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Short Communication

STUDY OF EMULSION PRODUCTS STABILIZED WITH SURFACTANTS BASED ON RHAMNOLIPIDS *PSEUDOMONAS* SP. PS-17

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ABSTRACT

Objective: To study the effectiveness of the biocomplex of surfactants based on rhamnolipids *Pseudomonas* sp. PS-17 (biocomplex PS) as an emulsifier in emulsions for use in dermatology.

Methods: Technological methods of o/w emulsions manufacturing, ultrasonic dispersion, as well as microscopic studies of manufactured emulsions have been used.

Results: To select the concentration of biocomplex PS as an emulsifier, studies of the emulsifying properties of bio complex PS in comparison with polysorbate 80 have been performed, taking into account the similarity of the hydrophilic-lipophilic balance of these substances. Several compositions of emulsions have been studied in which the biocomplex PS has been used as an independent emulsifier in a concentration from 4 to 10%, as well as in combination with other emulsifiers-lanolin and glycerol monostearate.

Conclusion: The creation of stable o/w emulsions requires the use of high concentrations of biocomplex PS as an emulsifier, more than 10%, which is impractical and economically unreasonable. The use of biocomplex PS as a co-emulsifier with emulsifiers of the second type allows obtaining stable emulsions at a total concentration of complex emulsifier 7-10%.

Keywords: Biocomplex PS, Rhamnolipids, Emulsions, Emulsifiers, Biosurfactants

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A significant number of dermatological medicines for dermal use are emulsion forms, which make up about 90% of all cosmetic creams and are widely used in compositions of topical semi-solid preparations [1-3].

Emulsions belong to microheterogeneous systems, the stability of which is of great importance because it affects the accuracy of dosing of the active substance [4]. The stability of emulsions depends on many factors, primarily on the type of used stabilizers. Among the emulsion stabilizers, surfactants deserve the most attention due to their multifunctionality. Preference is given to surfactants that are insensitive to pH, safe for humans, and biodegradable. Surfactants of microbiological origin meet these requirements [5].

Biosurfactants can form foams, emulsions, microemulsions regulate the stability of dispersed systems with liquid dispersion medium (aqueous and organic). Such physicochemical properties of these substances allow stabilizing the functional properties of various products. Biosurfactants increase the solubility of sparingly soluble (insoluble) medicinal substances in water; increase the stability of heterogeneous systems; extend the shelf life of medicines. The structure of their molecules can explain the contribution of biosurfactants to the improvement of various drugs: dipole molecules are oriented in a certain way on the phase interface and allow other substances to be adsorbed on them or to interact with them [6]. The presence of surfactants in the composition of drugs affects the bioavailability and efficacy of drugs.

Biosurfactants produced by *B. subtilis*, *Pseudomonas* spp., *Streptomyces spp.* are currently commonly studied [7, 8]. Their advantage over synthetic surfactants is that they are biodegradable, have low toxicity, and are used in low concentrations [6].

This study evaluates the effectiveness of the bio complex of surfactants based on rhamnolipids *Pseudomonas* sp. PS-17 (biocomplex PS) as an emulsifier in emulsions for use in dermatology.

The bio complex PS is a biogenic surfactant complex synthesized by bacteria of the genus *Pseudomonas*, which is a viscous mass consisting of rhamnolipids, alginate, and water [9]. The substance was developed in the Department of Physical Chemistry of Combustible Minerals L. M. Lytvynenko Institute of Physico-Organic Chemistry and Coal Chemistry, NAS of Ukraine.

The biocomplex PS has been used as an independent emulsifier in o/w emulsions, as well as as a co-emulsifier in combination with emulsifiers of the second type-lanolin and glycerol monostearate. Grape seed oil has been used as the oil phase of emulsions.

Emulsions of the o/w type with 30% of oil phase have been made in the laboratory conditions using a laboratory homogenizer at a stirring speed of 2000 rpm, the homogenization time was 15 min.

Before the microscopic examination, the emulsions have been mixed and sonicated for 5 min using an ultrasonicator UZDN-A650T (Akademprylad, Ukraine).

