Original article

Influence of thermal water and its oligoelements in the stability and efficacy of dermocosmetics formulations

Influência da água termal e de seus oligoelementos na estabilidade e eficácia de formulações dermocosméticas

ABSTRACT

Background: It has been proposed that thermal water can be used as an anti-inflammatory and mild hydrating agent in the preparation of skincare formulations. However, due to the complex process and strict quality control associated with its extraction from the ground, an oligoelements complex that mimics the effects of thermal water on skin is being used instead.

Objective: To evaluate the stability and effects of thermal water and its oligoelements in the formulation of cosmeceuticals, as well as their immediate effects on the skin.

Methods: An aqueous gel, a gel cream and an emulsion were prepared with and without the addition of oligoelements and submitted to physical stability analysis. The more stable preparations were tested on study subjects' forearms. The objective evaluation of the effects on the skin's texture and hydration was conducted according to biophysical and image analysis techniques before, immediately after and 2 hours after application. Questionnaires assessed participants' subjective perceptions of the various formulations. The possible anti-inflammatory effects were not evaluated.

Results: The effects of the more stable gel formulations were assessed on 15 study subjects. The gels increased the content of the aqueous corneum stratum, while the waters only produced an improvement of the skin's texture.

Conclusion: The results obtained from the subjective and objective analyses coincided and suggested the usefulness of the oligoelements complex in cosmeceutical products for moisturizing the skin.

Keywords: oligoelements; cosmetics; wetting agents.

RESUMO

Introdução: *a água termal vem sendo proposta como agente anti-inflamatório e hidratante leve. Posto que há rígido controle de qualidade e, portanto, dificuldade para sua extração do subsolo, um complexo de oligoelementos tem sido utilizado com finalidade equivalente.*

Objetivo: *avaliar a estabilidade e a influência da água termal ou de seus oligoelementos em formulações cosmecêuticas, assim como os efeitos imediatos de sua aplicação na pele.* ***Métodos:*** *foram elaborados gel aquoso, gel-creme e emulsão acrescidos ou não de oligoelementos e submetidos à análise de estabilidade física. Na análise da eficácia, 15 voluntárias aplicaram nos antebraços as formulações que demonstraram melhor estabilidade. A avaliação objetiva dos efeitos imediatos e após duas horas, em relação à hidratação e textura da pele, foi realizada através de técnicas de biofísica e análise de imagem, antes e após a aplicação. A avaliação subjetiva foi obtida por questionário de percepção desses efeitos pelas voluntárias. Não foi avaliada a possível ação anti-inflamatória.*

Resultados: *os géis foram considerados mais estáveis e selecionados para os testes de eficácia; também proporcionaram aumento do conteúdo aquoso do estrato córneo, e as águas apenas melhoraram a textura da pele.*

Conclusão: *os resultados obtidos na avaliação subjetiva e objetiva foram coincidentes e sugeriram a utilidade do uso de cosmecêuticos com oligoelementos na hidratação da pele como coadjuvantes em tratamentos dermatológicos.*

Palavras-chave: *oligoelementos; cosméticos; umectantes.*

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INTRODUCTION AND OBJECTIVE

Thermal water is a peculiar type of underground water, enriched with minerals such as sodium, magnesium, zinc, boron and manganese that is present in subsoil rocks, which emerges on the surface in the form of fountains. Experimental studies have shown that these oligoelements stimulate the migration of keratinocytes and could contribute for the cellular renewal process.[1] Although thermal water fountains can be found almost everywhere in the world, thenecessary technology for its use is not always available, due to the rigid quality control needed after it is extracted from the ground. Therefore a synthetic oligoelements compound has been developed to mimic the effects of thermal water on the skin. The importance of several inorganic ions – such as calcium, sodium, zinc, magnesium, manganese and potassium – in the composition of the stratum corneum has been documented.[2] There is evidence that pure thermal water might have mild moisturizing and anti-inflammatory effects when used as a vehicle or an active principle in cosmeceutic formulations. In this way, natural thermal water or when used in the preparation of products including cosmeceuticals, may facilitate hydration of the skin, recovery from cosmetic procedures such as chemical peels and laser treatment, and to treat cutaneous aging, acne, rosacea and other inflammatory dermatoses.[3,4,5]

