

RESEARCH ARTICLE

Selected Essential Oils in Cosmetic Emulsions: Process Oriented Stability Studies and Antimicrobial Activity

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Abstract

Essential oils show self-preserving properties, which gives an opportunity for the cosmetic industry to reduce or even eliminate the application of synthetic preservatives like parabens, which are thought to be endocrine-disrupting compounds. The present study was focused on advancing of our understanding on antibacterial and antifungal properties of essential oils in cosmetic products. Especially, towards the development of practical and applicable aspects of abovementioned natural products. Presented procedures and results can be easily up-scaled and transferred to cosmetic industry. Results comprising procedure for preparation of a stable cosmetic emulsion using essential oils as natural preservatives, the evaluation of its stability by the turbidimetric method and estimation of preserving capabilities of essential oils including comparison with commonly used synthetic preservatives are presented in this present study. The stability evaluation of cosmetic emulsions revealed that the most stable emulsion is one comprising citrus oil (C= 2%) and vitamin E (C= 1.5%). The most unstable compositions are ones with rosemary oil (C= 2%), vitamin E (C= 1.5%) and citrus oil (C= 1%). Microbiological tests showed that the prepared emulsions consist of less than 10 CFU/g, which proves that the compositions are microbial clean and thus high quality.

These results confirm our hypotheses that essential oils can be effective natural preservatives in cosmetic formulations with concurrent stability and other features oriented on the possible commercial application.

Keywords: Essential oils, cosmeceuticals, emulsions, natural preservatives

Introduction

Essential oils show self-preserving properties, which gives an opportunity for the cosmetic industry to reduce or even eliminate the application of synthetic preservatives. Mutations of microorganisms leading to resistance towards conventional preservatives triggered research for finding alternative preserving systems in various branches of industry, including cosmetic industry (Sheldon, 2005). In addition, common preservatives – parabens (*p*-hydroxybenzoate esters) – possess estrogenic activity in various in-vitro and in-vivo tests (Table 1); are thought to act as endocrine disruptive compounds (Darbre and Harvey, 2008; Byford et al., 2008; Routledge et al., 1998) and induce proliferation of cancer cells (Byford et al., 2008; Darbre et al., 2003; Darbre et al., 2002).

Antimicrobial activity enables application of essential oils as food preservatives (Burt, 2004), crop cultivation growth stimulants (Calsamiglia et al., 2007) and as natural pesticides in ecological agriculture (ElShafei et al., 2010). Literature references point out their high activity against gram-positive bacteria and slightly lower activity against gram-negative (Burt 2004; Skočibušić et al., 2006). In addition to antimicrobial properties, essential oils exhibit therapeutic properties over a wide range of targets. Oils isolated from thyme, rosemary and lavender counteract alopecia areata. Chamomile and lavender oils show anti-inflammatory and anti-acne

activities. In addition, rosemary and lemon oils exhibit anti-wrinkle properties (Chaturvedi et al, 2007). It is worth to notice that essential oils may induce allergic reactions, despite their natural origin (Maddocks-Jennings, 2004). To minimize the risk of their occurrence, the concentration of essential oils in cosmetic formulations is limited to 1-2%. The selection of essential oils for purposes of our study was made based on an analysis of their specific activity and direct influence on the skin.

Table 1. Estrogenic activity of parabens.

Paraben	Estrogen receptor recombined in yeasts	Human receptor MCF-7	Rat ovarian estrogen receptor	Cells of ovarian cancer HeLa
Methylparaben	+	+	-	n/a
Ethylparaben	+	+	+	+
Propylparaben	+	+	+	+
Butylparaben	+	+	+	+
Isopropylparaben	+	+	n/a	n/a
Isobutylparaben	+	+	n/a	n/a
Benzylparaben	+	+	+	n/a

Nowadays, the nano-encapsulation of active substances is very important in cosmetic formulations. Some research has been made regarding the use of essential oils as preservatives in cosmetics (Varvaresou et al., 2009). Lemon and lavender oils were previously investigated as potential natural preservatives in cosmetics. Previous studies confirmed growth inhibitory properties of lemon oil towards *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* (Kunicka-Styczyńska et al., 2011). Another studies focused on antimicrobial and antifungal properties of sage, eucalyptus and laurel tree oils (Maccioni and Anchisi, 2002). Synergistic effects of combination of essential oils and low doses of synthetic preservative were observed. Our results also confirm this statement. All previously conducted studies focused on basic research that only proves potential of essential oils as constituents of cosmetics.

Presented study was focused on two directions in natural products research. The first was to advance our understanding on antibacterial and antifungal properties of essential oils in cosmetic products. Nevertheless, the main purpose of our study was directed towards practical and applicable aspects of abovementioned natural products. Presented procedures and results can be easily up-scaled and transferred to cosmetic industry. This study is also a continuation of our interest in preparation of new semisynthetic therapeutic agents (Siemieniuk et al., 1992a; Siemieniuk et al., 1992b) and novel nano-carriers and encapsulation processes (Jarzycka et al., 2013; Bazylińska et al., 2012a; Bazylińska et al., 2012b; Bazylińska et al., 2011; Wilk et al., 2009). We have taken an advantage of new technologies and methods for process-oriented preparation of stable cosmetic emulsions using essential oils as natural preservatives, the evaluation of their stability by the turbidimetric method and estimation of preserving capabilities of essential oils including comparison with commonly used synthetic preservatives. Results of this study bring answer to the question if essential oils can be effective natural preservatives in cosmetic formulations with concurrent stability and other features facilitating the possibility of commercial application.

