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The quality control of some dermo-cosmetic products

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Abstract This paper refers to the quality analysis of three dermo-cosmetic products: two face creams and a biphasic solution used for skin cleaning: a treatment cream for acne complexion, an anti-irritating soothing cream and a matifying purifying lotion. The following characteristics have been investigated: aspect, smell, colour, solubility, type of emulsion, stability test at certain temperatures (4°C and 40°C), pH, melting point, water, volatile substances and total fats contents, acidity, ester index, saponification index, iodine index, peroxide index, microbic carriage, metal traces (determined by ICP-MS method). All analysed dermo-cosmetic products have appropriate physico-chemical characteristics. The analyses made for determining the microbial charge have proven that the three dermo-cosmetic products do not contain any aerobic pathogen germens or micromicetes, *Staphylococcus aureus* and *Escherichia Coli*. The ICP-MS analysis has proven that the dermo-cosmetic products do not contain traces of Hg and Pb.

Keywords: dermo-cosmetic products, quality control, aerobic pathogen, *Staphylococcus aureus, Escherichia Coli*

1. Introduction

The "oil in water" (O/W) and "water in oil" (W/O) emulsions, representing the majority of cosmetic and pharmaceutical creams are thermodynamically unstable, usually splitting into two distinct phases. This instability could be manifested at different time rates and through a variety of physic-chemical processes, due to creaming (or sedimentation), flocculation, coalescence or phase inversion. From a commercial point of view, the new products should have a storage stability of several months at ambient temperature and under widely varying external influences. Nevertheless, the shelf life assessment of O/W or W/O emulsions remains one of the most time consuming and difficult issues for industrial scientists [1].

Photodynamic agents are toxic to organisms after UV- irradiation. Natural and synthetic chemicals can become toxic as a result of absorbtion of photons. Perception of phototoxic hazards to biological system is increasing predominantly due to expanding use of chemicals in agriculture, industry, medicine and the concomitant exposure to ultraviolet radiation [2].

A cosmeceutical is a logical evolutionary concept, given the advances in skin anatomy and physiology. Contemporary belief is that almost all compounds applied to skin have the ability to penetrate and exert changes to skin structure [3].

There is a growing concern about the physiological and behavioural effects of environmental metals traces in human population [4-5]. The toxicity of lead and mercury at high levels of exposure is well known, but a major concern is the possibility that continual exposure to relatively low levels may entail adverse health effects.

As a consequence, traces of heavy metals in cosmetic products represent serious health issues, especially for pregnant woman and neonates [6-7].

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The subject of this paper comprises in the quality analysis of three Romanian dermo-cosmetic products using conventional and modern methods.

2. Experimental

To assess the quality of the studied dermo-cosmetic products, the following characteristics have been investigated: aspect, smell, colour, solubility, type of emulsion, stability test at certain temperatures (4°C and 40°C), pH, melting point, water, volatile substances and total fats contents, acidity, ester index, saponification index, iodine index, peroxide index, microbic carriage, Cd, Hg, Pb and Ti concentration.

2.1. Samples and sample preparation

The studied cosmetic products, purchased from the local market were two face creams and a biphasic solution used for skin cleaning, named as follows:

- C I - a treatment cream for acne complexion;
- C II - an anti-irritating soothing cream;
- C III - a matifying purifying lotion.

Throughout the analysing process, the samples have been kept at room temperature in appropriate, sealed containers.

To analyse the metal concentrations, clear solutions of the samples have been obtained by microwave digestion.

2.2. Reagents and solutions

All reagents were of analytical-reagent grade (Merck) and all solutions were prepared using deionised water. The working solutions used for different determinations/ measurements were as follows: KOH 0.1 N for acidity index, HCl 0.5 N for saponification index, $Na_2S_2O_3$ 0.1 N for iodine index and $Na_2S_2O_3$ 0.01 N for peroxide index.

2.3. Equipments

The following equipments have been used to perform the quality control of dermo-cosmetic products: a thermostate Julabo F12 (20 – 100°C; ±0.02°C), a pH meter Oakton 310 (0-14; ±0.01 pH), an electric oven UNB 200 Memmert (25-200°C; ±0.5°C).

An Agilent 7500ce ICP-MS equipped with a concentric nebulizer was additionally used for metal concentration. The gas flow was 1.32L/min and the detector was electronic multiplier.