When developing cosmeceutic formulations one should consider, among other factors, the compatibility and stability of the active substances – in particular, the product's ability to maintain its physical characteristics and rheological behavior after having been manufactured and packaged.[6,7]

Additionally, the effectiveness of and consumer preference for such products must be investigated. The use of non-invasive techniques to evaluate the effectiveness of cosmeceutical formulations is recommended and considered sufficient for the registration of these products, as well as for cosmetics, by the regulatory agencies in Brazil, the United States and Europe – Anvisa, FDA and Colipa, respectively.[8] Although the term "cosmeceutic" is still not formally recognized by those agencies and causes some confusion, the development and offering of such products – also called functional cosmetics – has been quickly expanding. New substances are developed based on an improved understanding of the physiology of human skin and are introduced to the field of dermatology to address the demand for products that can effectively improve the appearance of the skin and complement other dermatological treatments.[9]

There are several water- and thermal water-based cosmeceuticals on the market. It is important to evaluate the real benefits of these elements for the skin using studies of good methodological quality. This information can help dermatologists decide whether to recommend these products to their patients.

This study evaluated the stability and influence of thermal water or its oligoelements in cosmeceutical formulations, as well as the immediate effects of its topical application in solutions or when delivered in dermocosmetics.

METHODS

Design: This was an experimental study involving healthy volunteers. The study cannot be considered an intervention analysis since the application of the formulations on the skin occurs only once and has no therapeutic purpose.

Formulations and Waters: A compound of oligoelements based on salts of sodium, magnesium, zinc and L-carboxy pyrrolidine acid manganese was used as the raw material. The waters subjected to testing were: (A1) distilled and isolated deionised water; (A2) distilled and deionized water containing 1% oligoelements compound; and (A3) a commercial thermal water. Three formulations were elaborated: (F1) an emulsion based on batyl alcohol and soy lecithin, polymers of acrylates and of methyl phenyl polysiloxane; (F2) a gel based on a polysaccharide of biotechnological origin; and (F3) a gel cream based on a polysaccharide of biotechnological origin and methyl phenyl polysiloxane. These formulations were evaluated in their isolated form (F1A, F2A and F3A), with the addition of 1% of the oligoelements compound (F1B, F2B, F3B) and with the addition of the thermal water (F1C, F2C, F3C).

Stability Evaluation: All formulations were subjected to centrifugation tests, pH determination and visual evaluation. The formulations selected in these tests were subjected to a rheological behavior study, accomplished with a Cone and Plate type (USES, Brookfield, Massachusetts) DV-III digital rheometer, for the evaluation of the parameters of viscosity, consistence index, flow index and thixotropy. The formulations were maintained in normal environmental conditions and in an oven at 37°C and 45°C. Samples (0.5g) were collected in intervals of 7 days for a period of 28 days.[7]

Efficacy Evaluation: The more stable formulations were selected. The samples were either left in their original state or were combined with a 1% oligoelements compound or with commercial thermal water, and were submitted for subjective (self-evaluation through questionnaires) and objective (non-invasive techniques) evaluation of effectiveness. After being granted approval by the Human Subject Institucional Review Board from the University of Pharmaceutics of Ribeirão Preto – University of São Paulo, volunteers with phototypes II and III skin were recruited. The volunteers' forearms were divided in three areas and thermal water or a formulation was applied in each area. The areas, the formulations and the water were chosen in a randomized way in order to minimize differences among the analyses. Skin condition was evaluated through biophysical and image analysis techniques before (basal values), immediately after and two hours after the application of the formulations and water. The basal measures were taken after 20 minutes of acclimatization in a controlled atmosphere, at temperatures ranging from 20 to 22°C and with relative humidity between 45% and 55%.

Measurements to determine the aqueous content of the corneum stratum were taken using a Corneometer CM 825 (Courage & Kazaka Electronic GmbH, Köln, Germany), which gauges the hydration level of the skin based on the principle of electric capacitance measurement.[8,10] To evaluate the function of the skin as a barrier, a Tewameter TM 210 (Courage & Kazaka Electronic GmbH) measured the evaporation of water on the skin's surface. [10] To assess the viscoelastic characteristics of the skin, a Cutometer SEM 575 (Courage & Kazaka Electronic GmbH) measured the ratios of viscoelasticity/skin elasticity (Uv/Ue) and biological elasticity (Ur/Uf) 11. Skin texture was evaluated by determining the cutaneous micro-relief using a Visioscan VC 98. This device measures the skin's rugosity using the technique of optical profilometry, which digitizes images obtained from a video camera equipped with a unit that emits ultraviolet light (SEsm - skin smoothness). [12,13]

After the application of the formulations and waters, the volunteers completed questionnaires that evaluated several parameters, including: sensation to the touch, viscosity, and spreadability of the formulations and waters; and the general aspect, softness, shine, texture and hydration of the skin. Additionally, study subjects were questioned about the perception of effectiveness of the formulations and purchase intention.