Materials and Methods

Material

Glycerine was purchased from PPH Stanlab; potassium hydroxide from POCH S.A.; stearin from cosmetic warehouse "ZrobSobieKrem"; tocopheryl acetate (vitamin E) and L-Ascorbic acid (vitamin C) from cosmetic warehouse e-Naturalne. Rosemary, lemon, lavender and eucalyptus oils were purchased from Avicenna-oil

and Phenonip (mixture of phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben and isobutylparaben) from cosmetic warehouse "mazidla.pl"

Pseudomonas aeruginosa ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231 were used for microbial quality control studies.

Sample preparation

Emulsions were prepared using Homogenizer Ultra Turrax T25 Basic, IKA. All cosmetic emulsions were prepared using hot-hot cold method. Choice of the method was based on different physical states of water and oil phases and temperature sensitivity of essential oils and vitamin E. Components of water and oil phases (Table 2) were weighed separately. Both phases were stirred vigorously and heated to 70-80°C. When both phases reached the same temperature, oil phase was transferred steadily into water phase. In the next step mixture was homogenized at 19000 rpm for 10 minutes and subsequently at 11000 rpm for 20 minutes. Emulsion was cooled down to room temperature, temperature-sensitive components were added and final mixture was homogenized at 19000 rpm for 10 minutes. Emulsions were stored in the refrigerator in closed containers.

Emulsion stability studies with freeze-thaw method

Samples of cosmetics emulsions were stored at -16°C for 16 h and then at room temperature for 8 h. Cycle was repeated four times.

Emulsion storage stability studies

Samples of cosmetics emulsions were stored at room temperature for 24h. Observations were made after 1, 2, 4, 6 and 24 h.

Emulsion stability studies by turbidimetry

TurbiscanLab^{expert}, Formulaction SA was used for emulsion stability studies with turbidimetric method. Emulsion samples in glass cuvettes were illuminated by a focused light beam (near infrared, $\lambda = 880$ nm). The entire length of the sample was scanned and transmission (180° from the light source) and backscattering (45° from the light source) data each 40 μ m were acquired. Measurements were made twice using single scan mode: 2 days and then 2 weeks after preparation of emulsions. Intensity profile of backscattered light along height of the cuvette was acquired.

Microbial quality control studies

Plate method was used for microbial quality control. Total count of mesophilic microorganisms and presence of specific bacteria and fungi like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* are measured according to the Polish Ministry of Health regulations from 23rd December 2002 (Polish Minister of Health decree "Dz. U. Nr 9. pos. 107" 2003 (<http://isap.sejm.gov.pl/DetailsServlet?id=WDU20030090107>)) and ISO standards PN-EN ISO 22718:2010, 22717:2010, 18416:2009, ISO 21149:2009 and 21148:2009.

Results and Discussion

Preparation of emulsions

All of presented studies were conducted according to the guidelines of the Polish Minister of Health and adequate ISO norms (Polish Minister of Health decree "Dz. U. Nr 9. pos. 107" 2003 (<http://isap.sejm.gov.pl/DetailsServlet?id=WDU20030090107>); Polish Norm PN-EN ISO 22718:2010; Polish

Norm PN-EN ISO 21148:2009; Polish Norm PN-EN ISO 22717:2010; Polish Norm PN-EN ISO 18416:2009; Polish Norm PN-EN ISO 21149:2009). The highest grade raw materials were used for the presented studies. A vanishing cream formula was chosen for our studies. It ensures in obtaining reliable results regarding the preserving properties of essential oils, without interference from other common cosmetic constituents. The vanishing cream formula is commonly used for the preparation of cosmetic products. It is a light, O/W type moisturizing cream that absorbs quickly into the skin. Standard formula of vanishing cream is based on stearin (Table 2).

Table 2. Cosmetic emulsion formula.

phase	materials	[% w/w]
water	glycerine	8
	potassium hydroxide	0,7
	water	76,3
oil	stearin	15

The prepared emulsion was easy-to-scoop, uniform, formed a cone and left a fine fatty film after application on the skin. Four essential oils with the best antimicrobial and antifungal properties (according to the literature) have been chosen: rosemary oil, lemon oil, eucalyptus oil and lavender oil. 1% and 2% concentration of essential oils are used for the preparation of the studied emulsions (Table 3). The first number after "AP" indicates the weight percent of an essential oil, and the second is the weight percent of vitamin E or phenonip.

