EXPERIMENTAL STUDIES ON SELECTION OF OPTIMAL GELLING AGENT AND ITS RATIONAL CONCENTRATION IN COMPOSITION OF VAGINAL GEL WITH ESSENTIAL OILS

Iryna Kryklyva, Olena Ruban, Tetyana Ponomarenko, Larisa Bobrytska, Vita Grytsenko

National University of Pharmacy of Ukraine

Introduction. Vaginal candidiasis is a relevant problem in gynecology, as every third woman in the world faces this disease, which is caused by yeast-like fungi of the genus Candida albicans, and is manifested by itching, redness, swelling of the genitals [7,9,14,17]. Today, there are many drugs for the treatment of these diseases (antifungal agents based on clotrimazole, natamycin, nystatin, fluconazole). Some of them are used locally (creams, gels, vaginal tablets, suppositories), others - internally (tablets, capsules) [11,12,16]. But synthetic drugs are not always effective, with long-term use they can cause irritation and addiction. In this regard, the development and creation of drugs based on medicinal plant raw materials is of great interest. Phytopreparations have a mild effect, can be used for a long time, and practically do not show side effects.

Semisolid medicinal products are easy to use, provide a long-term concentration of active pharmaceutical ingredients (API) directly at the place of application of the drug, can combine several APIs of multidirectional action in one dosage form, etc. In recent years, gels have gained considerable popularity, being a convenient dosage form for local therapy of vaginal candidiasis, well absorbed and distributed on the mucous membrane of the vagina. The above determines the high bioavailability of active pharmaceutical ingredients. Hydrophilic and lipophilic medicinal substances can be introduced into the composition of gels, and thanks to the gel base influence on their release and bioavailability, increase the effectiveness and safety of drugs, which is an important aspect for a dosage form which is applied to the vaginal mucosa. The gel is the optimal medicinal form for the introduction of an active complex of essential oils, as well as their gradual release to achieve an optimal therapeutic effect. The gel form contributes to the penetration of the active substance into the tissues, causes an additional cooling effect, is safe for the skin and mucous membranes, and is convenient to use. The advantages inherent in gels make them very promising in the development of a topical preparation for application to the mucous membrane. Such processes as the release and absorption of active pharmaceutical ingredients depend on the type of carrier, physico-chemical and structural-mechanical properties of both active and auxiliary substances, so the goal of our work was to select a rational gelling agent and its concentration in the composition of the vaginal gel [15].

The purpose of this work was to choose the optimal gelling agent in the composition of the vaginal gel.

Materials and methods

In order to select the optimal gelling agent in the composition of the vaginal gel, we studied the following gelling agents - Aristoflex AVC (Ammonium Acryloyldimethyltaurate / VP Copolymer) - France, Clariant, Licigel – Sodium acrylates copolymer (and) Lecithin) – France, Lucas Meyer, Sepimax ZEN (Polyacrylate Crosspolymer-6) - Seppic, France. To determine the rational concentration of the gelling agent, gel samples were prepared with different concentrations of gelling agents - 1.0%, 1.5%, and 2.0%, and their structural and mechanical properties were studied.

The samples were made in laboratory conditions at a temperature of 25 °C, the speed of rotation of the stirrer was 100 rpm. A gelling agent was added to purified water and left to swell for 20 minutes. A solution of essential oils in alcohol and lactic acid were added to the gelling agent solution and mixed thoroughly until a homogeneous gel was formed.

A Proof of the therapeutic effectiveness of medicinal products is not the only goal of formulation development research. It is necessary to study the technological parameters of gel samples that affect the process of their production and ease of use. The main technological parameters of gels are rheological properties. Structural and mechanical properties have a significant impact on the process of releasing active pharmaceutical ingredients from gels and determine their consumer characteristics (extrusion from tubes, spreadability, adhesion and preservation of their original properties during storage and transportation) [2,5,15].

During rheological studies, samples are subjected to mechanical destruction with the help of an inner cylinder at an increasing rate. This process mimics the one that occurs during application to the surface of the skin or mucous membranes during its use or manufacturing processes (mixing, dosing and transportation). Therefore, an ideal sample can be considered one that is easily dosed and extruded from the tube, easily applied and distributed on the surface of the skin and mucous membrane [5,6,13].

