EVALUATION OF HYDROPHILIC GLOBULES WITH SODIUM ALGINATE CONTAINING A LACTIC ACID–CHITOSAN COMPLEX

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Abstract

An important issue in the treatment of vaginitis is the amount of time the drug remains on the vaginal mucosa. If the contact time is too short, the drug cannot work effectively to ensure the correct pH in the vaginal environment. This study evaluated formulations of globules containing sodium alginate, lactic acid and chitosan with different pH and rheological properties. The experimental studies revealed 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 appropriate concentration of sodium alginate produces a preparation with excellent properties to coat the surface of the vaginal mucosa.

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

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1. Introduction

Studies on vaginal forms of drugs have not been fully satisfactory. One important problem is the time the drug remains on the vaginal mucosa. If the contact time is too short, the drug cannot ensure the appropriate pH in the vaginal environment. pH outside the physiological range causes inflammation and enables the development of pathogenic flora [1-3]. Results from prior studies have encouraged further research possibilities to optimise the pharmaceutical properties of gynaecological preparations [4-14]. These studies have revealed that sodium alginate has a significant effect on the physicochemical properties of gynaecological powders that transform into gels in an aquatic environment. The rheological assessments revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan and sodium alginate compared with gels without sodium alginate. Each of the tested hydrogels showed thixotropic properties, creating hysteresis loops in the graphs, depending on the shear rate and shear stress. With the increase in shear rate, the value of the dynamic viscosity of the tested formulations decreased. This outcome proved the pseudoplastic nature of the tested gels made of powders [15]. The positive results of the research have encouraged work on another form of the drug that is convenient for application.

Given the encouraging results of globules containing sodium alginate, lactic acid and chitosan, the aim of this study was to investigate the influence of selected polymers on the physicochemical properties of globules for gynaecological purposes. The formulations had different pH and rheological properties. A device was used to simulate the conditions in the vagina to study the adhesion and movement of the gel on the mucosa of the organ. *In vitro* studies demonstrated that the gels obtained from the globules were maintained at the application site. After application, the gel covered the surface of the apparatus. The gels showed high dynamic viscosity. A wide pH range of the gels allows for the selection of the optimal 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% and a viscosity of 15 mPa*s, 1% in acetic acid (20°C) (Sea Fisheries Institute, Gdynia, Poland); methylcellulose with a viscosity of 4000 mPa*s, 2% in H_2O (20°C) (Sigma-Aldrich Chemical Company Ltd. Gillingham, England); gelatine (LOBA Chemie, Wien, Austria), polyethylene glycol 200 (PEG-200; Sigma-Aldrich Chemie GmbH, Germany), sodium alginate (Sigma-Aldrich Chemie GmbH); and aqua purificata as required by FP XII.

2.2. Apparatuses

– CX 742 pH meter (Elmetron, Poland)
– 2 MLW Viscosimeter Rheotest (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 three stages: (1) complexing lactic acid and chitosan, (2) obtaining the recipient and (3) preparing the test gels.

First, to prepare the lactic acid–chitosan complex (stoichiometric weight ratio of 1:1 and 2:1), the required amount of powdered chitosan (0.83 g) was added to a known amount

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

Second, obtaining the excipient involved three steps: (a) preparation of gel from methylcellulose and sodium alginate, (b) preparation of gel from gelatine and (c) preparation of the excipient and pouring it into the form. A gel was obtained from methylcellulose and sodium alginate by adding a known amount of this compound to the solution of hydrophilising substance in water. 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. Gelatine was left with water until swelling was completed and then dissolved by heating. The lactic acid–chitosan complex was added to a liquid gelatinous gel and heated until a homogenous gel was obtained. Distilled water was added to obtain the initial mass. Gels prepared from methylcellulose (4.0 g), sodium alginate (0.5, 0.7 or 1.0g) and gelatine (16.0 g) with the lactic acid–chitosan complex were combined into a homogenous excipient; the mass was adjusted to 100.0 g with distilled water. The excipient was poured into a form that had been previously covered with a thin layer of PEG-200.

Finally, a gel was obtained by heating the globules in a water bath.

2.3.2. Analytical Methods

2.3.2.1. pH Measurement

The potentiometric method was used to measure the pH of the gels. Specifically, a combined electrode integrated into an ELECTRON CX-742 multifunctional computer meter was immersed into the investigated gel. All gels were tested three times, and the results are reported as the average of three measurements at 37°C.

