Ana Catarina da Silva Marques

Biometrologic Evaluation of Cosmetic Products

Monografia realizada no âmbito da unidade de Estágio Curricular do Mestrado Integrado em Ciências Farmacêuticas, orientada pelo Professor Doutor António José Ribeiro e apresentada à Faculdade de Farmácia da Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

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Coimbra, 06 de Setembro de 2016.

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(Ana Catarina)

The tutor

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(António José Ribeiro)

The student

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(Ana Catarina da Silva Marques)

Thanks

To my professor adviser, António Ribeiro, for the aid and availability, as well as the illustrious guidance.

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To the real city of student, of love and longing, Coimbra.

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List of abbreviations and acronyms

EEMCO – European Group for Efficacy Measurements on Cosmetics and Other Topical Products

EU – European Union

ICH – International Conference on Harmonization

LED – Light Emitting Diode

NMF – Natural Moisturizing Factor

NIR – Near-Infrared

PCA – Pyrrolidone carboxylic acid

RRT – Ressonance Running Time

TEWL – Transepidermal Water Loss

UV – Ultra Violet

Abstract

Given the growing importance that cosmetic products have on human's health and in our daily life, it is important to increase the control of these products, both in terms of safety and effectiveness. Taking into account that conducting animal tests for the production and validation of cosmetic products is prohibited by law, producers of these products have to resort to alternative methods. Biophysical methods have gained an important highlight in the scientific community, in particular the non-invasive methods. They allow a safe and faster evaluation of cosmetics.

The purpose of this work is to describe some methods and equipments used at national and European level to test the effectiveness of cosmetic products and correlate the parameters evaluated with the alleged properties in the products. The methods include evaluation tests of the following skin properties: hydration, elasticity, coloring, sebum production and perspiration.

Keywords: claims, coloring, cosmetics, effectiveness, elasticity, hydration, legislation, methods, sebum production, tests, transepidermal water loss.

Resumo

Tendo em conta a importância crescente que os produtos cosméticos têm assumido na saúde humana e no dia-a-dia do Homem, é necessário aumentar o controlo destes produtos, tanto a nível de segurança como de eficácia. Desta forma, a legislação, nomeadamente a legislação europeia, define que, de forma a proteger o consumidor, é necessária a realização de vários testes que comprovem a segurança e eficácia dos produtos comercializados. Tendo em conta que a realização de testes em animais para a produção e validação de produtos cosméticos é proibida por lei, os produtores destes produtos têm de recorrer a métodos alternativos. Os métodos biofísicos têm ganho um relevante destaque no meio científico, em particular, os métodos não-invasivos, pois permitem uma avaliação segura e mais rápida aos indivíduos que se voluntariam para esses testes.

O objectivo deste trabalho é a descrição de alguns métodos e equipamentos utilizados a nível nacional e europeu para testar a eficácia dos produtos cosméticos e correlacionar os parâmetros avaliados com as propriedades alegadas nos produtos. Os métodos incluem testes de avaliação das seguintes propriedades da pele: hidratação, elasticidade, coloração, produção de sebo e transpiração.

Palavras-chave: alegações, coloração, cosméticos, eficácia, elasticidade, hidratação, legislação, método, sebo, testes, transpiração.

Introduction

From the earliest records of man's life in earth it is possible to see his concern with his image, because even at that time the *Homo sapiens sapiens* created decorative objects and used natural materials to embellish himself. With the knowledge of human anatomy, histology and the development of technology, the skin and factors related to it began to gain importance in the field of human's health. The skin is the largest organ of the human body and establishes the first contact with the exterior environment, due to this, taking care and protect it became necessary to reach a healthy life.

The use of cosmetic products goes back to prehistory as it is seen in rock paintings. Later on, the Egyptians were the ones that gave most importance to body care, beauty and cosmetics as they were associated to being similar to the gods. Since then, dermatology and cosmetics started to evolve in a continuous way and it will continue to evolve so it can be able to fulfill the human needs (HERNANDO, 2016).

In the 21st century, a cosmetic product is defined as "any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition" (INFARMED, 2016). These products are available in countless forms of presentation and due to the fact that they are so required they become "ordinary" in a certain way. However it is important not to forget that these products are in touch with our body and even if, by definition, they do not get to the bloodstream, they are still able to modify our skin properties. Due to these facts, and to protect the consumer, several laws have been developed to oblige the producers to execute tests to ensure safety and the effectiveness of these products. According to the Regulation (CE) N.° 1223/2009 of the European Parliament and of the Council of 30 november 2009 on cosmetic products, these tests stopped being executed in animals (with some exceptions described in the regulation). In this sense, the laboratories started to develop alternative methods.

The consumers' demands tend to increase proportionally with the technological development and they want to ensure that they buy a product that satisfies their requirements and, above all, that comply with the claims associated with it. According to the

law, the companies must justify their products claims and they do it resorting to tests, usually biophysics (EUR-LEX, 2009).

