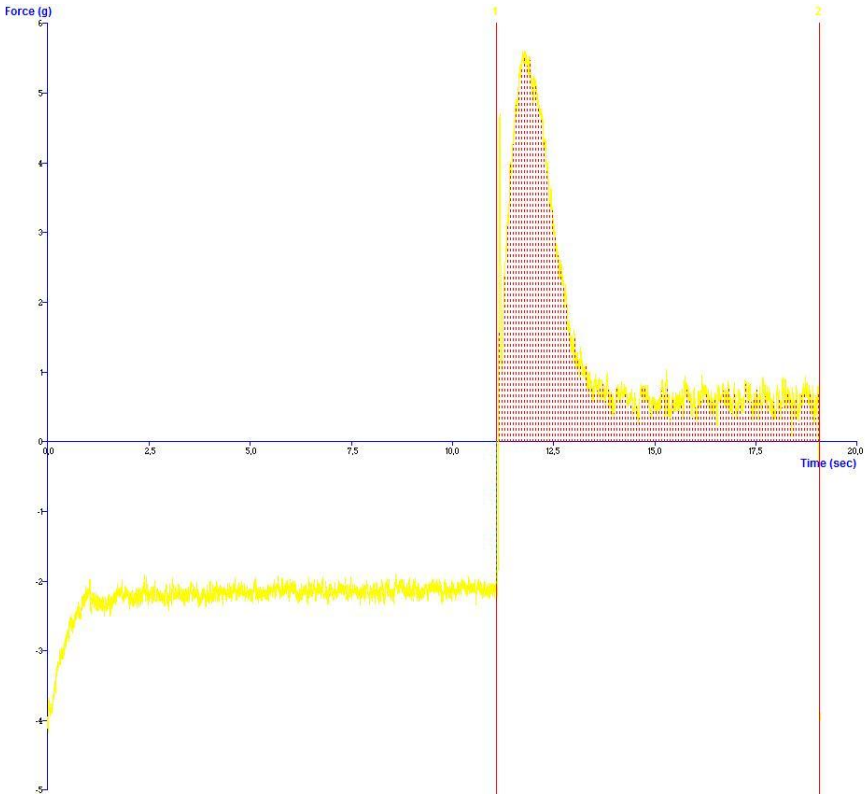

Figure 2. Measurement of texture of thermosensitive gel with addition methylcellulose 4000 mPa*s and stoichiometric ratio lactic acid to chitosan 1:1 with poloxamer 407

4. Conclusions

1. The investigations showed that the addition of a thermosensitive polymer poloxamer 407 increases the adhesive properties of the investigated gels, but at the same time it increases their pH.
2. The addition of hydroxypropylmethylcellulose reduces the pH and maintains high dynamic viscosity.
3. The investigations revealed that it is possible to obtain gels with high dynamic viscosity and to obtain physiological range pH thermosensitive gynaecological gels.
4. The thermosensitive gels obtained from thermosensitive powders possess the work of adhesion with different values.

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