Recovery of Skin Protective and Barrier Function

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ABSTRACT

Thesis deals with the skin and the recovery of its protective and barrier function. The aim of the work was to carry out a corneometric measurement of moisturizing properties of cosmetic products on a group of volunteers. Recovery of skin protective and barrier function was carried out by application of moisturizing cosmetic products to the skin damaged by solution of sodium lauryl sulfate.

After the corneometric measurement of moisturizing properties of the skin was found out that moisturizing effects of cosmetic products are on a good level. Cosmetic products with olive oil and urea had the best moisturizing effects.

Keywords: skin, moisturization, humectant, emollient

ABSTRAKT

Práce se zabývá problematikou kůže a obnovou její ochranné bariérové funkce. Cílem bylo provést korneometrické měření hydratačních účinků kosmetických přípravků na skupině dobrovolníků. Obnova ochranné bariérové funkce byla provedena aplikací hydratačních přípravků na kůži narušenou roztokem laurylsulfátu sodného.

Po provedení korneometrického měření hydratačních vlastností kůže bylo zjištěno, že kosmetické přípravky vykazují dobré hydratační účinky. Nejlepší účinky vykázaly krémy s obsahem olivového oleje a močoviny.

Klíčová slova: kůže, hydratace, humektant, emolient

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INTRODUCTION

Human skin is one of the largest and the most complex organs in the body. The skin is composed of two main layers – the epidermis and the dermis.

Skin covers the body and divides internal organs from the external environment. It also fulfills many important functions. One of the most important function is that the skin protects against pathogens and damage from external environment and against the water loss. It means that the skin is a personal barrier of human body.

Also the water content is very important for the good function of skin as a barrier. The moisturization of the skin is daily affected by using of many detergents and cleansing cosmetic products. The skin has a natural mechanism of its moisturization because the main layer of epidermis contains a specific mix of hygroscopic low molecular weight compounds that keep the dead skin cells hydrated.

The importance of water presence in the skin is very significant and because of this reason the methods for the measurement of the skin moisturization were invented. They are based on measurements of conductance, capacitance and impedance of the skin. One of the most using method is corneometric method which is based on the fact that dielectrical constants of water and other materials are different.

It is clear of these facts that the skin moisturization problem is very important and interesting and that was the main reason why this theme was chosen. The aim of this thesis is general treatise on the skin and its functions and to carry out corneometric measurement of moisturizing properties of cosmetic products.

I. THEORETICAL PART

1 HUMAN SKIN

Skin mediates the most important transactions of our lives. Skin is the key to our biology, our sensory experiences, our information gathering, and our relationships with others. [1] Human skin is one of the largest and the most complex organs in the body. It contains all of the structures of the body and forms a functional barrier between internal and external environments. [2] Simply put, skin is the flexible, continuous covering of the body that safeguards our internal organs from the external environment. It protects us from attack by physical, chemical, and microbial agents and shields us from most of the harmful rays of the sun, while it works hard to regulate our body temperature. Far from being an impervious barrier the skin is a selectively permeable sheath. It is constantly at work as a watchful sentinel, letting some things in and others out. Skin is also home for hundreds of millions of microorganisms, which feed on its scales and secretions. But our skin is more than a defensive shield, a gatekeeper, and a personal zoo. [1]

The functionality of skin depends on the establishment and maintenance of the delicate compositional and organisational balance of the dermal matrix. There are well-recognised age-related changes in the dermis, and molecular aberrations in structure or expression of matrix molecules, particularly collagen and elastin fibres, result in a range of phenotypically recognizable cutaneous diseases. In addition, skin is particularly susceptible to modification by environmental insults such as ultraviolet irradiation. [2]

The complexity of skin is highlighted by the hierarchy of cellular and matrix arrangements that give rise to the outer epidermis, the inner matrix-rich dermis, and the dermal-epidermal junction which provides the adhesion between these compartments and underpins the integrity of skin. [2]

1.1 Structure of skin

Skin is a formidable physical barrier that protects us from the environment. It has become particularly adapted to withstand desiccation, allowing us to live in a non-aqueous environment, and it is essential for thermal regulation of body temperature. [3]

The skin is a continuous sheet covering the entire body surface. It is composed of two main layers: the epidermis and the dermis. [3]

The relative and the total thickness of the two layers varies over different regions of the body. The epidermis is the thickest on the palms and the soles of the feet. The dermis is the thickest on the back and the thinnest on the palms. [3]

The structure of the skin is shown in Fig. 1. [4]

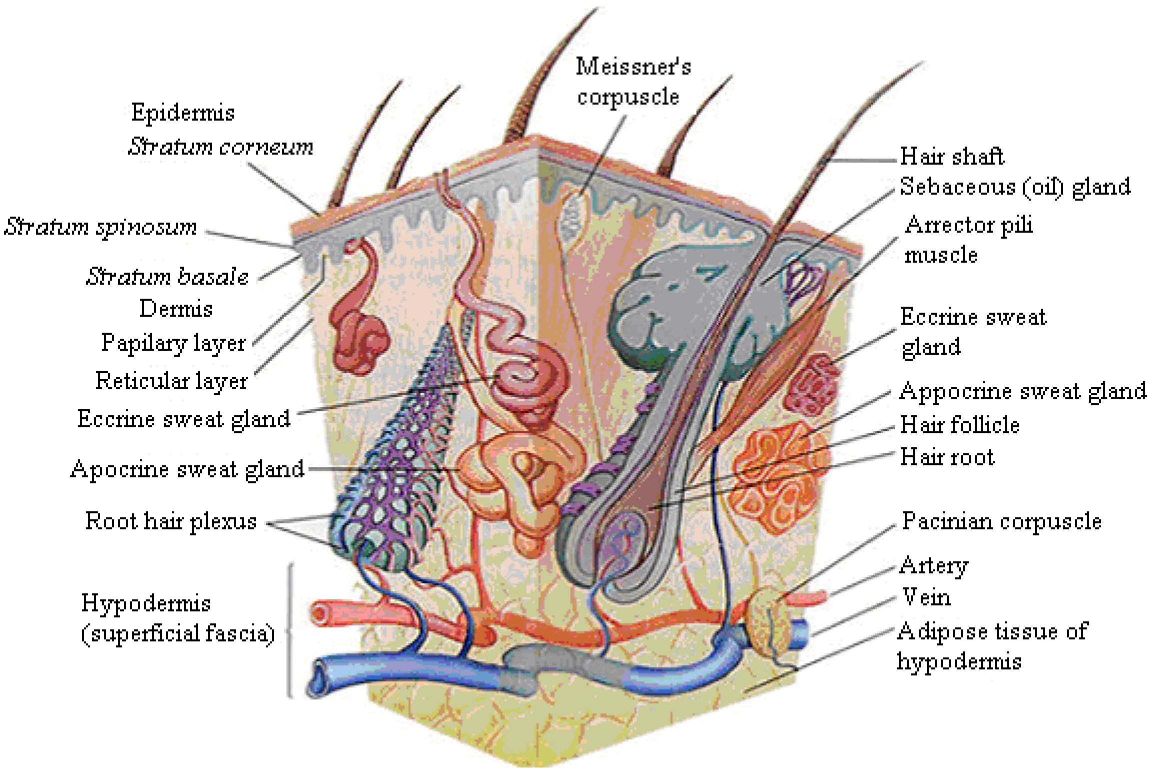

Fig. 1: *The structure of the skin*

Skin contains about 64 % of water, 22 % of proteins, 13 % of lipids, 0,5 % of carbohydrates, 200–300 mg of chlorides, 118–408 mg of natrium, 80 mg of kalium, 4–8 mg of magnesium, 5 mg of calcium, 3.13–6.6 mg of organic binded phospohorus, 60 mg of sulphur, 1.5–5 mg of ferrum, 0.1 mg of cuprum, 0.5 mg of zinc, 0.3 mg of manganese and 0.03 mg of iodine. [5]

One cm^2 of human skin contains about: [5]

- 6 million cells;
- 15 sebaceous glands;
- 100 cm blood vessels and veins;
- 5 hairs;
- 100 sweat glands;
- 5000 haptic corpuscles;
- 400 cm nerve fibres;
- 25 pressure receptors;
- 200 pain receptors;
- 12 cold receptors;
- 2 thermic receptors.

1.1.1 Epidermis

Epidermis (Fig. 2) is comprised of *stratum corneum* (horny layer), which is the outermost layer exposed to the environment. Next layers are *stratum lucidum*, *stratum granulosum* (granular layer), *stratum spinosum* (prickle cell layer) and *stratum basale* (basal layer). [6]

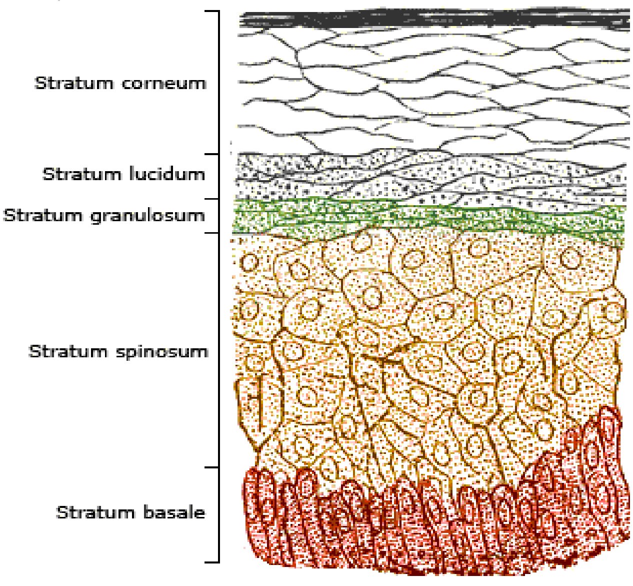

Fig. 2: *Epidermis*

1.1.1.1 Stratum corneum

The major function of the epidermis is the formation of a complete permeability barrier, which is mediated by lipids sequestered in the intercellular domains of the *stratum corneum*. These lipids contain approximately equal quantities of ceramides, cholesterol, and free fatty acids, as well as lesser amounts of non-polar lipids and cholesterol sulfate, arranged as broad membrane bilayers in the *stratum corneum* interstices. The *stratum corneum* is often schematically represented as a brick wall, the terminally differentiated, keratin-filled corneocytes are the bricks, while the lamellar intercellular lipid domains represent the mortar. This thin, the outermost epidermal layer of the skin, as the principal regulatory barrier governs the transcutaneous traffic of water and exogenous substances. [7]

This tissue typically consists of 10–15 layers of flattened, keratinized dead cells embedded in a lipid-rich matrix and they may be about 10 μm when dry but usually swells to several times this thickness when hydrated. The barrier properties of the *stratum corneum* are controlled by its composition: 75–80 % proteins, 5–15 % lipids and 5–10 % unidentified material on a dry weight basis. [8]

The composition of this matrix is different compared to other biomembranes, because polar lipids such as phospholipids, the major bilayer-forming lipids in almost all biomembranes, are absent in the *stratum corneum*. In comparison to the other biomembranes the cholesterol content is rather high. A minor cholesterol derivative in the *stratum corneum* is cholesterol sulfate, with its highest concentration between the granular layer and the innermost layer of the *stratum corneum*. Detection of triglycerides as described in the literature has been explained either by contamination with sebum glycerides or by extraction of subcutaneous fat. Therefore triglycerides are not supposed to play an important role within the *stratum corneum* lipids. However, free fatty acids represent 10–25 % of the *stratum corneum* lipids. These fatty acids are both saturated and unsaturated and they are of the length of chain C16 and C18. The major part of the *stratum corneum* lipids are ceramides. They are known to be the most important component of the *stratum corneum* multilamellar lipid structure with definite physicochemical properties necessary for the barrier function of the skin. [9]

Table 1 summarizes the composition of the *stratum corneum* from different references. [10, 11, 12]

Table 1: *Lipid composition [% (w/w)] of human stratum corneum lipids according to the literature*

Lipid class	Lampe et al. [10]	Elias [11]	Wertz [12]
Free fatty acids	19.3	25.0	10.0–15.0
Cholesterol	14.0	20.0	25.0
Triglycerides	25.2	Trace	Trace
Sterolesters	5.4	10.0	5.0
Ceramides	18.1	35.0	50.0
Others	18.0	10.0	-

The ceramides, which belong to the family of sphingolipids, consist of a sphingoid base with an amide-linked fatty acid chain. They are produced in a catabolic process during the latest stage of epidermal differentiation when the lamellar body contents are extruded into the extracellular space via removing the sugar from glucosyl- and galactosylceramides (cerebrosides) which are found in the *stratum basale* and the *stratum granulosum* of the deeper layers of the skin. Representative molecular structures and relative proportions of the *stratum corneum* are shown in Fig. 3. [9]

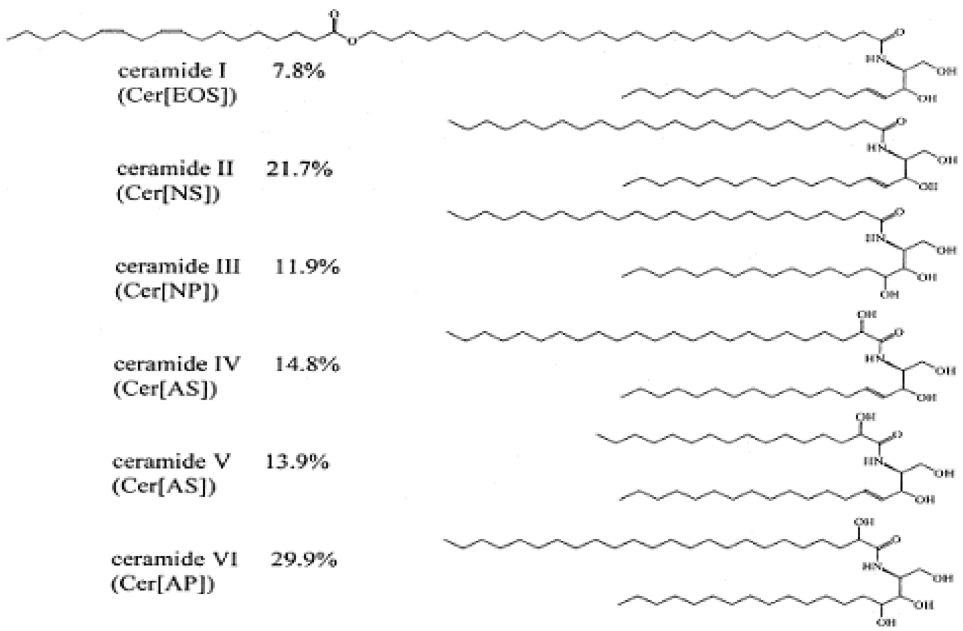

Fig. 3: *Structures and relative proportions of the stratum corneum ceramides*

Despite of exhaustive scientific research, the molecular architecture of the *stratum corneum* and the mechanisms that control transepidermal water loss or influence of the percutaneous absorption of compounds with low molecular weight are not yet well described. Information about the molecular organization of the *stratum corneum* is important for the treatment of skin diseases, transdermal drug delivery and the cosmetic maintenance of the skin. [13]

1.1.1.2 Stratum lucidum

Stratum lucidum is a layer of a few rows of dead cells which lies beneath the *stratum corneum*. [14] It is also called the transparent layer and it is present only in the thick skin of the palms of the hands and the soles of the feet. [15]

1.1.1.3 Stratum granulosum

In spite of being a viable epidermal layer, the next layer, *stratum granulosum* (1–3 cell-layers thick) contains enzymes that have the potential to degrade vital cell organelles such as nuclei. By synthesizing keratin and degrading cell organelles, the keratinocytes in this layer gradually differentiate into the corneocytes of the *stratum corneum*. The keratinocytes also synthesize membrane coating granules that carry the precursors for intercellular lipid lamellae of the the *stratum corneum*. [6]

