RESEARCH ON DEVELOPMENT OF GEL PATCH CONTENT FOR CORRECTION OF SKIN AGE-RELATED CHANGES

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Abstract

Patches are thin disks on fabric or hydrogel base used as express masks for face skin care. Active agents having anti-aging properties are peptides. One of peptides, especially Acetyl Hexapeptide-8 (argireline) has myorelaxation properties, therefore its incorporation to anti-aging patch composition is vital. The objective of the development is the creation of a safe and easy-to-use preparation to fight against mimic wrinkles in a form of patches with argireline.

Subjects of the research are experimental samples of hydrogel patches with argireline.

For the identification of an active agent in patch composition a standard Pharmacopoeia determined reaction with ninhydrin for amino acid derivatives was used. Quantitative analysis was conducted using the same reaction with ninhydrin by the absorption spectrophotometry method in visible region.

For the hydrogel base samples the organoleptic, physical and chemical as well as structural and mechanical properties research has been conducted. Organoleptic properties researches have shown that the experimental samples have homogeneous surface, are colorless and clear, the odour is peculiar and faint. The samples have positive results of colloidal and thermal stability research; pH value is within acceptable limits (5,0-9,0). The results of structural and mechanical properties research show the availability of acceptable thixotropic properties, strength; prove that the system has a plastic type of flow.

Based on preformulation studies an optimal composition of patches with argireline was developed as well as the patches quality factors research was conducted. External appearance, color, odour, colloidal and thermal stability, pH value were determined in accordance with the requirements of Standard of Organization of Ukraine (SOU/COY) 24.5-37-103:2004 «Cosmetic gels. Common technical specifications». External appearance – a homogeneous gel-like mass free from foreign impurities; colorless, clear; odour – faint, peculiar; pH 6,3±0,1. Colloidal and thermal stability specifications are met. It has been proven that active agent addition to the patch composition does not have an effect on organoleptic and physical and chemical properties of the research samples.

Qualitative analysis was conducted using the Pharmacopoeia determined reaction of ninhydrin solution to amino acids. Its positive effect determined its use for the quantitative analysis by the absorption spectrophotometry method in visible region. It has been determined that active agent release within 30 minutes is more than 80%, and inessential changes in release values are observed from 30 to 60 minutes.

Keywords: anti-aging therapy, hydrogel patches, peptides, argireline, Acetyl Hexapeptide-8

Introduction

Under the existing circumstances anti-aging products, notably beauty products against mimic wrinkles, are used starting from 25-27 years old already, and there is an increasing tendency of their use at a young age. It is conditioned by modern way of life (decreased physical activity, unhealthy diet, etc.), ecology, decorative cosmetics of insufficient quality, intensive ultraviolet irradiation from the sun and other factors affecting state of health and skin youth [8]. The most popular products are in the form of cream, gel, serum and also patches, which were chosen by us for our study. Patches are thin disks on fabric or hydrogel base used as express masks for face skin care. Active agents having anti-aging properties are peptides. Decorinyl regulates collagen synthesis, therefore decrease in wrinkle depth is observed. Palmitoyl Tetrapeptide-7 (Пальміто леїл-тетрапептид-7) enhances protective functions of epidermis, decreases negative effect of the environment and ultraviolet rays, extends cell lifetime, slows down wrinkle formation. Matrixyl stimulates elastin, collagen and glycosaminoglycan synthesis. Acetyl Hexapeptide-8 (argireline) has a myorelaxation effect. Palmitoyl Hexapeptide-12 (Пальміто леїл-гексапепид-12) stimulates the synthesis of collagen and other basic proteins of extracellular matrix [9]. In light of this, incorporation of argireline to patch composition to fight against mimic wrinkles is vital. It is a synthetic low-molecular-weight peptide formed by a short chain of natural amino acids. Argireline is a safe substitution for a botulinum toxin, it does not have a neuroparalytic effect, does not provoke allergic reactions and does not require necessarily injection method of introduction [10].

The aim of the research is the development of content and technology of therapeutic and cosmetic preparation in a form of patches with argireline as well as the development of technique for active agent identification and quantitative analysis.

Methods

Subject of the research is the experimental samples of hydrogel patches with argireline.

The research of quality factors was conducted in accordance with the requirements of Standard of Organization of Ukraine (SOU/СОУ) 24.5-37-103:2004 «Cosmetic gels. Common technical specifications» [1]. Organoleptic properties: external appearance and color were evaluated by visual examination of a specimen placed in a thin even layer on a glass slide or a piece of white paper; homogeneity was evaluated by touch in a way of slight rubbing of a specimen; odour was evaluated by an organoleptic method in a specimen after external appearance evaluation [2].

