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# Justification of Composition of the Cream with Sapropel Extract

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## Abstract

**Aim:** The aim of the study was to work out the composition of cream with extract of sapropel from Prybych deposits, Volyn region, Ukraine, and to determine its organoleptic, physicochemical indices, structural and mechanical (rheological) parameters, colloidal and thermal stability, as well as to justify the choice of effective preservatives. **Materials and Methods:** The following materials were employed: Extract of sapropel from Prybych deposits; emulsion base, containing corn oil, emulsifier №1, cetylstearyl alcohol, and purified water. To carry out the research a set of methods (centrifugal, thermal, and potentiometric), to analyze colloidal and thermal stability, and to determine pH values of the tested samples were used. Rheological properties of the samples were determined on the rotating viscometer. **Results:** There was determined the influence of the extract on physicochemical and rheological properties of the emulsion base; all the experimental samples remain thermally and colloidally stable and have satisfactory organoleptic properties; incorporation of SE into an emulsion base in a concentration of up to 20% retains structural and mechanical properties of the base. **Conclusion:** As the results of the carried out experimental investigations, the effect of the SE on physicochemical and rheological properties of its emulsion base. Microbiological research showed that the maximum reduction of the number of viable cells of microorganisms was observed applying as preservatives euxyl K 100 (0.1%) and nisin (0.01%).

**Key words:** Effectiveness of antimicrobial preservatives, emulsion base, extract of sapropel, rheological properties

## INTRODUCTION

Among promising raw materials for preparation of medical, veterinary and cosmetic remedies are sapropel, which is a unique natural organic material, containing a large number of carboxylic, humic and amino acids, vitamins, micro- and macro-elements, and possessing a wide range of pharmacological effects, as well anti-inflammatory, reparative, antibacterial, and antioxidant.<sup>[1-4]</sup>

In scientific literature, the term “sapropel” has been used mostly in a generic sense for describing organic-rich fine-grained sediments deposited in stagnant water. In respect to physical and chemical properties, sapropels are diverse materials with different content of organic substances and mineral nutrient concentration.<sup>[5]</sup>

The current age of sapropel sediments in lakes does not exceed 12,000 years. The composition and properties of sapropel from different

locations vary in wide ranges, due to the productivity of the parent pond, the properties of the surface currents and climatic conditions, etc. An important role in the formation of bottom sediments plays the factor of the lake fluidity.

In Belorussia, Russia and Ukraine sapropel is already used as a raw material for cosmetic applications, balneology, livestock farming, agriculture, and other areas. Sapropel thermal capacity provides a long and deep tissue heating and normalizes blood pressure, assisting the treatment in joints, peripheral nervous system, skin, and inflammation of female genitals. The heated sapropel applications successfully treat phlegmons, mastitis,

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furunculosis, chronic gastritis, gastric ulcer, and duodenum diseases, because these applications stimulate the phagocytes activity, and leading to intensive tissue regeneration.<sup>[1]</sup>

Despite the undeniable prospects of applications and certain achievements in their investigation, today the products of sapropel processing are not widely used in pharmacy, medicine, including veterinary and cosmetology. In our point of view, it is caused by the chemical composition of sapropel, which depends on the location, conditions of its formation, the diversity of flora and fauna<sup>[3]</sup> and, as a consequence, the difficulties in standardization of the natural raw material.

A rich composition of sapropels allows to use them successfully in cosmetology. Sapropelic therapeutic muds exhibit the anti-inflammatory and anti-allergic effect, protect the skin from the damaging effects of free radicals, slow down the aging processes, providing for skin a freshness, firmness, and elasticity, also improve cellular regeneration, moisturize, and increase elasticity and thickness of the keratoid layer of the epidermis.<sup>[4,6]</sup> The uses of native products and individual components of sapropels for many years have shown their effectiveness, accessibility, convenience, and safety.

The aim of this research is to work out the composition of cream with SE and to determine its organoleptic, physicochemical indices, structural and mechanical parameters, colloidal and thermal stability, as well as justify the choice of effective preservatives to provide the accordance of the developed cream with the European Pharmacopoeia (EP) requirements, related to its microbiological purity.