1.1.1.4 Stratum spinosum

Stratum spinosum, the next viable epidermal layer consists of 2–6 layers of columnar keratinocytes that modify themselves into polygonal shapes. The keratin in this layer aggregates to form filaments called tonofilaments that on further condensation produce cell membrane connecting structures called desmosomes. The *stratum spinosum* together with the lower *stratum basale* layer is known as the Malphigian layer. [6]

1.1.1.5 Stratum basale

Stratum basale is the layer with all the typical cell organelles and it is the only layer that is capable of cell division. The keratinocytes in this layer are connected with the basement membrane (or dermo-epidermal membrane) by proteinaceous structures called hemidesmosomes and with cells of the *stratum spinosum* layer by desmosomes. In addition to the keratinocytes, other specialized cells present in the basal layer are known as melanocytes, Langerhans cells and Merkel cells. Melanocytes secrete melanosomes containing melanin (eumelanin or phaeomelanin) that protects the skin from ultraviolet radiations and free radicals. Langerhans cells are derived from bone-marrow and as part of the immune system function as antigen presenting cells (APC) of the skin. Merkel cells together with nerve endings are present in the dermis and they are responsible for cutaneous sensation.[6]

1.1.2 Dermis

Dermis is 3–5 mm thick and it is composed of numerous connective tissues, especially collagen fibrils and elastic tissues that respectively provide support and flexibility to the dermis. It is supplied with a reticulate network of blood vessels, lymphatic vessels, nerve endings and numerous appendages. There are three main appendages namely, hair follicles, sebaceous glands and eccrine glands present in the skin that originate from the dermis. [6]

1.1.2.1 Hair follicles

Hair follicles cover the entire body surface except soles of feet, palm of hands and lips. [6] The mature hair follicle is a complex structure consisting of specialized cells of both epithelial and dermal origin. Each follicle has several concentric layers of differentiated epithelial keratinocytes, known as root sheaths, surrounding the central hair shaft structures consisting of an outer cuticle, a cortex, and a central medulla. The basal portion of hair follicle, namely, the hair bulb, engulfs a cluster of differentiated dermal cells at its base, forming the dermal papilla, which are thought to play an important role in regulating the growth and differentiation of the hair. [16]

Hair follicle in the human skin is shown in Fig. 4. [17]

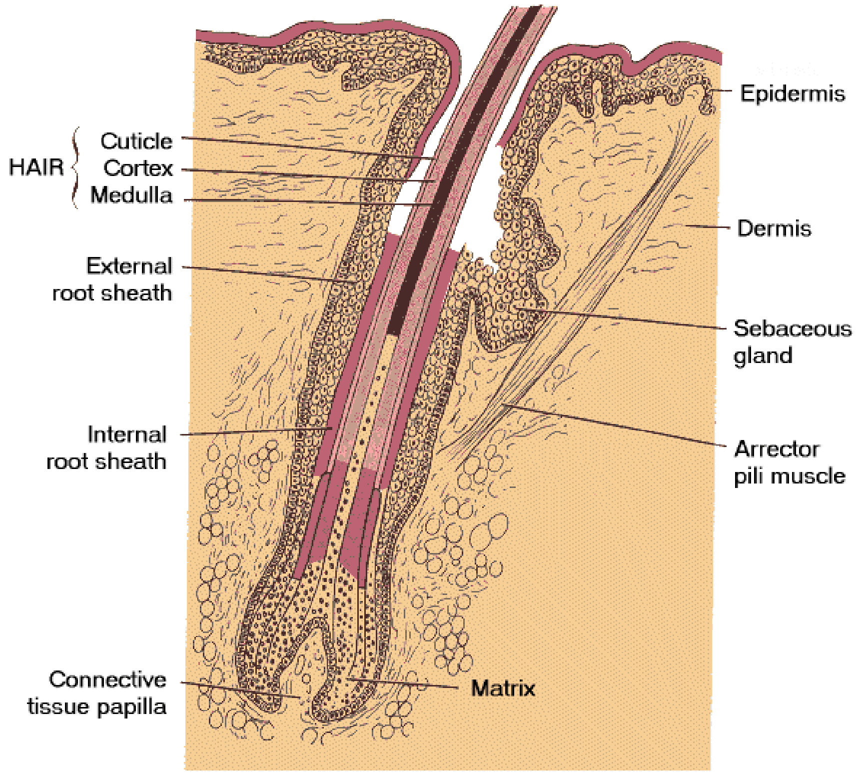

Fig. 4: *Hair follicle*

Hair follicle development begins during embryogenesis and it is mediated by a series of complex signaling interactions between the mesenchymal cells (dermis) and overlying ectodermal cells (epidermis). [16]

In mammalian skin, hair follicles produce hairs that fulfill a number of functions including thermoregulation, collecting sensory information, protection against environmental trauma, social communication, and mimicry. During postnatal life, hair follicles show periodic changes in their activity; phases of intensive growth and hair shaft production (anagen) are followed by apoptosis-driven regression (catagen) and relative resting (telogen). Capacity of the hair follicles to periodically activate a program of hair shaft formation is strikingly important for many aspects of mammalian biology including cell-cell communications, tissue regeneration, carcinogenesis, aging, and psychosocial behavior. [18]

1.1.2.2 Sebaceous glands

Sebaceous glands are embedded in the dermis over most of the body surface. Sebaceous glands, which are not found in the glabrous skin of the palms and soles, are acinar glands that usually have several acini opening into a short duct. This duct usually ends in the upper portion of a hair follicle; in certain regions, such as the glans penis, glans clitoridis, and lips, it opens directly onto the epidermal surface. The acini consist of a basal layer of undifferentiated flattened epithelial cells that rests on the basal lamina. These cells proliferate and differenciate, filling the acini with rounded cells containing increasing amounts of fat droplets in their cytoplasm. Their nuclei gradually shrink, and the cells simultaneously become filled with fat droplets and burst. [19]

The sebaceous gland is an example of a holocrine gland, because its product of secretion is released with remnants of dead cells.[19] Sebum secreted by sebaceous glands associated with the follicles is composed of free fatty acids, triglycerides and waxes and it plays a vital role in lubricating the skin surface and maintaining a surface pH of approximately 5. [6]

1.1.2.3 Sweat glands

Sweat glands are widely distributed in the skin except for certain regions, such as the glans penis. The merocrine sweat glands are simple, coiled tubular glands whose ducts open at the skin surface. Their ducts do not divide, and their diameter is thinner than that of the secretory portion. The secretory part of the gland is embedded in the dermis, it measures approximately 0.4 mm in diameter and it is surrounded by myoepithelial cells. Contraction of these cells helps to discharge the secretion. Two types of cells have been described in the secretory portion of sweat glands. Dark cells are pyramidal cells that line most of the luminal surface of this portion of the gland. Their basal surface does not touch the basal lamina. Secretory granules containing glycoproteins are abundant in their apical cytoplasm. Clear cells are devoid of secretory granules. [19]

The eccrine glands originate in the dermis secrete sweat (a dilute salt solution of pH 5) in response to physical and emotional stress. [6] Eccrine sweat glands open directly onto the skin and regulate body temperature. Innervated by sympathetic nerves, these sweat glands are distributed throughout the body, except for the lips, ears, and parts of the genitalia. They secrete a hypertonic solution made up mostly of water and sodium chloride; the prime stimulus for eccrine gland secretion is heat. [20]

Specialized glands known as apocrine glands are also present and they are located in the dermo-epidermal layer in selective regions. All appendages act are known as shunt routes for permeants that can enter the lower layers of the skin without traversing the intact barrier of the *stratum corneum*.[6]

Apocrine sweat glands appear chiefly in the axillae and genitalia; they are responsible for producing body odor and are stimulated by emotional stress. The sweat produced is sterile but undergoes bacterial decomposition on the skin surface. These glands become functional after puberty. [20]

Ceruminous glands, located in the external ear canal, appear to be modified sweat glands and secrete a waxy substance known as *cerumen.* [20]

1.1.3 Hypodermis

The hypodermis (or subcutaneous fat layer) connects the dermis with the underlying organs. It provides insulation to the body and it protects the body from mechanical shock. [6]

The hypodermis is the innermost and thickest layer of the skin. It invaginates into the dermis and is attached to the latter, immediately above it, by collagen and elastin fibres. It is essentially composed of a type of cells specialised in accumulating and storing fats, known as adipocytes. These cells are grouped together in lobules separated by connective tissue . [21]

The hypodermis acts as an energy reserve. The fats contained in the adipocytes can be put back into circulation, via the venous route, during intense effort or when there is a lack of energy providing substances, and they are transformed into energy afterwards. When it is spoken of burning up calories, it is spoken of burning up fats in particular. The hypodermis participates, passively at least, in thermoregulation since fat is a heat insulator. [21]

The anatomical position of the hypodermis is clearly a sexual characteristic. While the hypodermis is distributed over the entire body, it has a tendency to accumulate above the belt over the abdomen and shoulders in men, and in women, below the waist around the thighs, hips and buttocks. [21]

1.2 Functions of skin

The skin fulfills a number of important functions: [3]

- Protection against:

  - physical trauma;
  - penetration of chemicals, drugs and environmental insults;
  - water loss;
  - ultraviolet radiation;
  - infection.

- Provides sensory information (hot, cold, sharp, smooth);
- Insulates and provides special mechanism for temperature control (vasodilatation and vasoconstriction; evaporative sweating);
- Sythesizes vitamin D;
- Provides containment for internal organs (made effective by elasticity);
- Contributes to our physical and sexual identity through its color, texture and it bears the amount of hair;
- Communication functions (as above) and through blushing, pallor and sweating. Is a tactile link with the outside world.

1.2.1 Skin as a barrier

The epidermal layer not only protects us from environmental pathogens but also acts as a barrier to water loss. The protein-lipid layer, located in the upper layers of the epidermis, is necessary for establishment and maintenance of a water barrier. [22]

Also components of intercellular junctions, termed tight junctions, play an essential role in development of barrier function in the skin. [22]

The epidermis of the skin is a self-renewing, stratified epithelium that functions as the interface between the human body and the outer environment. The epidermis protects against mechanical, chemical and microbial attack and functions as a permeability barrier by preventing water loss from the dermis. The complex organization of cells in the epidermis and the way keratinocytes interconnect contribute to these important skin functions. In particular, cell division in the basal layer of the epidermis gives rise to daughter keratinocytes that undergo a specific program of differentiation as they pass through the various layers within the epidermis. Towards the outer surface of the skin, a series of biochemical events results in keratinocyte death and formation of an insoluble layer termed the *stratum corneum*. The latter consists of extracellular lipids, organized as lamella, together with a mixture of proteins that are covalently crosslinked into a rigid scaffold through the action of transglutaminase 1. The crosslinked remains of keratinocytes are termed the cornified cell envelope. The major cell envelope reinforcement proteins are loricrin crosslinked to small amounts of certain proline-rich proteins. In addition, transglutaminase 1 attaches ceramide lipids by ester linkages to cell-surface molecules, including involucrin, envoplakin and periplakin. There is evidence that cell envelope assembly is necessary for barrier development in the skin. [22]

Development of the epidermal barrier helps prevent attack from outside world and water loss from the inside of the body. Tight junction can be added to the cell envelope and extracellular lipids as a major player in epidermal barrier assembly and maintenance. In fact, the tight junction in the granular layer of the skin might function as the first line of defense against water loss, while the cell envelope plays an equally essential role as a second permeability barrier within the complex stratified layers of the epidermis. [22]

2 MOISTURIZATION

Nearly everyone has used a skin moisturizer product. In fact many people use a moisturizer every day of their life. [23] Moisturizers are used to alleviate symptoms of clinically and subjectively dry skin. Recent studies suggest that certain ingredients in creams may accelerate the recovery of a disrupted barrier and decrease the skin susceptibility to irritant stimuli. [24] The products on the moisturizer shelves appears to be much the same, a variety of creams and lotions. There are sprays and foams, gels and serums, oils and jelly, balms and lipsticks, foundations and mascara, and even sunscreens, all labeled as moisturizing, etc. Each of the products mentioned before has a label (pack copy) that describes the product, lists the ingredients, provides instructions for use, and describes the benefits to be expected. Some moisturizers appear to contain one or more special moisturizing ingredients, whereas other products that claim to be highly effective skin moisturizers do not. Some moisturizers are described as natural in a way that suggest that naturalness is important. But many moisturizers seem not to be natural and yet are apparently excellent moisturizing products. Then there are moisturizers described for different types of skin, for different parts of the body, for different times of the day, for younger or older consumers, and for different ethnic groups. [23]

2.1 Natural mechanism of skin moisturization

The structure of the *stratum corneum* is often likened to the bricks and mortar of a wall. The bricks are the dead skin cells of the *stratum corneum* (corneocytes), and these are embedded in a matrix of intracellular lipid bilayers (the mortar). Corneocytes are flat pancakelike protein structures approximately 1 μm thick and 50–80 μm in diameter. The protein matrix of corneocytes contains a specific mix of hygroscopic low molecular weight compounds that keep corneocytes hydrated. The main components of this mix, collectively known as skin’s natural moisturizing factor (NMF), are lactic acid, urea, various salts, and amino acids derived from degradation of the protein filaggrin in the lower regions of the *stratum corneum*. There are three types of lipid that combine to form the intercellular lipid matrix of the *stratum corneum*. These are fatty acids, ceramides, and cholesterol. Each lipid type is bipolar with a hydrophilic (water loving) head group/region and hydrophobic (water hating) side chain/region. These lipids spontaneously form alternating lipid layers of hydrophilic and hydrophobic regions. These alternating lipid bilayers form the water barrier of the *stratum corneum*. The layers control the movement of water through the *stratum corneum*, measured as transepidermal water loss (TEWL) and also form a seal around each of the corneocytes, locking in the NMF, which being water soluble would otherwise diffuse away. [23]

Skin has two mechanisms for retaining moisture: [23]

1. Natural moisturizing factor within the protein matrix of corneocytes;
2. Triple lipid bilayers around and between corneocytes.

Moisture is required in the *stratum corneum*, particularly in the superficial layers, to keep the *stratum corneum* soft, supple, and flexible and to activate desquamation (exfoliation). [23]

Desquamation, the shedding of corneocytes from the skin surface, is an enzymic process (degradation of desmosomes) which requires an optimum water activity. If desquamation is impaired, superficial corneocytes remain attached to those below and pile up as visible flakes on the skin surface and they are responsible for the characteristic dullnes, white scaly appearance, roughness, and flaking of dry skin. [23]

2.2 Skin moisturizers

In the simplest terms a moisturizer is a product designed to restore and maintain optimum hydration of the *stratum corneum*. Notwithstanding the thousands of moisturizing products available to consumers there are only two posibilities how to moisturize the skin: [23]

1. The first way is to increase water-holding capacity of the *stratum corneum* by external application of hygroscopic ingredients, collectively known as humectants. These ingredients serve to replace skin NMF that has been washed away or otherwise depleted. Humectants act in the same way as NMF, and indeed some of the humectants commonly used in moisturizers are components of the skin NMF, e.g. lactic acid and urea;
2. The second way is to keep water in the *stratum corneum* by depositing an impermeable layer of water-insoluble oily material on the skin surface. Oily materials mimic the effect of the natural lipid bilayers of the skin to restrict evaporation from the surface and to seal NMF/humectants in corneocytes. These oily emollient mate-

rials also help to restore impaired water barrier function in regions where natural skin lipids have been lost.

