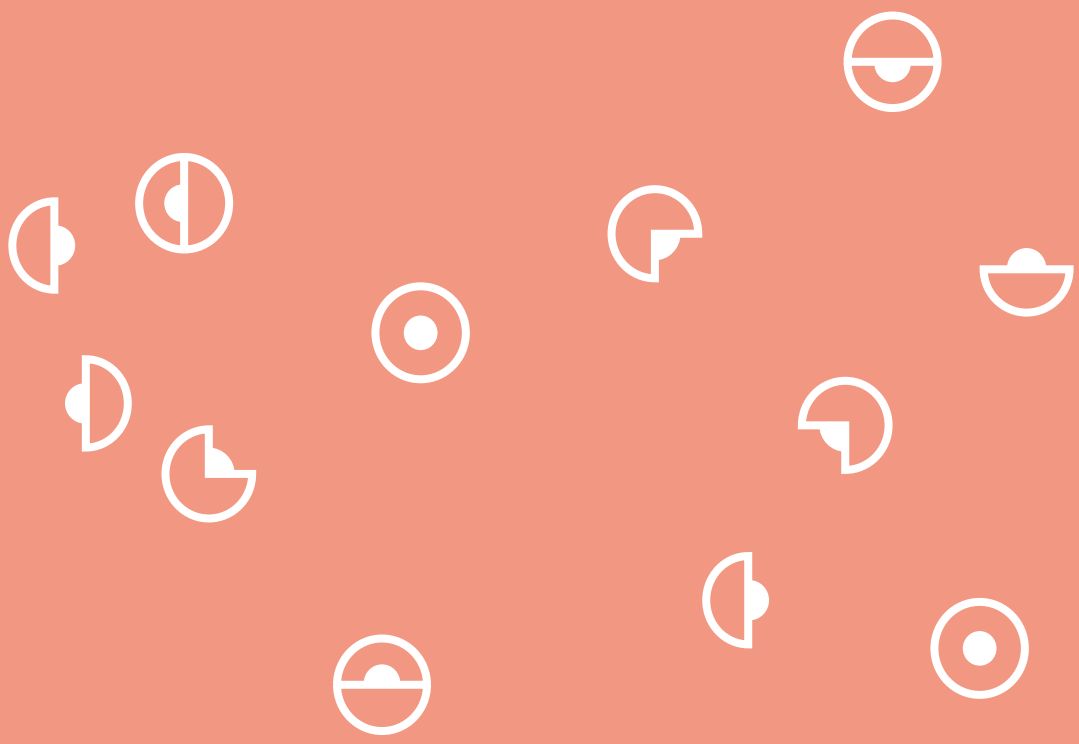
Selected skin delivery systems



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Kraków 2020

Cracow University
of Technology

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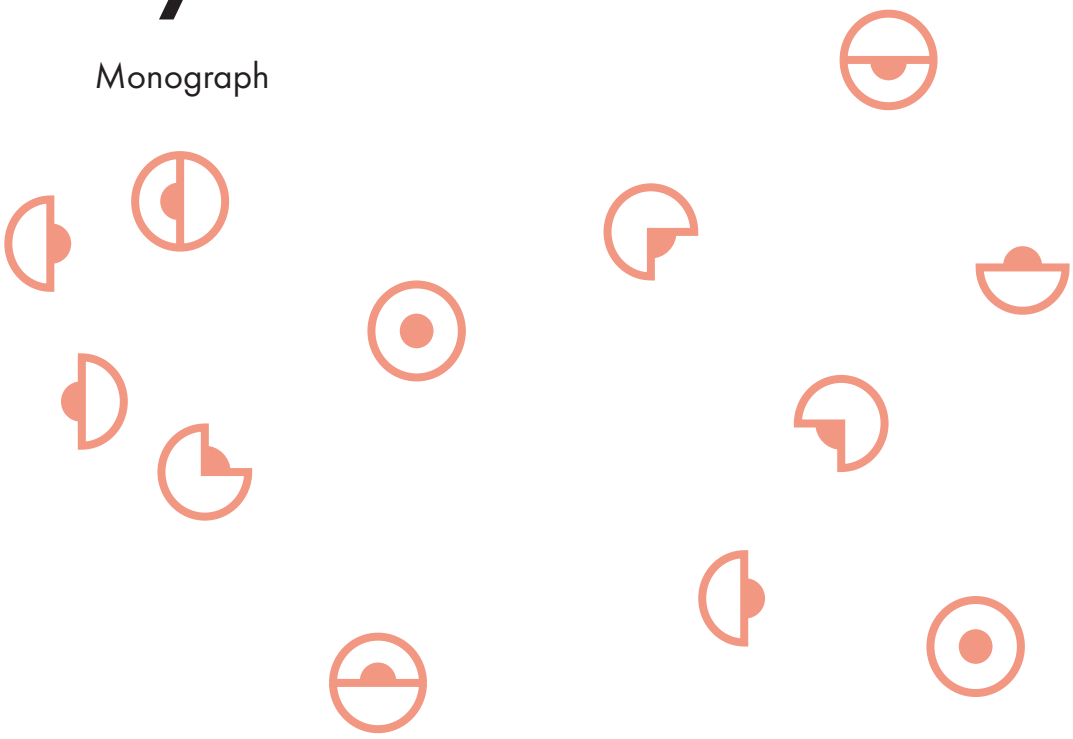
Monograph



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INTRODUCTION

The beauty and cosmetic industry is one of the most dynamically growing branch of an industry. The demands of the modern market regarding cosmetic products are increasingly high. There is a growing need for multifunctional, safe, innovative ingredients, especially of natural origin, and products which are fully safe and with maximum efficacy.

Cosmetic product means any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odors (Regulation (EC) no 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetics Products). Modern cosmetics are a source of biological active substances not only intended to "protect the skin and keep it in good condition" they also influence some physiological processes, working in a similar manner to drugs.

One of the preconditions of producing the optimum efficacy of the cosmetic product is to develop its formulations which facilitate delivery of the active substances into the skin. It is important to achieve optimum skin penetration by the actives. For this reason, an elaboration of novel formulations requires a skill of anticipating the substances capacity to penetrate the skin. The major barrier to the active compounds on their way while penetrating the skin is the *stratum corneum*; its lipophilic nature and a cohesive structure prevent the skin permeation. Therefore, the key factor of cosmetics efficacy is the ability of the actives penetration through the *stratum corneum*.

It is assumed that the diffusion of active substances through the skin has two possible ways: intracellular and intercellular. One of the most important factor influencing the active substance permeation through the skin are physical and chemical properties of the very substance, such as a molecular mass, their lipophilicity and polarity. As the affinity of the active substance for the lipid layer of the epidermis increases, so does its potential to penetrate the *stratum corneum*. Whereas, most of the hydrophilic compounds feature a poor potential of dermal penetration. Y.B. Choy and M.R. Prausnitz (2011) applied the "Rule of Five" to evaluate the potential for transdermal delivery of the active agents. The requirements of the "Rule of Five" are as follows: molecular weight less than 500, number of H-bond donors less than 5, number of H-bond acceptors less than 10, and values of octanol/water partition coefficient logarithm ($\log P_{o/w}$) between 1–5 or few polar centers. Moreover, it is ideal to have a topical active substance with a melting point of less than 200°C and a reasonable aqueous or oil solubility of more than 1 mg/mL. To sum up, the permeation of the molecules through the *stratum corneum* are maximal for small, non-ionic molecules, with a minimum hydrogen bonding groups.

Another important factor to obtain the effective cosmetic product is well designed base formulation: a vehicle, a carriers or a skin delivery system of the active substances.

The physicochemical form and a composition of the cosmetic, as well as, pharmaceutical product may increase the activity of the active substance. For example, an addition of so call "skin permeation enhancers" to the base formulation can modified the physical and chemical properties of the *stratum corneum*. They cause a reversible change in the structure of intercellular cement by improving its permeability.

Most studies reported in the reference literature were done to optimise the composition and the form of the delivery system to improve the skin penetration by the active components.

Among others, emulsions are widely used in cosmetic products as carriers of active substances. They are the cosmetic form which effectively supports the retention of water and lipid balance of the skin, while demonstrating an ability to transport active ingredients (polar and nonpolar alike) into deeper layers of the skin; this effect increases the efficacy of cosmetics. It is known that the type of emulsion (W/O vs. O/W emulsion), droplet size of an internal phase, nature of applied emollients, surfactant/co-surfactant system and the presence of permeation enhancers may affect the percutaneous absorption. Currently on the market, apart from classical forms of cosmetic products such as emulsions, there are available a number of modern carrier systems, such as: multiple emulsions, micro- and nanoemulsions, nanodispersions, gels, microcapsules, liposomes, nanostructured lipid carriers (NLC) or polymeric encapsulates. These kinds of formulations not only allow the delivery of the active substance from cosmetic to the desired site in the skin but also reduce potential skin irritations or protect unstable raw materials against damage caused by environmental factors.

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I. SKIN FUNDAMENTALS

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ABBREVIATIONS

AS	–	active substance
ATR-FTIR	–	attenuated total reflectance-fourier transform infra-red
GAG	–	glycosaminoglycans
IR	–	infrared spectroscopy
LC-MS	–	liquid chromatography – mass spectrometry
$\log P_{o/w}$	–	logarithm of octanol/water partition coefficient
NLC	–	nanostructured lipid carriers
NMF	–	natural moisture factor
O/W	–	oil in water emulsion
PBS	–	phosphate buffer solution
SB	–	*stratum basale*
SC	–	*stratum corneum*
SG	–	*stratum granulosum*
SL	–	*stratum lucidum*
SLB	–	skin lipid barrier
SMILES	–	simplified molecular input line entry system
SP	–	*stratum papillare*
SPME	–	solid-phase microextraction method
SR	–	*stratum reticulare*
SS	–	*stratum spinosum*
TEWL	–	transepidermal water loss
UV	–	ultraviolet radiation
W/O	–	water in oil emulsion

1. STRUCTURE OF THE SKIN

The skin is the largest organ of the human body, acts as a major barrier between the body and an environment and provides it with overall protection [1, 2]. The total surface area of the skin in an adult can be as much as 2 m^2, with the skin thickness ranging from 0.5 to 5 mm, and a total weight of approximately 3 kg (and 20 kg with the subcutaneous tissue) [2–4].

The skin is made of multiple layers of cells and tissues, which are held to underlying structures by connective tissue. There are three main layers of the skin [1–5]:

- the epidermis,
- the dermis,
- the subcutaneous fatty tissue.

The skin also houses certain appendages, such as nails, hair and sebaceous and sweat glands. The skin-associated structures serve a particular function including sensation, protection or heat loss. Figure 1 is a schematic diagram of the human skin structure.

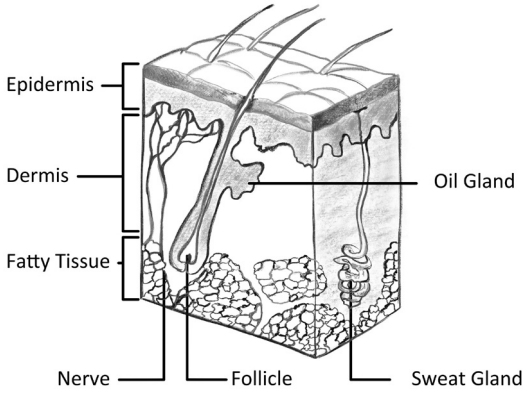

Figure 1. Schematic diagram of skin structure (by author)

The human skin plays many roles in the functioning of the body, and the most important ones follow [2, 4]:

- protection against microorganisms (bacteria, viruses and fungi), provided by the skin lipid barrier (or SLB), which features a slightly acidic pH, and the specific structure of the *stratum corneum*,
- protection against damage, provided by the fibres in the connective tissue and the subcutaneous fatty tissue,
- protection against UV exposure: the skin reflects the light with the *stratum corneum*, whereas the part of the radiation spectrum which penetrates the skin is absorbed by melanin, a chemical synthesised in melanocytes,
- chemical protection: the SLB on the surface and the specific structure of the *stratum corneum* largely reduce the skin permeation by chemicals, especially the hydrophilic ones,

- a systemic thermoregulation component: the skin either retains or facilitates radiation of heat to the outside by operation of perspiratory glands and a specific blood flow; the subcutaneous tissues act as a thermal insulation barrier,
- participation in respiration,
- participation in metabolism: the dermal adnexa (the sebaceous and perspiratory glands) excrete metabolites.

1.1. EPIDERMIS

The epidermis is the outermost layer of the skin. In terms of anatomic, it is a continuously regenerating multilayered *epithelium*. The *stratum corneum* it the outermost layer of the epidermis which is incessantly exfoliated. The exfoliation cycle takes approximately 28 days from the division of the cells, located in the deeper and live strata to the exfoliation of the cells past their death [2–5]. The epidermis consists of five layers: *stratum basale, stratum spinosum, stratum granulosum, stratum lucidum*, and *stratum corneum* (Figure 2). The *stratum corneum* (SC) is made of the necrotic (dead) epidermal cells; the *stratum basale* (SB) and the *stratum spinosum* (SS) are made of live cells which undergo physiological processes. Among the two latter strata is the *stratum granulosum* (SG), in which most vital functions have ceased in the cells. The interface of the *stratum corneum* and the *stratum granulosum* is separated with a thin layer called the *stratum lucidum* (SL), which is only visible in the keratinized epidermis of the palms and the feet [2, 3].

SC pays most important role in the skin barrier function as a most outermost layer of the epidermis. It is possible due to its structure. The *stratum corneum* consists of 10–15 layers of corneocytes, and varies in thickness from approximately 10–15 μm in the dry state to 40 μm when hydrated [2, 6, 7]. It comprises a multilayered "brick and mortar" (Figure 2B) like structure of keratin-rich corneocytes (bricks) in an intercellular matrix (mortar) composed primarily of ceramides, free fatty acids, triglycerides, cholesterol, cholesterol sulphate and sterol/wax esters [8–10]. The hydrocarbon chains are arranged into regions of crystalline, lamellar gel and lamellar liquid crystal phases (Figure 2C), thereby creating various domains within the lipid bilayers (Figure 2D).

The correct hydration of the intercellular cement is also maintained by hydrophilic molecules, capable of binding water and located in the aqueous areas of the intercellular cement; they are called the ingredients of Natural Moisture Factor (NMF). It is a combination of free amino acids, pyroglutamic acid and its sodium salt, lactic acid salt, urea and other substances [11–14]. The interactions between nonpolar components and more specifically, between the alkyl chains of lipids, as well as the formation of hydrogen bonds between the molecules of water and polar fragments of the lipids, build a cohesive structure of the intercellular cement [10, 11].

The compact structural makeup lets the *stratum corneum* form a protective layer which prevents water loss from the living and deeper layers of the skin. The *stratum corneum* allows

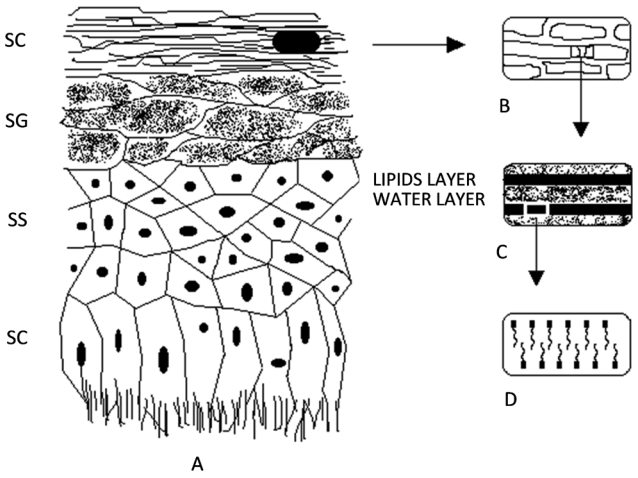

Figure 2. Model of the epidermis structure [5]

only for a low diffusion of water, which is called the Transepidermal Water Loss (TEWL). TEWL is a measure of integrity of the epidermal barrier. It is also a useful tool in evaluating skin barrier function and their health. TEWL is expressed in milligrams per cm^2 per unit of time. The standard TEWL range of a healthy skin is 0.2 to 0.4 mg/cm^2/h, or approximately 300 g of water a day [4]. The vapour of water via this route is designed to reduce the gradient of concentration in the *stratum corneum*, provide a proper course of physiological processes in other epidermal layers, and moisturise the top layers of the skin [11, 12].

The deepest layer of epidermis, directly connected to the dermis is *stratum basale* (SB). SB is composed of columnar cells (Figure 2) attached to the *stratum spinosum* layer via hemidesmosomes. Some of the cells are the stem cells which divide to form the keratinocytes of the *stratum spinosum*. Additionally, among cells in *stratum basale* there are melanocytes, the cells producing melanin, the pigments which protect the dermis from harmful effects of UV radiation. The radiation spectrum, which penetrates the skin, is absorbed by the melanocytes and triggers chemical reactions by which tyrosine, an amino acid is converted in to melanin, what makes the skin look darker, providing what is known as sun tan [4].

1.2. DERMIS

The dermis thickness ranges from 3 to 5 mm [15]. The layer consists of a mixture of fibrous proteins (collagen and elastin) and an interfibrillar gel of glycosaminoglycans, salts, and water. In the structure of the dermis there is distinguished two layers: the *stratum papillare* (SP), which includes the papillae with multiple thin blood vessels, and the *stratum reticulare* (SR), which is adjacent to the subcutaneous tissue and characterised with a more cohesive arrangement of collagen fibres [2, 3].

The dermis includes collagen fibres, elastic fibres and reticulin fibres, cellular components, and the intercellular ground substance. Embedded in the dermis are blood vessels, lymphatic vessels, perspiratory and sebaceous glands, hair follicles, and nerve endings [3, 15]. The dermis is largely a backbone of the skin. It determines the mechanical strength of the skin, as well as its elastic and flexible properties. The collagen fibers are approximately 75% of the dry mass of the dermis, occupying nearly 30% of the dermis volume [3, 15]. The collagen fibres are a heterogeneous protein which features high contents of proline and glycine with hydroxyproline, an amino acid. The elastic fibres account for 2 to 4% of the dry mass of the dermis and just 1% of the dermis volume [16]. By virtue of their mechanical properties, they resemble the performance of elastomeric materials and endow the skin with elasticity. The main constituent of the elastic fibres is elastin, a protein with a high cross linking ratio [3]. The elastin present in the skin is decomposed by proteolytic enzymes, elastases. Fibroblasts produce new strands of trophoblastic which are bonded into new elastic fibres. The fibres regenerate slowly, and the rate is reduced greatly as the ageing progresses [4].

The dermis features the blood vessels which transport nutrients to the skin. The dermis also features multiple receptors and nerve endings interconnected with the brain. The dermis has perspiratory glands and the follicles which grow hair. The follicles are the outlets of sebaceous glands. The hair follicles are attached to the arrector pili muscles [2, 3].

The space between the fibres of collagen and elastin is filled with a gel-structured substance which features glycosaminoglycans (GAG) [17]. These polysaccharides contain molecules of aminoglucose with multiple sulphate and carboxylic radicals. This group of chemicals in the dermis include chondroitin sulphate and dermatan sulphate, which are bound to proteins, and hyaluronic acid [18]. The hygroscopic properties of GAG maintain a constant concentration of water [17, 18]. Collage and elastin retain their three--dimensional structure only when hydrated [3]. The high volume of water in the dermis is essential for the maintenance of proper mechanical properties of the elastic collagen fibres. When the water concentration decreases, the structure of the collagen fibres changes along with their physical properties. The initial stages of the changes are reversible; however, further dehydration may result in permanent denaturation. The quantity of GAG is largely reduced as ageing progresses. A prominent reduction is observed in the chondroitin sulphate content, the quantity of which at the age of 60 years is lower by over 80% [8, 18]. An effect is the reduced mechanical properties of the skin, as evidenced by wrinkles.

1.3. SUBCUTANEOUS TISSUE

Beneath the dermis is the subcutaneous tissue, made from fatty cells and fibrous tissue, which form partitions between the fat lobules. The fatty layers is the thermal insulation which protects the body from cold (by reducing the heat loss from the system). The fat also provides an energy reserve during sudden elevations in energy demand. Beneath the fatty tissue layer are internal organs, vessels, and nerves [3].

2. SKIN PERMEATION ROUTES

The *stratum corneum* is the main barrier to percutaneous adsorption of the most of the actives used in cosmetic and pharmaceutic products [1, 2].

In the 1980s, the first kinetic model of the skin was developed which featured the *stratum corneum* defined as a lipophilic membrane which inhibited diffusion of hydrophilic compounds, especially in the ionic group [19]. A few years later, the model which took into consideration two skin permeation routes: lipophilic (or through the intercellular cement) and hydrophilic (or through micropores) was published. Currently, it is accepted that the transepidermal delivery of active substances can be achieved via the intercellular cement lipids, in between corneocytes (which is a intercellular route), and through the corneocytes (which is the transcellular route) [1, 15, 20–23] and the hydrophilic micropore route is postulated as the pathway for transdermal diffusion of ionic compounds [22].

Apart from these two routes: through the *stratum corneum* (the transepidermal route) and transfollicular, the active compounds from cosmetics or pharmaceutics may penetrate the skin through the its appendages (the sweat glands, the sebaceous glands, and the hair follicles). Nevertheless, if it consider that the skin appendages represent only 0.1% of the total surface of the human skin the contribution of this route for permeation flux of active substances is small [1, 20].

Figure 3 presents the schematic diagram of the delivery routes of the active substances through the epidermis. As was already mentioned above, the predominant diffusion route for active substances through the skin is the intercellular voids (the intercellular route). It is not possible to circumvent the barrier formed by the lipid matrix and deliver an active substance via the transcellular route only. An active substance must always pass the intercellular lipophilic matrix which is the major determinant of the skin permeation process [1, 15, 20–26].

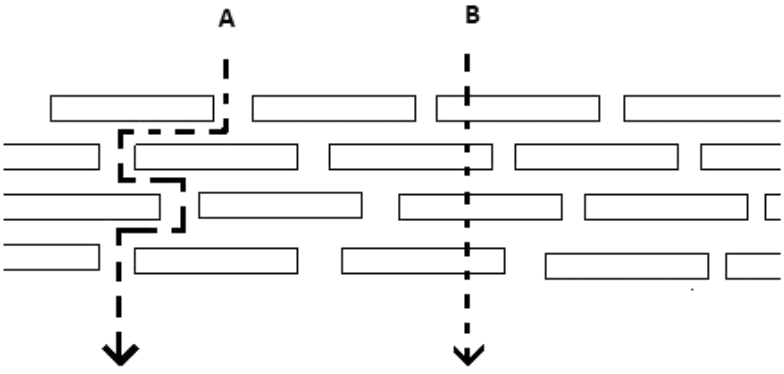

Figure 3. Penetration routes of active substances in the *stratum corneum* (A – intercellular; B – transcellular) (by author)

To conclude, the skin permeation routes include transport across the epidermis and skin appendages, particularly the hair follicles and sweat glands that form an alternative pathway to the intact epidermis. The major route of skin permeation is through the *stratum corneum*. Most active substances penetrate the skin via the hydrophobic intercellular cement, which is why lipophilic compounds can rather easily pass the outer layer. The transport of hydrophilic compounds, especially the ionized ones, is significantly limited. The route through the skin appendages contributes little to the rate of skin absorption of most actives in the steady state; however, this route enables permeation of charged molecules and large polar compounds e.g. peptides [21, 22, 25].

2.1. MECHANISM OF SKIN PERMEATION

There are multiple stages involved in the penetration of an active substance (AS) from its vehicle (or the base formulation) into the skin (see Figure 4). The stages of the processes in their order of performance are [19, 27, 31]:

- dissolution of the AS in the vehicle and down to the outer skin surface,
- diffusion of the AS within the vehicle and down to the skin surface,
- distribution of the AS between the vehicle and the lipids in the *stratum corneum*,
- diffusion within the *stratum corneum*,
- distribution between the lipophilic *stratum corneum* and the deeper hydrophilic and vivid epidermal layers,
- diffusion through the hydrophilic epidermal layers and into the *dermis*,
- penetration through the capillary vessel walls and into the bloodstream (applies to medicinal substances administered on the skin).

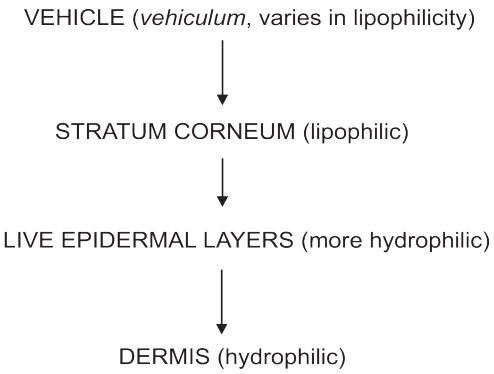

Figure 4. Route of an active substance from the applied cosmetic product to the skin (by author)

The active substance delivery mechanisms may have different modes. Several modes of penetration of chemical compounds through biological membranes are classified. Most often, the skin permeation by compounds is based on passive diffusion [20, 27–29]. The active

substance passes the membrane without any energy expenditure, since an equalization of concentration is at play. The direction of movement of the molecules follows the concentration gradient, which depends on physical forces only: diffusion, osmosis, and the kinetic energy of the molecules.