## MATERIALS AND METHODS

In accordance with investigated organoleptic and consumer properties, thermal and colloidal stability, pH values, structural and mechanical characters of prototype samples, for further experiments concerning development of composition of a cream with sapropel extract was selected the emulsifying base containing 15% of corn oil, 6% of emulsifier No. 1 (Lanette SX), 1% of cetostearyl alcohol and purified water.<sup>[7]</sup> The following materials were employed: Extract of sapropel from Prybych deposits, located in Volyn region; emulsion base, containing 15% corn oil, 6% emulsifier №1 (Lanette SX, mixture of cetearyl alcohol, sodium lauryl sulfate, and sodium cetearyl sulfate), 1% cetyl stearyl alcohol, and purified water to 100 g.

In development process in terms of the SE cream composition, there were considered basic principles and requirements of the following applicable regulatory items: EP 8.0., 2013 (monographs 04/2010:0132; 5.1.3; 5.1.4)<sup>[8]</sup> and the State Standard of Ukraine No. 4765:2007 “Cosmetic creams. General technical conditions”, 2008.<sup>[9]</sup>

The organoleptic characteristics of the samples of creams were investigated for their appearance, odor, color, consumer

properties, and possible development of features of physical instability (coalescence, flocculation, aggregation of particles, and coagulation).

Determination of acid-base balance (pH values) was conducted by the potentiometric method, applying pH meter pH-150 MI (“Measurement Techniques Ltd.,” Russian Federation) in 10% aqueous solution of the cream in accordance with the EP 8.0., 2013.<sup>[8]</sup>

Determining the colloidal stability was used the laboratory centrifuge MPW-210 (“Mechanika precyzyjna,” Poland). Test tubes were filled with 2/3 volume (approximately 9 g) by investigated samples, weighted with an accuracy of 0.01 g and centrifuged for 5 min at speed 5000 rpm; the relative strength of the centrifuge was around 5000 g.<sup>[9]</sup>

To determine the thermal stability, there were taken 6 glass tubes with a diameter of 15 mm and a height of 150 mm, which were filled with 2/3 the volume of the studied samples and placed in the thermostat TC-80M-2 at 40–42°C for 24 h. The bases were considered as stable, if the formation of the water phase was not observed in any glass tubes.<sup>[9]</sup>

Determination of the structural and mechanical (rheological) parameters, according to applicable regulations of the EP 8.0., 2013 (monograph 2.2.10), was conducted on the rotating viscometer Brookfield HB DV-II PRO (the USA), applying the rotating type adapter with coaxial cylinders in the range of shear rate from 3.0 to 93.0 s<sup>-1</sup> (spindle SC4-21 for 8.3 g camera). For determining its structural viscosity, the mass of the sample was placed in a tunk and immersed in the liquid spindle, which turned and fixed the value of viscosity ( $\eta$ ), shear stress ( $\tau$ ), and shear rate (Dr) that automatically were presented on the display of the device. Determination of structural and mechanical properties of the developed drug was carried out at temperature 13°C, 20°C, and 34°C. Required temperature levels were provided using the ultra-thermostat, which is included in the package of viscometer.<sup>[10,11]</sup>

To study the effectiveness of preservatives in the cream’s experimental samples with sapropel extract in conditions *in vitro*, as test strains were used American Type Culture Collection (ATCC) reference strains: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404. In accordance with the recommendations of the EP 8.0., 2013,<sup>[8]</sup> the test-strains of bacteria and fungi were grown individually on the appropriate nutrient media: *S. aureus*, *Pseudomonas aeruginosa*, and *B. subtilis* on the casein soya bean digest agar (at 30–35°C for 18–24 h); The test strains of fungi on plate of Sabouraud Dextrose Agar at 20–25°C (*Candida albicans* was incubated for 48 h, *A. brasiliensis* - for 5 days). The purity of each microorganism culture was confirmed by their typical morphological, tinctorial, cultivation, and biochemical properties.