2.2.1 Humectants

Humectants are cosmetics ingredients intended to increase the water content of top layers of the skin. They are hygroscopic substances generally soluble in water; these moisture attractants maintain an aqueous film at the skin surface. [25]

They are able to attract water from the atmospehere (if atmospheric humidity is greater than 80 %) and from underlying epidermis. Although humectants may draw water from the environment to help hydrate the skin, in low-humidity conditions, they may take water from the deeper epidermis and dermis, resulting in increased skin dryness. For this reason, they work better when they are combined with occlusives. Humectants are also popular additives to cosmetic moiusturizers because they protect product against evaporating and thickening, which increase the shelf life of products. Some humectants have bacteriostatic activity as well. Humectants draw water into the skin, causing a slight swelling of the *stratum corneum* that gives the perception of smoother skin with fewer wrinkles. As a result, many moisturizers are touted as antiwrinkle creams even though there is no long-term antiwrinkling effect. Examples of commonly used humectants include glycerin, sorbitol, sodium hyaluronate, urea, propylene glycol, α-hydroxy acids, and sugars. [26]

2.2.1.1 Glycerin

The primary used humectant in personal care products is glycerin; it tends to provide a heavy and tacky feel which can be overcome by using it in combination with other humectants such as sorbitol. [25] Glycerin is a strong humectant and has a hygroscopic ability that closely resembles that of natural moisturizing factor. NMF is naturally present within corneocytes. It is made up of amino acids and their derivatives, as well as lactate, urea, citrate, and sugars. NMF is made of very water-soluble chemicals so that it can absorb large amounts of water, even when humidity levels are low. This also allows the *stratum corneum* to retain a high water content even in a dry environment. Many attempts have been made to mimic the NMF in moisturizing products. [26]

2.2.1.2 Propylene glycol

Propylene glycol is the second most widely used humectant in cosmetic products. It reduces viscosity of surfactant solutions and tends to depress the foam. [25] Propylene glycol is an odorless liquid that functions as both a humectant and an occlusive. It has antimicrobial and keratolytic properties. Propylene glycol has been shown to enhance the penetration of drugs such as minoxidil and steroids. Propylene glycol is known to be a weak sensitizer itself but may contribute to contact dermatitis by enhancing penetration of another allergen. [26]

2.2.1.3 Urea

Urea is a component of the NMF. It has been used in hand creams since the 1940s. In addition to being a humectant, urea has a mild antipruritic effect. Although there is some disagreement in the literature, several studies show that combining urea with hydrocortisone, retionic acid, and other agents increases penetration of these agents. [26]

2.2.1.4 Hydroxy Acids

α-Hydroxy acids (AHAs) are naturally occuring organic acids that function as humectants as well as having exfoliating properties. Glycolic (Fig. 5) and lactic (Fig. 6) acids, derived respectively from sugar cane and sour milk, are the most commonly used types in moisturizing products. [26]

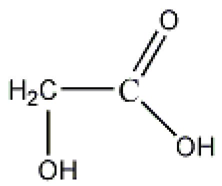

Fig. 5: *Glycolic acid*

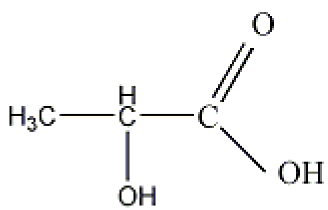

Fig. 6: *Lactic acid*

Other AHAs include malic acid (Fig. 7) derived from apples, citric acid (Fig. 8) derived from acid fruits, and tartaric acid (Fig. 9) derived from grapes. [26]

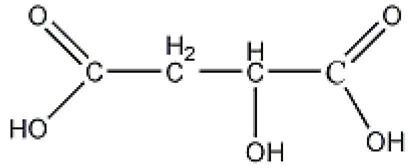

Fig. 7: *Malic acid*

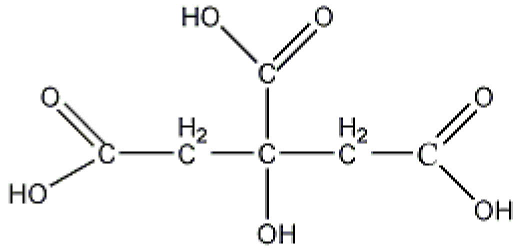

Fig. 8: *Citric acid*

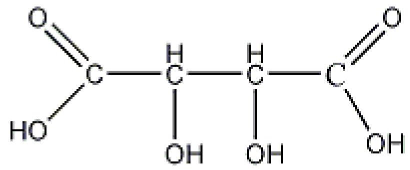

Fig. 9: *Tartaric acid*

Topical preparations that contain AHAs have long been known to exert significant influence on epidermal keratinization. Salicylic acid (Fig. 10), a chemical exfoliant and the lone β-hydroxy acid (BHA), is derived from willow bark, wintergreen leaves, and sweet birch, but is also available in synthethic form. [26]

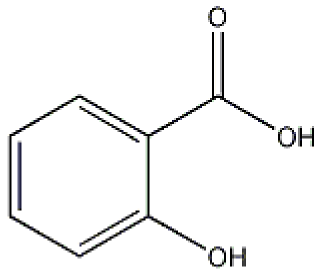

Fig. 10: *Salycilic acid*

The cosmetic effects of hydroxy acids include normalization of the *stratum corneum* exfoliation, resulting in increased plasticization and decreased formation of dry scales on the surface of the skin. AHAs and BHA function by degrading the desmosomes and allowing desquamation to proceed. They also influence corneocyte cohesiveness at the basement levels of the *stratum corneum*, where they affect its pH, and improve desquamation. The application of AHAs and BHA in high concentrations leads to detachment of keratinocytes and epidermolysis; application at lower concentrations degrades intercorneocyte cohesion directly above the granular layer, which furthers desquamation and thinning of the *stratum corneum*. A thinner *stratum corneum* is more flexible and compact, giving the skin more youthful appearance. This increased flexibility that is obtained after the use of AHAs has been shown to persist even in low-humidity situations. This thinner, more compact *stratum corneum* is also desirable because it better reflects light, making the skin appear more luminous. [26]

2.2.1.5 Lactic acid

Lactic acid is unique because it is an AHA as well as a component of the NMF. This means that it offers the same benefit as other AHAs by promoting desquamation, but may have other beneficial effects as well. The benefits of lactic acid on photoaged skin are well understood as demonstrated by a double-blind vehicle-controlled study that found that an 8% α-lactic acid formula was superior to vehicle for the treatment of photoaged skin. Statistically, significant improvements were seen in skin roughness and signs of photodamage (mottled hyperpigmentation and sallowness). However, the benefits of lactic acid in dry skin are just being elucidated. Lactic acid (especially α-isomer) has been found *in vitro* and *in vivo* to increase the production of ceramides by keratinocytes. In addition, application of the α-isomer of lactic acid to keratinocytes not only increased the ceramide content but appeared to increase the ratio of ceramide 1-linoleate to ceramide 1-oleate. This is likely an important finding because a reduced ratio of ceramide 1-linoleate to ceramide 1-oleate is seen in diseases such as atopic dermatitis and acne. The increased levels of ceramides, particularly ceramide 1-linoleate, may partly explain why patient treated with the α-isomer of lactic acid showed an improved water barrier with less TEWL after a surfactant patch test than did patients treated with vehicle alone. [26]

Low molecular weight polyethylene glycols (PEGs from about 10 to 200 PEG units), amino acids (AA), and other constituents of skin natural moisturizing factors like sodium pyrrolidone carboxylic acid (PCA) and sodium lactate are also applicable for use in surfactant based skin cleansing products. [25]

Humectants are not substantive to the skin, they are easily rinsed off after cleaning; consequently, skin feel improvement is not obvious to perceive, and their efficacy in terms of skin moisturization is difficult to document. Glycerin, propylene glycol, 1,3-butylene glycol, or sorbitol are typically used in body washes, bubbles baths, shower gels, or soaps to prevent the desiccation of the product itself and the formation of a dry layer at the surface. They also ensure stability and clarity of liquid cleansers at cold temperatures. Few substantive humectants can be mentioned. They are cationic in nature which makes them adsorbing to the negatively charged skin surface. In the quaternized polyalkoxylated methyl glucose derivative (lauryl methyl gluceth-10 hydroxypropyldimonium chloride) the hydrophilic moiety delivers humectant properties; the hydrophobic chain at the cationic end of the molecule ensures both substantivity and skin conditioning. Chitosan-PCA is another example. Chitosan is a polycationic (at acidic pH) high molecular weight polymer produced by deacetylation of chitin, the major constituent of invertebrate exoskeletons. Combining chitosan with PCA leads to a highly substantive, film forming humectant material. [25]

2.2.2 Emollients

These are substances added to cosmetics to soften and smooth the skin. They function by filling the spaces between desquamating corneocytes to create a smooth surface. These products provide increased cohesion, causing a flattening of the curled edges of the individual corneocytes. This leads to a smoother surface with less friction and greater light refraction. Many emollients function as humectant and occlusive moisturizers as well. Lanolin, mineral oil, and petrolatum are examples of occlusive ingredients that also confer an emollient effect. Lanolin has a reputation for being a common sensitizer, prompting several manufacturers to label their products as lanolin-free. This reputation may be unwarranted because it is actually a very weak allergen. [26]

The most known emollients are:

2.2.2.1 Animal fats

The animal-derived fats used in emollients have changed over the years. Beef and mutton fat were extensively used at one time, but have been supplanted by aesthetically superior materials. The most important source of animal fat for the cosmetic and pharmaceutical industries is tallow, obtained directly from animal carcasses by the process of rendering. Lanolin (sheep wool fat) has been extensively used in the past, but is rarely used now in its unmodified form because of its propensity to cause allergic contact dermatitis. Various chemically modified lanolins are now available which are much less liable to cause sensitivity problems. The use of whale oils in emollients enjoyed a fashinable popularity until conservationists made us aware of the barbarities of whaling. [27]

2.2.2.2 Vegetable oils

Vegetable oils are much more popular with the manufacturers of emollients. Indeed the unmodified oils of certain seeds, nuts and fruits (e.g. peanuts, olives and sunflower seeds) are often included in the final formulations. Such vegetable oils used in their natural form are much appreciated by some patients but they have quite a greasy feel to them and they are mostly employed as emulsions or as bath preparations which are formulated so that the oils form a dispersion in the bath. Examples of vegetable-based oils are coconut oil, palm kernel oil, groundnut oil, olive oil and cotton seed oil. [27]

2.2.2.3 Mineral oils

Oils that derive from oils found deep in the earth are termed mineral oils – even though they come originally from minute sea creatures alive millions of years ago. The mineral oils used for emollients are products of petroleum distillation and contain a large number of organic compounds, but especially long and branched chain aliphatic hydrocarbons. The refining process includes not only distillation, but also solvent extraction, crystallization, alkali neutralization and bleaching, and produces both petroleum jelly and light liquid paraffin (white oil). So-called light paraffin oil is often employed as a major constituent of medical emollients. [27]

2.2.2.4 Synthetic oils

Apart from the animal, vegetable and mineral sources, lipids are also derived from man-made compounds. Synthetic silicone oils seem to have ideal properties for emollients and are often included. [27]

2.2.2.5 Waxes

Waxes are complex semi-solid lipid mixtures that also derive from animal, vegetable or mineral sources. Some regard lanolin as a wax, but beeswax from honeycombs, carnauba wax from carnauba palm tree and paraffin wax are among the most frequently used. Spermaceti wax was obtained from the head oil of whales, but for obvious reasons is no longer used. [27]

2.3 Measurement of moisturization

Recently, several *in vivo* studies using modern bioengineering techniques have been performed to evaluate the relationship between the mechanical properties and water content of the epidermis. The instruments that have been used for assessing epidermal hydration are based on measurements of conductance, capacitance and impedance of the skin. [28]

2.3.1 Methods for measurement of skin moisturization

There are used different methods for detection of skin moisturization. Generally any of these methods is not available for practical usage because these methods are too expensive, inexact or they give only too general findings. [29]

Using methods: [29]

1) Electrical measurement of impedance, rezistance and phase angle – it is influenced by the effects of polarization, electrolytes in the skin and transition resistance. It needs galvanic contact between measuring circuit and measured subject;
2) Infrared spectroscopic measurement – there is measured absorbing of infrared rays by the skin and there are analysed peaks characteristic for a water;
3) Resonant frequency measurement – elasticity of the skin is affected by the skin moisturization and it leads to a different transfer of mechanical vibrations between

transmitter and acceptor of signal located on the skin. There are analysed changes in bonds;

4) Fotoacustic method – it is based on the measurement of acustic signals which originate in textus and they are caused by the pressure changes which are caused by periodic light;

5) Evaporating methods – quantity of water evaporated from the skin surface is measured. These methods are analysed by electrical measurement or by absorbing of water to a measuring material. They are used only for indirect determination of the skin moisturization because they evaluate only relative part of evaporated water.

2.3.1.1 Corneometric method

Optimal solution of all problems connected with the measurement of skin moisturization is corneometric method where capacitance through the *stratum corneum* layer is measured. Method is based on the fact that dielectrical constants of water (which is 81) and other materials (dielectrical constant is usually minor than 7) are different. Measuring condensator reacts to the changes of water quantity by the change of final capacitance in the zone of measurement. These changes of capacitance are automatically recorded. There are many advantages of this method. Mainly there is no electric current in the measured subject because in this method is not galvanic contact between measuring equipment and measured subject. Obtained values are practically not affected by polariazation or ionic conduction and measurement is more quick. [29]

3 THE AIMS OF THE WORK

The aim of this thesis was to make a literature research on the topic the skin, its structure, each layers of the skin, its function, protection and moisturization.

Further, to carry out a corneometric measurement of moisturizing effects of cosmetic products in defined time intervals on a group of volunteers. Cosmetic products will be applied to a skin damaged by 0.5% solution of sodium lauryl sulfate.

Obtained values will be processed, compared and discussed.

II. PRACTICAL PART

4 METHODICS

4.1 List of chemicals

Sodium chloride (NaCl): (99.9%, deliverer: Petr Lukeš, Czech Republic)

Sodium lauryl sulfate (SLS): (Dodecylsulfate Sodium Salt for Molecular Biology, deliverer: Merck, CAS number: 151-21-3)

Distilled water

Sample 1 – Hand Cream

Sample 2 – 24-Hour Hydrating Cream for All Skin Types

Sample 3 – Moisturizing Hand Cream with Liquid Crystals

Sample 4 – Eye Cream with Caviar

Sample 5 – Day Cream

Sample 6 – Oil Free Moisturizer

Sample 7 – Dermatology Hand Cream

Sample 8 – Dermatology Moisturizing Face Cream

Sample 9 – Olive Moisturizing Face Cream

Sample 10 – Treatment Cream – 16 % of Healing *Cannabis Sativa L.*

Sample 11 – Treatment Cream – 27 % of Healing *Cannabis Sativa L.*

Sample 12 – Hemp Seed Oil

4.2 List of equipment

Corneometer CM820 PC (Courage + Khazaka, Köln – Germany)

Laboratory thermometer

Syringe (Medilab, Czech Republic)

Filter paper – blue – speed of filtration 120 s (Filpap, Czech Republic)

Glass for chemical ware

Tear sticking strip (Henkel – Pritt, Germany)

Computer programme UNISTAT 5.5 (UNISTAT LTD, United Kingdom)

4.3 Description of equipment

4.3.1 Corneometer CM820

Corneometer CM820 PC consists of console and measuring sonde. The sonde is connected with the console by a cabel of 1 m lenght and with a special adaptor. Measured value of moisturization is displayed as a maximum three-decimal number on a display sized 40 x 18 mm. [29]

Measuring sonde is square shaped and its active part, which is covered by a special glasses, is mobile along the axis and has a upthrow 3 mm. Principle of measurement requires a perfect contact of sonde with a constant pressure and because of it a forehead of sonde is small – 7 x 7 mm. Interior mobile part – active part of sonde – is held down against the epidermis by the strenght of 3.5 N. [29]

Corneometer CM820 PC works automatically. Results of measurement can be affected by these factors: [29]

1) incorrect contact of measuring sonde with the epidermis (too high pressure, sloping position, scabrous skin surface, hair below the sonde);
2) measurement is done immediately after the physical or psychical strain;
3) measurement is done in extreme temperature and/or humidity conditions in the room;
4) measurement is done longer (effect of occlusion);
5) impurities and humidity in a sonde, defective sonde.

4.3.1.1 *Technical parameters*

Technical parameters of measuring equipment are in Table 2.

