RESEARCH OF HYDROPHILIC GLOBULES CONTAINING A LACTIC ACID-CHITOSAN COMPLEX

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Abstract

Gynecological scientific journals report that traditional therapeutic regimens used to treat vaginal inflammation recommended by global treatment centers and health organizations are not producing satisfactory results. Insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH conditioning the physiological biocenosis of the organ. The conducted research and obtained positive results encourage further research possibilities in the field of optimization of pharmaceutical properties of the examined gynecological preparations. Assuming the above assumptions tested impact of polyvinylpyrrolidone K-15, polyvinylpyrrolidone K-30 and polyvinylpyrrolidone K-90 in the properties of the globules. The study was prepared formulations with different pH and rheological properties. The device simulating the conditions in the vagina, studied the adhesion and movement of the gel on the mucosa of the organ. Globules after application of the apparatus simulates the natural conditions in the gel passes and cover the surface. A wide range of pH of the gels allows the selection of the optimum formulation.

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

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1. Introduction

Insufficient duration of the drug's contact with vaginal mucosa does not provide adequate pH conditioning the physiological biocenosis of the organ. Traditional therapeutic schemes recommended by world treatment centres and health organizations do not bring satisfactory results [1-3]. The conducted research and obtained positive results encourage further research possibilities in the field of optimization of pharmaceutical properties of the examined gynecological preparations [4-14].

Aim of this study was to investigate the influence of selected polymers on the physico-chemical properties of globules for gynecological purposes.

Assuming the above assumptions tested impact of polyvinylpyrrolidone K-15, polyvinylpyrrolidone K-30 and polyvinylpyrrolidone K-90 in the properties of the globules. The study was prepared formulations with different pH and rheological properties. The device simulating the conditions in the vagina, studied the adhesion and movement of the gel on the mucosa of the organ. Globules passed in gels were examined for their properties. In vitro studies have demonstrated that the gels obtained from the globules are maintained at the application site. Globules after application of the apparatus simulates the natural conditions in the gel passes and cover the surface. As a result of studies, the dynamic viscosity of the gels obtained from globules. The test shows thixotropical properties of gels. A wide 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%, viscosity of 15 mPa*s, 1% in acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland), methylcellulose viscosity of 4000 mPa*s, 2% in H_2O (20°C) (Aldrich Chemical Company Ltd. Gillingham, England), gelatin (LOBA – Chemie, Wien – Fishamend), polyethylene glycol 200 [PEG-200] (Sigma-Aldrich Chemie GmbH, Germany), polyvinylpyrrolidone K-15, K-30, K-90 (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), aqua purificata as required by you FP XI.

2.2. Apparatus

- pH meter Elmetron - CX 742 (Elmetron Poland)
- Viscosimeter Rheotest - 2 MLW (Medingen Dresden Germany)
- Device simulating conditions in the vagina

2.3. Methods

2.3.1. Preparation of hydrophilic intravaginal globules

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

1. Preparation of the lactic acid - chitosan complex (stoichiometric weight ratio of 1:1 and 2:1).

The required amount of powdered chitosan (0.83g) was added to a known amount of lactic acid 89% (0.56g for 1:1 or 1.12g for 2:1) and was mixed. The mixture was left for 24 h until a clear, thick fluid was formed [4].

2. Obtaining the excipient:

a) Preparation of gel from methylcellulose and polyvinylpyrrolidone K-15, K-30, K-90

A gel was obtained from methylcellulose and polyvinylpyrrolidone K-15, K-30, K-90 by adding a known amount of this compound to the solution of hydrophilizing substance in water. In order to enhance the process of gelation, the mixture was cooled to 5-10 °C.

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The homogenous gel was weighed and enough distilled water was added to obtain the initial mass.

b) Preparation of gel from gelatin

Gelatin was left with water until swelling was completed and then dissolved by heating. The lactic acid - chitosan complex was added to liquid gelatinous gel and heated until an homogenous gel was obtained. Distilled water was added to obtain the initial mass.

c) Preparation of the excipient and pouring into the form

Gels prepared from methylcellulose (4.0g), polyvinylpyrrolidone K-15, K-30, K-90 (5.0g) and gelatin (16.0g) with the lactic acid - chitosan complex were combined into a homogenous excipient and supplemented with distilled water. The excipient was poured into a form that had been previously covered with a thin layer of polyoxyethylene glycol – 200.