Physical and chemical properties: in order to confirm colloidal stability two test tubes were filled to 2/3 of their capacity immediately after gel base preparation and weighed (mass difference between filled test tubes should not exceed 0,2 g), kept in a thermostat for 20 min at 22-25 °C. After the samples structurization they were centrifuged for 5 min with a revolution rate 100 s^{-1}. The samples are considered to be stable, if there is not more than a drop of water released in a test tube after 5 min of centrifugation.

Immediately after base preparation, in order to evaluate thermal stability, three 25 cm^3 cylinders were filled to 2/3 of their capacity with gel, plugged with stoppers and placed into a thermostat at 40-42 °C. The samples were kept in a thermostat for 24 hours, and then their stability was evaluated by the absence of aqueous phase release [4].

pH measurements were conducted according to the following technique. 10,0 g of the research sample was supplemented by 90 cm^3 of distilled water, stirred. An aqueous phase was separated by the decantation method and transferred to a 100 cm^3 glass, where electrodes were then placed. And after the instrument readings got stable, pH value readings were taken according to the instrument scale [3].

Structural and mechanical properties measurement: the research was conducted using Rheolab QC rotational rheometer (Anton Paar, Austria) with coaxial cylinders CC27/S-SN29766. Immediately after the gel preparation a batch of the sample of around 20,0 (± 0,5) g was placed into a tank of an outer cylinder. After the samples structurization the examination was conducted at 25 °C. The values of shear stress, shear rate and viscosity were registered automatically by the instrument [5].

Argireline quantitative analysis in patches was conducted according to the technique as follows.

Precise batch of patches (2,5 g) in a beaker was supplemented by 50,0 ml of purified water and left for 30 min at room temperature. Then supernatant liquid was transferred quantitatively into a 100,0 ml graduated flask and made up to volume with purified water. 10,0 ml of the resulting solution was placed into a 25,0 ml graduated flask and supplemented by 11 ml of 0,2% ninhydrin alcohol solution. The mixture was heated in water bath for 10 min at 100°C; then it was cooled off and made up to volume with purified water. Technique approbation was conducted on 6 series of the research samples.

At the same time the solution with 0,100 g of argireline sample (SigmaAldrich or other) was being prepared. For this purpose 0,100 g of argireline sample (SigmaAldrich or other) was placed to a 100,0 ml graduated flask and dissolved in water making up to volume with purified water. Then 10,0 ml of the resulting solution was placed into a 25,0 ml graduated flask and supplemented by 11 ml of 0,2% ninhydrin alcohol solution. The mixture was heated in water bath for 10 min at 100°C; then it was cooled off and made up to volume with purified water.

Compensation solution: 1,1 ml of 0,2% ninhydrin alcohol solution was mixed with 1,0 ml of water and heated under the same conditions as the research samples. The solution was cooled off, placed into a 25,0 ml graduated flask and made up to volume with purified water.

Absorption spectra of the research solutions as well as optical density of analytical solutions were registered with the help of Evolution 60s spectrophotometer. The measurements of optical density were conducted using quartz glass cuvettes with a 10 mm layer thickness comparing a standard sample solution with a compensation solution [6,7].

Quantitative content of argireline in patches was defined using a formula:

$$X, g = \frac{A \cdot m_{b_{SS}} \cdot m_{av.} \cdot \%_{SS}}{A_{st} \cdot m_b \cdot 100},$$

where:

A – optical density of the research sample solution;

A_{st} – optical density of argireline standard sample (SS) solution;

$m_{av.}$ – average weight of patches, g;

$m_{b_{SS}}$ – batch weight of argireline SS;

m_b – patch batch weight taken for analysis;

$\%_{SS}$ – percentage of active agent in argireline SS.

Results

When making a selection of auxiliary substances, we have taken into consideration that they should be chemically and pharmacologically indifferent, should provide expected consumer properties, component safety, process temperature (the temperature should not exceed 40°C, since peptide substance is present) and economic feasibility. For the experimental samples preparation sodium alginate, starch, polyvinylpyrolidone, gelatin, guar gum were used as elastomers (**Tab. 1**).

One out of six model samples was chosen for further researches by means of comparison of their consumer properties by such major characteristics as strength, adhesion, lustre, smoothness, elasticity, tactile sensations. Based on a poll of twenty respondents (women from 25 to 40 years old), it has been determined that an optimal composition of a base is a sample No.6, which has the best consumer properties (strong enough and elastic, well adheres to skin, has excellent adhesive properties, pleasantly tactile, has smooth, lustrous surface).

Organoleptic as well as physical and chemical properties researches were performed on the series of three research samples of composition No.6. Organoleptic properties of the research samples are acceptable. The results of hydrogen index measurement have shown that pH value is within acceptable limits (5,0-9,0). Colloidal and thermal stability specifications are met (**Tab. 2**).