Table 2: *Technical parameters of Corneometer CM820* [29]

Power supply	220 V 50/60 Hz or 110V	
Principle of measurement	capacitance	
Measured area	49 mm^2	
Pressure power	3.56 N	
Proportions	depth	235 mm
	width	180 mm
	high	75 mm
Weigh	1.4 kg	

4.4 Description of using cosmetic products

For the whole measurement were used 12 samples of cosmetic products.

4.4.1 Hand Cream

This silky cream contains natural moisturizers, Avocado and Aloe extracts which nourishes skin.

This product was made in USA.

Ingredients

Aqua, Propylene Glycol, Dimethicone, Behentrimonium Methosulfate, Cetearyl Alcohol, Polyquaternium-37, Propylene Glycol Dicaprylate/Dicaprate, PPG-1 Trideceth-6, Allantoin, Tocopherylacetate, *Aloe Barbadensis* Leaf Extract, *Persea Gratissima* (Avocado) Oil, Ethylhexylpalmitate, Lecithin, Caprylic/Capric Triglyceride, Benzophenone-4, Isopropylparaben, Isobutylparaben, Butylparaben, Methylparabens, Alcohol, Glyceryl Stearate, Oleic Acid, Ascorbyl Palmitate, Tocopherol, Parfum/Fragrance, Cinnamal, Limonene, Linalool, CI 15985 (Yellow 6), CI 42090 (Blue 1).

4.4.2 24-Hour Hydrating Cream for All Skin Types

This light fine cream contains grapefruit extract, Aloe Vera and vitamin E to enhance skin elasticity and firmness and protect against free radicals. This cream provides the skin with long term hydration and the cream is suitable for day and night treatment of all skin types.

This product was made in Czech Republic.

Ingredients

Aqua, Paraffinum Liquidum, Caprylic/Capric Triglyceride, Isohexadecane, Isopropyl Myristate, *Aloe Barbadensis*, Polygyceryl-3 Methylglucose Distearate, Propylene Glycol, Ethylhexyl Methoxycinnamate, Cetearyl Alcohol, Tocopheryl Acetate, *Citrus Grandis* Seed Extract, Butylmethoxydibenzoylmethane, Phenoxyethanol, Methylparaben, Ethylparaben, Propylparaben, Isobutylparaben, Disodium EDTA, Parfum, Citronellol, Hexylcinnamal.

4.4.3 Moisturizing Hand Cream with Liquid Crystals

Cream contains natural moisturizers and liquid crystals, which elongate moisturizing effect and effectively control active substance release. Cream enhances skin elasticity and firmness and after that hands are smooth and soft.

This product was made in Slovakia.

Ingredients

Aqua, PPG-15 Stearyl Ether, Isohexadecane, Steareth-2, Steareth-21, Stearic Acid, *Cera Alba*, Cetearyl Alcohol, Dimethicone, EDTA, Urea, Lactic Acid, Triclosan, Methylparaben, Ethylparaben, Propylparaben, Butylparaben, Parfum, Phenoxyethanol.

4.4.4 Eye Cream with Caviar

Special product for eye care, which nourishes, stretches, hydrates, smoothes and protects against wrinkles. Cream contains highly valuable caviar extract.

This product was made in Czech Republic.

Ingredients

Aqua, Paraffinum Liquidum, *Persea Gratissima* Oil, Acrylamide Copolymer /and/ C13-14 Isoparaffin /and/ Laureth-7, Glycerin, Panthenol, Glyceryl Stearate, Caviar Extract, Disodium EDTA, Methylparaben, Propylparaben, Methylchloroisothiazolinone, Methylisothiazolinone Parfum.

4.4.5 Day Cream

Non-scented moiustizing cream for dry and very dry skin, which smoothes fine lines.

This product was made in Switzerland.

Ingredients

Aqua, Isopropyl Isostearate, Glycerin, Cetearyl Isononanoate, Butylene Glycol, Cyclopentasiloxane, Squalene, *Butyrospermum Parkii* (Shea Butter), Triethylhexanoin, Panthenol, Polymethyl Methacrylate, Tocopheryl Acetate, Phenoxyethanol, Cetyl Alcohol, Cetearyl Olivate, Creatine, Dimethicone, Potassium Cetyl Phosphate, Propylene Glycol, Sorbitan Olivate, Sorbitol, Sodium Polyacrylate, Polyacrylate-13, Ethylhexylglycerin, Polyisobutene, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Arginine, Collagen Amino Acids, Xanthan Gum, Disodium EDTA, Carbomer, Sodium Hyaluronate, Polysorbate 20, Palmitoyl Pentapeptide-4.

4.4.6 Oil Free Moisturizer

This laboratory sample was prepared in UK.

Ingredients

Aqua, Corn Starch Modified, Coclopentasiloxane, Coclomethicone, Dimethicone Crosspolymer, Dimethicone, Cyclohexasiloxane, Cetearyl Alcohol, Cetearyl Glucoside, Ceteareth-20, Beta Glucan, Tocopheryl Acetate, Phenoxyethanol, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, *Citrus Aurantium Dulcis* (Orange) Oil, Ethylhexylglycerin, Limonene, Tetrasodium Glutamate Diacetate, Citric Acid, Sodium Hydroxide, C13-14 Isoparaffin, Laureth-7, Polyacrylamide.

4.4.7 Dermatology Hand Cream

Combination of active substances – 5 % olive oil and 3 % urea – smoothes and recovers dry and rough skin. Cream reduces itching. It is ideal subsidiary care in case of *neurodermitis*. Cream is colorant free and silicon and parrafin oil free.

This product was made in Germany.

Ingredients

Aqua, *Olea Europaea* Oil, Polyglyceryl-3 Distearate, Cetearyl Alcohol, Panthenol, Urea, Caprylic/Capric Triglyceride, Glycerin, Dacaprylyl Carbonate, Sodium Lactate, *Cera Alba*, Neopentyl Glycol Diethylhexanoate, Lactic Acid, Octyldodecanol, *Echium plantagineum* Seed Oil, *Cardiospermum Halicacabum* Leaf Extract, *Helianthus Annuus* Seed Oil Unsaponifiables, Phenoxyethanol, Sodium Cetearyl Sulfate, Benzyl Alcohol, Potassium Sorbate, Tocopherol, Sodium Hydroxide, Parfum, Hydrogenated Palm Glycerides Citrate.

4.4.8 Dermatology Moisturizing Face Cream

Dermatology moisturizing cream with 3 % of olive oil and 5 % of urea moisturize dry skin. It is ideal subsidiary care in case of *neurodermitis* and sensitive skin. Cream reduces itching and it is colorant free and silicon and parrafin oil free.

This product was made in Germany.

Ingredients

Aqua, Urea, Caprylic/Capic Triglyceride, *Olea Europaea* Oil, Cetearyl Alcohol, Polyglyceryl-3 Distearate, Glyceryl Stearate Citrate, Panthenol, Neopentyl Glycol Diethylhexanoate, Sodium Lactate, Octyldodecanol, Silica, Lactic Acid, *Echium Plantagineum* Seed Oil, *Cardiospermum Halicacabum* Leaf Extract, *Helianthus Annuus* Seed Oil Unsaponifiables, Phenoxyethanol, Benzyl Alcohol, Potassium Sorbate, Sodium Cetearyl Sulfate, Xanthan Gum, Tocopherol, Sodium Hydroxide, Parfum.

4.4.9 Olive Moisturizing Face Cream

Olive moisturizing face cream with special moisturing vitamin complex and urea provides 24 hours intensive care to a skin. Skin is visibly smooth again.

This product was made in Germany.

Ingredients

Aqua, Urea, *Olea Europaea* Oil, *Butyrospermum Parkii* Butter, Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Ethylhexyl Palmitate, Panthenol, Tocopheryl Acetate, Phenoxyethanol, Benzyl Alcohol, Potassium Sorbate, Sodium Hyaluronate, Tocopherol, Parfum, Triacetin, Citric Acid, Butylphenyl Methylpropional, Linalool, Citronellol, Limonene, Benzyl Salicylate, Eugenol, Geraniol.

4.4.10 Treatment Cream - 16 % healing *Cannabis Sativa L.*

Treatment cream from pure hemp seed oil, myrrh, marjoram, dead sea salt and with gently acid pH (4,5) for special care about the skin with eczema. Contained active substances maintain natural regeneration of skin cells, recovery moisture and pH balance of epidermis.

This product was made in Czech Republic.

Ingredients

Aqua, *Cannabis Sativa* Seed Oil, *Butyrospermum Parkii*, Glycerin, Polyglyceryl-3 Methylglucose Distearate, Glyceryl Stearate, Stearic Acid, Panthenol, Glucose, *Commiphora Myrrha* Extract, *Majorana hortensis*, Dead Sea Salt, Tocopheryl Acetate, Bisabolol, Allantoin, Glucose Oxidase, Lactoperoxidase.

4.4.11 Treatment Cream - 27 % healing *Cannabis Sativa L.*

Treatment cream from pure hemp seed oil with glycerin, allantoin and vitamin E for all-day treatment of smooth children's skin. Contained active substances maintain natural regeneration of skin cells, recovery moisture and pH balance of epidermis.

This product was made in Czech Republic.

<u>Ingredients</u>

Aqua, *Cannabis Sativa* Seed Oil, Polyglyceryl-3 Methylglucose Distearate, Glyceryl Stearate, Stearic Acid, Glycerin, Panthenol, Tocopheryl Acetate, Sodium Lactate, Sodium PCA, Glycine, Fruktose, Urea, Niacinamide, Inositol, Lactic Acid, Glucose, Bisabolol, Allantoin, Glucose Oxidase, Lactoperoxidase.

4.4.12 Hemp Seed Oil

Hemp Seed Oil – cold pressed on 7th January 2010 at Institute of Chemical Technology Prague, Deparment of Dairy and Fat Technology.

4.5 Experiment

4.5.1 Preparation of solutions

Physiological solution (0.85% solution of NaCl in distilled water)

For the preparation of 300 ml physiological solution was weighed 2.55 g of NaCl (calculation you can see further) and it was dissolved, quantitatively transferred into the flask and completed with distilled water to the volume of 300 ml.

Calculation of NaCl: 0.85 g…………100 ml

↑x g……………..300 ml↑

$$x = \frac{300}{100} \cdot 0.85 = 2.55 \text{ g}$$

0.5% solution of SLS (SLS + physiological solution)

For the preparation of 250 ml 0.5% solution of SLS was weighed 1.25 g of SLS (calculation you can see below) and it was dissolved, quantitatively transferred into the flask and completed with physiological solution to the volume of 250 ml.

Calculation of SLS: 0.5 g……………..100 ml

↑x g……………….250 ml↑

$$x = \frac{250}{100} \cdot 0.5 = 1.25 \text{ g}$$

4.5.2 Preparation of material for testing

Ten cosmetic products – creams – were bought in a trade network for the measurement and two samples were prepared in a laboratory – one in a laboratory in United Kingdom and one in a laboratory at Institute of Chemical Technology in Prague. All tested samples were taken into 12 syringes (volume of syringe was 1 ml) and used for testing by test method described below in capture 4.5.4.

4.5.3 Volunteers

The selection of volunteers and the test methods were carried out in accordance with the Declaration of Helsinki (1964) and the International Ethnical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, 2002). The study was approved by the Ethical Review Committee of the National Institute of Public Health.

A group of 5 healthy white female volunteers with the same type of skin, age 39–59, participated in this study. All volunteers had been informed about the scope of the study. They completed a medical history form and confirmed their participation in the study by signature on individual informed consent. The study included all ethical principles of biomedical research involving human volunteers.

4.5.4 Test method

The experiment was carried out in the room with the temperature 22.2°C. Acclimatization of volunteers took 20 minutes before each measurement.

Left and right volar forearms of each volunteer were divided to completely eight parts (four parts on the left and four parts on the right forearm) – filter paper sized 2 x 4 cm served as a template and small signs were signed on the forearms around the filter paper with permanent marker to know where to apply 0.5% solution of SLS (sodium lauryl sulfate) and samples of creams.

Afterwards, 0.5% solution of SLS was taken into a syringe and seven filter papers of the same size as template were put into the Petri dish where they were modified by the 0.5% solution of SLS from syringe and they were saturated approximately 20 seconds. After that, filter papers were placed (by the help of sticking strip) to the seven of eight parts of the skin on volar forearms for 4 hours. One of eight parts served as a control, it means that this part was not irritaded by 0.5% solution of SLS. Filter papers were removed after 4 hours and all tested areas of volar forearms were measured by corneometric sonde five times (it means that they were measured in time 0 h). The degree of hydration was measured by Corneometer CM 820 (Courage + Khazaka, Germany). Measurements were performed on the test parts and on the control part in parallel.

Six samples of creams were applied immediately after measurement in time 0 h. Each of the cream was taken into the syringe (the volume of syringe was 1 ml) and 0.1 ml of each cream was applied from the syringe onto one tested area of volar forearm of each volunteer. Scheme of tested areas on the left and right volar forearms is shown in Fig. 11 and real representation of tested areas on the left and right volar forearms can be seen in Fig. 12. Afterthat the cream was spreaded 30 seconds by the finger on the whole tested area. First area served as a control again, it means that this part was not irritated by 0.5% solution of SLS and it was not covered by cream. Second area was unprotected, it means that this part was irritated by 0.5% solution of SLS and it was not protected by cream. Corneometric measurement was carried out again, it means that all tested areas of volar forearms were measured by corneometric sonde five times and measurement intervals were 1 h, 2 h, 3 h, 19.5 h, 20.5 h, 21.5 h, 22.5 h, 23.5 h, 24.5 h, 25.5 h and 26.5 h for the samples 1–6 and 1 h, 2 h, 3 h, 20 h, 21 h, 22 h, 23 h, 24 h, 25 h, 26 h and 27 h for the samples 7–12 after application of cream.

Obtained values in corneometric units were noted and averaged out for each tested area of each volunteer. Average values were converted into the percentual expression and standard deviations for the measurement in each time were calculated by the formula below: [30]

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{(n-1)}}$$

where: s – standard deviation

x – each score

$\bar{x}$ – the mean or average

n – the number of values

Σ – the sum of the values

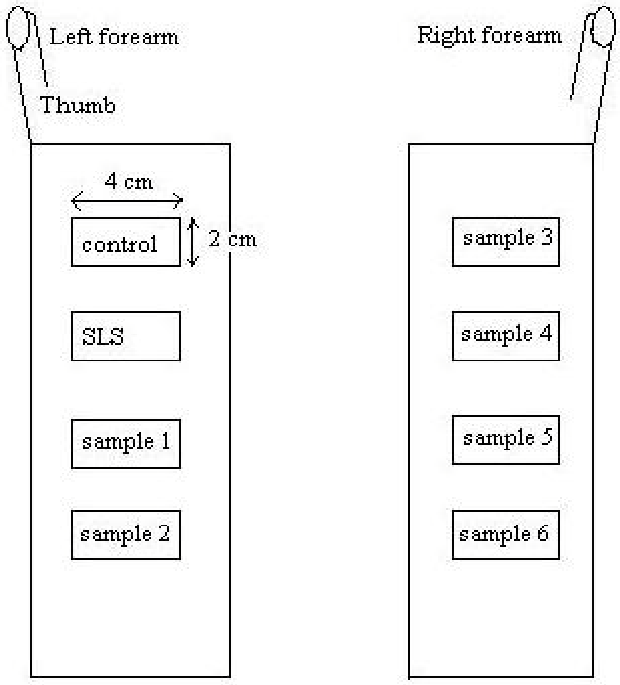

Fig. 11 *Schematic representation of left and right volar forearm* [31]

Fig. 12: *Real representation of left and right volar forearms*

5 EVALUATION AND DISCUSSION

5.1 Determination of the moisturizing effect of tested samples

Skin moisturization was measured separately for samples number 1–6 and for samples number 7–12. Results were separated into two series. Obtained values were expressed in corneometric units. Each tested area of volar forearms of each volunteer was measured five times. It was necessary for evaluation to average out values for each tested area of each volunteer. Average values were converted into the percentual expression – values for 0.5% solution of SLS were calculated to a percentage of control and values for samples number 1–12 were calculated to a percentage of 0.5% solution of SLS. Results were summarized into the tables (Table 3 and Table 4) and these were used for next graphical representation.