This work has the purpose to discriminate and explain the tests generally used by cosmetic products laboratories to evaluate their products effectiveness and justify their claims, in a national and European level. In all the methods we can find described in the literature (books, articles, magazines and journals) were selected those which are referenced in a standard manner by different cosmetics production laboratories, as Expanscience Laboratories, with which I had the opportunity to contact, and external evaluation laboratories that evaluate cosmetic products, such as Inovapotek, a Portuguese company located in Porto.

Considering the parameter to evaluate, the indicated tests can be divided in tests for evaluation: of the cutaneous hydration, cutaneous elasticity, cutaneous coloring, cutaneous sebum and cutaneous sweat.

Cosmetics legislation and regulation

Only cosmetic products for which a legal or natural person is designated within the Community as responsible person shall be placed on the market. The responsible person is usually the manufacturer established within the Community except if the manufacturer designates a person to be the responsible person or when a cosmetic produced is exported and imported back into the Community. In that case, the responsible person is each one of the importers. Also the distributors shall be the responsible person when they place a cosmetic product on the market under their name or trademark or modify a product already placed on the market in such a way that compliance with the applicable requirements may be affected (EUR-LEX, 2009). The responsible person is responsible for the safety of their products and must ensure that all their products accomplish the cosmetics legislation applied in EU (EUROPEAN COMISSION, 2016).

In Portugal, *Infarmed* is the national entity responsible for the regulation and supervision of the cosmetic market allowing the consumers and health professionals to have safe and quality cosmetic products.

In order to try to protect consumers from misleading hype, gross exaggeration and preposterous claims, the European Group for Efficacy Measurements on Cosmetics and Other Topical Products (EEMCO) has produced a number of guidance papers and introductory reviews on the use of non-invasive methods for efficacy documentation of cosmetics, allowing the marketers of cosmetic products to use non-invasive technology to

substantiate "scientifically" claims of efficacy. Panels of experienced investigators have now provided explicit guidelines for measuring transepidermal water loss, laser Doppler imaging, dermoscopy, stratum corneum hydration, tristimulus colorimetry, optical profilometry, and others.

The prohibition of animal tests using the final cosmetic products since 2004 and the prohibition of ingredients tests in animals since 2009 has led to the prohibition of any kind of cosmetic product tested in animals in the European Union. Therefore, the Commission has determined alternative methods, *in vitro* or *in silico*, that should be validated to be accepted by the responsible entities (COMISSÃO EUROPEIA, 2013).

The first step for testing cosmetic products is to choose the proper kind of study, so when a study is initiated we can follow a checklist like the following example (SERUP, 2006):

"1. Is the study endpoint truly quantitative in nature, narrow enough for specific study, and truly suited to support the idea?

2. Which structure or function is actually being measured?

3. When should measurements be performed?

4. Interperson, intraperson, and intralesion variation, and if possible, variability data from normal and healthy skin?

5. Influence of gender, age, and race?

6. Statistical evaluation of the design and the size of the sample studied?

7. Studies and literature validating the instruments applied?

8. Guidelines and legal requirements, including ethical aspects?

9. Are environmental conditions such as temperature and humidity under control and expected to remain constant during the study period?

10. Needs for preconditioning of study subjects?

11. Calibration, maintenance, and control of instruments before, during, and at end of study?

12. How to conclude and report the study?"

The skin function and structure

The skin consists of three layers of tissue: the epidermis, an outermost layer that contains the primary protective structure, the stratum corneum; the dermis, a fibrous layer that supports and strengthens the epidermis; and the subcutis, a subcutaneous layer of fat

beneath the dermis that supplies nutrients to the other two layers and that cushions and insulates the body (FREINKEL & WOODLEY, 2001).

The regional variations are of great importance because they can influence skin behavior and thus susceptibility to disease. The major anatomical differences related to body placement involve stratum corneum thickness, distribution of appendages and melanocytes, variation in the structure of the dermoepidermal junction and of the dermis, and changes in blood supply (FREINKEL & WOODLEY, 2001).

The skin is subject to the influence of solar radiation, temperature, humidity, domestic and occupational contactants, therapeutic agents, and a host of environmental agents.

Skin aging, more or less a physiological event, is characterized by several biological and histopathological changes: transepidermal water loss (TEWL) and skin hydration both decrease; corneocytes size and thickness of the stratum corneum both increase leading to desquamation of the skin; corneum hydration is decreased in elderly subjects and moisture content is reduced in exposed areas (JORGEN SERUP, 2006).

The skin colour change is a natural defense mechanism from the sun light, more specifically, from the UV-A and UV-B radiation. That mechanism results in the production of the pigment melanin by the melanocytes (LE PHYSIQUE, 2015).

Anatomical changes often induce functional changes that can be quantified with combined non-invasive techniques that allow the assessment of skin function relative to sex, age, and race.

I- The epidermis

The epidermis is formed by four distinct layers: 1) basal layer, where the keratinocytes, or corneocytes are continuously formed by mitosis and where are the melanocytes, a specialized skin cells responsible for the production of the pigment melanin; 2) spinous layer which is immediately peripheral to the basal layer; 3) granular layer, with granules of keratohyalin contained in the cells; 4) stratum corneum which is peripheral to the granular layer and has keratinocytes that have lost their nuclei and most of their organelles and contents, including the keratohyalin granules. They become progressively flattened and filled with keratin and are ultimately desquamated (FREINKEL & WOODLEY, 2001).

