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STUDY OF THE HYALURONIC ACID SOLUBILITY FOR DEVELOPMENT OF THE VAGINAL GEL COMPOSITION

Topicality. Hyaluronic acid is widely used in cosmetic and medical products as a moisturizing, anti-inflammatory and reparative component. Currently, the urgent issue is the expansion of the assortment of medicines of native production, which include hyaluronic acid.

Aim. The research is devoted to the choice of the optimal solvent for the insertion of hyaluronic acid into a composition of vaginal gel.

Materials and methods. The object of the study was low molecular weight hyaluronic acid with a molecular weight of 0.09×10^6 Da. Microscopic analysis using a Microscope Konus Academi has been carried out to study the dissolution of the hyaluronic acid powder in water, peach oil, the most common hydrophilic non-aqueous solvents and mixtures thereof.

Results and discussion. In the course of the study, the shape and size of the hyaluronic acid particles in various solvents and their mixtures were determined.

Conclusions. It has been established that the hyaluronic acid is unlimitedly swells in water at a temperature of 20 °C. Addition of PEO-400 and propylene glycol improves its wetting and distribution of particles in solvent volume. The addition of tween-80 to purified water has allowed increasing the speed and completeness of the hyaluronic acid dissolution.

Key words: *hyaluronic acid; vaginal gel; solubility*

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Вивчення розчинності кислоти гіалуронової для розробки складу гелю вагінального

Актуальність. Кислота гіалуронова є речовиною, що широко застосовується у складі косметичних та лікарських препаратів як зволожуючий, протизапальний та репаративний компонент. На теперішній час актуальним питанням є розширення асортименту лікарських препаратів вітчизняного виробництва, до складу яких входить кислота гіалуронова.

Мета роботи. Дослідження присвячено визначенню оптимального розчинника для введення кислоти гіалуронової до складу гелю вагінального.

Матеріали та методи. Об'єктом дослідження була низькомолекулярна кислота гіалуронова з молекулярною масою $0,09 \times 10^6$ Да. Визначення розчинності проводили за допомогою мікроскопічного методу, який дозволяє дослідити зміну форми та розміру часток упродовж часу. Мікроскопічний аналіз проводився за допомогою лабораторного мікроскопу Konus Academi виробництва Італії. У якості розчинників були використані: вода очищена, гліцерин, пропіленгліколь, ПЕО-400, олія персикова, спирт етиловий, твін-80 та їх суміші.

Результати та їх обговорення. В ході дослідження було визначено форму та розмір часток кислоти гіалуронової в різних розчинниках та їх сумішах.

Висновки. Встановлено, що кислота гіалуронова необмежено набухає у воді при температурі 20 °C. Додавання ПЕО-400 та пропіленгліколю покращує її змочування та розподіл частинок в об'ємі розчинника. Додавання до води очищеної твіну-80 дозволило підвищити швидкість та повноту розчинення кислоти гіалуронової.

Ключові слова: *кислота гіалуронова; гель вагінальний; розчинність*

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Изучение растворимости кислоты гиалуроновой для разработки состава геля вагинального

Актуальность. Кислота гиалуроновая широко применяется в составе косметических и лекарственных препаратов как увлажняющий, противовоспалительный и репаративный компонент. В настоящее время актуальным вопросом является расширение ассортимента лекарственных препаратов отечественного производства, в состав которых входит кислота гиалуроновая.

Цель работы. Исследование посвящено определению оптимального растворителя для ввода кислоты гиалуроновой в состав геля вагинального.

Материалы и методы. Объектом исследования была низькомолекулярная кислота гиалуроновая с молекулярной массой $0,09 \times 10^6$ Да. Определение растворимости проводили с помощью микроскопического метода, который позволяет исследовать изменение формы и размера частиц на протяжении времени. Микроскопический анализ проводился с помощью лабораторного микроскопа Konus Academi производства Италии. В качестве растворителей были использованы: вода очищенная, глицерин, пропиленгликоль, ПЭО-400, масло персиковое, спирт этиловый, твин-80 и их смеси.

Результаты и их обсуждение. В ходе исследования были определены форма и размер частиц кислоты гиалуроновой в различных растворителях и их смесях.

Выводы. Установлено, что кислота гиалуроновая неограниченно набухает в воде при температуре 20 °C. Добавление ПЭО-400 и пропиленгликоля улучшает ее смачивание и распределение частиц в объеме растворителя. Добавление к воде очищенной твина-80 позволило повысить скорость и полноту растворения кислоты гиалуроновой.