Each container of the examined sample was inoculated with suspension, which contained one of the test-strains, providing the inoculum of not  $<10^5$ – $10^6$  colony-forming units (CFU) in 1 ml. From each container were removed after inoculation the samples at appropriate intervals, indicated in the EP 8.0., 2013<sup>[8]</sup>: 2, 7, 14, and 28 days; the effectiveness of preservatives was evaluated by the  $\log_{10}$  reduction in the number of viable micro-organisms against the value, obtained for the inoculum during the subjected period after inoculation. In accordance with EP 8.0., 2013,<sup>[8]</sup> the presence of not  $> 100$  CFU of aerobic microorganisms (total aerobic microbial count, TYMC) and 10 CFU yeast and mold fungi (total combined yeasts/molds count, TYMC) is allowed in 1 g of the nonsterile product for cutaneous application; *P. aeruginosa* and *S. aureus* must be absent.

During our previous investigations, there was worked out the emulsion base, containing 15% corn oil, 6% emulsifier № 1 (Lanette SX), 1% cetyl stearyl alcohol, and purified water to 100 g. To study the influence of the extract and the pH value of the developed base were prepared 5, 10, 15, and 20% samples of SE. A liquid extract of sapropel was obtained from the substance with the content of organic matters varying from 40% to 97%. It was processed with 0.1 N alkali and applied the cavitation with the speed 3000 rev/min for 60 min at 50–60 °C to obtain a homogeneous suspension, then the extract was filtered, evaporated in a vacuum evaporator to a ratio of 1:2 and adjusted to pH 7.0 with the citric acid.

Test samples of creams with SE were prepared by the method of phase inversion. Emulsifiers of the oil were melted at 70–80°C. Water was heated (about 10%, according to the method of phase inversion) separately to the same temperature and added into the oil phase. To obtain the water-in-oil emulsion, the homogenizer Polytron PT 3100 D

(“Kinematica AG”, Switzerland) for 5 min at a speed of 3000 rev/min (average speed) was used. The rest portion of a water phase of the same temperature then was added at 55–65°C; a phase inversion occurred. Mixing was continued till the emulsion cooling to room temperature.

## RESULTS AND DISCUSSION

Investigations concerning the choice of appropriate constituents, including the basis and suitable excipients, for semi-solid preparations for cutaneous application are significant research items within the development of the composition of promising pharmaceuticals and cosmetics.<sup>[12-15]</sup>

Test samples of SE cream, yielding various content of sapropel in cream (0%, 5%, 10%, 15%, and 20%), were assessed for organoleptic properties, pH values, thermal indices, and colloidal stability. The dependence of the structural and mechanical properties of the cream from the extract concentration was also determined. The research outcomes are presented in Table 1.

The results of the carried out experiments show that an increase in the amount of sapropel extract in emulsion bases leads to a slight increase in pH value and a decrease of the viscosity of the cream. Test samples of different quantitative content of SE in the emulsion bases are thermally and colloidally stable, exhibiting satisfactory organoleptic properties.

The rheograms [Figure 1] demonstrate the ability of extract to dissolve the base in a small degree, but the cream base provides the appropriate structural and mechanical properties - plasticity and minor thixotropic characteristics. It allows using the developed base without extra correction of the composition under incorporation of SE in concentrations of up to 20%.

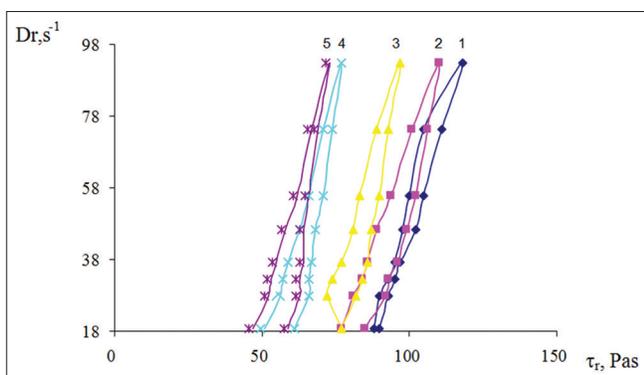
**Table 1:** Properties of experimental samples of emulsion bases,  $n=5$