1.1- The hydrated state of skin

The integrity of the stratum corneum of the epidermis influences the passage of water to the external environment and, consequentially, determines the water retention which will contribute to the skin elasticity. The water binds with the stratum corneum by soluble metabolites, structural proteic components and sebum components. The water evaporation from the skin surface is prevented by ceramides and intercellular lipids present in the stratum corneum and sebum (R. DARLENSKI, 2011).

The lipids and the natural moisturizing factor (NMF) maintain the skin hydration. This factor acts like a "sponge" that keeps water in stratum corneum providing to the skin a soft sensation. The composition of the NMF is described in the table 1.

Amino Acids	40%
Ammoniac, uric acid and other organic acids	1.5%
Pyrrolidone carboxylic acid (PCA)	12%
Ions Na+ , K+ , Ca2+, Mg2+, PO4 3- , Cl-)	18.5%
Urea	7%
Lactate	12%
Citrate	0.5%
Sugar, organic acids, peptides	8.5%

Table 1 - Composition of the NMF (SOLER, 2005)

- **non-invasive evaluation of the skin hydration**

Nowadays, the methods used to determine the skin hydration are based on the measurement of electrical capacitance, impedance and conductance (TAGAMI, 2006). By definition, impedance is related to the electric opposition suffered by the skin when exposed to an alternating current. Conductance is the skin capacity for transferring electrical current. In turn, capacitance is an electrical quantity which is determined by the amount of electrical energy that can be accumulated by the skin and the amount of alternating current determined at a certain frequency (Tagami, 2006). This kind of measure is of the most used methods for the evaluation of the hydric content. The water has a high dielectric constant and the stratum corneum is considered a dielectric medium that when hydrated leads to changes in dielectric properties. Thus, the capacitance changes proportionally as a function of the degree of skin hydration. For measure capacitance we can use equipments like Corneometer® from Courage Khazaka and MoistureMeter® from Delphin Tecnologies Ltd

(GABARD, CLARYS , & BAREL, 2006). The operation principle of the Corneomoter® is represented in the figure below.

FIGURE 1 - Corneometer® operation principle (with permission from Esther Bász, 2016) (COURAGE-KHAZAKA, 2016)

The Corneometer® CM 825 consists of gold electrodes interdigitated on a grid and covered with a thin layer of vitrified low dielectric constant insulation. During measurement, an electric field penetrates the surface layers of the skin (stratum corneum), by a probe applied vertically on the skin, and the dielectric constant is measured. The measure of capacitance is obtained 1 second after application and converted in arbitrary units (a.u.) from 0 to 130. Values of hydration below 30 a.u. correspond to very dry skin; between 30 and 40, dry skin; and higher than 40, normal skin. The capacitance results are affected by many factors as the age, sex, body area (palms and forehead are the most hydrated areas and inferior members and abdomen are the less hydrated areas), the environment (22°C e 50±5% for temperature and humidity, respectively) and the equipment (bad position of the probe, higher pressure of the probe into the skin or successive measurements on the same anatomical site may cause false results) (Cosderma-Laboratoire, 2016) (GABARD, CLARYS, & BAREL, 2006).

Another method used to evaluate the dynamic evolution of the skin hydration is the Raman spectroscopy. A probe is applied to the skin emitting a laser on it and detecting the scattered light (the majority of the scattered light is of the same frequency as the excitation source) (InPhotonics, 2012). This method has been applied for measurement of the depth profiles of different molecular concentrations of elements, e.g. water and free amino acids, in the skin in vivo, allowing to characterize and quantify the natural moisturizing factors (Egawa & Tagami, 2007) (CROWTHER & Et. Al., 2008).

This technique has shown promising results comparing to the previous techniques, like near-infrared (NIR) that only obtain viable values in the most superficial layers of the skin. Studies suggest that the variations in the concentration depth profiles of water, free amino acids and lipids in the skin are related to age, season and site, so these parameters can

influence the results obtained with Raman spectroscopy and should be taken into account (Egawa & Tagami, 2007).

1.2- Skin friction

Another parameter used to measure the skin hydration is the coefficient of friction. In theory, a surface is brought into contact with another and moved relative to it. When the two surfaces are brought into contact, the perpendicular force is defined as the normal force (N). The friction force (F) is the force that opposes relative movement between the two surfaces. In practice, a probe is pressed onto the skin with a known normal force and then is detected the skin's frictional resistance to the movement of the probe.

The Frictiometer FR 700 has a probe which contains a motor, a steering unit and the friction head with a plain, smooth Teflon disk. A constant rotational speed is applied onto the skin by the friction head. The torque is measured and the result is displayed as Frictiometer units (COURAGE KHAZAKA, 2016). The operation principle is represented in the figure below.