Transdermal delivery of a substance is a passive kinetic process along a concentration gradient, where Fick's first law is commonly used to describe the steady-state permeation through the skin (eq. 1) [1, 20, 26–28, 30, 31].

$$J = \frac{DK(c_v - c_R)}{h} = k_p(c_V - c_R) \quad (1)$$

with:

- J – steady-state flux ($\mu g \cdot cm^{-2} \cdot s^{-1}$),
- D – diffusion coefficient ($cm^2 \cdot s^{-1}$),
- K – partition coefficient of the permeant between the vesicle and the *stratum corneum*,
- h – diffusional path length, thickness of the *stratum corneum* (cm),
- k_p – permeation coefficient of the permeant in the *stratum corneum*,
- $c_V - c_R$ – concentration gradient of permeant between the vesicle and the *stratum corneum* ($\mu g \cdot cm^{-3}$).

In most circumstances $c_R << c_V$ equation (1) can be simplified to equation (2)

$$J = \frac{DKc_v}{h} = k_p c_V \quad (2)$$

with:

- J – steady-state flux ($\mu g \cdot cm^{-2} \cdot s^{-1}$),
- D – diffusion coefficient ($cm^2 \cdot s^{-1}$),
- K – partition coefficient of the permeant between the vesicle and the *stratum corneum*,
- h – diffusional path length (cm),
- k_p – permeation coefficient of the permeant in the *stratum corneum*,
- c_V – concentration of permeant in vesicle ($\mu g \cdot cm^{-3}$).

From equation (2) it is deduced that the flux of permeant across the skin may be enhanced by concentration of the permeant in the vehicle. Hence, the higher the concentration of the penetrant in a base formulation is, the more effective is the diffusion process. Thus the concentration of the active agent in the vehicles should be as high as possible, i.e. at its solubility limit. However, a limitation to bear in mind is the affinity of overly concentrated active substances to skin irritation. Apart from the actives concentration, other diffusion limiting parameters also include the lipophilicity of the active substance

(i.e. the partition coefficient) [31] and its molecular mass, (the diffusion coefficient of the molecule is inversely proportional to the molecular mass) [1, 20, 32].

A transmembrane transport of molecules against the laws of diffusion is active transport and this delivery requires an energy input. To facilitate it, vehicles are used with an affinity for the carried active substance. The energy required to activate the vehicle can be generated from ATP oxidation or decomposition. If the vehicle-based delivery follows the concentration gradient, it requires no energy input, which is called a facilitated delivery. For small molecules solved in the medium which penetrates the pores of a membrane, the phenomenon of delivery is based on convective delivery. The higher the differential concentration on both sides of the barrier and the pore clear diameter are, the faster is the rate of convective delivery. This type of transport is viable only with molecules no larger than 400 Da. Delivery via ion couples is a special case of passive diffusion by which the diffusing particles are heavily dissociated and coupled with organic ions, which in turn are solvent in the lipids of the membrane. Pinocytosis is another mechanism of transmembrane molecular delivery. Pinocytosis is absorption of fat droplets or solid particles by a membrane. The membrane becomes concave at the point of absorption, forming small vesicles which contain the substance in transit. The contents of the vesicles passes the membrane, leaving the vesicles to fade out slowly [1, 26, 28–31].

3. FACTORS AFFECTING TRANSDERMAL PERMEABILITY

There are several factors which influence the efficacy of active substances permeation through the skin. They can be classified into main four groups:

a) properties of the permeant, (lipophilicity, polarity, and molecular mass) [33–36],
b) properties of vesicles [20, 31, 32, 37–45],
c) biological factors (skin health, skin thickness, age, and hydration of the *stratum corneum*) [3, 4],
d) physical factors, e.g. temperature, massage, electrophoresis, or microneedling, which favour skin permeation [26, 46, 47].

3.1. EFFECT OF THE ACTIVE AGENTS PROPERTIES

Among others factors, the efficacy of skin permeation is dependent on the size (or volume) of each penetrated molecule [1, 19]. As shown in equation (2), the efficacy of skin permeation by a substance is proportional to its diffusion coefficient (D), which itself is inversely proportional to the molar mas of the molecule (eq. 3). Therefore, the diffusivity of the molecules in the *stratum corneum* being maximal for small molecules (of molecular weight less than 500 Daltons) [1, 19, 33, 37, 46, 48].

$$D \sim \frac{1}{M} \tag{3}$$

with:

D – diffusion coefficient ($cm^2 \cdot s^{-1}$),

M – molecular weight.

Moreover, the penetrant solubility and its melting point have an impact on the skin penetration. The ideal topical active substance should be characterized with a melting point of less than 200°C, reasonable aqueous or oil solubility of more than 1 mg/mL and the presence of no or few polar centres [1, 20, 33, 35].

The most important property of the active is its lipophilicity. As already was discussed, the specific structure of the *stratum corneum* determine a high potential of lipophilic substances of penetrating the outer layer, while most hydrophilic compounds feature a poor potential of a dermal penetration [1, 19, 33, 49].

Since lipophilicity significantly determines the active substance penetration through the skin, the permeability is often improved by modifying the chemical structure of the penetrant. A good example of such modification is an increase in the lipophilicity of the substances used as antioxidants: ascorbic acid (by conversion it into ascorbyl palmitate), or gallic acid (by conversion into esters, e.g. octyl gallate). The addition of alkyl and aryl chains to the original compound increases the lipophilicity of the substance; those substituent groups capable of dissociation or which feature electronegative atoms (like nitrogen or oxygen) reduce lipophilicity. A modified substance can permeate the *stratum corneum* more easily, and next it can be converted to its original form (e.g. by hydrolysis of the lipophilic precursor) [49, 50].

The molecule lipophilicity or its affinity to the lipid phase, is express as a decimal logarithm of the partition coefficient ($\log P_{o/w}$) in between two immiscible solvents. In the case of cosmetic raw materials, their lipophilicity is measure using n-octanol as the non-polar solvent, while water is used as the polar one. The relation which defines the n-octanol/water partition coefficient (eq. 4) results from the Nernst's Distribution Law (Partition Law). The n-octanol/water partition coefficient can also be calculated based on the chemical structure of the tested substance [51].

$$P_{o/w} = \frac{c_{octanol}}{c_{water}} \tag{4}$$

with:

$P_{o/w}$ – n-octanol/water partition coefficient,

$c_{octanol}$ – concentrations of a substance distributed in n-octanol, ($g \cdot cm^{-3}$),

c_{water} – concentration of a substance distributed in water, ($g \cdot cm^{-3}$).

For substance which demonstrate a high performance in skin permeation, the values of octanol/water partition coefficient logarithm ($\log P_{o/w}$) should be between 1 and 5 (Figure 5) [33, 49].

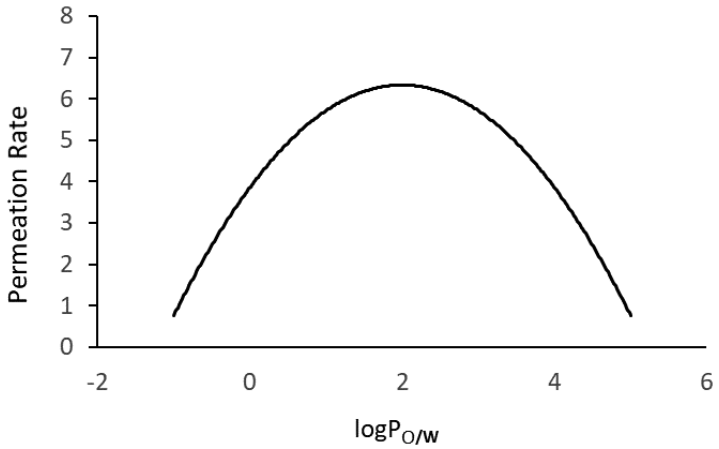

Figure 5. Relationship between skin permeation and the substance lipophilicity [49]

Results of Potts and Guy studies [52] demonstrated that the permeability of a compound from an aqueous solution through the *stratum corneum* could be estimated from its physicochemical properties, mainly two parameters, the octanol/water partition coefficient and the molecular size (molecular volume or molecular weight). Their analysis indicated that the molecule lipophilicity (expressed as the partition coefficient) more influences the skin permeation than size factors.

3.1.1. DETERMINATION OF ACTIVE SUBSTANCES LIPOPHILICITY

Several methods exist to determine the n-octanol/water partition coefficient. $LogP_{o/w}$ can be determined theoretically (by calculation or with computer software) or experimentally measured [55].

The methods of theoretical determination of n-octanol/water partition coefficient are utilized when the chemical structure of the compound is known. Alternatively, $logP_{o/w}$ can be calculated with computer software, e.g. Chemsketch [54], or with reference databases, like Molinspiration property calculation service FAQ [55] and ALOGPS [56].

Moreover, the theoretical methods include those based on software and hardware aided procedures, and databases with experimental test results. The structure of the substance of concern is input to the software, preferably as a SMILES code (Simplified Molecular Input Line Entry System), which differentiates between specific functional groups of molecules and their interrelations. Other methods of data input, include drawing the molecules or specifying the CAS numbers. The software automatically divides the molecule into groups of atoms (e.g. $-CH_2-$, -C=O, -OH), each assigned a specific coefficient value (which depends on the specific group's nature: hydrophilic or lipophilic). The values of correction factors are included (e.g. cyclicity or aromaticity of the molecule). All coefficient values are totalled to derive $logP_{o/w}$ for the whole molecule [57]. Examples of the theoretical methods include the π-Fujita-Hansch method [57], the Rekker method [58] or the Hansch-Leo method [59].

Examples of experimental test methods include: the Shake Flask method [60], the Slow–Stirring method [61], and SPME (solid-phase microextraction method) [62]. The Shake Flask method comprises determination of the distribution of the tested molecule between water and the non-polar solvent, such as n-octanol. It is applied to pure chemicals which $\log P_{o/w}$ is between –2 and 4 (or sometimes up to 5, which depends on the limit of detection of the applied test method). This method does not apply to testing of surfactants. Dissociating substances are investigated with buffer solutions instead of water [60, 64]. The Slow–Stirring method allows determining the n-octanol/water partition coefficient of highly lipophilic substances in the $\log P_{o/w}$ range of 5 to 8.2. Here, a mixture of water, n-octanol and the test substance is stirred slowly in a thermostatically controlled reactor to prevent the formation of microdroplets, a usual phenomenon in the Shake Flask method. The exchange between the phases is accelerated by the stirring process [61]. SPME is suitable for $\log P_{o/w}$ determination in substances, the lipophilicity of which cannot be tested with the foregoing methods. In SMPE, the partition coefficient is determined with oil and water by application of solid phase microextraction. The substance concentration in both phases are determined by chromatography, e.g. LC-MS [62].

3.2. EFFECT OF THE VEHICLE ON TRANSDERMAL DELIVERY

As it was already mentioned, penetration (diffusion) of the active substances through the skin depends on its diffusion coefficient. Therefore, at the constant temperature, apart from the active molecule properties, also the interaction between a vesicles and the active substance and properties of the carriers are important [1, 2, 31, 32]. Well-designed base formulation significantly influences the efficiency of cosmetic or pharmaceutical products. Both, the physicochemical form of the vehicle and its composition may either increase or decrease the activity of the active substance [1, 15, 32, 38]. It is very important that the active compound does not have an overt affinity to the base formulation; otherwise its release into the skin can be completely inhibited [1].

Many studies reported in the reference literature were done to optimise the composition of the vehicle formulations to improve the skin permeation by active components. The most popular form, among others delivery systems, are emulsions, because of their excellent solubilizing properties for both lipophilic and hydrophilic active ingredients.They can help achieve a homogeneous configuration between many immiscible ingredients, including hydrocarbons, fats, waxes, odoriferous substances, water, and hydrophilic substances. Emulsions effectively supports the retention of water and lipid balance of the skin, while demonstrating an ability to transport active ingredients (polar and nonpolar alike) into deeper layers of the skin [15, 21, 32, 36, 38, 63].

It is known that the type of emulsion (W/O vs. O/W emulsion) [15, 32], nature of applied emollients [1, 31, 41], surfactant/co-surfactant system [38, 40, 42] and the presence of permeation enhancers may affect the percutaneous absorption [1, 20, 37,

44, 45]. Additionally, the influence of an emulsion droplet size on the delivery rate of the active ingredients through the skin is also a subject of fundamental and applied interest, but remains a matter of controversy. Most authors believe that the delivery of active ingredients can be influenced by droplet size [65–68] but some studies [69, 70] have shown no significant effect.

Sikora at al [71] studied the skin permeation of Forskolin, a diterpene isolated from *Coleus forskohlii.* As delivery formulations they applied O/W emulsions, differing in droplet size (0.38 μm and 10 μm). Their results showed no correlation between the emulsion droplet size and the penetration of forskolin through the skin. The actives permeation through human skin from both of the emulsions was similar. These results [60–71] support the view that penetration through the skin is a molecular diffusion controlled process. It is confirmed also by results of other studies [72–76] which indicate that viscosity of the base formulation affecting the release of the actives from the carrier system, as an effect its skin penetration.

For particles or large molecules in a viscous fluid (usually a liquid solution), the Stokes–Einstein equation (5) can be applied.

$$D = kT/6\pi\mu r \tag{5}$$

with:

D – diffusion coefficient ($m^2 \cdot s^{-1}$),
T – temperature (K),
k – Boltzmann constant ($J \cdot K^{-1} = kg \cdot m^2 \cdot s^{-2} \cdot K^{-1}$),
μ – fluid viscosity ($kg \cdot m^{-1} \cdot s^{-1}$),
r – radius of the diffusing particle (m).

From the equation (5) it follows that the particles diffusion in the viscous fluid is inversely proportional to its viscosity. Therefore, the rheological properties of delivery systems significantly influence the release profile of the active substance, and that it is a crucial step for the penetration process [72–76]. Generally, an increase in viscosity of the carrier systems decreases the release of the active substance Transdermal delivery enhancer.

The permeation of a substance through the skin structure can be improved by applying absorption promotors (or transdermal delivery promotors) in the form of base formulation additives. The promotors interact with the skin structures to modify their properties and improve permeability. The promotors are usually good solvents for the active substance, which further promotes skin permeation.

The authors of numerous works stressed out that transdermal delivery promotors are very important to cosmetic and pharmaceutical formulations, given the contribution to efficacy. The promotors work by converting the laminar system structure in the lipid matrix of the epidermis, by which the diffusion of active substances can be facilitated or accelerated through the *stratum corneum*. The most commonplace sorption promotors include: alcohols, fatty acids and esters thereof (e.g. alcohols with oleic acid, isopropyl myristate, or isopropyl palmitate); alcohols (propylene glycol, isopropanol, or ethanol), urea, turpentine and the derivatives thereof [35, 37].

Lipophilic promotors operate the hydrophobic chains in lipids. Hydrophilic and amphiphilic promotors operate the hydrophilic groups in the intercellular cement lipids. The effect of this is the relaxation of the stratified structure of the epidermal lipids, which increases its fluidity and the diffusion rate of an active substance into the skin. However, sorption promotors can operate more than just the intercellular cement lipids. The main section of the *stratum corneum* included corneocytes, or cells the main building blocks of which are keratin and small amounts of other proteins with hydrophilic low-molecular compounds, and water [8–11].

The hydrophilic substances which increase the penetration by active ingredients may have an intracellular mechanism of action and operate the proteins of corneocytes mainly by reacting with the polar groups of α-keratin. This most likely results in the formation of a system of microscopic pores, through which the hydrophilic route of deliver is possible [23]. Outside the corneocytes and within the liquid crystal structure of the intercellular cement, non-uniformly distributed aqueous areas are located. The water is retained in the areas by interactions with highly hydrophilic compounds, such as NMF components, and the polar fragments of peptides and lipids of the cement. Here, the hydrophilic promotors, like glycerine, penetrate the aqueous volumes in the intercellular cement and increase their surface area by the hygroscopic effect [28]. An optimum selection of the sorption promotor in a formulation can highly improve the diffusion of an active substance through the skin.

3.3. TRANSDERMAL DELIVERY ENHANCER

The authors of numerous works stressed out that the permeation of a substance through the skin can be improved by applying absorption promotors, (also called transdermal delivery enhancers), into the base formulation composition [1, 20, 37, 44, 77]. These raw materials are very important to cosmetic and pharmaceutical formulations, given the contribution to efficacy.

The promotors are usually good solvents for the active substance. They work by converting the laminar system structure in the lipid matrix of the *epidermis*, by which the diffusion of active substances can be facilitated or accelerated through the *stratum corneum* [19–21].

The classification of transdermal delivery enhancers is frequently based on their chemical structure. The most commonplace sorption promotors include: fatty acids (oleic acid, undecanoic acids); alcohols (glycerol, propylene glycol, polyglycols, isopropanol, or ethanol), and esters thereof (e.g. isopropyl myristate, or isopropyl palmitate); amines, amides (e.g. urea), phospholipids, terpenes, terpenoids and essential oils, sulfoxides (dimethyl sulfoxide, dodecyl methyl sulfoxide) and surfactants (polysorbates, sodium lauryl sulphate, quaternary ammonium salt) [1, 20, 44, 45, 77].

Lipophilic promotors operate the hydrophobic chains in lipids. Hydrophilic and amphiphilic promotors operate the hydrophilic groups in the intercellular cement lipids. The effect of this is the relaxation of the stratified structure of the epidermal lipids, which

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The hydrophilic substances which increase the penetration by active ingredients may have an intracellular mechanism of action and operate the proteins of corneocytes mainly by reacting with the polar groups of α-keratin. This most likely results in the formation of a system of microscopic pores, through which the hydrophilic route of deliver is possible [28]. Outside the corneocytes and within the liquid crystal structure of the intercellular cement, non-uniformly distributed aqueous areas are located. The water is retained in the areas by interactions with highly hydrophilic compounds, such as NMF components, and the polar fragments of peptides and lipids of the cement. Here, the hydrophilic promotors, like glycerine, penetrate the aqueous volumes in the intercellular cement and increase their surface area by the hygroscopic effect [13].

An optimum selection of the sorption promotor in a formulation can highly improve the diffusion of an active substance through the skin. Nowadays, apart from chemical transdermal delivery enhancers and different forms of delivery systems, to improve the skin permeation, some transdermal transport technology (e.g. microneedle array, phonophoresis, iontophoresis, electroporation) have been developed and commercialized [77].

4. METHODS OF PERMEABILITY TESTING

In vitro and *in vivo* testing methods are used most often in medical sciences and cosmetology to investigate the permeability of biologically active compounds through the skin. The permeation coefficients are determined for the tested substances by application of model membranes, tissue cultures which imitate the human skin, or actual preparations from the human skin. Systems are also commonly used apply model membranes, for example a liposomal membrane model, placed in a Franz diffusion cell.

The permeability tests are carried out in a variety of conditions with different types of model membranes, and with certain modifications of the model membrane pre-treatment processes. The human skin is the perfect model which ideally represents the conditions in which the tested cosmetic product is expected to potentially perform. Animal skin or surrogate skin made by application of biotechnological engineering are the viable alternatives to the human skin [78–82]. Different thickness ranges of the membranes are used, with the higher end of the range at 1620 ± 580 μm and the lower end of the range at 390 ± 80 μm. Thinner membranes are better in testing the absorption of lipophilic compounds, where thicker membranes could be impenetrable. However, the membrane thickness is insignificant to the test results [83].

The *in vitro* testing of *stratum corneum* permeability were a topic of high interest already in the 1960s. Form the decades of accumulated experimental data, a staggering database now exists in which permeability of hundreds of chemicals can be referred to in pharmaceutical and cosmetic applications, as well as when investigating the risks and toxicity of chemicals [84–88].

The permeability of chemical compounds is tested in dedicated diffusion chambers. The most popular type are the Franz static diffusion chamber and the Bronaugh flow diffusion chamber. The principle of an operation of the diffusion chamber is testing of a compound's diffusion through the skin or another type of membrane. Both diffusion types related to the chambers, meaning the static and flow (or dynamic) diffusion, are methods officially recognised as viable replacements for *in vivo* testing. The donor and receptor parts are separated with a skin or the membrane. The diffusing substance is collected from the receptor part continuously or in defined time intervals. The receptor part contains a receptor medium. The receptor compartment solution should provide sink conditions. The tested compound can be dosed to a limited extent (< 10 µl/cm^3 or 10 µg/cm^3) liberally (> 10 µl/cm^3 or 10 µg/cm^3). With liberal dosing, the skin receives the maximum dose of the tested substance. This ensures the repeatability of test results [78, 88, 89].

Temperature is controlled in the chamber with a water jacket; alternatively, warm air chambers are often used. Ideally, testing should be performed at 32°C, which corresponds to the *in vivo* test temperature [78].

4.1. FRANZ DIFFUSION CELL

The Franz chamber (diffusion cell) and its modifications are the most popular apparatus in testing of a skin permeation by substances [20, 71, 78, 89–93]. The Franz cell comprises of two thermostat-controlled parts: the donor part and the receptor part, separated by a membrane (see Figure 6).

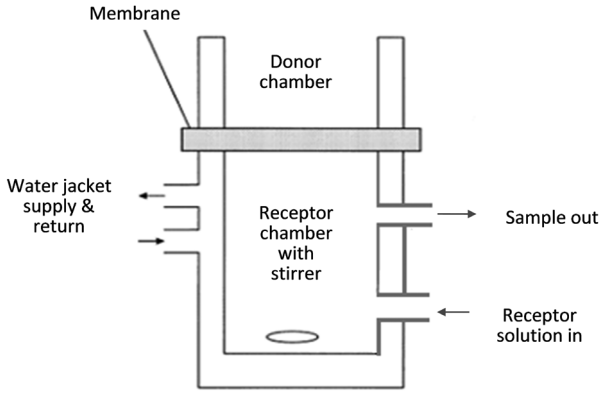

Figure 6. Schematic diagram of the Franz Diffusion Cell (by author)

In the donor part the sample of a vehicle containing the active substance is placed. The receptor part is below the membrane and filled with a receptor media, which is continuously agitated by a magnetic stirrer. Among its numerous advantages, the Franz diffusion cell boasts a high accuracy, ease of operation, and a fast and precise sampling. The samples of the receptor solution are taken with the lateral arm of the chamber, while the decrement of the receptor solution is replenished from a fresh stock. A typical and often problem with this test setup is the poor solubility of some of the tested compounds. In case of the hydrophilic substances a phosphate buffer solution (PBS), with the physiological pH at 7.4 is used. In the case of lipophilic molecules this problem is solved by addition of organic solubility improvers, such as non-ionic surfactants, ethanol, proteins, or cyclodextrins. However, it is necessary to remember that, the addition of the solubilizing additive may affect the membrane properties and permeability.

Figure 7 shows the automated test system (Microette Plus®) includes: 6-cell drive system, 6 vertical diffusion cells, programmable circulating water bath, Vision® Microette™ autosampler and AutoFill™ collector. The system provides precise sampling from the cells for extensive unattended tests and collection the receptor solution samples into HPLC or UPLC vials for their analysis.

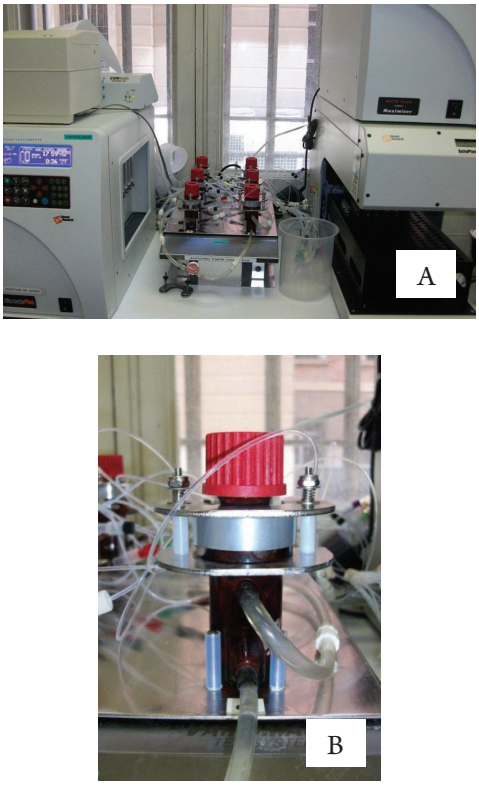

Figure 7. Microette Plus® (A), comprising of six Franz Diffusion Cell (B) (by author)

4.2. FLOW DIFFUSION CHAMBER

Figure 8 shows a flow diffusion chamber. Unlike in the Franz diffusion chamber, the receptor solution is continuously pumped through the receptor part (chamber) of the cell [78].

The flow diffusion chamber shown in Figure 8 is used for testing the substances with the absorption in the upper part of the range and with a poor miscibility in the receptor phase. It is important here to determine the minimum phase flow rate to ensure a good mixing coupled with continuous removal of the absorbed compound from the diffusion chamber, and to minimise the dwelling of poorly mixed layers. For most tested substances, the increase in the flow rate is largely negligible to absorption, with the sole exception of very poorly soluble compounds.