The Olympus BX51 microscope (Olympus, Japan) has been used to study emulsions. Emulsion samples have been examined using a 40x, 0.75 NA high-aperture lens and a LUMC-B11/Sony camera (Labtron, WB). The size of the dispersed particles has been determined using the software complex ImageJ, (NIH, USA); statistical analysis of the particle size distribution in the emulsions was calculated by ANOVA in the software complex GraphPad Prizm (GraphPad Software, USA).

During the development of drugs or cosmetics in the form of emulsions, an important step is the choice of excipients that would allow obtaining high-quality and safe products. Such key excipients that require special attention are emulsifiers, which are preferably surface-active whose molecules have a diphilic structure. They form a boundary layer on the surface of immiscible liquids, which reduces the surface tension, and thus increases the possibility of emulsion formation [10].

The development of emulsions, in particular the choice of the concentration of emulsifiers, is mainly carried out based on experimental studies, which are quite expensive and require a long time to conduct research.

Hydrophilic-lipophilic balance (HLB) of surfactants is crucial for the stabilization of emulsions and the optimal choice of emulsifier. If a mixture of surfactants is used for stabilization, the number of HLB is equal to their arithmetic value. The stability of emulsions also depends on such factors as the nature of the dispersion medium and the dispersed phase, the magnitude of the surface tension, and the viscosity of the system [11, 12].

Most emulsifiers, which are surfactants, can cause side effects, including allergic reactions [13]. Therefore, promising, in this case, is the study of new excipients that would be safe for humans.

The Department of Drug Technology and Biopharmaceutics of Danylo Halytsky Lviv National Medical University is conducting studies of the biocomplex PS as a new auxiliary component, in particular as a preservative and emulsifier.

The biocomplex PS is a biosurfactant synthesized by Pseudomonas sp. PS-17. The composition of the biocomplex PS includes mono-and dirhamnolipids, alginate, and water. The advantages of this form of the product are its concentrated form, the possibility of long-term storage, ease of transportation, and use. This biosurfactant has low values of surface (27.9-29.8 mN/m) and interfacial tension (0.01-0.5 mN/m), high emulsifying and suspending activity (emulsification index-65-85%) [9].

The biocomplex PS can also be used to produce rhamnolipids by extraction, which are highly effective surfactants and can be used in medicine and cosmetics as independent components. According to the literature, the HLB of monorhamnolipid is 13, and dirhamnolipid-21 [6]. This indicates that they can form stable emulsions of the o/w type, thus can be used as an emulsifier.

At the first stage of our study, an empirical approach to the choice of biocomplex PS concentration has been applied, in particular, several compositions of o/w emulsions with 30% of oil phase have been investigated, in which biocomplex PS has been used as an independent emulsifier, and emulsions in which biocomplex PS has been used as a co-emulsifier in combination with emulsifiers of the II type-lanolin and glycerol monostearate.

Since the studied emulsifier is similar to polysorbates in HLB indicator, for selection of the concentration of biocomplex PS needed to obtain stable emulsions, studies of the emulsifying properties of biocomplex PS in comparison with polysorbate-80 have been performed. Nonionic surfactants, especially polysorbate-20 and polysorbate-80, are most commonly used to introduce hydrophobic particles into aqueous solutions and to make oil-in-water emulsions. Existing studies of polysorbates in the drug delivery system show polysorbates as more biocompatible, minimally toxic, less hemolytic, and with low levels of cell surface irritation that can maintain physiological pH in solutions [14-16]. It is known that for stabilization of emulsions polysorbate-80 is used in the amount of 1.0-2.0 g per 10.0 g of oil [6], so in the studied emulsions biocomplex, PS as an independent emulsifier is used in a concentration from 4 to 10%. Taking into account the well-known approaches to the use in o/w emulsions 70% emulsifier of the II type and 30% emulsifier of the I type, in emulsions in which the biocomplex PS is used as a co-emulsifier, a complex emulsifiers lanolin anhydrous: biocomplex PS or glycerol monostearate: biocomplex PS is used in a ratio 70:30 with a total content of emulsifiers from 4 to 10%.