All data were submitted to statistical analysis for the test of Analysis of Variance using MINITAB software.

RESULTS

Formulations' Stability: All formulations analyzed presented stability in the preliminary stability tests, having been selected for the rheological behavior evaluation. From the rheological behavior evaluation, it was possible to observe that formulation F1 presented higher values of apparent viscosity in relation to the other formulations and, when 1.0% of the oligoelements compound was added, a decrease in the viscosity values could be observed (Table 1). Formulation F2 presented the smallest variation in the values of apparent viscosity.

All formulations presented flow indices less than 1, indicating a pseudoplastic behaviour (Table 2). Formulation F1 presented the highest thixotropy values and, additionally, the larger fluctuations in those values. The addition of oligoelements to this formulation further elevated its thixotropy (Tables 3 and 4, respectively).

Formulations' Effectiveness: Volunteers with phototypes II and III skin (n=15) aged 25-35 years were selected for this study. The results obtained in the evaluation of immediate effects showed that all gels (F2A, F2B and F2C) caused a significant increase in the corneum stratum's aqueous content 2 hours after application when compared with basal values (Figure 1). The different types of water studied did not produce statistically significant differences in this parameter.

The parameters related to evaluating the function of the skin as a barrier (Figure 2) –viscoelasticity/elasticity and biological elasticity ratios, and the skin's micro-relief (Figure 3) – did not present any statistically significant alteration 2 hours after the application of the formulations under assessment.

No significant differences among the parameters were observed in the sensorial evaluation and effectiveness perception test after a single application, when comparing the differences in the gels' formulations (F2A, F2B and F2C) and waters (A1, A2 and A3) amongst themselves.

When comparing the gels with the waters, it was observed that in some parameters (sensation to the touch, viscosity and sensation in the skin five minutes after the application) (Figure 4), the waters scored significantly higher than the gels. The gels were more effective in the hydration parameter, according to the volunteers' self-evaluations (Figure 5).

DISCUSSION

The topical application of thermal water, both in its pure state and as a vehicle or active principle in formulations, has been increasing due to the growing interest for products that act as anti-inflammatory and moisturizers.

The development of cosmeceutic products containing thermal water or its oligoelements – if in their stable form and thus guaranteeing their effectiveness – may represent an important contribution to the prevention and control of dry skin. Effective products may serve as complementary therapy for some inflammatory dermatoses, post cosmetic procedure reactions and irritations resulting from dermatological treatments of conditions such as acne and melasma. Therefore, studies must clearly and objectively evaluate both the effectiveness and safety of such products, since products marketed as cosmeceuticals must conduct safety studies that prove the product does not cause adverse reactions.

Current standards require that such formulations undergo an experimental phase, with rigorous tests, followed by non-invasive, in vivo evaluations that demonstrate a high degree of

Table 1 - Minimum apparent viscosity (cP) of the formulations studied (F1, F2 and F3) containing 0% or 1% oligoelements compound (FO1, FO2 and FO3), when kept in the initial environment (baseline) and subjected to 45ºC for 7, 14, 21 and 28 days. Values calculated at the maximum point of shear.

	Minimum apparent viscosity (cP)				
Form.	Initial environment	45ºC			
		7 days	14 days	21 days	28 days
F1	1194.000	1185.000	1042.000	1027.000	970.700
FO1	914.200	970.700	901.900	889.600	872.400
F2	987.900	946.100	975.600	1020.000	1052.000
FO2	951.100	963.300	983.000	926.500	973.200
F3	1044.000	1037.000	1003.000	1140.000	1106.000
FO3	842.900	978.100	960.900	987.900	1005.000

Table 2 - Flow Index of the formulations studied (F1, F2 and F3) containing 0% or 1% oligoelements compound (FO1, FO2 and FO3), when kept in the initial environment (baseline) and subjected to 45ºC for 7, 14, 21 and 28 days.