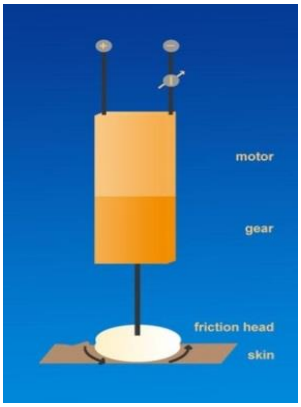

FIGURE 2 -Frictiometer operation principle (with permission from Esther Bász, 2016) (COURAGE-KHAZAKA, 2016)

Hydration studies revealed that dry skin has lowered friction while hydrated skin has an increased amount of friction. However, the skin response is more complex, because very wet skin also has a lowered friction coefficient, much like the characteristics of dry skin (SIVAMANI RK1, 2003).

Studies realized by Prall and Nacht *et al.* (NACHT, 1981) showed that the use of emollients and moisturizer creams make the friction coefficient rise in a similar fashion to water. However, the effects of the creams lasted for hours, while the water effects lasted for about 5 to 20 minutes (SIVAMANI & et al, 2006).

1.3 - Transepidermal Water Loss (TEWL)

The transepidermal water loss (TEWL) reflects the skin barrier integrity because when the water of the dermis arrives to the surface it is going to evaporate. We lose about 300 to 400 mL/24hours of water by evaporation (DMITRIEVA & BURG, 2011).

A healthy and non-damaged skin has a normal TEWL. However, people with a pathology that affects the skin barrier, like the atopic dermatitis, suffers an increased loss of skin water by evaporation (SEIDENARI & et al, 2006).

- **non-invasive evaluation of the Transepidermal Water Loss (TEWL)**

Many cosmetics producers have used the measurement of TEWL to support claims in their products like "mildness", "reduction in irritative skin reactions", "skin hydration", "skin repair", "protective effect against UV damage" and "anti-perspirant". This test can also screen ingredients that have a beneficial effect on the barrier function and offer the possibility to monitor *in vivo*, on human skin, the effect of topical treatment in an objective and non-invasive way (FLUHR & et al., 2011) (ROGIERS & GROUP, 2001).

TEWL measurement also has interest in studies for anti-inflammatory and moisturizing creams for atopic dermatitis. In fact, studies showed that certain moisturizers improve water barrier function and skin susceptibility to irritants in atopic patients (SEIDENARI & et al, 2006).

Nowadays there are different types of instruments capable of measuring TEWL using the open-chamber technique, such as the Tewameter®, TM210 and TM300, (Courage and Khazaka, Cologne, Germany) and DermaLab (Cortex Technology, Hadsund, Denmark). (TUPKER & PINNAGODA, 2006).

The Tewameter® TM 300 is the worldwide most accepted measuring device for the assessment of the Transepidermal Waterloss (TEWL) (COURAGE KHAZAKA, 2016).

The open chamber method of TEWL measurement is based on the diffusion principle in an open chamber,

$$\frac{Dm}{Dt} = -D.A.dp/dx$$

where diffusion flux, Dm/Dt (g/h/m^2) , represents the mass (m) of water transported in grams at a given time (t), and it's directly proportional to the water vapor diffusion coefficient in atmospheric air , D (mmHg), to the contact surface area, A (m^2) and to the

dp/dx, corresponding to atmospheric vapor pressure, p (mmHg), taking into account the length between the measuring site and the site of the skin surface, x (m)

The probe is applied perpendicularly to the skin and consists of a hollow cylinder with two pairs of sensors measuring temperature and humidity, one pair slightly higher than the other. It measures the moisture at two different sites and from this the TEWL can be calculated.

A high number of variables affecting TEWL measurements have been identified: age, ethnicity, anatomical position, skin temperature, sweating, circadian rhythm, skin health and external factors (temperature, humidity, direct light, etc). These should be rigorously taken into consideration, collected and reported with study results, to ensure meaningful communication of results (PLESSIS et al., 2013). However, in Tewameter® TM300, the small size of the probe head minimizes the influence of air turbulences inside the probe and the low weight of the probe ensures easy handling for the operation and no influence on surface structure of the skin. To work under standardized conditions is of the utmost importance to obtain reliable and reproducible results (COURAGE KHAZAKA, 2016).

1.4- Skin coloration

The skin color determination is important to the cosmetic producers to test the effectiveness of tanning agents, with or without sun, and sun screen protectors, make-ups, whitening products, decorative cosmetics, hair and carotene food supplements (COURAGE-KHAZAKA, 2016) (HERNANDO, 2016).

- **non-invasive evaluation of the skin color**

The color changing observation is a subject method that depends of the watcher and is influenced by factors like skin pigmentation and blood perfusion. For that reason, the industry needed to develop a more objective and reproducible equipment. Then they started to use sensitivity of human skin to UV radiation. In 1976, the International Illumination Commission defined CIELAB (CIE L*a*b*) that are parameters proposed for the unambiguous communication of skin-color information: L* for lightness, a* axis for red-green opponent colors and b* axis for the yellow-blue opponent colors (WESTERHOF, 2006).