Despite regenerating the activity of vitamin C on vitamin E it wasn't possible to include it into our emulsion formula. Vitamin C is miscible in water but very sensitive to high temperature. The necessity of heating the oil phase to melt stearin and the water phase to acquire uniform emulsion disenabled the use of vitamin C in our formula. Phenonip was used as a synthetic preservative. It consists of phenoxyethanol (72,6%), methylparaben (15,48%), butylparaben (4,04%), ethylparaben (3,95%), propylparaben (1,95%) and isobutylparaben (1,84%).

Table 3. List of emulsions.

[% w/w]	Rosemary oil	Lemon oil	Lavender oil	Eucalyptus oil
1	1-AP-1	6-AP-1	8-AP-1	9-AP-1
2	1-AP-2	6-AP-2	8-AP-2	9-AP-2
Vitamin E	1-AP-2-1,5E	6-AP-2-1,5E	-	-
	1-AP-1-1,5E	6-AP-1-1,5E	-	-
Phenonip	1-AP-2-0,5P	6-AP-2-0,5P	-	-
	1-AP-1-0,5P	6-AP-1-0,5P	-	-

Quality control of cosmetic emulsions

Quality control of cosmetic emulsions was carried out according to norm (Polish Norm BN-64/6140-02 1964). Table 4 shows the results of organoleptic tests: the evaluation of appearance, colour and scent, temperature stability in room temperature and in freeze and thaw conditions.

Table 4. Results of quality control of emulsions

	1-AP-1	6-AP-1	8-AP-1	9-AP-1	1-AP-1-1,5E	6-AP-1-1,5E	1-AP-1-0,5P	6-AP-1-0,5P
Appearance	Smooth, uniform, without lumps and impurities							
Colour	Uniform white							
Scent	agreeable	agreeable	sharp	sharp	agreeable	agreeable	agreeable	agreeable
21°C	+	+	-	-	+	+	+	+
- 16°C	+	+	-	-	+	+	+	+
	1-AP-2	6-AP-2	8-AP-2	9-AP-2	1-AP-2-1,5E	6-AP-2-1,5E	1-AP-2-0,5P	6-AP-2-0,5P
Appearance	Smooth, uniform, without lumps and impurities							
Color	Uniform white							
Scent	agreeable	agreeable	sharp	sharp	agreeable	agreeable	agreeable	agreeable
21°C	+	+	-	-	+	+	+	+
- 16°C	+	+	-	-	+	+	+	+

+no changes in emulsion during test time

-emulsion changed during test time

None of emulsions changed colour or fragrance. During the temperature stability studies changes of organoleptic properties were observed for eucalyptus and lavender oil (emulsions 8-AP and 9-AP) – they were hard-to-scoop and did not form a cone. In addition, they were characterized with an inconsistent, lumpy texture and air bubbles were detected during application. The observed changes result from temperature sensitivity of the studied samples. The optimal temperature for emulsion storage ranges between 4 °C and 20 °C. Above 20 °C the process of delamination of the emulsion is enhanced due to lowered viscosity of the liquid and faster sedimentation of dispersed micelles. The dispersing phase in O/W emulsions freezes at temperatures below 0 °C. The following emulsions were chosen for stability studies using the turbidimetric method (Table 5).

Table 5. Emulsions chosen for stability studies with turbidimetric method.

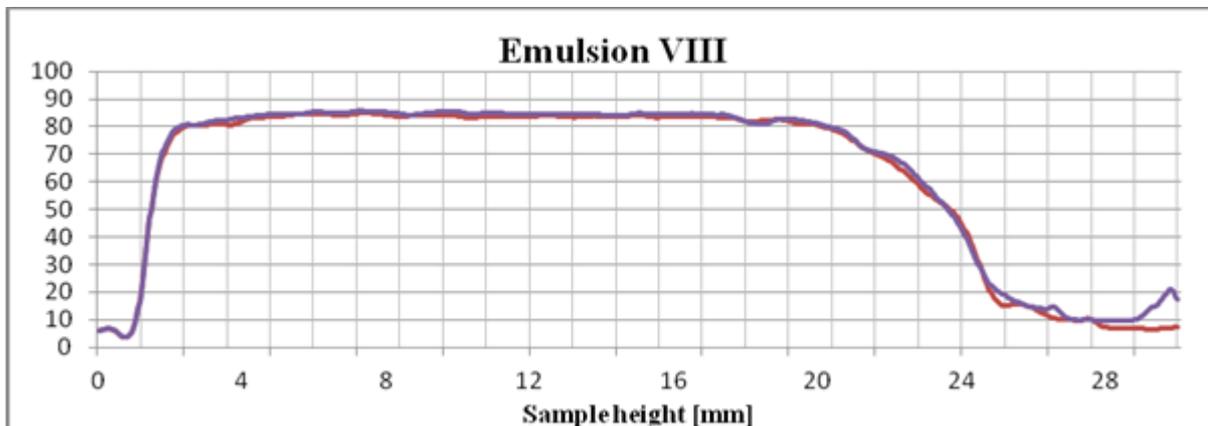
Emulsion nr	Label	Preservatives
I	1-AP-1	1% rosemary oil
II	1-AP-2	2% rosemary oil
III	1-AP-1-1,5E	1% rosemary oil, 1,5% vitamin E
IV	1-AP-2-1,5E	2% rosemary oil, 1,5% vitamin E
V	6-AP-1	
VI	6-AP-2	2% lemon oil
VII	6-AP-1-1,5E	1% lemon oil, 1,5% vitamin E
VIII	6-AP-2-1,5E	2% lemon oil, 1,5% vitamin E
IX	6-AP-1-0,5P	1% lemon oil, 0,5% Phenonip

