The effect of tensides on the skin hydration

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Téma práce: **The effect of tensides on the skin hydratation**

Zásady pro vypracování:

I. Theoretical part

1. Write a literary research on the theme: Skin structure and barrier function.

2. Write a literary research on the theme: Surfactant and skincare.

II. Experimental part

1. Compare the effects of products on the skin.

2. Use the experimental method: Corneometry.

3. Achieved results evaluate and critically discuss.

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[1] SCHLOSSMAN, M. L. The chemistry and Manufacture of Cosmetics. Volume I. ISBN: 9781932633474.
[2] FÖRSTER, T., Cosmetic lipids and the Skin Barier. New York: Marcel Dekker, 2002, ISBN: 0824706641.
[3] ZOLLER, U. Handbook of detergents, Part E: Applications. 2008, ISBN: 9781574447576.
[4] PAYE, M., BAREL, A., MAIBACH, H. Handbook of Cosmetic Science and Technology.Informa Health Care. 2001.

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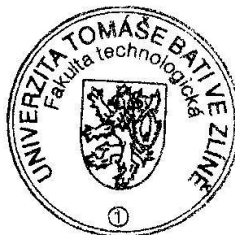
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ABSTRAKT

Je obecně známo, že vliv tenzidových prostředků na *Stratum corneum* se liší podle jejich složení. Cílem této práce je porovnat účinek pěti komerčních sprchových gelů na hydrataci kůže. Tyto produkty se liší jak složením, přídavkem hydratačních látek, tak cenou. Pro srovnání jsou použity dodecylsulfát sodný a neošetřená pokožka. K měření míry hydratace je použit přístroj Corneometer CM 820. Dle výsledků je míra hydratace změněna po použití všech vzorků, avšak největší rozdíly mezi výrobky jsou zřetelné až po aplikaci vyšší koncentrace. Hydratace je sledována 26 hodin a asi po 24 hodinách je vidět samovolné obnovování přirozené hydratace kůže. Tato studie také poukazuje na to, že pouhé tvrzení na výrobcích a ani uvedené složení negarantují jejich účinek. Ten velmi závisí také na kvantitativním složení, které je ale pro konzumenta většinou neznámé.

Klíčová slova: tenzidové prostředky, dodecylsulfát sodný, corneometer, *Stratum corneum*, hydratace, hydratační látky

ABSTRACT

It is well known that the effect of surfactant-based products on the *Stratum corneum* (SC) varies according to their composition. The present work aims to compare five commercial cleansing products with respect to their effects on the SC hydration. The products differ in ingredients, added moisturizing agents and price. Sodium lauryl sulfate (SLS) and untreated skin serve as reference controls. The skin capacitance is assessed by Corneometer CM 820. The samples induce the SC hydration in all cases, albeit to various extent. The results show marked differences among the samples only when higher concentrations are used. Nevertheless, the skin hydration tends to recover in one day after the application. This study shows that the claims on the products and listed ingredients do not guarantee their real efficacy as it depends also on the quantitative composition which is usually unknown to consumers.

Keywords: surfactants, sodium lauryl sulfate, corneometer, *Stratum corneum*, hydration, moisturizing agents

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I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

Skin is a complex system and demands every day care. Keeping it clean and hydrated helps to maintain skin in a good state [1]. Nevertheless, it is known that frequent use of soaps and synthetic detergents to cleanse the skin may lead to dry, chappy appearance or eczema. But there are individual differences in susceptibility and various products are less well tolerated than others [2]. The whole process also depends on the length of exposition.

The maintaining of water in the *Stratum corneum* - the outermost layer of the skin that creates the interface between the organism and the environment - is influenced especially by the presence of lipids of intracellular matter, by the skin barrier in global and by hygroscopic substances that occur in corneocytes. These substances are called natural moisturizing factors (NMF) [3- 6].

Recently, there has been growing interest in products with moisturizing effect that contain substances reducing the negative effect of anionic surfactants, the main components of shower gels. These substances are called humectants and cosmetics containing these active ingredients are supposed to increase the quantity of water in horny layers. Humectants are hygroscopic hydrophilic substances that are water soluble; they can bind water, prevent it from evaporation and retain it in horny cells [7].

The aim of the thesis is to measure and evaluate in defined intervals the hydrating effect of cosmetic products on the skin after the application of certain products and sodium lauryl sulfate in different concentrations.

I. THEORETICAL PART

1 SKIN HISTOLOGY AND PHYSIOLOGY

Skin is one of the largest organs in the body covering area about 2 m^2 and making up about 16% of body weight. It has many functions, the most important of which is a barrier to protect the body from noxious external factors and to keep the internal system intact [8]. Skin is composed of three layers; the epidermis, the dermis and the subcutis (see Fig. 1). But for the cosmetic industry only the first two layers are important.
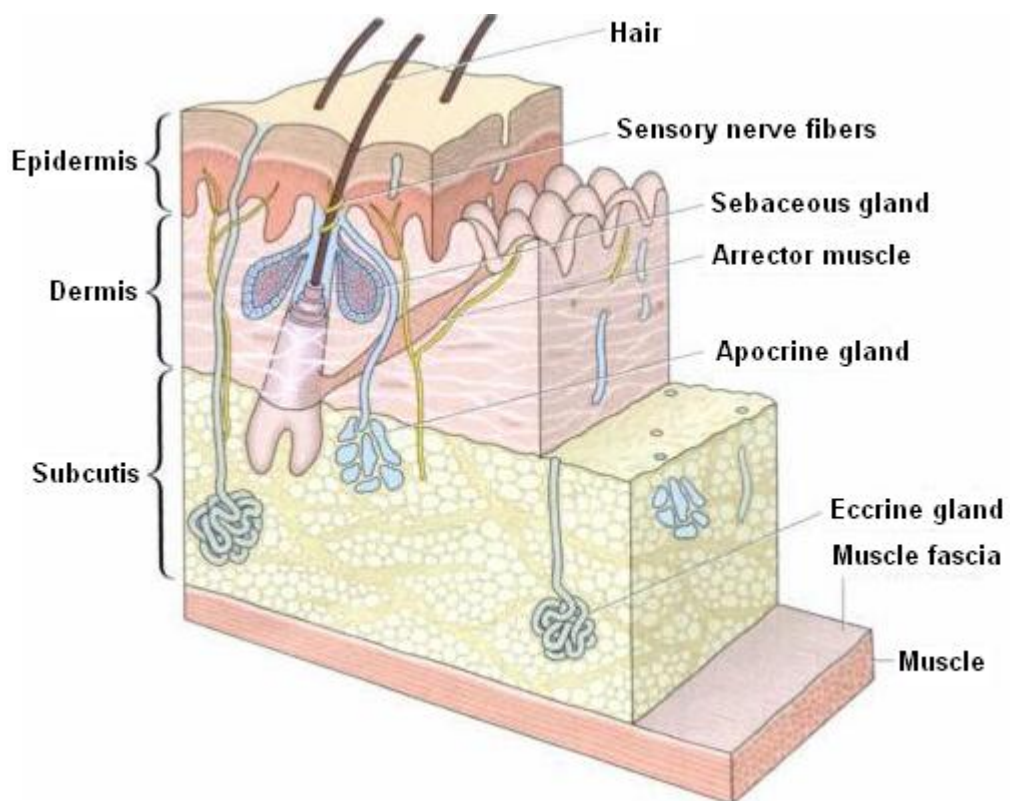
Fig. 1: Structure of the skin [9]

1.1 Epidermis

The epidermis is a stratified squamous epithelium which is about 0.1- 1.4 mm thick. Its main function is to act as a protective barrier. The main cell of the epidermis is

keratinocyte, which produces the protein keratin. The four layers of the epidermis (see Fig. 2) represent the stages of maturation of keratin [8].
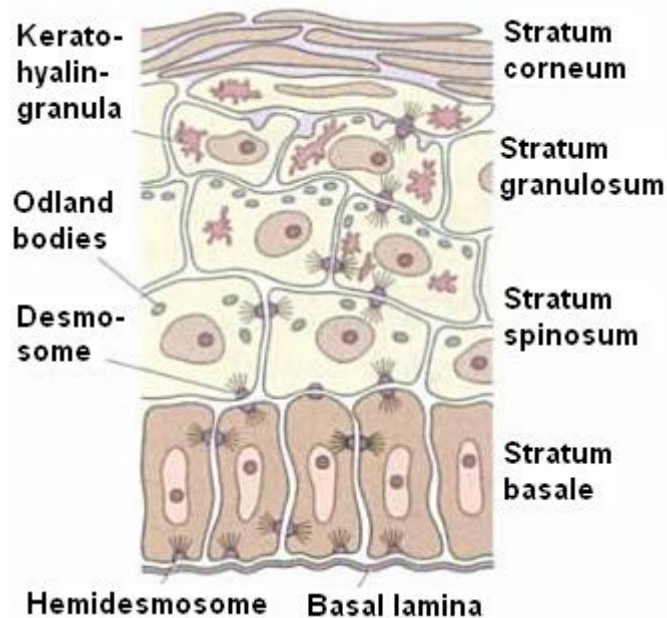
Fig. 2: Layers of epidermis [9]

1.1.1 Stratum basale

The basal cell layer is composed mostly of keratinocytes. Its cells contain keratin tonofibrils and are fixed to the basement membrane by hemidesmosomes. Five to ten percent of the basal cell population is made up by melanocytes. Melanocytes synthetize melanin and transfer it via dendritic processes to neighboring keratinocytes. The biggest quantity of these cells occur on the face and other exposed sites and they are of neural crest origin. Also Merkel cells are found in the basal cell layers. These cells are closely associated with terminal filaments of cutaneous nerves and seem to have a role in sensation. Their cytoplasm contains neuropeptide granules, neurofilaments and keratin [8].

1.1.2 Stratum spinosum

This layer is formed by daughter basal cells that migrated upwards and generate polyhedral cells which are interconnected by desmosomes. Keratin tonofibrils form a supportive mesh in the cytoplasm of these cells. Langerhans cells are found mostly

in this layer [8]. These cells are part of immune system and prevent or impede penetration of foreign substances through the skin [10].

1.1.3 Stratum granulosum

In this layer cells become flattened and lose their nuclei. The cells of this layer are characterized by keratohyalin granules and arise from the spiny cells. Keratohyalin granules are seen in the cytoplasm together with membrane-coating granules (which expel their lipid contents into the intercellular space) [8, 11].

1.1.4 Stratum lucidum

The *Stratum lucidum* occurs just above the *Stratum granulosum*. The cell structure can be seen only by a microscope. Keratinizing cells contain eleidin which has very high refractive ability and due to this characteristic this layer looks like transparent. The *Stratum lucidum* is a coherent layer and abound in thick skin [12, 13].

1.1.5 Stratum corneum

Terminally differentiated keratinocytes in this layer are remade into corneocytes; the keratinocytes plasma membrane is transformed into the cornified envelope as a result of extensive cross-linking of structural proteins by an enzyme [14]. Corneocytes have no nucleus, they are dead keratinocytes that provide strength and rigidity to the *Stratum corneum*.

The keratin fibers are stacked thanks to fillagrin which is derived from keratohyalin granules and is finally degraded in the upper layers to form amino acids, pyrolidone carboxylic acid and lactic acid in the *Stratum corneum*. These substances act as natural moisturizing factors and will be described later in another section [15].

The *Stratum corneum* is the outermost layer of the epidermis and is made up of approximately 15 layers of corneocytes that are completely filled with keratin - therefore it is often called horny layer. The corneocytes are packed tightly together and are surrounded by a layer of lipids. This complex is often compared to bricks and mortar - providing structural strength through the keratin-filled bricks and water proofing through the layer of lipid mortar [16].

The cells in the outer horny layer of the *Stratum corneum* flake off and are replaced by new cells from below. The process of new cells differentiating and reaching the *Stratum corneum* takes approximately 28 days [14]. It is also estimated that each day, one layer of the *Stratum corneum* is peeled off and one is synthesized from the *Stratum granulosum* below [16]. The rate of addition of new keratinocytes to the *Stratum corneum* is equilibrated with the rate of loss of dead keratinocytes from its surface. This balance of poliferation and differentiation is thought to maintain a healthy skin barrier [17]. This cycle of **keratinization** - the flaking of the dead cells and replacing them by new cells - is important because of variety of skin problems. However, the cycle might be disturbed by a variety of factors (cosmetics, low relative humidity, cold weather, hormones, drugs and diseases).

1.2   Dermis

The dermis (or corium) is located just below the basal membrane of the epidermis and extends into the subcutis. While the boundary between the epidermis and the dermis is very clear, the boundary between the dermis and the subcutis is less clear. The dermis is composed of the upper layer called the *Stratum papillare* that forms an undulating border with the epidermis, and the lower layer − the *Stratum reticulare*, transitioning into the subcutis layer. Undulating boundary between the epidermis and the dermis increases the contact area between the two layers and provides optimal nutrition of basal cells from blood cells passing through the *Stratum papillare* [18].

The dermis is a thick layer of viscoelastic tissue containing blood vessels, nerve endings, hair follicles and sweat glands. The structure of this layer comprises a relatively small number of cells, located in proteins fibers network and surrounded by an amorphous jelly called ground substance [19].

The major cells within the dermis are **fibroblasts** [14], which primary function is to synthesize two main fibrous proteins [20]: collagen and elastin.

Collagen

Collagen is the most abundant component of dermal tissue, comprising approximately 70-80% of its dry weight and forms a rigid scaffold running throughout the dermis [16, 17].

Elastin

Elastin makes up approximately 2% of the total volume of the dermis. It is a flexible fiber which is responsible for the elasticity and stretch of the skin [17].

Ground substance

This fiber consists of several components and the major one is a class of molecules called **proteoglycans**. These molecules are part protein and part sugar. They have a number of roles within the skin including [17]:

- maintenance of water and ion balance

- support for other dermal components

- mediate attachment of fibroblasts and growth factors involved in dermal repair

1.3 Subcutis

The subcutis (about 14% of the body weight = circa 10 kg) fixes the skin (the dermis) on the surface of the body. The subcutis and also the dermis contain large blood and lymph glands and fat, which is enclosed in grape-shape cells, arranged in parallel [21].

The subcutis layer performs three important functions [18]:

- isolation of the body from cold and heat

- lobes of fat cells form a layer that acts as a shock absorber on the fascia (fibrous wrapping muscles) and muscle tissue, which is located underneath

- fat cells serve as an important place to store nutrients; once the body needs it, the nutrients are transported from the fat cells into the surrounding blood vessels that supply nutrients to other body parts

1.4 Substructures of the skin

Several important substructures or appendages are associated with the skin: hairs and their associated sebaceous glands, nails, and sweat glands.

1.4.1 Nails

Nails are a modified type of the epidermis. They consist of closely compacted layers of dead, keratinized epithelial cells. The nail is a phylogenetic remnant of the mammalian claw. Its main role is to protect the finger tip and facilitate grasping and tactile sensitivity in the finger pulp. The nail matrix contains dividing cells which mature, keratinize and move forward to form the nail plate. The nail plate is thick approximately 0.3- 0.5 mm and grows at a rate of 0.1 mm per day. Toenails grow more slowly. The nail bed produces small amount of keratin and is adherent to the nail plate. Due to the adjacent dermal capillaries the pink color of the nail is made. The white lunula is the visible distal part of the matrix and the hyponychium is the thickened epidermis which underlies the free margin of the nail [8].