As an equipment example there is Colorimeter® from Courage Khazaka. The probe sends out white LED (Light Emitting Diode) light that is scattered in all directions. Part of

these travels through the skin layers and another part is scattered out of skin. The light reflected from the skin is measured in the probe. The raw data of the probe is corrected with a special color matrix to adapt closely to standard values and is expressed accordingly. The Skin-Colorimeter probe is specially designed to detect small color changes in the skin, thus ideal for comparison measurements (COURAGE-KHAZAKA, 2016). In the figure below is demonstrated in a schematic way the Colorimeter® principle.

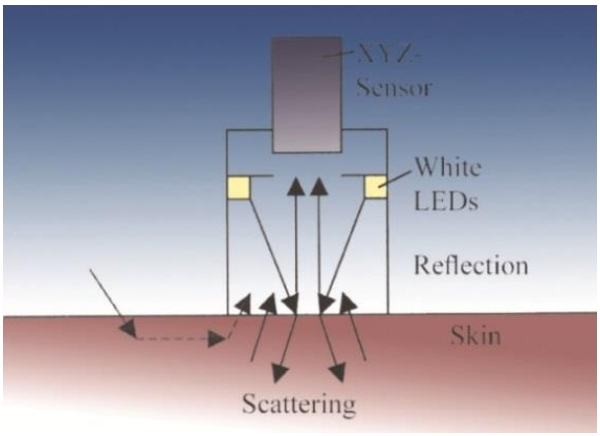

FIGURE 3 - Colorimeter® principle (with permission from Esther Bász, 2016) (COURAGE KHAZAKA, 2016)

Another methodology is available to measure the skin color change based in spectrophotometry. The skin color is influenced by erythema and pigmentation and these parameters can be determined with the quantification of hemoglobin and melanin indices, respectively (TUPKER & PINNAGODA, 2006). The next picture represents what happens in this kind of methodology.

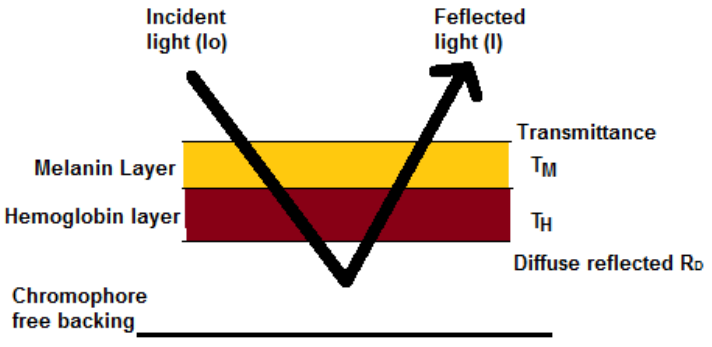

FIGURE 4 - Optical skin model of three-layered structure with an outer melanin layer, an inner hemoglobin layer and a backing representation chromophore-free dermis, where I0- intensity of incident light, I- Intensity of reflected light, TM- Transmittance of the.

Transmittance is, by definition, the ratio of energy transmitted through and emerging from a body to the total flux incident on it. Diffuse reflectance is the ratio of the total amount of radiation reflected by a surface to the total amount of radiation incident on the surface (THOMAS, 2006).

The Mexameter® MX 18 is a very easy, quick and economical tool to measure melanin and hemoglobin by reflectance. The measurement is based on absorption/reflection. The probe of the Mexameter® MX 18 emits 3 specific light wavelengths. A receiver measures the light reflected by the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. The melanin is measured by specific wavelengths chosen to correspond to different absorption rates by the pigments. For the erythema measurement specific wavelengths are also used, corresponding to the spectral absorption peak of hemoglobin and as a way to avoid other color influences (*e. g.* bilirubin) (COURAGE KHAZAKA, 2016).

However, this method has some error factors: erythema index increases in an apparently linear fashion as the melanin index increases and is influenced by the body site in study; the melanin index may be affected by the oxygen saturation level of hemoglobin; the influence of external factors like temperature and seasonal period (TAKIWAKI, 2006).

II- The dermis

The dermis is composed of an association of fibers, mainly collagen, with materials known as glycosaminoglycans, which are capable of holding a large amount of water, thus maintaining the turgidity of the skin (FREINKEL & WOODLEY, 2001).

2.1- Skin elasticity

The viscoelasticity of skin surface is determined by the elastin and collagen fibers. In younger skin these fibers are dispersed beneath the skin surface keeping it firm, supple and elastic. All the skin layers (epidermis and dermis) are involved in the skin viscoelasticity and that is mainly affected by the skin hydration. With aging, the skin loses its elastic properties, resulting in the appearance of wrinkles. Wrinkles are formed by reduction of adipose tissue and subepithelial thickening of the stratum corneum and manifest aesthetically shaped "folds". Its formation is due to many factors, the main one being chronological age and the effect of the decreased level of certain hormones and hormone receptors at the cutaneous level. The exposure to the ultraviolet radiation has an important role in their appearance –

known as photoaging – and also the mimetic origin (habit of gesturing). More skin aging factors are nicotine, alcohol, genetic predispositions, and diseases (HERNANDO, 2016) (COURAGE KHAZAKA, 2016).