3. Preparation of the tested gel.

A gel was obtained by dissolved by heating the globules in water bath.

2.3.2. Analytical methods

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

2.3.2.2. 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.

- shear stress for the range Ia: $\tau = c \cdot \alpha_{(1\text{-}12)} = 85.0 \cdot \alpha_{(1\text{-}12)}$
- viscosity for the range Ia: $\eta = \frac{\tau \qquad \tau}{D(1-12)D(1-12)} \cdot 100 = \frac{85.0 \cdot \alpha(1-12)85.0 \cdot \alpha(1-12)}{D(1-12) \qquad D(1-12)} \cdot 100$
- shear stress for the range IIa: $\tau = c \cdot \alpha_{(1\text{-}12)} = 820.2 \cdot \alpha_{(1\text{-}12)}$
- viscosity for the range IIa: $\eta = \frac{\tau \qquad \tau}{D(1-12)D(1-12)} \cdot 100 =$

$$\frac{820.2 \cdot \alpha(1-12)820.2 \cdot \alpha(1-12)}{D(1-12) \qquad D(1-12)} \cdot 100$$

Meaning of symbols:

- τ [N/m^2] – shear stress
- η [mPa*s] – viscosity
- α [°] – shear angle
- D [1/s] – shear rate

2.3.2.3. Measurement of the ability to coat a surface with a gel

Due to the lack of a suitable measuring device, a model simulating conditions in the vagina was constructed (Figure1). Measurement of the displacement ability of gels formed from vaginal globules.

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Gels displacement studies were carried out in a model that simulates vaginal conditions (Figure1). It is a glass tube with a round bottom. From the outside, it is surrounded by a glass wall connected to the tube at its outlet. In the outer glass wall there are two tubes: water inlet and outlet from the "Remontar" type UTU 5 thermostat. The space between the inner glass and outer wall forms a water jacket surrounding the inner measuring part. Water flows continuously, maintaining a temperature of 37° C (body temperature). The inner measuring part of the model simulating the conditions in the vagina is 30 cm long and 3 cm in diameter. There is a scale on the outer glass wall. Adhesion measurement determines the ability of gels to move. For this purpose, 3 cm^3 of gel was measured with a medical syringe and applied to the upper part of the model simulating the conditions in the vagina. After 5, 10, 15 and 20 minutes from application, the gel flow length in cm was read. Each gel was tested six times and the result is given as the average of these measurements. Measurement uncertainty (u) for each measurement (x) was estimated based on the accuracy of the scale division of the model used to simulate the conditions in the vagina, u (x) = 0.5 cm. The results are collected in Table 3.

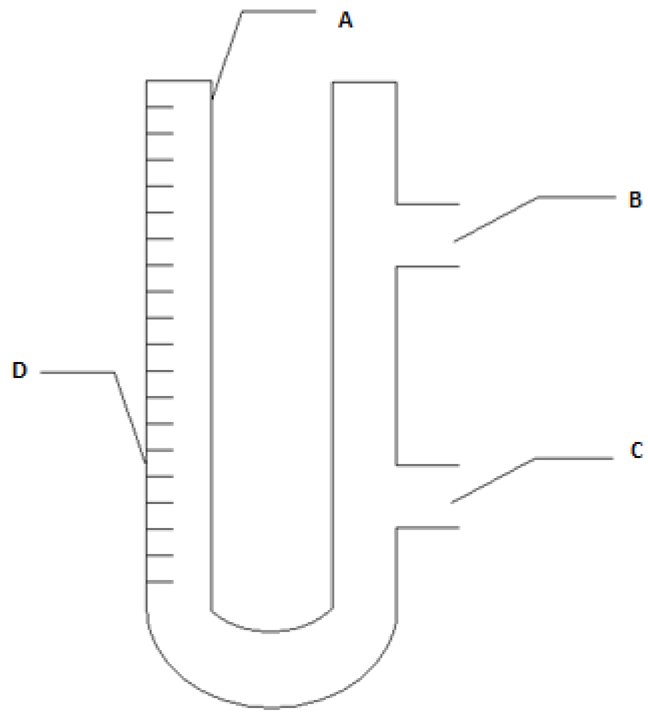

Figure 1. Diagram of a model simulating the conditions in the vagina
A – glass tube – place of application of the preparation (gel)
B – water outflow
C – water supply
D – glass wall with scale