1.4.2 Hairs

Hairs consist of heavily keratinized cells and grow from deep recesses of the epidermis known as follicles. Hair follicles' main function is to produce insoluble fibers of hair. The follicles are dynamic structures that undergo cyclical growth changes. The cycle consists of three distinct phases: anagen - a period of active growth, catagen - a period of changes and restructuring, and telogen - a rest period before resuming the growth. The follicle grows out of the epidermis at an angle. Follicle is bounded by a basement membrane similar to the epidermis. The outer root sheath is the most peripheral of the cellular components. The next compartment is the inner root sheath. Innermost is the hair shaft, which contains three compartments: the cuticle, the cortex that forms the bulk of the hair shaft, and the medulla, which is variable. The follicle also contains structures like the sebaceous gland and the bulge which are the site of insertion of the arrector pili muscle which is responsible for "goose bumps" [22].

1.4.3 Glands

The skin glands comprise sweat, musk (large sweat glands in the armpits and the genital area), sebaceous and mammary glands. Sebaceous glands are almost always associated with hair follicles that bringing the sebum on skin surface. Gland size and quantity of sebum brought out on the surface differ in various parts. E.g. glands on the face are larger than the glands on the arms and legs [18].

2 SKIN BARRIER AND NATURAL MOISTURIZATION

The *Stratum corneum* (SC) as the uppermost layer performs the most important role in the protection of the body against externalities. In addition, the SC limits the amount of water absorbed and removed by the epidermis. This function is performed by the horny layer by means of various mechanisms: the binding of water, epidermal lipids, desquamation and regeneration [18]. Chemical composition of SC is the cause and basis of the barrier function. This is determined particularly by its physical-chemical properties. The *Stratum corneum* is composed of 75 - 80% proteins, 5 to 15% lipids and the rest are other organic compounds and water, including natural moisturizing factors (amino acids, urea, lactams, etc.) that keep the skin soft, pliable and supple. Proteins are found primarily in keratinocytes (about 70% α-keratin, and approximately 10% β-keratin) and about 15% of proteins have different peptide structures, including skin enzymes. The keratinocytes are highly insoluble and very resistant to chemicals [23].

2.1 Skin lipids

The *Stratum corneum* is often modeled as a brick wall. The SC corneocytes with their resistant cell envelopes and keratin microfibrils are considered to be the bricks, and the layers of lipids found between the cells are considered to be the mortar [24]. The lipid ''mortar'' is the main barrier to water passing out through the SC [25]. There are two sources of skin lipids; both of these sources provide different lipid composition. These two sources are sebum and epidermal lipids.

2.1.1 Epidermal lipids formation

Precursors of epidermal lipids are formed in the Golgi complex of keratinocytes in the *Stratum spinosum*. They are stored in the form of bilayer lipid membranes in organs called Odland granules. In the upper parts of the *Stratum granulosum* the lipid double layers are forced out by exocytosis into the intercellular space. During this maturation process polar glycolipids, phospholipids and sterol esters are enzymatically converted to less polar lipids such as ceramides and free fatty acids, creating a semipermeable lipid barrier of the horny layer [18]. This mechanism is illustrated in figure 3.
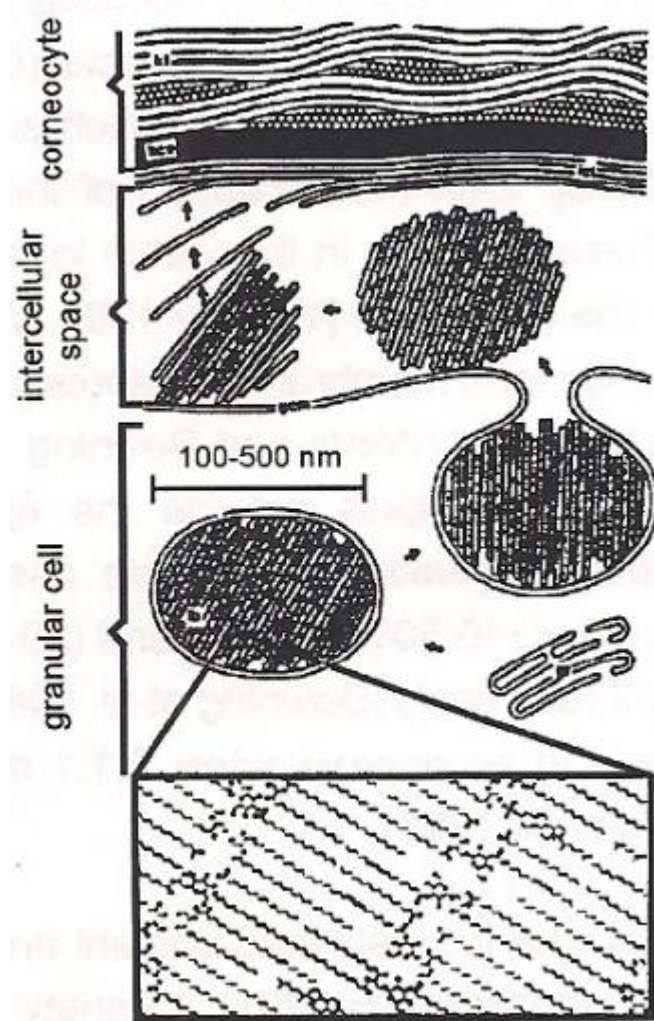
*Fig. 3: Lipids in Odland granules are weakened
and finally displaced into the intercellular space
of the upper granular layers* [26]

2.1.2 Sebum production

Sebaceous glands are usually accompanied by the hair follicle. Sebaceous glands are on the surface of the skin open and are surrounded by connective tissue. The sebum is formed by splitting of dead cells that are rich in lipids. Cells producing lipids differentiate and become denucleated. After this process the cell structures are disintegrated and lipids are secreted by sebaceous ducts on the skin surface. In human skin, sebaceous glands are concentrated on the face, forehead and scalp, but completely absent on the palms and soles [27].

2.1.3   Lipids composition

During the differentiation of epidermal cells on their way from the basal layer, through the granular layer up to the horny layer, changes in the composition of lipids occur. As Fig. 4 demonstrates this process, the quantity of phospholipids increases at the beginning, but is completely missing in the horny layer. The same applies in the case of glucosyl ceramides. A significant increase in the content of hydrophobic ceramides and fatty acids is also notable, especially in the final step of differentiation. Also the cholesterol content increases strongly. The concentration of other components changes much less and there is a great implication of three substances - ceramides, cholesterol and free fatty acids - so that the lamellar lipid structure in the *Stratum corneum* can be created. This lamellar lipid structure is a basic condition of a highly effective barrier function against penetration of water [27].
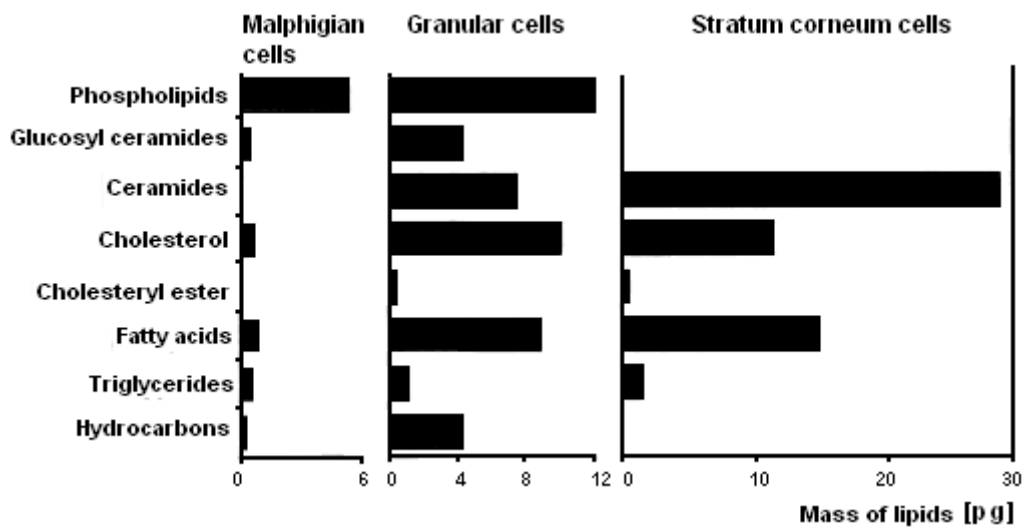
Fig. 4: Change in lipid composition during epidermal cells differentiation [28]

The composition of sebum is very different from the epidermal lipids. There are no ceramides, there is only a small amount of cholesterol, but there is a significant proportion of squalene, which can serve as a sebum indicator. The main components of sebaceous lipids are triglycerides - about 60%. But during their passage through the sebaceous channel they are hydrolyzed into free fatty acids, glyceryl, mono- or

diacylglyceryl. Polar lipids are represented by phospholipids, principally by phosphatidylcholine. Its composition is described in table 1. The sebum on the skin is mixed with the products generated by biochemical transformation of epithelial cells (keratinocytes). The mixture of skin lipids is made up of keratinocytes lipids and the products of keratin degradation (daily production is around 20 mg / day) [29].

Table 1: Human sebum composition [30]

Lipid	Composition of sebum on the skin surface (%)
Triglycerides	42
Free fatty acids	15
Wax esters	25
Squalene	15
Cholesterol esters	2
Cholesterol	1

2.1.4   Ceramides

The main polar lipids of the *Stratum corneum* consist of 9 types of ceramides (described in 2003 [16]), differing in polar head construction and the average length of chains. The basis of the ceramide molecule is a basic alcohol, which is represented by sphingosine, fytosphingosine or 6-hydroxysphingosine. A fatty acid is bound to their amino group in position 2. This fatty acid may contain hydroxyl group in the α or ω position. The length of fatty acids varies from 16 to 34 carbons in the ω- hydroxy-ceramides, the most frequently occurring acids contain 24 carbons. Three types of ceramides containing ω-hydroxyacid of around 30 carbons are especially important - there is ester-like binding essential fatty acid on the terminal hydroxyl. These ceramides serve as the molecular link between the lamellas. In general, the ceramide molecule contains a polar head and two hydrophobic chains, like phospholipids. Unlike phospholipids forming a normal cell membrane, the polar heads of ceramide are significantly smaller and allow closer layout of lipids in lamellas. Ceramides of the

Stratum corneum have considerably longer hydrophobic chain and do not create bilayers, but a multilayer interconnected lamellar structure (see figure 5). These structural features explain why the permeability of ceramides lamellas of the *Stratum corneum* are thousands times lower than the permeability of phospholipids bilayers. In lamellar granules the ceramides are presented in the form of glucosyl ceramides and sphingomyelins that probably represent the less lipophilic transport form of skin ceramides. For the release of ceramides from these precursors the glucocerebrosidase, respectively sphingomyelinase are required.
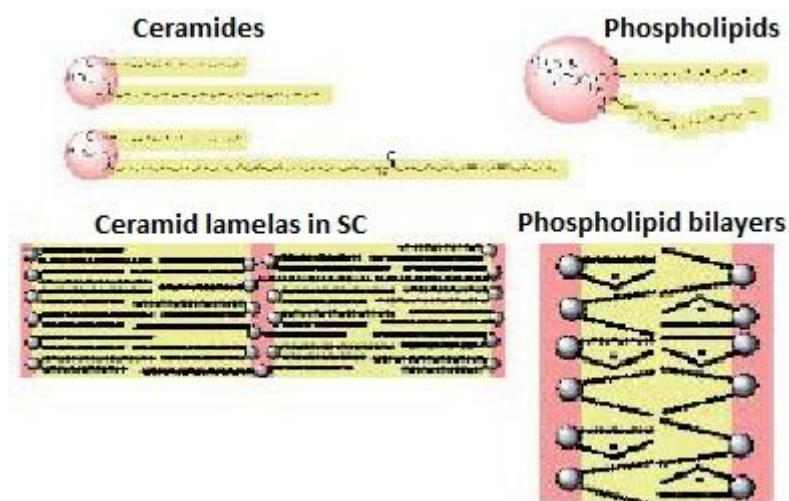
Fig. 5: Comparison of ceramides and phospholipids and their structures in the Stratum corneum [31]

2.2 Water binding

Normally the *Stratum corneum* must be as impermeable as possible except for a small amount of water loss hydrating the outer layers of the *Stratum corneum*, which is necessary to maintain its flexibility and to provide enough water to allow enzyme reactions that facilitate the *Stratum corneum* maturation events, together with corneodesmolysis and ultimately desquamation [32].

Compared with other aqueous tissues, the upper layers of the *Stratum corneum* usually contain very little water (5-15% by weight). But with the combined action of the hygroscopic molecules, it has the ability to imbibe 5-6 times its own weight in water resulting in swelling of the *Stratum corneum*. Moreover, the hydration

of the *Stratum corneum* is dependent on the ambient relative humidity (RH) and temperature. With increasing RH, the amount of water bound in the tissue increases. The bonding of water within the *Stratum corneum* differs according to the water content in the tissue. About 10-15% is tightly bound on polar groups of the structural proteins and is essentially unavailable for hydrolytic processes, but with increasing water content, the water is less tightly bound [33].

The *Stratum corneum* uses three main mechanisms to hold water [34]:

- the intercellular lamellar lipids whose physical conformation provide a tight and semi-permeable barrier to the passage of water through the tissue

- the presence of fully matured corneodesmosomes and ceramide-hydrophobed corneocytes which influence the tortuosity of the SC and thereby the diffusion path length of water

- the presence of both intracellular and extracellular hygroscopic materials - natural moisturizing factors (NMF)

In biological sense, the NMF allow the outermost layers of the *Stratum corneum* to retain moisture against the drying action of the environment. Traditionally, it was believed that this water plasticized the *Stratum corneum*, keeping it proof by preventing cracking and flaking which might occur due to mechanical stresses. From a physical chemistry perspective, the specific ionic interaction between keratin and NMF, accompanied by a decreased mobility of water, leads to a reduction of intermolecular forces between the keratin fibers and increased elastic behavior. Recent studies have emphasized that it is the neutral and basic free amino acids in particular, that are important for the plasticization properties of the *Stratum corneum* [34].

Table 2 provides the composition of NMF. Substances as amino acids, pyrrolidone carboxylic acid, urea, uric acid, glucosamin, creatinin, phosphates, lactates, chlorides, citrates, carbohydrates, peptides, etc. belong among NMF [5, 35, and 36]. Hyaluronic acid and glyceryl have been shown to be present naturally in the SC, both of them derived from sebaceous triglyceride breakdown. Recent data also indicate that lactate plays a critical role in influencing the physical properties of the SC. Lactate and potassium were found to be the only components of the NMF analyzed that correlated significantly with the state of hydration, stiffness and pH, of the SC. Lactate may also be

derived in part from sweat. The generation and maintenance of an acid pH within the SC, also called "acid mantle", is critical to the proper functioning of this tissue. Studies have pointed out an essential role of free fatty acids, especially urocanic acid that plays a vital role in the regulation of the *Stratum corneum* pH [37-40].

Table 2: Composition of NMF [41]

Substrates	Amount (%)
amino acids	40.0
pyrrolidone carboxylic acid	12.0
lactate	12.0
urea	7.0
ammonia, uric acid, glucosamine creatine	1.5
citrate	0.5
Na, K, Ca, Mg, PO_4^-, Cl^-	18.5
sugars, organic acids, peptides, unidentified materials	8.5

2.3   Protective acid mantle

Due to the presence of weak acid components, the aqueous part of hydrolipid film makes up the protective acid mantle which performs three important functions [18]:

1. supporting of development and maturation of epidermal lipids, which help maintain the barrier function

2. indirect protection against penetration of microbial pathogens

3. direct protection against alkaline substances

Protective acid mantle contains lactic acid and various amino acids from sweat, free fatty acids from sebum, amino acids and pyrrolidone carboxylic acid resulting from the cornification process of cells. The value of physiological pH of skin is between 5.4 and 5.9. In this pH range, the skin is colonized with normal skin flora and pathogenic microorganisms than cannot multiply. Acidic pH of the horny layer plays a key role in creating of epidermal lipids and together with them forms a permeable barrier [18].