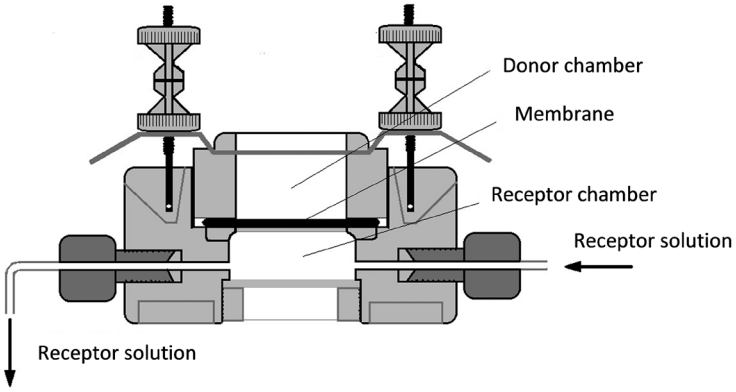

Figure 8. Flow diffusion chamber [78]

The continuous flow of the receptor solution elutes hydrophobic substances from the skin. The skin becomes much more permeable, due to this modification of its properties. Flow diffusion requires pre-testing to determine the optimum flow rate [20, 78].

4.3. SEGMENTATION OF THE *STRATUM CORNEUM*

Another example of *in vitro* testing method of skin permeation is segmentation of the *stratum corneum*, also called tape-stripping [20, 78, 93]. The method, established by Wagner et al [93], is used to determine the substance concentration after absorption by the *stratum corneum* and in different parts of the epidermis. During a tape stripping test, the successive layers of the *stratum corneum* are removed and tested for the concentration levels of the compound absorbed.

A tape-stripping tester allows determination of the scale and intensity of permeation by the test compound within the individual layers of the skin. Unlike the diffusion chambers discussed before, the skin is the receptor for the permeant. An advantage of this method is that

it eliminates excessive skin hydration. There is no risk of change in the skin quality from the flow of the receptor solution. Depending on the specific tested substance, the tape stripping test is completed when the active substance reaches the innermost skin layer. In the method, a portion of skin is placed on filter paper infused with a Ringer solution. Following a suitable treatment, the successive layers of the skin are delaminated and the substance concentration in each separated layer is determined with a suitable analytical method (e.g. HPLC). During a tape--stripping test, the successive layers of the *epidermis* are removed and tested for the absorbed concentration levels. Figure 9 shows the process flow chart of tape stripping.

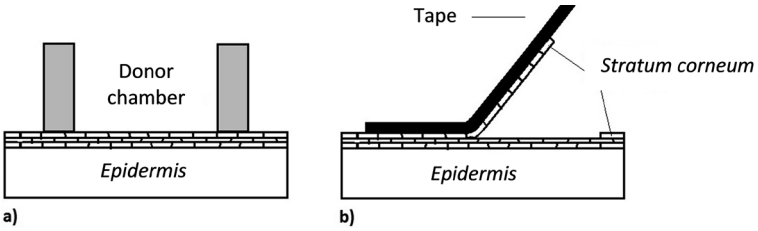

Figure 9. Process flow chart of tape stripping: When the test formulation is applied in the donor part (a) and then removed, the *stratum corneum* is removed by tape stripping (b) [20]

4.4. ATR-FTIR

Alternative solutions existing to the model systems of testing skin permeation by substances are in *situ* permeation studies. Infrared spectroscopy (IR) is a technique that has been developed to study diffusion through the skin [94–96]. One alternative is ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infra-Red), which allows direct testing of permeation of substances through the *stratum corneum*. The *stratum corneum* is mounted between a crystal and the donor solution which contains the test substance (see Figure 10).

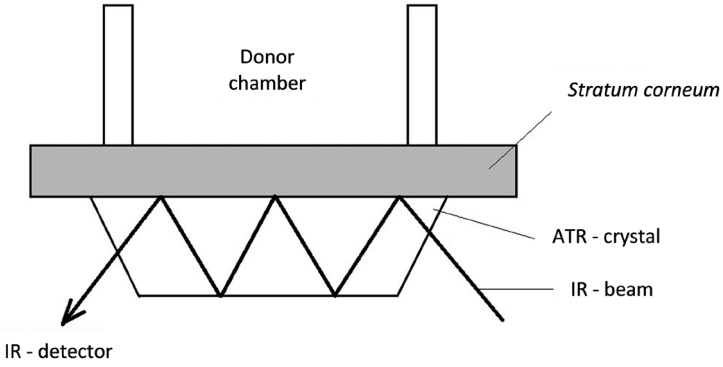

Figure 10. Schematic diagram of the ATR-FTIR test method of *stratum corneum* permeability [20]

The diffusion of the test substance inward the barrier layers is monitored by measuring absorbance versus time. The absorbance result is converted to concentration. The test substance delivered from the vehicle in the donor chamber moves to the deeper layers of the epidermis and it is measured by an IR beam scanner [94, 96].

To conclude, experimental methodology to determine *in vitro* skin permeation evolves from classical methods, based on diffusion cells, to the ATR-FTIR and the skin stripping methods, which appear more sensitive and sophisticated procedures. The stage is set, therefore, for objective selection of raw materials and the optimization of a formulation composition, for obtaining efficient skin delivery systems for cosmetic and pharmaceutic applications.

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II. NANOEMULSIONS

Małgorzata Miastkowska

ABBREVIATIONS

DLS – dynamic light scattering
EPI – emulsion phase inversion
HLB – hydrophilic-lipophilic balance
O – oil
O/W – oil-in-water
P – packing parameter
PCS – photon correlation spectroscopy
PIC – phase inversion composition
PIT – phase inversion temperature
S – surfactant
SEM – scanning electron microscopy
SLN – solid lipid nanoparticles
SNEDDS – self-nanoemulsifying drug delivery systems
TEM – transmission electron microscopy
UV – ultraviolet
W – water
W/O – water-in-oil

1. CHARACTERISTIC OF NANOEMULSIONS

Nanoemulsions are colloidal systems composed of an aqueous phase and an oil phase, stabilised with surfactants, and sometimes with addition of auxiliary surfactants. The size of the dispersed phase droplets is in the range from 20 to 500 nm. In the literature they are also referred to as submicron emulsions, ultrafine emulsions or miniemulsions [1–6]. Similarly to emulsions, they can be divided into two types: oil-in-water O/W and water-in-oil W/O [7].

These systems demonstrate the following properties [8–15]:

- transparent or semi-transparent appearance when the size of the dispersed phase droplets is smaller than the wavelength of the visible light spectrum,
- large interphase surface,
- low interphase and surface tension,
- stability against creaming and sedimentation (this property significantly extends the product's shelf life),
- kinetic stability,
- contrary to microemulsions, they can be produced using less surfactant (ca. 5–10%), which allows to obtain systems safer for the organism,
- they pass through the corneal layer of the epidermis, which enables to introduce higher concentrations of active substances into deeper skin layers,
- they protect active substances from external factors, and, as a consequence, from chemical degradation due to processes such as oxidation or hydrolysis,
- they increase the bioavailability of the active substances insoluble in water,
- they reduce adverse effects of medicines, due to a controlled release.

Due to their unique properties, nanoemulsions are widely used in cosmetic, pharmaceutical and food industries.

2. THERMODYNAMIC STABILITY OF NANOEMULSIONS

From the point of view of thermodynamics, nanoemulsions are non-equilibrium systems, which may lead to flocculation, coalescence and/or Ostwald ripening [10]. However, very small sizes of the internal phase particles result in a significant reduction of the forces of gravity, and Brownian motion protect the system against creaming and sedimentation, thus increasing its kinetic stability [5, 9].

The difference in thermodynamic stability between microemulsions and nanoemulsions is illustrated by Gibbs equation (1):

$$\Delta G = \Delta A\gamma - T\Delta S \tag{1}$$

where:

ΔG – is the change in the system's free energy [J],
ΔA – is the change in the interphase surface [m^2],
ΔS – is the change in the entropy [J/K],
γ – is the interphase tension [J/m^2],
T – is the absolute temperature [K].

Microemulsion can form spontaneously only when the value of the system's free energy ΔG is negative. Due to a significant change of the interphase surface (ΔA) this condition can only be met for extremely low values of total interphase tension (γ). Due to a high content of surfactants, in microemulsions the interphase tension is lower than 10^{-3} mN · m^{-1}. It counters the high value of the system's entropy resulting from a high degree of fragmentation of the internal phase, and affects the thermodynamic stability of microemulsions [16].

Comparing the stability of nanoemulsions and microemulsions it is worth emphasizing that the stability of nanoemulsions is considerably dependent on the method of production and the order of combining the ingredients of a composition. Moreover, nanoemulsions are stable in time, regardless of temperature fluctuations [5, 16, 17], whereas microemulsions may be destabilised due to temperature changes or addition of the external phase [16, 18]. A study by Fernandez [5] suggests that O/W nanoemulsions may be diluted with water without affecting the size of particles, whereas addition of the active substance can result in a change in the size of the internal phase droplets [19, 20].

As previously mentioned, the main destabilisation process observed in nanoemulsions is coalescence and Ostwald ripening [3]. The process is determined by the "solubility" of the oil phase in water. The higher polarity of the oil, the easier the diffusion of droplets into larger ones – resulting from the difference in the Laplace pressure between droplets of various sizes [10] – via the continuous phase [21].

Equation (2) describes the process of Ostwald ripening [3, 5, 22]:

$$\omega = \frac{dr^3}{dt} = \frac{8}{9} \cdot \frac{C(\infty)\gamma V_m D}{\rho RT} \tag{2}$$

where:

ω – rate of Ostwald ripening [m^3/s],
r – mean radius of a droplet after time t [m],
ρ – density of the internal phase[kg/m^3],
t – time of formation of increasingly large dispersed phase droplets [s],
γ – interphase tension [J/m^2],
D – diffusion coefficient of the dispersed phase in the continuous phase [m^2/s],
V_m – molar volume of the internal phase [m^3/mol],
$C(\infty)$ – solubility of infinitely large droplets [kg/m^3],
R – gas constant [J/mol · K],
T – absolute temperature [K].

The equation (2) demonstrates that the rate of formation of increasingly large dispersed phase droplets is directly proportional to the cube of their radius.

The size of internal phase droplets may be affected, with time, by coalescence (eq. 3) [3]:

$$\frac{1}{r^2} = \frac{1}{r_0^2} - \left(\frac{8\Pi}{3}\right)\omega t \tag{3}$$

where:

r – mean radius of a droplet after time t [m],
r_0 – mean radius of a droplet in time $t = 0$ [m],
ω – rate of coalescence [$m^{-2} \cdot s^{-1}$],
t – time of formation of increasingly large dispersed phase droplets [s].

Although Ostwald ripening is the principal mechanism of destabilisation, after longer periods of time coalescence may be observed simultaneously [3].

3. COMPOSITION OF NANOEMULSIONS

The interest in nanoemulsions as a base for active substances, both in medicines and in cosmetics, is increasing. Table 1 presents examples of substrates used to prepare nanoemulsions, described in the literature.

In their studies, many authors use low-polarity oils as the oil phase of nanoemulsions, including decane [1], hexadecane [9], isohexadecane [9, 24, 25] or paraffin oil [5]. Higher-polarity oils frequently found in publications include isopropyl myristate [3, 22, 26] and capric and caprylic acid triglycerides [19, 27, 28, 29]. Recently, the interest in natural oils, such as rapeseed oil [30], soy oil [31, 32] or castor oil [33] has been increasing.

The data in Table 1 clearly demonstrate that non-ionic emulsifiers with a high HLB are used for stabilisation of nanoemulsions. The popularity of this group of surfactants results from their good usability (they are resistant to changes in pH or addition of electrolytes, they can be used in combination with co-emulsifiers), and dermatological properties (they do not demonstrate an irritating effect on the skin). Sorbitan fatty esters (Spans) [1], polyoxyethylated esters of sorbitan and fatty acids (Tweens, Polisorbats) [1, 28, 30, 34–36], oxyethylated alcohols and fatty acids [5, 9, 24, 25], as well as oxyethylated derivatives of rapeseed oil [3, 19, 22, 37] are also very popular. Scientist increasingly often use emulsifiers of natural origin: soy lecithin [27, 31], hen egg lecithin, sucrose stearate [26] or proteins [38].

Table 1. A review of substrates used for preparation of nanoemulsions [23]

Aqueous phase	Oil phase	Emulsifier/ Co-surfactant [INCI name]	Active substance	Emulsion type	Reference
Water	Paraffinic hydrocarbons	Laureth-4	–	O/W and W/O	**9**
		Ceteareth-6, Ceteareth-25	–	O/W	**5**
		Laureth-4, Laureth-6	–	O/W	**24**
		Laureth-2, Laureth-4	–	W/O	**25**
		Sorbitan monolaurate, Sorbitan monooleate, Polysorbate 20, Polysorbate 80	–	W/O	**1**
	Isopropyl myristate	PEG-7 hydrogenated castor oil, PEG-35 castor oil	–	W/O	**3**
		Polysorbate 80, Sorbitan monolaurate/ 1,2-Octanediol	Ibuprofen	–	**39**
		PEG-60 hydrogenated castor oil/Ethanol	–	O/W	**22**
		Egg lecithin, Sucrose Stearate; Ɣ-Cyclodextrin	Fludrocortisone acetate, Flufenamic acid	O/W	**26**
	Medium-chain triglycerides	Soybean lecithin, PEG-35 castor oil, Polysorbate 80	Carbamazepine	–	**27**
		Polysorbate 20, 40, 60, 80	β-carotene	O/W	**34**
		Polysorbate 20, 40, 60, 80, and 85	Tocopheryl acetate	O/W	**28**
Water, Glycerine		Polysorbate 80, Phospholipids, Poloxamer 188	α-Tocopherol	O/W	**29**
Buffer solution	Short-, medium- and long-chain triglycerides	β-Lactoglobulin	Curcumin	O/W	**38**

Water	Medium-chain triglycerides, natural oils	Polysorbate 20, 80 and 85	–	O/W	**35**
		PEG-35 Castor Oil	Lidocaine	O/W	**19**
	Natural oils	Polysorbate 80	Tocopherol	O/W	**30**
Water, propylene glycol		Soybean lecithin, Polysorbate 80, Poloxamer407	Camphor, Menthol, Methyl salicylate	O/W	**31**
Water		Polysorbate 80, Phosphatidylocholine	α-, δ-, γ-Tocopherols	–	**32**
		Polysorbate 80, Polyethylene-polypropylene glycol	Tocopheryl acetate	O/W	**40**
		Polysorbate 80	Thalidomide	O/W	**36**
		Sorbitan oleate/PEG-30 castor oil	–	O/W	**33**
		Salmon lecithin	Coenzyme Q10	O/W	**43**
		Sorbitane trioleate, Polyoxyethylene (20) oleyl ether	–	O/W	**41**
	Caprylic monoester of propylene glycol	PEG-35 castor oil/ Diethylene glycol monoethyl ether	Ramipril	O/W	**37**
			Aspirin		**43**
	Lauroyl glycerol derivatives oxyethylated with 6 mol of ethylene oxide + Glycerine triacetate	Polysorbate 80/ Diethylene glycol monoethyl ether	Aceclofenac	O/W	**44**
	Glycerine triacetate + Isopropyl myristate	Polysorbate 80/ PEG-400	Nimodipine	O/W	**45**
	Caprylic and capric diesters of propylene glycol + Glycerine triacetate	Polysorbate 80/ Diethylene glycol monoethylether	Glibenclamide	O/W	**46**
	Oleic acid + Isopropyl myristate	Polysorbate 20/Carbitol	Carvedilol	O/W	**47**
	Thistle oil	polysorbate 80, decyl glucoside, and a polyglyceryl-4 ester blend	–	O/W	**48**

4. METHODS OF PRODUCTION OF NANOEMULSIONS

The methods of production of nanoemulsions described in the literature include both the high- and low-energy processes (Figure 1). The high-energy methods consist in using mechanical energy, usually from mechanical devices such as high-revolution mixers, high--pressure homogenizers and ultrasound generators. Low-energy methods are based on the chemical energy stored in the emulsion ingredients (mainly in surfactants) [49]. The presence of emulsifiers results in reduced interphase tension, and thus in reduction of the energy required for fragmentation of the particles [9].

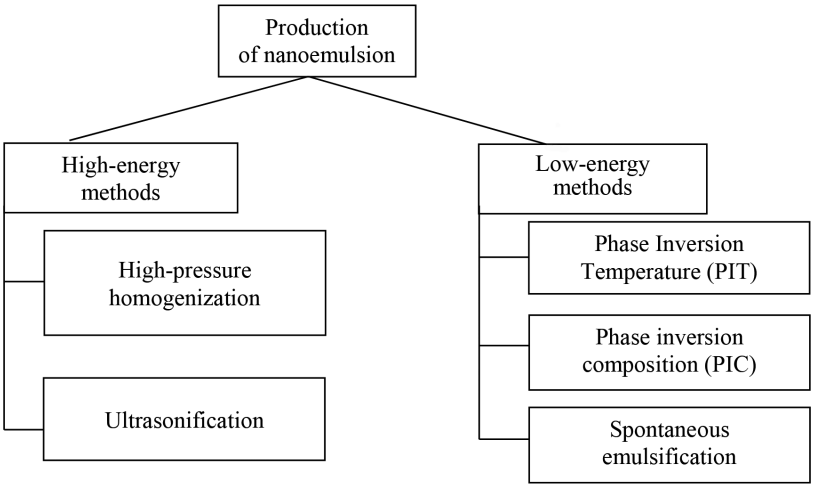

Figure 1. Methods of production of nanoemulsions [6, 23]

4.1. HIGH-ENERGY METHODS

During the emulsification process, various phenomena are observed: fragmentation of particles, surfactant adsorption on the interphase surface, or particle collisions. The particles are fragmented when the deformation forces are higher than the Laplace pressure [9].

Following Laplace equation (eq. 4), the smaller the size of the internal phase particles we want to obtain, the higher energy input or quantity of surfactant is required. Numerous authors confirmed that the size of particles decreases with the increase of the surfactant concentration in the emulsion [5, 19, 24], or with the increase of the homogenisation pressure [31, 34].

$$p = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right) \tag{4}$$

where:

p – Laplace pressure [N/m^2],
R_1, R_2 – basic radiuses of the droplet's curve [m],
γ – interphase tension [N/m].

The most frequently used high-energy method is high-pressure homogenisation, due to the effectiveness of the process, and homogeneity of the obtained systems. Pre--emulsion undergoes repeated pressure homogenisation, when it is pressed through narrow gap valves under a very high pressure of several dozen MPa [50]. The degree of fragmentation of the internal phase is determined by the pressure, number of cycles and the temperature of homogenisation [31, 34].

4.2. LOW-ENERGY METHODS

The phase inversion temperature (PIT) emulsification is a method described for the first time by Shinode and Saito in 1969 [51]. It is based on a temperature-induced change in the solubility of polyoxyethylated, non-ionic surfactants. As the temperature rises, the surfactants become lipophilic, due to dehydration of the polyoxyethylated chain. In low temperatures, the polyoxyethylated, hydrophilic emulsifier produces O/W emulsions. Along with the increase of the temperature, the curve of the interphase surface changes from positive to negative, the phases are inversed, and a W/O emulsion is produced. In the intermediate temperature, also known as HLB temperature, the curve of the surfactant equals zero, and a bicontinuous phase and/or lamellar liquid crystal structures are formed (Figure 2) [5, 7].

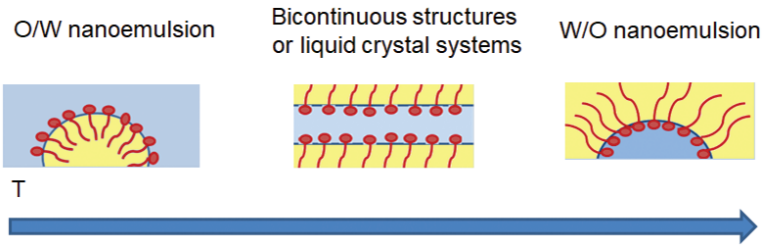

Figure 2. Temperature-induced change of the emulsion type [6, 23]

The phase inversion composition method consists in a phase transition due to a change in the composition following addition of one of the phases. The emulsification process is conducted in a fixed temperature. This method can be applied in two ways:

a) by gradually adding the oil phase to the water/surfactant mixture at a fixed temperature [3, 52],
b) by gradually adding the aqueous phase to the oil/surfactant mixture (Figure 3) [19, 22].

In the PIC method, as in the PIT method, the curvature of the interphase surface changes from negative (W/O) to positive (O/W); however, in this case it is a result of

the increase in the aqueous phase content. Also here we observe a transition through the multiphase region, and liquid crystal structures are formed [55, 56]. Some authors refer to this method as emulsion phase inversion (EPI) [33, 35].

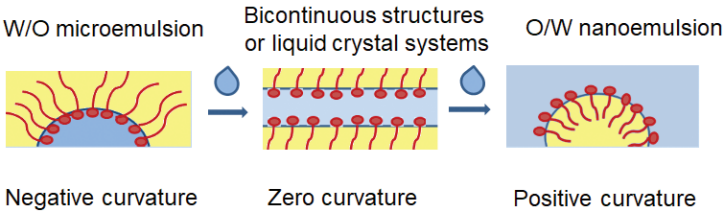

Figure 3. Change of the emulsion type following addition of the aqueous phase [6, 23]

Another method of producing nanoemulsions is spontaneous emulsification [7, 39]. In this case, nanoemulsion is produced as a result of rapid diffusion of the surfactant and/or solvent particles from the dispersed phase into the continuous phase. The process does not involve a change in the surfactant curvature (without transition between the phases).

Botet explains formation of nanoemulsion without a surfactant by "Ouzo effect", i.e. addition of water to a ternary, homogeneous solution containing water, alcohol and essential oil (soluble in ethanol, insoluble in water). During dilution, part of alcohol passes from the organic phase to the aqueous phase, which results in a spontaneous formation of oil droplets in the solution [10, 55, 56].

4.3. CONSTRUCTION OF PHASE DIAGRAM

Depending on the number of the degrees of freedom in a given system several types of phase diagrams may be created. The Gibbs' phase rule allows determining the number of degrees of freedom depending on the composition of the system and the conditions of obtaining it and is described by the equation (5) [57–59].

$$F = C - P + 2 \tag{5}$$

with:

F – number of the degrees of freedom,
C – number of independent chemical components,
P – number of phases present in the system.

In processes of low-energy emulsification, a helpful method of description of the composition of phases contained in a colloidal arrangement is creation of triangular phase diagrams. They involve ternary systems comprising: oil (O), water (W) and surfactant (S) or quaternary systems comprising a mixture of surfactant/co-surfactant at a constant weight ratio, which may be illustrated by the means of so called pseudo-triangular phase diagrams.

They allow demonstration of the quantitative and qualitative composition primarily the microemulsion systems, in constant temperature and pressure environment. However, many authors use phase diagrams to illustrate regions of nanoemulsion occurrence, despite the fact that these systems are not in a thermodynamic equilibrium [4, 25, 33, 37, 40, 45, 46].

During the process of generation of nanoemulsion the existence of various phase systems can be observed: W/O microemulsions, crystalline systems or co-continuous structures, micellar solutions or O/W microemulsions (Figure 4).

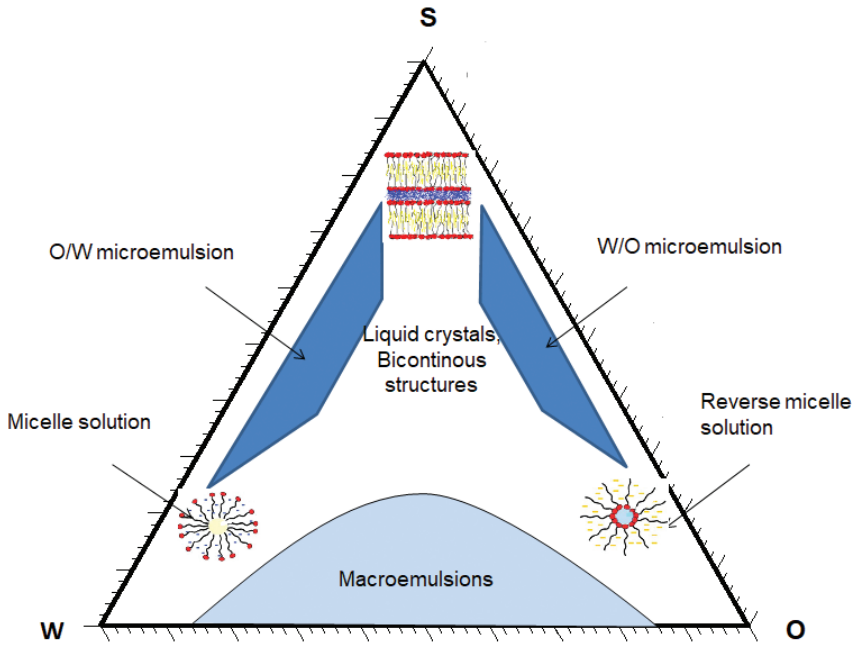

Figure 4. Diagram showing a ternary phase diagram [6]

Microemulsions and nanoemulsions, similarly to macroemulsions are divided into O/W and W/O systems. Windsor introduced another classification, which considers the influence of the surfactant with water and oil phases, i.e. R indicator [60, 61].

$$R = \frac{A_{CO}}{A_{CW}} \tag{6}$$

or

$$R = \frac{A_{CO} - A_{OO} - A_{LL}}{A_{CW} - A_{WW} - A_{HH}} \tag{7}$$

with:

A_{ww} – cohesion energy between water particles,
A_{OO} – cohesion energy between oil particles,

A_{CW} – cohesion energy between particles of surfactant and water,
A_{CO} – cohesion energy between particles of surfactant and oil,
A_{LL} – surface energy between hydrophobic parts of surfactant,
A_{HH} – surface energy between hydrophilic parts of surfactant.