Ключевые слова: *кислота гиалуроновая; гель вагинальный; растворимость*

INTRODUCTION

Hyaluronic acid is a multifunctional component in the treatment of various diseases. Depending on the length of the polysaccharide chain, the hyaluronic acid is classified into low, medium and high molecular weight, each of which differs by action and is used to treat various diseases [1-4]. Low molecular weight hyaluronic acid shows a moisturizing, reparative and anti-inflammatory effect, penetrating into the deep layers of the epidermis, may exhibit the functions of active transport and be the carrier of various active ingredients [5, 6]. We are developing a gel formulation for the treatment of urogenital symptoms in climacteric, infectious diseases of female genital organs and during gestation. These conditions are accompanied by the appearance of such unpleasant symptoms as dryness, itching, irritation and pain when urinating [2, 5]. Low molecular weight hyaluronic acid is the active ingredient in the gel's composition, as it is able to show a moisturizing, anti-inflammatory and regenerative effect on the mucous membranes of female genital organs [7, 8]. Therefore, the purpose of our work was to determine the rational method for introducing hyaluronic acid into the composition of the vaginal gel.

MATERIALS AND METHODS

The object of the study was low molecular weighted hyaluronic acid with a molecular weight of 0.09×10^6 Da. Determination of solubility was carried out using a microscopic method, which allows investigating the change in the shape and size of particles over time. Microscopic analysis was performed with the help of a laboratory microscope Konus Academi produced in Italy. For powder particles observed in the microscope field, the correct geometric shape was selected and measured for its length and width, considering the magnification [9]. To characterize the degree of powder particles isometricity, the form factor was calculated according to the formula:

$$K = W / L,$$

where: W – average width of particles, μm ; L – average length of particles, μm .

As solvents were used: purified water, glycerol, propylene glycol, PEO-400, peach oil, ethyl alcohol, tween-80

and mixtures thereof. The determination was carried out within 5 minutes after the contact of the substance and the solvent [10]. The ratio of the substance and solvent was as 1 : 2.

RESULTS AND DISCUSSION

In the first stage of the study, the shape and size of the hyaluronic acid particles were determined (Fig. 1).

The results shown in Fig. 1 indicate that the powder is capable of agglomeration. Clusters of particles have the form of polyhedra with a non-uniform rough surface, with a form factor of about 0.8 and a size of 0.7 to 1.2 microns. The obtained data allow concluding that agglomerates of hyaluronic acid have a large specific surface in comparison with individual particles, which, in turn, allows predicting unsatisfactory wetting of the substance.

With the help of microscopic analysis, the solubility of hyaluronic acid in the solvent media, which are most often used in the technology of soft dosage forms, has been studied (Fig. 2-8). The analysis of data shown in Fig. 2 indicates that in the mixture of hyaluronic acid with glycerol there is a change in the shape of particles throughout the surface, in the field of view there are both agglomerates and individual particles with a linear size of 0.01 to 0.5 μm .

With the addition of propylene glycol (Fig. 3), an increase in solid phase volume was observed, indicating a long-term swelling with formation of an unsolvated dispersion.

Addition of PEO-400 (Fig. 4) facilitates the beginning of the edge wetting process with subsequent change in shape and linear dimensions. In the field of view, both swollen particles and agglomerates are observed. Linear sizes range from 0.01 to 0.5 μm . The results obtained indicate a long-term dissolution process with limited swelling.

Adding peach oil to hyaluronic acid (Fig. 5) contributed to the formation of dense agglomerates with the inclusion of air bubbles. Wetting was observed only in separate particles. The obtained results testify to the inappropriateness of the use of oil in the composition of dosage forms with hyaluronic acid due to moderate solubility and poor wetting.

The addition of ethanol to the hyaluronic acid (Fig. 6) did not lead to a change in the linear size and shape, but the distribution of particles in the volume of solvent was observed.

A change in the picture of dissolution was observed when purified water was added (Fig. 7). In the field of