3 SURFACTANTS: DEFINITION, CLASSIFICATION, APPLICATION

A surfactant (a contraction of the term surface-active agent) is a substance that, when present at low concentration in system, has the property of adsorbing onto the surface or interfaces of the system and of altering to a marked degree the surface or interfacial free energies of those surfaces (or interfaces). The term interface indicates a boundary between any two immiscible phases. The interfacial free energy is the minimum amount of work required to create that interface. The interfacial free energy per unit area is that, what is measured when the interfacial tension between two phases is determined. Surfactants as a group have the ability to modify interface between various phases and lower the interfacial tension.

Each surfactant molecule has one hydrophilic and one lipophilic part (see Fig. 6). As a rule, surfactant is soluble in at least one of the containing phases and is used to perform one or more of the following tasks: clean (detergency), wet, emulsify, solubilize, disperze, or foam. The most useful and widely accepted classification of surfactants is based on the nature of the hydrophilic segment of the surfactant molecules. This approach creates four large groups of chemicals: amphoterics, anionics, cationics, and nonionics [42, 43].
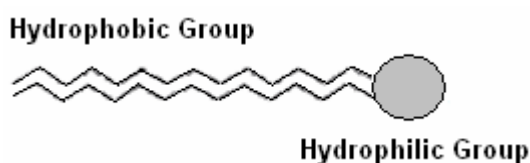
Fig. 6: Hydrophobic hydrocarbon "tail" and a hydrophilic "head" group of a surfactant

Soap is historically the first known surface-active skin cleanser. But anionic surfactants represent the largest volume of surfactants produced so far. New surfactants, particularly derived from natural products such as sugar, were extensively developed in the last decade in search for biocompatible and biodegradable properties. The production of

mild nonionic and amphoteric surfactants is expected to grow rapidly in the coming years as well [44].

3.1   Surfactants in body cleansing liquid formulations

Shower products can be divided up to two categories differentiated by consumer habits. The first category contains products, which do not contain soap and are favored in Europe and in the United States; the products of the second category contain soap and are favored in Asia. This thesis is focused especially on the first category and liquid products.

The first category of cleansing formulations sold in Europe and in the Unites States is based on synthetic surfactants. These surfactants are much milder to skin than the soap-based surfactants and can induce neutral pH on the skin. Products formulated with these surfactants are much more compatible with human skin when used in a wide variety of skin care agents.

Liquid cleansing products may have a single anionic surfactant, or may be based on a mixture of various surfactants. The most common surfactant used in liquid products is **alkyl ether sulfate**, with different levels of ethoxylation having different solubilizing cations, such as sodium, potassium, and ammonium. Surfactants such as **acyl isothionates, sarcosinates, sulfosuccinates, alkyl phosphates, sugar esters, sulphoacetates, lauramide diethanolamine (DEA), cocamide DEA, amine oxides,** and **amphoacetates** are used as cosurfactants to improve mildness and lathering properties of the products. Also **cocamidopropyl betaine** as an amphoteric surfactant is used for these properties in many cases [45].

3.2   Effects of surfactants on the skin

3.2.1   Changes in skin surface pH

The skin surface is naturally acidic (pH 5.4-5.9) and this aspect is in contrast to the pH of the internal environment of near neutrality. This acid mantle is important in preventing microbial and fungal infections. The use of soaps and detergents in general

leads to increases in pH, i.e. the skin becomes more alkaline [46]. However, the degree of change and the time to recovery varies with different products [47].

3.2.2 Transepidermal water loss

Transepidermal water loss (TEWL) is a measure of the barrier function of the skin and a sensitive index of damage to that barrier. Prolonged contact with surfactants may lead to cutaneous inflammation with increased TEWL [48]. The nature of these changes is not clear but undoubtedly involves the initial interaction of the surfactant with intercellular lipids of the *Stratum corneum* leading to penetration of the surfactant into the viable epidermal cell layer underneath. The surfactant may then cause cell damage or even cell lysis resulting in the development of a clinically obvious irritant reaction [49]. Figure 7 illustrates the influence of water, synthetic detergent and soap on TEWL increase.
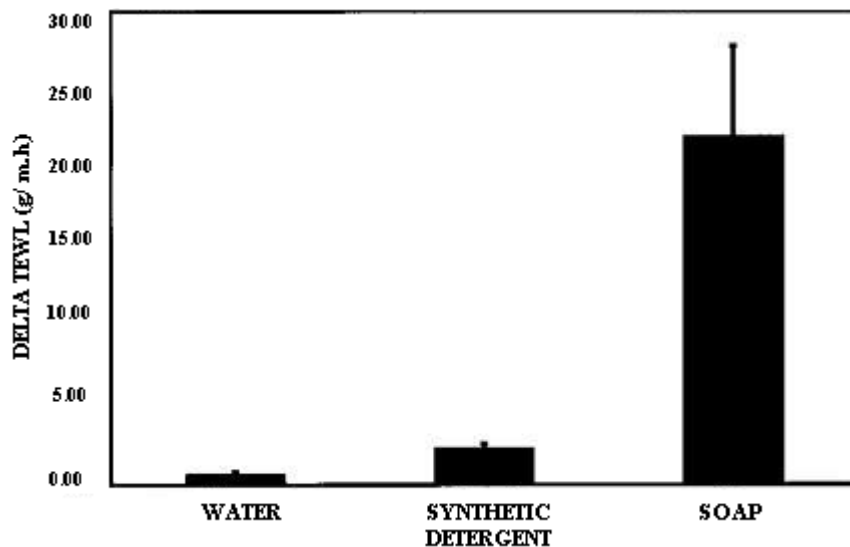

Fig. 7: Changes of TEWL after 15 minutes of washing with water, synthetic detergent and soap. 15-times 2-minutes washings [50]

3.2.3 Proteins in detergency

Interactions with keratin. It is generally agreed that penetration of surfactants within the keratin matrix of the *Stratum corneum* or cuticle cells of the hair fibers is the first step of skin/hair damage and that only monomeric surfactants can penetrate, while the hydrated micelles are too large to enter the tight network of keratin. When in the *Stratum corneum* and hair cuticle, surfactants are supposed to cause denaturating effects on the keratin structure. This partial denaturation is supposed to be based on one or combination of mechanisms: the hydrocarbon tail of surfactants penetrates into apolar regions of the keratin replacing the conformation-stabilizing hydrophobic interactions by ligand-segment interactions; the ionic head of surfactants produces attraction-repulsion forces on the charges groups of keratin disordering its architecture; the formation of excess of positive or negative charges causes additional pressure, the matrix swelling and high permeability may occur [51].

Interactions with collagen. Collagen is the most abundant protein in the skin, connective tissue and other mammalian body parts. The density of collagen net is too low and the structure of collagen fibers in dermis is not as strong as in the layer of keratin. Isoelectric point of collagen is higher (pI ~ 7) than of keratin. Collagen is able to bind anionic surfactants by electrostatic and nonpolar interactions at pH ~ 6. Interactions of collagen with surfactants are associated with changes in volume and weight of collagen fibers. These interactions are dependent on the pH of reaction components, ionic strength, and surfactant concentration. In the environment, where pH is lower than the isoelectric point of collagen, the process of swelling increases [52].

3.2.4 The influence of surfactants on the lipid barrier

The protective lipid barrier of the skin is composed of highly organized lipid layers located between the cells of the SC. In order to disorganize these lipids and alter the skin barrier function, surfactants have to integrate into lipid layers that are mostly hydrophobic. Because of their small size, surfactant monomers can easily reach the intercellular lipids and disturb the skin barrier function. This effect depends on the relative proportion of monomers in solution [53]. Morganti [54] reported that washing the skin with water decreased surface lipids by about 24%, while washing with soap reduced surface lipids by about 35%. Surprisingly, washing with a synthetic

detergent bar reduced surface lipids by even a greater amount, about 53%. Lipid removal was linked to a decreased ability of the skin to retain natural moisturizing factors and ultimately lead to dry skin.

4 STRATEGIES TO MOISTURIZE THE SKIN

Resulting from the previously mentioned problems caused by surfactants and the fact that exogenous water alone is insufficient to moisturize the skin, moisturization of the skin has become an issue for the cosmetic industry. Formulations of shower and bath products have been improved with addition of special substances.

Moisturization is defined as adding water to the skin, retaining water inside the skin, eliminating the feeling of skin dryness, reducing/ eliminating the development of dry skin scales, or enhancing the lipid barrier function. Moisturization can be achieved in several ways: with the usage of emollients (to mask the rough scaly condition), occlusive agents (to reduce water loss from the skin), humectants (to help retain water in the skin), lipid fluidizing agents and enhancers of barrier repair [55, 33].

4.1 Humectants

Humectants are hygroscopic compounds capable to retain moisture in a product for an extended time. They should have low volatility. Humectants are divided into three classes: inorganic, organic and metal-organic. Organic humectants include polyols, amino acids and polysaccharides. An example of inorganic humectants is calcium chloride. Metal organic salts are represented by lactate and glycolate salts. The group of organic humectants is particularly used in the cosmetic industry and will be described in this chapter [41].

4.1.1 Choice of humectants

There are several factors that influence the choice of humectants:

Equilibrium hygroscopicity: is a state when an aqueous solution neither gains nor loses water at a given relative humidity. In other words it can be explained as the weight percentage of water in a solution in equilibrium with the given relative humidity.

Dynamic hygroscopicity: is the speed at which a compound or its aqueous solution will gain or lose moisture while approaching equilibrium. It is expressed in comparison with another material.

Volatility: is a tendency of the substance to evaporate. Volatility is important under conditions of practical use, usually at room temperature.

Other important properties of humectants that influence the choice are: low cost, good compatibility, lack of toxicity, color, odor, taste, lack of corrosive action and low freezing point [41].

4.1.2   Polyols

Polyols are organic compounds with more than one hydroxyl group; but due to their toxicity only some of them have importance in body care. Polyols considered safe include dipropylene glycol, sorbitol solution, glyceryl, honey, polyethylene glycols, glucose syrup, propylene glycol, invert sugar and 1,3- butylene glycol, where glyceryl, propylene glycol and sorbitol are the most widely used in cosmetics. Nevertheless, even these three substances can be used in cosmetic products only in limited concentrations [41].

Of these three polyols, sorbitol is the least volatile and propylene glycol most volatile. Table 3 shows the humectant properties of some polyols at 30, 50 and 70% relative humidity (RH) [41].

Table 3: Humectant properties of common polyols at 30, 50, and 70% RH

	Equilibrium hygroscopicity, % solids vs. RH (%)		
Name	**30**	**50**	**70**
Glyceryl	89	90	65
Glucose	94	88	79
Sorbitol (85%)	96	87	75
Sorbitol (70%)	X	X	75
Propylene glycol	91	82	68
Sodium lactate	84	68	50

4.1.3 Amino acids and NMF

Urea and alpha hydroxyl acids, such as lactic acid, are examples of the group of amino acids. They can alter the hydrogen bonding properties of the protein in the skin and improve rheological properties and skin elasticity. Also pyrrolidone carboxylic acid is available as a cosmetic raw material in the form of its sodium salt. Sodium pyrrolidone carboxylic acid has been shown to be superior as a humectant to glyceryl, propylene glycol and sorbitol in certain relative humidity.

Many combinations of sodium pyrrolidone carboxylic acid, lactate salts and hydrolyzed collagen with other amino acid are available too. They possess very good humectancy and moisturizing abilities. Frequently used amino acids include proline, glycine, alanine, threonine, arginine and glutamic acid [55, 41].

4.1.4 Polysaccharides

Hyaluronic acid in the form of its salt, sodium hyaluronate, is one of the most frequently employed polysaccharides. Chemically it is composed of repeating disaccharide units of N-acetyl- D-glucosamine and D-glucuronic acid. It is a component of the intercellular matrix of connective tissue. Sodium hyaluronate maintains the skin moisture content, has a high water-holding capacity, excellent skin-lubrication properties and also an ability to soften and smooth the skin.

Chitosan - product of chitin deacetylation - has excellent humectant properties similar to hyaluronic acid [41].

Seaweeds and algae extracts can be also found in bath products. They are humectants with good moisturizing properties [56]. Some of the representatives include: *Laminaria saccharina, Chondrus crispus, Palmaria Palmata, Ascophyllum Nodosum, Fucus vesiculosus, etc.*

4.1.5 Other types of recently discovered humectants

Oleyl glyceryl ether, known also as selachyl alcohol has been reported to have moisturizing properties as well. It is in the form of liquid crystals and at the temperature

of about 35 °C these liquid crystals exhibit a very high water retention that persists for a long time [57].

Excellent moisturizing properties are exhibited by a composition comprising trihydric or more water soluble **polyhydric alcohol, lecithin and 3-methyl-1, 3-butylene glycol**. This composition was described in a Japanese patent [58].

Other patents describe for example **crosslinked protein polymers** or **silicone polyester polymers** as durable humectants. These silicone polyester humectants are substantive to hair and skin due to their structure [59, 60].

4.2 Emollients

Emollients are agents that soften and smooth the skin. The essential component of any emollient is a lipid. The lipid may be animal, vegetable-derived or obtained from mineral oils. Respectively, the lipid may be synthetic in origin. The fatty acids of the lipids used are mostly 8-18 carbon atoms [61].

Some representatives of emollients are given in table 4. These substances are usually added to the product as smoothing ingredient, antistatic additive, solvent, or skin protection additive. Cosmetic emollients should be resistant to hydrolysis and oxidation. From the consumer point of view, spreadability, solvent properties, rate of absorption through the skin, oily feeling and self-softening effect are also considered. However, they should not hinder the passage of certain components through the skin [61, 10].

Table 4: Classification of the emollients according to the polarity

Nonpolar:	paraffinum liquidum, squalene
Polar:	dimethicone, isopropyl palmitate, isopropyl stearate, triglycerides
Strongly polar:	almond oil, sunflower seed oil, decyl oleate, avocado oil
Very strongly polar:	PPG-15 stearyl ether

4.3 Occlusive agents

Occlusive agents are substances that can completely preclude evaporation of water from the skin when applied on the skin in a thin layer. Sometimes they are classified as emollients as well. The main representatives are: paraffinum, high molecular waxes, oils with more unsaturated fatty acids (linoleic acid, linolenic acid), apricot kernel oil, avocado oil, citrus grandis seed extract, jojoba oil, rosa canina fruit oil, sesamum indicum oil [10].

5   PRINCIPLES OF SKIN HYDRATION MEASUREMENT

Various methods are used for evaluation of SC hydration [62]. In this thesis, the corneometer was chosen for skin hydration measurement. Principles and brief description of the available methods are given in the following list.

 List of known methods:

- **Electrical measuring of impedance, resistance, and phase angle.** It is influenced by effects of polarisation, electrolytes in the *Stratum corneum* and contact resistance. This process requires galvanic contact through measured circuit and measured object.

- **Infrared spectrophotometric measurements.** The absorption of infrared rays through the horny layer of the skin is measured and characteristic peaks for water are evaluated.

- **Resonant frequency measurements.** Elasticity of the skin affected by its moisture content results in different mechanical vibration transmission between the transmitter and receiver signal placed on the skin. Changes in bonds are evaluated.

- **Photo-acoustic method.** This method is based on measurement of acoustic signals accrued in tissue due to pressure changes caused by periodic light.

- **Evaporation methods.** In these methods the amount of evaporated water from the skin surface is measured. The evaluation is carried out by electrical measurements or absorption of water into the measuring substance. These methods are for determining of moisture content of the skin and can be used only indirectly, because only part of the evaporated water from the skin is evaluated; moreover this amount only approximately corresponds with real moisture content.