Skin thickness was a widely used parameter to evaluate the influence of different factors on skin aging. This parameter is studied by employing high-frequency ultrasound.

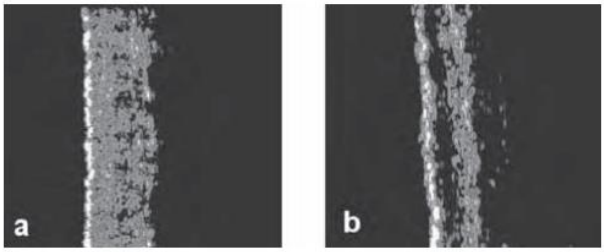

FIGURE 5 - Twenty-megahertz ultrasonographic skin images of the dorsal aspect of the forearm (a) in a young woman (aged 21) and (b) in an elderly woman (aged 83) with marked sun exposure during the lifetime, by JORDEN SERUP, 2006.

- **non-invasive evaluation of the skin elasticity**

Nowadays cosmetic producers have multiple equipments to provide support to prove "anti-aging", "firmness" and "anti-celullite" claims (HUA, XIE, CHEN, & LI, 2013). The most used equipments are based on viscoelasticity measure, like Reviscometer® and Cutometer® from Courage Khazaka. These provide valuable information on physiological and pathological changes of human skin as well as the efficacy of topical treatment.

The Cutometer® is a suction chamber method (represented in the Figure 6). Negative air pressure is applied to the skin surface through the probe aperture. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor, as well as two prisms facing each other, which project the light from transmitter to receptor. The light intensity varies due to the penetration depth of the skin. The resistance of the skin to the negative pressure (firmness) and its ability to return to its original position (elasticity) are displayed as curves (penetration depth in mm/time) in real time during the measurement, enabling to objectively quantify (COURAGE KHAZAKA, 2016).

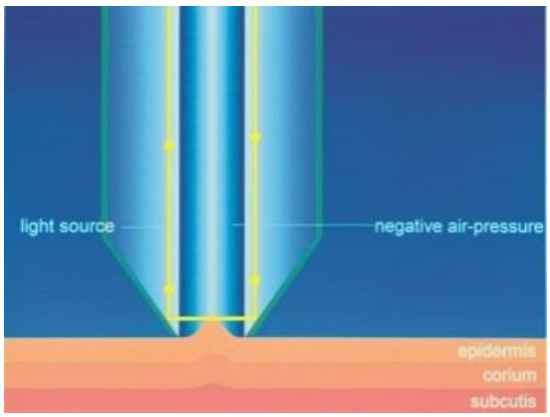

FIGURE 6 - Cutometer® operation principle(with permission from Esther Bász, 2016) (COURAGE KHAZAKA, 2016)

Probes in an aperture diameter of 4 or 6 mm are for the study of the outer skin layers, and the 8 mm for measurement of full-thickness elasticity. The probe is connected to the main unit with an air tube and an electric cable. The pressure can be adjusted between 50 and 500 mbar and can be built up immediately or gradually at a controlled rate, as decided. The suction time and relaxation time can be changed from 0.1 to 60 seconds, and the number of measurement cycles from 1 to 99. Two measuring modes can be chosen, a stress–strain technique and a time–strain technique (TUPKER & PINNAGODA, 2006).

The Reviscometer® uses acoustic wave propagation to study the mechanical behavior of the skin. This has two probes in skin contact: one needle probe emits an acoustic shock wave and the propagation time needed to reach the receiver needle probe is determined – Ressonance Running Time (RRT). The shock wave propagation will be affected by the isotrophy of the skin (direction of the collagen and elastic skin fibers and the stratum corneum hydration level). With aging, the wave propagation time increases because the skin fibers direction changes. The relation between RRT and skin hydration has been shown by studies realized by Paye and his contributors in dry skin people, showing that the wave propagation time increases in a dehydrated skin (PAYE & et al., 2007).

III- Cutaneous appendages

3.1- Sebaceous glands

Sebaceous glands are predominantly distributed on the face and scalp and don't exist in the palms of the hands and the soles of the feet. They are usually attached to hair follicles and secrete a mixture of fats (triglycerides, wax esters, squalene, and cholesterol) and cellular debris, which is discharged as sebum. Sebum helps not only to form the slightly

greasy surface film of the skin but also keeps the skin flexible and prevents the skin's loss of absorption of excessive amounts of water.

The sebaceous glands are involved in the development of the common adolescent skin disorder known as "acne vulgaris". That occurs when the outlet from the gland to the surface of the skin is plugged, allowing sebum to accumulate in the follicle and sebaceous duct. The chemical breakdown of triglycerides in the sebum, by bacterial action, releases free fatty acids, which in turn trigger an inflammatory reaction producing the typical lesions (pimples) of acne (FREINKEL & WOODLEY, 2001).

- **non-invasive evaluation of the skin sebum**

Measurement of oily skin is earning importance in cosmetics studies especially in cleansers, anti-acne products, shampoos and hair care, products for oily skin. The producers of these cosmetic products have used a non-invasive method that helps them to support their claims and efficacy tests (HUA, XIE, CHEN, & LI, 2013).