The content of sapropel in cream, %	Organoleptic characteristics	Thermo-stability	Colloidal stability	pH value of 10% extract	Structural viscosity ( $\eta$ ) MPa·s, 20 rev/min
0	Homogeneous cream white, odorless, creamy consistency	Stable	Stable	6.5±0.2	6900±10
5	Homogeneous cream, cream-colored, odorless, creamy consistency	Stable	Stable	6.7±0.1	6810±10
10	Homogeneous cream, light brown, odorless, creamy consistency	Stable	Stable	6.9±0.2	6700±10
15	Homogeneous cream, brownish color, with a specific (soil) odor, creamy consistency	Stable	Stable	7.0±0.1	6540±10
20	Homogeneous cream, brown color with a specific (soil) odor, creamy consistency	Stable	Stable	7.1±0.2	6290±20

**Table 2:** The effectiveness of preservatives in the composition of experimental samples of creams with sapropel extract

№ of the sample	Samples of sapropels	Tested micro-organism	Control of tested culture	Log reduction				
				Initial concentration	2 days	7 days	14 days	28 days
1	Cream with extract of sapropel	<i>S. aureus</i>	$7.7 \times 10^5$	1.01	1.01	1.01	n/i	n/i
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	1.23	1.02	0.98	n/i	n/i
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	2.06	1.49	1.42	0.82	0.83
		<i>C. albicans</i>	$1.43 \times 10^5$	1.23	0.99	0.94	0.62	0.62
2	Cream with 0.01% nisin	<i>S. aureus</i>	$7.7 \times 10^5$	1.01	1.30	2.54	2.58	n/i
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	2.03	1.48	2.13	n/d	n/d
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	2.26	2.78	3.11	n/d	n/d
		<i>C. albicans</i>	$1.43 \times 10^5$	3.10	0.86	0.83	0.74	0.74
3	Cream with 0.01% nisin and 0.8% germaben	<i>S. aureus</i>	$7.7 \times 10^5$	2.01	2.31	3.10	n/d	n/d
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	2.13	2.48	n/d	n/d	n/d
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	1.36	2.01	2.34	2.58	n/d
		<i>C. albicans</i>	$1.43 \times 10^5$	1.22	2.12	2.82	n/d	n/d
4	Cream with 0.8% germaben	<i>S. aureus</i>	$7.7 \times 10^5$	2.00	2.28	3.02	n/d	n/d
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	2.11	2.40	3.28	n/d	n/d
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	1.21	2.00	2.29	2.25	n/d
		<i>C. albicans</i>	$1.43 \times 10^5$	1.18	2.03	2.61	n/d	n/d
5	Cream with 0.01% nisin, 0.1% euxyl K100	<i>S. aureus</i>	$7.7 \times 10^5$	2.01	2.35	3.32	n/i	n/d
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	1.1	2.48	n/d	n/d	n/d
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	1.58	2.17	2.31	2.72	n/d
		<i>C. albicans</i>	$1.43 \times 10^5$	1.53	2.12	2.68	n/d	n/d
6	Cream with 0.1% euxyl K100	<i>S. aureus</i>	$7.7 \times 10^5$	2.01	2.31	3.16	n/i	n/d
		<i>P. aeruginosa</i>	$7.2 \times 10^5$	1.1	2.31	n/d	n/d	n/d
		<i>A. brasiliensis</i>	$2.80 \times 10^6$	1.58	2.17	2.35	2.46	n/d
		<i>C. albicans</i>	$1.43 \times 10^5$	1.53	2.07	2.72	n/d	n/d

*A. brasiliensis*: *Aspergillus brasiliensis*, *S. aureus*: *Staphylococcus aureus*, *C. albicans*: *Candida albicans*, ND-viable cells of the test organisms were not detected, NI - the number of viable cells of microorganisms did not increase



**Figure 1:** Rheogram of the dependence of shear stress ( $\tau_r$ ) on shear rate ( $Dr$ ) of the cream test samples at 20°C, where 1 - emulsion base; 2 - the sample of the 5% SE cream; 3 - the sample of the 15% SE cream; 4 - the sample of the 10% SE cream; 5 - the sample of the 20% SE cream

The next stage of the investigation was to study the choice of the preservative for cream prototypes in accordance with the requirements of the EP 8.0., 2013.<sup>[8]</sup> The results of studies, carried out by other research groups,<sup>[12,16,17]</sup> caused the choice of the antimicrobial preservatives as possible ingredients of the composition of the cream with sapropel extract in the current investigation.