- **Corneometer.** The instrument measures the electrical capacitance of the SC. The measuring probe works as a condenser (see Fig. 8), whose capacity is influenced by a change in the dielectric constant of any material with which it comes into contact.
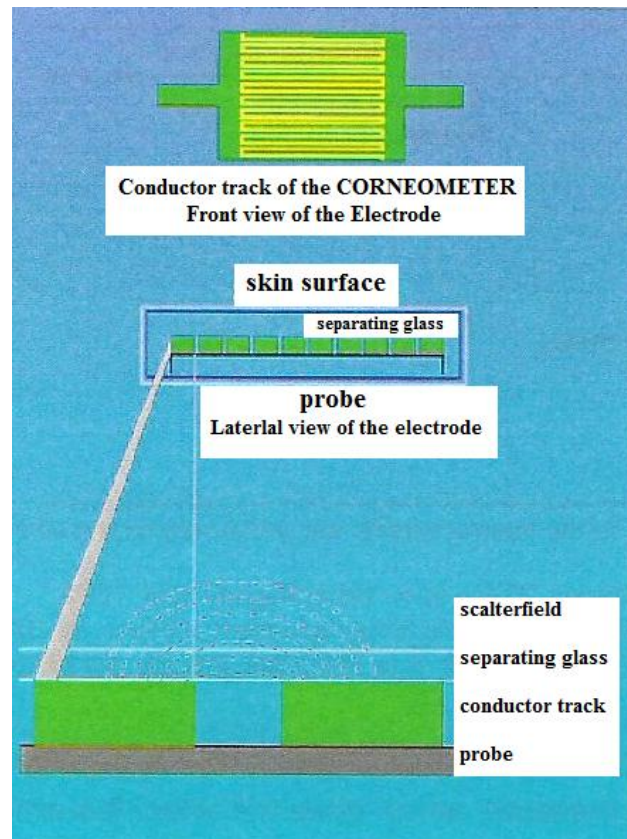
Fig. 8: Schematic representation of the corneometer probe head consisting of ceramic tiles with numerous parallel-located gold lines. The even and odd lines, respectively, are connected to each other to form two conductor tracks separated by an insulating material, i.e. to form a capacitor. An electric scatter field of variable frequency is established in the SC and the upper layers of the epidermis. High water contents increase the capacity of the probe resulting in changes of the values displayed [63, modified]

The following equation is applied:

$$C=C_0+C_x \qquad\qquad (E\text{-}1)$$

> Where: $\quad$ C= capacity of condenser (F)
>
> $\quad\quad\quad\quad\quad$ C_0= basic capacity
>
> $\quad\quad\quad\quad\quad$ Cx= measurement capacity as a function of the relative dielectric constant of material present in the electrical field of the condenser

Frequencies of about 1 MHz are applied. The device determines the water content of the SC down to a depth of about 0.1 mm. Since water has a high dielectric constant (81) compared to other materials (mainly less than 7), an increase in the water content will raise capacitance values. Presence of salts and ions may affect the results. Values are expressed in arbitrary units.

Relationship between electrical conductance and the skin water content is not linear, but depends on the hydrogen binding state of water molecules to the keratin chains. According to their strength of binding to keratin, three types of water can be defined: "tightly bound water", for water contents from 0 to around 7%, "bound water" (7-35%) and "free water" (less than 35%). Consequently, substances or treatments that interact with the keratin-water network may modify conductance without changing the actual water content [64- 67].

6 JUSTIFICATION OF THE RESEARCH

Optimal skin hydration is one of the important factors that keep skin in a healthy state. Naturally, water binding and skin moisturization performed in the *Stratum corneum* are based on three main factors: intercellular lamellar lipids, presence of corneodesmosomes and ceramide-hydrophobed corneocytes and natural moisturizing factors.

Every day the skin is exposed to various body cleansing formulations. Some of them contain as a major surfactant an anionic surfactant - sodium lauryl sulfate. Sodium lauryl sulfate is usually accompanied by other cosurfactants to improve mildness and lathering properties of the products. Nevertheless, the surfactants may have quite negative influence on the health condition of the skin including its hydration. Thus the formulations of shower products are improved by addition of other substances such as humectants, emollients or occlusive agents. Humectants are hygroscopic compounds that can bind the moisture and retain it in the upper layers of the *Stratum corneum*.

The aim of this work is to find out how various liquid cleansing products influence the degree of hydration of the SC. Five different commercial products are compared with a certain concentration of sodium lauryl sulfate. The products are applied on the volar forearm of human volunteers. In defined intervals the hydration of the skin is measured by corneometer.

II. EXPERIMENTAL PART

7 MATERIALS

7.1 Chemicals and commercial cosmetic products

- Distilled water

- Sodium lauryl sulfate:

    - molecular formula: $C_{12}H_{25}NaO_4S$

    - molecular weight: 288.38 g. mol^{-1}

    - purity: ± 99%

    - producer: Merck

- Commercial sample No. 1: Liquid soap

Table 5: Liquid soap ingredients

Ingredients	Functions in the product /*
Aqua	solvent
Sodium Laureth Sulfate	cleansing, emulsifying, foaming, surfactant
Cocamidopropyl Betaine	antistatic, cleansing, foam boosting, hair conditioning, surfactants, viscosity controlling
Sodium Chloride	bulking, masking, oral care, viscosity controlling
Cistric Acid	buffering, chelating, masking
Styrene/ acrylates copolymer	film forming, opacifying
Parfum	perfuming
Hexyl Cinammaldehyde	denaturant, flavouring, perfuming
Linalool	deodorant, perfuming
Chloroacetamide	preservative
2-bromo-2-nitropropane-1,3-diol	preservative
CI 19 140	cosmetic colorant
CI 15 985	cosmetic colorant

/ All ingredients in the thesis and their functions are listed in accordance with Inventory and a common nomenclature of ingredients employed in cosmetic products (INCI), amended by Commission Decision 2006/257/EC of 09.02.2006*

- Commercial sample No. 2: Moisturizing shower gel for men

Table 6: Composition of Moisturizing shower gel for men

Ingredients	Funcions in the product
Aqua	solvent
Sodium Laureth Surfate	cleansing, emulsifying, foaming, surfactant
Cocamidopropyl Betaine	antistatic, cleansing, foam boosting, hair conditioning, surfactants, viscosity controlling
Cocamide DEA	emulsifying, emulsion stabilising, foam boosting, surfactants, viscosity controlling
Glycerin	denaturant, humectant, solvent, perfuming
Sodium Chloride	bulking, masking, oral care, viscosity controlling
Sodium PEG-7 Olive Oil Carboxylate	emulsifying, foam boosting, hydrotrope
Coco- Glucoside	surfactant, foaming
Glyceryl Oleate	emollient, emulsifying, perfuming
Hydrolyzed Algae Extract	skin conditioning
Polyquaternium-7	antistatic, film foaming
Panthenol	antistatic, skin conditioning, hair conditioning,
Menthol	denaturant, masking, rereshing, soothing
Lactic Acid	buffering, humectant, skin conditioning
Benzophenone- 4	UV absorber, UV filter
Benzyl Alcohol	preservative, solvent, viscosity controlling, perfuming
Methylchloroisothiazolinone	preservative
Methylisothiazolinone	preservative
Parfum	deodorant, masking, perfuming
CI 42 090	cosmetic colorant

- Commercial sample No. 3: Olive liquid soap

Table 7: Olive liquid soap ingredients

Ingredients	Functions in the product
Aqua	solvent
Sodium Laureth Sulfate	cleansing, emulsifying, foaming, surfactant
Cocamidopropyl Betaine	antistatic, cleansing, foam boosting, hair conditioning, surfactants, viscosity controlling
Sodium Benzoate	anticorrosive, masking, preservative
Sodium Chloride	bulking, masking, oral care, viscosity controlling
Styrene/ acrylates copolymer	film forming, opacifying
Parfum	perfuming
butylphenyl methylpropional	perfuming
PEG-10 Olive Glycerides	emulsifying
Benzyl Alcohol	preservative, solvent, viscosity controlling, perfuming
Methylchloroisothiazolinone	preservative
Methylisothiazolinone	preservative
CI 47 005	cosmetic colorant
CI 42 051	cosmetic colorant

- Commercial sample No. 4: Kids shower and shampoo

Table 8: Kids shower and shampoo ingredients

Ingredients	Functions in the product
Aqua	solvent
Sodium Laureth Sulfate	cleansing, emulsifying, foaming, surfactant
Cocamidopropyl Betaine	antistatic, cleansing, foam boosting, hair conditioning, surfactants, viscosity controlling
Sodium Cocoamphoacetate	cleansing, foaming, hair conditioning, surfactan
Coco- glucoside	surfactant, foaming
Cocamide DEA	emulsifying, emulsion stabilising, foam boosting, surfactants, viscosity controlling
Propylene Glycol	solvent
Chamomilla Recutita Flower Extract	masking, skin conditioning
Sodium Benzoate	anticorrosive, masking, preservative
Sorbic Acid	preservative
Potassium Chloride	viscosity controlling
Methylparaben	preservative
Sodium Chloride	bulking, masking, oral care, viscosity controlling
Benzyl Alcohol	preservative, solvent, viscosity controlling, perfuming
Methylchloroisothiazolinone	preservative
Methylisothiazolinone	preservative
Triethylene Glycol	masking, solvent, viscosity controlling
Propylene Glycol	humectant, skin conditioning, solvent, viscosity controlling
Magnesium Nitrate	hair conditioning
Magnesium Chloride	viscosity controlling
Parfum	perfuming
CI 16 035	cosmetic colorant
Lactic Acid	buffering, humectants, skin conditioning
Niacinamide	smoothing

- Commercial sample No. 5: Shower gel with ¼ moisturizing cream

Table 9: Ingredients of Shower gel with ¼ moisturizing cream

Ingredients	Functions in the product
Aqua	solvent
Sodium Laureth Sulfate	cleansing, emulsifying, foaming, surfactant
Glycerin	denaturant, humectant, solvent, perfuming
Cocamidopropyl Betaine	antistatic, cleansing, foam boosting, hair conditioning, surfactants, viscosity controlling
Cocamide MEA	emulsifying, emulsion stabilising, foam boosting, surfactants, viscosity controlling
Parfum	deodorant, masking, perfuming
Isopropyl Palmitate	antistatic, binding, emollient, skin conditioning, solvent, perfuming
Cucumis Sativus Fruit Extract	emollient, skin conditioning
Acrylates Copolymer	antistatic, biding, film forming
Guar Hydroxypropyltrimonium Chloride	antistatic, film forming, skin conditioning, viscosity controlling
Glycol Distearate	emollient, emulsifying, opacifying, skin conditioning, viscosity controlling
PPG-12	skin conditioning
Laureth-4	antistatic, emulsifying, masking, surfactant
Sodium Chloride	bulking, masking, oral care, viscosity controlling
Citric Acid	buffering, chelating, masking
Sodium Benzoate	anticorrosive, masking, preservative
Alpha- Isomethyl Ionone	skin conditioning, perfuming
Benzyl Alcohol	preservative, solvent, viscosity controlling, perfuming
Butylphenyl Methylpropional	perfuming
Citronellol	perfuming
Hexyl Cinnamal	denaturant, flavouring, perfuming
Hydroxyisohexyl 3- Cyclohexane Carboxaldehyde	perfuming
Limonene	deodorant, solvent, perfuming
Linalool	deodorant, perfuming
CI 19 140	cosmetic colorant
CI 61 570	cosmetic colorant

7.2 Materials

- Filter paper (producer: WHATMAN; W&R BALSTON, Ltd., England)

- Plaster fixation

- Pipettes (2 ml)

- Black marker

- Petri dishes

7.3 Preparation of materials for measurement

Sodium lauryl sulfate (SLS) was dissolved in distilled water and a reference 0.5% solution was prepared. Each cosmetic product was dissolved in distilled water as well and 2.5% and 5.0% solutions were made.

The concentration of aqueous solution of SLS was chosen on the base of previous studies [68, 69] as this concentration represents the highest possible concentration which elicits minimal or no skin irritation in the form of erythema. Visually undamaged skin was required for measurement in this study. Since most commercial products contain about 20% of surfactants, some of them namely SLS, 2.5% aqueous solution of the cosmetic products was suggested for testing as it ensures approximately the same concentration of SLS as the reference 0.5% aqueous SLS. For comparison the twice higher concentration of aqueous solution of the cosmetic product was used as well.

Eleven Petri dishes were marked with numbers and each of them was filled with 0.5% aqueous SLS or solution of commercial cleansing products with concentration 2.5% or 5.0% of the product in distilled water. Table 10 provides a clear division of individual products, concentrations and numbering of Petri dishes. The numbering starts with number 2 as number 1 indicated blank (untreated site) on the skin. The numbering corresponds with numbering on the forearms of the volunteers.

Filter paper was cut into fifty-five squares (approximately 2x4 cm) as the number of volunteers was 5 and 11 patches were needed for each. Patches of the filter paper were put into the Petri dishes with individual samples and let to absorb the aqueous solutions.

Table 10: Division of samples in Petri dishes

Petri dish No	Concentration [%]	Sample
2	0.5	Aqueous SLS
3	2.5	No. 1: Liquid soap
4	5.0	No. 1: Liquid soap
5	2.5	No. 2: Men moisturizing shower gel
6	5.0	No. 2: Men moisturizing shower gel
7	2.5	No. 3: Olive liquid soap
8	5.0	No. 3: Olive liquid soap
9	2.5	No. 4: Shower and shampoo for children
10	5.0	No. 4: Shower and shampoo for children
11	2.5	No. 5: Shower gel with ¼ moisturizing cream
12	5.0	No. 5: Shower gel with ¼ moisturizing cream

7.4 Instrumental equipment used for measurement

Hydration of the *Stratum corneum* was determined with non-invasive, skin capacitance meter (Corneometer CM 820, Courage + Khazaka, Germany) (see Fig. 9).

Fig. 9: Corneometer CM 820, Courage + Khazaka

8   STUDY PROCEDURE

8.1   Formal rules of the study

Aim of the study: Assessment of the degree of skin hydration after application of various commercial liquid cleansing products, determined by corneometer

Testing facility: National Reference Center for Cosmetics, National Institute of Public Health, Prague, Czech Republic

Study period: The study was performed within the period of 15. 3. 2010- 16. 3. 2010.

The test was carried out in compliance with: Cosmetic Product Test Guidelines for Assessment of Human Skin Compatibility, Colipa, Bruxelles 1995 [70], Guidelines for the Evaluation of the Efficacy of Cosmetic Products, Colipa, Bruxelles 1997 [71], (COLIPA= The European Cosmetic, Toiletry and Perfumery Association).

Volunteers selection: The selection of volunteers and the test methods were carried out according to the Declaration of Helsinki (1964) [72] and the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS, 2002) [73]. The study was approved by the Ethical Review Committee of the National Institute of Public Health.

A group of 5 healthy volunteers, at the age of 25-65, participated in the 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 was conducted observing all ethical principles of biomedical research involving human volunteers.

8.2 Preconditioning

During the one-day preconditioning period the volunteers had to refrain from using any skincare products as well as special skincare cleansing products on their forearms.