Winsor classification in accordance of the value of R indicator lists four types of microdispersion:

- **Winsor I:** two-phase area, O/W microemulsion coexists with oil phase; R < 0,
- **Winsor II:** two-phase area, W/O microemulsion coexists with water phase; R > 0,
- **Winsor III:** three-phase system, O/W and W/O microemulsion exists in parallel to water and oil phases; R = 1,
- **Winsor IV:** homogenic, single-phase (isotropic) O/W or W/O type microemulsion system [17, 61–63].

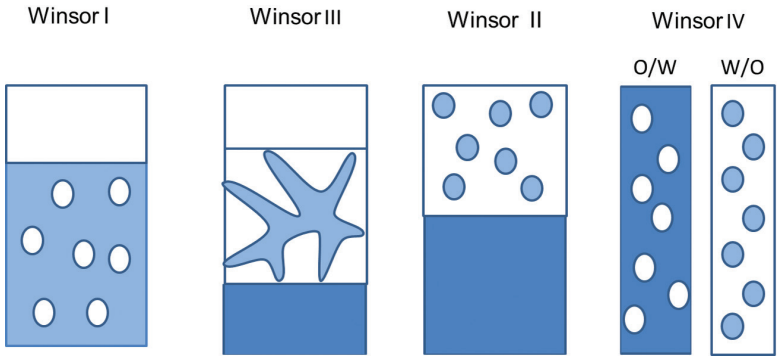

Figure 5. Microemulsion types – in accordance to Winsor classification [23, 61]

In order to understand the type of occurring microstructures is the so called surfactant packing parameter *P*, described by the equation (8):

$$P = V/a_0 \cdot L_c \tag{8}$$

with:

V – volume of hydrophobic alkyl chain of the surfactant particle [m^3],
L_c – length of hydrophobic alkyl chain of the surfactant particle [m],
a_0 – surface area of the polar group of the surfactant [m^2].

If:

- P < 1, the phase border is a positive curve, O/W type microemulsion or spherical micelles are created;
- P > 1, the phase border is a negative curve, W/O type microemulsion or reverse micelles are created;
- P = 1, the phase border displays both positive and negative trend, lamellar structures are created (Figure 6).

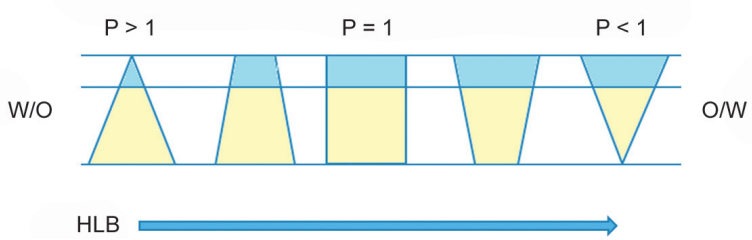

Figure 6. Influence of the P value and surfactant construction on the type and structure of emulsion systems [23, 58, 63, 64]

5. METHODS OF ANALYSIS OF THE SIZE OF PARTICLES AND MORPHOLOGY OF EMULSION SYSTEMS

The assessment of nanoemulsion properties encompasses, among other things, the analysis of the size of the droplets and studying the morphology of emulsion system structures. Furthermore, studied are: viscosity, surface tension and interfacial tension as well as pH of obtained systems.

The most commonly applied methods of analysis of the size of the internal phase in nanoemulsions involves the technique of dynamic light scattering (DLS) also known as photon correlation spectroscopy (PCS). Nanoemulsion structure is examined with scanning (SEM) and transmission (TEM) electron microscopy. Examining the type of nanoemulsion is conducted with a conductometric method.

5.1. DYNAMIC LIGHT SCATTERING TECHNIQUE (DLS)

Dynamic light scattering (DLS) technique measures Brownian motion and relates this to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. The velocity of the Brownian motion is defined by a property known as the translational diffusion coefficient (D). The speed at which the particles are diffusing is measured. Brownian motion, which constantly affect particles, cause changes in intensity of scattered light $I(t)$, which is continually fluctuating. The rate at which these intensity fluctuations occur will depend on the size of the particles The analysis of the intensity of scattered light as a function of time provides information on motions of dispersed particles. Assuming that the non-interacting spherical particles are dispersed in a continuous medium of η viscosity, we can calculate the particle diffusion coefficient D, on the basis of Stokes–Einstein equation (9) [65, 66]:

$$D = kT/3\,\pi\eta d(H) \tag{9}$$

where:

D – diffusion coefficient [m^2/s],
k – Boltzmann constant [J/K],
T – absolute temperature [K],
η – continuous medium viscosity [Pa · s],
$d(H)$ – particle diameter [m].

The diameter of particles that is measured in DLS is a value that refers to how a particle diffuses within a fluid so it is referred to as a hydrodynamic diameter. The hydrodynamic diameter also refers to the velocity of the particle in liquid and is calculated from the measurement of the diameter of the sphere, which has the same diffusion coefficient as the measured particle [65].

5.2. TRANSMISSION AND SCANNING ELECTRON MICROSCOPY

Electron microscopes have emerged as a powerful tool for the characterization of a wide range of materials. Their versatility and extremely high spatial resolution render them a very valuable tool for many applications. The two main types of electron microscopes are the Transmission Electron Microscope (TEM) and the Scanning Electron Microscope (SEM).

There are a variety of similarities between those methods. Both are types of electron microscopes and give the possibility of seeing and investigating small, subatomic particles or compositions of sample. The main difference is that as a result of the interaction of the sample with the high-energy electron beam, different signals are generated. The method used in SEM is based on scattered electrons while TEM is based on transmitted electrons. The scattered electrons in SEM are classified as backscattered or secondary electrons. Moreover SEM focuses on the sample's surface and its composition whereas TEM provides the details about internal composition. Therefore TEM can show many characteristics of the sample, such as morphology, crystallization, stress or even magnetic domains. On the other hand, SEM shows only the morphology of samples [67].

Because nanoemulsions have liquid form, the best method of analysing their structure is so called cryo – SEM or cryo-TEM. Preparation of samples for analysis is carried out by quick immersion of the sample in coolant e.g. liquid nitrogen. The frozen sample is coated by a layer of metal to increase the conductivity of electrons. Its fragmentation is carried out in vacuum in at the temperature of liquid nitrogen with the use of microtome cooled by liquid nitrogen. In the following stage of sample preparation the ice is removed from its surface by sublimation in vacuum. Then, the replicas are shadowed by dusting the surface of the sample with platinum and carbon. The replica layer is removed from the surface of the sample by chemical etching with acid or detergent solutions, rinsed, dried and transferred to TEM or SEM analysis. The analysis tells us i.a. the information regarding the occurrence of aggregates

in the sample structure, which tells us of its homogeneity [68]. It should be noted that the sample does not always have to be sprayed. Currently, it is possible to use low-vacuum electron microscopes or specialized in-situ measurements attachments.

6. THE EFFECT OF NANOEMULSIONS ON THE RELEASE OF ACTIVE INGREDIENTS

Active ingredient permeation is also significantly affected by the physicochemical form in which it is administered. In their studies, many authors compare the effectiveness of different bases as active ingredient carriers.

Shakeel et al. [44] investigated aceclofenac (nonsteroidal anti-inflammatory drug and painkiller) permeation into skin from six nanoemulsions with different fragmentation levels, from a gel nanoemulsion and from a classic gel. The nanoemulsion was made up of a mixture of Triacetin (INCI: Glycerol Triacetate) and Labrafil (INCI: Oleoyl Macroglycerides), serving as the oil phase, Tween 80 as a surfactant, Transcutol-P (INCI: Diethylene glycol monoethyl ether) as a surfactant, and distilled water. The authors of the study noted that the nanoemulsion that released the highest amount of aceclofenac, contained the least amount of oil phase and was characterized by the smallest size of internal phase particles (approximately 35 nm). During the next stage of the study, the profiles of aceclofenac release from the selected nanoemulsion, gel nanoemulsion and classic gel were compared. It was found that in this case as well, aceclofenac permeation occurs the most effectively when released from the nanoemulsion, and the least – from a classic gel.

Levels of carbamazepine (dibenzazepine derivative, second-generation anti-epileptic drug) release from its aqueous solution and O/W nanoemulsion to an acceptor solution (phosphate buffer, pH = 7.4) were studies by Kelmann et al. [27]. The nanoemulsion was obtained using the spontaneous emulsification method and was characterized by particle size of approx. 150 nm. A mixture of ricin oil and medium-chain triglycerides, stabilized with soy lecithin served as the oil phase in the nanoemulsion. It was observed that the active ingredient is released from the nanoemulsion at a slower rate than from the aqueous solution carbamazepine. The authors of the study explain this by the fact that carbamazepine diffusion from they hydrophobic internal phase of the nanoemulsion is more difficult due to poor water solubility of the lipophilic carbamazepine.

Baboota et al. [69] studied skin permeation of a nonsteroidal drug with an anti--inflammatory and painkilling effect, Celecoxib. Nanoemulsion, nanoemulgel and gel were used as carriers for the drug. The nanoemulsion was obtained using the phase inversion composition method. It was characterized by an internal phase particle size of approx. 34.6 nm. It was composed of 10% Sefsol 218 (INCI: Propylene glycol mono caprylic ester) and Triacetin (INCI: GlycerolTriacetate) as oil phase. A mixture of Tween 80

(INCI: Polysorbate 80) and Transcutol-P (INCI: Diethylene glycol monoethyl ether) served as surfactants. Furthermore, the nanoformulation included 40% water and 2% of the drug. The nanoemulgel was obtained by adding 1% gelling agent Carbopol-940 (INCI: Carbomer) to the nanoemulsion. The gel formulation contained 1% Carbopol-940, 10% isopropyl alcohol, 10% PEG-400, 10% propylene glycol, 0.5% triethanolamine, 2% active ingredient and water. As a result of the *in vitro* celecoxib skin permeation test, it was observed that it was characterized by the highest permeation rate (Jss) and coefficient (Kp) when released from the nanoemulsion, compared to gel and nanoemulgel.

Kotyla et al. [30] compared the rate of transdermal transport of δ tocopherol from microemulsion and nanoemulsion. The emulsion systems contained water, rapeseed oil as the oil phase, and Polysorbate 80 as the emulsifier. The only difference between the test preparations was the method of their preparation; the nanoemulsions were made using a pressure homogeniser, while microemulsions were produced with a magnetic stirrer. The size of dispersed phase particles for the nanoemulsion was 65 nm, while for the microemulsion – 2,788 nm. Tests were performed on a group of golden hamsters. Two hours after administering the nanoemulsion, a 36-fold increase in δ tocopherol levels in hamster skin was observed. With the microemulsion used as a base for the test preparations, only a 9-fold increase of vitamin E in rodent skin was noted. Three hours after application, the increase was 68-fold, while for the microemulsion, tocopherol concentration in hamster skin only increased 11-fold.

No permeation promoters were used during the tests in order to demonstrate a direct relation between the degree of emulsion internal phase fragmentation, forming the base for the active ingredient, and the active ingredient rate of skin permeation. Test results confirmed that nanoemulsions are a physicochemical form that enables a substantial increase in δ tocopherol availability in transdermal application. O/W nanoemulsions solubilize the active ingredient with poor water solubility, enabling its transport to live layers of skin, where it is released.

Wang et al. [39] studied ibuprofen (nonsteroidal anti-inflammation drug) release from self-nanoemulsifying drug delivery systems (SNEDDS) with different internal phase particle sizes (58 nm, 96 nm, 143 nm) and from a traditional drug form (pills) as a reference sample. The oil phase in the nanoemulsions was composed of isopropyl mirystate, 1,2-octanodiol served as a co-surfactant, and the entire system was stabilised with Tween 80 (INCI: Polysorbate 80) and Span 20 (INCI: Sorbitan monolaurate). All these formulations contained 10 wt. % of the active ingredient. Tests demonstrated that ibuprofen release from the test formulations depends on their level of dispersion. For the first 30 minutes, the most ibuprofen was released from the nanoemulsion with the smallest particle size (58 nm), while the least – from the nanoemulsion with the lowest dispersion degree and from the pill.

Klang et al. [26] compared transdermal permeation of lipophilic (fludrocortisone acetate and flufenamic acid) and hydrophilic compounds (fluconazole and minoxidil) of drugs released from O/W nanoemulsions stabilized with lecithin (nanoemulsion particle size was approx. 180 nm) and from systems where sucrose stearate served as an emulsifier

(internal phase particle size was approx. 140 nm). The oil phase was a mixture of cetearyl ethylhexanoate and isopropyl mirystate. *In vitro* tests demonstrated that hydrophilic drugs permeated quicker and in much greater amounts than hydrophobic drugs from either of the test O/W nanoemulsions, which the scientists explain by much better solubility of hydrophilic drugs in an aqueous acceptor solution. As concerns the effects of the emulsifier used on test drug permeation through skin, the effects were comparable for lipophilic drugs. For hydrophilic drugs, however, significantly better skin permeation was achieved for lecithin-stabilised formulations, probably due to the microscope-confirmed presence of liposomal structures, in which active ingredients could partially be incorporated. Furthermore, it was observed that an addition of γ-cyclodextrin markedly increased the degree of skin penetration by fludrocortisone acetate. This is related to the fact that dextrins increase steroid drug solubility by creating inclusive complexes with them.

Araujo et al. [36] compared the release of thalidomide (a drug with antiemetic and painkilling effects) from its acetonitrile solution and from a nanoemulsion with particle size of approx. 200 nm, obtained using the spontaneous emulsification method. The emulsion system was composed of water, ricin oil (10 wt. %) as the oil phase, with soy lecithin (3.0 wt. %) and Polysorbate 80 (0.5%) serving as emulsifiers. Active ingredient release tests demonstrated that thalidomide (0.1 wt. %) release profiles from both carriers were similar, and the amount of substance released was close to 80%.

Harwansh et al. [70] tested the effects of nanoemulsion and gel on skin permeation by glycyrrhizin, a triterpenoid isolated from licorice root, which has anti-inflammatory, antibacterial and anti-swelling effects. The nanoemulsion was composed of 32.4% Span 80 (INCI: sorbitan monooletae), 3.7% Brij 35 (INCI: Laureth-23), 10% isopropyl alcohol, 46.5% soy oil, 6.45% water and 1% active ingredient. It was obtained using the PIC method, and the internal phase particle size was 22 nm. In turn, the gel formulation contained 1% Carbopol-940, 10% isopropyl alcohol, 10% PEG-400, 10% propylene glycol, 0.5% triethanolamine, 1% active ingredient and water. As a result of the *in vitro* glycyrrhizin skin permeation test, it was observed that it was characterized by a significantly higher permeation rate (Jss) and coefficient (Kp) when released from the nanoemulsion, compared to gel.

Pratap et al. [47] studied the effects of nanoemulsions on bioavailability of topically administered carvedilol, a 3rd generation β-blocker. After oral administration, this compound is quickly absorbed by the gastrointestinal tract (80%), but its biological availability remains low due to it being intensively metabolised by the liver during the first pass effect. The nanoemulsion was produced using the spontaneous emulsification method. A mixture of oleic acid and isopropyl mirystate, combined at a weight ratio of 3:1 and in the amount of 4%, served as the oil phase. Tween 20 (INCI: Polysorbate 20) in the amount of 12% was used as a surfactant and 24% Carbitol (INCI: Diethylene Glycol Monoethyl Ether) as a co-surfactant. Aqueous phase content was 60%. The preparation contained 6 mg carvedilol. The particle size of the resulting nanoformulation was 71.8 nm. *In vitro* carvedilol permeation tests demonstrated that the nanoemulsion significantly improved the drug's bioavailability, and skin surface application eliminated the first pass effect.

The effects of nanoemulsion composition on transdermal transport of tamoxifen citrate (a selective estrogen receptor modulator, used mainly in nipple cancer treatment) were studied by Pathan et al. [71]. For this purpose, the aqueous titration method was used to produce a series of O/W nanoemulsions, which were composed of oleic acid as the oil phase, RH 40 (INCI: PEG-40 Hydrogenated Castor Oil) as a surfactant, ethanol as a co-surfactant, and distilled water. To improve drug permeation through skin, essential oils were added to the formulation: dill oil, lemon oil, or coriander oil, at a 5 wt. % concentration. The resulting systems were characterized by an internal phase particle size of less than 100 nm. The addition of oils as permeation promoters resulted in a minor change of physicochemical properties of the base emulsion (size, polydispersity, viscosity), while it significantly improved transdermal transport of tamoxifen, compared to the control sample (nanoemulsion without an essential oil). The best effects were achieved for the formulation containing dill oil, which at the same time was characterized by the lowest particle size (25.5 nm) and the lowest viscosity.

Chavda et al. [72] utilised the ultrasonification method to obtain liquid and solid SNEEDS systems as carriers of isotretinoin (a retinoid used in acne treatment). Transcutol P (glycol monoethyl ether) served as the oil phase. Tween 80 (polyoxyethylene sorbitol monooleate) was used as a surfactant, while PEG 400 (polyethylene glycol) served as a co-surfactant. The formulations differed from each other in the weight ratio of oil phase (O) to the surfactant/co-surfactant mixture (S/CoS = 50:50), O:S/CoS from 40:60 to 50:50. The resulting systems were characterised by particle size within the 29.53–64.71 nm range. Tests demonstrated that there is no significant difference in isotretinoin release profiles from liquid or solid SNEDDS systems. Both nanosystem forms released 100% of their active ingredients, while the control sample (pure drug) only released 14%.

Badran et al. [73] studied transepidermal transport of meloxicam, a lipophilic nonsteroidal anti-inflammatory drug, from ultra-fine self-emulsifying drug delivery nanosystems (UF-SNEDDS). Solubility tests demonstrated that meloxicam was characterized by the best solubility in Cremophor RH 40 (INCI:PEG-40 Hydrogenated Castor Oil), Tween 60 (INCI: Polysorbate 60), and Capmul MCMC8 (INCI: Glyceryl Caprylate) surfactants, and in PEG 400 (INCI: PEG-8) co-surfactant. As a result, 6 formulations differing in the type of surfactant used were prepared: SNEDD-F1 (Cremophor RH 40), SNEDDS-F2 (a mixture of Cremophor RH 40 and Tween 60), SNEDDS-F3 (a mixture of Cremophor RH 40 and Capmul MCM C8), SNEDDS-F4 (a mixture of Cremophor RH 40 and PEG400), and SNEDDS-F5 (a mixture of Cremophor RH 40, Tween 60, Capmul MCM C8 and PEG 400). Each system was characterized by particle size lower than 50 nm (thus the ultra-fine designation) A meloxicam solution in phosphate buffer (pH = 7.4) was used as the control sample. *In vitro* tests of drug permeation through skin showed that nanoformulations markedly increased meloxicam permeation through skin, compared to the control sample. The test drug exhibited the highest permeation rate (Jss) and coefficient (Kp) when released from the SNEDSS-F5 formulation.

Kinetics of isotretinoin release from coconut oil-based O/W nanoemulsions were tested by Miastkowska et al. [74]. The formulation produced was stabilized by Polysorbate 80 and was characterized by internal phase droplet size of approx. 21 nm. The release test was conducted in thermostated diffusion chambers. The reference sample was an isotretinoin solution in coconut oil. Tests confirmed that the test substance was released from the nanoformulation (10.39 wt. %) in significantly greater amounts than from oil (1.85 wt. %) Furthermore, it was found that isotretinoin release from nanoemulsion occurs according to zero order kinetics, meaning that the release rate is independent of component concentration. As systems with extended active ingredient release, nanoemulsions enable extending the duration for which substance concentration remains in the therapeutic range at a lower maximum concentration of the ingredient. This enables application frequency to be reduced.

The literature data shown indicate that in general, nanoemulsions are a more effective form of active ingredient transport, compared to classic emulsions, gels or microemulsions, due to the high level of internal phase particle dispersion and high interface surface area. However, it must be noted that some authors find no significant differences in the transport of active ingredients from nanoemulsions with different degree of dispersion, or in their effectiveness, compared to other bases. This is related to the fact that the process of active ingredient release from the carrier and the rate of its permeation to the cornified layer of epidermis are affected by lipophilicity of the compound, its molar mass, solubility and the drug's affinity to the base and the acceptor fluid. Composition of the carrier base itself is not insignificant either, such as the presence of permeation promoters or substances improving drug solubility [23].

7. APPLICATION OF NANOEMULSIONS IN PHARMACEUTICAL AND COSMETIC INDUSTRY

The use of nano emulsions as forms of administering active substances in drugs and cosmetics is increasingly applicable and the interest in that field of science has grown in the recent years.

The liquid nature of the emulsion system provides nanoemulsions with appropriate application properties, possible to sense on skin, which makes them attractive products for cosmetic industry [21, 52]. Using nanoemulsion as a base in a cosmetic allows optimisation of the manner of application of active ingredients and fragrances in hair and skin care products. Many authors point out the fact that in comparison to microemulsions, liposomes, solid lipid nanoparticles (SLN), classic emulsions or gels, nanoemulsions are more effective active substance vehicles [9, 31, 75, 76]. Nanoemulsions, similarly to microemulsions, increase the useful life of many products thanks to resistance to sedimentation and creaming.

Their additional advantage over microemulsions is the significantly lower surfactant content (about 5–10%), which allows maintaining proper stability of the system and makes the preparations harmless to human body [6, 15]. Examples of cosmetics in form of nanoemulsion available on the market are UV-filter hairspray from Korres, Nanocream from Sinerga, Nanogel from Kemira, Vital Nanoemulsion A-VC serum from Marie Louise, Bepanthol Ultra face cream from Bayer or NanoVital facial fluid from Vitacos Cosmetics [77, 78]. Additionally, an international concern, L'Oreal, patented a line of cosmetic recipes in form of nanoemulsion [15, 77, 79]:

- US Patent number: US6274150B1 (Nanoemulsion based on phosphoric acid fatty acid esters and its uses in the cosmetics, dermatological, pharmaceutical, and/or ophthalmological fields),
- US Patent number: US6464990B2 (Nanoemulsion based on ethylene oxide and propylene oxide block copolymers and its uses in the cosmetics, dermatological and/or ophthalmological fields),
- US Patent number: US5753241A (Transparent nanoemulsion less than 100 nm based on fluid non-ionic amphiphilic lipids and use in cosmetics or in dermopharmaceuticals),
- US Patent number: US6689371B1 (Nanoemulsions based on sugar fatty ethers and its uses in the cosmetics, dermatological and/ophthalmological fields),
- US Patent number: US6335022B1 (Nanoemulsions based on oxyethylenated or non--oxyethy-lenated sorbitan fatty esters and its uses in cosmetics, der-matological and ophthalmological fields).

Nanoemulsion used as medicine vehicles are intended to solve the issue involving conventional system of administering medicines, namely the low biocompatibility. The ideal system for administering medicine should maximise the therapeutic effect, while minimising toxicity of the system to the organism [80]. The capability of nanoemulsions to dissolve significant amounts of hydrophobic compounds, including drugs, while maintaining mutual biocompatibility and the capability to protect the medicines from destruction as a result of enzymatic hydrolysis makes them effective vehicles for drugs in human organism. Additionally, their implementation allows controlling the progress of releasing the drug into the organism, e.g. over extended period, which may lead to reducing the frequency and amount of drug doses. Apart from that, nanoemulsion as a system of large interface surface allows rapid diffusion of the active substance of a medicine e.g. through skin [15, 74, 81–83]. Because of the aforementioned properties, nanoemulsions found use in pharmacy as so called SNEDDS (self-nanoemulsifying drug delivery systems). Thanks to them it was possible to improve the absorption of i.a. drugs such as: Sandimmune® iSandimmun Neoral® (cyclosporin A), Norvir® (ritonavir) and Fortovase® (saquinavir) [83], Sporanox® (itraconazole) [84], Limethason® (deksametazon), Diprivan® (propofol) [15], ketoprofen, dapsone, ibuprofen, meloxicam or nystatin [85]. Because they provide a perfect alternative to conventional vehicles of medicines (ointments, gels, creams) nanoemulsions are used in treatment of skin inflammation, psoriaris or atopic dermatitis [85].