Evaluation of emulsions stability using turbidimetric method

TurbiscanLab^{expert} is an optical device that allows the characterization of the actual state of dispersion and the long-term analysis of the destabilization processes in emulsion. It is the most complex test that allows the detection of migration phenomena and even the slightest stability changes. The results of carried experiments are depicted as intensity profiles of backscattered light along test tubes. Emulsion VIII that comprises 2 % of lemon oil and 1.5 % of vitamin E is the most stable (Fig. 1). Lowering of the profile isn't

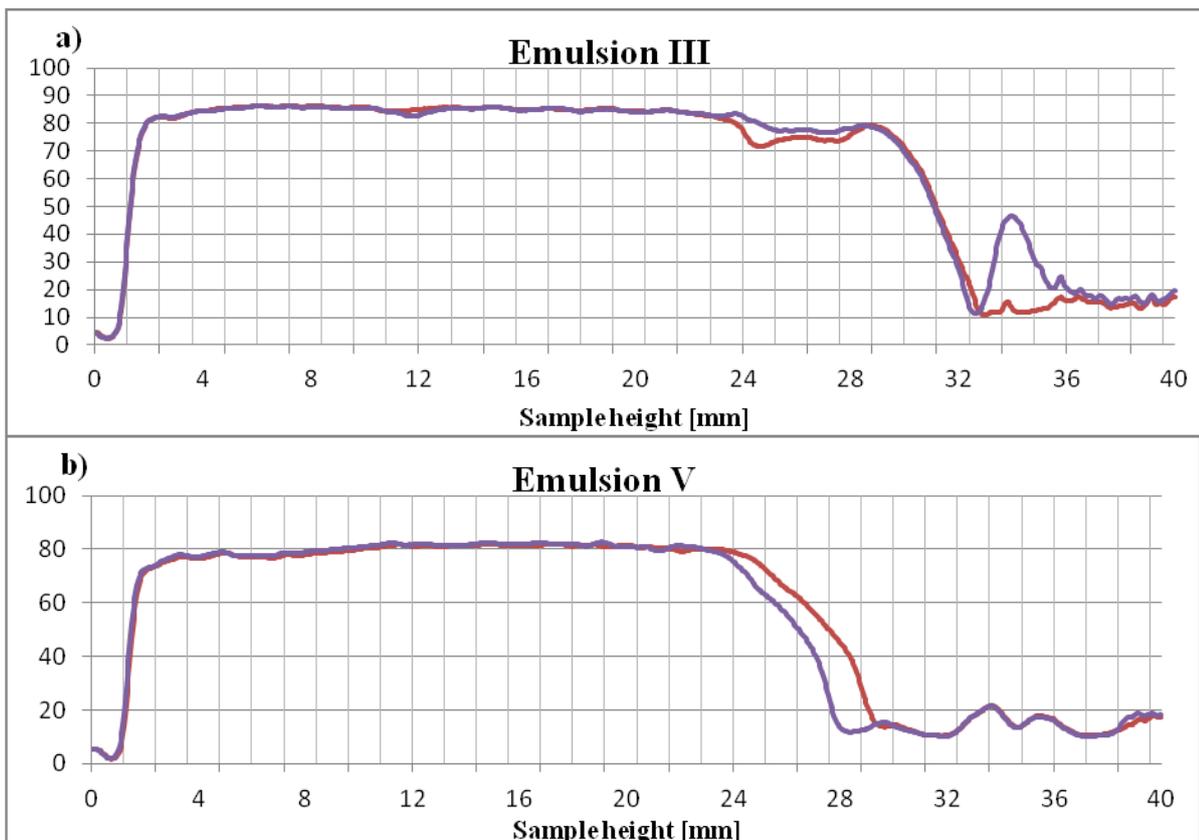
observed and no interferences in the lower and upper parts of the sample are detected. A scan of the profile after 24 h from the preparation of the emulsion overlaps with the profile's scan after 2 weeks which proves the stability of the emulsion VIII.