Based on the findings of structural and mechanical properties investigations conducted the graphs were built showing the dependence of shear stress (τ) on shear rate (γ) and viscosity (η) on shear rate (γ) (**Fig. 1**).

Occurrence of hysteresis loops denotes the availability of acceptable thixotropic properties. Shear stress value, that is necessary for system breakdown, is indicative of the strength of a structured gel. The graph shows that structural viscosity of the research samples gradually decreases as far as the shear rate increases. Such kind of dependence is descriptive of the systems with a plastic type of flow and proves that the research samples are structured disperse systems.

Based on preformulation studies, the following composition of therapeutic and cosmetic

preparation in a form of patches was developed: argireline – 5%, sodium alginate – 1%, glycerin – 5%, guar gum – 0,5%, polyvinylpyrolidone – 1%, preservative (Euxyl PE 9010) – 0,5%, aroma compound (J'Adore) – 0,2%, purified water – q.s. to 100. The research of quality factors (external appearance, color, odour, colloidal and thermal stability, pH value) was conducted in accordance with the requirements of Standard of Organization of Ukraine (SOU/COУ) 24.5-37-103:2004 «Cosmetic gels. Common technical specifications». External appearance – a homogeneous gel-like mass free from foreign impurities; colorless, clear; odour – faint, peculiar; pH 6,3±0,1. Meets colloidal and thermal stability specifications. It has been proven that active agent addition to the patch composition does not have an effect on organoleptic and physical and chemical properties of the research samples.

In terms of chemical structure argireline is an amino acids derivative, so the Pharmacopoeia determined reaction of ninhydrin solution to amino acids was used for qualitative analysis. Positive effect of the reaction determined its use for the quantitative analysis of an active agent in patches by the absorption spectrophotometry method in visible region.

For the purpose of development of the present technique 0,1 % argireline aqueous solution was made and reaction with 0,2% ninhydrin alcohol solution was performed. The pattern of absorption spectrum of colored solution absorption has been studied in 400 nm to 700 nm region. The maximum of colored argireline solution with ninhydrin is observed at 571 nm wave length (**Fig. 2**).

For further use of the reaction for a quantitative analysis the reaction time, temperature setting, solution stability, active agent to ninhydrin ratio and the obedience of a colored reaction product to Bouguer-Lambert-Beer law were determined. It has been determined that the reaction goes in boiling water bath for 10 min with active agent to ninhydrin molecular ratio (0,000011 mole : 0,00012 mole). Solution stability under these conditions is one hour, and Bouguer-Lambert-Beer law obedience is observed within argireline concentration range of 0,02 % to 0,05 % (**Fig. 3**).

The present technique was transferred for the determination of time of active agent release from patches. The monitoring had been conducted every 5 minutes (**Fig. 4**).

The results of calculations have shown that active agent release within 30 minutes is more than 80%, and inessential changes in release values are observed from 30 to 60 minutes.

Discussion

Optimal composition of hydrogel base has been developed and substantiated. Organoleptic, physical and chemical as well structural and mechanical properties of the research samples have been investigated. The composition of hydrogel patches with argireline has been developed and substantiated. The identification and quantitative analysis techniques for the developed therapeutic and cosmetic preparation have been elaborated and taken as a basis for active agent release profile determination; it has been determined that active agent release for 30 minutes is more than 80%. Suggested techniques can be used for stability investigation, shelf life determination as well as can be included to the developed preparation quality control techniques in the future.

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Table 1. Elastomer combinations in the research samples

Elastomer	Concentration, %					
	№1	№2	№3	№4	№5	№6
Sodium alginate	1,0	-	0,5	1,0	-	1,0
Polyvinylpyrolidone	1,0	1,0	1,0	-	-	1,0
Starch	-	-	5,0	5,0	5,0	-
Gelatin	-	10,0	-	-	10,0	-
Guar gum	-	-	-	-	-	0,5

Table 2. Organoleptic and physical and chemical properties of the experimental samples

№	Organoleptic properties			Physical and chemical properties	
	External appearance	Color	Odour	pH	Colloidal and thermal stability
1	homogeneous, lustrous surface	colorless, clear	faint, peculiar	6.9±0,1	stable
2	homogeneous, lustrous surface	colorless, clear	faint, peculiar	6.2±0,1	stable
3	homogeneous, lustrous surface	colorless, clear	faint, peculiar	6.1±0,1	stable

