INFLUENCE OF A PECTIN ON THE PROPERTIES OF HYDROPHILIC POWDERS CONTAINING A LACTIC ACID-CHITOSAN COMPLEX

Katarzyna Małolepsza-Jarmołowska

Chair and Department of Pharmaceutical Technology, Faculty of Pharmacy The "Silesian Piasts" memorial Medical University of Wroclaw,
50-556 Wrocław, Borowska 211A Street, Poland.
e-mail: katarzynamj@poczta.onet.pl

Abstract

Gynecological powders are designed to correct abnormal pH environments of the vagina and the stabilise the pH to the physiological state 3.5-5.0. Powders were transformed into gels and were tested for their properties. Formulations were prepared with varying pH and rheological properties. The test showed the work of adhesion of gels. All gels with ratios of 1:1 and 2:1 of lactic acid to chitosan showed a pH in the physiological range at 37°C. Additional pectin and excipients allows various formulations with a wide range of pH to be obtained. Rheological investigation revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and pectin in comparison to the gels without pectin. Studies of work of adhesion showed the effect of glycerol, 1,2-propylene glycol and their concentration on the value of the work of adhesion. The results obtained in the experimental studies demonstrated that it is possible to produce a preparation with optimal pharmaceutical and application properties.

Key words: *lactic acid-chitosan complex, physiological environment of vagina, hydrophilic powders, vaginal mucosa, anti-inflammatory drugs, vaginal infections.*

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1. Introduction

Anatomical and physiological conditions in the vagina do not facilitate easy application of the drug or its maintenance at the site of application. Insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH conditioning of the physiological biocenosis of the organ. 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.

The use of a hydrophilic base for lactic acid complexed with alkaline polymers have enabled the production of powders passing under natural conditions in the gel with rheological properties of vaginal discharge: dynamic viscosity above 100 mPa*s. The gel remains at the site of application and provides adequate environmental pH 3.5-5.0. Thus, the continuation of studies on the optimization of the powder compositions in order to improve their adhesion properties and thus increase the efficacy of the therapy is fully substantiated. Gynecological powders are designed to correct the abnormal pH environment of the vagina and to stabilise the pH to the physiological state 3.5-5.0 [1 - 12].

The aim of this work was to study the pharmaceutical properties of powders for gynecology. These powders must pass in a gel that expresses properties close to those of the natural conditions of the vagina to cover the vaginal mucosa. Therefore, the present study investigated the most important parameters influencing the properties of the tested powders: pH, dynamic viscosity, adhesion.

This work also investigated the effects of pectin additives on the properties of the powders. These powders, passing in gels, were examined for their properties. The formulations were prepared with varying pH and rheological properties. The test shows the work of adhesion of gels . The results allowed the dynamic viscosity of the gels obtained from powders to be defined. A range of pH of the gels allows the selection of the optimum formulation .

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in the experiments: lactic acid (P.Z.F. Cefarm, Wrocław, Poland), chitosan with a deacetylation degree of 93.5% (Sea Fisheries Institute, Gdynia, Poland), methylcellulose (Aldrich Chemical Company Ltd. Gillingham, England), glycerol (Sigma-Aldrich Chemie GmbH, Germany), 1,2-propylene glycol (Sigma – Aldrich Chemie GmbH, Germany), lemon-apple pectin (Aldrich Chemical Company Ltd. Gillingham, England), aqua purificata, acc. to FP X.

2.2. Methods

2.2.1. Preparation of hydrophilic intravaginal powder

The preparation of powder containing lactic acid complexed with chitosan consisted of the following stages:

1. Preparation of the lactic acid - chitosan complex.

The required amount of powdered chitosan was added to a known amount of lactic acid and was mixed. The mixture was left for 24 h until a clear, thick fluid was formed. This could be joined with methylcellulose [4].

2. Preparation of powder from methylcellulose and pectin**.**

The pectin was mixed with a known amount of methylcellulose. Next, the mixture was added to the lactic acid complexes with chitosan and glycerol or 1,2-propylene glycol. The resulting powder was thoroughly pulverized. A homogenous powder was obtained

by sieving through a mesh of size 0.16 mm.
3. Preparation of the tested gel.
A gel was obtained by mixing the powder with a known amount of distilled water and was cooled to 5 - 10 °C to enhance the process of gelation. The homogenous gel was weighed and an additional amount of distilled water was added to obtain the initial mass.