To select an effective preservative for the SE, we have produced the samples with the following preservatives: The sample № 2 contained nisin in a concentration of 0.01%; the sample № 3 - nisin 0.01% and germaben II International Nomenclature of Cosmetic Ingredients (INCI): Propylene glycol, diazolidinyl urea, methylparaben, and propylparaben - 0,8%; the sample № 3 - germaben 0.8%, the sample № 4 - nisin 0.01% and euxyl K 100 (INCI: Benzyl alcohol, methylchloroisothiazolinone, and

methylisothiazolinone) - 0.1%; and the sample №5 - euxyl K 100 0.1%. The sample № 1 did not contain a preservative. Results of the carried out investigations are shown in Table 2.

Therefore, experimentally was demonstrated that the sample № 1, which contained no antimicrobial preservatives, had not an antimicrobial action. This was evidenced by an increase of the number of viable cells (fungi of the genus *Candida*) and its maintaining on a constant level (*S. aureus*, *P. aeruginosa*, and *Aspergillus brasiliensis*) for a 28-day-research.

The inclusion of a preservative nisin into the sample composition provides the effective antimicrobial action against the bacteria *S. aureus*, but at the same time, it does not provide the protection of the samples from the fungi of the genus *Candida* and *Aspergillus* and possesses a low efficiency against *P. aeruginosa* (the sample № 2).

A comparative study of the antimicrobial action of the combinations of nisin and germaben, nisin and euxyl K 100 showed almost the lack of difference in the manifestation of antimicrobial effect (the examples № 3 and № 5). The combination of these preservatives provides a synergistic effect of antimicrobial action against the test strains of bacteria (*S. aureus* and *P. aeruginosa*) and protection from the fungi of the genera *Candida* and *Aspergillus*.

It should be noted that the use of germaben and euxyl K 100 also provides the effective antimicrobial effect on experimental strains of microorganisms. Despite the fact, that for these samples, the log reduction of the number of bacteria was slightly lower, compared with a combination of preservatives, they meet criteria of the EP 8.0., 2013.<sup>[8]</sup>

Conducted microbiological research shows that samples of the SE cream, containing germaben, euxyl K 100 and their combination with nisin, meet the criterion "A", in accordance with the EP requirements (5.1.3) for medicinal products, applied externally. The analysis of the *log reduction* of the number of viable cells of micro-organisms showed that maximum reduction was observed applying in the SE cream composition as preservatives euxyl K 100 and nisin (the sample №4).

## CONCLUSION

The uses of native products and individual components of sapropels for many years have shown their effectiveness, accessibility, convenience, and safety. Sapropelic therapeutic muds exhibit the anti-inflammatory and anti-allergic effect, protect the skin from the damaging effects of free radicals, slow down the aging processes, providing the skin freshness, firmness, and elasticity.

As the results of the carried out experimental investigations, the effect of the SE on physicochemical and rheological properties of its emulsion base, consisting of 15% corn oil,

6% emulsifier №1, and 1% cetyl stearyl alcohol and purified water to 100 g, has been determined. The organoleptic characteristics of the samples of creams were investigated for their appearance, odor, color, consumer properties, and possible development of features of physical instability.

According to the outcomes of the investigation on organoleptic and physicochemical parameters, it has been established that increasing the concentration of SE in the emulsion basis results in an increase of pH value and a decrease in the viscosity of the cream; all the experimental samples remain thermally and colloiddally stable and have satisfactory organoleptic properties.

As a result of the studied rheological properties of emulsion bases with sapropel extract, it was established that the incorporation of sapropel extract into an emulsion base in a concentration of up to 20% retains structural and mechanical properties of the base and, therefore, does not require any further correction of the composition.

The performed microbiological research showed the effectiveness of incorporation into SE cream composition as preservatives 0.1% euxyl K 100, 0.8% germaben, and their mixtures with 0.01% nisin; maximum reduction of the number of viable cells of micro-organisms was observed applying as preservatives euxyl K 100 (0.1%) and nisin (0.01%).

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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