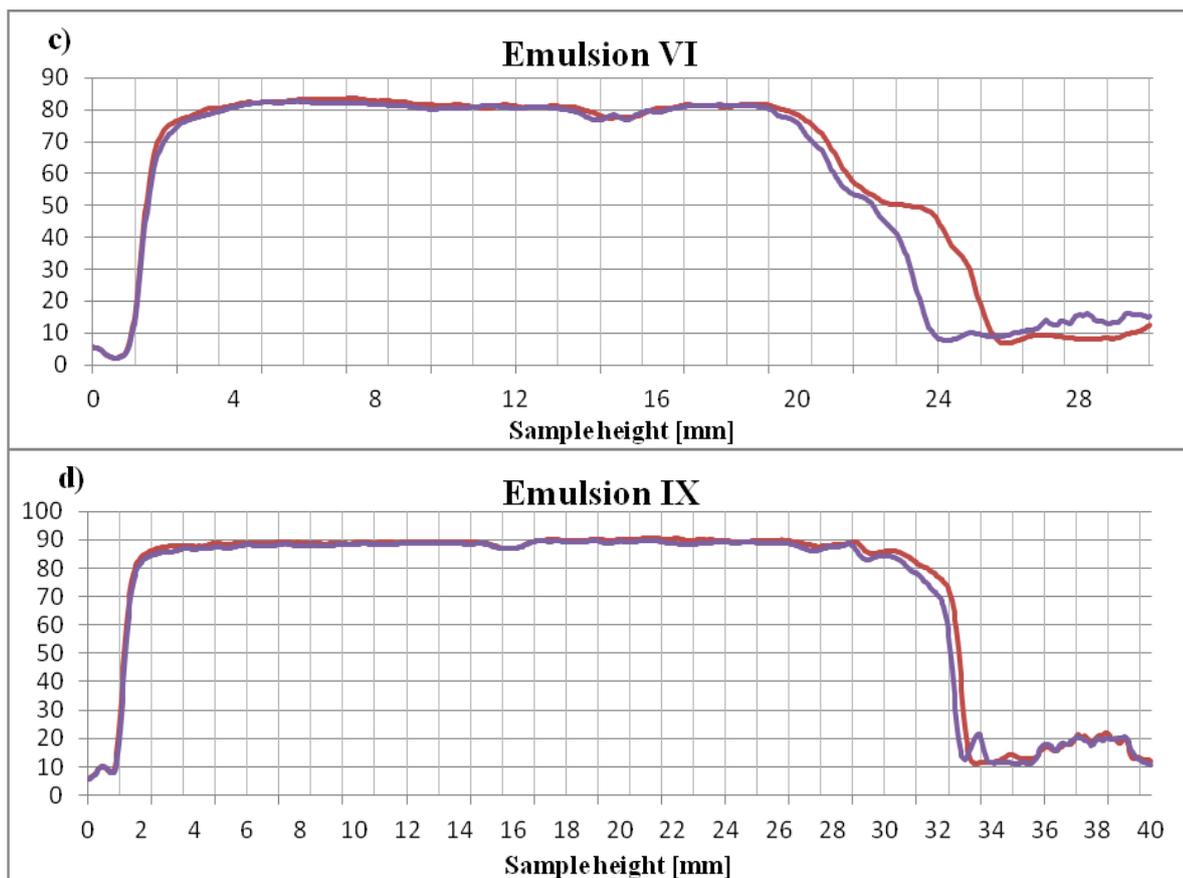
Figure 1. Intensity profile of emulsion VIII.



Similar characteristics of backscattering intensity profile are observed in emulsions III, V, VI and IX (Fig. 2a-d).

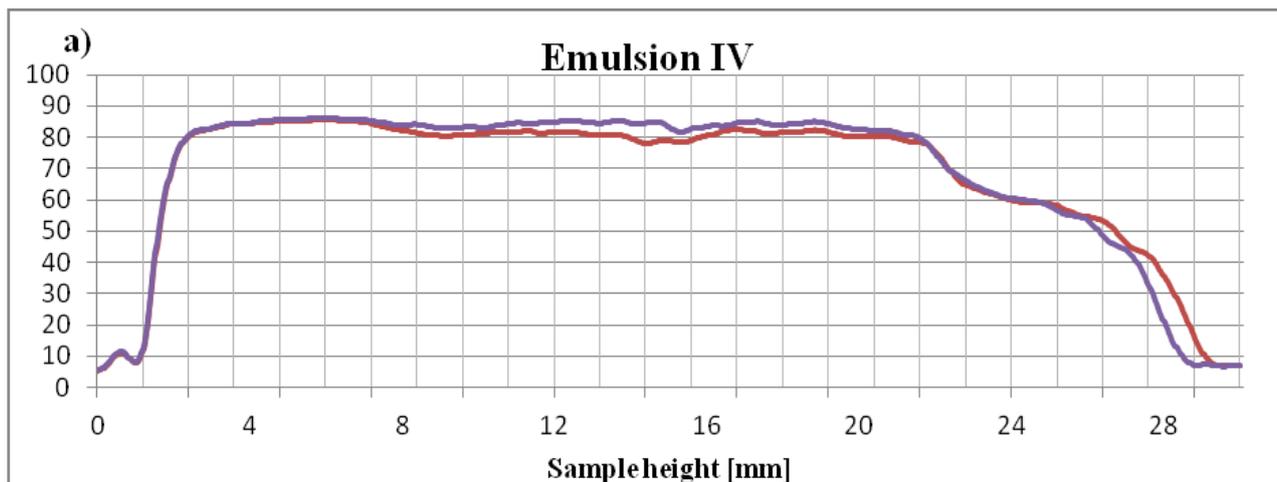
Figure 2a-d. Intensity profiles of emulsions III, V, VI and IX.