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III. MULTIPLE EMULSIONS

Małgorzata Miastkowska

ABBREVIATIONS

CARS	–	coherent anti-stokes raman scattering
DLS	–	dynamic light scattering
EE	–	encapsulation efficiency
HLB	–	hydrophilic-lipophilic balance
O_1	–	internal oil phase
O_2	–	external oil phase
O/W	–	oil-in-water
O/W/O	–	oil-in-water-in-oil
W_1	–	internal water phase
W_2	–	external water phase
W/O	–	water-in-oil
W/O/W	–	water-in-oil-in-water
$W_1/O/W_2$	–	water (W_1)-in-oil-in-water (W_2)
$O_1/W/O_2$	–	oil (O_1)-in-water-in-oil (O_2)

1. CHARACTERIZATION OF MULTIPLE EMULSIONS

In respect of the number of dispersed phases, emulsions systems are classified as simple emulsions and multiple emulsions. The simple emulsions are two-phase liquid systems, composed of a continuous phase and a dispersed phase. In respect of the dispersed phase type, they are classified as oil-in-water (O/W) and water-in-oil (W/O) systems. The multiple emulsions are systems of which the structure is more complex: their original (simple) emulsion in them is additionally dispersed in the external phase forming an emulsion in another emulsion [2]. This type of systems, which was first reported by Seifriz in 1925, is also called double emulsions. Their size is in the range from 0.1 to 100 μm [3]. The multiple emulsions are classified as either of the two types of systems [4–6]:

1) $W_1/O/W_2$ – in which the W_1/O is dispersed in the water phase (W_2); if the compositions of the internal and external water phases are the same, this type of emulsion can be described as W/O/W;
2) $O_1/W/O_2$ – in which the O_1/W emulsion is dispersed in the oil phase (O_2); if the compositions of the internal and external oil phases are the same, this type of emulsion can be described as O/W/O.

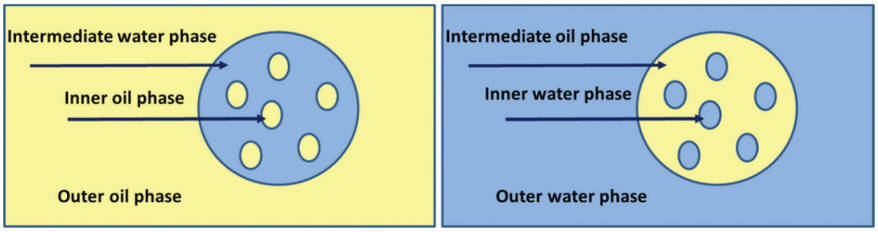

Figure 1. Schematic presentation of the two types of double emulsion droplets. On the left – a typical O/W/O multiple droplet; on the right – a typical W/O/W multiple droplet (by author)

Moreover, the multiple emulsions can be classified by the number and size of the internal-phase droplets (Figure 2) [4, 5]:

1) Type A – systems comprising large, single internal phase droplets,
2) Type B – systems comprising more than one internal phase droplet,
3) Type C – system comprising numerous, small internal phase droplets.

In comparison with the classic emulsions, the multiple emulsions have the following advantages [3, 7]:

1) ability to simultaneously encapsulate the hydrophilic and lipophilic active components,
2) controllable release of active substances,
3) protection of the active components, incorporated in the internal phase, from oxidation and enzymatic degradation,
4) higher bioavailability of active components,
5) ability to mask the disagreeable taste or smell of foodstuff components.

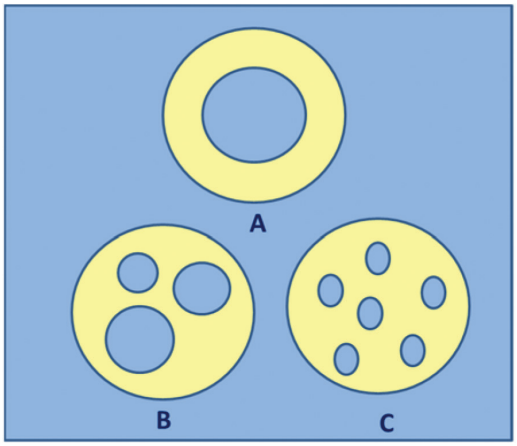

Figure 2. Classification of multiple emulsions by the number and size of internal phase droplets (by author)

2. PHYSICO-CHEMICAL PROPERTIES OF MULTIPLE EMULSIONS

The principal problem encountered in the production of double emulsions is that they are thermodynamically unstable, because of the excess free energy, connected with the two interfaces: W_1/O and O/W_2 in the $W_1/O/W_2$ emulsions, and O_1/W and W/O_2 in the $O_1/W/O_2$ emulsions [4, 8, 9]. Therefore, these systems are not resistant to destabilization processes such as: coalescence, flocculation and creaming.

Moreover, in the case of $W_1/O/W_2$ systems, their stability is governed by these three factors [10]:

1) difference of pressures between the internal (W_1) and external water phase (W_2).
2) interactions between emulsifiers with a low/high HLB on the O/W_2 interface,
3) interactions between the polymer thickener and the hydrophilic emulsifier in the external water phase (W_2).

The precondition for obtaining a stable $W_1/O/W_2$ multiple emulsion is to obtain a stable, highly-dispersed homogeneous W_1/O emulsion [9, 10]. It is also very important to select the appropriate type and amount of surfactants of the external and internal phase and to prevent their interactions. The optimum HLB is ≤ 8 and ≥ 12 for the lipophilic and hydrophilic emulsifiers, respectively [3, 11]. Moreover, low-molecular emulsifiers are preferably used for an W_1/O emulsion which is the dispersed phase (for instance, polyglycerol polyricinoolaete or sorbitan monooleate), combined with the high-molecular emulsifier (such as proteins) or polymer emulsifier in the external water phase [6, 8]. The polymer emulsifiers account steric stabilization and the proteins (such as sodium caseinate

provide an electrostatic barrier between the internal phase droplets, which prevents their coalescence [12]. Some of the most frequently used polymer emulsifiers are: cetyl PEG/PPG-10/1 dimethicone and PEG 30-dipolyhydroxystearate [13]. The stability of the multiple emulsions can also be increased by making a simple emulsion with the highest obtainable degree of dispersion (for instance, microemulsion), and increasing the viscosity of the internal (water or oil) phase [6].

In addition, the volume of the water phase and its components are very important in the obtaining of multiple emulsions. According to the literature, the optimum content of the simple emulsion ought to be in the range 20–50%. At higher concentrations, the system has a higher viscosity and the internal phase droplets grow bigger, leading to a destabilization of the system. Moreover, above a certain concentration of the simple emulsion, phase inversion may take place [3]. On the other hand, addition of electrolytes into the internal phase of the $W_1/O/W_2$ emulsion is of importance because of the osmotic pressure between the internal (W_1) and the external (W_2) water phases.

3. THE COMPOSITION OF MULTIPLE EMULSIONS

Table 1 shows representative compositions of the respective phases of the $W_1/O/W_2$, $O_1/W/O_2$ multiple emulsions and their active components. The concentration of the simple emulsion, a parameter which determines the stability of the whole system, is also given.

Table 1. A survey of raw materials comprised in multiple emulsions

Emulsion type	W_1 phase	Oil phase	W_2 phase	Simple emulsion W_1/O [%]	Encapsulant	Ref.
W/O/W	Water, alginate	Sunflower oil; polyglycerol polyricinoleate	Water, lecithin	–	–	9
	Water, ethanol, sodium chloride	Caprylic/Capric Trigliceride; Polyglycerol polyricinoleate	Sodium chloride; sodium caseinate or sodium carboximethylcellulose, Polysorbate 20 or Polysorbate 80	20	Trans-resveratrol	4
	Water, sodium chloride	Heavy paraffin oil; Sorbitan monooleate	Water, steareth-10 with cetosteareth-30 or PEG-8 stearate with polysorbate 20	40	–	8

W/O/W	Water, heptahydrate of magnesium sulfate	Octyloctanoate, paraffinum liquidum and polyethylene; polyglyceril-2-sesquiisosterate and methylglucose dioleate	Water, saccharose monolaurate and saccharose, D(+)-glucose, xantan gum	36.53	Mandelic acid	**7**
	Water, heptahydrate of magnesium sulfate	Caprylic/Capric Trigliceride; PEG/PPG-18/18 methicone; Cetyl dimethicone copolyol	Monohydrated glucose solution; Poly(ethylene oxide-b-propylene oxide-b-ethylene oxide)	45	–	**14**
	Water, heptahydrate of magnesium sulfate	Caprylic/Capric Triglyceride, Cetyl dimethicone copolyol and PEG–30 dipolyhydroxystearate	Poloxamer 407, water	80	–	**13**
	Water, magnesium sulfate, gelatin	Refined soybean oil; Tetraglycerin monolaurate condensed ricinoleic acid ester	Water, magnesium sulfate, glucose, Decaglycerol monolaurate	30	L-Ascorbic Acid	**15**
	Water, heptahydrate of magnesium sulfate	Parafin oil; Cetyl dimethicone copolyol	Polyoxyethylene 20 cetyl ether; water	80	Grean Tee Extract	**16**
	Water	Soybean oil; Polyglycerol polyricinoleate	Water, whey protein isolate	40	–	**17**
	Water, magnesium sulfate	Paraffin oil; Cetyl dimethicone copolyol	Water; Polysorbate 80	90	L-Ascrobic acid	**18**
	Water, sodium chloride	Parafin oil; cethyl dimethicone copolyol	Water; block copolymer of ethylene and propylene oxide	75	Paromomycin	**19**
	Water	Caprylic/Capric Triglyceride; PEG-7 Hydrogenated Castor Oil	Water, Polysorbate 80	–	Diclofenac sodium	**20**
	Water, magnesium sulfate	Heavy paraffin oil, Sorbitan oleate and soy lecithin	Water, Steareth-20	40	DNAzyme	**21**
	Water, sodium Chloride	Cetyl palmitate or Isopropyl myristate or jojoba oil or; cetyl dimethicone copolyol or sorbitan stearate	Water; cocamidopropyl betainę and/or Polysorbate 80; acrylic acid polymer	50	Clotrimazole	**22**

O/W/O	liquid paraffin	Water; Ceteth 10	Liquid parafin; sorbitan monooletae	–	–	**23**
	liquid paraffin	Water; copolymer of ethylene and propylene oxides	Liquid parafin, hydroxyoctacosanyl hydroxystearate and microcrystalline wax; glycerol sorbitan fatty acid Ester	–	Hydrocortisone	**24**
	Paraffin oil and microcrystalline wax	Water; copolymer of ethylene and propylene oxides	Liquid parafin, hydroxyoctacosanyl hydroxystearate and microcrystalline wax; glycerol sorbitan fatty acid ester	–	Asiatic acid, madecassic acid and asiaticoside	**25**
	Canola oil	Water, sodium caseinate, lecithin	Palm-cotton stearin	–	–	**26**

The data in Table 1 indicate that, in the case of the $W_1/O/W_2$ emulsion, Polyglycerol polyricinoleate and Cetyl dimethicone copolyol are used as the predominant lipophilic emulsifiers [4, 9], whereas compounds of the group comprising ethoxylenated esters of sorbitan and fatty acids are used as the hydrophilic emulsifiers. In the $O_1/W/O_2$ emulsions, the lipophilic emulsifiers are mainly esters of sorbitan and fatty acids.

The internal water phase often comprises electrolytes such as sodium chloride [8, 15] or magnesium sulfate [7, 14, 16]. The most frequently used emulsion stabilizers are: sodium alginate [15] and sodium caseinate [4], gelatin, xanthan gum and guar gum [10]. Salts and sugars are used for controlling the osmotic pressure between the internal (W1) and external water phases (W2) [6].

The oil phase is usually composed of caprylic and capric triglycerides [4, 13, 14], paraffin oil [7, 23] or natural oils [9, 17, 26]. On the other hand, natural-oil based emulsions are less stable than those containing paraffin oil, because of their higher affinity for the water phase [27].

4. METHODS TO OBTAIN MULTIPLE EMULSIONS

There are two main methods for the formation of multiple emulsions: one step emulsification and two step emulsification [27].

The one step emulsification is based on mechanical mixing and phase inversion. The phase inversion involves the gradual addition of an aqueous solution of hydrophilic emulsifier to a W/O emulsion, formed previously by mechanical agitation. The W/O/W emulsion is

formed as an intermediate mesophase stage before the system completely inverts into an O/W emulsion. Inversion is most likely when the dispersed water phase is closely packed. Inversion is possible at the internal water phase concentrations of more than 70%. Using a reverse procedure results in oil-in-water-in-oil ($O_1/W/O_2$) multiple emulsion formation. [11, 27–29]

The two-step emulsification (Figure 3) is the most widely used method to obtain multiple emulsions. It is based on the re-emulsification of the simple emulsion. The first step is to obtain the simple emulsion using an excess of the hydrophilic emulsifier (O_1/W emulsion) or lipophilic emulsifier (W_1/O emulsion). In the second step, the simple emulsion is homogenized with the external phase containing a rather low concentration of the emulsifier, dedicated to that phase. The efficiency of that method is determined by the obtaining of a stable simple emulsion [23, 27, 30]:

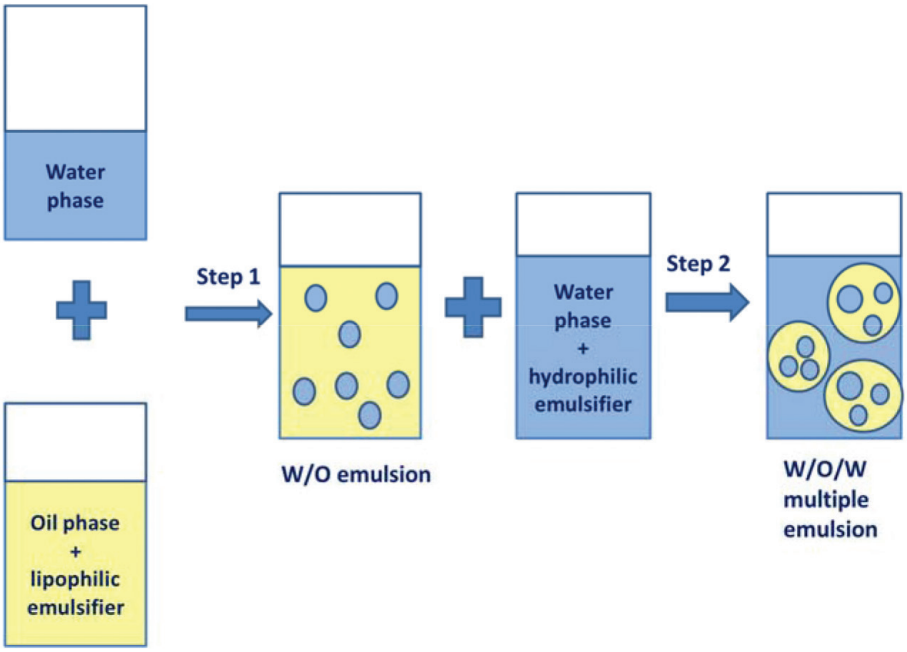

Figure 3. Schematic representation of two-step emulsification method for obtaining a W/O/W multiple emulsion (by author)

Also the choice of the method of homogenization, especially for the second step of the two-step process, is essential for the obtaining of a stable system [6]. It is worth noting that the introduction of too much energy into the system in the second step of homogenization may lead to its destabilization [31]. The following methods of homogenization are typically used in the obtaining of multiple emulsions: mechanical homogenization, high-pressure homogenization, sonification, and membrane emulsification.

The high-pressure homogenization is based on high-pressure pumping the sample through small inlet openings. The high-pressure homogenization equipment operates in the

range from 50 to 100 MPa [32]. Owing to the simultaneous action of shear forces, turbulent flow, and cavitation, particles of the size of less than 1nm can be obtained. The parameters which contribute to reducing the size of the internal phase particles in this method include: pressure, number of cycle, and process temperature [33–35].

Micro-fluidization is a high-pressure homogenization technique. It is carried out in micro-fluidizers, which comprise positive displacement pumps, operated at high pressures ranging from 150 to 650 MPa. Moreover, the device comprises a high--pressure pump and a mixing chamber which is composed of micro-channels [32, 36]. The emulsion is made to flow through that chamber by the high-pressure pump [33, 37]. The sample is separated to form two streams in the micro-channels. The streams collide which each other, causing a shear force effect. The resulting emulsion system is filtered under nitrogen to eliminate any larger, non-homogeneous particles of the emulsion. The size of the droplets, stability and viscosity of the obtained formulation is affected by the homogenization pressure and number of cycles [37, 38].

In the membrane emulsification method, the emulsions are forced to flow through a microporous membrane using a high-pressure pump. As the result of the turbulent and laminar flow taking place in the process, the emulsion droplets break up, forming smaller droplets. The resulting emulsion is characterized by monodispersity and resistance to creaming and sedimentation [38, 39].

5. METHODS OF ANALYSIS OF THE PHYSICO-CHEMICAL PROPERTIES OF MULTIPLE EMULSIONS

The multiple emulsion systems are characterized by analysing their physicochemical properties such as: structure, particle-size distribution, emulsion type, viscosity, stability and ability of encapsulation of the active components.

5.1. ANALYSIS OF PARTICLE SIZE AND MORPHOLOGY

The size of the multiple emulsion particles can be assessed by means of an optical microscope, scanning electron microscope, and particle size analyser of which the functioning is based on laser diffraction **(these methods are described in chapter II. 5.)**.

DLS technique can be used for assessing the size of the internal water phase particle of the W_1/O simple emulsion. For the emulsion $W_1/O/W_2$, the oil phase particle size is usually assessed by static light dispersion [6].

The morphology of these systems is usually analysed by polarization microscopy, contrast phase microscopy. Naturally, optic microscopy is not sufficient if the external and

internal water phase have the same refraction coefficient. The CARS method (coherent anti--stokes raman scattering) can be used as an alternative in that case. It enables the contrast imaging of non-colored samples. In this method of microscopy, a near-IR light is generated by the laser and it interacts with vibrating particles in the sample, causing a change of their energy and CARS signal emission. The signal is used for the specific visualization of various fractions of the particles in the formulation. This enables the morphological analysis and compositional analysis of the sample [40, 41].

5.2. EMULSION TYPE ANALYSIS

The characterization of a multiple emulsion must start from identification of the emulsion type, that is, either O/W/O or W/O/W. The identification methods are based mainly on the examination of the physico-chemical properties of the system, to find out which of the two – the water phase or the organic phase – is the continuous phase. The following methods are used for the purpose [42]:

a) Dilution method – the method is based on the observation of the behavior of emulsion droplets on contact with the water phase and the oil phase. It shows that the external emulsion phase is or is not mixed with these phases. However, sometimes, the method provides ambiguous results, in which case some extra tests using other methods must be carried out.
b) Indicator method – two types of colorants are used, one of which is soluble only in the oil phase and the other – only in the water phase. In practice, sudan IV is often used for the oil phase and methyl orange is used for the water phase. The tests are carried out by either one of the two methods. The first method is to color one of the phases before obtaining the dispersed system, this is followed by microscopic observation to find out whether the colored phase is the continuous phase or the dispersed phase. In the other method, the colorant is added to the emulsion in a test tube, mixed gently, and then a droplet of the emulsion is placed on the microscope slide and observed using a microscope to make sure that the external phase is colored. The method detects the presence of simple emulsions or multiple emulsions in the system.
c) Conductivity method: the method is based on the measurement of electrical conductivity (S/cm) of emulsion systems. For emulsion systems in which the external phase is a polar solvent (usually water phase with addition of some electrolytes), the value of conductivity is high (more than 20 μS/m, depending on the electrolyte and the water phase concentration) [18]. In contrast, when the continuous phase is a non-polar solvent, then the emulsion has a very low electrical conductivity (less than 10 nS/m) [43].

5.3. MEASUREMENTS OF ENCAPSULATION EFFICIENCY

Double emulsions have the potential for encapsulation of both hydrophobic as well as hydrophilic drugs, cosmetics, foods and other high value products. Techniques based on double emulsions are commonly used for the encapsulation of hydrophilic molecules, which suffer from low encapsulation efficiency because of rapid drug partitioning into the external aqueous phase when using single emulsions [44].

Encapsulation is the process of entrapping biologically active substances inside the vesicle of the sphere structure. It provides an overall protections to the agent from external factors, improves its stability, activity, targeted delivery and sustained release. Encapsulated material can be released for a specified time at a predetermined rate, depending on the process conditions for release-controlled or modified release [45]. Various techniques are employed to form the capsules, among others spray drying, spray cooling, single and double emulsion- solvent evaporation, liposome entrapment, coacervation, suspension polymerization and emulsion polymerization [46, 47].

The efficiency of encapsulation (EE) in the appropriate phase of the dispersed emulsion in the production process is another important value which characterizes the multiple emulsion as an active component carrier. The efficiency of encapsulation defines the active substance content and is found as the difference between the component weight that is introduced into the system in the emulsion production process and the component weight that remains in the continuous phase of the obtained multiple emulsion, referred to the introduced weight [48, 49].

The parameter can be calculated from equation (1) [17, 48]:

$$EE = 100[1 - C^*V^*/C_wV_w] \tag{1}$$

where:

- C^* – concentration of active component in the external water phase of the W/O/W emulsion,
- V^* – volume of the external water phase of the W/O/W emulsion,
- C_w – known concentration of active component, introduced into the internal phase of the W/O simple emulsion,
- V_w – volume of the internal water phase of the W/O simple emulsion.

The efficiency of encapsulation would be 100% if the concentration of the internal water phase and the active component concentration introduced into the W/O simple emulsion were the same during the re-emulsification, that is, during the second step of the process to obtain the multiple emulsion [50].

6. THE EFFECT OF MULTIPLE EMULSIONS ON THE RELEASE OF ACTIVE COMPONENTS

According to the literature, the multiple emulsions are an interesting form of cosmetic and pharmaceutical formulations, because they have a number of advantages, including their ability to simultaneously encapsulate the hydrophilic and lipophilic active components, and their controllable release.

The effect of the emulsion type (O/W, W/O and W/O/W) on the transdermal absorption of the hydrophilic active components having medium (metronidazole) and high polarity (glucose) was studied by Ferreira et al. [51]. The emulsion systems they studied had the same qualitative and quantitative compositions: 20% paraffin oil, 3.2% lipophilic surfactant (Hypermer A60, a modified polyester), 0.8% hydrophilic surfactant (Synperonic PE/F127, block copolymer of ethylene oxide and propylene oxide), 0.5% heptahydrate of magnesium sulfate, 0.5% active component, and water. The difference was the emulsification method. The multiple emulsion was obtained by homogenizing (700 rpm for 40 min) 80% of a W/O simple emulsion (obtain in the first step) with 20% of the external water phase comprising a hydrophilic emulsifier. The O/W and W/O simple emulsions were prepared in the classic way. The aqueous phase containing heptahydrate of magnesium sulfate and metronidazole were added to the oil phase. The skin permeability tests indicated that in the case of metronidazole, a medium polarity medicine, the transdermal transport was fast and comparable for all the emulsion types tested. For the high polarity compound (glucose), the rate of absorption from the O/W emulsion was much higher than for the other carriers.

Doucet et al. [52] compared the permeability of caffeine from the O/W classic emulsion and the W/O/W multiple emulsion through different membrane models (fragments of human skin obtained from plastic surgery, synthetic cellulose membranes and a model of human epidermis). The W/O simple emulsion was built of the oil phase composed of cetearyl octanoate, vaseline oil and a lipophilic emulsifier (cetyl dimethicone copolyol), and the water phase composed of heptahydrate of magnesium sulfate and 1.25% of an active component. The multiple emulsion was obtained by mixing gently (200–900 rpm) 80% of the simple emulsion with the external water phase comprising a hydrophilic surfactant (ethylene oxide/propylene oxide block copolymer). The composition of the O/W emulsion and that of the multiple emulsion were the same. The difference was that in the case of the classic emulsion, the water phase and the oil phase were combined by vigorous mixing with a homogenizer at 10,000 rpm. The permeability tests indicated that, regardless of the membrane type, the caffeine absorption was 2.6 times as slow as that from the simple emulsion. Also the t diffusion rate (flux) and the permeability coefficient of caffeine were much lower than those for the O/W classic emulsion.

Carlotti et al. [7] studied the release of mandelic acid (alpha-hydroxy acid having an anti--acne effect, among other things) from the internal and external water phase of a W/O/W

emulsion. The multiple emulsion was obtained by the two step method. In the first step the original W/O emulsion was obtained in which the internal phase was an aqueous solution of heptahydrate of magnesium sulfate, and the oil phase comprised a mixture of paraffin oil, polyethylene and octyloctanoate as well as lipophilic emulsifiers (polyglyceryl-2--sesquiisostearate and methylglucose dioleate). The simple emulsion was then homogenized with the external water phase comprising hydrophilic surfactants (saccharose monolaurate and saccharose monostearate) as well as D(+)-glucose, and xanthan gum. Mandelic acid was added into the internal or external water phase at a concentration of 0.025%, after which the release of the active agent from each phase was compared. The study indicates that the rate of the release of mandelic acid from the external phase of the multiple emulsion was 2.5 times as fast as that from the internal water phase of the W/O/W emulsion. This confirms that these are complex systems and that their three-phase structure enables the controllable release of their active components.