2.2.2. Analytical methods

2.2.2.1. pH-measurement

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. All gels were tested three times, and the results were reported as the average of three measurements at 37°C.

2.2.2.2. Dynamic viscosity measurement

Rheological investigations were performed using a rotational viscosimeter Rheotest 2 Medingen Dresden. The determinations were performed in I a and II a range on a K-1 cone with a diameter of 36 mm and a 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. All gels were tested three times, and the results were reported as the average of three measurements. The values of the shear stress and viscosity were calculated from measurements at 37°C.

2.2.2.3. Measurement of adhesion

A test for texture profile analysis (TPA) was performed with Exponent Stable Micro Systems Texture Analyzer TA-XT 2 plus.
The measurements were conducted in order to illustrate the influence of the type of methylcellulose on the adhesion strength of the prepared gels.
To perform the measurements, a probe (P/1S) in the shape of a ball, built in stainless steel, with a diameter of 1 inch was used.
The measurement parameters were as follows: speed of downward movement of the probe during the test was 0.5 mm /s, and the lifting speed of the probe was 10 mm /s, the maximum permissible force was 100 g, the dwell time of the probe in the gel was 10 s, and the height at which the probe was raised above the surface of the gel was 40 mm.
The measurement was started by placing the gel in a cylindrical vessel with a transparent plexiglass texturometer. Then, the probe was lowered just above the surface of the gel so that there was direct contact between them (the probe remained in this position for 10 seconds). After selecting the appropriate parameters of the program, the measurement started. The probe began to rise at a speed of 10 mm /s at a height of 40 mm above the surface of the gel after contact with the surface of the gel. All gels were tested three times, and the results were reported as the average of three measurements at 37°C.

3. Results and Discussion

Gels obtained from powders containing lactic acid complexed with chitosan revealed stoichiometric ratios of 1:1 and 2:1lactic acid to chitosan and 4% methylcellulose (4000 mPa*s). Their pH ranged from 3.92 for 1:1 gels to 3.48 for 2:1gels [13].
The addition of 5-25% glycerol increased the pH ranged from 4.40 to 4.94 for 1:1 gels and from 3.86 to 4.41 for the 2:1 ratio gels. Further addition of 1.0%, 2.0% and 3.0% of

pectin decreased the pH from 4.10 to 4.75 for 1:1 gels and from 3.50 to 3.90 for the 2:1 ratio gels in relation to the pH range of powders with the addition of glycerol (Table 1).

Table 1. Influence of glycerol and pectin on the pH of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of glycerol [%]	pH of gels with glycerol	pH of gels with glycerol and 1.0% pectin	pH of gels with glycerol and 2.0% pectin	pH of gels with glycerol and 3.0% pectin
1:1	5	4.40	4.35	4.16	4.10
1:1	10	4.44	4.37	4.20	4.15
1:1	15	4.54	4.47	4.26	4.19
1:1	20	4.84	4.63	4.32	4.22
1:1	25	4.94	4.75	4.40	4.35
2:1	5	3.86	3.62	3.58	3.50
2:1	10	4.06	3.75	3.62	3.57
2:1	15	4.16	3.80	3.70	3.64
2:1	20	4.31	3.86	3.76	3.71
2:1	25	4.41	3.90	3.82	3.79

The addition of 5-25% of 1,2-propylene glycol increased the pH from 4.49 to 4.97 for 1:1 gels and from 3.90 to 4.50 for 2:1 gels. Further addition of 1.0%, 2.0% and 3.0% of pectin decreased the pH from 4.26 to 4.85 for 1:1gels and from 3.68 to 4.34 for 2:1gels in relation to the pH range of powders with the addition of 1,2-propylene glycol (Table 2).