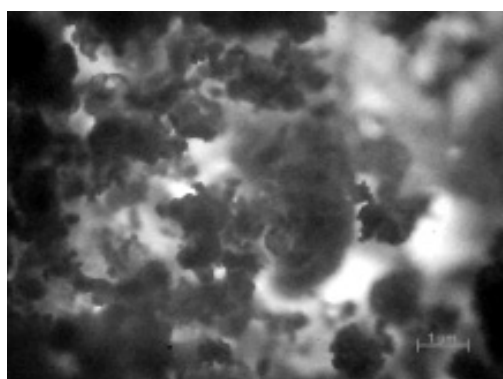


Fig. 1. Photomicrograph of dry hyaluronic acid powder

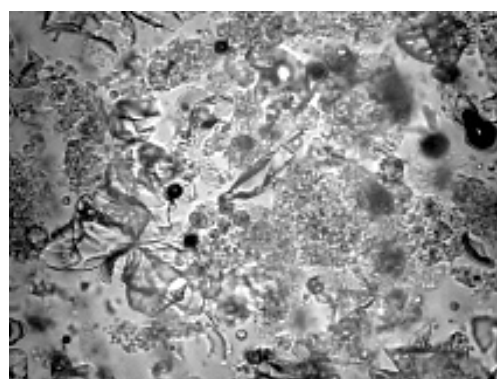


Fig. 2. Photomicrography of hyaluronic acid in glycerol

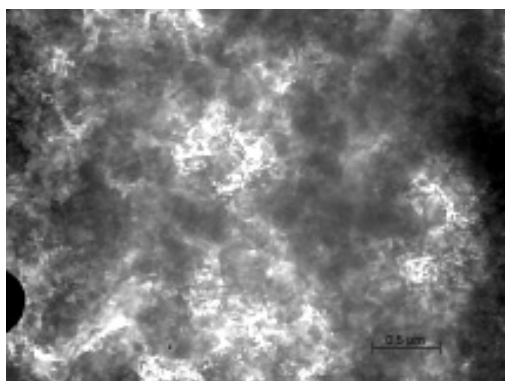


Fig. 3. Photomicrography of hyaluronic acid in propylene glycol

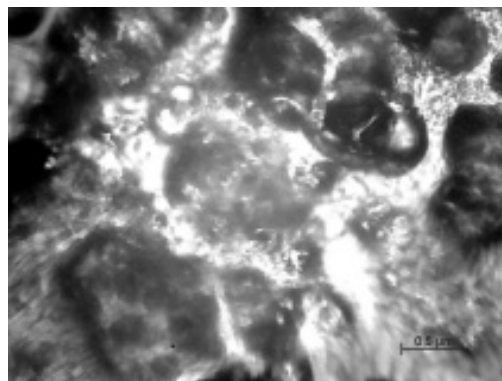


Fig. 5. photomicrography of hyaluronic acid in peach oil

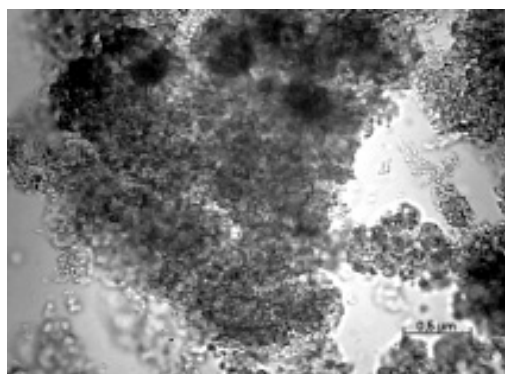


Fig. 4. Photomicrography of hyaluronic acid in PEO-400

view, individual particles were observed with the simultaneous swelling of the formed agglomerates. The particles were transparent, bulky, with fragments on a smooth surface. Linear size of particles was $0.5\ \mu\text{m}$ with a form factor of 0.7. To improve the solubility, it was decided to conduct the study at a temperature of $60\ ^\circ\text{C}$. As can be seen from Fig. 8, there was observed a decrease in the linear sizes from 0.01 to $0.03\ \mu\text{m}$, redistribution of particles in the volume of solvent without the process of gel formation.

Thus, according to the results of previous studies, it can be concluded that the most uniform distribution of particles with a change in the linear dimensions and shape is observed in ethanol 96 %, water purified $60\ ^\circ\text{C}$, but the

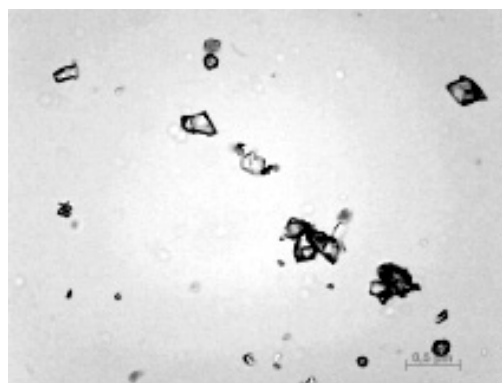


Fig. 6. Photomicrography of hyaluronic acid in ethanol

swelling process is better when added to the purified water of $20\ ^\circ\text{C}$, PEO-400, propylene glycol and glycerol.

For further determination of the rational conditions for the preparation of hyaluronic acid solution, studies were carried out in mixtures of purified water with glycerin, propylene glycol, ethanol and PEO-400 at a temperature of $20\ ^\circ\text{C}$ in a ratio of 10 : 1, taking into account the possible content of hydrophilic non-aqueous solvents in the composition of dosage form (Fig. 9).