The rheological characteristics of the model samples were measured on a rotational viscometer Myr 3000 V2R (Viscotech, Spain) in a system of coaxial cylinders following the SPU method (2.2.10) in a wide range of shear rates. The study was carried out at a temperature of (37 ± 0.1) °C.

For the study, a suspension of the experimental sample (about 30.0 g) was taken and placed in the capacity of an external stationary cylinder, after which the cylinder was attached to the device frame, including the internal movable cylinder. As a result, the studied sample filled the annular gap of the coaxial cylinders. At certain speeds of rotation of the inner cylinder, the indicator indicators of the device were recorded. The determination was made with an increase in the speed of rotation of the cylinder and in the opposite direction

Based on the measurement results, rheograms of the dependence of the shear (r) on the gradient of the shear rate (Dr) were built [10].

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Results and discussion

At the first stage of research, three types of model gel samples were produced using Aristoflex AVC, Sepimax ZEN and Lecigel. The choice of the indicated gelling agents was justified by the fact that they are technological and do not require neutralization; swell quite quickly; are stable to degradation under yield value shear stress; compatible with polar organic solvents; ensure obtaining gels of different viscosity, which can vary depending on the amount of polymer used. The composition of the test samples is shown in Table 1.

Table 1. Composition of model samples, %

Substances	Model samples		
	No.1	No.2	No.3
Tea tree essential oil	2.0	2.0	2.0
Lavender essential oil	2.0	2.0	2.0
Lecigel	2.0	–	–
Sepimax Zen	–	2.0	–
Aristoflex AVC	–	–	2.0
Lactic acid	to pH 4.5		
Ethyl alcohol 96%	10.0	10.0	10.0
Purified water	to 100.0		

Samples of gels were made according to the generally accepted technology: the required amount of a gelling agent (Aristoflex AVC, or Sepimax ZEN, or Lecigel) was weighed on the scales and the required amount of purified water was added, periodically stirring until dissolved. The required amount of purified water was measured separately, the required amount of lactic acid preweighed on an electronic scale was added and mixed. The required amount of essential oils of tea tree and lavender was dissolved in 96% ethyl alcohol, constantly stirring until complete dissolution. An alcoholic solution of essential oils and a solution of lactic acid were added to the gelling agent solution to a pH of 4.5. Stirred the mixture until a homogeneous, uniform gel was obtained without visible mechanical inclusions. The prepared samples were evaluated for organoleptic and consumer properties, their colloidal and thermal stability and pH level were determined. The results are given in Table 2.

Table 2.Organoleptic and physicochemical properties of model samples (n=5, P=95 %)

Sample No.	Organoleptic indicators	Colloidal stability	Thermal stability	pH
1	The gel of uniform consistency, without impurities, opaque, white with a characteristic smell and delicate texture. Not sticky.	Stable	Stable	4.43± 0.09
2	The gel has a homogeneous consistency, without impurities and inclusions, opaque. It has a smell characteristic of essential oils. Distributes evenly on the skin, does not leave a sticky feeling.	Stable	Stable	4.45±0.07
3	The gel has a homogeneous consistency, without impurities, opaque. It has a characteristic smell. The texture is not dense. Spreads well when applied to the skin, does not leave a sticky feeling	Stable	Stable	4.50±0.06

The data in Table 2 show that the test samples met the quality criteria for vaginal gels. At the next stage, the antifungal properties of gel samples were investigated. The results are shown in Figure 1.