For graphical representation of the samples number 1–6 served Table 3 where the results of the measurement of these samples are summarized.

Table 3: *Average results in time 0–26.5 h in percentual expression*

	% of control	**% of 0.5% solution of SLS**					
time (hours)	**0.5% solution ofSLS**	**1**	**2**	**3**	**4**	**5**	**6**
0	92.31	100.00	100.00	100.00	100.00	100.00	100.00
1	89.61	114.60	109.72	112.62	113.03	110.58	98.71
2	88.37	114.74	116.14	118.67	120.11	121.99	103.71
3	93.02	103.84	108.57	110.37	114.69	112.90	100.92
19.5	91.62	105.99	109.68	105.47	112.23	112.73	104.88
20.5	94.01	106.23	109.71	105.36	111.25	109.13	101.24
21.5	95.30	106.50	107.25	102.05	109.50	109.41	102.84
22.5	97.31	105.32	104.46	99.79	106.59	105.21	97.89
23.5	95.45	102.81	107.36	104.61	107.54	107.15	100.08
24.5	97.74	101.53	103.20	102.02	103.37	102.75	98.65
25.5	96.90	104.91	105.15	104.75	109.23	107.93	103.30
26.5	96.08	102.97	103.36	104.03	105.46	105.23	99.63

As already has been mentioned above, these results were used for graphical representation of time addiction of moisturization, where time is indicated on the *x* axis (expressed in hours) and moisturization is shown on the *y* axis. Symbol 0.5% SLS means 0.5% solution of SLS in all graphs.

Graphic addiction of 0.5% solution of SLS calculated to a percentage of control is presented in Fig. 13. It is evident that skin moisturization of parts of the skin damaged by 0.5% solution of SLS after 4 hours of exposure was lower (skin moisturization in time 0 h was 92.31 %) in comparison with control part of the skin. There was a decrease of 3.94 % from time 0 h to time 2 h and an increase of 4.65 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h and there a skin moisturization was 91.62 %. The increase of moisturization was 5.68 % from time 19.5 h to time 22.5 h. Then there was a decrease and again increase to time 24.5 h when the skin moisturization mounted to its natural level of moisturization.

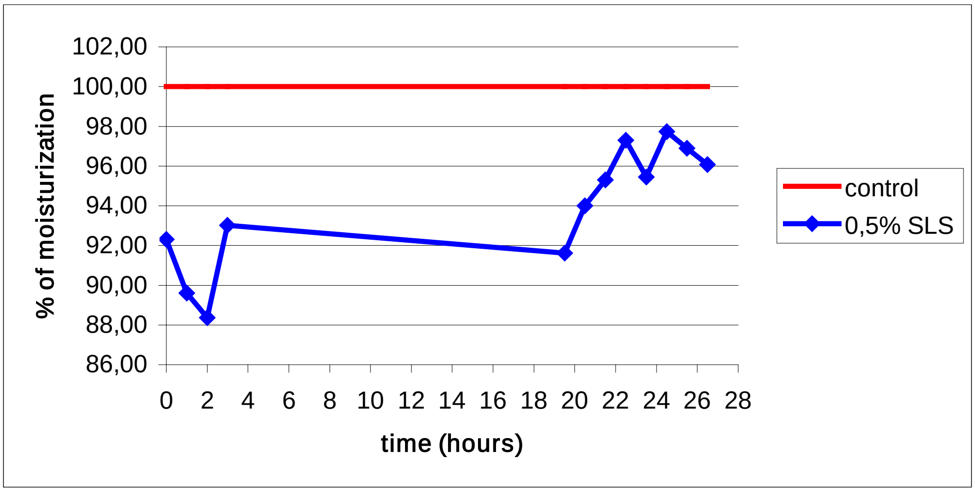

Fig. 13*: Graphic addiction of 0.5% solution of SLS calculated to a percentage of control – 1. measurement*

Graphic addiction of the sample number 1 calculated to a percentage of 0.5% solution of SLS is presented in Fig. 14. It is evident that the sample number 1 has the highest moisturizing properties in first two hours after application of cream onto the skin because the moisturization of the skin rapidly increased to 114.74 % at this time. Then the moisturization decreased to 103.84 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h when the moisturization was 105.99 % and moisturization started to decline to its natural level in time 21.5 h.

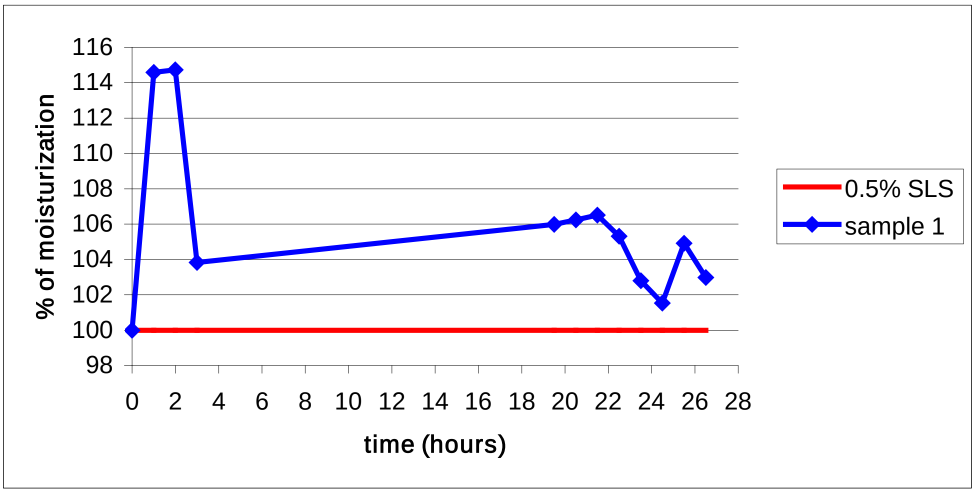

Fig. 14: *Graphic addiction of the sample number 1 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 2 calculated to a percentage of 0.5% solution of SLS are shown in Fig. 15. Moisturization of the skin steadily increased in first two hours to 116.14 %. Then there was a decrease of 7.57 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h and its moisturization was 109.68 %. Moisturization started to mount to its natural level from time 20.5 h. It is visible from the graph that the highest moisturizing properties of the sample number 2 were two hours after application of cream.

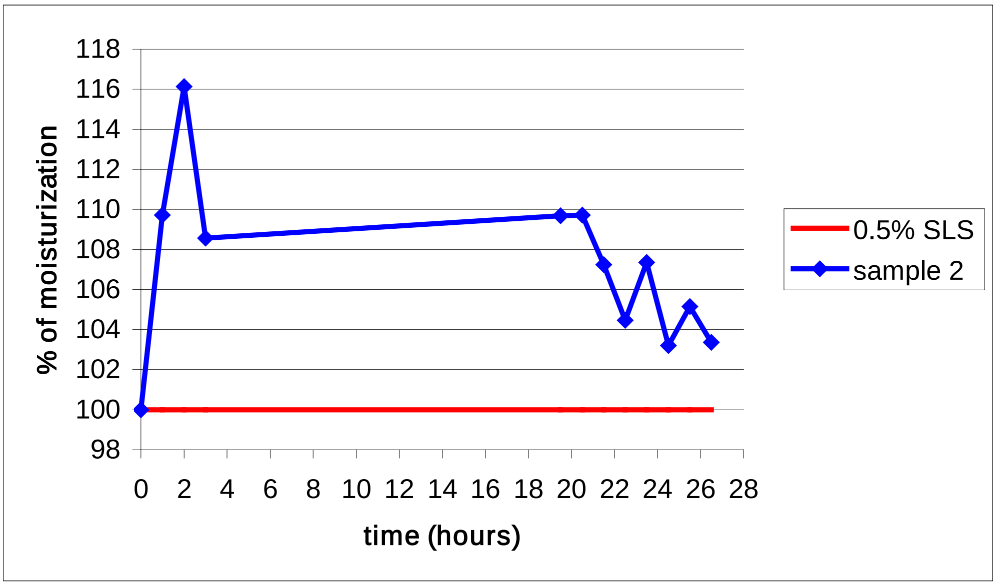

Fig. 15: *Graphic addiction of the sample number 2 calculated to a percentage of 0.5% solution of SLS*

Graphic addiction of the sample number 3 calculated to a percentage of 0.5% solution of SLS is presented in Fig. 16. Graphic representation of moisturizing properties of the sample number 3 is quite similar to graphic representation of moisturizing properties of the sample number 2. Moisturization rapidly increased in first two hours of measurement to 118.67 % and decreased to 110.37 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h when the moisturization was 105.47 %. There was a decrease of 5.68 % from time 19.5 h to time 22.5h. Then there was an increase of 4.82 % from time 22.5 h to time 23.5 h and the moisturization started to mount to its natural level from time 23.5 h.

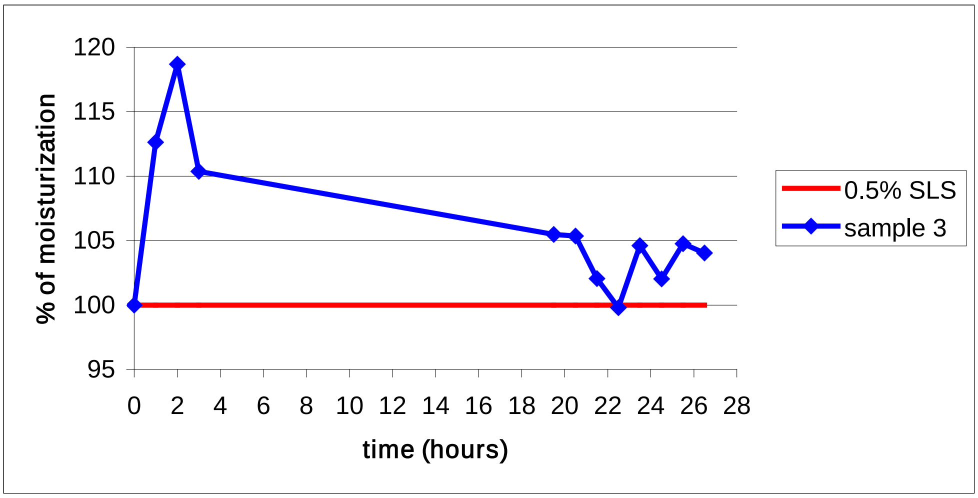

Fig. 16: *Graphic addiction of the sample number 3 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 4 are shown in Fig. 17. There was an increase of 20.11 % from time 0 h to time 2 h. Moisturization decreased to 114.69 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h when the moisturization was 112.23 % and from this time the moisturization started to decline to its natural level.

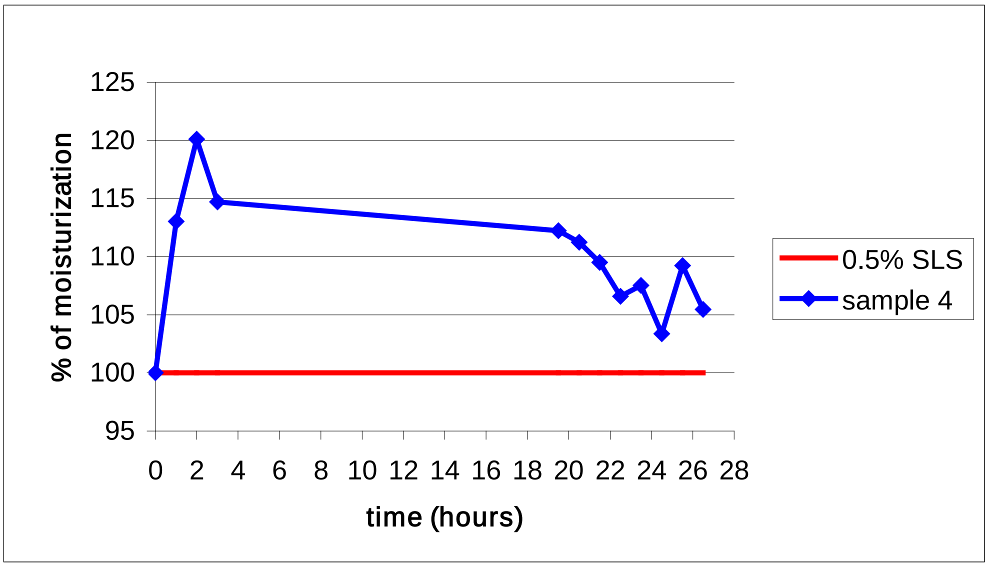

Fig. 17: *Graphic addiction of the sample number 4 calculated to a percentage of 0.5% solution of SLS*

Graphic addiction of the sample number 5 calculated to a percentage of 0.5% solution of SLS is presented in Fig. 18. It is evident that the moisturization steadily increased to 121.99 % in first two hours of measurement. There was a decrease of 9.09 % from time 2 h to time 3 h. Next measurement was carried out in time 19.5 h when the moisturization was 112.73 % and it started to decline to its natural level from this time.

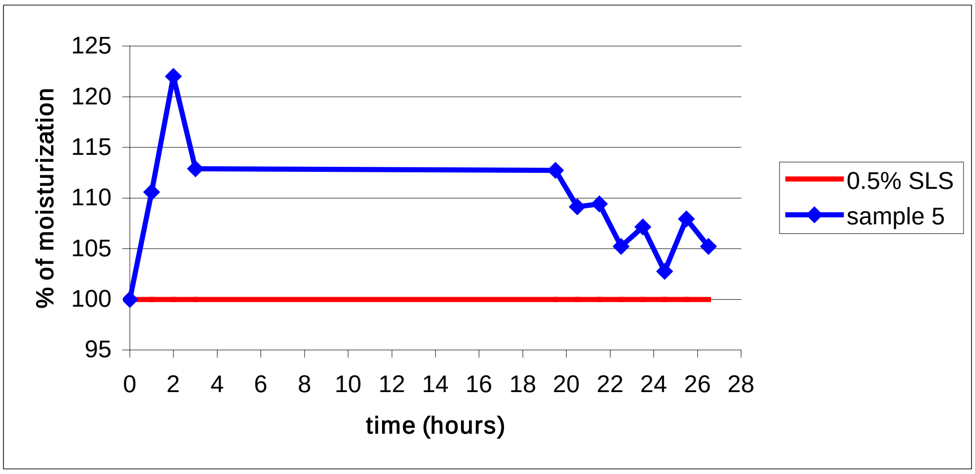

***Fig. 18:** Graphic addiction of the sample number 5 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 6 are shown in Fig. 19. Course of a curve of moisturization is imbalanced. This imbalance can perhaps be explained by deficient homogenization because this product was made at laboratory conditions.

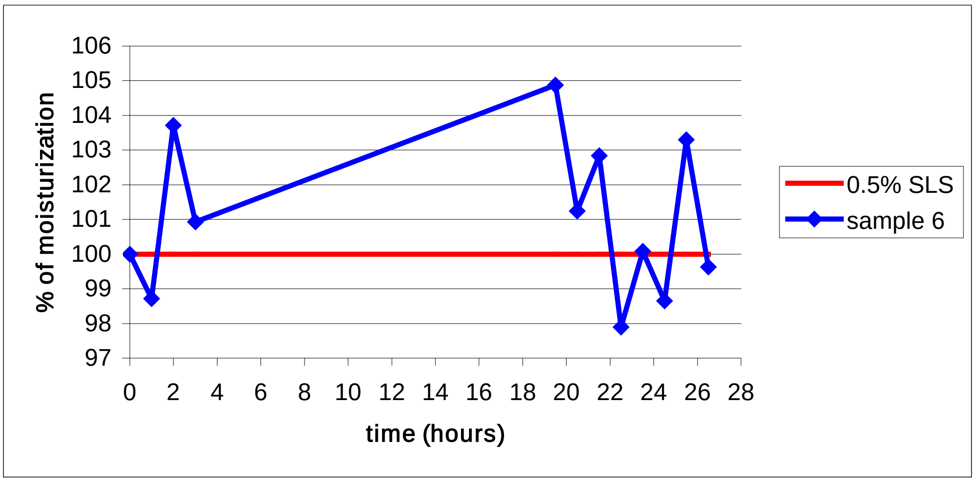

***Fig. 19:** Graphic addiction of the sample number 6 calculated to a percentage of 0.5% solution of SLS*

For graphical representation of the samples number 7–12 served Table 4 where the results of the measurement of these samples are summarized.