2.4. Methods of analysis

The measurements were in accordance to 10998-88 Romanian standard [8] and Romanian Farmacopeea [9].

Solubility is determined by measuring the volume of solvent (in mL) needed for dissolving 1g of solid substance, at the temperature of 20±2°C. The solubility of creams was tested using methanol, CCl_4, hot water, benzene, oil ether, HCl [35%].

For *stability test* at 4°C and 40°C a thermostat was used. A weighing bottle is filled with 5g of substance and closed; it is kept in a thermostat for eight hours at a constant temperature of 4°C. After the weighing bottle is taken out and the substance is examined, the sample is reintroduced in the thermostat and kept for eight hours at a constant temperature of 40°C. Finally, the sample is re-examined. It is considered stable, if the separation of phases does not occur.

The *melting point* is defined as the temperature that a substance needs to reach in order to melt entirely. If the melting process implies decomposition, the temperature at which the substance changes its aspect (becoming brown or with gas bubbles) is considered the melting or the decomposing point.

Water, volatile substances and total fats contents, acidity, ester index, saponification index, iodine index, peroxide index methods of determination were presented in a previous paper [10].

The bacteriological tests determine the detection of pathogen microorganisms found on the exposed surface of seeding in specific cultivated environments or in the case of complex colouring techniques. Schliesser T has used referential microorganisms, whose use is even nowadays being approved not only for the characterisation of certain substances' bactericidal capacity, but also for the necessity of decontamination's current control of efficiency [11]. These test organisms are: *Staphilococcus aureus, Streptococcus faecium, Pseudomonas aeruginosa, Proteus mirabilis, Mycobacterium avium, Candida albicans,* the

zygotes of *Eimeria tenella* and the non-embryonated eggs of *Ascaris suum*.

The test of the total number of aerobic mesophil germens (T.N.A.M.G.) and the total number of micromicetes (N.T.M.) was executed following the protocol indicated by international norms [12]. Sterilised tampons have been used collecting the samples previously kept in tubes; before using them, these tubes have been made moist by introducing 1mL of sterilised physiologic solution in each one. At least ten samples (of 1g each) have been collected: five from the inferior part and five from the lateral sides. The collecting has been made by wiping 1cm^2 surface from each chosen spot; each tube being numbered, the tampon has afterwards been introduced in the corresponding tube. The place of each collection has also been noted down.

The seeding of samples has been executed by wiping with a tampon the surface of the Plate Count agar, divided in Petri plates in order to determine TNAMG, and then wiping the surface of the Sabouraud agar with antibiotics (chloramphenicol) for micromicetes. By dividing six triangular areas, up to six samples can be seeded on the surface of a Petri plate; the number of each sample has to be written in the corresponding seeded sector using eyeliner. The incubation time is 24-36 hours at a temperature of 37°C for TNAMG and 4-5 days at a temperature of 25-30°C for micromicetes. The corresponding colonies for each sample have been counted after incubation.

Surviving population of *Staphilococcus aureus* was determined by standard plating methods [13]. At each sampling time, colonies of *Staphylococcus* were selected, Gram-stained, and observed for catalase and oxidase reactions to confirm the presence of *Staphilococcus aureus*.

The traces of Ti, Cd, Pb and Hg from the analysed dermo-cosmetic products have been determined using the ICP-MS method.

3. Results and Discussions

Some samples characteristics: aspect, smell, color were determined in order to establish if these products are in accordance with standards demands (**Table 1**):

It has been noticed that all samples have a homogeneous aspect and a specific smell. The treatment cream for acne complexion (C I) is yellow, whilst C II and C III are white.