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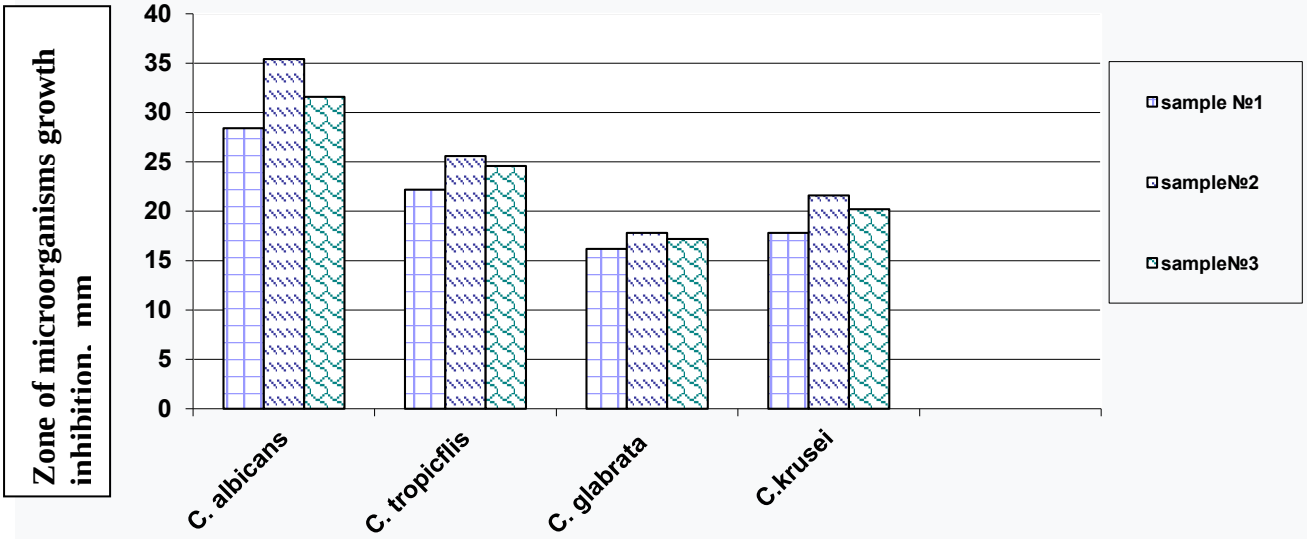

Fig.1Results of the antifungal activity study of vaginal gel samples (n=5, P=95 %)

The data shown in Figure 1 demonstrate that samples 1-3 have an average level of antifungal activity (the diameter of the growth inhibition zones of microorganisms is 15-25 mm) in relation to the fungi C. glabrata and C. krusei, the culture of C. tropicalis is sensitive to samples No. 1 and No. 3 (the diameters of growth inhibition zones are 22.2±0.4 and 24.6±0.5, respectively). The test strains of C. albicans ATCC 885-653 are the most sensitive (the diameter of the growth inhibition zone exceeded 25 mm) to the developed samples of the semisolid dosage form (diameters of the growth inhibition zones 28.4±0.5mm, 35.4±0.5mm, 31.6±0.5mm, respectively) as well as C. glabrata to the sample No.2 (diameter 25.6±0.5mm)/

Thus, it has been established that samples No. 2 and No. 3 have the greatest antifungal activity against yeast-like fungi of the genus Candida, so they were chosen for further research.

The next stage of our research was the study of the antimicrobial activity of the gel samples selected above. The results are shown in Table 3.

The data presented in Table 3 show that the studied samples No. 2 and No. 3 have moderate antimicrobial activity in relation to the used test strains. The studied sample No. 3 shows higher activity in relation to all used cultures of microorganisms - *S. aureus* ATCC 25293 - 21.2±0.4; *B. Subtilis* ATCC 6633 - 22.2±0.4; *E. coli* ATCC 25922 - 24.8±0.4; *Ps. aeruginosa* ATCC 27853 - 21.8±0.4. Thus, it should be noted that the release of active substances from sample No. 3 is more complete, as evidenced by the larger diameters of growth inhibition zones. Therefore, sample No. 3, produced using Aristoflex AVC, was selected for further research.

Table 3 Antimicrobial activity of vaginal gel samples (n=5, P=95 %)

Sample gel	**Cultures of microorganisms**			
	***S. aureus* ATCC 25293**	***B. subtilis* ATCC 6633**	***E. coli* ATCC 25922**	***Ps. aeruginosa* ATCC 27853**
	Diameters of the growth inhibition zone of microorganisms, mm			
No. 2	13.8±0.4	15.8±0.4	20.6±0.5	15.6±0.5
No. 3	21.2±0.4	22.2±0.4	24.8±0.4	21.8±0.4

In order to choose the optimal concentration of Aristoflex AVC in the composition of the vaginal gel, the next stage of our research was the determination of the structural and mechanical properties of the experimental samples that contained the gelling agent in a concentration of 1%, 1.5% and 2%.

Based on the measurement results, rheograms of the dependence of the shear stress (τ) on the shear rate gradient (Dr) were constructed, and the type of flow, yield strength and presence of thixotropic properties were determined (Figure 2).