Form.	Flow index				
	Initial environment	45ºC 7 days	14 days	21 days	28 days
F1	0,270	0,310	0,330	0,330	0,330
FO1	0,330	0,320	0,350	0,340	0,340
F2	0,180	0,150	0,140	0,170	0,130
FO2	0,200	0,190	0,170	0,160	0,190
F3	0,200	0,180	0,190	0,160	0,170
FO3	0,240	0,230	0,170	0,160	0,160

reliability.[15] Dermatologists should understand the approval process in order to effectively evaluate new products.

The formulations of this study were submitted to the pH evaluation and preliminary tests of physical stability. Evaluating the pH was important for the stability of the formulation as well as for its compatibility with the skin's acid pH 16; the formulations presented pH values of between 6 and 7, a band considered appropriate for the purposes of this study.

The evaluation of the rheological behavior provided important information on the physical stability of cosmetics and cosmeceuticals, given that the consistence and the spreadability of the products must not vary among lots, assuring the technological quality of the finished product.[6]

All formulations presented a pseudoplastic behavior, meaning that a flow index less than 1 – an interesting characteristic for gels and emulsions implying a reduced viscosity and application of force – facilitating their spreadability on the skin.[16]

Regarding the thixotropy, a formulation that presents high values requires longer to reconstitute its structure after the interruption of a force.[17] Formulation F1 presented high thixotropy values and was thus considered less stable than the others.

The analysis of the group of variables involved in the rheology of the formulations showed that formulation F2 was the most stable, therefore it was included in the effectiveness evaluation.

The objective evaluation of the formulations' effectiveness using non-invasive methods makes it possible to conduct studies under real use conditions on human skin. The evaluation of the immediate effects is important because it allows verification of reported alterations in the skin's condition and to have influence in the adherence to the use of cosmeceutic products.

The forearm region was chosen for the evaluation of cosmeceutical products' effectiveness because they present advantages such as the volunteers' greater adherence due to the ease of application and evaluation, as well as lower interference from environmental conditions and lifestyle.[8,11,15]

Regarding the stratum corneum's aqueous content, the results obtained in the evaluation of the short-term effects showed that all gels caused a significant increase two hours after application, providing a significant hydrating effect, whereas the waters did not present that effect.

In the evaluation of the function of the skin as a barrier, both the gels and the waters decreased the TEWL (transepidermal water loss) two hours after a single application; however, those alterations were not statistically significant. It is possible that prolonged use could have contributed to the improvement of the protecting role of the skin. According to some authors, thermal waters in the area of Saint-Gervais, France, rich in boron and manganese, stimulate the migration of keratinocytes in vitro, being capable of improving the healing of wounds.[1,18] The zinc acts in the cutaneous physiology, modulating inflammations, accelerating the reepithelialization process and the proliferation of keratinocytes and fibroblasts.[19] Manganese and cop-

Table 3 - Area of hysteresis of the formulations studied (F1, F2 and F3) containing 0% or 1% oligoelements compound (FO1, FO2 and FO3), when kept in the initial environment (baseline) and subjected to 45ºC for 7, 14, 21 and 28 days.

Form.	Area of hysteresis ($dyne/cm^2 . s$)				
	Initial environment	45ºC 7 days	14 days	21 days	28 days
F1	4077,484	2858,564	2669,828	2866,428	2492,888
FO1	4981,844	3743,264	4529,664	4454,956	4683,012
F2	169,076	-271,308	1380,132	2107,552	1045,912
FO2	-896,496	1.226,784	1309,356	1498,092	1301,492
F3	711,692	1.183,532	-994,796	1942,408	817,856
FO3	-672,372	-287,036	-931,884	-2.760,264	-1686,828

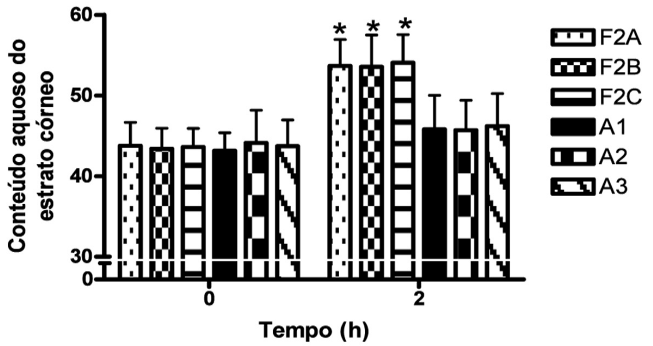

Figure 1: Aqueous content of corneum stratum on subjects' forearms before and 2 hours after application of formulations: F2A (vehicle), F2B (vehicle containing 1% oligoelements compound), F2C (vehicle containing 1% commercial thermal water) and A1 (distilled and deionized water), A2 (distilled and deionized water containing 1% oligoelements compound) and A3 (commercial thermal water).