8.3 Test areas and application of samples

The measurement was performed on both forearms. The test sites were marked with black marker and patches with absorbed aqueous sample solutions were placed on them as figure 10 demonstrates.
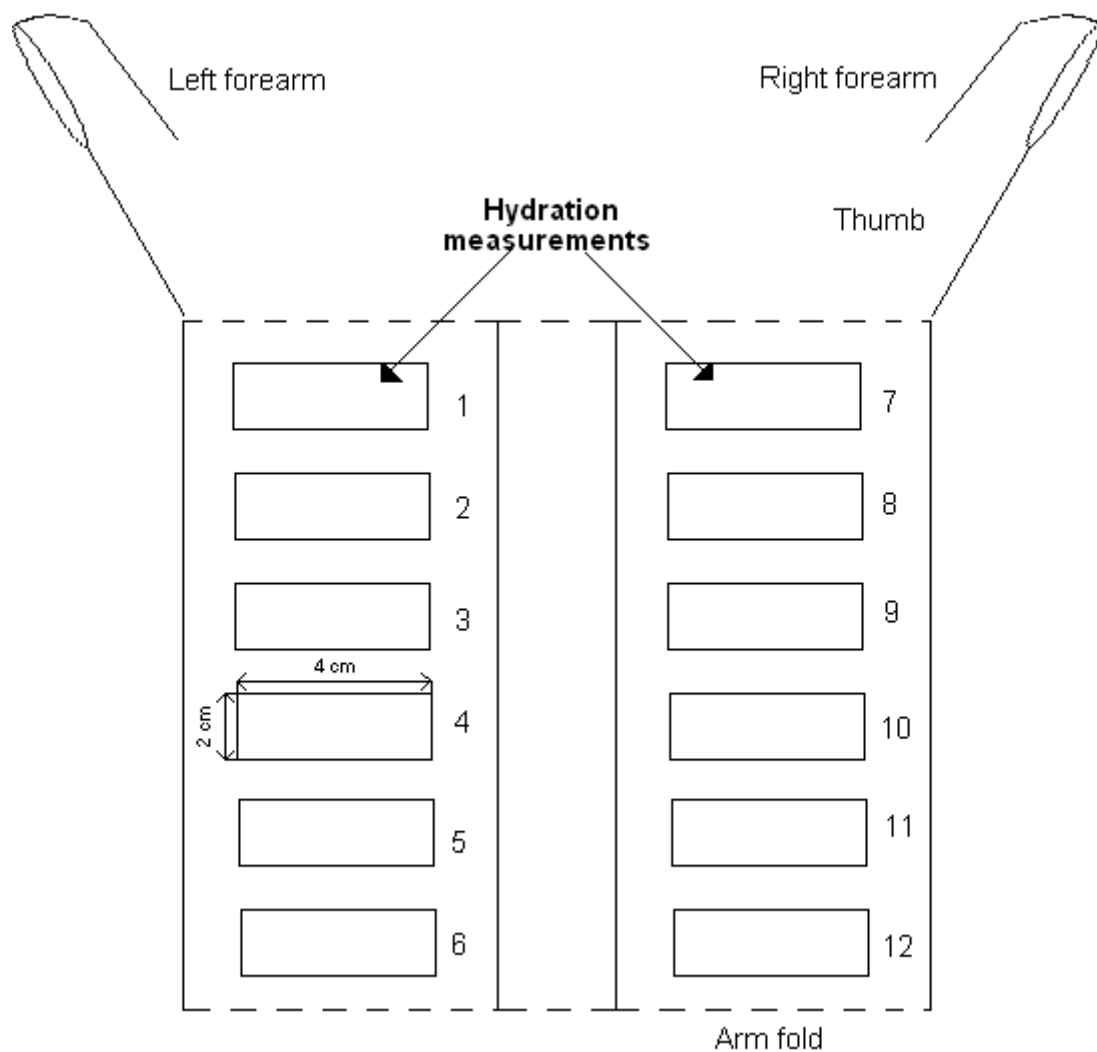

Fig. 10: Schematic representation of the test sites on both forearms [63, modified]

Then the patches were fixed with a plaster. The application took 4 hours. It was initiated at 8:00am and ended at 12:00pm on 15th March 2010. After the four-hour application the plasters with patches were removed.

8.4 Intervals and conditions of the measurement

The bio-instrumental measurements to assess the skin moisture were taken at following times: t0:   before the application of the products

                    t1:   immediately after the four- hour application

Then the measuring time points were: 1h (t2), 2h (t3), 3h (t4) after the application and continued the following day: 20h (t5), 22h (t6), 24h (t7) and 26h (t8) after the application. Table 11 summarizes the study schedule for the first measured subject, the other subjects followed so that the time intervals were observed.

Table 11: Study schedule

Day	Time		Action
Day -1 **14th March 2010**			Preconditioning
Day 1 **15th March 2010**	7:30- 8:00	**t0**	Acclimatization, informed consent of the subject, marking areas, corneometry
	8:00		Treatment
	12:00	**t1**	End of the application, corneometry
	12:55- 13:00		Acclimatization
	13:00	**t2**	Corneometry
	13:55- 14:00		Acclimatization
	14:00	**t3**	Corneometry
	14:55- 15:00		Acclimatization
	15:00	**t4**	Corneometry
Day 2 **16th March 2010**	7:55- 8:00		Acclimatization
	8:00	**t5**	Corneometry
	9:55- 10:00		Acclimatization
	10:00	**t6**	Corneometry
	11:55-12:00		Acclimatization
	12:00	**t7**	Corneometry
	13:55- 14:00		Acclimatization
	14:00	**t8**	Corneometry, end of the study

The measurements were performed on the test and control sites in parallel. Each corneometer measurement was performed five times per test area. All measurements were done by the same study investigator. Within the two days the skin on forearms of the volunteers was not treated by any other cosmetic product. Acclimatization of the volunteers lasted 5 minutes before each measurement. The measurements were performed in a laboratory with temperature $21.0 \pm 1.0°C$ and $52.5 \pm 2.5\%$ relative humidity.

9   RESULTS AND DISCUSSION

A twenty-six-hour test for assessment of the effect of surfactant products on the skin hydration was performed. Commercial products commonly available on the Czech market were used for the experiment. These products varied in composition and price. Two of them claimed moisturizing effect, but without any further information. Their ingredients were listed without information on the exact quantity in the products. For comparison, the first test area was untreated and served as a negative control (blank). The reference 0.5% aqueous dilution of SLS was applied on the second test area. The corneometry was done according to the time schedule and procedure written in the previous chapter.

Statistics. Five values from one test area were obtained. The average value of the five measurements was calculated. Since five volunteers were used, five arithmetic means were obtained. These were also averaged before the analysis and standard deviation was calculated. All calculations in the thesis were evaluated by means of Microsoft Office Excel and Microsoft Office Word.

The mean was calculated by the following equation:

$$\bar{x} = \frac{1}{n}.(x_1 + x_2 + ... + x_n) = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad \text{(E-2)}$$

Where: n….. …………..number of measurements

$x_1, x_2, …, x_n$…..population

The standard deviation (SD) was calculated by the following equation:

$$s = \sqrt{\frac{1}{n-1} \cdot \left(\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2\right)} \qquad \text{(E-3)}$$

Due to the fact that the experiment had to be done by one investigator using the same instrumentation for all measurements and it was required to perform the test on the same

subjects at the same time, it was decided to use a low number of subjects (5). Measuring of all 12 test areas of one subject took circa 5-10 minutes. The number of volunteers could not be higher because the time intervals of the first day would be impossible to observe. Therefore, because of the low number of subjects, it was pointless to do further statistical analysis.

Experimental results are presented in two different sections. The efficacy of every individual product is discussed separately and in conclusion all the results are compared. Changes in analyzed parameters are shown as a percentage towards the negative control (blank) and 0.5% SLS as a reference surfactant. In both cases blank and reference control represent 100% and the values are taken from the same time point as for the sample. The results are given in tables and graphs because all specifications and details cannot be included in graphs without a loss of clarity.

9.1   The effect of 0.5% SLS on the skin hydration

Sodium lauryl sulfate has historically been the most common anionic surfactant used in personal cosmetic cleansing products. The reason of such a large usage is very low price and good cleansing, emulsifying, foaming and surfactant functions. It is also widely used as a positive control in various tests for skin irritation in vitro and in vivo [74]. It was chosen as a reference surfactant for this study.

Aqueous SLS at the concentration of 0.5% was applied on the second test area of the left forearm. After the 4-hour application the area was without any visual changes and erythema. Changes in the skin hydration were monitored during the following 26 hours and compared with untreated skin of the first test area on the left forearm. Table 12 summarizes obtained values of % hydration of the control. In this case blank served as the negative control and represented 100%.

As can be seen in figure 11, immediately after the end of the application of the SLS, the skin hydration rapidly dropped about 7.2% and during the first 4 hours slightly increased, but still the value of the skin hydration was approximately about 5% lower than without treatment.

As revealed by the graph, after 20 hours the values of the skin hydration went above the level of untreated skin. The skin hydration on the test area was 100.6% after

20 hours. Two hours later the hydration increased by 5.5%. At the end of the time period, the skin hydration decreased and remained constant, values were moving very close to the values of the control site without application.

The results reflected that healthy skin had the ability to recover its barrier after application of 0.5% SLS within one day without using any further recovering products.

All the deviations and fluctuation were probably caused by different working place of the volunteers, especially different room temperature, relative humidity and also personal stress. An air-conditioned room was unavailable for the measurement. And consequently Barel et al. [75] point out changes in long-term applications due to climatic influences, and mentioned temperature and humidity as the decisive factors for biological fluctuations.

Table 12: Changes in the degree of the skin hydration after application of 0.5% SLS; compared with blank

Time points	Time after the application (h)	% of control /**		
t1	0	92.8	±	3.44
t2	1	95.6	±	9.25
t3	2	93.9	±	3.86
t4	3	94.6	±	4.25
t5	20	100.6	±	4.25
t6	22	106.1	±	4.55
t7	24	99.5	±	4.64
t8	26	99.8	±	2.38

*/** Mean values ± standard deviations are given for each time point and are expressed as a percentage of the control site (negative or reference control), arbitrarily set at 100%; used for all tables within the chapter of results and discussion*
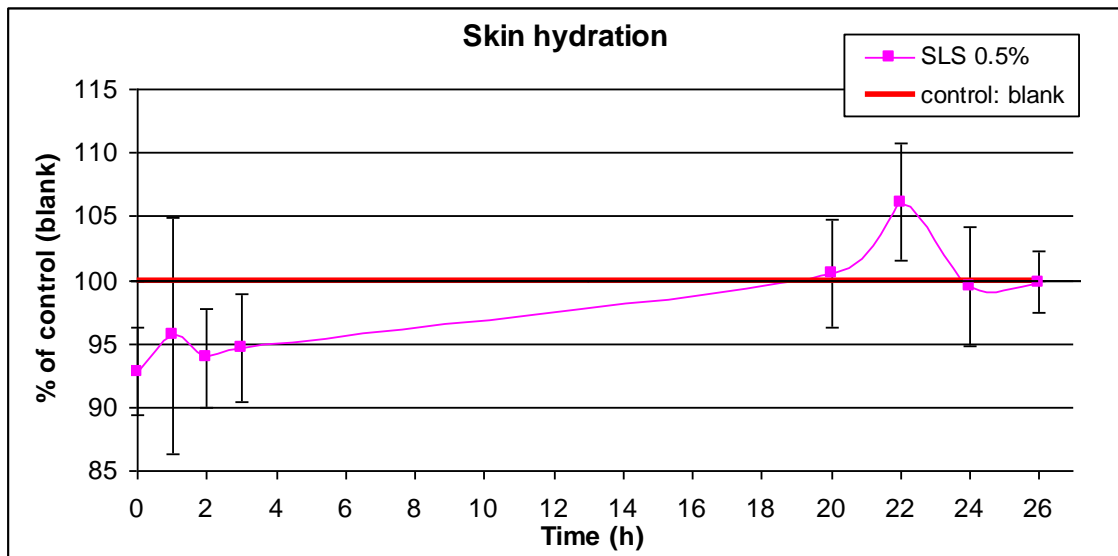
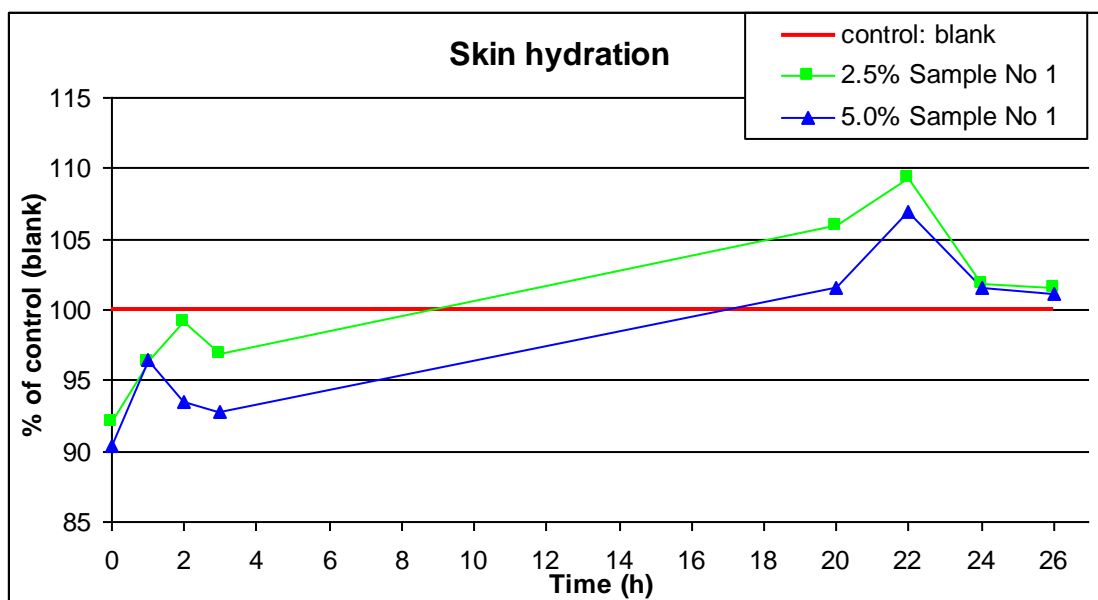*Fig. 11: Stratum corneum hydration as evaluated by capacitance measurement after application of 0.5% SLS; compared with blank*

9.2   The effect of sample No 1 on the skin hydration

The sample No 1 whose composition was described in chapter 8.1 in table 5 belongs among the cheapest cleansing products on the Czech market. The producer declares that the product serves for frequent hand cleansing. The liquid soap contains no humectants, emollients, nor occlusive agents that could help to retain the skin moisturization.

The product was applied on the left forearm in two concentrations. The 2.5% aqueous solution was applied on the third site and the fourth site was treated with the 5.0% aqueous solution of the liquid soap No 1.

Both concentrations were compared in each time point with blank and reference control, respectively, arbitrarily set at 100%.

9.2.1   Comparison with blank

Table 13 summarizes obtained values of % hydration in comparison with the control. In this case blank served as the negative control and represents 100%. As can be seen in figure 12, the hydration of the test sites treated with the sample No 1 was decreased in comparison with untreated skin. During the first four hours after the application, the degree of SC hydration stayed under the level of untreated skin. The sample

at the concentration 5.0% showed moderately stronger dehydrating effect than the sample with lower concentration. Since time point t5 the hydration seemed to have recovered as the values of capacitance occurred slightly above the control line.

Table 13: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 1; compared with blank

Time points	Time after the application (h)	2.5% Sample No 1 % of control			5.0% Sample No 1 % of control		
t1	0	92.0	±	6.5	90.4	±	4.6
t2	1	96.4	±	10.9	96.5	±	6.4
t3	2	99.2	±	4.0	93.5	±	5.0
t4	3	96.8	±	6.1	92.7	±	5.1
t5	20	105.9	±	6.7	101.6	±	7.8
t6	22	109.3	±	7.6	106.9	±	8.9
t7	24	101.8	±	6.0	101.6	±	5.7
t8	26	101.6	±	5.6	101.1	±	5.8

Fig. 12: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 1; compared with blank /***

/*** Error bars are not included for clarity (for SD see the appropriate table); used for all other graphs within the chapter of results and discussion.