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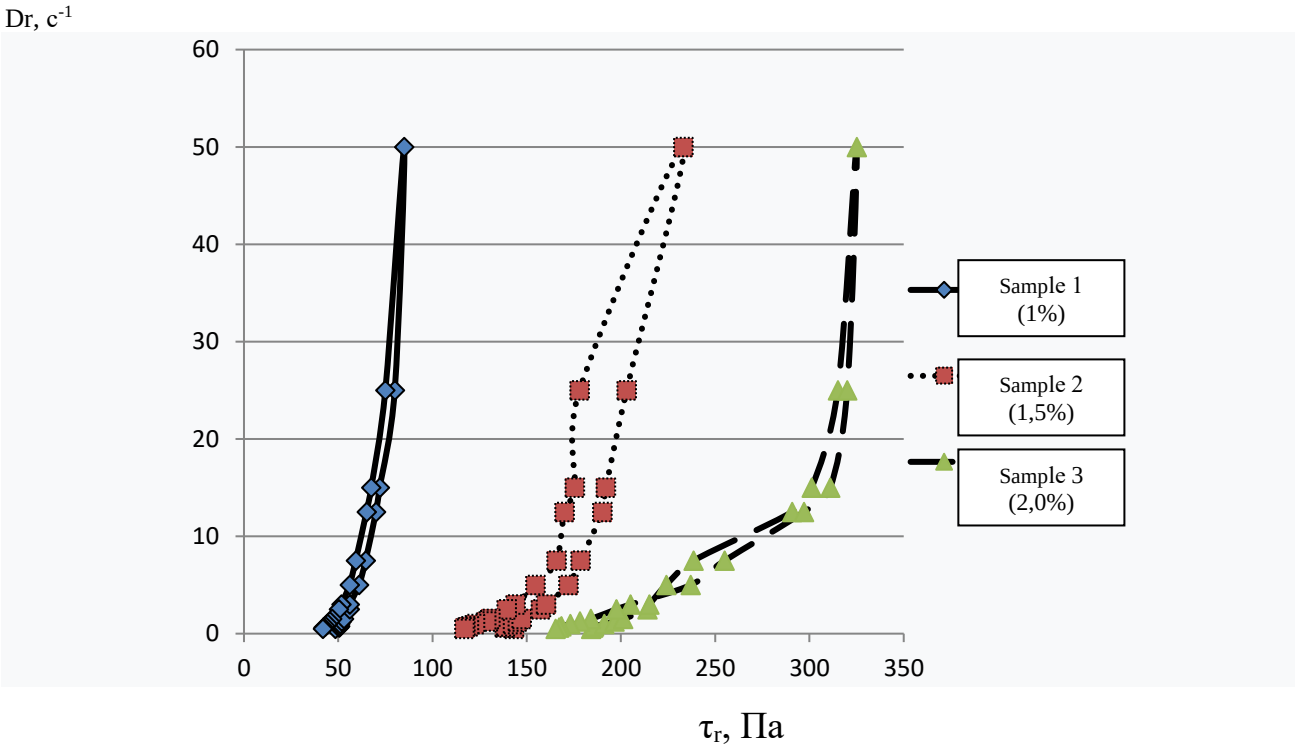

Fig.2 Flow rheograms of test samples of vaginal gel based on Aristoflex AVC gelling agent (n=5, P=95 %)

As can be seen from Figure 2, the descending and ascending curves form hysteresis loops on the rheograms, which confirms the thixotropic nature of the studied samples. Analyzing the loops, it can be concluded that all test samples have sufficient thixotropy, which is evidenced by the area of their surfaces.

The use of a gelling agent in the amount of 2% (sample No. 3) helps obtaining a gel structure with high viscosity, which makes it possible to predict the inconvenience of its application, packaging, as well as extrusion from tubes.

The use of a gelling agent in the amount of 1.5% (sample No. 2) causes partial destruction of the sample structure with gradual recovery when the shear stress is reduced.

The area of the hysteresis loop of sample No. 1 with a concentration of 1% was smaller than the loop areas of the other samples and has a smooth ascending and descending curve. As can be seen in the graph, the sample has a directly proportional relationship between viscosity and speed of rotation, therefore, as the spindle speed increases, the structural viscosity decreases.