** significant difference in relation to the baseline (p < 0.005).*

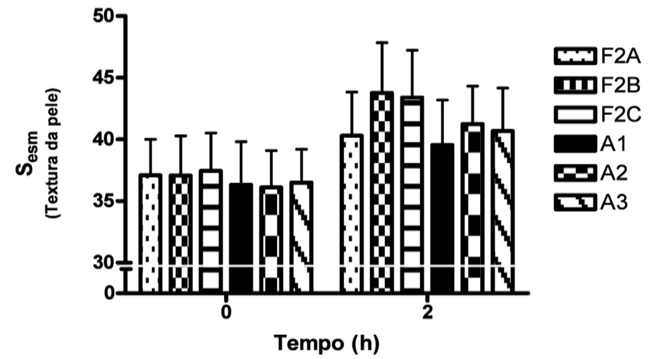

Figure 3: SEsm (skin smoothness) in the volunteers' forearm area, before and 2 hours after application of formulations: F2A (vehicle), F2B (vehicle containing 1% oligoelements compound), F2C (vehicle containing 1% commercial thermal water) and A1 (distilled and deionized water), A2 (distilled and deionized water containing 1% oligoelements compound) and A3 (commercial thermal water).

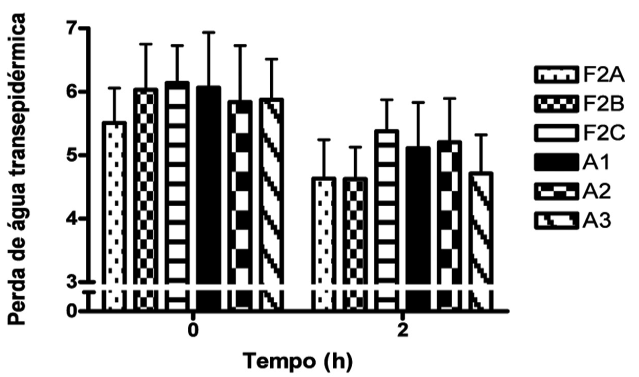

Figure 2: Transepidermal water loss in the volunteers' forearm area, before and 2 hours after application of formulations: F2A (vehicle), F2B (vehicle containing 1% oligoelements compound), F2C (vehicle containing 1% commercial thermal water) and A1 (distilled and deionized water), A2 (distilled and deionized water containing 1% oligoelements compound) and A3 (commercial thermal water).

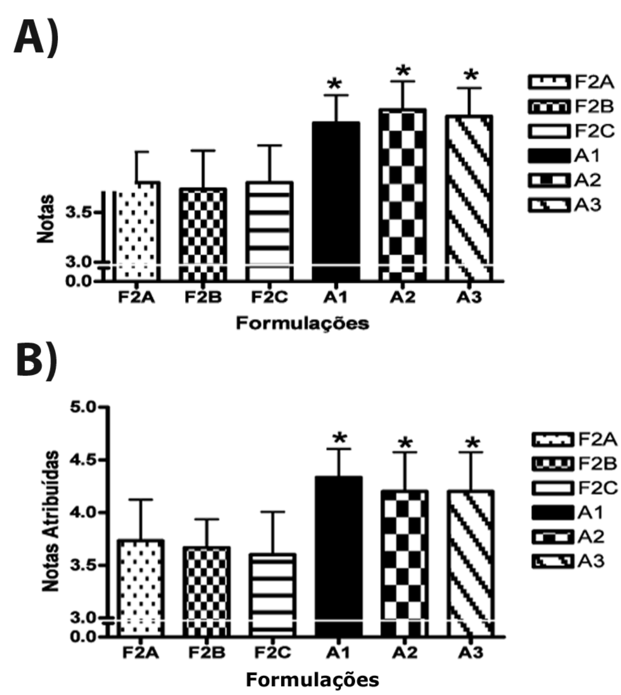

Figura 4: Sensorial evaluation of parameters "sensation to the touch and viscosity" (A) and "sensation after five minutes" (B), of formulations: F2A (vehicle), F2B (vehicle containing 1% oligoelements compound), F2C (vehicle containing 1% commercial thermal water) and A1 (distilled and deionized water), A2 (distilled and deionized water containing 1% oligoelements compound) and A3 (commercial thermal water).