Table 2. Influence of 1,2-propylene glycol and pectin on the pH of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of 1,2-propylene glycol [%]	pH of gels with 1,2-propylene glycol	pH of gels with 1,2-propylene glycol and 1.0% pectin	pH of gels with 1,2-propylene glycol and 2.0% pectin	pH of gels with 1,2-propylene glycol and 3.0% pectin
1:1	5	4.49	4.45	4.30	4.26
1:1	10	4.52	4.50	4.34	4.31
1:1	15	4.60	4.56	4.42	4.39
1:1	20	4.88	4.70	4.55	4.44
1:1	25	4.97	4.85	4.67	4.51
2:1	5	3.90	3.86	3.79	3.68
2:1	10	4.11	3.90	3.81	3.75
2:1	15	4.22	4.19	3.88	3.79
2:1	20	4.39	4.26	3.90	3.86
2:1	25	4.50	4.34	3.99	3.93

Rheological studies demonstrated that the gels obtained from powders possessed a dynamic viscosity of 398 mPa*s for the 1:1 stoichiometric ratio in the complex and of 356 mPa*s for the 2:1 ratio [13].
The enrichment of the composition of the tested powders with 5-25% glycerol resulted in increased dynamic viscosity of the formulation from 591 to 681 mPa*s for 1:1gels and from 615 to 699 mPa*s for 2:1 gels (Table 3).
A modification of the composition of the tested powders with 1.0%, 2.0% and 3.0% of pectin increased the dynamic viscosity of formulations from 627 to 825 mPa*s for 1:1gels and from 654 to 778 mPa*s for 2:1 gels (Table 3).

Table 3. Influence of glycerol and pectin on the viscosity of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	**Concentration of glycerol [%]**	**Dynamic viscosity of gels with glycerol [mPa*s]**	**Dynamic viscosity of gels with glycerol and 1.0% pectin [mPa*s]**	**Dynamic viscosity of gels with glycerol and 2.0% pectin [mPa*s]**	**Dynamic viscosity of gels with glycerol and 3.0% pectin [mPa*s]**
1:1	5	681	694	735	825
1:1	10	654	681	692	771
1:1	15	623	664	679	744
1:1	20	599	645	656	725
1:1	25	591	627	636	699
2:1	5	699	735	746	778
2:1	10	663	708	729	765
2:1	15	645	680	693	750
2:1	20	629	671	682	749
2:1	25	615	654	680	744

The addition of 5-25% of 1,2-propylene glycol increased the dynamic viscosity from 609 to 699 mPa*s for 1:1gels and from 629 to 726 mPa*s for 2:1 gels. Further addition of 1.0%, 2.0% and 3.0% of pectin increased the dynamic viscosity from 630 to 840 mPa*s for 1:1gels and from 664 to 822 mPa*s for 2:1 gels (Table 4).

Table 4. Influence of 1,2-propylene glycol and pectin on the viscosity of gels obtained from investigated powders containing 4% methylcellulose

Stoichiometric ratio of lactic acid to chitosan	Concentration of 1,2-propylene glycol [%]	Dynamic viscosity of gels with 1,2-propylene glycol [mPa*s]	Dynamic viscosity of gels with 1,2-propylene glycol and 1.0% pectin [mPa*s]	Dynamic viscosity of gels with 1,2-propylene glycol and 2.0% pectin [mPa*s]	Dynamic viscosity of gels with 1,2-propylene glycol and 3.0% pectin [mPa*s]
1:1	5	699	722	760	840
1:1	10	667	699	735	825
1:1	15	634	675	680	803
1:1	20	615	653	667	799
1:1	25	609	630	641	791
2:1	5	726	765	789	822
2:1	10	678	743	767	816
2:1	15	655	688	740	800
2:1	20	636	679	722	799
2:1	25	629	664	699	780

Gels obtained from the powders possessed the work of adhesion - the adhesiveness of the gel to the probe was 57.85 g/s for gels obtained with 5.0% glycerol and 3.0% pectin (Figure 1), and 82.64 g/s for gels obtained with 5.0% 1,2-propylene glycol and 3.0% pectin (Figure 2).