Figure 1. The graph of dependency of shear stress on shear rate and viscosity on shear rate
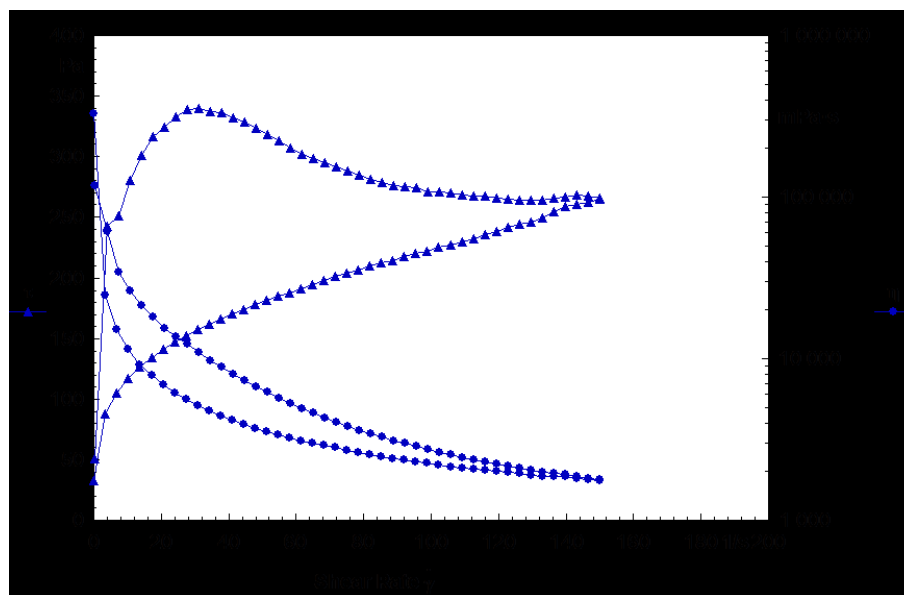
Figure 2. Absorption spectrum of 0,04% argireline aqueous solution absorption after the reaction with ninhydrin
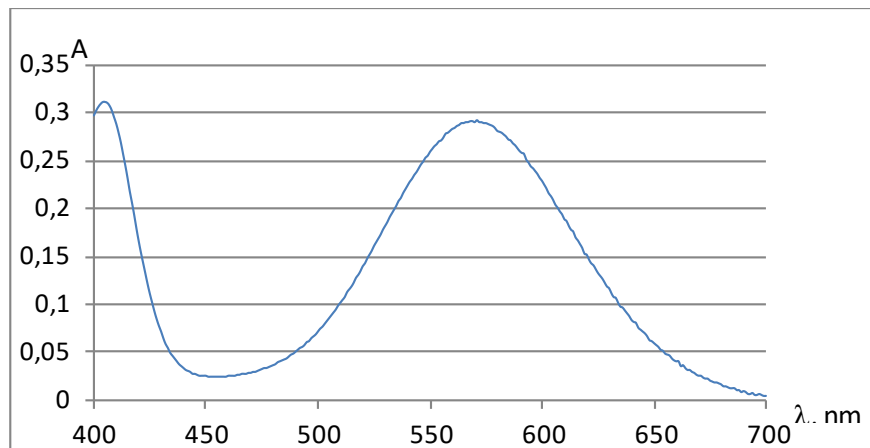
Figure 3. The graph of dependency of optical density on argireline concentration in ninhydrin reaction product at 571 nm wave length
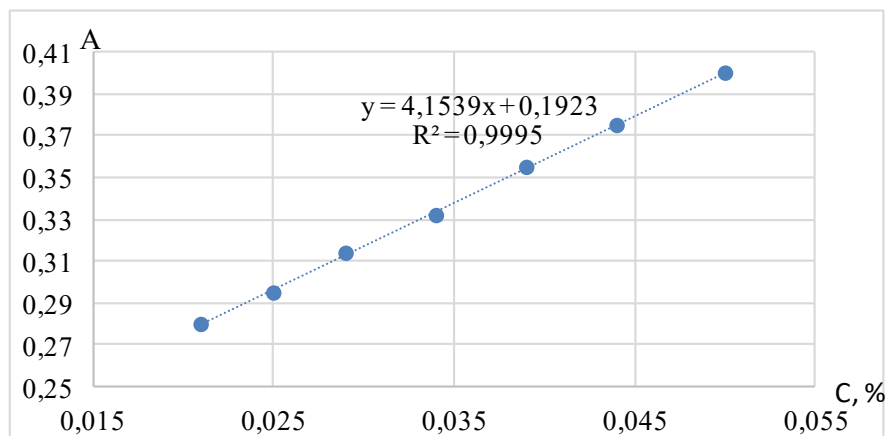

$$y = 4{,}1539x + 0{,}1923$$
$$R^2 = 0{,}9995$$

Figure 3. Active agent release from patches within one hour