** significant difference in relation to the formulations F1, F2 and F3 (p<0,005).*

per salts can also stimulate the proliferation of keratinocytes, accelerating the recovery of the cutaneous barrier.[18]

In the parameters related to the viscoelasticity/elasticity ratio, the biological elasticity and the skin's micro-relief, although no statistically significant alterations were observed, the results obtained for the gels showed an improvement in the cutaneous micro-relief as compared to the parameter texture (SEsm - skin smoothness). The water solutions also produced improvement in the skin's texture 2 hours after application. It was also observed that when either in solutions or delivered in gels, the oligoelements showed even better effects in the texture of the skin.

Sensorial analysis is important in the development of a product because the user can vary the frequency and amount of use, depending on her or his higher or lower acceptance of its characteristics, which may have an impact on its effectiveness.[20] However, no significant differences were observed between the formulations in the sensorial evaluation and effectiveness perception.

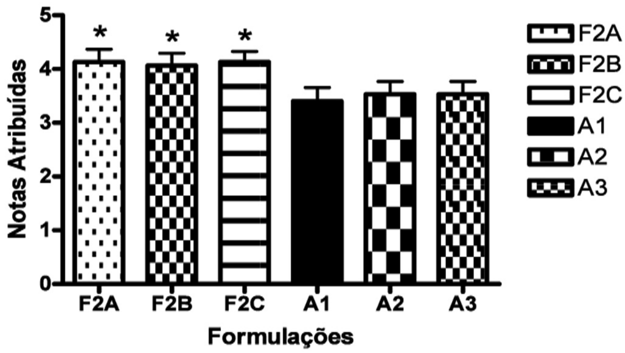

Figura 5: Perception of efficacy (subjective evaluation) regarding the hydration provided by the application of formulations: F2A (vehicle), F2B (vehicle containing 1% oligoelements compound), F2C (vehicle containing 1% commercial thermal water) and A1 (distilled and deionized water), A2 (distilled and deionized water containing 1% oligoelements compound) and A3 (commercial thermal water).

** significant difference in relation to the the waters A1, A2 e A3 (p<0,005).*

A study of sensorial evaluations with thermal waters of different origins has shown that, independently of the concentration of minerals, all caused a pleasant sensation in the skin five minutes after application, which coincides with the results obtained in the present study. However, according to the authors, the smaller the concentration of minerals in the water, the more intense the sensation of softness in the skin ten minutes after application. [21]

When comparing gels and waters, it was possible to observe that, in the parameters of sensation to the touch, viscosity and sensation in the skin five minutes after application, the waters obtained significantly higher marks than the gels, whereas in the parameter of hydration, the gels were more effective according to the volunteers' self-evaluation.

In this manner, in what concerns to the hydration of the skin, this study has showed that the subjective evaluation was coincident with the objective evaluation, once the gels increased the aqueous content of the stratum corneum and the volunteers, when evaluating the perception of effectiveness, have attributed significantly higher marks for the gel formulations.

CONCLUSION

In the experimental conditions of this study it was possible to conclude that:

1) as for the rheological behaviour, the formulation in gel based on polysaccharide of biotechnological origin presented the highest stability

2) in the objective evaluation, the formulations in gel provided a significant increase in the values of the aqueous content of the corneum stratum, with a tendency of improvement in the texture and the skin's function as a barrier

3) in the sensorial evaluation, after 5 minutes of the application, the waters presented a better acceptance by the volunteers when compared to the gels

4) as for the perception of the effects about the hydration of the skin, the formulations in gels obtained the highest marks in the subjective evaluation

5) regarding the moisturizing effect of the gels, both the subjective and the objective analyses were coincident

6) in the objective evaluation, the thermal waters did not show significant immediate effects regarding the hydration; only an improvement tendency in the texture and function of the skin as a barrier; the sensorial perception was adequate