9.2.2 Comparison with SLS

Table 14: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 1; compared with SLS treated test site

| Time points	Time after the application (h)	2.5% Sample No 1 % of control			5.0% Sample No 1 % of control		
t1	0	99.2	±	4.9	97.6	±	6.8
t2	1	100.7	±	3.1	101.4	±	6.8
t3	2	105.8	±	4.0	99.6	±	2.3
t4	3	102.3	±	4.8	98.0	±	4.0
t5	20	105.3	±	4.4	101.1	±	6.9
t6	22	103.0	±	5.9	100.7	±	6.4
t7	24	102.4	±	4.8	102.3	±	6.7
t8	26	101.9	±	7.2	101.4	±	7.6
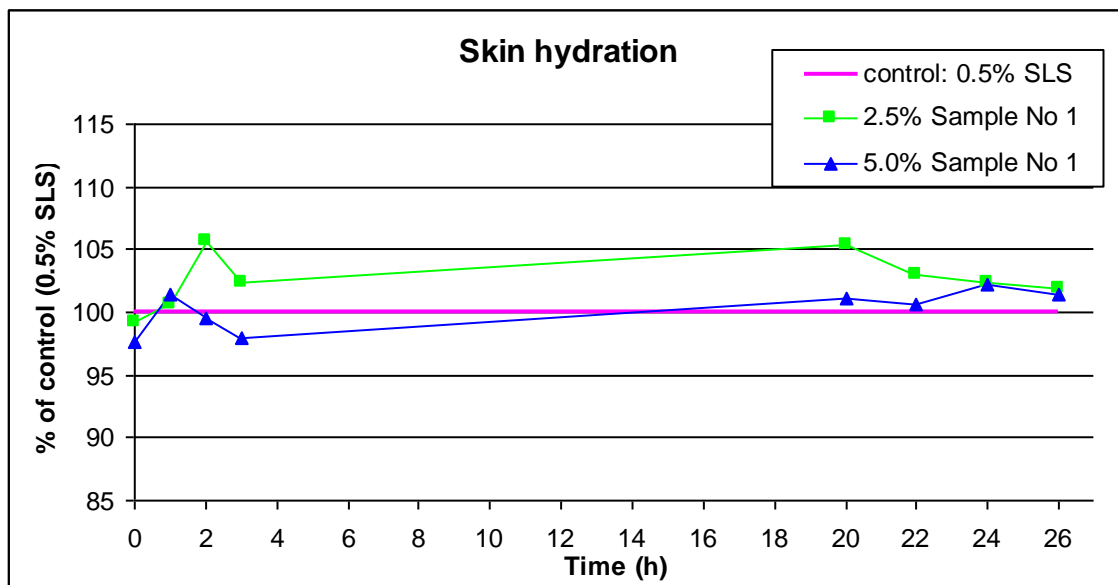
Fig. 13: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 1; compared with SLS treated test site

According to the results, both concentrations of the liquid soap had higher dehydrating effect than 0.5% aqueous SLS immediately after the application at time point t1. In the first 4 time points the values of hydration after treatment with 5.0% solution of sample No1 were lower than after SLS treatment. In the case of the 2.5% solution

the values increased from 99.2% up to 105.8% within the first three hours (see table 14 and fig. 13).

As became evident, in the second day of measuring the differences between the two concentrations were still high. Greater dehydration was caused by the more concentrated solution; nevertheless the SC water content was higher than after SLS treatment in both cases. In the last two intervals of the measuring, the values of hydration were levelled in both test areas and the hydration was more than 1% higher than in the test area treated with SLS.

9.3   The effect of sample No 2 on the skin hydration

The sample No 2 is declared to be a moisturizing shower gel for men. Its description was given in table 6. This formulation contains also ingredients helping to retain natural skin moisture. Humectants are represented by glycerin and lactic acid. Glyceryl oleate belongs among emollients and sodium PEG-7 olive oil carboxylate is a hydrotropic substance. The sample No 2 in concentrations of 2.5% and 5.0% was applied on the test sites 5 and 6, respectively.

9.3.1   Comparison with blank

Table 15: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 2; compared with blank

| Time points	Time after the application (h)	2.5% Sample No 2 % of control			5.0% Sample No 2 % of control		
t1	0	93.6	±	10.2	90.8	±	8.2
t2	1	97.5	±	12.2	94.5	±	9.7
t3	2	97.4	±	7.8	98.7	±	12.6
t4	3	99.8	±	11.3	96.9	±	11.7
t5	20	103.6	±	6.2	103.3	±	9.6
t6	22	108.6	±	6.9	105.0	±	6.4
t7	24	97.4	±	3.9	100.4	±	6.4
t8	26	96.3	±	7.0	100.5	±	6.8
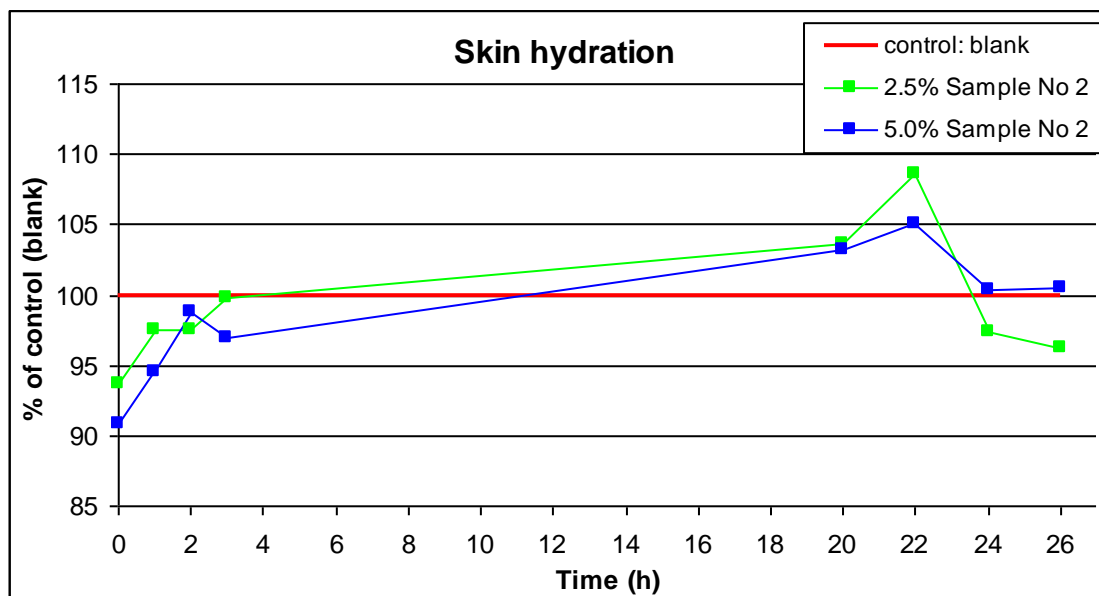
Fig. 14: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 2; compared with blank

The effect of the sample on the skin hydration is given in table 15 and figure 14. The results show that after application the hydration was considerably decreased. During the following hours it tended to increase.

The highest values of hydration were obtained 22 hours after the application. Since this point, the hydration tended to decline again.

9.3.2 Comparison with SLS

In this case the level of hydration after the application was very similar to the hydration of skin treated with SLS (see table 16 and figure 15). The only difference occurred at the beginning, when lower dehydration could be seen after the treatment with 2.5% solution of the sample No 2.

These results were very interesting as a moisturizing effect was claimed on the cosmetic product and four moisturizing agents were added into the formulation. Despite of the unknown amount of individual ingredients in the product, it might be assumed that the effect of detergency was stronger than the effect of the moisturizing agents. It is highly likely that the concentrations of the humectants and the emollients in the formulation were too low.

Table 16: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 2; compared with SLS treated test site

| Time points	Time after the application (h)	2.5% Sample No 2 % of control			5.0% Sample No 2 % of control		
t1	0	101.0	±	11.6	98.1	±	10.5
t2	1	103.4	±	11.3	100.3	±	10.4
t3	2	100.0	±	7.4	100.9	±	9.2
t4	3	105.5	±	10.9	102.2	±	10.0
t5	20	102.7	±	5.3	102.3	±	9.2
t6	22	102.4	±	6.5	99.1	±	7.4
t7	24	96.7	±	8.5	99.7	±	10.3
t8	26	98.2	±	7.3	102.4	±	7.1
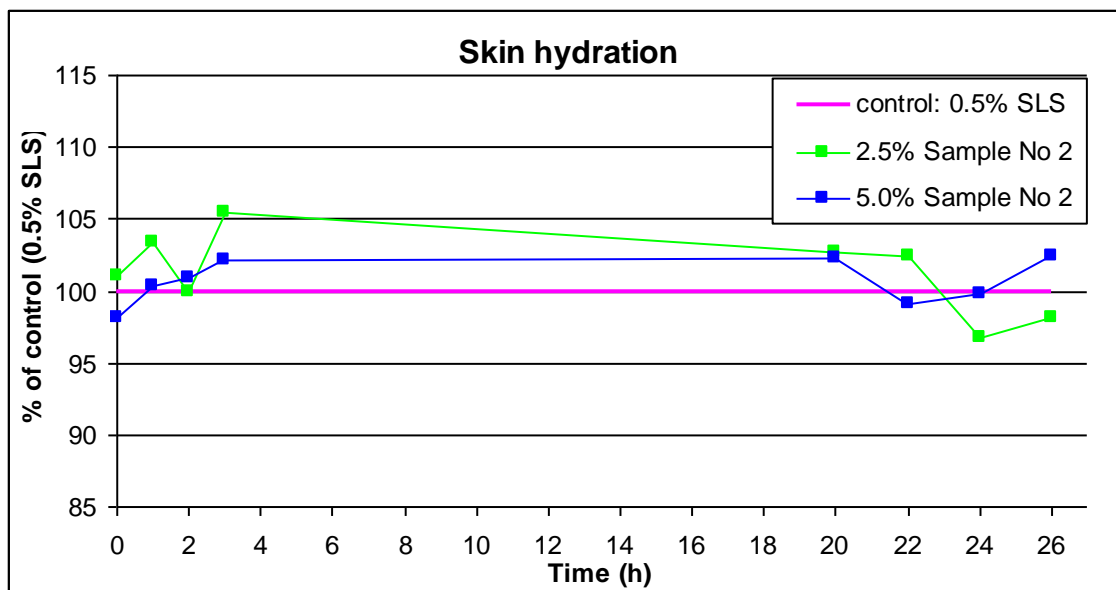
Fig. 15: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 2; compared with SLS treated test site

9.4 The effect of sample No 3 on the skin hydration

This commercial product is called Olive liquid soap. It is a product of lower price and no moisturizing agents are present in the formulation. Test site 7 was treated with 2.5% solution and test site 8 with 5.0% solution of sample No 3. Both the sites were on the right forearm.

9.4.1 Comparison with blank

As shown in figure 16 and in table 17, this product was mild to the skin regarding the hydration. Except few time points, all values were mostly higher than 100%.

Although the basic surfactant composition was similar or identical to other products and no other moisturizing agents were added, the dehydrating effect of surfactants was less aggressive to the skin in comparison with previous samples.

Table 17: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 3; compared with blank

| Time points	Time after the application (h)	2.5% Sample No 3 % of control			5.0% Sample No 3 % of control		
t1	0	99.5	±	12.5	95.1	±	12.4
t2	1	104.3	±	16.7	101.4	±	12.6
t3	2	102.5	±	9.2	101.4	±	12.8
t4	3	100.8	±	14.4	99.7	±	10.7
t5	20	101.9	±	20.3	100.6	±	10.2
t6	22	104.1	±	12.1	106.4	±	7.8
t7	24	96.8	±	9.5	97.7	±	7.1
t8	26	100.2	±	9.8	102.1	±	5.3
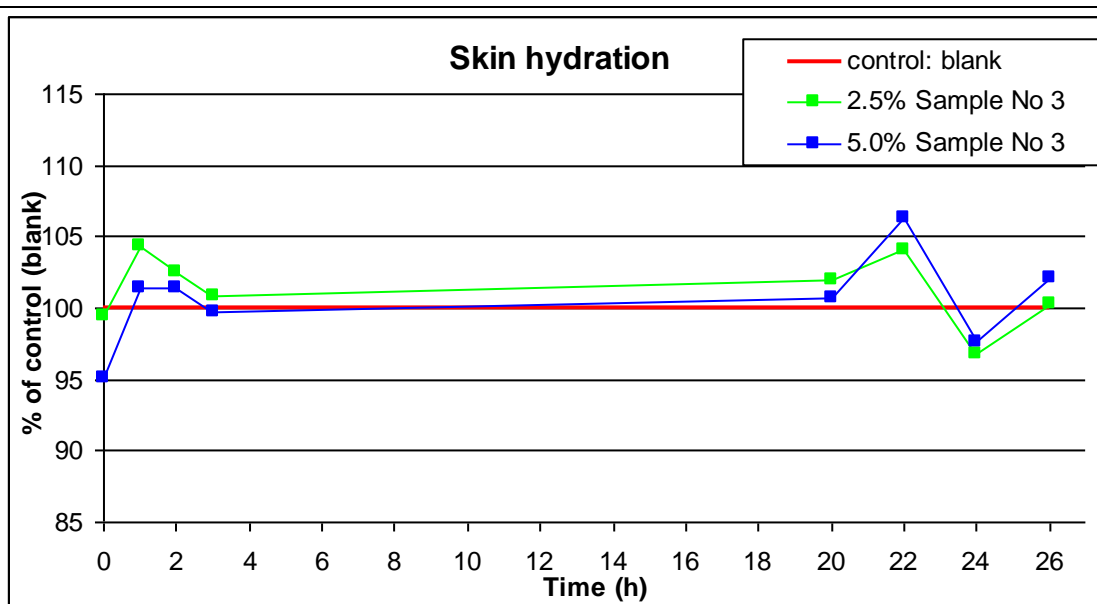
Fig. 16: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 3; compared with blank

9.4.2 Comparison with SLS

Table 18: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 3; compared with SLS treated test site

| Time points	Time after the application (h)	2.5% Sample No 3 % of control			5.0% Sample No 3 % of control		
t1	0	107.0	±	10.6	102.5	±	12.2
t2	1	108.8	±	10.0	106.0	±	6.9
t3	2	109.4	±	10.6	108.1	±	13.2
t4	3	106.2	±	10.7	105.2	±	7.9
t5	20	101.5	±	20.5	100.2	±	10.8
t6	22	98.3	±	12.8	100.3	±	7.1
t7	24	97.7	±	12.0	98.4	±	8.2
t8	26	100.6	±	11.7	102.4	±	7.5
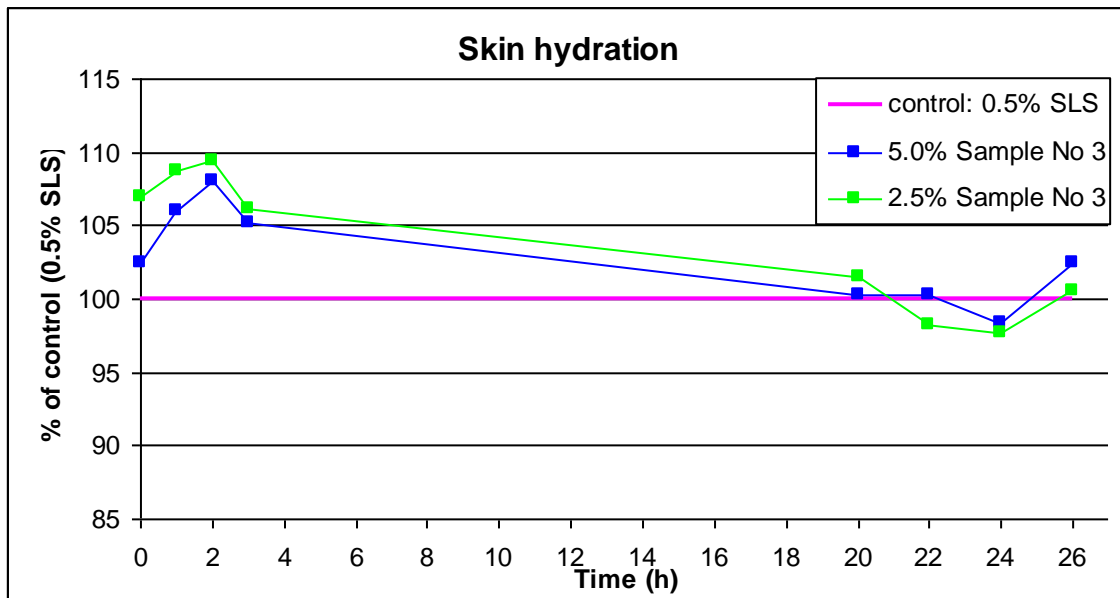
Fig. 17: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 3; compared with SLS treated test site

Table 18 and figure 17 demonstrate that the hydration at t1 was up to 7% higher in comparison with reference control after application of sample No 3 at concentration of 2.5%. In the case of 5% solution, the hydration was 102.5%. During the first 3 hours of measuring the hydration tended to increase. After this point the hydration started to decrease and the second day measurement results almost corresponded with the values of SLS treated skin.