Table 4: *Average results in time 0 – 27 h in percentual expression*

	% of control	% of 0.5% solution of SLS					
time (hours)	**0.5% solution of SLS**	**7**	**8**	**9**	**10**	**11**	**12**
0	85.20	100.00	100.00	100.00	100.00	100.00	100.00
1	86.56	113.03	119.44	123.79	89.71	87.57	95.96
2	91.60	118.61	122.78	122.71	108.21	111.90	110.58
3	92.09	122.31	124.16	120.05	113.82	113.85	110.56
20	96.84	109.57	105.55	107.11	108.28	108.72	104.03
21	98.30	110.29	105.88	104.94	106.98	108.13	105.96
22	98.42	107.61	106.50	104.82	104.98	106.84	102.54
23	95.58	109.28	107.37	103.85	109.59	109.58	104.92
24	96.76	107.32	106.32	106.03	108.15	108.21	102.85
25	96.47	108.22	107.17	104.80	108.00	106.88	101.73
26	96.15	107.62	105.70	101.04	106.89	108.76	100.48
27	95.64	108.76	107.93	101.73	105.96	108.44	104.15

As already has been mentioned before, these results were used for graphical representation of time addiction of moisturization, where time is indicated on the *x* axis (expressed in hours) and moisturization is shown on the *y* axis, symbol 0.5% SLS means 0.5% solution of SLS in all graphs again.

Graphic addiction of 0.5% solution of SLS calculated to a percentage of control is presented in Fig. 20. It is evident that the skin moisturization of parts of the skin damaged by 0.5% solution of SLS after 4 hours of exposure was lower (skin moisturization in time 0 h was 85.20 %) in comparison with control part of the skin. It is shown in this graph that the moisturization steadily increased and mounted to its natural level during the whole measurement. It is visible from the graph that the graphic addiction presented in Fig. 20 is similar to a graphic addiction presented in Fig. 13. In both graphs we can see that the skin moisturization after application of 0.5% solution of SLS first rapidly decreased in comparison with control part and during the next measurement the skin moisturization increased and mounted to its natural level.

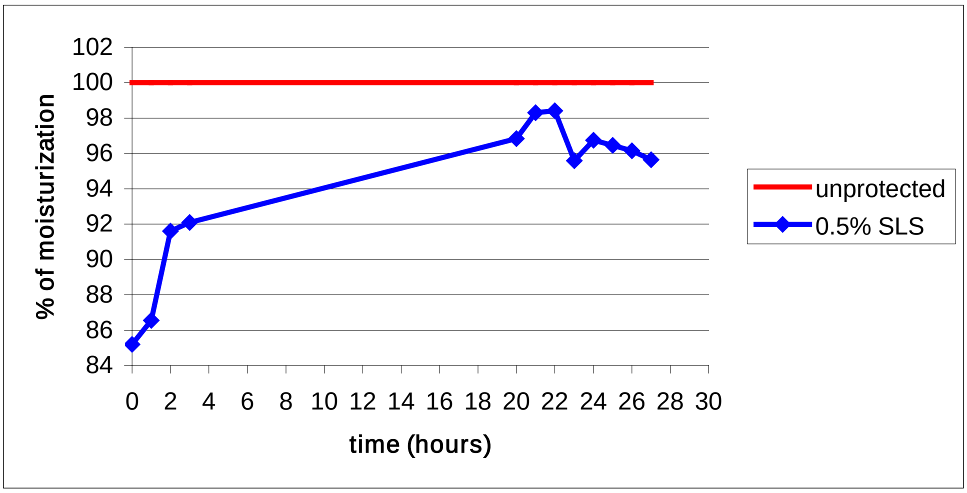

Fig. 20*: Graphic addiction of 0.5% solution of SLS calculated to a percentage of control – 2. measurement*

Moisturizing properties of the sample number 7 are shown in Fig. 21. It is visible from this graph that the highest moisturizing properties of the sample number 7 were in first three hours of measurement because the moisturization rose steadily to 122.31 %. Next measurement was carried out in time 20 h when the moisturization was 109.57 %. Graphic addiction from time 20 h to time 27 h shows that the moisturization was on a level at 107.32–110.29 %.

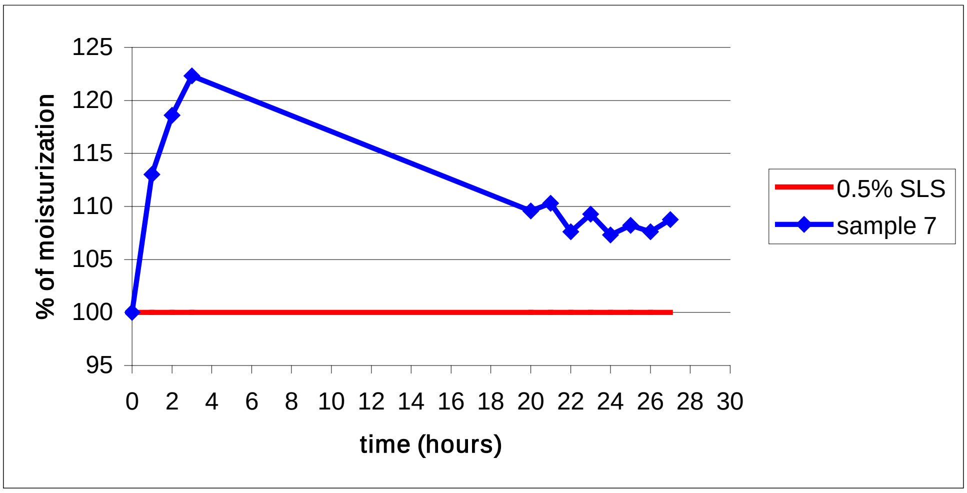

Fig. 21: *Graphic addiction of the sample number 7 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 8 are shown in Fig. 22. It is evident in graphic addiction that there was an increase of 24.16 % in first three hours of measurement. Next measurement was carried out in time 20 h when moisturization fell sharply to 105.55 % and next course of curve of moisturization was on a level at 105.55–107.93 %.

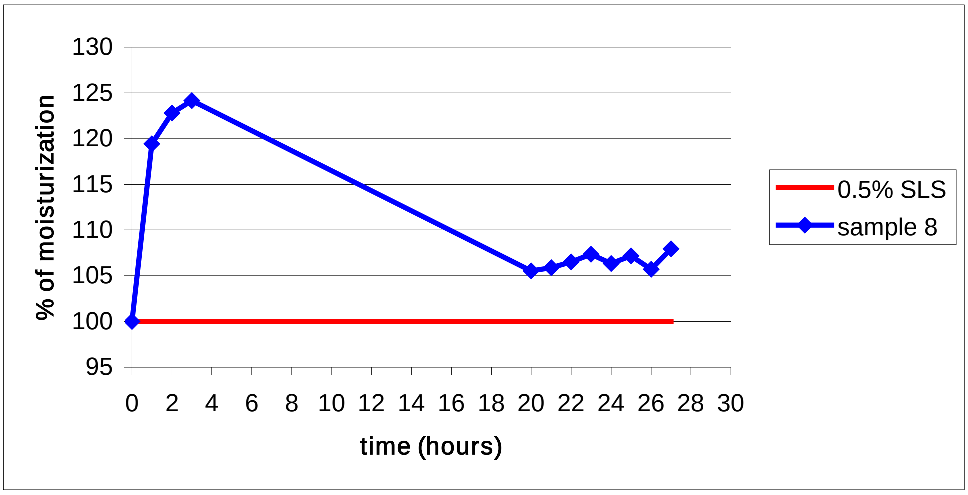

Fig. 22: *Graphic addiction of the sample number 8 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 9 are shown in Fig. 23. The moisturization increased considerably to 23.79 % one hour after application of cream and then decreased steadily to its natural level from time 1 h.

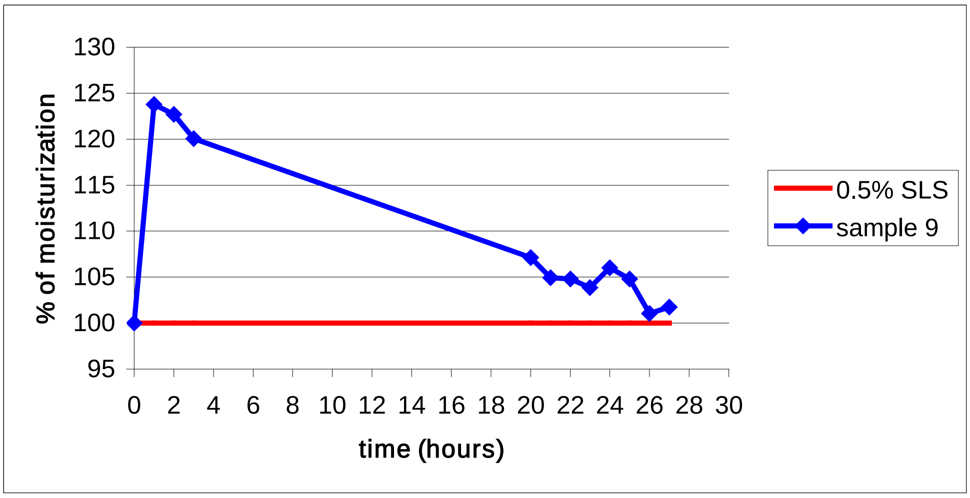

Fig. 23: *Graphic addiction of the sample number 9 calculated to a percentage of 0.5% solution of SLS*

Graphic addiction of the sample number 10 calculated to a percentage of 0.5% solution of SLS is presented in Fig. 24. There was a decrease of moisturization 10.29 % one hour after application of cream. This decrease can perhaps be explained by the fact that the sample contains large amount of oily components which block the pores of the skin and after that it is disabled to the corneometric sonde to measure the skin moisturization.

The skin moisturization increased considerably to 113.82 % from time 1 h to time 3 h. This increase can be caused by the fact that cream penetrated into the skin and it was possible to measure the skin moisturization with corneometric sonde again. Next measurement in times 20–22 h showed that there was a decrease of moisturization from 108.28 % to 104.98 %. The moisturization increased again to 109.59 % from time 22 h to time 23 h and then it started to decline to its natural level.

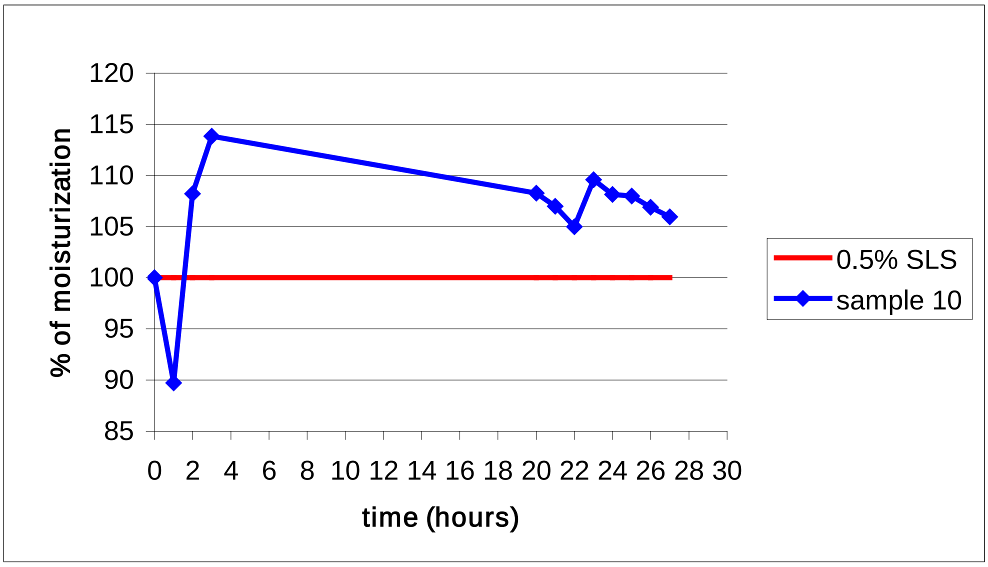

Fig. 24: *Graphic addiction of the sample number 10 calculated to a percentage of 0.5% solution of SLS*

Moisturizing properties of the sample number 11, which are shown in Fig. 25, are quite similar to moisturizing properties of the sample number 10. Moisturization fell dramatically to 87.57 % one hour after application of cream what can be caused by large amount of oily components in the cream again and then it increased to 113.85 % from time 1 h to time 3 h when the cream probably penetrated into the skin and it was possible to measure the moisturization with corneometric sonde.

Next measurement was carried out in time 20 h and the moisturization was 108.72 %. Course of a curve from time 20 h to time 27 h shows that moisturization was on a level at 106.84–109.58 %.

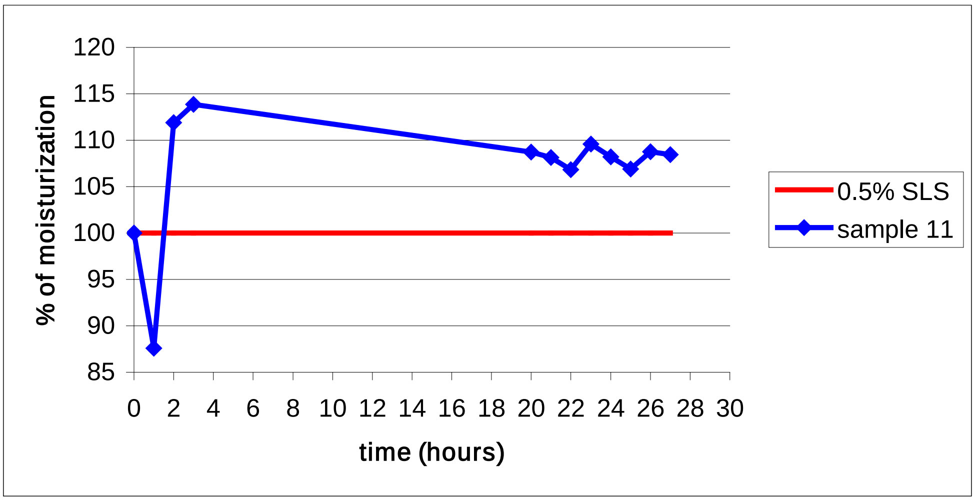

Fig. 25: *Graphic addiction of the sample number 11 calculated to a percentage of 0.5% solution of SLS*

Graphic addiction of the sample number 12 calculated to a percentage of 0.5% solution of SLS is shown in Fig. 26. There was a decrease of 4.04 % one hour after application of the sample. This decrease can be explained by the fact that this sample represented cold pressed hemp seed oil and the penetration to the skin took more time and it was not possible to measure skin moisturization with corneometric sonde exactly at the time 1 h.

The moisturization rapidly increased to 110.58 % from time 1 h to time 2 h. It is visible from graphic addiction that the moisturization decreased slightly to its natural level during the next measurement.

