PHARMACEUTICAL ASSESSMENT OF GYNAECOLOGICAL POWDERS WITH THE ADDITION OF HYDROPHILISING SUBSTANCES CONTAINING LACTIC ACID COMPLEXED WITH CHITOSAN

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Abstract
Research on gynaecological preparations followed that of optimising their pharmaceutical properties, which is beneficial for gynaecological powders. Gels obtained from powders containing lactic acid complexed with chitosan revealed a stoichiometric ratio 1:1, 2;1, 3:1, 4:1 and 8:1. Their pH ranged from 3.92 to 4.44 for 1:1 gels and from 2.36 to 2.84 for the 8:1 ratio. The addition of 5% polyethylene glycol-400 increases the pH from 4.52 to 4.92 for 1:1 gels and from 2.54 to 3.41 for the 8:1 ratio. A modification of the composition of the tested powders containing 5% polyethylene glycol-400 increased the range of the dynamic viscosity of formulations suitably from 78 to 520 mPa·s for 1:1 gels and from 39 to 423 mPa·s for the 8:1 ratio. Measurements performed in a biopharmaceutical model revealed that the gels obtained from powders containing lactic acid complexed with chitosan are able to move from 25 to 28 cm.

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

DOI: 10.15259/PCACD.19.11

1. Introduction

Commonly applied drug forms tend to leave the vagina when the patient assumes an upright position.

The use of hydrophilic gels with high adhesion properties and an ability to spread over the vaginal mucosa enable the 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].

Research on gynaecological preparations followed that of optimising their pharmaceutical properties, which is beneficial for gynaecological powders. The test powders pass under natural conditions in the gel covering the vaginal mucosa.

The aim of this study was to investigate the effect of selected substances hydrophilising on the physico-chemical properties of powders for gynaecological purposes.

The influence of the hydrophilising substances such as polyethylene glycol-200 and polyethylene glycol-400 were assessed on the pharmaceutical properties of powders tested. Formulations were prepared with varying pH and rheological properties.

Adhesion tests were carried out, and movement of the gel on the mucous membrane, in the simulated device, in the vagina. Powdered gels were examined for their properties.

The results of *in vitro* studies showed that the gels formed from powders remain at the application site. Backfill occurs when applied to the instrument, which imitates the natural conditions in the vagina, and passes the gel covering the surface.

The resulting gels exhibit thixotropic properties, characterised by a defined dynamic viscosity. A wide range of pH tested gels allows the optimum formulation to be selected.

2. Materials and Methods

2.1. Materials

The following chemicals of analytical grade were used in experiments: 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), polyoxyethylene glycol 200 (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), polyoxyethylene glycol 400 (Aldrich Chemical Company Ltd. Gillingham – Dorest SP 84 SL, England), and aqua purificata, acc. to FP IX.

2.2. Methods

2.2.1. Measurements of physical properties

2.2.1.1. Rheological investigations (dynamic viscosity)

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 the ascend-

ing 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 experiments are presented in Table 2.

2.2.1.2. Determination of adhesion

The determination of adhesion was performed on a biopharmaceutical model imitating the conditions in the vagina. This was a 30 cm long and 3 cm diameter, calibrated glass tube attached to a REMONTAR type UTU5 ultrathermostat. Water, at a constant temperature of 37 °C, was flowing continuously through the water jacket in the biopharmaceutical model. The measurement of adhesion determines the ability of gels obtained from powders to move. For this reason, 3 cm of gel was attached to a syringe and placed in the upper part of the model, imitating an artificial vagina. The distance of the gel flow in cm was read 5, 10, 15 and 20 minutes after application. Each gel was investigated three times and the final result was a mean of the measurements. The results obtained in the experiments are presented in ***Figure 1***.

2.2.2. 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 24 h until a clear, thick fluid was formed that could be joined with methylcellulose [4].

2. Obtaining the excipient-preparation of powder from methylcellulose.

A powder was obtained from methylcellulose, by adding a known amount of this compound to the lactic acid complexes with chitosan and polyethylene glycol-200 or polyethylene glycol-400. The resulting powder was thoroughly pulverised. Homogenous powder was obtained by being passed through a sieve with 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 of distilled 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.

3. Results and discussion

Gels obtained from powders, containing lactic acid complexed with chitosan, reveal stoichiometric ratios of 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 (***Table 1***).

The addition of 5% polyethylene glycol-200 increases the pH range from 4.40 to 4.80 for 1:1 gels and from 2.39 to 3.29 for the 8:1 ratio (***Table 2***).

Table 1. *Influence of methylcellulose viscosity on pH gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	3.92	3.96	4.17	4.25	4.44
2:1	3.48	3.82	4.04	4.19	4.25
3:1	3.17	3.25	3.44	3.65	3.90
4:1	2.75	2.87	2.90	2.95	3.09
8:1	2.36	2.58	2.65	2.78	2.84

Table 2. *Influence of viscosity of methylcellulose and 5% polyethylene glycol-200 on pH of gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	4.40	4.47	4.62	4.66	4.80
2:1	3.85	4.19	4.53	4.62	4.61
3:1	3.48	3.70	3.92	4.04	4.49
4:1	3.05	3.12	3.46	3.56	3.77
8:1	2.39	2.95	3.02	3.14	3.29

The enrichment of the composition of the tested powders containing 5% polyethylene glycol-400 resulted in an increased pH range of the formulation to 4.52 to 4.92 for 1:1 gels and from 2.54 to 3.41 for the 8:1 ratio (***Table 3***).