9.5 The effect of sample No 4 on the skin hydration

Kids shower and shampoo was used as sample No 4. The complete composition of the formulation was written in table 8. The humectant properties of the product were supported by propylene glycol and lactic acid in the formulation.

The product was topically applied at two concentrations as the previous samples on test sites 9 and 10.

9.5.1 Comparison with blank

Skin hydration values measured after single application of sample No 4 at concentrations of 2.5% and 5.0% are shown in figure 18 and mean values with standard deviations are given in table 19.

No considerable differences in hydrating capacity of the less and more concentrated samples after application were found. A small decrease of the hydration values after application was almost identical for both tested solutions. Following the time point t2, the hydration state of the forearm test sites 9 and 10 was improved and in comparison with untreated control skin the hydration slightly increased. The best results were obtained between time intervals t5 and t6. Within the following hours of measuring the hydration tended to decrease and return to values similar to those of the untreated skin.

Table 19: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 4; compared with blank

| Time points	Time after the application (h)	2.5% Sample No 4 % of control			5.0% Sample No 4 % of control		
t1	0	96.7	±	8.0	94.7	±	5.2
t2	1	102.2	±	9.8	100.4	±	8.5
t3	2	101.8	±	6.1	100.8	±	5.8
t4	3	100.8	±	8.5	100.9	±	4.2
t5	20	104.3	±	9.1	105.5	±	8.3
t6	22	102.1	±	8.6	108.2	±	9.9
t7	24	97.3	±	8.7	100.1	±	8.1
t8	26	103.2	±	10.2	103.1	±	9.4
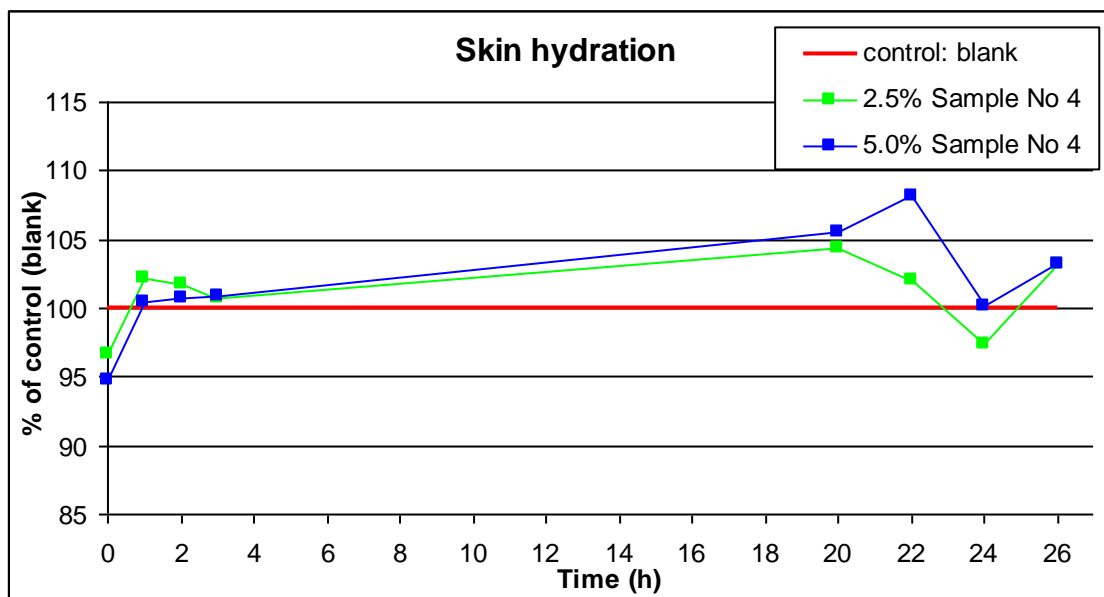
Fig. 18: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 4; compared with blank

9.5.2 Comparison with SLS

Table 20: Changes in the degree of the skin hydration after applications of 2.5% and 5.0% sample No 4; compared with SLS treated test site

| Time points	Time after the application (h)	2.5% Sample No 4 % of control			5.0% Sample No 4 % of control		
t1	0	104.1	±	6.9	102.2	±	5.7
t2	1	107.1	±	6.3	105.2	±	4.5
t3	2	108.6	±	8.1	107.4	±	4.2
t4	3	106.4	±	5.8	106.7	±	3.5
t5	20	103.9	±	9.9	105.1	±	9.6
t6	22	96.2	±	7.4	101.9	±	7.5
t7	24	98.1	±	10.4	100.9	±	10.2
t8	26	103.6	±	12.4	103.6	±	11.7
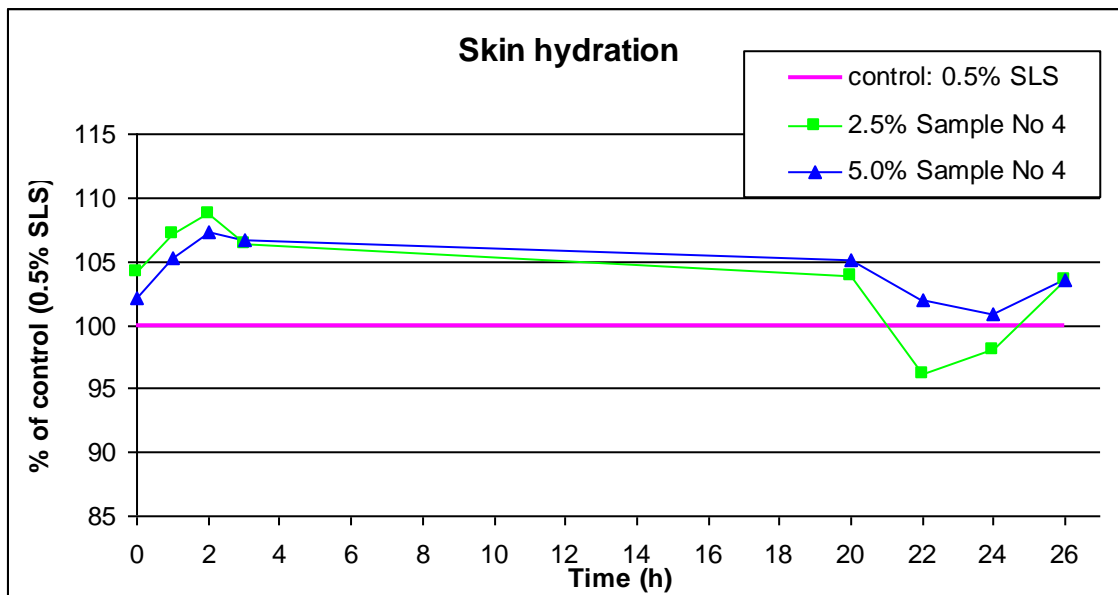
Fig. 19: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 4; compared with SLS treated test site

The increase in the SC hydration after four-hour application of Kids shower and shampoo was very probably caused by the residues of humectants from the product in the *Stratum corneum*. The high values stayed at the same level for approximately 20 hours. Then the capacitance rapidly collapsed and in the case of 2.5% solution the values were even lower than in the case of skin treated by SLS (see table 20 and figure 19). But as can be seen, the more concentrated solution of the sample consistently improved skin hydration. And in comparison with the sample No 2, which was also enhanced with moisturizing agents, it is evident that the moisturizing agents were added into the formulation in an ideal amount so that their effect could be performed.

9.6   The effect of sample No 5 on the skin hydration

The last two test sites on the right forearm were treated with shower gel declaring addition of ¼ of moisturizing cream. The ingredients of the formulation (see table 8) were supplemented with emollients and humectants: glycerin (denaturant, humectant, solvent, perfuming agent), cucumis savitus fruit extract (emollient, skin conditioning) and glycol distearate (emollient, emulsifying, opacifying, skin conditioning, viscosity controlling), which were supposed to increase or retain the hydration capacity of the *Stratum corneum*.

Test site 11 was treated with the solution of 2.5% concentration and test site 12 was used for 5.0% dilution of sample No 5.

9.6.1   Comparison with blank

Little differences could be detected between the values after the application of 2.5% and 5.0% sample No 5. The treatment of the skin with the less concentrated solution of the sample decreased the hydration under the level of untreated skin hydration at t1. Hydration of the test site 12 had higher values of capacitance immediately after the application.

Afterwards, values for both concentrations increased and 22 hours after application reached the highest values. From this time point the capacitance in test sites 11 and 12 started to slump, however the values stayed above the level of negative control.

This product gave the best results. In comparison with the previous samples, the highest degree of skin hydration was obtained and what more, except the time interval t1, all lines were above the level of natural skin hydration.

Table 21: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 5; compared with blank

Time points	Time after the application (h)	2.5% Sample No 5 % of control			5.0% Sample No 5 % of control		
t1	0	97.9	±	4.8	102.0	±	6.4
t2	1	103.3	±	8.4	106.3	±	10.2
t3	2	102.1	±	7.5	102.8	±	4.8
t4	3	101.3	±	5.2	104.2	±	7.4
t5	20	103.7	±	5.7	108.1	±	11.3
t6	22	105.8	±	8.9	110.1	±	4.5
t7	24	100.3	±	4.1	101.4	±	2.8
t8	26	103.6	±	10.9	103.7	±	9.9
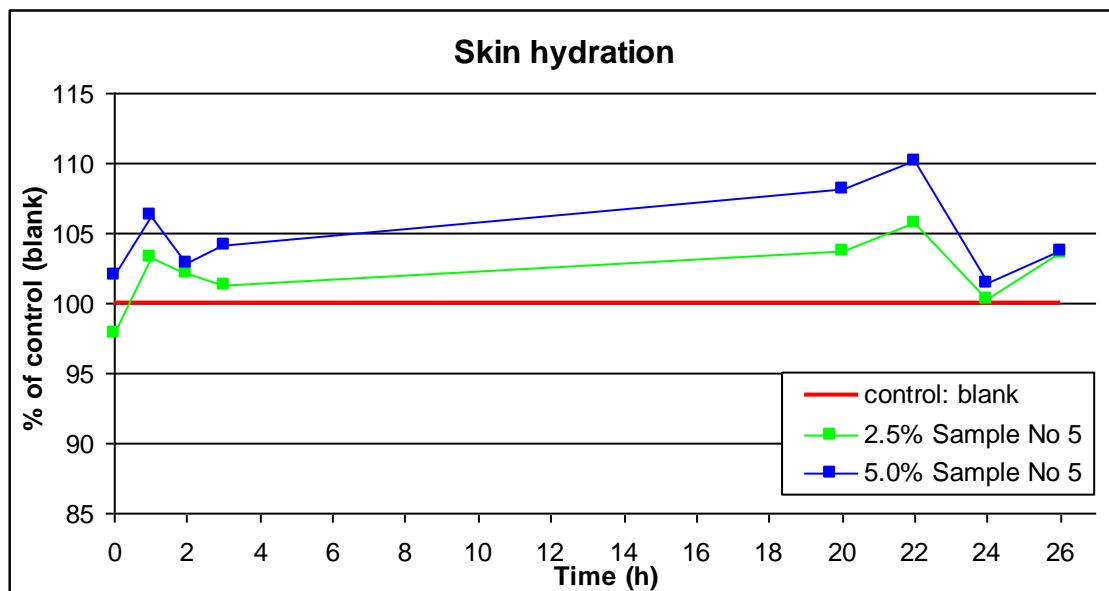
Fig. 20: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 5; compared with blank

9.6.2 Comparison with SLS

Table 22 and figure 21 summarize the results of effect of sample No 5 on skin hydration compared with the hydration after SLS application. The application of the fifth sample increased the SC water content as evidenced by a 105.8% rise in capacitance after 2.5% solution application and by a 110.1% rise after 5.0% solution application in the first time point t1 comparing with SLS treatment. When following the SC hydration over time, a moderate increase of the capacitance was observed up to 22 hours after application, then the hydration tended to decrease.

During the second day testing, the values were approaching the values of skin capacitance after SLS application. As can be seen, the moisturizing effect was stronger in the test site treated with 5.0% dilution of sample No 5. It is possible that a higher amount of moisturizing agents of the formulations remained in the SC.

Table 22: Changes in the degree of the skin hydration after application of 2.5% and 5.0% sample No 5; compared with SLS treated test site

Time points	Time after the application (h)	2.5% Sample No 5 % of control			5.0% Sample No 5 % of control		
t1	0	105.8	±	8.0	110.1	±	8.3
t2	1	108.4	±	7.0	111.5	±	8.1
t3	2	108.8	±	6.1	109.6	±	3.7
t4	3	107.1	±	5.0	110.1	±	5.6
t5	20	103.3	±	6.3	107.5	±	10.8
t6	22	99.6	±	5.5	103.9	±	5.5
t7	24	101.0	±	5.9	102.1	±	4.5
t8	26	104.0	±	13.1	104.1	±	12.0
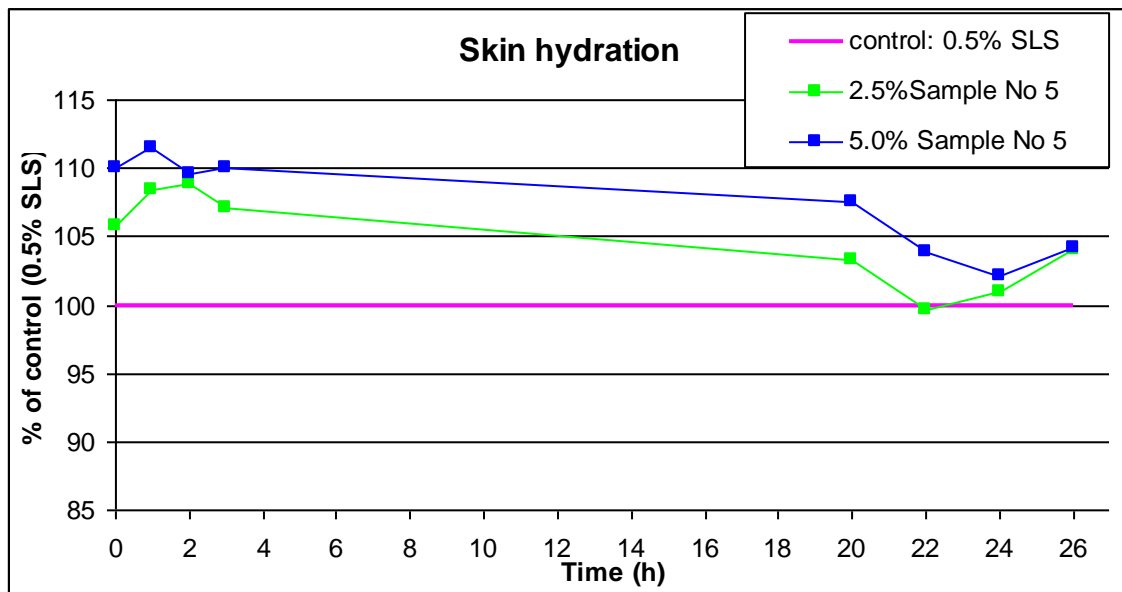
Fig. 21: Stratum corneum hydration as evaluated by capacitance measurement after application of sample No 5; compared with SLS treated test site

9.7 Overall assessment and comparison of the products with regard to their effect on skin hydration

In the present study, five different surfactant-based cosmetic products available on the Czech market were tested for their hydrating capacities. Products varied in composition, presence of moisturizing agents and finally in price (see table 23).