7) in this study, it was not possible to identify significant alterations in the elasticity and relief of the skin neither suggest any anti-inflammatory activity

8) in spite of the favourable stance of sensorial perception towards the waters, the cosmeceutic formulations in gels provided a better hydrating effect, suggesting the usefulness of that class of products for the hydration of the skin, as well as for adjuvant use in dermatological treatments ●

REFERENCES

1. Chebassier N, Ouijja EH, Viegas I, Dreno B. Stimulatory effect of boron and manganese salts on keratinocyte migration. Acta Derm Venereol. 2004;84(3):191-4.
2. Nakagawa N, Sakai S, Matsumoto M, Yamada K, Nagano M, Yuki T, et al. Relationship between NMF (lactate and potassium) content and the physical properties of the stratum corneum in healthy subjects. J Invest Dermatol. 2004;122(3):755-63.
3. laquieze S, Czernielewski J, Baltas E. Beneficial use of Cetaphil moisturizing cream as part of a daily skin care regimen for individuals with rosacea. J Dermatolog Treat. 2007;18(3):158-62.
4. Hashizume H. Skin aging and dry skin. J Dermatol. 2004;31(8):603-9.
5. Draelos ZD, Ertel KD, Berge CA. Facilitating facial retinization through barrier improvement. Cutis. 2006;78(4):275-81.
6. Leonardi GR, Maia Campos PMBG. Estabilidade de formulações cosméticas. Int J Pharm Compounding. 2001;3(4):154.
7. Gaspar LR, Maia Campos PMBG. Rheological behavior and the SPF of sunscreens. Int J Pharm. 2003;250(1):35-44.
8. Leonardi GR, Gaspar LR, Maia Campos PMBG. Application of a non-invasive method to study the moisturizing effect of formulations containing vitamins A or E or ceramide on human skin. J Cosmet Sci. 2002;53(5):263-68.
9. Draelos ZD. The cosmeceutical realm. Clin Dermatol. 2008;26(6):627-32.
10. Dal' Belo SE, Gaspar LR, Maia Campos PMBG. Moisturizing effect of cosmetic formulations containing Aloe vera extract in different concentrations assessed by skin bioengineering techniques. Skin Res Technol. 2006;12(4):241-6.
11. Dobrev H. Use of cutometer to assess epidermal hydration. Skin Res Technol. 2000;6(4):239-44.
12. De Paepe K, Lagarde JM, Gall Y, Roseeuw D, Rogiers V. Microrelief of the skin using a light transmission method. Arch Dermatol Res. 2000;292(10):500-10.
13. Koh JS, Kang H, Choi SW, Kim HO. Cigarette smoking associated with premature facial wrinkling: image analysis of facial skin replicas. Int J Dermatol. 2002;41(1):21-7.
14. Rodrigues L, Pinto P, Silva N, Galego N, Quaresma P, Fitas M, et al. Caracterização da eficácia biológica de hidratantes por análise dinâmica do conteúdo hídrico epidérmico e profilometria de transmissão luminosa. Cosmet Toiletr. 1997;9(2):44-9.
15. Rogiers V, Balls M, Basketter D, Berardesca E, Edwards C, Eisner P, et al. The potential use of non-invasive methods in the safety assessment of cosmetic products - The report and recommendations of ECVAM/EEMCO Workshop 36. Altern Lab Anim. 1999;27:515-37.
16. Martin A, Bustamante P, Chun AHC. Rheology. 4th ed. Philadelphia: Lea & Febiger; 1993.
17. Dahms GH. Einflu, der thixotropie auf die lichtschutzwirkung von sonnenschutzemulsionem. Parfum Kosm. 1994;75(10):675-9.
18. Tenaud I, Leroy S, Chebassier N, Dreno B. Zinc, copper and manganese enhanced keratinocyte migration through a functional modulation of keratinocyte integrins. Exp Dermatol. 2000;9(6):407–16.
19. Dreno B. Oligoelements et peau. Dermatologie Pratique 1996;182(1):1-3.
20. Distante F, Pagani V, Green B, et al. Objective evaluation of placebo effect in cosmetic treatment [CD-ROM]. Florence: Cosmetic Conference; 2005.
21. Bacle I, Meges S, Lauze C, Macleod P, Dupuy P. Sensory analysis of f our medical spa spring waters containing various mineral concentrations. IntJ Dermatol. 1999;38(10):784-6.