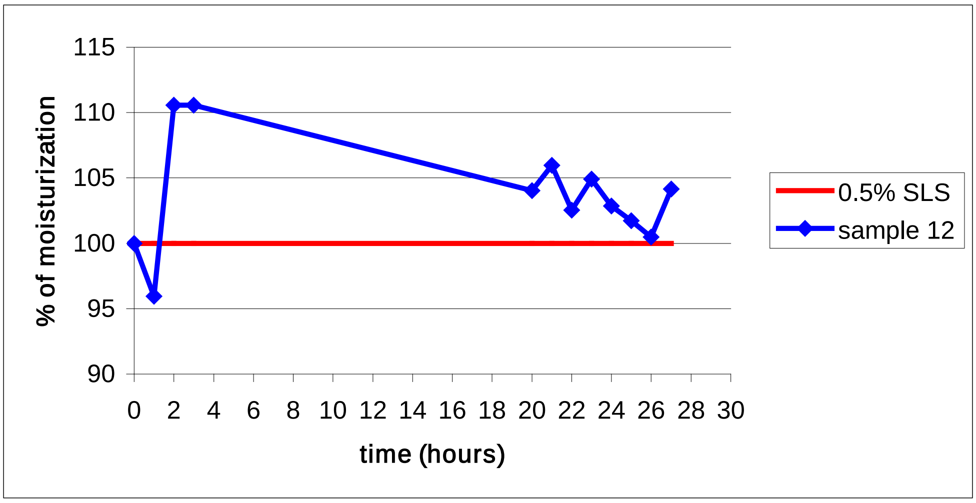

Fig. 26: *Graphic addiction of the sample number 12 calculated to a percentage of 0.5% solution of SLS*

5.2 Moisturizing effects comparison according to a way of use

To compare the moisturizing effects of tested samples we came up in the next step to comparison of these products according to a way of use.

Graphic comparison of hand creams is shown in Fig. 27. It is visible from graph that the sample number 3 and the sample number 7 had almost the same moisturizing properties in first two hours of the measurement and these properties were better than moisturizing properties of the sample number 1. Meanwhile the moisturization after application of the sample number 7 increased from time 2 h to time 3 h, the moisturization after application of the sample number 1 and the sample number 3 decreased dramatically from time 2 h to time 3 h and the sample number 1 had the worst moisturizing effect from this group of creams. Next measurement showed that long-term moisturizing properties were almost the same at the sample number 1 and the sample number 3 and these were worse than moisturizing properties of the sample number 7. Moisturizing effect of the sample number 7 was the best during the whole measurement.

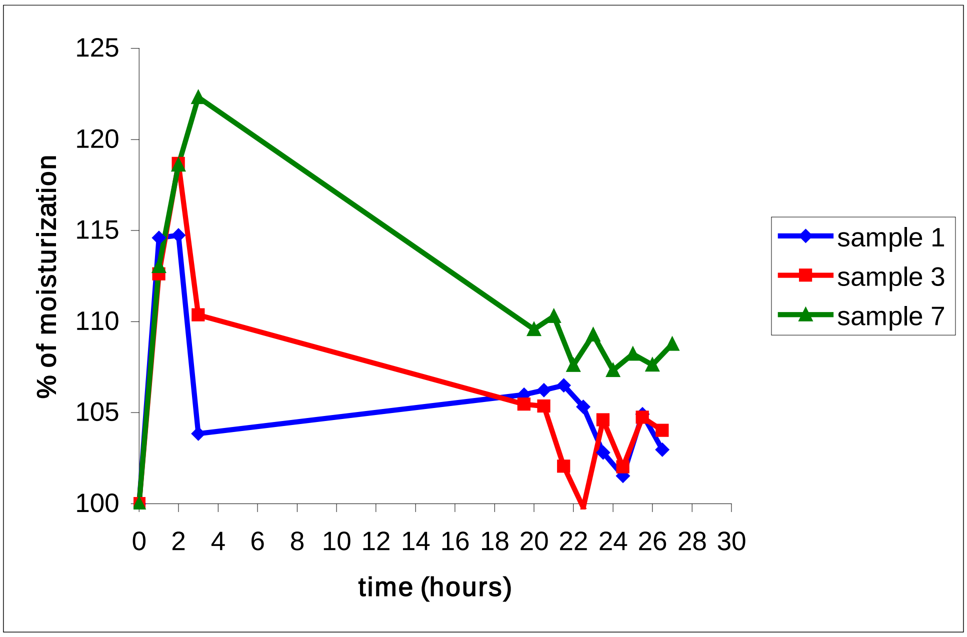

Fig. 27: *Graphic comparison of hand creams*

Graphic comparison of face creams is shown in Fig. 28. Graphic addiction shows that moisturizing properties one hour after application were the lowest at the sample number 2 and at the sample number 5 and the highest at the sample number 9. Moisturizing effect of the sample number 2 was worse two hours after application of creams than moisturizing effects of the samples number 5, 8 and 9 which were on the same level. Meanwhile the moisturizing properties of the sample number 8 increased from time 2 h to time 3 h, the moisturizing properties of the samples number 2, 5 and 9 decreased.

The measurement in time 20 h shows that moisturizing effect of the sample number 5 was the highest and the moisturizing effect of the sample number 8 was the lowest. Moisturizing properties of the samples number 2 and 5 were on the same level in time 21 h as well as moisturizing properties of the samples number 8 and 9 which were lower. Moisturizing effect from time 22 h is almost the same at all samples.

It is evident from the graph that moisturizing properties of the samples number 8 and 9 fell dramatically from time 3 h to time 20 h while the moisturizing properties of the samples number 2 and 5 remained unchanged from time 3 h to time 20 h.

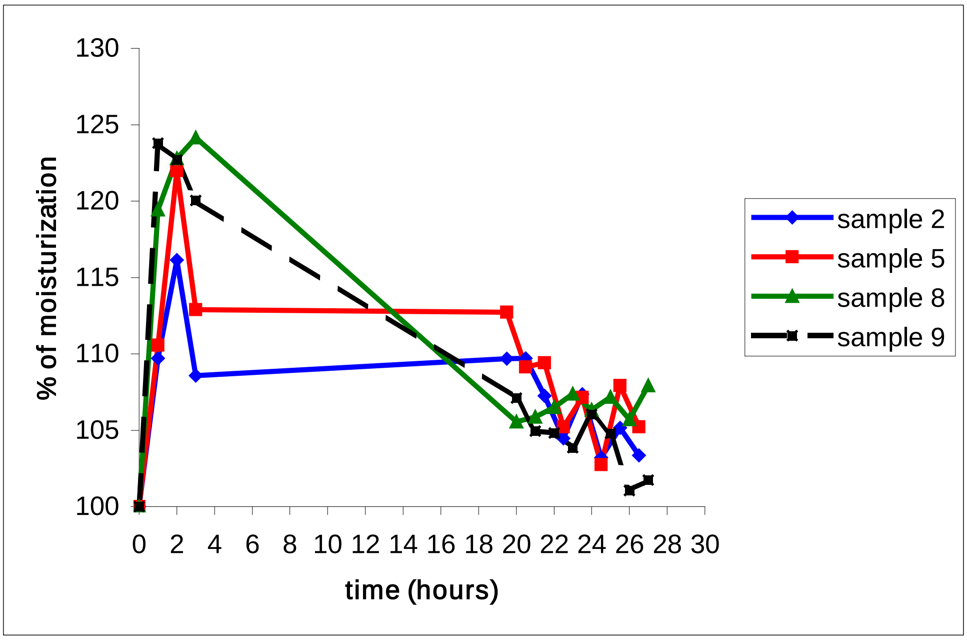

Fig. 28: *Graphic comparison of face creams*

Graphic comparison of products containing *Cannabis Sativa L.* is shown in Fig. 29. Moisturizing properties of all samples decreased first hour after application but the decrease was not so rapid at the sample number 12 which represented cold pressed hemp seed oil. It is visible from the graph that all samples needed more time for the penetration into the skin because two hours after application the skin moisturization increased (the most at the sample number 11 and the least at the sample number 10). The moisturization of the samples number 10 and 11 was at the same level three hours after application and the moisturization of the sample number 12 was the lowest three hours after application. Next measurement showed that the long-term moisturizing properties of the sample number 12 were worse than the long-term moisturizing properties of the samples number 10 and 11. It is visible from the graph that moisturizing properties of the samples number 10 and 11 are very similar during the whole measurement and they are higher than the moisturizing properties of the sample number 12.

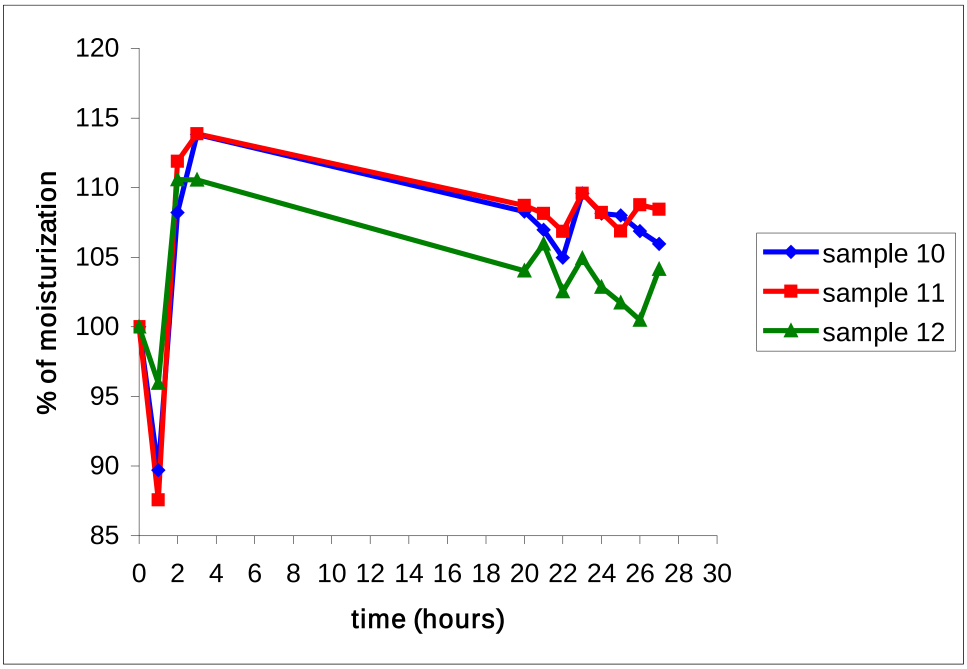

Fig. 29: *Graphic comparison of products containing Cannabis Sativa L.*

5.3 Statistical evaluation

The classical experiment Analysis of variance, where the obtained values represent independent parameter, was used for the statistical evaluation. Computer programme UNISTAT 5.5 was used for this evaluation. The results of statistical evaluation of obtained values are shown in Table 5.

Table 5: *Analysis of variance*

Source of variation	Sum of squares	Degree of freedom	Average square	F value	Significance
Main effects	232661.724	27	8617.101	120.383	0,0000
Time	94036.499	11	8548.773	119.429	0,0000
Volunteer	69812.486	4	17453.121	243.825	0,0000
Sample	68868.834	12	5739.070	80.176	0,0000
Second-order interaction	185750.623	224	829.244	11.585	0,0000
Time × Volunteer	43973.061	44	999.388	13.962	0,0000
Time × Sample	73483.054	132	556.690	7.777	0,0000
Volunteer × Sample	68336.307	48	1423.673	19.889	0,0000
Third-order interaction	88954.559	528	168.475	2.354	0,0000
Time × Volunteer × Sample	88954.559	528	168.475	2.354	0,0000
Explained	507366.907	779	651.305	9.099	0,0000
Error	223331.412	3120	71.581		
Total	730698.319	3899	187.407		

As already has been mentioned above, the results of statistical evaluation are shown in Table 5. Significance is very important value because it predicates about the deviation – the smaller significance, the greater importance of deviation.

The main effects of the measurement were time, volunteers and samples. The significance in the Table 5 shows that the moisturization level was different in each time, at each volunteer and also at each sample.

Second-order interaction predicates about the relation between two effects. It follows from this fact that moisturization level of each volunteer was different in each time, moisturizing effects of each samples were different in each time and moisturizing effects of each samples were different on each volunteer.

Third-order interaction predicates about the relation among all three main effects. It follows from this fact that moisturizing effects of samples were different on each volunteer and they were also different in each time.

The value *explained* shows us how much has been explained and the value *error* shows how much has not been explained. It is evident from the value *error* that there would be required larger statistical population (bigger group of volunteers) for more precise determination of moisturizing effects of cosmetic products.

CONCLUSIONS

This thesis deals with the human skin and its protective and barrier function which is closely related with the skin moisturization. The aim of this thesis was first to make a literature research on the theme of the skin, its structure, functions, protection and moisturization and second to carry out the corneometric measurement of moisturizing properties of cosmetic products.

In this study twelve samples of cosmetic moisturizing products were used for the corneometric measurement which was carried out first three hours after application of cream and then 19.5–26.5 h (20–27 h) after application of cream. On the basis of the corneometric measurement was found out that:

- ten cosmetic products bought in trade network and cold pressed hemp seed oil prepared in laboratory really moisturize the skin;
- oil free moisturizer prepared in laboratory does not have good moisturizing properties what can perhaps be caused by the deficient homogenization of the sample;
- hand cream containing the combination of olive oil and urea has the best moisturizing properties in comparison with hand creams containing generally used humectants and emollients (e.g. glycerin) or liquid crystals;
- face creams containing the combination of olive oil and urea have better short-term moisturizing effect than face creams containing other generally used humectants and emollient but long-term moisturizing effect is very similar at all tested samples;
- cosmetic products containing *Cannabis Sativa L.* have good short-term as well as long-term moisturizing effect which is better than the moisturizing effect of pure cold pressed hemp seed oil.

In fine it should be mentioned that chosen time intervals would be better for the long-term moisturizing effect detection which could be very important for example at anti-wrinkle cosmetic products. But for the corneometric measurement of moisturizing properties of all generally used cosmetic products like hand creams or face creams would be better to measure mainly in time interval one hour to ten hours after application of cream because these cosmetic products are applied to the skin repetitively during the day or they are applied at least twice a day in case of face creams. It follows directly from the statistical evaluation that for more precise determination would be required larger statistical population (bigger group of volunteers).

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LIST OF SYMBOLS AND ABBREVIATIONS

E	Ester-linked fatty acids
O	ω-OH fatty acids
S	Sphingosine
N	Non-OH fatty acids
P	Phytosphingosine
A	α-OH fatty acids
NMF	Natural moisturizing factor
TEWL	Transepidermal water loss
PEG	Polyethylenglycol
AA	Amino acids
PCA	Pyrrolidone carboxylic acid
AHA	α-hydroxy acid
BHA	β-hydroxy acid
SLS	Sodium Lauryl Sulfate

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Symbol s means standard deviation in all Tables, 0.5% SLS means 0.5% solution of SLS in all Tables.