The Sebumeter® of Courage Khazaka is the most used method to reproducibly and accurately determine the sebum level of the skin surface, as well as on scalp and hair. The measurement is based on grease spot photometry. The mat tape of the Sebumeter® SM 815 is brought into contact with skin or hair. It becomes transparent in relation to the sebum on the surface of the measure area. Then the tape is inserted into the aperture of the device and the transparency is measured by a photocell. The light transmission represents the sebum content (COURAGE KHAZAKA, 2016).

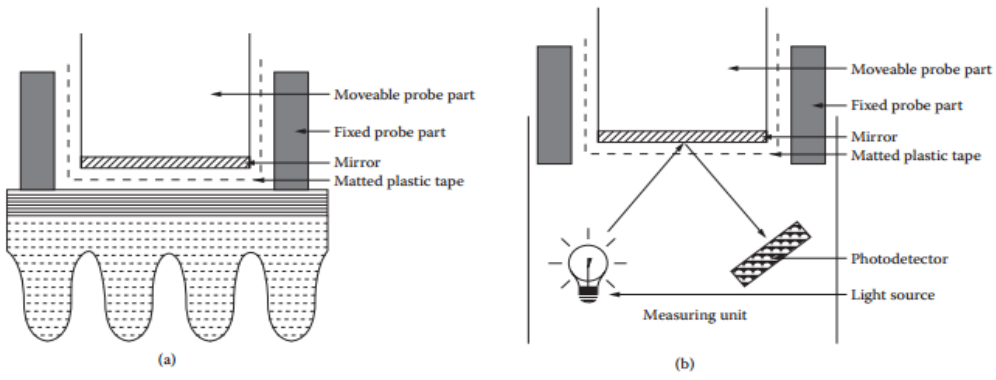

FIGURE 7 - (a) Sebum collection step of the Sebumeter. (b) Sebum measure step of the Sebumeter. (From Elsner, P., in Bioengineering of the skin: Methods and Instrumentation, Berardesca, E.et al. Eds., CRC Press, Boca Raton, FL1995, p.83)

The sebum measure is influenced by factors like age (sebum production increases after puberty, reaching a peak in both sexes between 30 and 40 year); sex (the secretion of sebum

is greater in males than females except in the age group 10 to 15 years); endocrine status; skin temperature; time of the day (secretion of sebum is maximal in the middle morning and minimal during the late evening and early morning); body site (sebaceous glands are found predominantly on the scalp, face, chest, and back) and diseases (O'GOSHIi, 2006).

The gloss is usually associated to an oily skin. So it is useful for cosmetic producers to have an equipment that helps them to support claims like "skin shine reducing". This kind of test is also useful in hair care and decorative cosmetics (lipsticks, make-up etc.).

The Skin-Glossymeter® of Courage Khazaka is an example of equipment that can be used to measure gloss and his principle is based on reflection.

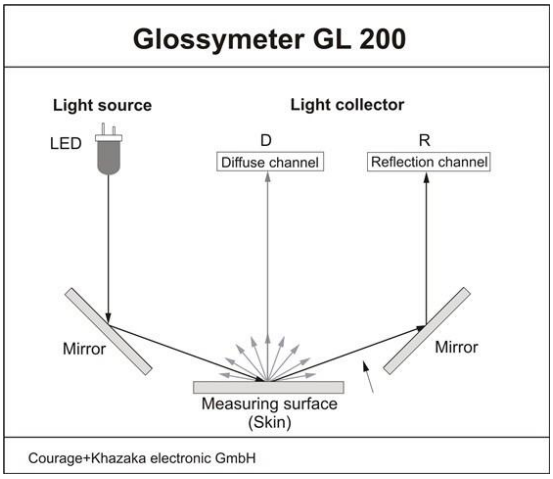

FIGURE 8 - Schematic design of Skin-Glossymeter technology (with permission from Esther Bász, 2016) (COURAGE KHAZAKA, 2016)

Parallel white light is created by LEDs in the probe head. To be able to emit light at 60° in a relatively small and uniquely designed measurement head, light is sent out at 0° and reflected by mirrors to 60°. Two separate measurement channels measure the direct reflected light (again guided by a mirror in the same angle into the reflection channel) and the diffuse reflected (scattered) light. The scattered/diffuse reflected light is measured at 0° (completely vertically above the measured surface) under the assumption that light is scattered in the same way over all degrees (diffuse channel). So the Skin-Glossymeter GL 200 expresses both, the portion of directly reflected light (gloss) and the diffuse scattered portion from the skin surface. This equipment stands out from the others gloss measurements because, according to Courage Khazaka, it's the only one that can obtain a gloss value mostly free from influence of skin color, by using a deduced special formula. Skin color may affect the gloss determination because some part of the light will penetrate into the upper layers and will get scattered depending mainly on the skin color (dark skin scatters less than light skin) (COURAGE KHAZAKA, 2016).