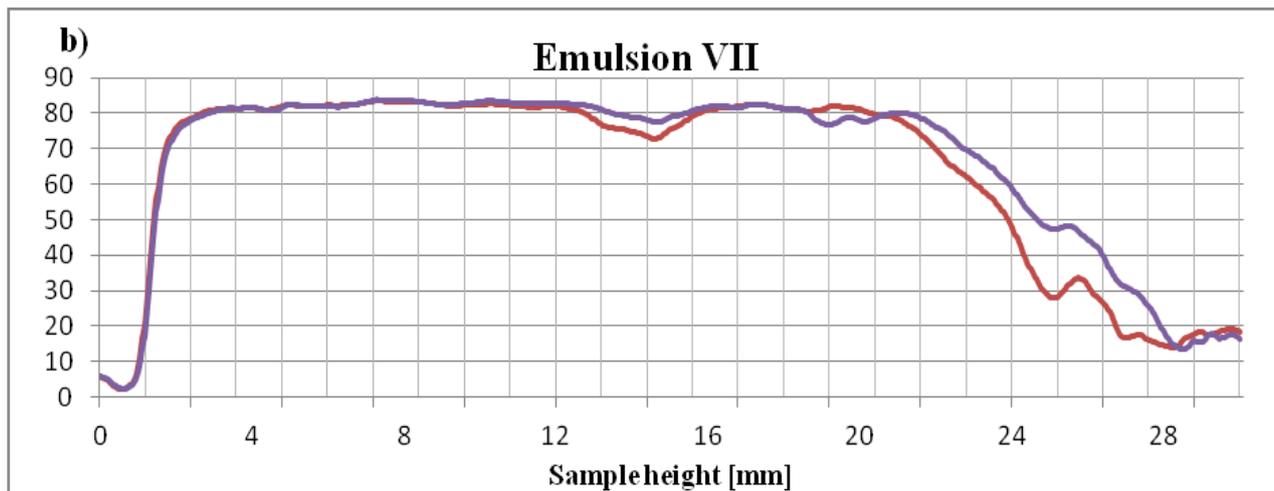




Scans overlap within the range 0 to 20 mm of test tubes height which mean that the structure and average drop dimensions do not change over time. In the upper part of the test tubes, the intensity profile lowers which may indicate that creaming or other aggregation processes start. The most unstable systems are emulsions IV and VII (Fig. 3a,b) which comprise 2% of lemon oil.

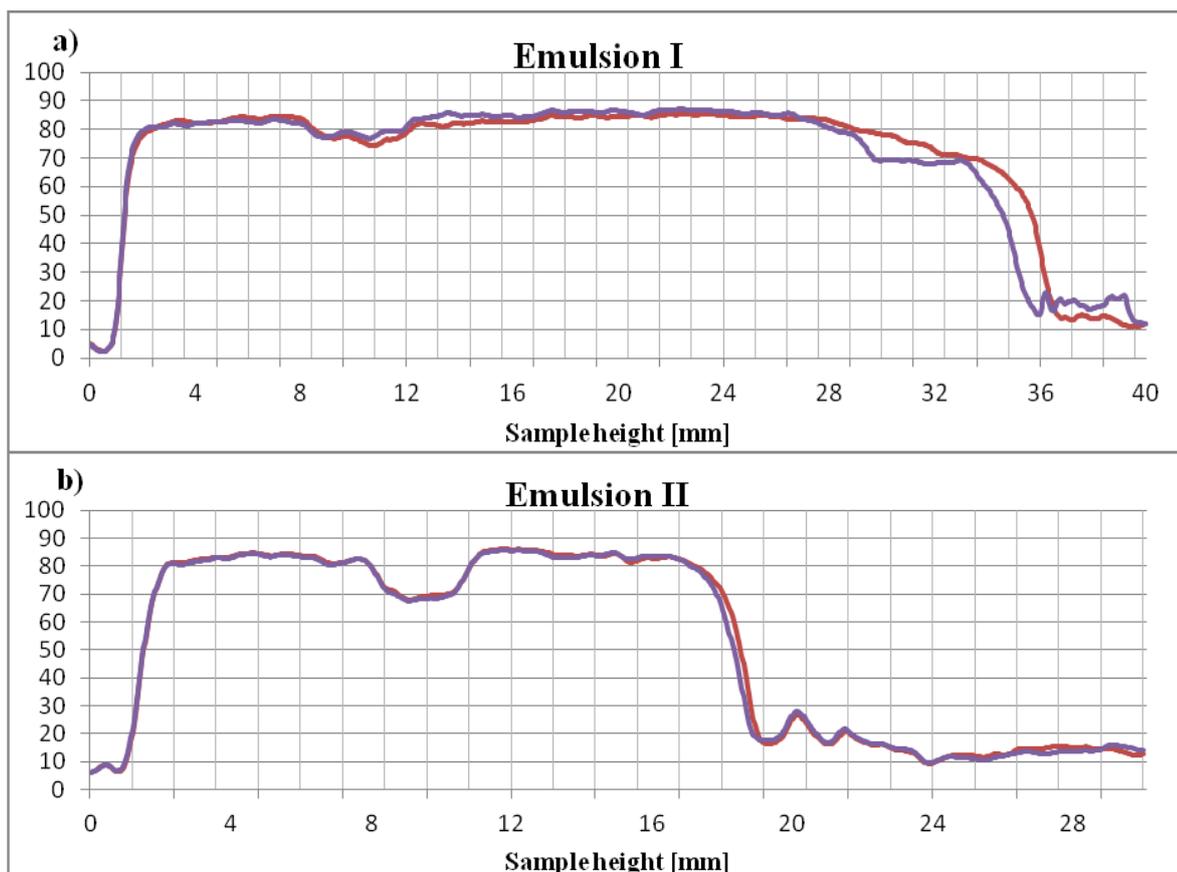
Figure 3a,b. Intensity profiles of emulsions IV and VII.