Table 23: Basic samples differences

Sample	Average price per 1 l (CZK)	Moisturizing agents
No 1	30	-
No 2	240	glycerin, sodium PEG-7 olive oil carboxylate, glyceryl oleate, lactic acid
No 3	38	-
No 4	210	propylene glycol, lactic acid
No 5	280	glycerin, cucumis sativus fruit extract, glycol distearate
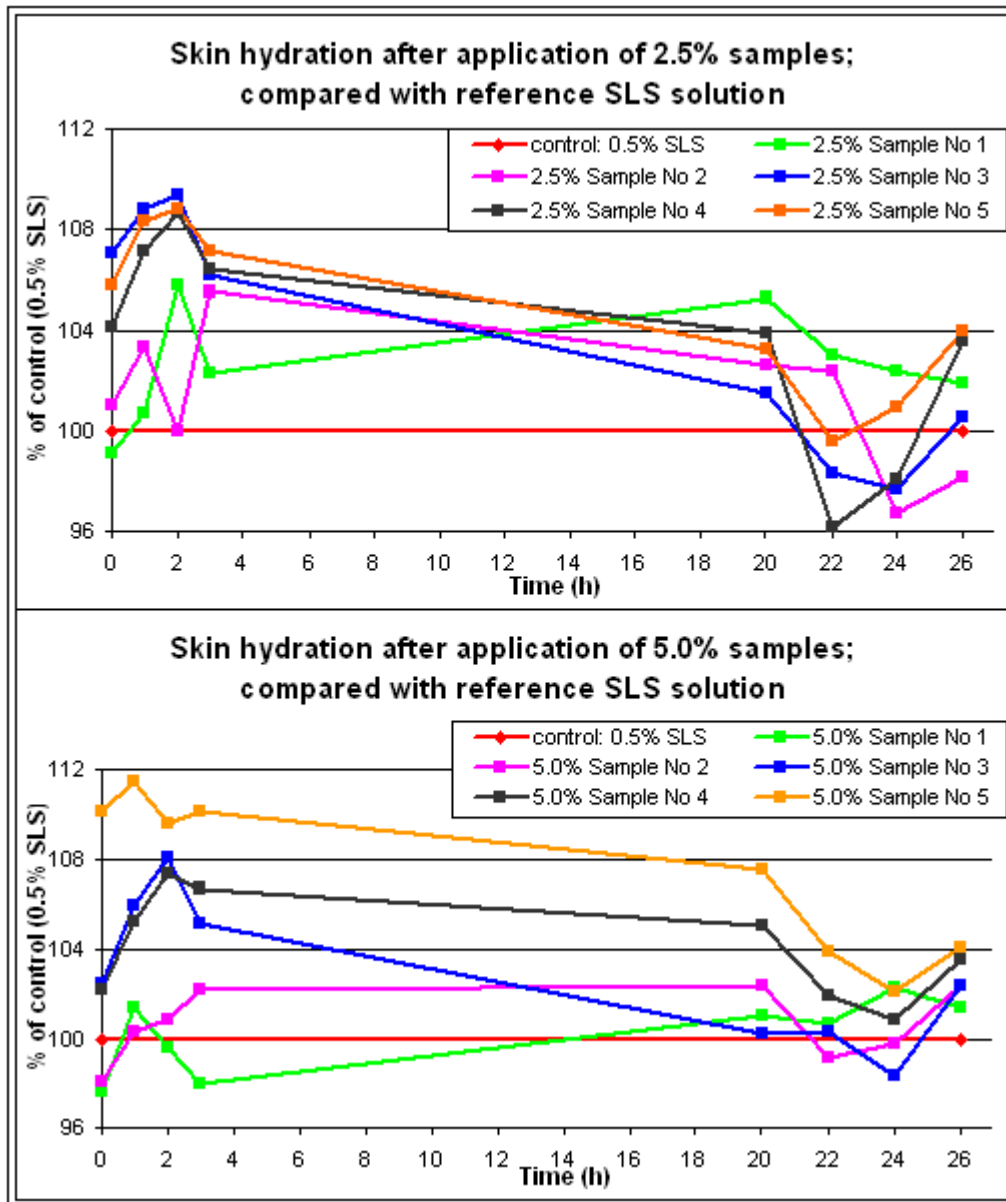
Fig. 22: The effect of the products on the skin hydration; compared with SLS treated test site

Comparison with SLS. In figure 22 there are two graphs depicting the skin hydration as a function of time. Hydration was evaluated by capacitance measurement after 4-hour application of various products at concentrations of 2.5% and 5.0%. The values recorded at the experimental test sites were expressed as percentage of the values recorded at the control site treated by 0.5% SLS, arbitrarily set as 100%.

The comparison of 2.5% dilutions of the samples is in the upper graph. As can be seen, except sample No 1, whose hydration decreased after the application under the level of

100%, all the other samples had lower dehydrating effects than SLS and reached values above the level of SLS. During the first four hours, the hydrating effect was increased in case of all samples. Afterwards, the hydration tended to decrease and in the last time intervals all values fluctuated around the 100% line.

When using 5% dilutions of the samples, marked differences among the products could be seen. Clearly the highest value of capacitance immediately after application was recorded in case of sample No 5, followed by samples No 3 and 4. More pronounced dehydrating effect could be seen after the application of samples No 1 and 2; the plot of their lines was during next 20 hours more stable. The skin hydration after the treatment with products 3, 4 and 5 was decreased after 20 hours. It is evident, that after one day the skin hydration values after the application of the products tended to level off and were reaching values reflecting the natural capacity of human skin for regeneration.

Samples No 4 and No 5 had the best effect on the skin hydration increase. Both of them belonged among expensive products and their formulations were enhanced by moisturizing agents. Paradoxically, very low values of skin hydration were obtained after application of sample No 2, which was also quite expensive, contained humectants and emollients and was designated as "Moisturizing shower gel for men". However, in case of this sample at the beginning and at the end of the measurement the capacitance was lower than after 0.5% SLS treatment.

It is likely that the samples at the concentration 2.5% contained such a low amount of the moisturizing ingredients in the aqueous solution that they were not able to perform their functions properly.

Comparison with blank. Greater differences among results occurred in the case when 5% dilutions of products were used (see figure 23). Immediately after application only sample No 5 reached higher values of skin hydration than the untreated skin. Hydration tended to increase in all test sites. The highest values of the SC hydration were reached 22 hours after application. From this time point hydration declined and returned to the state before treatment. The best results were obtained after the application of 5% dilutions of samples No 5 and 4. In contrast, samples 4 and 5 reached considerably lower values of the SC hydration when in 2.5% concentration.

Samples No 1 and 2, which had worse effect on hydration at the concentration of 5.0%, dehydrated skin in a less extent when used in 2.5% concentration, nevertheless there are minimal differences among the SC hydration in the upper graph of the figure.

Finally, it should be mentioned that neither the composition of the products, price, nor claimed effects guarantee the real effect of the product on skin. Usually the quantitative composition of the formulations is unknown as was the case of this work. Thus e.g. the amount of the moisturizers may be so low that they might be rendered ineffective. On the other hand, the content of surfactants might be too high, then the ratio between them and other ingredients is inadequate and the performance of the active ingredients is almost suppressed.

Another fact that has to be taken into account is the fact that this study was based on measurements in vivo and every organism reacts to the same material by different way. Because of the short time intervals, only 5 volunteers could be used and an air-conditioned laboratory was unavailable for this measurement. It is possible that all these facts, especially unstable temperature and relative humidity, were the cause of the deviations and fluctuation of the final results.

This study proved the necessity to perform proper clinical studies supporting the claims on cosmetic products such as "moisturizing, hydrating", because only tests in a group of human volunteers can confirm the efficacy of such products [76].
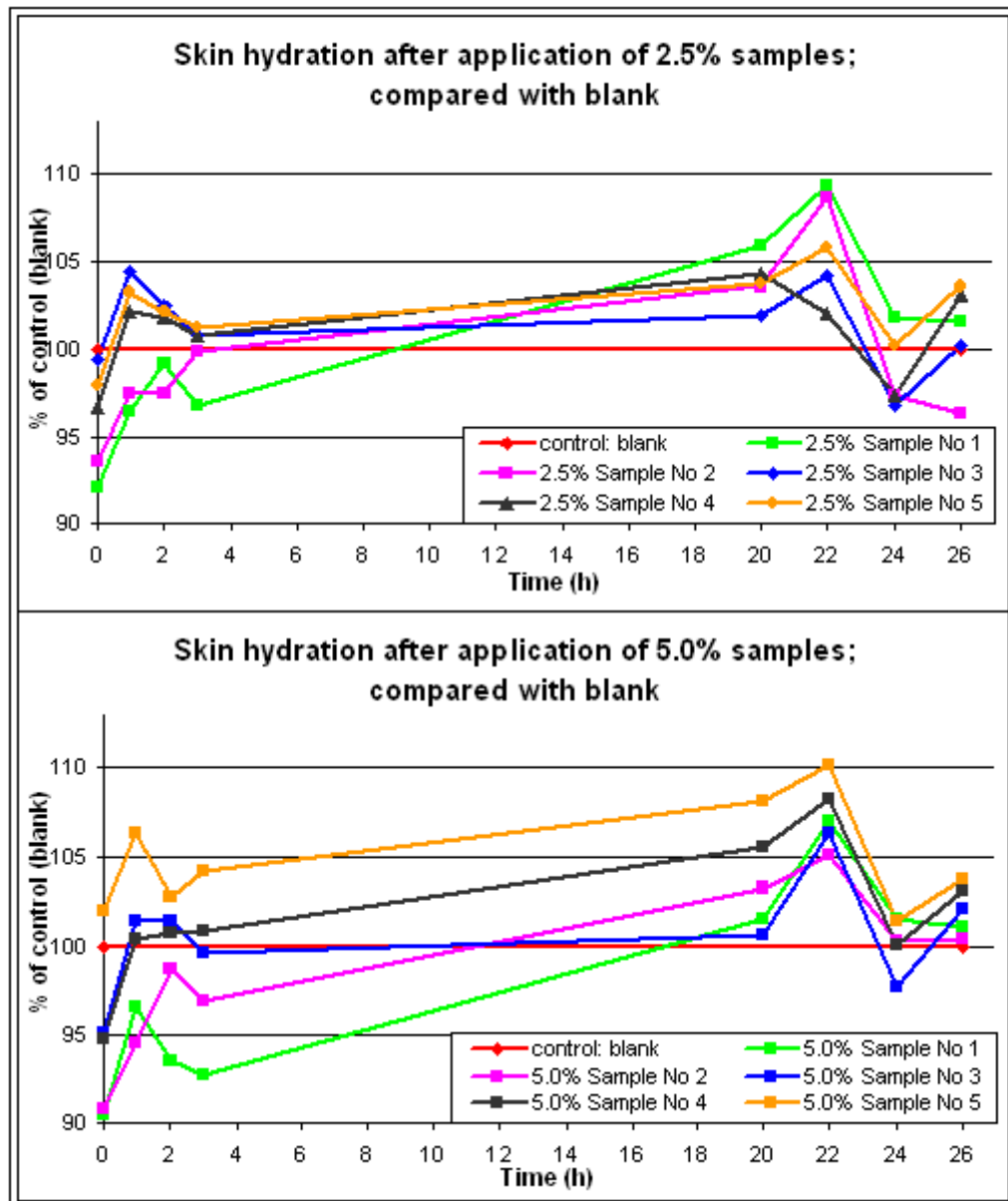
Fig. 23: The effect of the products on the skin hydration; compared with blank

CONCLUSION

- This thesis deals with the effect of surfactant-based products on the *Stratum corneum* hydration. Five commercial shower gels differing in prices and formulations were tested. All of them were applied on both forearms of human volunteers at 2.5% and 5.0% concentrations. For comparison, one test area was untreated and served as negative control. One test site was treated with sodium lauryl sulfate, historically most frequent surfactant in skin cleansing products, which served as reference standard. The application lasted four hours. The skin moisture was consequently assessed by Corneometer CM 820 in 8 time intervals within 26 hours after application.

- In most cases, immediately after the removal of the patches with absorbed samples, the skin hydration was higher than after 0.5% SLS treatment or the values were very similar. Within the second day of measuring there were already minimal differences between the hydration of product-treated sites and the reference test site treated with SLS.

- Compared to untreated skin, an opposite effect was recorded. Immediately after application, the skin hydration was lower than in the case of the blank, then it tended to increase. The highest values were obtained 22 h after application, afterwards it started to decline and the values of test sites skin hydration were similar to the value of untreated skin.

- In case of the comparison both with reference control and negative control, distinguishable results were obtained when using 5% dilutions of the samples. The best result regarding skin hydration was obtained in case of product No 5 declaring an addition of ¼ of moisturizing cream. This product was quite expensive and contained added humectants and emollients. Higher values of the SC hydration were obtained also after treatment with a "kids shower and shampoo", its price was quite similar to the previous product and the formulation was enhanced by moisturizing agents as well.

- The lowest values of skin hydration were obtained after application of the cheapest product. This product did not contain any ingredient retaining or even recovering skin hydration. Unexpectedly, very low values of skin hydration

were recorded after application of a product labelled "moisturizing shower gel for men". This product contained humectants and belonged among the most expensive shower gels.

- Overall, the samples induced the SC hydration to some extent in all cases, particularly in the time interval 20-24 hours. Nevertheless, the skin hydration tended to return to normal values in one day after the removal of the patches with absorbed solutions.

- It should be noted that this study has been focused only on the *Stratum corneum* hydration and it is impossible to determine from the obtained data what effect the tested products had on the skin barrier as a complex. Furthermore, these results cannot be taken as an evidence for performance of other shower gels with similar composition because the exact quantities of the ingredients in the products were unknown.

- Moreover, the effect of the skin cleansing products was induced by factors including climatic conditions and personal physical and psychical state of the volunteers participating in the study.

- This study showed the importance of confirmatory clinical studies as the claims on the products and listed ingredients do not guarantee their real efficacy for consumers.

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LIST OF ABBREVIATIONS

NMF   natural moisturing factors

SC   *Stratum corneum*

DEA   diethanolamine

TEWL   transepidermal water loss

pI   isoelectric point

RH   relative humidity

SLS   sodium lauryl sulfate

SD   standard deviation

C   capacity of condenser

C_0 basic capacity

Cx   measurement capacity as a function of the relative dielectric constant of material present in the electrical field of the condenser

$\bar{x}$ arithmetic mean

x_1, x_2, x_n population

n number of measurements

s   standard deviation

h   hour(s)

CZK   Czech crowns

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