Gomes et al. [19] studied the effect of the carrier type (O/W or W/O/W emulsions, ointment) in the *in vitro* release study as well as the transdermal permeation of paramomycin – an aminoglycoside antibiotic having a bactericidal and anti-parasitic effect and used for treating skin leishmaniasis. The multiple emulsion and the classic emulsion had the same qualitative and quantitative compositions (22% paraffin oil, 3% Cetyl PEG/PPG-10/1 Dimethicone, 1% block copolymer of ethylene and propylene oxide, 1% paramomycin sulfate and 73% 0.08 M sodium chloride), but they were obtained by different emulsification methods. To obtain the multiple emulsion, in combining the original W/O emulsion with the external water phase (the second step of emulsification) the mixing rate was much slower (400 rpm) than in the obtaining of the original W/O emulsion and the classic O/W emulsion (8000 rpm). The ointment was prepared by mixing the medical preparation with paraffin oil. The release tests were performed only for the O/W emulsion – a multiple emulsion with paramomycin in the internal water phase – and for a multiple emulsion with the antibiotic in its external water phase. It was proved that the release of the antibiotic from the O/W emulsion was the fastest and the highest, but its release from the multiple emulsion with paramomycin in the internal water phase was the lowest. It is believed that the oil membrane phase is the factor which limits the release of hydrophilic paramomycin from the internal water phase of the multiple emulsion. Also the transdermal permeation of the medical preparation was much higher in the case of the O/W emulsion (87.1% ± 3.9), in comparison with the W/O/W emulsion (14.7% ± 0.5) and the ointment (16.6% ± 0.1).

Kim et al. [53] studied the effect of the multiple emulsions on the bioavailability of apigenin – a bioactive compound of a flavonoid group having anti-inflammatory properties. The internal water phase of the emulsion was obtained by dissolving apigenin in ethanol. The oil phase was obtained by dissolving the emulsifier (Polysorbate 80 or Polyglycerol polyricinoleate) in soybean oil or orange oil. Distilled water was the external water phase of the multiple emulsion. Apigenin release tests showed that the release of the flavonoid (53.99%) from the multiple emulsion comprising orange oil and Polysorbate 80 was the

highest and the lowest release was obtained from the emulsion comprising Polysorbate 80 and soybean oil (33.4%). The authors believe this is attributable to differences in the particle sizes of the respective multiple emulsions. The emulsion comprising Polysorbate 80 and orange oil was characterized by the lowest particle size. Moreover, the bioavailability of apigenin was higher, in comparison with the flavonoid suspension in DMSO (Dimethylsulfoxide) (49.2%).

Miastkowska and Wójtowicz [54] studied the effect of the emulsion type (O/W, W/O and O/W/O) on the release of dexamethasone, corticosteroid used to treat many different inflammatory conditions such as allergic disorders and skin conditions. Olive squalane was used as the oil of the emulsions. The Phytocream 2000 (INCI: Potassium Palmitoyl Hydrolyzed Wheat Protein, Glyceryl Stearate, Cetearyl Alcoho) played the role of a hydrophilic emulsifier, while the Cithrol PG3PR (INCI: Polyglyceryl-3 Polyricinoleate) was the lipophilic emulsifier. The multiple emulsion were composed of 80% primary emulsion, 5% of W/O emulsifier and 15% of squalene. The drug release test was used for evaluation of the obtained formulations. The released amount of dexamethasone through dialysis membranes from multiple emulsion was 15%. This value was lower than that obtained in a reference tests (18% from O/W emulsion and 23% from W/O emulsions). The release profile of dexamethasone from multiple emulsion confirmed that this system can play a role as a carrier with delayed release of the active substance.

The above information from the literature indicates that active substances are released at a much lower rate from the multiple emulsions, in comparison with the classic emulsions. This is connected with their three-phase structure, owing to which the active components can be released in a controllable manner. Of course, as for the other carriers, the active component type and its interactions with the emulsion base are also of importance.

7. APPLICATION OF MULTIPLE EMULSIONS

For many years, multiple emulsions have been used in the food-processing industry as low--fat bases for nutrients (such as sodium ascorbate, Omega-3 polyunsaturated fatty acids, vitamin B_{12}, curcumin, folic acid, *trans*-resveratrol) and as carriers for edible flavors which are able to mask their disagreeable taste [6].

Moreover, probiotics such as the bacterial strain *Lactobacillus acidophilus, Lactobacillus salivarius* or *Lactobacillus delbrueckii* have been encapsulated in them in order to extend the life of the bacterial cells. Santos et al. used them successfully for extending the duration of the cooling effect of menthol and extending the taste of xylitol in chewing gum [6].

As regards the ability of multiple emulsions to protect active components from the effect of external factors and to be released in a controllable manner, they are used in the pharmaceutical industry [18, 31, 55] as carriers for adjuvants [56], hormones [57, 58], steroids [24, 59] and enzyme immobilization systems [60].

In the cosmetic industry, they are emulsion bases for a number of bioactive components, such as mandelic acid [7], α-arbutin, lactic acid, and niacinamide [61], vitamin C and wheat protein [18, 62], the palmitate of vitamin A, liposoluble sun filters, natural or synthetic ceramides [63].

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IV. SUB-MICRON VEHICLES OF BIOLOGICALLY ACTIVE SUBSTANCES

Elwira Lasoń

ABBREVIATIONS

AFM – atomic force microscopy
CMC – critical micelle concentration
DL – drug loading
DLS – dynamic light scattering
EE – encapsulation efficiency
GUV – giant unilamellar vesicles
HPH – high pressure homogenization
LUV – large unilamellar vesicles
MLV – unilamellar vesicles
NLC – nanostructured lipid carriers
O/W – oil in water emulsion
OLV – oligolamellar vesicles
PCS – photon correlation spectroscopy
PI – polydispersity index
PIT – phase inversion method
SEM – scanning electron microscopy
SLN – solid lipid nanoparticles
SUV – small unilamellar vesicles
TEM – transmission electron microscopy
W/O – water in oil emulsion
ZP – zeta potential
HPLC – high-performance liquid chromatography

1. SUB-MICRON VEHICLES OF BIOLOGICALLY ACTIVE SUBSTANCES

Colloidal vehicles of biologically active substances, like liposomes or nanoparticles, can be applied to improve the therapeutic index of existing and new medical drugs, as well as increase their efficacy and/or reduce their toxicity. If the systems are carefully designed for the location and route of administration, they may provide solutions to issues caused by new medical drug classes (including peptides, genes, and oligonucleotides). They may also serve as alternatives to traditional, highly hydrophobic drugs. In these systems, the *in vivo* fate of an active substance will no longer depend on the properties of its medicinal drug, it will also depend on the vehicle system which should facilitate a controlled and targeted release of the active substance according to the therapeutic design [1–8]. These reasons back the recent popularity of sub-micron colloidal vehicles, especially in localised applications (on the skin).

The delivery of therapeutic substances without chemical penetration enhancers, like surfactants and organic solvents, is extremely desirable when it is necessary to maintain the normal protective functions of the skin and prevent its irritation or damage. The vehicles of active compounds and drugs, including liposomes, polymer or lipid nanoparticles, inorganic nanoparticles, and micro or nanoemulsions, offer an alternative to chemical penetration enhancers. Their intended use includes prevention of skin damage and enhancement of active substance penetration with a controlled release [9–11].

Innumerable types of cosmetics, demonstrating various properties and containing many active substances are available on the market. However, from a consumer's point of view, it is the effectiveness of cosmetic products that counts. The fact that they contain active substances with a potentially perfect effect on the skin does not mean that they will fulfil the manufacturer's promises. Properties of a cosmetic product are determined primarily by the active substance's ability to act: the more of it penetrates through the corneum, the more effective is the product. At the time when interest in cosmetology is increasing, together with expectations of consumers, understanding of effective active substance carriers is essential to providing successful products, appreciated by users.

1.1. POLYMER NANOPARTICLES

Polymer nanoparticles are attractive vehicles for localised skin application since they can control the release rate of active substances by diffusion from the polymer matrix and penetration of its layers. Nanocapsules and nanospheres are among the most popular polymer nanoparticles in skin penetration applications [12–16]. Research has also been carried out into polymer vesicles [17] and polymer core-multishell dendritic vehicles [18].

Nanoparticles are most often made from cellulosics, poly (alkyl cyanoacrylates), poly (methylidene malonates), polyorthoesters, polyanhydrides, and polyester compounds,

including poly (lactic acid), poly (glycolic acid), and polycaprolactone and their copolymers [6]. The most popular manufacturing processes of nanoparticles include coacervation, evaporation and diffusion of solvents, interfacial polymerization, and shear degradation (i.e. high-pressure homogenization).

In general terms, nanoparticles are highly stable due to a relatively rigid matrix which retains its form for a long period of time. Unlike emulsions or liposomes, nanoparticles can provide highly effective protection of incorporated, sensitive active compounds and, as said before, controlled released of drugs from polymer matrices [19–21]. However, nanoparticles as drug vehicles are encumbered with certain deficiencies, including retention of organic solvent residues from manufacturing processes, the toxicity of the polymer, and difficult large-scale production of the vehicles. Polymers often suffer from erosion, diffusion of the drug substance from the matrix, or surface desorption; the concentration levels of produced polymer suspensions is relatively low and rarely exceeds 2% [22, 23].

1.2. MICRO- AND NANOEMULSIONS

Microemulsions are dispersive systems usually with the droplet size below 100 nm and which do not tend to coalesce [24, 25, 36, 37]. Microemulsions are spontaneously formed systems in presence of a hydrophilic ingredient, a lipophilic ingredient, a surfactant and a co-surfactant [26, 27, 38, 39]. The systems are characterised by certain and very specific physicochemical properties, including transparency, optical isotropy, low viscosity, and thermodynamic stability [26, 28, 38, 40]. Microemulsion are widely used as high-efficacy drug vehicles for percutaneous and localised on-skin delivery of active substances [29, 41].

Most microemulsions have a very low viscosity, which may inhibit their application for percutaneous delivery of active substances due to certain utility drawbacks [30, 42]. The main mechanism behind the benefits of microemulsions in percutaneous drug delivery include: a high solubility potential of hydrophilic drugs in microemulsion systems, enhancement of penetration of microemulsion ingredients, and improved thermodynamic activity of drugs in these vehicles [26–28, 38–40].

Nanoemulsions are isotropic dispersed systems of two non-miscible liquids. A nanoemulsion usually comprises an oil phase dispersed in an aqueous (water) phase or vice versa; the resulting droplets are sized in nanometres. Unlike microemulsions, nanoemulsions are thermodynamically unstable; their formation alone usually requires high-energy processing [31, 43]. Nanoemulsions are susceptible to Ostwald ripening, which results in instability of the emulsion, e.g. creaming of flocculation. It is, however, possible to obtain nanoemulsions which are metastable for extended periods by very low droplet dimensions and application of suitable surfactants. Nanoemulsions are vehicle systems which are non-irritating, non-toxic, and can be applied on the skin and the mucous membranes; they can also be used in enteral and parenteral applications. Nanoemulsions are suitable as vehicles for hydrophobic and hydrophilic drugs alike; they have been most

popular in cosmetics. Nanoemulsions can be manufactured in three different ways: by high--pressure homogenization, ultrasonic dispersion, and phase inversion temperature (PIT) method. Percutaneous administration of active ingredients in nanoemulsions has seen a decline in applications recently due to inherent stability problems of this administration form. Examples of compounds administered in nanoemulsion vehicles include γ-tocopherol, caffeine, DNA plasmides, methyl salicylate, aspirin, and insulin [32, 44]. Nanoemulsion applications as vehicles of analgesics, corticosteroids and anticarcinogenics is critical; these drug types administered in nanoemulsions can act immediately without the obstacle of administration barriers [33–36, 45–48].

1.3. INORGANIC NANOPARTICLES

Inorganic nanoparticles feature unique chemical, physical, mechanical and optical properties which has made them widely popular in different scientific and industrial fields: from catalytic processing to solar panels and sensors, and nanomedicine [37–39, 49–51]. Several types of inorganic nanoparticles have been recently successful in percutaneous and on-skin delivery of drugs, thanks to rational design of the vehicles. The inorganic make-up of nanoparticles provides long-term stability.

Inorganic nanoparticles generally feature certain universal properties, making them feasible in dermatological applications: a large availability, a good biocompatibility in most cases (exception is nanosilver), a potential for targeted delivery (e.g. with selective destruction of cancer cells without damage to normal tissues) and controlled release of active ingredients [40, 52]. The benefits make inorganic nanoparticles feasible for application in cosmetic anti-aging products, acne treatment, skin moisturisers and skin care products, and treatment of dermal disorders, including skin cancer or vitiligo (acquired leucoderma). The prime examples of inorganic nanoparticles are: zinc dioxide (ZnO) and titanium dioxide (TiO_2) used in UV filters. These mineral compounds can absorb and disperse UV radiation to protect the skin from its harmful effects. Much research proved that nanoparticles of TiO_2 and ZnO cannot penetrate into the skin without penetration enhancers, irrespective of the size and the coating of these molecules [41, 42, 53, 54].

Metal nanoparticles, including silver, have gained a general acclaim due to unique antibacterial and antifungal properties [43, 55]. Silver nanoparticles have a huge potential for prevention of inflammation of wounds and can promote the wound healing processes by localised application. Localised administration of preparations with silver nanoparticles requires assessment of safety and percutaneous penetration capability. The penetration capability of silver nanoparticles is much lower than in other inorganic metal nanoparticles, like gold; the high ratio of free ions is precipitated as Ag-S in the outermost layer of the *stratum corneum* [44, 56].

Gold nanoparticles are ideal intracellular target vectors; their size can be easily adjusted between 0.8 nm and 200 nm [45]. The surface of gold nanoparticles can be modified to

enable different functions and a good biocompatibility. It is also possible to trace the trajectory of these non-toxic nanoparticles in cells [45, 57].

Although rich in benefits, such as feasibility as active substance vehicles and a successful history of localised and transdermal drug delivery, inorganic nanoparticles may feature harmful properties which still have not been evaluated in full. Note that inorganic nanoparticles may demonstrate various nanotoxic properties and differences in skin penetration dependent on the presence of additional components, shape, size, cargo, or hydrophilicity.

2. LIPOSOMES

Liposomes, described for the first time by Bangham et al. in 1960s [46], for over five decades became an integral element of scientific and clinical research on delivery of active substances. Their potential benefits have been especially valued by cosmetic and pharmaceutical industries [47–49]; the substances have also found use in the food industry [50, 51]. Liposomes also have been broadly used in medicine. As carriers, they effectively deliver drugs to blood circulation, slowly releasing the active substance, and thus protecting the organism against the adverse effects. Liposomes can be used to administer therapeutic agents in antineoplastic treatment, and to stimulate expression of signal proteins in regenerative medicine. They also play an important role as particles that reach the places unavailable for endoscopic tests. Liposomes have also been used as non-viral vectors in gene therapy [52].

Due to the ability to enclose different substances in liposomes, the research is regarding these delivery systems in developing in two key directions: using liposomes as simple and convenient models of the biological membrane, and using them as the structures to transport and deliver the enclosed substances to cells [53]. The first studies exploring possible external uses of liposomes as carriers of active substances were published in the 1980s. The first marketed cosmetic products containing liposomes was Capture by Dior. Since then, the cosmetic industry demonstrates an increasing interest in these delivery systems.

2.1. STRUCTURE OF LIPOSOMES

Liposomes are sub-micron vesicles containing a bilayer of naturally amphiphilic phospholipids, composed of hydrophobic chains forming the inside of the membrane, and of hydrophilic polar groups on the outside the membrane (Figure 1). Immersed in an aqueous solution, at concentrations above their critical micelle concentration (CMC), these particles create bilayer membranes. This specific structure of a liposome allows it to incorporate and protect hydrophilic and lipophilic active ingredients from chemical and biological degradation.

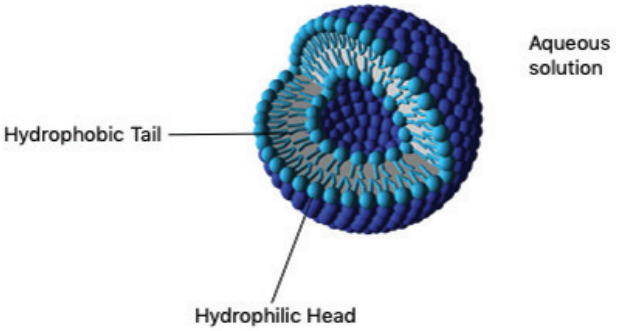

Figure 1. Liposome structure (by author)

The most important materials used in liposome production include lecithins, sphingolipids and, to a limited extent, non-ionic surfactants [54].

Lecithin is a mixture of phospholipids with various fatty acid residues, depending on its source. It is usually obtained form soy or rape seeds, but egg yolk is also a rich source of lecithin. Lecithins are produced in the process of oil hydrogenation, by clearing the viscous, yellow-brown lecithin slime. Lecithin slime is soluble in fats and organic solvents. Soy beans constitute the principal source for industrial lecithin production, as egg yolks processing is too expensive [55, 56].

Soy lecithin contains mostly phospholipids, triglycerides, sterols, glycolipids, and a small quantity of fatty acids. The percentage share of individual ingredients is as follows:

- phosphatidylcholine ca. 33%,
- phosphatidylethanolamine ca. 14%,
- phosphatidylinositol ca. 17%,
- phosphatidyl acid ca. 6.4%.

The composition of lecithin obtained from egg yolks slightly different, and it phosphatidylcholine content is 66–76%.

Phosphatidylcholine (Figure 2), the principal component of lecithin, is a water-soluble substance that disperses creating multilayer micelles. It does not produce a molecular solution, but gathers the hydrophobic substances inside the micelles [53].

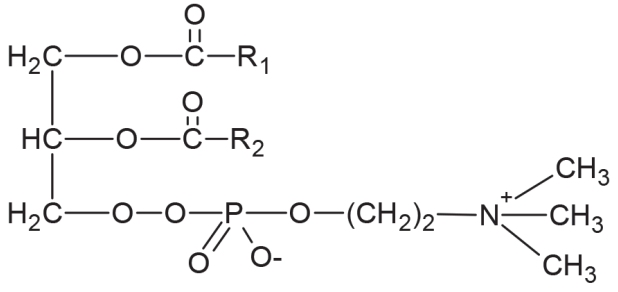

Figure 2. Phosphatidylcholine molecule (by author)

Lecithin is a very good emulsifier and biosurfactant. As an amphiphilic molecule, it is composed of a lyophilic part – "tail", in this case created by fatty acid residues, and "head", containing phosphoric acid residues and choline, as well as glycerol. Depending on the OH group that the phosphocholine group combines with, α-lecithins (primary group) and β-lecithins (secondary group) are formed (Figure 3) [56].

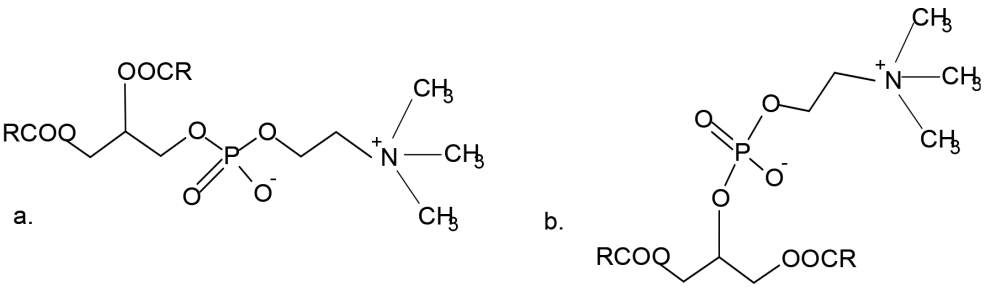

Figure 3. Chemical structure of α – lecithin (a) and β – lecithin (b) (by author)

2.2. CLASSIFICATION AND PROPERTIES OF LIPOSOMES

A dynamic development of liposome technology allowed to obtain various types of liposomes, whose membranes may be structurally modified using sterols, polymers, proteins or sugars [57]. Liposomes can be classified by size, the number of bilayers, and the composition (Table 1); in terms of cosmetic and pharmaceutical applications, efficacy requires a proper selection of the liposome due to the specific properties of these vehicles, by the administration route, and post-application pharmacokinetic processes [58, 59].

Table 1. Classification of liposomes [60]

Classification by size and phospholipid bilayer count	Classification by composition
• SUV (small unilamellar vesicles) sized 20–100 nm • LUV (large unilamellar vesicles) sized > 100 nm • GUV (giant unilamellar vesicles) sized > 1,000 nm • OLV (oligolamellar vesicles) sized 100–1,000 nm • MLV (unilamellar vesicles) sized > 500 nm	• Conventional liposomes • Long-circulating liposomes • Cationic liposomes • Stimuli-sensitive liposomes (pH, temperature, magnetic field) • Immunoliposomes

The most basic types of liposomes include SUV, LUV and MLV. The smallest (20–100 nm) liposomes are SUV, composed of a one layer of phospholipids. The external lipid layer is larger than the inner one, so it contains more lipid particles, which increases the membrane tension. It may result in unwanted fusions between particles. LUV are large vesicles of 100–400 nm, composed of a lipid monolayer. These particles are stable and do

not demonstrate any tendency to undergo fusion. They are characterised by a favourable distribution of lipids, both in the inner and outer layer. MLV are of 500 nm to a few μm in diameter, and they are composed of many concentric layers.

Regarding the volume of the aqueous space in a liposome, the most beneficial are LUV particles, as SUV and MLV can contain only a limited quantity of aqueous solution, compared to the amount of lipids required to encapsulate it, or the quantity of the solution is to low to be effective [61].

Liposome structures can exist in two thermodynamic states: as gel and in liquid form. They have different phase transition temperatures. In gel liposomes, the phase transition temperature is above the room temperature, and in the case of liquid structure, the phase transition temperature is higher than the room temperature. It determines the choice of lipids used for the liposome shell. For instance, liposomes composed of saturated phospholipids are solid, whereas those containing unsaturated phospholipids are liquid in room temperatures. Therefore, it is possible to regulate liquidity and membrane permeability by using polyunsaturated phospholipids containing linoleic and linolenic acids [57].

In the context of liposome structure, another important issue is their durability. Regarding the physical properties, liposomes rehydrated after lyophilisation, dehydration or freezing preserve their stability, and do not demonstrate changes in transparency. Only very intensive mixing may damage the bilipid layer. To protect the membrane, liposomes should not come in contact with surfactants, especially the anion ones, as it results in a change of charge on the membrane surface, and destabilisation of its structure, due to hydrolysis of phosphatidylcholine. Solvents, such as ethanol or salts with a high degree of dissociation, also demonstrate an adverse effect of liposome stability, Addition of substances that limit the mobility of hydrocarbon chains, e.g. cholesterol or the substances increasing the degree of solvation, helps to prevent destabilisation. Moreover, polyunsaturated lipid groups that are susceptible to oxidation can be protected using antioxidants. Oxidation of lipid structures increases permeability of membranes and loosens their structure, which may lead to destabilisation of the liposome structure, resulting in a release of the active substance. This is associated with a reduced durability and effectiveness of the cosmetic product. In addition, liposome stability can be affected by the type of formulation in which given structures are used. For instance, xanthan gum used in gel products may lead to destabilisation of liposome structures with time, and to a release of the active substances, which will significantly reduce the effectiveness of the product [57]. Nevertheless, using liposomes in hydrogels does not prevent their effective transportation into the deeper skin layers. However, it should be considered that hydrophilic polymers may affect the physical stability of certain liposomes, so the stability of liposomes should be controlled in every hydrogel-based formulation using these systems [62].

The ability to encapsulate active substances in liposomes depends on a few factors, such as the type of phospholipids used, the physicochemical properties of the active substances, and the techniques of producing capsules. Selection of proper phospholipids is essential for the stability and the release rate of the active substances from liposomes. Using polymerised

amphiphilic compounds increases the stability and resistance to changes in pH, effects of detergents or high temperatures. However, the assessment of the effects on the release rate is more complex. In some cases when polymerised phospholipids are used the permeability of the active substance through the liposome membrane is reduced, and conversely – in other cases the permeation increases. The polymerised chain of phospholipids may include vinyl, acetylene and diacetylene groups. In the case of diacetylene groups, which contain sp-hybridised carbons, durable liposomes are formed. After exposing them to UV radiation, a highly cross-linked membrane is formed. The mobility of the lyophilic chains is reduced, and they come closer, creating spaces through which the active substances can pass from the interior of the liposome. It increases the release rate of the active substances [63].

The type of phospholipids used certainly affects the quantity of the substance enclosed in liposomes. It appears that liposomes obtained form unsaturated phospholipids enable encapsulation of greater volumes of materials than those from saturated phospholipids. The number of unsaturated bonds in a phospholipid molecule affects the volume of the enclosed active substance to a higher degree than e.g. the length of the phospholipid chain [64].

Durability of liposomes may be increased by using substances that, after penetration of the lyophilic chains, reduce their mobility (e.g. cholesterol), or substances that increase the degree of membrane solvation (e.g. proteins). In addition, the durability of liposomes can be increased with antioxidants. Liposomes are sensitive to oxidation, and polar oxidation products result in loosening of the membrane structure. Tightly packed acyl groups theoretically protect the molecules against oxidation; however, additional strengthening of the structure via the use of antioxidants significantly improves the durability of liposomes. Therefore, e.g. tocopherol should be used to prevent membrane destabilisation [57].