As can be seen, the mixture of water with PEO-400 and propylene glycol significantly improved the solubility and wetting of hyaluronic acid (Fig. 9a, 9c). In the field of vision, the distribution of particles throughout the vo-

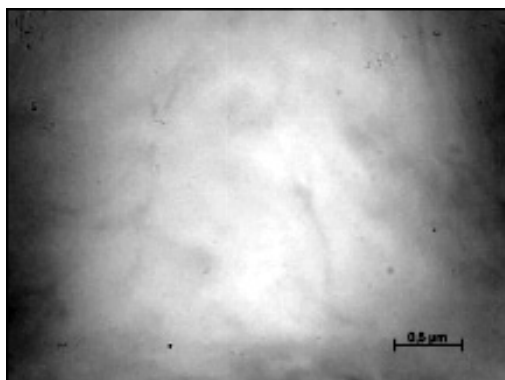


Fig. 7. Photomicrography of hyaluronic acid in water purified at $20\ ^\circ\text{C}$

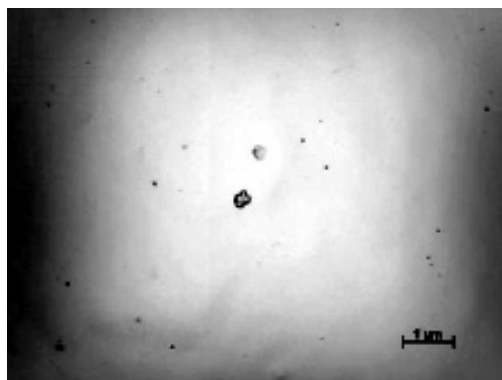


Fig. 8. Photomicrography of hyaluronic acid in purified water at $60\ ^\circ\text{C}$

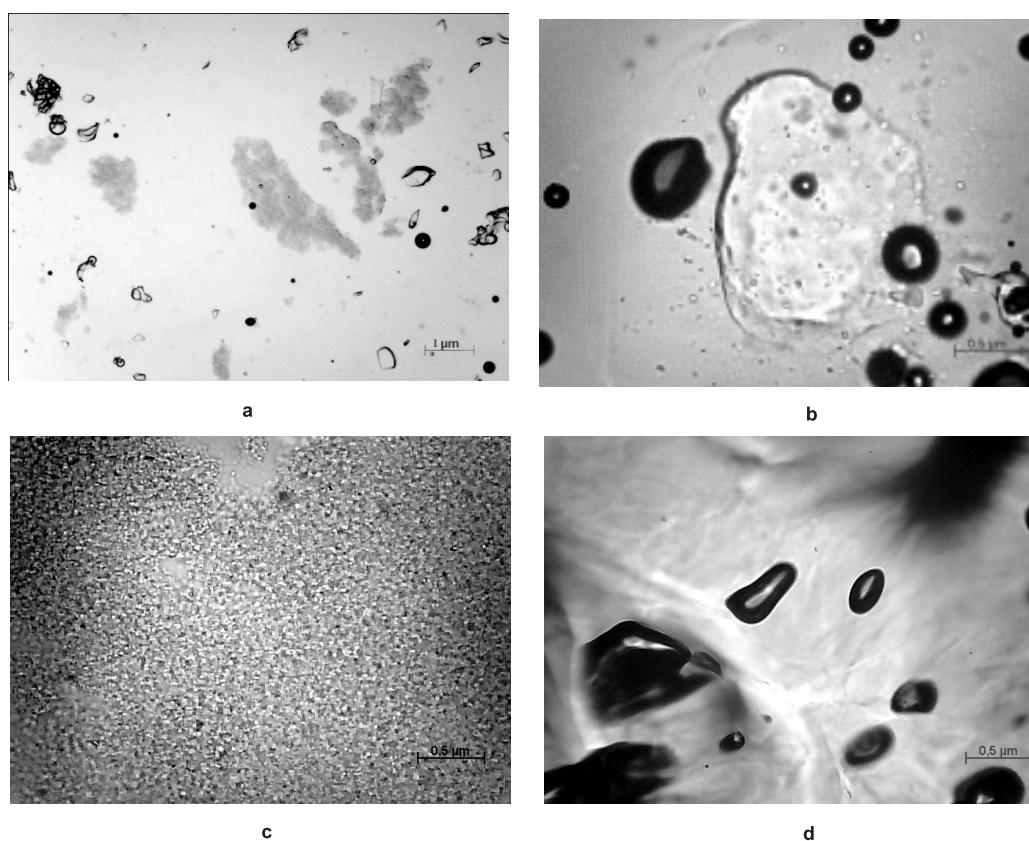


Fig. 9. Photomicrography of hyaluronic acid in a mixture of purified water and: a) PEO-400, b) glycerol, c) propylene glycol, d) ethanol