Rheological studies demonstrated that the research gels obtained from powders possess the dynamic viscosity ranging from 53 to 398 mPa·s for the 1:1 stoichiometric ratio in the complex and from 19 to 242 mPa·s for the 8:1 ratio (***Table 4***).

A modification of the composition of the tested powders containing 5% polyethylene glycol-200 has increased the range of the dynamic viscosity of formulations suitably from 69 to 465 mPa·s for 1:1 gels and from 29 to 354 mPa·s for the 8:1 ratio. Further addition of the 5% polyethylene glycol-400 resulted in a further increase in the dynamic viscosity of the formulations from 78 to 520 mPa·s for 1:1 gels and from 39 to 423 mPa·s for the 8:1 ratio.

Measurements performed in a biopharmaceutical model revealed that the gels obtained from powders containing lactic acid complexed with chitosan are able to move from 25 cm to 28 cm.

Statistical evaluation was performed. The analysis was carried out on the basis of multivariate analysis of variance (ANOVA). The level of significance was $\alpha = 0.05$. Differentiating factors were used in the study of different types of methylcellulose viscosity: 4000, 1500, 400, 25, 15 mPa·s and polyethylene glycol: 200, 400.

In these studies, it has been shown that the composition of each formulation affects the runoff and was considered statistically significant ($p < 0.0001$). The longest path flow, and

Table 3. *Influence of methylcellulose viscosity and 5% polyethylene glycol-400 on the pH of gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	pH gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	4.52	4.58	4.74	4.78	4.92
2:1	3.97	4.31	4.86	4.70	4.73
3:1	3.60	3.82	4.04	4.16	4.62
4:1	3.17	3.24	3.58	3.68	3.89
8:1	2.54	3.12	3.14	3.26	3.41

Table 4. *Influence of methylcellulose viscosity on rheological properties (dynamic viscosity η in mPa·s) of gels obtained from investigated powders.*

Stoichiometric ratio lactic acid to chitosan	η in mPa·s gels with addition methyl-cellulose				
	4000 mPa·s	1500 mPa·s	400 mPa·s	25 mPa·s	15 mPa·s
1:1	398	254	165	110	53
2:1	356	232	159	98	44
3:1	305	221	143	87	38
4:1	286	204	136	71	24
8:1	242	198	129	62	19

thus the greatest ability to move the gel under conditions simulating natural gels obtained were characterised with methylcellulose-based powders having a viscosity of 15 mPa·s + polyethylene glycol-200. The shortest route runoff showed gels formed from powders containing methylcellulose having a viscosity of 4000 mPa·s + polyethylene glycol-400 (***Figure 1***, see page 102).

All of the researches were performed at 37 °C.

The studies have shown that it is possible to obtain gels with high adhesion properties to vaginal mucous membrane. The use of methylcellulose with different values of the viscosity allows different formulations to be obtained with a wide pH range. Rheological investigations revealed an increase in the dynamic viscosity of preparations containing lactic acid complexed with chitosan, and revealed a stoichiometric ratio 1:1 in comparison to the gels with a ratio of 8:1.

Measurements performed in a biopharmaceutical model revealed that the gels obtained from powders containing lactic acid complexed with chitosan are able to move from 25 cm to 28 cm.

Results obtained in the experimental studies proved that it is possible to produce a preparation with optimal pharmaceutical and application properties.

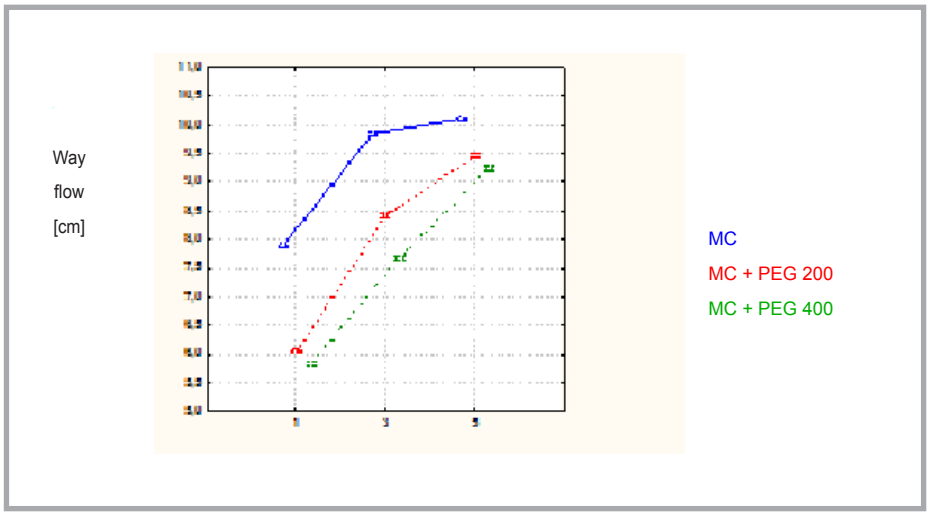

Figure 1. *Flow research gels obtained from powders with the addition methylcellulose 4000 mPa·s and stoichiometric ratio of lactic acid to chitosan of 1:1; MC - methylcellulose 4000 mPa·s, PEG-200 - polyethylene glycol-200, PEG-400 - polyethylene glycol-400.*

4. Conclusions

1. The researches demonstrated that methylcellulose with different values of viscosity significantly affect the adhesive properties of hydrophilic gels obtained from powders, but have a wide pH range.

2. The gels obtained from the powders were characterised by the specific dynamic viscosity.

3. Powders show the adhesion of the gel covering the surface of the apparatus simulates the conditions in the vagina.

5. References

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