The manufacture of emulsions involved the application of technological rules and techniques used in the manufacture of semi-solid preparations [17]. The compositions of the studied emulsions are shown in table 1.

Table 1: Emulsions stabilized with biocomplex PS

Name of components	Compositions								
	№ 1	№ 2	№ 3	№ 4	№ 5	№ 6	№ 7	№ 8	№ 9
Grape seed oil	30.0								
Biocomplex PS	4.0	7.0	10.0						
Complex emulsifier (lanolin anhydrous: biocomplex PS 70:30)				4.0	7.0	10.0			
Complex emulsifier (glycerol monostearate: biocomplex PS 70:30)							4.0	7.0	10.0
Aquae purificatae	till 100.0								

As a result of studying the stability of emulsions, it was found that emulsions stabilized only with biocomplex PS in the studied concentrations are not stable for a long time, they separate rapidly and require high concentrations of biocomplex PS, more than 10%, which is impractical and economically unreasonable. The separation time of the emulsions depends on the concentration of the biocomplex PS, the emulsions with 10% of biocomplex PS remained stable for the longest time. Stirring the samples for 5 min using an ultrasonic dispersant UZDN-A650T (Akademprylad, Ukraine) did not significantly improve the stability of the emulsions. Fig. 1 shows the microstructure of experimental samples № 1-6.

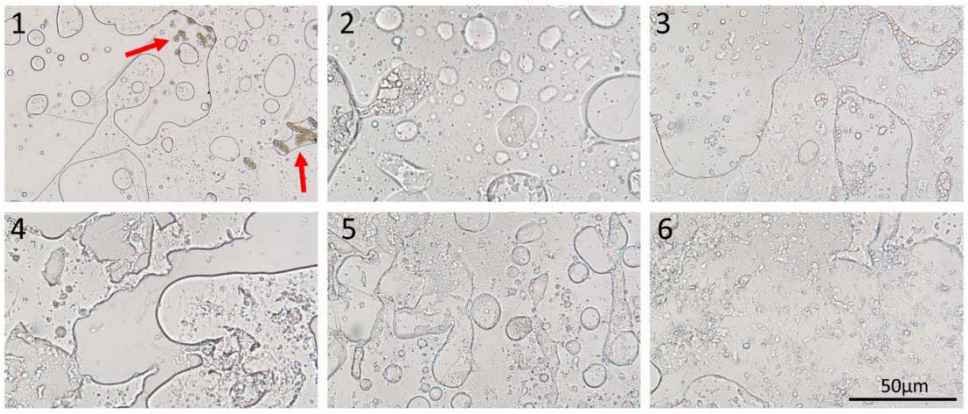

Fig. 1: Microphotos of the studied samples of emulsions № 1-6 (magnification 40x)

After 12 mo of storage of emulsions № 1-2, their separation and formation of dark inclusions have been observed, which indicates microbiological contamination and the need to introduce additional preservatives (fig. 1-1, red arrows). Such results confirm the results obtained earlier about the unsuitability of biocomplex PS use as the only preservative in o/w emulsions, but only as a part of complex preservative [18].

The combination of the biocomplex PS with other surfactants, in the ratio 30% of the I type emulsifiers (biocomplex PS) and 70% of the II type emulsifiers, allows obtaining more stable emulsions, the stability of which is additionally confirmed by microscopic studies. Fig. 2 shows the microstructure of experimental samples № 7-9 and the dimensional distribution of their particles.