Lowering of the intensity profile of backscattered light is visible along the whole test tube. It can be caused by flocculation or coalescence processes. Profiles of emulsions I and II (Fig. 4a,b) show interferences of rectilinear course in the middle part of the tube. First and second scan overlap so these interferences may be caused by an air bubble.

Figure 4a,b. Intensity profiles of emulsions I and II.



The sedimentation process is not observed in any of the samples – all profiles overlap at the lower part of the tube. The high density of the emulsions do not allow surface smoothing which manifests as interferences in the course of backscattered light intensity profiles.

Microbial Quality Control

According to guidelines included in the Polish Act on general product safety from 2007 (Polish Act on General Product Safety decree "Dz. U. Nr 35 pos. 215." 2007 (<http://isap.sejm.gov.pl/DetailsServlet?id=WDU20070350215>)) and the cosmetic Act from 30 March 2001 (Polish Act on Cosmetics decree "Dz. U. Nr 42 pos. 473." 2001 (<http://isap.sejm.gov.pl/DetailsServlet?id=WDU20010420473>)) every cosmetic product placed on the market must be safe for human health and for the environment. The safety assessment of cosmetics requires microbial quality studies. Six emulsions are evaluated. They comprise rosemary or lemon oil with vitamin E or phenonip. The studied emulsions are qualified to cosmetics category II (other cosmetics) according to a decree from the minister of health (Polish Minister of Health decree "Dz. U. Nr 9. pos. 107" 2003 (<http://isap.sejm.gov.pl/DetailsServlet?id=WDU20030090107>)) in 2002. This regulation states that the maximal limit of aerobic mesophilic microorganisms in the sample is 5000 colony forming units per 1.0 g of emulsion (CFU/g). In addition, category II cosmetic cannot contain *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Microbial quality tests of emulsions are summarized in Table 6.

Table 6. Results of microbial quality studies

	Ministry of Health req ²⁷	Emulsion II	Emulsion IV	Emulsion V	Emulsion VI	Emulsion VIII	Emulsion IX
Total count of aerobic microorganisms in 1.0 g of the sample	max 5000 CFU/g	Less than 10 CFU/g	Less than 10 CFU/g	Less than 10 CFU/g	Less than 10 CFU/g	Less than 10 CFU/g	Less than 10 CFU/g
<i>Pseudomonas aeruginosa</i> ATCC 15442	Not present	Not present	Not present	Not present	Not present	Not present	Not present
<i>Staphylococcus aureus</i> ATCC 6538	Not present	Not present	Not present	Not present	Not present	Not present	Not present
<i>Candida albicans</i> ATCC 10231	Not present	Not present	Not present	Not present	Not present	Not present	Not present

The evaluated emulsions contain less than 10 CFU/g, which proves their high microbial quality. This purity is a result of using raw materials that meet quality norms, carrying out the production process with good hygienic standards and antimicrobial and antifungal efficiency of natural preservatives.

Discussion

Emulsions are basic form of active substance carriers in cosmetic products. It's easier to maintain microbial purity in emulsions W/O due to the low amount of water that is necessary for the growth of microorganisms. On the other hand, emulsions O/W facilitate skin penetration by active substances like essential oils (Polish Norm PN-EN ISO 22717:2010 2010). The evaluation of the preserving properties of essential oils requires the preparation of a base emulsion with a limitation on the basic ingredients. During the course of our studies, vitamin E and vitamin C were used as natural preservatives. There are 8 forms of vitamin E from which 4 belong to a tocopherol class of compounds (Dweck, 2003). The most active biological form is α -tocopherol. Tocopherols are fat-miscible antioxidants that neutralize free radicals (Fryer, 1992). Stronger antioxidant activity may be achieved with the addition of vitamin C, which regenerates oxidized forms of vitamin E (Burke, 2007). These activities made vitamin E and vitamin C valuable for the cosmetic industry as natural preservatives.

The selection of a preservative and its concentration are one of the most important factors for the cosmetic industry. Preserving substances are components which are required in formulations with higher amounts of water, especially in O/W emulsions (Dweck, 2003). Preservatives should especially protect the water phase of the emulsion – proliferation and growth of microorganisms occur in the water phase. The factors determining the selection of a proper preserving substance include: pH, partition between oil and water, reactivity with other cosmetic ingredients and the container (Chaturvedi, 2007). The proper preservative is characterized by the lack of toxicity, irritant and sensitization effects, high activity against a broad spectrum of microorganisms [G(+) and G(-) bacteria and fungi including yeasts], high activity at low concentrations, solubility in water, lack of a perceptible fragrance, colour and taste. Moreover, it should be resistant to deactivating action of other cosmetics ingredients and the container. In addition, light, oxygen and elevated temperature shouldn't affect its properties (Dweck, 2003).