In most cases, liposomes tend to aggregate and fuse, which results in a leak of the enclosed substance, especially hydrophilic small molecules. A solution to this problem involves combining active substances with stable nanoparticles of e.g. chitosan, to form a permanent core inside the liposome, which might have a beneficial effect on the stability and control over the release of the active substances [65].

It is possible to produce liposomes containing phosphatidylcholine and cholesterol, with increased stability due to the association of amphiphilic polymers. It has been demonstrated that when liposomes introduced into an O/W emulsion, the ones with associated polymers are more stable, as they do not undergo fusion, and the active substances do not leak from the capsule interior [66]. One of such polymers is polymethyl methacrylate. The thickness of the capsule wall may be modified by changes in the polymer concentration in the oil phase of the emulsion before emulsification of phases. This phenomenon can be observed using a scanning microscope [67]. Internal wool lipids (IWL) are also used in liposome technology, as they are rich in cholesterol, free fatty acids, cholesterol sulfate and, importantly, ceramides. It has been demonstrated that using IWL in the production of liposomes, especially due to the presence of sterol sulfates, provides vesicles of high stability [68].

2.3. PREPARATION OF LIPOSOMES

Depending on the type of liposome required for a particular use, appropriate preparation methods are applied. Important factors include the size of liposomes, the number of layers and the production scale (Table 2). In a laboratory-scale production 1–100 cm^3 of suspension at 10–100 mg/cm^3 can be obtained, on a semi-laboratory scale 10 dm^3 of suspension at 100–300 mg/cm^3 can be produced, and on an industrial scale 10 dm^3 of suspension at 400 mg/cm^3 can be obtained [69].

The most important methods of liposome preparation include:

- Dry lipid film hydration,
- Ultrasonic disintegration,
- Ethanol injection,
- Reversed- phase evaporation,
- Dehydration-rehydration,
- Freeze-and-thaw (FAT) procedure,
- Calibration (extrusion through pores of certain sizes),
- Using French press.

Table 2. Methods of preparation and the use of various classes of liposomes

Type of liposomes	Usage	Preparation method
MLV	To enclose lyophilic solutions or when the effectiveness of enclosure of hydrophilic solutions is insignificant	Laboratory-scale production: thin lipid film hydration in aqueous solution, Industrial-scale production: lyophilisation, spray-drying, injection of a hydrophobic solution into the hydrophilic solution, with simultaneous solvent evaporation
SUV	When the effectiveness of encapsulation of aqueous solutions is insignificant	Disintegration of MLV liposomes with ultrasounds French press
LUV	For effective encapsulation of aqueous solutions	Detergent removal method Reversed – phase evaporation (the most efficient one)

2.3.1. DRY LIPID FILM HYDRATION METHOD

The hydrophobic substance to be enclosed inside the vesicle should be dissolved in chloroform or a chloroform-ethanol mixture, and added to the solution of the phospholipid mixture. Adequate volume (i.e. 1 cm^3) of the solution is placed in the evaporator round bottom flask and it is evaporated until a dry lipid film forms on the flask walls. The obtained lipid film is dried for 2–6 hours using calcium chloride in vacuum extractor. After that

period, approximately 1 cm^3 of a hydrophilic solution is added to initiate the process of lipid hydration. The flask is shaken manually until a milky suspension composed of MLV liposomes is formed. It is important to remove the part of the hydrophilic solution that has not been enclosed, using gel filtration on a column with molecular sieves.

2.3.2. ULTRASONIC DISINTEGRATION

The MLV liposome suspension is sonicated using an immersion disintegrator or a high-energy bath. The process is continued until the solution is clear. To avoid the risk of overheating, a cycle involving 2 minutes of sonication, and a 3-minute break should be used. After the process is completed, gel filtration should be performed if the enclosed compound is hydrophilic.

2.3.3. ETHANOL INJECTION METHOD

In this method adequate volume (i.e.10 cm^3) of the compound to be encapsulated in liposomes is mixed on a magnetic stirrer and heated above the phase transition temperature of the lipid mixture. Minimal volume (up to 750 mm^3) of ethanol lipid solution is injected vigorously into the warmed-up mixture, using Hamilton syringe. The ethanol residues are eliminated from the solution via a triple dialysis. The obtained mixture is thickened by ultrafiltration, and gel filtration is used to remove the aqueous phase that has not been encapsulated.

2.3.4. REVERSED-PHASE EVAPORATION

Adequate volume (i.e. 1 cm^3) of the prepared solution of lipids in ethanol is placed in a round bottom flask of a rotary vacuum evaporator, and solvents are evaporated. The obtained lipid film is dissolved in 6 time more volume of ethyl ether, or in the same amount of chloroform with isopropyl ether, mixed at 2:1 ratio. The mixture is placed in a beaker, together with 3 cm^3 of the compound to be encapsulated, warmed up to approximately 40°C, and sonicated for ca. 10 minutes. When a homogeneous emulsion is obtained, the solvents are evaporated on a vacuum evaporator, very carefully, as the mixture may froth. After the process stops, the liposome mixture should be gel-filtered.

2.3.5. DEHYDRATION-REHYDRATION METHOD

The MLV liposomes prepared using dry lipid film hydration method are lyophilised, and the obtained material is agitated out of 1 cm^3 of distilled water for rehydration, and then gel-filtered.

2.3.6. FREEZE-AND-THAW (FAT) PROCEDURE

This method increases the effectiveness of MLV liposome closure. During this process the number of liposome layers is reduced, and the amount of the water-soluble substance to be encapsulated increases. To achieve this goal, the MLV mixture is alternately frozen in dry ice with acetone or in liquid nitrogen, and warmed up in a water bath at 50–60°C.

2.3.7. EXTRUSION TECHNIQUE

This technique is preceded by the FAT procedure. After FAT, liposomes are calibrated using a manual calibrator, or a compressed nitrogen-powered calibrator. The calibration is performed 7–10 times, and gel filtration is applied. This technique is used for preparation of VET liposomes. The MLV-FAT-VET technique is presently the most frequently recommended one for preparation of liposomes, as it minimises the use of organic solvents, and reduces the probability of their presence in the final mixture, which would potentially result in degradation of lipids [69].

2.3.8. MASS PRODUCTION OF LIPOSOMES

The traditional methods of liposome production involve four basic steps: dissolution of lipids in organic solvents, suspension of lipids in an aqueous solution, purification of the obtained liposomes, and analysis of the final product. However, the residues of organic solvents in lipids or in the aqueous phase may result in toxicity of liposomes. Therefore, methods that would enable preparation of liposomal vesicles without the use of volatile organic solvents are being researched.

The development of methods that would allow to prepare liposomes of reproducible and clearly defined properties in a significant problem in a mass production of liposomes. Another important question is the stability of liposomes obtained in an industrial-scale process. Moreover, to produce liposomes on a mass scale, a proper equipment that meets the requirements of pharmaceutical industry is necessary. As the vesicles penetrate into the deep layers of the skin, i.e. Pass into the organism, a strict microbiological control is required. Sterilisation is an important issue in this context, as it may damage the delicate liposomal structures, especially as a result of heating. Another obstacle in manufacturing of liposomes is a high production cost. Therefore, the methods that require the smallest possible number of stages and devices, while preserving a high product quality, s well as offering a fast and reproducible production, are becoming increasingly popular. However, many conventional preparation methods do not meet these criteria.

It should be emphasised that only a few techniques enable to encapsulate a large quantity of hydrophilic substances. Bioactive agents can be introduced into liposomes primarily using the reversed-phase technique, and ether injection method. These processes enable efficient preparation of heterogeneous mixtures of LUV and MLV liposomes, usually without the need for additional methods, such as extrusion through polycarbon filters.

The majority of techniques used in preparation of liposomes require volatile organic compounds (VOC), mainly chloroform, ether or methanol, for dissolution of lipids. These solvents not only affect the structure of the active substances encapsulated in liposomes, but also have effects on toxicity and stability of liposomes, which has consequences for health and the environment. Therefore, it is recommended to remove solvent residues from the obtained liposomes, using appropriate techniques including gel filtration, vacuum method ad dialysis. However, even following the application of these

processes, trace amount of solvents remain in liposomal systems. Thus, the development of techniques for liposome preparation that do not require volatile organic solvents or detergents is very important [70].

2.4. LIPOSOMES ANALYSIS METHODS

2.4.1. SIZE ANALYSIS

There are a few methods that enable a precise measurement of the size of liposomal particles, and each of them describes different parameters of a given particle. They include: particle mass, its surface, maximal and minimal length and volume. Dimensional homogeneity of liposomal particles is important. The calibration processes do not always ensure production of identical particles characterised by low polydispersity. To determine precisely the size of liposomes, special methods are used.

One of them is microscopic observation, which, due to computer image analysis, also allows to determine quickly and accurately the shape and accumulation of liposomes. However, despite its numerous benefits, it is a time-consuming method, and its precision is limited; the analysed sample is only a part of the whole product, so it may not represent fully the entire material, and as a consequence the measurements can be imprecise.

Dynamic light scattering (DLS) is a more accurate method. Due to its universality and precision, the whole volume of the sample can be tested, the particles are not destroyed, the range of measurement is broad, and the method is not time-consuming. It enables testing of particles from 1 nm to 2,000 μm [64, 69].

2.4.2. ANALYSING THE VOLUME OF THE ACTIVE SUBSTANCE ENCAPSULATED IN LIPOSOMES

Testing methods can also be used to determine the volume of the active substance encapsulated in the liposome. For this purpose the unencapsulated substance is separated from the liposomal vesicles with encapsulated substance, and molecular filtration is applied. Spectrophotometry is another method that can be used. It involves sample breaking with the use of detergent, and releasing the active substance due to the dissolution of the lipid layers. Next, the phosphorus content and the quantity of the active substance are determined using UV-VIS spectroscopy [69].

2.4.3. OTHER METHODS OF ANALYSIS

Zeta potential is also an important parameter. It requires the use of electrophoretic technique. However, zeta potential is not found directly on the surface of a liposome, but very close to it, so it is useful to know the value of this parameter. Zeta potential values can provide insight into the stability of the liposomal suspension. If the particles attract, they might aggregate, which may result in destabilisation of the suspension. When particles repel each other, the suspension remains stable, and liposomes do not fall out of the solution [69].

Photon correlation spectroscopy (PCS) is another method of measurement of liposomal properties. It allows to measure the size of a liposomal particle in the range from a few nanometres to 1 μm. The measurement is performed using a laser, supported by photomultiplier tubes and a correlator, due to a relatively weak signal on the detector. This method consists in the measurement of quantitative changes in the light dispersed by the liposomal particles at certain time intervals. In the case of small liposomal particles, the diffusion movements are fast, which results in rapid fluctuations of the dispersed light, whereas larger particles are associated with much slower diffusion movements. The information about the changes in light intensity captured by the detector helps to determine precisely the sizes of liposomal particles [69].

2.5. THE USE AND BENEFITS OF LIPOSOMES

Liposomes have been applied and tested as universal chemical carriers in different administration routes: from parenteral and oral to skin and percutaneous. They have also found different medical indications, including therapies of cancer, infections and disorders of the skin [60].

The main benefits of localised liposome application in dermatology include:

- reduction of significant side effects caused by improper localisation of the drug in skin layers with prevention of systemic absorption;
- increased drug concentration within the skin due to the liposome structure, which imitates the composition of the epidermis, which makes liposomes substantive on biological membranes;
- non-toxic properties and biodegradability;
- easy large-scale production;
- feasible encapsulation of compounds soluble in water and lipid solvents;
- moisturisation and reconstruction of essential lipids in the skin layers [71, 72].

2.5.1. COSMETIC INDUSTRY

The mechanism of action of liposomes on the skin surface is widely used in cosmetic formulations. Adsorption of liposomes on the surface of epidermis protects it from the harmful effects of weather conditions, and their degradation process creates a protective filter, preventing water loss from the deeper skin layers, and maintaining a constant degree of moisturisation. The adsorbed liposomes gradually release the active substance with a specific effect. Cosmetic formulations offer a variety of active substances to satisfy different skin needs [70].

The cosmetic industry was the first one to introduce liposomal products on a mass scale, which involved the use of encapsulated active substances demonstrating milder effects than those used in pharmaceutical products. While developing formulas it is important to consider the effectiveness of ingredients, as well as their safety and stability. Long-term

ageing tests conducted before the products are introduced to the market can provide the relevant data. To obtain the optimal results, a few principles should be followed:

- avoid using surfactants and the substances that can affect lipids in the liposomes, such as solvents, as they destroy the capsule structure;
- avoid substances with a high dissociation rate, e.g. Salts that destabilise liposomes,
- avoid solvents that reduce the amount of water stabilising the surface of the liposomal layer, e.g. ethanol,
- excessive mixing may also result in destabilisation of liposomes,
- liposomes should be introduced into the cosmetic formula at the temperature intended for sensitive ingredients, i.e. under 45°C, without simultaneous homogenisation, and the optimal concentration of liposomes in a given cosmetic product should be 5–15%.

Liposomal stability is preserved in the presence of up to 10% of glycerine, up to 5% of propylene glycol, and up to 5% of ethanol, whereas a 1% NaCl solution or sodium lauryl sulfate destabilise lipid membranes. As for oils, they do not damage the structure of liposomal layers, due to a strong hydration of lecithin which prevents its dissolution by the oils used in the formula [72].

It is possible to produce liposomes containing phosphatidylcholine and cholesterol, with increased stability due to the association of amphiphilic polymers. It has been demonstrated that when liposomes introduced into an O/W emulsion, the ones with associated polymers are more stable, as they do not undergo fusion, and the active substances do not leak from the capsule interior [73]. One of such polymers is polymethyl methacrylate. The thickness of the capsule wall may be modified by changes in the polymer concentration in the oil phase of the emulsion before emulsification of phases. This phenomenon can be observed using a scanning microscope [67].

2.5.2. PHARMACEUTICAL INDUSTRY AND MEDICINE

Liposomes are composed of natural compounds, structurally corresponding to the epidermal lipids. It is very important, especially for the pharmaceutical use of liposomal systems. Due to this characteristic, liposomes are not toxic or harmful in intramuscular administrations, as they are well tolerated by the skin, and create a barrier that structurally resembles the natural one. It is very useful when the permeability of the epidermal barrier is increased, in inflammatory conditions, and in the case of excessively dry, flaking skin [74].

The greatest advantage of liposomes in the context of pharmaceutical and medical use is their targeted action. Moreover, they offer a slow release of the active substances, which prolongs and optimises their effective duration. In addition, they demonstrate immunostimulating effects, without any toxicity to the organism. They even reduce the adverse effects of some active substances, so certain harmful agents could be used as therapeutic products only due to the use of the liposomal technology [75].

The therapeutic substances introduced to the organism in liposomes can be classified into two groups. The first one comprises lyophilic substances encapsulated in liposomes due to the problems with their intravenous administration. The second group comprises medicines

with hydrophilic properties that in a traditional form cannot pass the blood-brain barrier, but administered in liposomes can do it due to the lyophilic nature of their shell [76].

To optimise the effects of liposomes, their surface is modified, e.g. it is covered with polyethylene glycol which extends the half-life of the product, as well as protects from solvents, prevents binding of the blood cells with these particles, and prevents potential interactions. In addition, liposomes can be modified so that they target precisely determined sites. The effect can be obtained by adding ligands, specific for individual cell types, that interact with a particular enzyme of receptor [76].

Liposomes are also used in therapy of malignant neoplasms. Thanks to the use of liposomal systems, substances that are very harmful for the organism can be released only in the cancer cell, reducing the adverse effect of the therapeutic agents. Liposomes are also used in gene therapy. It usually involves introducing specific genes into cationic liposomes, and transporting them to particular cells in the organism. This therapeutic field offers interesting options [77].

Doxorubicin is an example of a substance used in the liposomal form in the treatment of neoplasms, such as breast cancer and ovarian cancer. A medicine, as a hydrophilic substance, is transported in the aqueous phase of liposomes. The liposomal form eliminates the risk of hypersensitivity, since the lipid membrane has been modified so that the particles are "invisible" for the immune system [76].

An interesting type of liposomes are those that can transport antibodies, their fragments, peptides and other ligands added to the micelles composed of polyethylene glycol. This method enables to produce liposomes with targeted activity, selected individually for each patient [78].

The use of liposomes in immunotherapy is also researched. They are already used in this field for encapsulating allergens, but this application is controversial, due to problems with standardisation of preparations. However, using liposomes in immunology could improve the outcomes of treatment in patients with various allergies [79].

Liposome in pharmaceutical products are extensively studied for controlled drug release. One of the possible solutions is to incorporate proper receptors into the structure of the liposomal shell. IT applies primarily to antineoplastic products, based on the antigen--antibody interaction. The release of the active substance in this case will modify the permeability of the cancer cell membrane, resulting in destruction of the cell [63].

Despite a significant success in the pharmaceutical field, which paves the way to the development of a wide spectrum of medicinal products, liposomology still encounters numerous problems, such as monitoring of the drug release rate and finding materials that could pass through biological barriers, to ensure the optimal therapeutic effectiveness. Another important aspect is production of spatially stable liposomes, and obtaining a high and stable lipid-to-active substance ratio. Other problems associated with the development of liposomal technology for the transportation of medicines include quality of phospholipids used in production, low physicochemical profile of liposomes, low loading potential, very limited stability in time, and lack of safety data regarding a long-term use of liposomal drugs [80].

Summing up, using liposomes in cosmetic and pharmaceutical products is associated with a number of benefits, including:

- increased stability of active substances and products due to the separation of interacting ingredients,
- penetration of the substances into the deeper layer of the skin, and supplying them in the specific skin areas,
- protection of the sensitive ingredients from external factors: sunlight, air or moisture,
- prolonged time of action of the substance due to a slow, controlled release from the liposome shell,
- capsulation of substances with various properties: hydrophilic, lyophilic or amphiphilic in the appropriate segments of the liposomal particle [74].

2.5.3. OTHER USES OF LIPOSOMES

Liposomes are also used in many other scientific areas, e.g. in the studies on topology and biophysics of membranes, in photophysics and photochemistry, in the research on colloidal interactions and cell functions, as well as in many other fields.

Apart from applications in exact science, liposomes are also used in ecology. Liposomes that contain membranes with chelating substances are used for cleaning liquids from toxic or radioactive substances, e.g. for cleaning the water from nuclear reactors.

Liposomal systems are also used in food industry. Liposomes containing specific enzymes can shorten the time of fermentation and improve the quality of food products, which is used mainly in the production of long-ripening cheeses. Using enzymes encapsulated in liposomes reduces the maturation period by 30–50%, which has a significant economic impact, especially if the cheese has to mature for a year or linger [81].

2.5.4. CERTAIN PROBLEMS ASSOCIATED WITH THE USE OF LIPOSOMAL PRODUCTS

Aside from their undisputed and unique benefits, liposomes applied as active substance vehicles feature certain deficiencies, including low stability, relatively poor encapsulation efficiency, high manufacturing costs, degradability by hydrolysis or oxidation, and sedimentation, aggregation or fusion of the liposomal capsules during storage [82]. It must also be noted that the benefits and restraints of liposomes as active compound carriers strictly depend on their physicochemical and colloidal properties, including size, composition, capacity, stability, and biological interaction with cellular membranes [83].

A few aspects should be considered before liposomal products are used. They are recommended especially to middle-aged and elderly people, as young skin does not need strong cosmetics. Since the requirement for skin nutrients increases with age, liposomal products are intended rather for more mature consumers. It should also be emphasised that some people may develop allergic reaction, such as redness or skin tightness, due to strong effects of these products. One should remember the principles of using preparations with liposomes. The optimal effects are obtained when the product is used for the night, as it is

better absorbed and penetrates into the deep skin layers. Liposomal products should not be applied directly before leaving the house, because liposomes can absorb harmful products of fuel combustion, as well as heavy metals which, transported to the deep layers of the skin could induce adverse effects.

2.6. FORMULATIONS WITH LIPOSOMES – COSMETIC MARKET

Presently, many products rich in liposomes are available on the cosmetic market. They are primarily skin care products: day and night creams and serums, eye creams and serums, lotions, formulations containing active forms of vitamins A, B, C, E. To be effective, the active form of the vitamin in the cosmetic formulation must reach the deep layers of the skin. Enclosing vitamins in capsules helps to stabilise them, ensures their proper concentrations and the release of appropriate amounts. Cosmetics for mature dry or dehydrated skin containing moisturising substances. They include primarily hyaluronic acid and other hydrophilic substances. They can bind with water, and due to liposomal shells, they act not only on the skin surface, but also in its deep layers. Tanning cosmetics with dihydroacetone provide a controlled and balanced release of the active substance, resulting in an even and natural-looking tan. Encapsulated in the liposomes are peptides, such as collagen, elastin, spleen extract, thymus extract, proteins, as well as synthetic compounds including caffeine, panthenol, allantoin and other substances, such as co-enzyme Q10, heparin, glutathione, carnitine or fibronectin [54].

The range of cosmetic products with liposomal capsules includes also care products: aftershaves, mild, alcohol-free facial liquids, anti-stretch mark oils and after bath skin care oils. Many cosmetics contain substances of natural origin, such as herbal extracts, e.g. propolis, hop, thyme, aloe vera, wheat sprouts, garlic extract or Ginkgo biloba.

Cosmetics that contain liposomes are not ordinary products, so they should be used with special caution due to their wide spectrum of action on the skin [54, 84].

3. LIPID NANOPARTICLES

Lipid nanoparticles are colloidal vehicle systems for biologically active ingredients and an alternative to traditional drug vehicle systems, such as liposomes or polymer and inorganic nanoparticles. Lipid nanoparticles are usually between several dozen and several hundred nanometres. They are different from nanoemulsions in that the lipid core is in the solid form (Figure 4). The core can feature a single lipid or a mixture of lipids. Here, lipids can be triglycerides, fatty acids, waxes, or steroids.

Surfactants are required in the formulation of lipid nanoparticles to prevent aggregation of lipid particles and produce a stable system. Colloidal vehicles with a solid lipid core

usually comprise 0.1 to 30% of the fatty phase dispersed in an aqueous solution, plus 0.5 to 5% of a surfactant. Active substances are solved or slightly dispersed in the lipid matrix of the vehicle.

Two types of lipid nanoparticles are classified: the 'first generation' solid lipid nanoparticles (SLN) and the 'second generation' nanostructured lipid carriers (NLC).

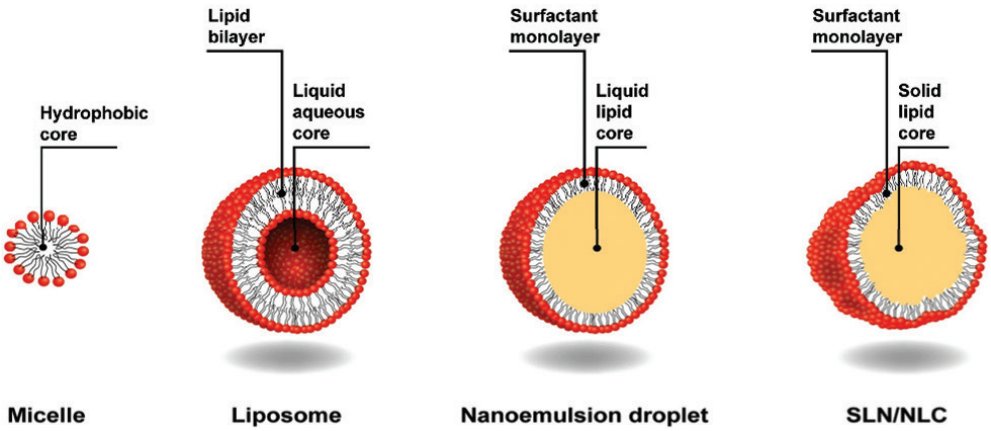

Figure 4. Comparison between micelles, liposomes, nanoemulsions and solid lipid nanoparticles [85]

3.1. SOLID LIPID NANOPARTICLES (SLN)

SLN comprise solid lipids and surfactants non-toxic to the human organism. In SLN, oil droplets are replaced with solid lipids [86, 87] present in the solid phase both at ambient and body temperatures [86, 88]. SLN facilitate controlled release of lipophilic substances and may carry higher active substance volumes than conventional vehicles. The only solvent in SLN is water, which is non-toxic. This eliminates the need for organic solvents; an added value is the easy large-scale production of SLN.

It generally assumed than SLN may comprise up to 30% of solid lipids by weight and dispersed in the aqueous phase. The fatty phase dispersion in the aqueous phase is fixed with amphiphilic compounds. Application of surfactants in SLN can only be restricted by the route of administration of the finished product. The selection of emulsifiers is relatively more restricted in parenteral administration applications. Mixtures of surfactants can provide a very high and durable stabilization of formulations [88].