2.3.2.2. Dynamic Viscosity Measurement

Rheological investigations were performed using a Rheotest 2 rotational viscosimeter. The determinations were performed in the Ia and IIa ranges 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 the ascending direction and 11 rates in the descending direction. All gels were tested three times, and the results are reported as the average of three measurements. The values of the shear stress and viscosity were calculated from measurements at 37°C using the following equations:

- 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:

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

For the above equations, τ is the shear stress (N/m^2), η is the viscosity (mPa*s), α is the shear angle and D is the shear rate (1/s).

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 [16]. It was used to examine the displacement of gels formed from vaginal globules. 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: a water inlet and a water 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. The adhesion measurement indicates the ability of gels to move inside the apparatus. For this experiment, 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. The gel flow length was read 5, 10, 15 and 20 min after application. 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.

3. Results and Discussion

3.1. pH Measurement

Gels were obtained from globules containing lactic acid complexed with chitosan in a stoichiometric weight ratio of 1:1 and 2:1 and 4.0% methylcellulose. Their pH ranged from 3.92 for 1:1 gels to 3.48 for 2:1 gels [13]. The addition of 5%-25% PEG-200 increased the pH, with a range from 4.43 to 4.95 for the 1:1 gels and from 3.42 to 3.68 for the 2:1 gels (compared with the previous range of 3.92 to 3.48). The addition of 0.5%, 0.7% or 1.0% sodium alginate decreased the pH of the 1:1 gels, with a range from 4.50 to 4.02 (compared with the previous range of 4.95 to 4.43), and increased the pH of the 2:1 gels, with a range from 3.80 to 4.39 (compared with the previous range of 3.42 to 3.68) (Table 1).

The use of methylcellulose and sodium alginate yielded various preparations with a wide pH range. All gels prepared with the lactic acid–chitosan complex showed a pH in the physiological range of 3.5-5.0 at 37°C. The addition of sodium alginate and excipients yielded various preparations with a wide pH range. The preparations containing the 2:1 lactic acid–chitosan complex showed a lower pH, which is an important feature and can be used in the treatment of advanced bacterial vaginosis. Most of the tested preparations had a pH of 3.5-4.5, which is preferred by gynaecologists.

3.2. Rheological Tests

The gels obtained from globules had a dynamic viscosity that depending on the formulation. Specifically, it was 139.16 to 354.41 mPa*s for the 1:1 lactic acid–chitosan complex and from 216.27 to 368.14 mPa*s for the 2:1 lactic acid–chitosan complex. The addition of 0.5%, 0.7% or 1.0% of sodium alginate to the globules increased the dynamic viscosity of formulations, with a range from 456.23 to 645.43 mPa*s for 1:1 gels and a range from 549.22 to 776.40 mPa*s for the 2:1 gels (Table 2).

The rheological evaluation revealed an increase in dynamic viscosity of preparations containing the lactic acid–chitosan complex and enriched with sodium alginate compared with gels without sodium alginate. The dynamic viscosity changed depending on the

Table 1. Influence of PEG-200 and sodium alginate on the pH of gels obtained from globules containing 4.0% methylcellulose and 16.0% gelatine

Lactic acid to chitosan stoichiometric ratio	PEG-200 concentration (%)	pH of gels with PEG-200	pH of gels with PEG-200 and 0.5% sodium alginate	pH of gels with PEG-200 and 0.7% sodium alginate	pH of gels with PEG-200 and 1.0% sodium alginate
1:1	5	4.43	4.12	4.10	4.02
1:1	10	4.48	4.24	4.12	4.07
1:1	15	4.55	4.37	4.14	4.12
1:1	20	4.87	4.42	4.27	4.17
1:1	25	4.95	4.50	4.32	4.22
2:1	5	3.42	4.06	3.83	3.80
2:1	10	3.46	4.12	3.87	3.82
2:1	15	3.51	4.15	3.89	3.85
2:1	20	3.63	4.27	3.92	3.92
2:1	25	3.68	4.39	4.01	3.95

Abbreviation: PEG-200, polyethylene glycol 200

Table 2. Influence of PEG-200 and sodium alginate on the viscosity of gels obtained from investigated globules containing 4.0% methylcellulose and 16.0% gelatine