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3. Results and Discussion

3.1. pH measurement

Gels obtained from globules containing lactic acid complexed with chitosan revealed stoichiometric weight ratio of 1:1 and 2:1 lactic acid to chitosan and 4.0% methylcellulose. Their pH ranged from 3.92 for 1:1 gels to 3.48 for 2:1gels [13].

The addition of 5-25% PEG-200 increased the pH ranged from 4.43 to 4.95 for 1:1 gels and from 3.42 to 3.68 for the 2:1 ratio gels (in compare to previous range from 3.92 and 3.48). Further addition of 5.0% of polyvinylpyrrolidone K-15, 5.0% of polyvinylpyrrolidone K-30, 5.0% of polyvinylpyrrolidone K-90 decreased the pH from 4.53 to 3.83for 1:1 gels (in compare to previous range from 4.95 to 4.43) and increased the pH from 3.80 to 4.20 for the 2:1 ratio gels (in compare to previous range from 3.42 to 3.68) in relation to the pH range of gels with the addition of PEG-200 (Table 1).

Table 1. Influence of PEG-200 and PVP K-15, K-30, K-90 on the pH of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration [%] PEG-200	pH gels with addition PEG-200	pH gels with PEG-200 and addition 5.0% PVP K-15	pH gels with PEG-200 and addition 5.0% PVP K-30	pH gels with PEG-200 and addition 5.0% PVP K-90
1:1	5	4.43	3.83	4.17	4.22
1:1	10	4.48	3.88	4.20	4.29
1:1	15	4.55	3.92	4.26	4.35
1:1	20	4.87	3.97	4.30	4.46
1:1	25	4.95	4.00	4.35	4.53
2:1	5	3.42	3.80	3.84	3.91
2:1	10	3.46	3.85	3.89	3.93
2:1	15	3.51	3.92	4.05	4.10
2:1	20	3.63	3.99	4.11	4.16
2:1	25	3.68	4.10	4.14	4.20

PVP – polyvinylpyrrolidone

The use of methylcellulose and polyvinylpyrrolidone makes it possible to obtain various preparations with a wide pH range. All gels with the lactic acid - chitosan complex with a weight ratio of 1: 1 and 2: 1 showed a pH in the physiological range of 3.5-5.0 at 37° C. The addition of polyvinylpyrrolidone and excipients allowed obtaining various preparations with a wide pH range. The preparations containing the complex in a 2: 1 weight ratio showed lower pH, which is an important feature and can be used in the treatment of advanced bacterial vaginosis. Most of the tested preparations were in the range of pH = 3.5-4.5 most preferred by gynecologists.

3.2. Rheological tests

Rheological studies demonstrated that the gels obtained from globules possessed a dynamic viscosity of the formulation from 139.16 to 354.41 mPa*s for the 1:1 stoichiometric ratio in the complex and from 216.27 to 368.14 mPa*s for the 2:1 ratio.

A modification of the composition of the tested globules with 5.0% of polyvinylpyrrolidone K-15, 5.0% of polyvinylpyrrolidone K-30, 5.0% of polyvinylpyrrolidone K-90 increased the dynamic viscosity of formulations from 468.11 to 657.39 mPa*s for 1:1gels and from 561.86 to 788.62 mPa*s for 2:1 gels (Table 2).