3.2- Sweat glands

The **eccrine sweat glands** secrete a hypotonic solution to plasma, with variable obligatory amounts of electrolytes, mainly sodium, chloride and potassium together with other compounds in very small amounts, namely: lactate, urea, ammonia, proteins and peptides (AQUALYTE) (FREINKEL & WOODLEY, 2001). Its principal function is thermoregulation and its second principal function is to moisturize the skin and maintain the skin pH. As an excretory organ, delivers to the skin substances with an important physiological function, like lactate and urea. The eccrine sweat can be measure by two non-invasive ways: the Filter Paper Technique and the Macroduct System.

The Filter Paper Technique consists in apply a preweighed filter paper over the skin area that was exposed to pilocarpine, place a plastic sheet over this area and seal airtight with surgical tape. Allow the sweat to accumulate on the gauze or filter paper, waiting approximately 20 to 30 min. The appearance of droplets on the plastic sheet indicates that enough sweat has accumulated and these droplets must be included in the collection. Next step is removing the filter paper with forceps, placing it immediately into the weighing bottle, and stopper. Handle the bottle with tissues as above. Weigh the bottle accurately (within 1 mg) to determine the weight of the filter paper and calculate the amount of sweat by difference, assuming that 1 g sweat equals to 1 ml sweat. This method only allows obtaining results with a considerable quantity of sweat but this disadvantage can be overcome by using the Macroduct system, which allows accurate analysis on very small quantities of sweat (BARTH, 2006).

The Macroduct System is composed by the Webster Sweat Inducer, and the Macroduct® Sweat Collector. The Webster Sweat Inducer stimulates the sweat in a skin area by pilocarpine iontophoresis. The Macroduct Sweat Collector is a disposable plastic device with a shallow concave undersurface that covers the skin area. At the apex, a small aperture leads to a spirally configured plastic capillary tube that withdraws the sweat as it pools in the concavity. This system has an important role in studies for cystic fibrosis (ELITECHGROUP, 2016) (BARTH, 2006).

FIGURE 9 - Macroduct system (with permission from Dennis Briscoe, 2016) (ELITECHGROUP, 2016)

The **apocrine sweat glands** are usually associated with hair follicles and are concentrated in the underarm and in genital regions. They secrete a fatty sweat into the gland tubule, stimulated by hormonal changes like emotional stress causes. This stimulation leads to the contraction of the tubule wall, expelling the fatty secretion to the skin, where local bacteria break it down into odorous fatty acids (FREINKEL & WOODLEY, 2001).

Several methods have been developed that employ either absorbent material to collect the secretions or their volatile products or direct cannulation of the apocrine duct to collect the secretions. The choice of technique will depend on the question to be solved. However, since the products of the apocrine, sebaceous and eccrine glands intermingle it is practically impossible to obtain pure apocrine sweat from an *in vivo* technique.

A method suggested to overcoming this problem is the "plaster-of-Paris disc". This disc, when applied in body areas that have eccrine and apocrine glands, like the axillary skin, adheres by polyethylene holders. Eccrine fluid is watery and is adsorbed by the discs, whereas the viscid lipid apocrine secretions collect on the disc surface, in a period of at least 3 to 4 hours. The apocrine droplets can be seen by fluorescence under ultraviolet light (TUPKER & PINNAGODA, 2006).

Conclusion

Cosmetic world is constantly changing and, nowadays, it has more legal control which makes the consumers and the producers more secure about the products. It has become clear now that there are a considerable number of instruments and methods to measure the efficacy of cosmetic products for a lot of parameters.

The harmlessness and quickness of the methods described through this work are their principal advantages, allowing the investigators and cosmetic producers obtain rapid results using different target populations (from babies to the elderly). Although, a non-clinical environment is a workplace environment that can be highly variable and difficult to control, presenting unique measure challenges not typically encountered in clinical settings. In order to obtain results that we can trust is necessary develop and implement basic guidelines and best practices to ensure meaningful and uniform communication of results, similar to what was made by an expert group in the 5th International Conference on Occupational and Environmental Exposure of Skin to Chemicals (OEESC) for the measurement of TEWL and skin hydration. Besides all the variations related to the work conditions, we have variations associated to the equipments. Nowadays many industries commercialized their own equipments what leads to the existence of many equipments in the market with the same function and objectives, differing only in the brand. When an investigator or product producer selects an equipment he needs to make sure that equipment is validated for the parameter that will be analyzed and should select the equipment who shows the best results. For example, studies demonstrated that to measure stratum corneum hydration the Corneometer 825, from Courage Khazaka, is more reproductible than Soft Plus, from Callegari S.p.A. (HUA, XIE, CHEN, & LI, 2013).

The biometrologic evaluation of cosmetic products was a giant step in the development of new products and allowed to the consumers and prescribers be assure of the security and effectiveness of the commercialized cosmetic products. In fact, many of the previous refered probes are daily applied directly with consumer. For example, many advisers from cosmetic laboratories carry with them a multi probe adapter equipment, that allows them to combine different probes, in consideration of their aims, like corneometer, sebumeter, frictiometer. They encourage the consumers to make a "cutaneous profile" and then they do an individual advice. Besides that, they can use that equipment to monitor the results of the products that their clients use, in a fast and safe way.

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