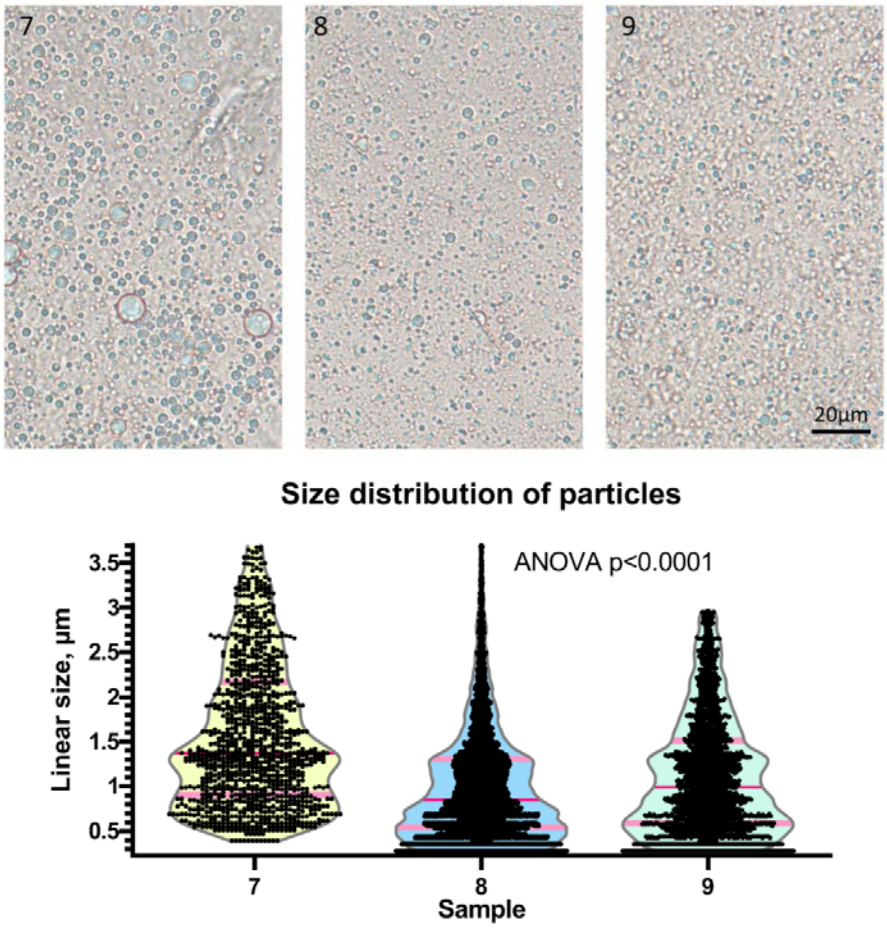

Fig. 2: Microphotos of emulsion samples № 7-9 and dimensional distribution of their particles (magnification 40x)

According to the results of the study of organoleptic and microscopic characteristics of emulsions samples with a complex emulsifier (lanolin anhydrous: biocomplex PS 70:30), the optimal concentration of the complex emulsifier is 10% (composition № 6); the used concentration of complex emulsifier in the amount 4% did not ensure the formation of stable emulsion (composition № 4)-the emulsion separated, was characterized by low viscosity; the use of 7% complex emulsifier contributed to the formation of an emulsion with a large particle size of the dispersed phase (composition № 5).

The use of a complex emulsifier (glycerol monostearate: biocomplex PS 70:30) in all studied concentrations provided the formation of stable emulsions (compositions № 7-9), but the use of 10% emulsifier contributes to excessive thickening of the emulsion (composition № 9). The optimal concentration of this complex emulsifier was 7% (composition № 8). The average particle size in the emulsions was 1.665±1.032 µm (mean±standard deviation) for sample № 7 with a median size 1.37 µm, n = 1123, for sample № 8 the average particle size was 1.024±0.670 µm with a median size distribution 0.85 µm, n = 6869, and for sample № 9 the average particle size was 1.123 µm±0.653 µm with a median size distribution 0.992 µm, n = 2154. Samples № 7 -9 significantly differed in particle size distribution in emulsions (fig. 2) determined by the ANOVA method, p<0.0001).

Thus, the creation of stable o/w emulsions requires the use of high concentrations of biocomplex PS as an emulsifier, more than 10%, which is impractical and economically unreasonable. The use of biocomplex PS as a co-emulsifier with emulsifiers of the II type allows obtaining stable emulsions o/w at a total concentration of complex emulsifier 7-10%.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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