Table 2. Influence of PEG-200 and PVP K-15, K-30, K-90 on the viscosity of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concen-tration [%] PEG-200	Dynamic viscosity of gels with addition PEG-200 [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 5.0% PVP K-15 [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 5.0% PVP K-30 [mPa*s]	Dynamic viscosity of gels with PEG-200 and addition 5.0% PVP K-90 [mPa*s]
1:1	5	354.41	498.65	573.26	657.39
1:1	10	334.17	487.32	569.37	648.28
1:1	15	280.02	480.51	560.22	636.63
1:1	20	234.41	476.99	556.31	628.52
1:1	25	139.16	468.11	548.49	615.31
2:1	5	368.14	599.68	628.54	788.62
2:1	10	250.02	586.65	598.59	611.84
2:1	15	233.15	580.23	591.43	608.56
2:1	20	224.56	576.11	580.87	593.00
2:1	25	216.27	561.86	575.22	580.32

PVP – polyvinylpyrrolidone

Rheological studies have shown an increase in dynamic viscosity of preparations containing lactic acid complexed with chitosan enriched with polyvinylpyrrolidone compared to gels without polyvinylpyrrolidone. The dynamic viscosity increased with the change in the type of polyvinylpyrrolidone. The lowest dynamic viscosity value was shown by gels formed from globules containing K-15 PVP. The increase in dynamic viscosity value was noticeable with the addition of PVP K-30. The highest dynamic viscosity value was represented by gels formed from globules containing K-90 PVP.

3.3. Measurement of the ability to coat a surface with a gel

The tested gels had the ability to coat the surface with gel at 37 ° C. The tested surface can be covered with gel. Gels containing lactic acid complexed with chitosan in a stoichiometric ratio of 1: 1 and 2: 1 and 5-25% PEG-200 content are able to move from 25 to 30 cm. The modification of the composition of the tested gels with the addition of 5.0% PVP reduced their mobility to 25.0-21.9 cm for PVP K-15, 23.9-21.0 cm for PVP K-30 and 23.0-20.0 cm for PVP K-90 in a 1: 1 ratio. Enrichment of the composition of the tested gels with the addition of 5.0% PVP containing lactic acid complexed with chitosan in a stoichiometric ratio in a 2:1 ratio reduced their mobility to 28.0-22.0 cm for PVP K-15, 24.0-21.9 cm for PVP K-30 and 23.8-20.8 cm for PVP K-90 (Table 3).

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Table 3. Influence of PEG-200 and PVP K-15, K-30, K-90 on able to move of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatin

Stoichiometric weight ratio lactic acid to chitosan [0.56g/1.12g : 0.83g]	Concentration [%] PEG-200	Able to move gels with addition PEG-200 [cm]	Able to move gels with PEG-200 and addition 5.0% PVP K-15 [cm]	Able to move gels with PEG-200 and addition 5.0% PVP K-30 [cm]	Able to move gels with PEG-200 and addition 5.0% PVP K-90 [cm]
1:1	5	25.0	21.9	21.0	20.0
1:1	10	26.5	22.8	22.6	21.5
1:1	15	27.9	23.6	22.9	22.3
1:1	20	28.9	24.5	23.2	22.9
1:1	25	29.4	25.0	23.9	23.0
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

PVP – polyvinylpyrrolidone

The presented studies have shown that it is possible to obtain gels with high adhesion to the vaginal mucosa. Examination of the surface's ability to coat the gel showed that PVP K-15, PVP K-30 and PVP K-90 affect the gel's ability to adhere to the surface. Gels with 5.0% PVP K-15 showed high adhesion compared to gels without PVP. Measurements carried out under simulation conditions in a vaginal model showed that the addition of 5% PVP K-30 to gels containing lactic acid complexed with chitosan more significantly reduces their mobility. Compositions containing 5.0% PVP K-90 are very well maintained on the surface being tested, ensuring even coating.

4. Conclusions

As a result of the research, it was found that the types of polyvinylpyrrolidone used affect the pH, dynamic viscosity and adhesion of methylcellulose gels. The ratio of lactic acid to chitosan in the complex has an additional effect. The preparations obtained have a pH in the desired physiological range and have high viscosity and adhesion. Gels showed good adhesion. The results obtained in experimental studies have shown that it is possible to produce a preparation with optimal pharmaceutical and application properties. The use of an appropriate ratio of lactic acid to chitosan in the complex and the right type of polyvinylpyrrolidone makes it possible to obtain a preparation with excellent coating properties that simulate the surface of the vaginal mucosa.

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