lume of solvent and the beginning of the swelling process was observed. Also noted was the disappearance of large amounts of air in the system of hyaluronic acid – solvent. The glycerin mixture did not contribute to increased solubility, but significantly increased wetting of hyaluronic acid particles (Fig. 9b). In the sample, there was the formation of air bubbles observed. The linear particle size fluctuated within 0.5 microns. In the ethanol-water mixture (1 : 1) there was observed a swelling process, but with the redistribution of particles in the volume of the solvent there was a change in its shape and linear dimensions without formation of a homogeneous system (Fig. 9d).

Thus, studies conducted have shown that the addition of the mixture of said solvents to hyaluronic acid does not result in the uniform distribution of particles throughout the medium, followed by swelling and complete dissolution. Therefore, at the next stage of the study, we were tasked with investigating the influence of surface-active substances on the indices of hyaluronic acid solubility. For the study, we had selected tween-80, which has a liquid consistency, does not require a temperature increase that will have a good effect on the conditions of swelling and solubility of the substance. For the study that was carried out immediately after preparation and after 5 minutes (Fig. 10), 3 % solution of tween-80 in water was used.

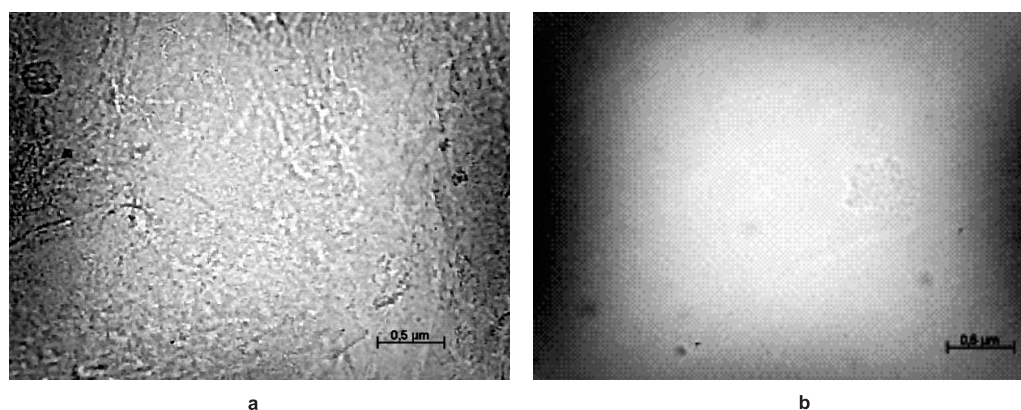


Fig. 10. Photomicrography of hyaluronic acid in a mixture of water with tween-80: a) immediately after the preparation of the solution, b) 5 minutes after the preparation of the solution

As can be seen from Fig. 10 with the addition of hyaluronic acid to a mixture of purified water and tween-80 in the field of view, a complete change in the appearance of solid particles was observed, with the formation of dispersion. Thus, at interaction of the solvent system with the hyaluronic acid, the formation of a dispersion with the simultaneous swelling process took place. After a period of time, a homogeneous system is formed, as evidenced by the data shown in Fig. 10b.

CONCLUSIONS

1. According to the results of microscopic studies, it was found that mixture of hyaluronic acid and purified water has the best indicator of solubility. The obtained results allow to concluding of unlimited swelling of the hyaluronic acid in an aqueous medium at a temperature of 20 °C with the formation of a homogeneous system over a certain period of time.
2. The use of such solvents as PEO-400 and propylene glycol, leads to moderate wetting and redistribution

of hyaluronic acid particles in solvent volume, which may contribute to solubility in this medium.

3. The addition of glycerin contributes to reducing the linear size and shape of the powder, its wetting, but unsatisfactorily affects the dissolution process. The use of peach oil and ethyl alcohol is not feasible when creating dosage forms of hyaluronic acid due to practical insolubility and poor wetting. The results of determining the solubility of hyaluronic acid in mixtures of water purified with hydrophilic non-aqueous solvents indicate an uneven distribution of its particles throughout the volume.
4. The addition of tween-80 to purified water significantly increases the dissolution rate and promotes the formation of a homogeneous system: hyaluronic acid - water purified.
5. It has been found that hyaluronic acid should be administered to the composition of the dosage form as a solution in purified water and tween-80.

Conflict of Interests: authors have no conflict of interests to declare.

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