Table 1. The organoleptic characteristics of dermo-cosmetic products

Product	Aspect	Smell	Colour
C I	Homogeneous, easily spreadable	Specific	Yellow
C II	Homogeneous, consistent, easily spreadable	Specific	White
C III	Biphasic solution	Specific	White

The solvents which can dissolve dermo-cosmetic products are shown in **Table 2:**

Table 2: Solubility of studied samples

Solvent	C I	C II	C III
Methanol (methyl alcohol)	Entirely soluble	Insoluble	Insoluble
CCl_4	Insoluble	Insoluble	Insoluble
Hot water	Entirely soluble	Partially soluble	Insoluble
Benzene	Insoluble	Insoluble	Insoluble
Oil ether	Insoluble	Insoluble	Insoluble
HCl [35%]	Entirely soluble	Insoluble	Partially soluble

The analysed products are different when it comes to solubility: C I is soluble in methylic alcohol and HCl and insoluble in benzene and CCl_4. C II is only partially soluble in hot water and insoluble in CCl_4, benzene and HCl. C III is partially soluble in HCl and insoluble in CCl_4, benzene, oil ether and methylic alcohol.

Consequently to the stability test at 4°C and 40°C it has been concluded that all the analysed products are stable at 4°C.

The emulsion type, the pH and the melting point are shown in **Table 3.**

Table 3: Emulsion type, pH and melting point of dermo-cosmetic products

Product	Emulsion type	pH	Melting point (°C)
C I	O/W	4.54	62
C II	O/W	5.89	67
C III	O/W	4.67	61

It has been noticed that all the analysed dermo-cosmetic products imply the O/W emulsion type, but the pH varies: while C II has the highest pH, C I has the lowest one. This lowest pH value of C I is given by the glycolic acid content (indicated on the label). The concentration of glycolic acid in C I is 6%. Considering the value obtained using the pH-meter the closest to the skin pH is C II.

C III has the lower value of the melting point, while C II has the highest one. It is recommended that, in order to maintain the products' stability, they should be kept at a lower temperature, away from light.

The content of water and volatile substances, the acidity index and the saponification index in each case are shown in **Table 4.**

Table 4: The content of water and volatile substances, acidity and saponification indices in dermo-cosmetic products

Product	Content of water and volatile substances %	Acidity index mg KOH/g sample	The saponification index mg KOH / g sample
C I	72.22	16.26	19.18
C II	70.27	8.12	20.83
C III	50.00	11.92	19.7

Considering the water percentage has to be as high as possible, regardless the type of cream, C I is the most hydrating one. By opposition, C III has the lowest percentage of water. As the labels of creams C I and C II mention thermal water as the first ingredient, these results are reliable. The low water percentage of C III can be justified by the fact that the sample is a matifying purifying biphasic lotion from which the aqueous phase has been removed and that the content of water and volatile substances have been determined after this extraction.

According to the acidity index, meaning the KOH volume needed for neutralising the content of free fatty acids of the sample, C II and C III have the lowest acidity index and C I has the highest one. Considering that these products are used for treating acne and that the labels do not specify the presence of fatty acids, the results are reliable.

The saponification index, meaning the KOH volume needed for neutralising the fatty acids resulted from saponifying the analysed sample, has the lowest value at C I and the highest at C II. The results are in concordance with the acidity index indicated values. The results for the ester, the iodine and the peroxide indices are shown in **Table 5.**

Table 5. The ester, the iodine and the peroxide indices in dermo-cosmetic products

Product	Ester index mg KOH/g sample	Iodine index g iodine/100g sample	Peroxide index $mLNa_2S_2O_3/g$ sample
C I	2.92	0.51	19.54
C II	12.71	2.6	9.7
C III	7.78	2.44	9.771

The ester index, meaning the quantity (in mg) of KOH needed for neutralising the fatty acids resulted from the saponification of 1g of analysed sample, had the highest value at C II and the lowest one at C I.

The iodine index obtained for the three dermo-cosmetic products, meaning the content of unsaturated hydrocarbons, is the lowest for C I and the highest for C II. The quantity of iodine is low enough, indicating not only that there are no high concentrations of nonsaturated hydrocarbons.

The peroxide index has been obtained in higher quantity for C I and lower for C III, meaning that C I can be considered the best product due to its antibacterial and kerolitic effect of peroxides; this aspect has been also confirmed by the 0.1% content of retinaldehyde mentioned on the product's label.

In **Table 6** the total number of aerobic mesophil germens and the total number of micromicetes in the investigated dermo-cosmetic products are presented.