Lactic acid to chitosan stoichiometric ratio	PEG-200 concentration (%)	Dynamic viscosity of gels with PEG-200 (mPa*s)	Dynamic viscosity of gels with PEG-200 and 0.5% sodium alginate (mPa*s)	Dynamic viscosity of gels with PEG-200 and 0.7% sodium alginate (mPa*s)	Dynamic viscosity of gels with PEG-200 and 1.0% sodium alginate (mPa*s)
1:1	5	354.41	486.24	561.11	645.43
1:1	10	334.17	475.40	565.40	636.00
1:1	15	280.02	468.22	548.35	624.11
1:1	20	234.41	476.34	544.26	616.20
1:1	25	139.16	456.23	536.42	603.60
2:1	5	368.14	587.31	616.31	776.40
2:1	10	250.02	574.42	586.14	599.01
2:1	15	233.15	568.05	579.43	596.40
2:1	20	224.56	564.00	568.15	581.10
2:1	25	216.27	549.22	563.10	568.50

Abbreviation: PEG-200, polyethylene glycol 200

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sodium alginate concentration. The gels formed from globules containing 0.5% sodium alginate had the lowest dynamic viscosity. The increase in dynamic viscosity was noticeable with the addition of 0.7% sodium alginate. The highest dynamic viscosity value was represented by gels formed from globules containing 1.0% sodium alginate.

3.3. Measurement of the Ability to Coat a Surface With a Gel

The tested gels had the ability to coat the surface of the simulated vagina at 37°C. Gels containing the lactic acid–chitosan complex and 5%-25% PEG-200 moved from 25 to 30 cm. Considering the 1:1 lactic acid–chitosan complex, the addition of sodium alginate reduced their mobility to 22.8-26.4 cm for 0.5% sodium alginate, 22.2-25.8 cm for 0.7% sodium alginate and 21.3-24.9 cm for 1.0% sodium alginate. Considering the 2:1 lactic acid–chitosan complex, the addition of sodium alginate increased the mobility to 24.2-29.4 cm for 0.5% sodium alginate, 23.6-28.4 cm for 0.7% sodium alginate and 22.0-26.0 cm for 1.0% sodium alginate (Table 3).

These findings demonstrated that it is possible to obtain gels with high adhesion to the vaginal mucosa. Examination of the ability of the gel to coat the surface showed that sodium alginate affects the gel's ability to adhere to the surface. Gels with 0.5% sodium alginate showed high adhesion compared with gels without sodium alginate. Measurements carried out under simulation conditions in a vaginal model showed that the addition of 0.7% sodium alginate to gels containing the lactic acid–chitosan complex notably reduced their mobility. The gels containing 1.0% sodium alginate were very well maintained on the surface being tested, ensuring even coating. They also showed the

Table 3. Influence of PEG-200 and sodium alginate on the movement ability of gels obtained from globules containing 4.0% methylcellulose and 16.0% gelatine

Lactic acid to chitosan stoichiometric ratio	PEG-200 concentration (%)	Movement of gels with PEG-200 (cm)	Movement of gels with PEG-200 and 0.5% sodium alginate (cm)	Movement of gels with PEG-200 and 0.7% sodium alginate (cm)	Movement of gels with PEG-200 and 1.0% sodium alginate (cm)
1:1	5	25.0	22.8	22.2	21.3
1:1	10	26.5	23.9	23.0	22.4
1:1	15	27.9	24.6	23.8	23.0
1:1	20	28.9	25.8	24.6	23.7
1:1	25	29.4	26.4	25.8	24.9
2:1	5	26.9	24.2	23.6	22.0
2:1	10	27.7	25.9	24.5	23.7
2:1	15	28.6	27.3	25.9	24.9
2:1	20	29.8	28.5	27.2	25.8
2:1	25	30.0	29.4	28.4	26.0

Abbreviation: PEG-200, polyethylene glycol 200 best adhesion to the test surface. Preparations travelling over a distance of 21.3-26.0 cm provide good surface coverage. It can be concluded that these formulations would adhere well and would not flow out when applied to the vagina. The obtained results require confirmation with *in vivo* tests.

4. Conclusions

The conducted experimental studies have shown that sodium alginate affects the pH, dynamic viscosity and adhesion of methylcellulose gels. The ratio of lactic acid to chitosan in the complex also influences these parameters. The resulting preparations had a pH in the desired physiological range. The gels showed high dynamic viscosity and good adhesion. Overall, 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 appropriate concentration of sodium alginate produces a preparation with an excellent ability to coat the surface of the vaginal mucosa.

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