Thus, in all samples, the influence of gelling agent concentration on gel viscosity is observed. The obtained research results indicate a non-Newtonian type of flow in gel systems.

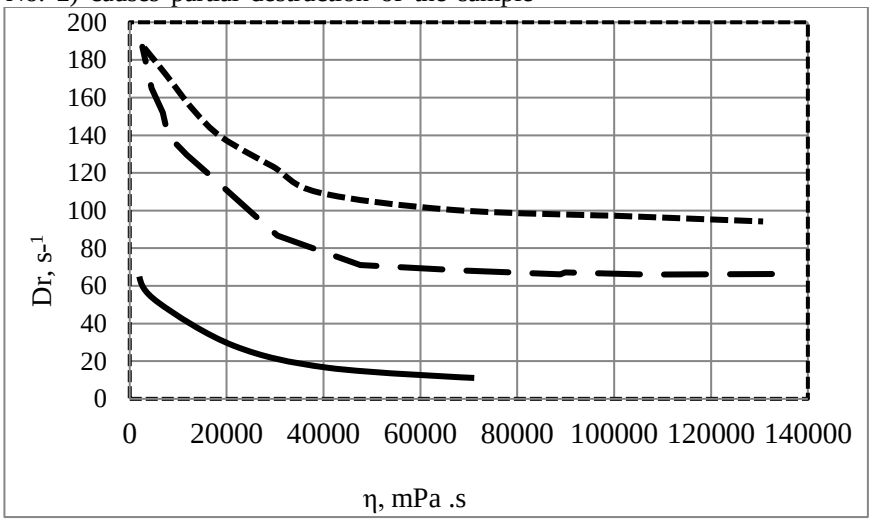

Fig. 3. Dependence of sample viscosity on shear stress (n=5, P=95 %)

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All studied samples demonstrate pseudoplastic flow, in which their viscosity decreases with increasing shear stress (Figure 3).

When the stress is reduced, the viscosity of the samples is gradually restored, which confirms the thixotropic properties of the studied gel samples. Accordingly, the Aristoflex AVC sample with a concentration of 1% had the lowest viscosity, and the sample with 2% Aristoflex AVC concentration had the highest viscosity.

Therefore, the obtained results allow us to state that the content of Aristoflex AVC gelling agent in a concentration of 1% (sample No. 1) is rational. This gel has the best plasticity, forms a steady state of equilibrium between the destruction of the structure and its restoration, so it will be easy to apply and distribute on the mucous membrane of the vagina and squeeze out of the tube, which indicates good consumer characteristics.

Conclusions

1. When determining the organoleptic properties of model samples prepared on gel bases (Aristoflex AVC, Lecigel and Sepimax ZEN), it has been established that all samples have good organoleptic properties and the required stability.
2. Based on the conducted microbiological studies, Aristoflex AVC was chosen as the optimal gelling agent in the vaginal gel being developed.
3. According to the results of structural and mechanical studies on the selection of the concentration of Aristoflex AVC in the composition of the vaginal gel, it has been proven that its optimal concentration is 1%.
4. The rheological properties of the gel formed by Aristoflex AVC have been investigated, and it was established that it has a plastic type of flow and is characterized as a structured dispersion system that will ensure an easy, painless, uniform distribution of the gel on the vaginal mucosa.

Experimental studies on selection of optimal gelling agent and its rational concentration in composition of vaginal gel with essential oils

Iryna Kryklyva, Olena Ruban, Tetyana Ponomarenko, Larisa Bobrytska, Vita Grytsenko