A Table 1: *Results of samples number 1–6 converted into percentual expression in time 0 h*

	4 hours after application of 0.5% SLS 8:30 - 12:30						
		% of control					
volunteer	0.5% SLS	1	2	3	4	5	6
I	96.11	75.56	78.06	90.28	92.22	96.67	96.39
II	100.00	94.17	98.89	100.00	99.44	100.00	98.61
III	86.63	93.32	89.31	95.99	89.57	84.49	83.16
IV	81.29	90.50	85.48	97.21	92.74	88.27	92.74
V	97.50	98.61	100.00	99.44	100.00	100.00	99.17
average	**92.31**	**90.43**	**90.35**	**96.58**	**94.80**	**93.89**	**94.01**
s	**7.13**	**7.88**	**8.27**	**3.47**	**4.17**	**6.36**	**5.88**

A Table 2: *Results of samples number 1–6 converted into percentual expression in time 1 h*

	1 hour after application of cream 13:30						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	90.56	110.43	97.55	100.92	108.90	109.51	105.83
II	100.28	99.17	99.44	100.00	100.00	100.00	96.39
III	89.94	113.04	109.01	118.63	112.73	111.80	99.69
IV	76.94	133.94	129.96	129.96	129.96	117.69	96.39
V	90.31	116.40	112.62	113.57	113.57	113.88	95.27
average	**89.61**	**114.60**	**109.72**	**112.62**	**113.03**	**110.58**	**98.71**
s	**7.43**	**11.27**	**11.60**	**11.26**	**9.74**	**5.93**	**3.85**

A Table 3: *Results of samples number 1–6 converted into percentual expression in time 2 h*

	2 hours after application of cream 14:30						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	88.02	108.54	113.92	132.60	142.72	138.29	113.29
II	97.78	102.27	102.27	102.27	102.84	102.27	94.89
III	85.76	119.60	119.27	119.60	118.27	120.27	93.69
IV	78.33	129.08	128.01	130.14	127.66	127.66	110.64
V	91.94	114.20	117.22	108.76	109.06	121.45	106.04
average	**88.37**	**114.74**	**116.14**	**118.67**	**120.11**	**121.99**	**103.71**
s	**6.47**	**9.20**	**8.36**	**11.78**	**14.09**	**11.75**	**8.04**

A Table 4: *Results of samples number 1–6 converted into percentual expression in time 3 h*

	3 hours after application of cream 15:30						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	87.22	101.91	114.33	128.03	138.22	121.02	107.64
II	100.00	94.17	100.00	98.89	102.78	100.00	88.89
III	95.18	103.57	107.14	104.76	107.14	106.85	94.35
IV	91.06	110.12	110.43	110.74	111.35	117.79	107.06
V	91.64	109.42	110.94	109.42	113.98	118.85	106.69
average	**93.02**	**103.84**	**108.57**	**110.37**	**114.69**	**112.90**	**100.92**
s	**4.31**	**5.80**	**4.85**	**9.76**	**12.36**	**8.10**	**7.80**

A Table 5: *Results of samples number 1–6 converted into percentual expression in time 19.5 h*

	19.5 hours after application of cream 8:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	84.36	103.31	117.22	119.21	119.21	119.21	119.21
II	97.22	98.86	89.43	93.71	98.86	99.43	91.43
III	93.22	105.70	112.34	101.58	112.03	113.92	103.48
IV	83.29	115.57	123.18	115.22	124.57	124.57	105.54
V	100.00	106.51	106.21	97.63	106.51	106.51	104.73
average	**91.62**	**105.99**	**109.68**	**105.47**	**112.23**	**112.73**	**104.88**
s	**6.73**	**5.48**	**11.56**	**9.99**	**9.08**	**8.93**	**8.82**

A Table 6: *Results of samples number 1–6 converted into percentual expression in time 20.5 h*

	20.5 hours after application of cream 9:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	84.99	111.00	118.67	118.33	120.00	120.00	109.00
II	97.18	104.07	97.67	92.15	103.20	94.19	90.99
III	95.28	104.02	111.46	105.57	109.29	107.74	96.28
IV	92.31	108.33	117.00	115.33	120.00	120.00	107.33
V	100.29	103.75	103.75	95.39	103.75	103.75	102.59
average	**94.01**	**106.23**	**109.71**	**105.36**	**111.25**	**109.13**	**101.24**
s	**5.20**	**2.93**	**7.96**	**10.41**	**7.46**	**9.91**	**6.77**

A Table 7: *Results of samples number 1–6 converted into percentual expression in time 21.5 h*

	21.5 hours after application of cream 10:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	88.58	107.23	107.55	107.23	114.15	113.21	105.97
II	98.33	100.85	94.35	91.53	96.89	94.63	91.81
III	98.42	111.90	115.43	108.68	113.18	114.15	106.11
IV	88.76	106.96	113.92	106.33	117.72	113.92	104.43
V	102.40	105.57	104.99	96.48	105.57	111.14	105.87
average	**95.30**	**106.50**	**107.25**	**102.05**	**109.50**	**109.41**	**102.84**
s	**5.61**	**3.54**	**7.53**	**6.80**	**7.45**	**7.46**	**5.55**

A Table 8: *Results of samples number 1–6 converted into percentual expression in time 22.5 h*

	22.5 hours after application of cream 11:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	88.80	107.57	108.20	112.93	113.56	113.25	99.05
II	99.72	100.28	91.92	96.94	99.72	92.20	93.04
III	95.27	104.97	109.01	95.34	105.90	106.83	94.10
IV	98.21	109.42	109.12	102.74	109.42	109.42	101.82
V	104.55	104.35	104.06	91.01	104.35	104.35	101.45
average	**97.31**	**105.32**	**104.46**	**99.79**	**106.59**	**105.21**	**97.89**
s	**5.21**	**3.11**	**6.54**	**7.57**	**4.68**	**7.14**	**3.67**

__A Table 9:__ Results of samples number 1–6 converted into percentual expression in time 23.5 h

	23.5 hours after application of cream 12:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	89.40	103.53	112.82	115.06	115.71	115.38	109.62
II	101.12	100.00	97.22	93.61	95.28	93.06	87.78
III	95.38	106.13	115.48	106.13	114.19	114.52	96.77
IV	89.39	104.38	111.25	109.06	112.50	112.50	105.94
V	101.98	100.00	100.00	99.17	100.00	100.28	100.28
average	**95.45**	**102.81**	**107.36**	**104.61**	**107.54**	**107.15**	**100.08**
s	**5.45**	**2.44**	**7.32**	**7.51**	**8.28**	**8.91**	**7.58**

__A Table 10:__ Results of samples number 1–6 converted into percentual expression in time 24.5 h

	24.5 hours after application of cream 13:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	94.46	102.35	104.69	104.40	105.57	105.57	101.76
II	99.17	100.84	95.80	98.60	100.84	98.60	97.48
III	100.00	104.78	107.46	102.99	102.39	101.49	90.75
IV	92.78	99.40	107.78	107.19	107.78	107.78	103.00
V	102.28	100.28	100.28	96.94	100.28	100.28	100.28
average	**97.74**	**101.53**	**103.20**	**102.02**	**103.37**	**102.75**	**98.65**
s	**3.55**	**1.89**	**4.58**	**3.76**	**2.87**	**3.41**	**4.36**

A Table 11: *Results of samples number 1–6 converted into percentual expression in time 25.5 h*

	25.5 hours after application of cream 14:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	85.20	111.48	113.77	122.62	132.46	130.16	118.03
II	100.00	100.00	98.89	93.89	99.72	96.11	95.56
III	100.30	108.13	108.13	108.43	108.43	108.43	105.42
IV	95.28	104.96	104.96	104.08	104.96	104.96	99.71
V	103.75	100.00	100.00	94.72	100.56	100.00	97.78
average	**96.90**	**104.91**	**105.15**	**104.75**	**109.23**	**107.93**	**103.30**
s	**6.44**	**4.51**	**5.46**	**10.51**	**12.03**	**11.88**	**8.06**

A Table 12: *Results of samples number 1–6 converted into percentual expression in time 26.5 h*

	26.5 hours after application of cream 15:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	1	2	3	4	5	6
I	85.83	101.94	109.71	113.59	116.50	116.50	111.00
II	97.50	102.28	97.72	98.29	102.28	99.72	96.30
III	101.43	100.56	97.74	101.41	96.89	98.31	88.70
IV	93.93	109.23	110.77	107.69	110.77	110.77	101.85
V	101.71	100.84	100.84	99.16	100.84	100.84	100.28
average	**96.08**	**102.97**	**103.36**	**104.03**	**105.46**	**105.23**	**99.63**
s	**5.86**	**3.20**	**5.74**	**5.80**	**7.14**	**7.15**	**7.28**

A Table 13: *Results of samples number 7–12 converted into percentual expression in time 0 h*

	4 hours after application of 0.5% SLS 8:00 - 12:00						
	% of control						
volunteer	0.5% SLS	7	8	9	10	11	12
I	80.90	90.75	82.09	107.46	104.78	106.57	94.03
II	78.44	83.29	80.05	87.06	82.48	71.70	76.82
III	93.00	85.67	80.67	108.00	99.00	99.00	93.33
IV	91.16	91.16	98.17	106.71	102.13	90.85	88.11
V	82.50	100.00	100.00	95.28	93.89	98.61	99.17
average	**85.20**	**90.17**	**88.20**	**100.90**	**96.46**	**93.35**	**90.29**
s	**5.79**	**5.75**	**8.93**	**8.37**	**7.87**	**11.91**	**7.59**

A Table 14: *Results of samples number 7–12 converted into percentual expression in time 1 h*

	1 hour after application of cream 13:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	81.02	118.22	95.54	134.57	88.48	85.87	97.40
II	90.83	107.95	112.23	125.69	87.77	66.06	83.49
III	93.70	98.78	111.01	112.84	94.80	74.62	88.07
IV	81.14	121.83	135.21	127.11	85.92	92.61	94.37
V	86.11	118.39	143.23	118.71	91.61	118.71	116.45
average	**86.56**	**113.03**	**119.44**	**123.79**	**89.71**	**87.57**	**95.96**
s	**5.09**	**8.51**	**17.37**	**7.44**	**3.14**	**18.05**	**11.33**

A Table 15: *Results of samples number 7–12 converted into percentual expression in time 2 h*

	2 hours after application of cream 14:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	82.04	131.32	130.94	156.60	134.72	132.08	132.83
II	96.76	117.60	108.10	105.31	103.35	101.40	100.56
III	95.65	104.85	109.09	120.61	95.76	103.94	106.67
IV	86.07	116.18	135.28	118.77	105.50	105.83	109.71
V	97.50	123.08	130.48	112.25	101.71	116.24	103.13
average	**91.60**	**118.61**	**122.78**	**122.71**	**108.21**	**111.90**	**110.58**
s	**6.32**	**8.69**	**11.70**	**17.78**	**13.64**	**11.28**	**11.55**

A Table 16: *Results of samples number 7–12 converted into percentual expression in time 3 h*

	3 hours after application of cream 15:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	81.41	128.72	122.84	141.52	125.95	124.57	123.88
II	102.50	120.05	113.01	106.78	107.05	105.42	99.46
III	97.00	114.24	115.48	113.00	111.15	111.76	108.05
IV	84.44	120.07	137.83	126.97	118.42	116.12	113.16
V	95.12	128.49	131.62	111.97	106.55	111.40	108.26
average	**92.09**	**122.31**	**124.16**	**120.05**	**113.82**	**113.85**	**110.56**
s	**7.93**	**5.56**	**9.42**	**12.65**	**7.41**	**6.35**	**7.99**

A Table 17: *Results of samples number 7–12 converted into percentual expression in time 20 h*

	20 hours after application of cream 8:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	90.33	112.54	109.79	116.82	114.07	122.32	110.40
II	91.39	109.42	98.48	103.34	108.81	102.43	103.95
III	99.04	115.76	116.08	112.86	115.43	115.76	105.79
IV	102.31	103.39	101.69	101.69	101.69	101.69	98.59
V	101.14	106.76	101.69	100.85	101.41	101.41	101.41
average	**96.84**	**109.57**	**105.55**	**107.11**	**108.28**	**108.72**	**104.03**
s	**5.01**	**4.32**	**6.46**	**6.48**	**5.93**	**8.68**	**4.00**

A Table 18: *Results of samples number 7–12 converted into percentual expression in time 21 h*

	21 hours after application of cream 9:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	87.25	117.28	116.28	118.94	119.60	123.92	119.60
II	95.75	106.21	93.79	99.11	103.25	98.82	98.22
III	103.03	119.61	117.65	109.15	111.76	117.65	113.07
IV	104.05	103.61	100.83	97.22	100.00	100.00	96.11
V	101.41	104.74	100.84	100.28	100.28	100.28	102.79
average	**98.30**	**110.29**	**105.88**	**104.94**	**106.98**	**108.13**	**105.96**
s	**6.23**	**6.75**	**9.42**	**8.11**	**7.61**	**10.53**	**8.98**

__A Table 19:__ Results of samples number 7–12 converted into percentual expression in time 22 h

	22 hours after application of cream 10:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	92.08	105.41	108.28	114.97	114.65	120.06	114.33
II	89.17	112.15	103.74	101.56	105.61	97.51	97.82
III	102.67	116.88	116.88	108.12	103.25	115.26	103.90
IV	105.60	101.40	100.56	99.44	100.56	100.56	96.37
V	102.56	102.22	103.06	100.00	100.83	100.83	100.28
average	**98.42**	**107.61**	**106.50**	**104.82**	**104.98**	**106.84**	**102.54**
s	**6.52**	**5.98**	**5.76**	**5.94**	**5.17**	**9.04**	**6.42**

__A Table 20:__ Results of samples number 7–12 converted into percentual expression in time 23 h

	23 hours after application of cream 11:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	97.75	112.17	113.49	113.82	118.42	119.08	115.13
II	92.44	109.09	99.09	97.58	106.67	104.55	99.70
III	90.53	117.65	117.65	103.27	115.69	117.65	106.54
IV	100.28	103.13	102.27	102.56	102.27	102.27	98.30
V	96.91	104.35	104.35	102.03	104.93	104.35	104.93
average	**95.58**	**109.28**	**107.37**	**103.85**	**109.59**	**109.58**	**104.92**
s	**3.58**	**5.30**	**7.02**	**5.37**	**6.31**	**7.23**	**5.97**

A Table 21: *Results of samples number 7–12 converted into percentual expression in time 24 h*

	24 hours after application of cream 12:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	90.21	109.83	115.25	130.85	122.03	127.12	119.32
II	92.70	103.64	97.88	102.73	105.76	96.06	97.27
III	97.44	118.42	118.75	99.34	113.49	118.42	102.63
IV	103.71	100.28	98.62	96.97	99.17	99.17	94.77
V	99.72	104.46	101.11	100.28	100.28	100.28	100.28
average	**96.76**	**107.32**	**106.32**	**106.03**	**108.15**	**108.21**	**102.85**
s	**4.84**	**6.34**	**8.85**	**12.54**	**8.60**	**12.28**	**8.65**

A Table 22: *Results of samples number 7–12 converted into percentual expression in time 25 h*

	25 hours after application of cream 13:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	89.26	115.12	118.56	124.05	123.71	123.71	119.59
II	96.05	104.71	98.24	97.65	105.00	92.06	93.24
III	98.41	116.50	116.50	102.59	108.74	115.53	100.32
IV	100.00	102.81	101.12	100.00	101.12	101.12	94.10
V	98.61	101.97	101.41	99.72	101.41	101.97	101.41
average	**96.47**	**108.22**	**107.17**	**104.80**	**108.00**	**106.88**	**101.73**
s	**3.82**	**6.28**	**8.56**	**9.75**	**8.33**	**11.27**	**9.50**

A Table 23: *Results of samples number 7–12 converted into percentual expression in time 26 h*

	26 hours after application of cream 14:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	89.71	99.36	102.87	114.01	112.10	114.97	100.32
II	94.87	107.51	98.20	97.90	105.11	101.80	98.50
III	98.66	122.03	122.37	91.86	115.25	121.69	106.44
IV	97.78	102.27	102.55	101.42	101.98	103.40	96.88
V	99.72	106.94	102.50	100.00	100.00	101.94	100.28
average	**96.15**	**107.62**	**105.70**	**101.04**	**106.89**	**108.76**	**100.48**
s	**3.60**	**7.81**	**8.51**	**7.26**	**5.86**	**8.12**	**3.24**

A Table 24: *Results of samples number 7–12 converted into percentual expression in time 27 h*

	27 hours after application of cream 15:00						
	% of control	% of 0.5% solution of SLS					
volunteer	0.5% SLS	7	8	9	10	11	12
I	87.39	113.77	114.10	118.03	118.36	127.21	118.03
II	95.00	105.26	109.94	97.95	103.80	99.71	94.15
III	94.96	112.50	113.13	94.06	105.94	112.50	104.06
IV	101.69	105.83	101.39	98.61	100.00	100.00	100.00
V	99.17	106.44	101.12	100.00	101.68	102.80	104.48
average	**95.64**	**108.76**	**107.93**	**101.73**	**105.96**	**108.44**	**104.15**
s	**4.86**	**3.61**	**5.63**	**8.39**	**6.52**	**10.47**	**7.87**