The results of our studies including the physicochemical tests facilitate the formulation of stable emulsions comprising essential oils as natural preservatives. Cosmetic emulsions with essential oils show very good functional features and meet all the requirements for commercial cosmetic products. In addition, commonly used industrial hot-hot cold technology is optimal for the preparation of cosmetic emulsions with essential oils. Temperature stability studies of emulsions show a lack of heat influence on organoleptic properties of products with lemon and rosemary oil. Slight changes of texture were observed for emulsions with lavender and eucalyptus oil. An emulsion consisting of 2% of lemon oil and 1.5% of vitamin E is the most stable. The application of essential oils as natural preservatives resulted in products with high microbial purity (<10 CFU/g). *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* are not present. The use of essential oils as natural preservatives provides the possibility of preparation of stable natural cosmetic emulsions with high microbial quality. This fact proves that commonly used parabens, which are estrogenic and harmful to aquatic environment, can be replaced with a natural, "green" alternative.

Generally, the essential oils are recognized as safe, although their properties vary between the groups. Hence, the most thorough research in terms of possible interactions is of a primary importance (Deepak et al, 2013). Specific oils, such as *Lavandula officinalis* and *Rosmarinus officinalis*, added to the formulation in concentration of 1.5%, demonstrated significant antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*.

According to the literature one may conclude that the plant essential oils, obtained from *Lavandula officinalis* and *Rosmarinus officinalis*, are suitable as natural preservatives in aqueous cream formulations and similar cosmetic products. In addition, the oils used in comparatively higher concentrations (as it is in our case) could (at the same time) perform a role of natural fragrances in the preparations (Muyima et al, 2002). On the other hand, strong and distinctive scent of some essential oils may limit their application in the cosmetic recipe (Varvaresou et al, 2009).

It has been also concluded that specific mixtures of essential oils prove to be very effective as growth inhibitors of a broad spectrum of microorganisms. It allows to decrease the amount of synthetic preservatives in the desired formulations. Maccioni et al. (2002) were able to decrease 8.5 times the concentration of synthetic preservatives. They found that *Laurus nobilis*, *Eucalyptus globulus* and *Salvia officinalis* oils added in the concentration of 0.025 % and 0.0125 % were 200-fold more active in combination with methylparaben.

The synergistic action of lavender, tea tree and lemon oils was tested in regard to the European Pharmacopoeia standards. Formulations containing only the essential oils met Criterion A.E.P. only for *Staphylococcus aureus*, *Candida sp.* and *Aspergillus niger*. The solubilizer addition in 5 % and the synthetic

preservative in 0.3 % enhanced the microbiological stability of formulation in accordance with Criterion A.E.P. (Siemieniuk et al., 1992b).

Conclusion

Microbial purity of cosmetics has major impact on their durability and safety of use. Generally, preservatives are used in small quantities and low concentrations. They still may pose a danger to human health causing allergies and in some cases, *i.e.* methylparaben, may lead to skin problems by producing free radicals when exposed to sunlight (Handa et al., 2006). Data show that despite being regarded as safe, parabens may incur various health problems with breast cancer (Kirchhof and deGannes, 2013) or oestrogen-like properties as examples. Therefore, there is a growing interest for production of preservative-free cosmetic products and also self-preserving formulations (where common preservatives have been replaced by other cosmetic ingredients with antimicrobial properties (Varvaresou et al, 2009).

Essential oils show a great potential for creation of a stabilized micellar systems which is meaningful in regard to their utilization in cosmetic formulations (Edris and Malone, 2012). We have acquired stable emulsion with high microbial purity, which is in the same time self-preserving and also preservative-free formulation, containing lemon oil and vitamin E.

Recent trends towards application of natural preservatives instead of synthetic ones in cosmetic industry prove a great potential of essential oils in this field. Literature data show that despite some issues, essential oils are promising alternative. They express high antimicrobial activity against bacteria and fungi, but on the other hand must be used in higher concentrations which limits the acceptable contact time with skin. It is necessary to carry on research determining possible interactions between components of cosmetic formulations due to their complexity (Kunicka-Styczyńska et al., 2011; Mulyaningsiha et al., 2010; Yorgancioglu and Bayramoglu, 2013; Bueno, 2015).

Large scale incorporation of natural ingredients of antimicrobial action into industry may have a great impact on natural environment as well as on human lifestyle. It has been reported that paraben residues have been found in rivers and drinking water (Handa et al., 2006). Green chemistry alternative seems to be a natural step leading towards improvement.

ACKNOWLEDGMENT

The work was financed by a statutory activity subsidy from the Polish Ministry of Science and Higher Education for the Faculty of Chemistry of Wrocław University of Technology.

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