Introduction. The deterioration of the demographic situation is one of the main problems of Ukraine. From 2008 to 2021, the country's population had decreased by 4.5 million, which is associated not only with the economic state of the country, but also with the state of women's health. Reproductive health is significantly affected by infectious diseases of the genital organs of women. One such disease is vaginal candidiasis. Due to the wide spread of this disease and its tendency to relapse, an important place in the complex of measures for its treatment is given to local intravaginal administration of drugs. In this regard, it is rational to use semisolid dosage forms (SDF), namely vaginal gels, which are able to tightly contact with the vaginal mucosa and quickly release active pharmaceutical ingredients at the site of application. The Department of Industrial Technology of Drugs of the National University of Pharmacy is developing the composition and technology of a new gel for the treatment of vaginal candidiasis, which contains essential oils of tea tree, lavender, and lactic acid. Essential oils provide a wide range of antibacterial and antifungal activity. The use of lactic acid allows maintaining a normal pH level of the mucous membranes of the female genital organs, and at the same time, the vital activity of lactobacilli, which suppress the pathogens of most female diseases. One of the main issues in the development of the gel is the choice of the gelling agent and its rational concentration. To conduct experimental studies on the selection of the optimal gelling agent in the composition of the vaginal gel, experimental samples have been made with gelling agents approved for medical use: Aristoflex AVC (Ammonium Acryloyldimethyltaurate / VP Copolymer) - France, Clariant, Licigel – Sodium acrylates copolymer (and) Lecithin) – France, Lucas Meyer, Sepimax ZEN (Polyacrylate Crosspolymer-6) - Seppic, France. The purpose of this work was to choose the optimal gelling agent and its concentration in the composition of the developed vaginal gel based on organoleptic, physicochemical, microbiological and structural-mechanical studies. **Materials and methods.** Using data from scientific literature, gelling agents Aristoflex AVC, Licigel and Sepimax ZEN were used in the composition of the samples at a concentration of 2%. The antimicrobial and antifungal activity of the test samples was studied in vitro by the agar diffusion method (the "wells" method). As test cultures pure cultures were used: gram-positive microorganisms Staphylococcus aureus ATCC 25293, spore culture Bacillus subtilis ATCC 6633, gram-negative cultures Candida tropicalis, Candida glabrata, Candida krusei. During the experiments, one-day suspensions of bacterial microorganisms and two-day suspensions of fungi in physiological saline were used. Microbial overload was 10^7 microbial cells in 1 mL of nutrient medium [10]. The rheological characteristics of the model samples were measured on a rotational viscometer Myr 3000 V2R (Viscotech, Spain) in a system of coaxial cylinders following the SPU method (2.2.10) in a wide range of shear rates. The study was carried out at a temperature of (37 ± 0.1) °C. Based on the measurement results, rheograms of the dependence of the shear (r) on the gradient of the shear rate (Dr) were built. The determination of the homogeneity of the analyzed eyes was carried out according to the methodology induced in DFU 1.0, p.511.Determination of colloidal stability. (GOST 29188.3-91 "Cosmetic products. Methods for determining the stability of emulsions"). The tubes, 2/3 filled with the sample, were placed in a water bath at a temperature of 45 ± 2°C for 20 min, after which they were centrifuged for 5 min at a speed of 6000 rpm. Stability was determined visually, in the presence of stratification - unstable, without changes - stable. Determination of thermal stability. (GOST 29188.3-91 "Cosmetic products. Methods for determining the stability of emulsions"). For determination, 5-6 glass test tubes with a diameter of 15 mm and a height of 150 mm were taken. The test tubes were filled with test samples (8-10) ml and placed in a thermostat brand TS-80M-2 with a temperature of 42.5±2.5 °C for 7 days. After that, the samples were transferred for 7 days to a refrigerator with a temperature

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of (6±2)°C and then kept at room temperature for 3 days. Stability was determined visually: if no delamination was observed in one tube, the sample was considered stable.Determination of the pH of aqueous gel solutions. The pH level of the studied samples was determined potentiometrically in accordance with the SPU method 2.0, Volume 1, p.2.2.3, p. 51–53. We used the pH-150 MI device. **Results and discussion.** The microbiological, structural-mechanical and organoleptic properties of vaginal gel samples with Aristoflex AVC, Licigel and Sepimax ZEN gelling agents have been studied. It has been established that the sample with Aristoflex AVC has good sensory properties: soft structure, is quickly and well adsorbed from the mucous membrane without leaving a feeling of stickiness and oily sheen, and also has a non-Newtonian pseudoplastic type of flow. The influence of different concentrations of Aristoflex AVC on rheological properties and microbiological activity has been studied. **Conclusions.** Based on the results of the experimental studies, Aristoflex AVC gelling agent at a concentration of 1% was chosen as the basis for the gel and the microbiological activity has been studied.

Key words: vaginal gel, gelling agent, organoleptic, microbiological, rheological and physicochemical studies.

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