Table 6. The total number of aerobic mesophil germens and the total number of micromicetes in dermo-cosmetic products

Product	The total number of aerobic mesophil germens	The total number of micromicetes (TNM)
C I	Less than 10 viable colonies/1 g of product	Less than 10 viable colonies/1 g of product
C II	Less than 10 viable colonies/1 g of product	Less than 10 viable colonies/1 g of product
C III	Less than 10 viable colonies/1 g of product	Less than 10 viable colonies/1 g of product

The results of analysing the *Escherichia Coli* microorganism and the *Staphylococcus aureus* are shown in **Table 7**.

Table 7. The results of analysing the content of *Escherichia Coli and Staphylococcus aureus* in dermo-cosmetic products

Product	The *Escherichia Coli* microorganism	The *Staphylococcus aureus*
C I	Negative	Negative
C II	Negative	Negative
C III	Negative	Negative

Results show the absence of these microorganisms in the analysed dermo-cosmetic products.

The results regarding the presence of Ti, Cd, Pb and Hg in studied products are shown in **Tables 8** and **9**.

Table 8. The content of Ti in dermo-cosmetic products

Product	Ti (μg/mL)	RDS (%)
C I	0.2	17.23
C II	0.243	15.94
C III	0.247	15.34

Table 9. The content of Cd, Pb and Hg in dermo-cosmetic products

Product	Cd (μg/mL)	Hg (μg/mL)	Pb (μg/mL)	RDS (%)
C I	0.23	0	0	11.59
C II	0.32	0	0	11.74
C III	0.22	0	0	15.30

Linearity of the ICP – MS method was assessed by carrying out calibration plots with six concentrations with coefficients of determination exceeding 0.9993. The recovery studies were carried out in triplicate. The percentage recoveries (99-101%) were ranged between the limits imposed by the Horwitz equation (85-110%) for the established concentration range, indicating the high accuracy of the method. Consequently to the ICP-MS analysis of three dermo-cosmetic products, it has been noticed that none of the products contain traces of Hg and Pb, and that the content of Cd is higher in C II, while C I and C III have relatively equal concentrations.

In our studied creams lead was not present while in Nigeria metal analyses in cosmetic emulsions show the following levels for lead: 0.01-0.9 mg/L [14]. This might be due to use of utility water obtained from lead pipes. The presence of lead in cosmetics has also been reported and thus the European Union (EU) law for cosmetic banned lead and lead compounds in cosmetics since 1976 [15], however trace amount of lead are unavoidable under conditions of good manufacturing practice [16].

Although the presence of cadmium in the samples were in trace amount (0.22-0.32 μg/mL) but the slow release of cadmium with low amount may also cause harmful effects to the human body. The presence of cadmium has also reported in various lipsticks [17] it does not have to be present in abundance in products to produce hypertension. In fact, when cadmium was injected directly into the subject it caused blood pressure to drop. So the small amounts are not safe. It targets blood vessel and heart tissue, the kidneys, lungs and brain, and results in heart disease, hypertension, liver damage, suppressed immune system and other nasty symptoms [18].

4. Conclusions

Three types of Romanian dermo-cosmetic products have been analysed: a treatment cream for acne complexion (C I), an anti-irritating soothing cream (C II) and a matifying purifying lotion (C III); their quality has also been tested. All these products are O/W emulsions, and the pH varies: C I and C III have similar values (4.54, respectively 4.67), whilst C II is the closest to the skin pH (5.84). As a result of testing the stability of the three products it has been noticed that they are stable starting from 4°C and even reaching temperatures between 40-60°C. Their melting points have been established to be 62°C for C I, 67°C for C II and 61°C for C III. As the water content has to be as high as possible, C I is considered to be the most appropriate; C III has the lowest content of water. C II has the lowest acidity index, whilst C I has the highest one, aspect also confirmed by the glycolic acid concentration (6%) mentioned on the product's label. C I has the lowest saponification index, while C II has the highest one. The results are in concordance with the values of the acidity index. While C I has the lowest iodine index, C II and C III have the highest one, indicating that C I has a higher stability at oxidation and polymerisation. The Ti content has similar values for all the three products. The analyses made for determining the microbial charge have proven that the three dermo-cosmetic products do not contain any aerobic pathogen germens and micromicetes, *Staphylococcus aureus* and *Escherichia Coli*. The ICP-MS analysis has proven that the studied dermo-cosmetic products do not contain traces of Hg and Pb; also, the Cd content is higher for C II, while C I and C III have relatively equal concentrations.

5. References

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