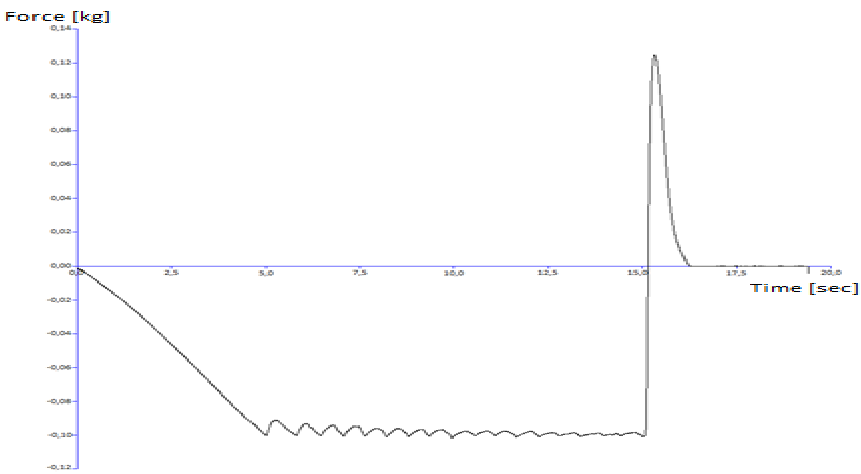

Figure 1. Measurement of texture of gels with 5.0 % glycerol and 3.0 % pectin with the addition of 4000 mPa*s methylcellulose and a stoichiometric ratio of lactic acid to chitosan of 1:1

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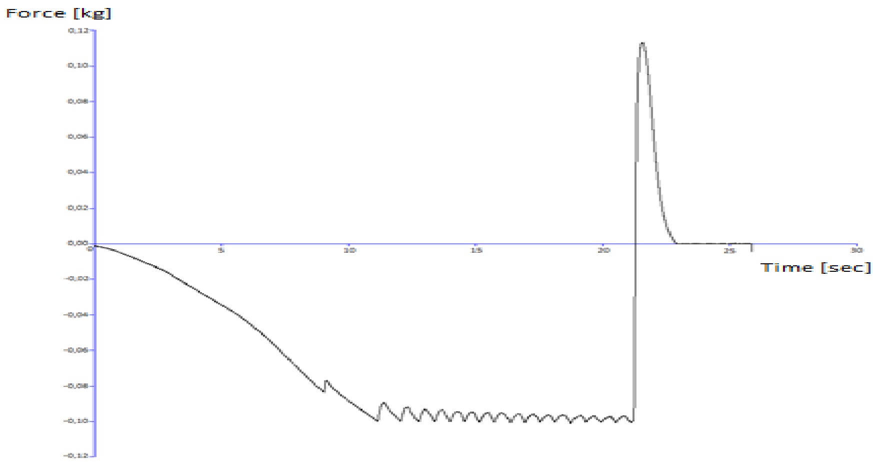

Figure 2. Measurement of texture of gels with 5.0 % 1,2-propylene glycol and 3.0 % pectin with the addition of 4000 mPa*s methylcellulose and a stoichiometric ratio of lactic acid to chitosan of 1:1

The presented studies have shown that it is possible to obtain gels with high adhesion to the vaginal mucous membrane. The use of methylcellulose and pectin allows various formulations with a wide range of pH to be obtained. The pH decreased with increasing concentration of pectin in gels obtained from powders. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and pectin in comparison to the gels without pectin. The dynamic viscosity increased with increasing concentration of pectin in gels obtained from powders. The study of the work of adhesion showed the effect of glycerol, 1,2-propylene glycol and their concentration on the value of the work of adhesion. The gels obtained from powders showed good adhesion.

The present study demonstrates the impact of the used excipients and the ratio of lactic acid to chitosan on pH, dynamic viscosity and the adhesiveness of methylcellulose gels obtained from powders.

All gels with the lactic acid–chitosan complex at 1:1 and 2:1 ratios showed a pH in the physiological range of 3.5–5.0 at 37°C. The addition of pectin and excipients allowed various formulations with a wide range of pH to be obtained. Formulations containing the complex at the ratio of 2:1 showed the lowest pH, which is an important feature and can be used in the treatment of advanced bacterial vaginosis.

Results obtained in the experimental studies demonstrated that it is possible to produce a preparation with optimal pharmaceutical and application properties. Due to the wide pH range, high dynamic viscosity and adhesiveness of the gels obtained from the powders, powders may be adapted to the individual needs of the patients.

4. Conclusions

The investigation demonstrated that the impact of pectin, used excipients and the ratio of lactic acid to chitosan affected the pH, dynamic viscosity and adhesiveness of methylcellulose gels obtained from powders. The obtained formulations have a pH in the desired physiological range and have high viscosity and adhesiveness.

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