SLN are highly stable, provided that they undergo certain processes. The steady formulation uniformity in storage and inability of crystallizing the lipid matrix are crucial. If the lipid matrix is crystallised, the ingredients enclosed in the nanoparticles are released which reduces the efficacy of the preparation [87, 89]. The storage of SLN

can cause problems with metastable structures which add dynamism to the formation of a β-modification, a structure with a higher order of organisation. This phenomenon removes the active ingredient contained in the SLN.

3.2. NANOSTRUCTURED LIPID CARRIERS (NLC)

SLN, the first generation lipid nanoparticles, feature many benefits, although not without certain deficiencies, of which the following should be noted [89]:

- a limited quantity of introduced active substance;
- removal of active ingredients during storage;
- insufficient concentration of SLN systems.

These problems prompted the development of the second generation of lipid nanoparticles, called 'nanostructured lipid carriers' (NLC). NLC comprise solid and liquid lipids. This provides a higher void volume in an NLC to nest in the active substance compounds. The void volume in an NLC comes from the imperfection of the crystal lattice in the structure caused by mixing of different types of lipids [87].

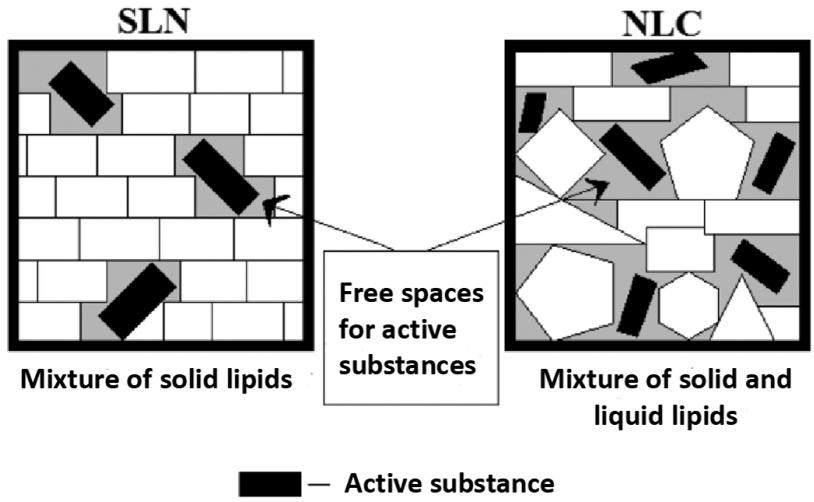

Figure 5. Diagram of the first generation SLN and the second generation NLC (by author)

The quantity of the active compound in SLN is limited due to the formation of lipid crystals. A release of the active compound may also occur, since lipids tend to reach an ideal crystalline state. Crystallization inhibition was developed and implemented by modifying the lipid phase composition of NLC by introducing a solid lipid matrix which is amorphous and does not undergo crystallization.

NLC comprise an oil phase with the ratio of solid lipids to liquid lipids between 70/30 and 99.9/0.1 [87, 90]. A high contributor to the durability of these aqueous dispersions is

the concentration, type and quantity of the emulsifier which must encapsulate the droplet surfaces of the lipids dispersed in the aqueous phase. The surfactants used in NLC must be highly biotolerated by the skin to prevent side effects during application. Several groups of surfactants can be classified as used for stabilization of NLC; the most often used include non-ionic surfactants, which do not irritate the skin, such as ETO (ethylene oxide) – based surfactants; other types include polyols and PEG (polyethylene glycol) derivatives. The stabilizers affect the crystallization of the lipid matrix by reducing the rate of polymorphic transformations [91].

3.3. PRODUCTION OF LIPID NANOPARTICLES

The techniques of production of nanostructural lipid carriers are similar to those use in preparation of SLN. Many of them were described in the literature: high-pressure homogenisation (HPH), ultrasonication, microemulsion technique, temperature--induced phase inversion technique (PIT), techniques involving the use of solvents: emulsification/evaporation, emulsification/diffusion, solvent injection, multiple emulsion and membrane technique [92, 93]. The most popular and frequently used one is high--pressure homogenisation, due to the facility of mass-scale production, absence of organic solvents, and a short duration of the process. The main alternative to HPH is ultrasonication, useful for laboratory-scale preparation of NLC, as it is fast and does not require expensive equipment [94].

3.3.1. HIGH-PRESSURE HOMOGENISATION (HPH)

This method is used for preparation of SLN and NLC. It has two technological versions: cold and hot. In both cases the first stage involves dissolution or dispersion of the active substance in a mixture of liquid lipids, and melted solid lipids (for NLC) [92–94].

In the process of hot HPH, the next stage consists in dispersing the melted liquids in an aqueous emulsifier solution by vigorous mixing (both phases at the temperature 5–10°C over the melting point of lipids). Hot pre-emulsion is formed, and then homogenised at the same temperature, using HPH, high-intensity ultrasonic bath/probe/stream, or microfluidiser. The product of this process is hot nanoemulsion. Next, nanostructural lipid particles are formed by cooling down the nanoemulsion for crystallisation of lipid droplets, and precipitation of lipid nanoparticles [92, 95]. This method has a few shortcomings [92, 96]:

- the high temperature used in the process can cause degradation of unstable active substances,
- surfactants usually have low cloud points (above the temperature phase separation is observed), and using high temperatures may reduce their ability to emulsify, which will eventually lead to NLC instability,

- during the homogenisation process, the hydrophilic groups of the active substance particle can cause penetration into the aqueous phase, which is associated with a low effectiveness of encapsulation in the NLC matrix. This may also increase the dissolution rate of the lipophilic compound in the hot aqueous phase.as a result, the compound may crystallise during the cooling down stage, and the structure of the NLC matrix prevents the return of the bioactive ingredient inside the carrier.

The cold high-pressure homogenisation method is more often used for SLN production than for NLC, but reports of such attempts are available in the literature [97]. In this option, after dispersion of the active substance in a mixture of melted lipids, the system is cooled down, e.g. with the use of liquid nitrogen. Subsequently, the lipid matrix is ground to microparticles (50–100 μm), e.g. in a ball mill. At this stage it is important not to exceed the meting point of lipids. The obtained microparticles are dispersed in cool, aqueous suspension of an emulsifier, and then homogenised to produce lipid nanoparticles at the room temperature or lower (to 0°C). Using the cold method, we obtain larger particles than those produced by hot HPH [92, 96]. The benefits of this method include [92]:

- a reduced risk of thermal degradation of the active ingredient,
- the high rate of cooling enables a balanced distribution of the active substance in the lipid matrix, simultaneously reducing its penetration into the aqueous phase,
- creation of the desired crystalline structure.

3.3.2. ULTRASONICATION

Ultrasonication is a dispersion method, used on a laboratory scale to produce nanostructural lipid carriers. Its advantages include lack of organic solvents and a limited use of surfactants and additives. Moreover, it is a fast method, that does not require any expensive equipment.

Dispersion of the lipid nanoparticles is obtained through homogenisation, followed by ultrasonic sonication of the pre-emulsion produced by dispersion of a mixture of melted lipids in a warm aqueous phase (both phases at the same temperature) containing emulsifier. In this method lipids are warmed up at 5–10°C above their melting point. After the ultrasonication process, which significantly determines the size of drops, the emulsion is cooled down to the room temperature. Cooling the emulsion below the crystallisation temperature of solid lipids allows to obtain lipid nanoparticles [96, 98]. This method has some shortcomings [96, 99]:

- the quality of dispersion of the lipid nanoparticles is reduced due to the presence of microparticles that result in physical instability of the preparation during storage,
- the long duration of sonication increases the risk of contamination of the sample with the metal from the tool used.

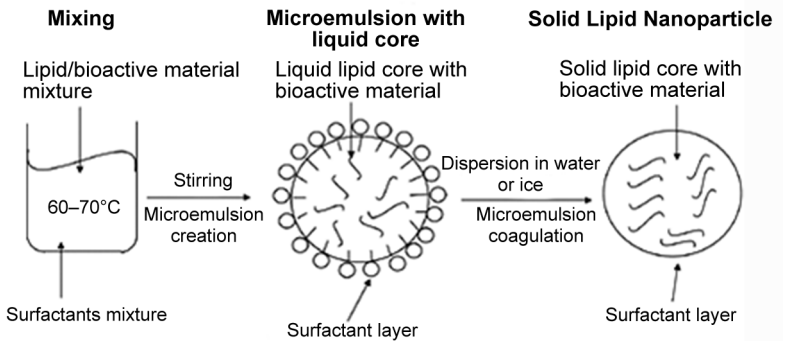

Figure 6. General process flow diagram of lipid nanoparticle production by ultrasonic dispersion (by author)

3.3.3. MICROEMULSION TECHNIQUE

This method of preparation of lipid nanoparticles consists in precipitation of small lipid drops by braking of microemulsion. Lipid nanoparticles are formed as a result of dispersion of hot O/W microemulsion in cold water (2–3°C) through gentle mechanical mixing. The volume ratio between the hot microemulsion and cold water ranges from 1:25 to 1:50. The main shortcomings of this method include a relatively high water content, difficulties with removing the excess of water, and using surfactants in high concentrations [96].

3.3.4. EMULSIFICATION – SOLVENT EVAPORATION TECHNIQUE

Using this method, NLC are obtained in three stages. In the first one, the organic phase is prepared by dissolution of lipids ad the active substance in an organic solvent that does not mix with water and has a low boiling point (e.g. methylene chloride, chloroform, cyclohexane). Next, the organic phase is dispersed in an aqueous solution of the emulsifier. In the last stage, the organic solvent is evaporated under a reduced pressure. The result of this process is nanodispersion of lipid particles in the aqueous phase. The advantage of this method consists in minimal exposure of the sample to high temperature, so it is recommendable for thermally sensitive compounds. The mean size of an NLC particle obtained using this technique is from 30 to 100 nm, depending on lipid concentration, type of emulsifier and manufacturing conditions [92, 98]. The main shortcomings of the method include [92]:

- presence of solvent residues in the final product, which may be associated with toxicological problems,
- the necessity to use a larger amount of surfactant than that required to stabilise NLC dispersion,
- low final NLC concentration, due to a limited solubility of lipids in the organic solvent used.

3.3.5. EMULSIFICATION – SOLVENT DIFFUSION TECHNIQUE

The lipid phase is dissolved in a solvent partially mixing with water (e.g. benzyl alcohol, isovaleric acid, tetrahydrofuran). Addition of water to the obtained organic phase results in separation of lipid nanoparticles. The mechanism of NLC production in this method is based on the diffusion of the solvent from the organic phase to the external aqueous phase. This technique has the same shortcomings than those observed in the emulsification-solvent evaporation method [96, 98].

3.3.6. SOLVENT INJECTION METHOD

In this method of nanoparticle production, the mixture of lipids is dissolved in an organic solvent mixing with water (e.g. methanol, isopropanol, acetone). After the melting of lipids, the organic phase is injected with an injection needle into the aqueous phase containing a surfactant. The mechanism of NLC formation is analogous to the solvent diffusion method, i.e. Nanoparticles crystallise as a result of a rapid diffusion of the solvent from the organic phase into the aqueous phase [96, 98].

3.3.7. MULTIPLE EMULSION TECHNIQUE

The multiple emulsion technique is used for production of SLN and NLC primarily when they are carriers for hydrophilic active substances and peptides. In the first stage, W/O nanoemulsion is formed by emulsification of the aqueous solution of the active substance in the melted lipid phase. The obtained nanoemulsion is then dispersed in the aqueous solution of a hydrophilic surfactant, to form a multiple W/O/W emulsion. The lipid nanoparticles are produced by crystallisation and filtration of lipids. The shortcoming of this technique consists in a relatively large size of the prepared particles [96, 98].

3.3.8. MEMBRANE TECHNIQUE

To prepare lipid nanoparticles with this method a cylindrical membrane module is used. The aqueous phase, together with the surfactant dissolved in it, circulates in the internal part of the module. The lipid phase is injected inside through the membrane pores which enable formation of small droplets that are subsequently dispersed in the aqueous phase. The temperature of the aqueous phase is maintained at the melting point of the lipids used. The lipid nanoparticles are produced by cooling down of the system to the room temperature. The advantage of this method consists in the ability to prepare particles of desired sizes, due to the use of membranes with appropriate pore sizes [98].

3.3.9. TEMPERATURE-INDUCED PHASE INVERSION TECHNIQUE (PIT)

This technique is based on a temperature-induced change in the properties of polyoxyethylated, non-ionic surfactants. As the temperature rises, the polyoxyethylated chain undergoes dehydration, and the surfactant becomes lyophilic (reduction of the HLB value). At the room temperature the emulsifier is hydrophilic and is used to produce O/W

emulsions. The increase of the temperature induces phase inversion, i.e. an O/W emulsion turns into a W/O emulsion. After cooling down, a reverse process is observed in the system. This method involves consecutive cycles of warming and cooling of the system in order to induce spontaneous phase inversion. After the process is completed, the obtained emulsion is diluted with cold water (0°C), which results in an irreversible breaking of the emulsion, and crystallisation of lipid nanoparticles [98].

3.4. CHARACTERIZATION OF LIPID NANOPARTICLES

A proper characterization of lipid nanoparticles is essential in the design and development of industrial-scale products. The quality control of produced nanovechicles is based on a variety of analytical methods which provide a good assessment of product properties. The characterization of lipid nanoparticles is problematic due to the complexity, colloid--range sizing and dynamic nature of the systems. The key parameters essential for proper characterization of NLC include [92, 100]:

- particle size and its distribution,
- particle charge and zeta potential,
- particle morphology,
- NLC stability in time,
- chemical stability of the included active substance,
- encapsulation efficiency and the release method of the active substance,
- levels of lipid crystallization and modification.

3.4.1. PARTICLE SIZE AND ITS DISTRIBUTION

The mean particle size and particle size distribution (usually expressed as the polydispersity index) are among the critical properties of NLC. These parameters condition the physical stability of NLC and affect the release rate of active substances. The particle size of a nanovehicle depends on multiple factors, including the composition of the preparation and the production method and conditions. The particle diameter in NLC is usually between 10 to 1,000 nm.

The particle size and the polydispersity index (PI) are usually determined with DLS (dynamic light scattering) analytical instruments. DLS enables accurate, reliable and repeatable determination of dispersion particle size at a sub-micron level. The required concentration of particles in the suspension ranges from 0.001% to 40%. DLS is applied to determine the diffusion coefficient of small particles suspended in a liquid medium. The diffusion coefficient is determined by light scattering intensity of the particles. The particles scatter light, which gives a fluctuation of scattering intensity versus time. A correlation of the fluctuations allows a determination of the particle size distribution. DLS techniques enable analysis of particles in the diameter range from several nanometres to approximately 3 microns; these particles exhibit perceptible Brownian movement. The NLC size data

is derived in the fluctuation rate of scattered light [10]. The polydispersity index (PI), a characteristic of particle size distribution, has a major impact on the physical stability of NLC. The maximum stability of the dispersion requires a PI value between 0.1 and 0.25 [92, 100].

Scientific literature features much research into the comparison between SLN and NLC particles produced in identical conditions. The research results vary; it is then impossible to ascertain if the application of mixtures of liquid and solid lipids in production of lipid nanoparticles (NLC) positively affects the nanoparticle size (i.e. reduces the particle diameter) or not [101].

3.4.2. ZETA POTENTIAL

Zeta potential (ZP) is an important parameter by which the stability of lipid nanoparticle dispersions is assessed. Zeta potential, or the electrokinetic potential, is present in the bilayer at the surface of dispersed particles. Zeta potential occurs where ions at the dispersed phase surface meet with the continuous phase ions, i.e. the slip interface. Zeta potential is a measure of electrostatic interaction between the particles which can move relative to each other. A high zeta potential reduces the likelihood of particle aggregation by electric repulsion. Lowering the zeta potential reduces the physical stability of a lipid nanoparticle dispersion. The minimum zeta potential which provides a stable vehicle system is ±30 mV. Values around ±20 mV provide short-term stability of the dispersion. If all particles in a system are at high positive or negative zeta potential; the homogeneous charge will cause repulsion and prevent particle aggregation. Many commercial zeta potential testers are integrated with DLS instruments to facilitate particle charge and size analysis with a single analytical package [92, 102].

3.4.3. MORPHOLOGY OF NANOPARTICLES

Advanced microscopic techniques, SEM (scanning electron microscopy), TEM (transmission electron microscopy) and AFM (atomic force microscopy) are typically used to determine the particle size, the particle size distribution, the surface topography, the morphology and the internal structure of NLC [92].

SEM provides three-dimensional image of a sample surface. In this method, the sample surface is bombarded with an electron beam; the test object morphology is determined by the emission of secondary electrons from the sample surface. A SEM analysis of NLC requires converting them into dry powder, followed by deposition of gold or platinum. The analysis is made in a vacuum environment [92].

TEM provides two-dimensional image of sample surfaces only. TEM has a higher image resolution than SEM and can provide insights into the internal structure of lipid nanoparticles. Not unlike in a SEM analysis, samples must be powdered. To enhance the image contrast, a powdered sample is dyed with heavy metal salts; the maximum thickness of the specimen must not exceed 100 nm [92].

AFM techniques has revolutionised our ability to characterise colloidal objects. AFM allows the visualisation of samples with sub-nanometre resolution in three dimensions in atmospheric or submerged conditions. This advanced microscopy technique, consists in moving a probe across a specimen. The probe operates with an atomic-scale acuity to reproduce a topological map of the particle. The AFM probe moves very close to the specimen surface. The AFM image is produced by measuring the interaction between the AFM probe's tip and the investigated sample surface. This provides a three-dimensional image with a resolution of 0.01 nm. In AFM, the sample's morphology, energy dissipation, height, etc. are recorded by various modes of operations like contact mode (static), non--contact (dynamic) mode and tapping mode. The sample holding substrates commonly used in the AFM are muscovite mica, gold slides, silicon wafers and glass slides. Mica is preferred for its flatness and transparency. For NLC the most common are mica plates [92, 103, 10].

3.4.4. CHEMICAL STABILITY OF INCORPORATED ACTIVE SUBSTANCE

Chemical stability of the active ingredient is critical for effective application of NLC in the pharmaceutical, food and cosmetic industries. It is the basis for the determination of encapsulation efficiency (EE) and drug loading (DL). Chemical stability can be determined by destructive or non-destructive testing.

Destructive testing consist in destruction of the carrier structure for a complete release of the active substance to a suitable medium. NLC structure destroyers and drug solvents include organic solvents, like methanol, acetonitrile, hexane, acetone, and tetrahydrofuran. A drawback of the method is that the extraction process with the specific solvent can affect the chemical stability of the active substance. The drug content following the extraction process is quantitatively determined by UV-VIS spectroscopy and HPLC (high-performance liquid chromatography). The chromatography-based techniques also permit monitoring the potential degradation of biosubstances and controlling the release of impurities and undesirable ingredients from the NLC [92, 104].

Non-destructive testing involves measurement of a physical property of NLC, the colour of NLC dispersion. This is done by colorimetry and absorbance measurement of the specimen at the wavelength λ_{max} specific to the active substance being tested. Non--destructive testing is straightforward and does not require solvent extraction. A deficiency of the method is the need to produce an accurate calibration curve.

If the active substance concentration in the NLC is known, further analysis is possible to determine the biological efficacy of the carrier (EE and DL) [92].

3.5. DERMATOLOGICAL AND COSMETIC APPLICATIONS

Lipid nanoparticles are attractive systems of colloidal vehicles for on-skin application due to a number of beneficial application effects. They are also suitable for application on damaged skin and the skin prone to inflammation, due to the aforementioned

non--irritating and non-toxic properties of lipids. SLN and NLC exhibit good occlusive properties which increase the moisturisation of the skin and reduce skin wrinkles; they also demonstrate an improved or targeted penetration of biologically active compounds into specific skin layers [96]. Enhanced skin penetration by encapsulated bioactive substances is caused by an intimate contact of the systems with the corneum from the extremely low, nanometre-scale size of the lipids [105]. If the undisputed benefits from the properties of lipid particles in dermatological applications are enhanced with the beneficial action of the active substance, preparations with wide spectra of action can be formulated, from typically cosmetic products to medicinal formulations.

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SUMMARY

Selected transdermal delivery systems is a monograph concerning the most modern forms of cosmetic and pharmaceutical products, such as nanoemulsions, multiple emulsions, liposomes and various types of nanoparticles used as vehicles of active substances. On the modern market of cosmetics and pharmaceutical products there is a growing demand for completely safe and effective products. One of the conditions for obtaining an effective products is to develop formulations that will allow the delivery of active substances to the right place in the skin.

The book is divided into several chapters. Chapter I is dedicated to the structure of a skin. The human skin is the main protective barrier, limiting the penetration of exogenous compounds into the body. During elaboration of new transdermal formulations, it is important to predict the ability of active substances to penetrate the skin. In the following sections of Chapter I important aspects related to the penetration of active compounds into the skin are discussed. Subsequently, transport routes through the epidermis and factors affecting the permeation process are presented. The last unit of the Chapter I is dedicated to selected in-vitro methods that allow assessing the permeability of the actives from the product to the skin, which are a helpful tool in the design of new formulations.

Chapter II of the monograph contains an information concerning nanoemulsions, their composition, thermodynamic stability, and methods of production. Additionally, methods for testing physicochemical properties of the nanosystems and their applications in cosmetic and pharmaceutic industry have been described.

In chapter III, the reader can broaden his knowledge of multiple emulsions as physicochemical forms that allow to control the release of active substances into the skin. Similarly to chapter II, the following subsections discussed physicochemical properties, methods of obtaining and characterization of the multiple systems.

The last part of the monograph, Chapter IV, is devoted to sub-micron vehicles of the active substances used in cosmetic and pharmaceutic products. Various types of nanoparticles from the group of inorganic, polymer and lipid (SLN and NLC) nano-carriers were discussed. Among others, also liposomes, as an effective form of delivery of active substances through the skin, are widely discussed. Similar to previous parts, in Chapter IV there are described the complete information on the methods of obtaining, testing the physicochemical properties and the application of the submicron carriers for biological active compounds.

STRESZCZENIE

Selected transdermal delivery systems stanowi opracowanie monograficzne poświęcone najbardziej nowoczesnym formom produktów kosmetycznych i farmaceutycznych, takim jak nanoemulsje, emulsje wielokrotne, liposomy oraz różnego rodzaju nanocząstki stanowiące nośniki dla substancji aktywnych emulsja. Współczesny rynek stawia produktom kosmetycznym i farmaceutycznym coraz wyższe wymagania. Rośnie zapotrzebowanie na całkowicie bezpieczne i efektywne produkty. Jednym z warunków uzyskania skutecznego preparatu jest opracowanie formulacji, które pozwolą na dostarczanie substancji aktywnych do odpowiedniego miejsca w skórze.

Książka podzielona jest na kilka rozdziałów. Zasadniczą część, opisującą poszczególne rodzaje formulacji, poprzedza rozdział I poświęcony budowie skóry jako podstawowej barierze ochronnej ograniczającej wnikanie związków egzogennych do organizmu człowieka. Przy tworzeniu nowych receptur ważna jest umiejętność przewidywania zdolności substancji do przenikania przez skórę, dlatego w kolejnych podrozdziałach pierwszej części monografii omówione zostały istotne aspekty związane z penetracją związków aktywnych do skóry. Kolejno przedstawiono drogi transportu przez naskórek oraz czynniki wpływające na proces przenikania. W ostatnim rozdziale tej części pracy opisane są wybrane metody *in vitro* pozwalające na ocenę przenikalności substancji aktywnej z produktu do skóry, które stanowią pomocne narzędzie w projektowaniu nowych formulacji.

Rozdział II zawiera najważniejsze informacje dotyczące: właściwości nanoemulsji, technologii ich wytwarzania, metod charakterystyki właściwości fizykochemicznych, a także przykłady zastosowania.

W III rozdziale czytelnik może poszerzyć swoją wiedzę na temat emulsji wielokrotnych jako form fizykochemicznych pozwalających na kontrolowane uwalnianie substancji aktywnych do skóry. Podobnie jak w rozdziale II w kolejnych podrozdziałach tej części omówiono właściwości fizykochemiczne, metody otrzymywania oraz charakterystyki emulsji wielokrotnych.

Rozdział IV poświęcony jest submikronowym nośnikom substancji aktywnych. Omówione zostały różnego rodzaju nanoczastki z grupy nieorganicznych, polimerowych i lipidowych (SLN i NLC) nanonośników. W tym rozdziale oczywiście nie zabrakło miejsca również na przedstawienie liposomów jako efektywnej formy dostarczania substancji aktywnych przez skórę. W rozdziale IV, podobnie jak w II i III, zawarte są kompletne informacje na temat metod otrzymywania, badania właściwości oraz zastosowania submikronowych nośników.

ZUSAMMENFASSUNG

Selected transdermal delivery systems ist eine Monographie über die modernsten Formen von kosmetischen und pharmazeutischen Produkten wie Nanoemulsionen, Mehrfachemulsionen, Liposomen und verschiedenen Arten von Nanopartikeln, die als Träger von Wirkstoffen verwendet werden. Auf dem modernen Markt für Kosmetika und pharmazeutische Produkte stellt eine wachsende Nachfrage nach absolut sicheren und wirksamen Produkten dar. Eine der Bedingungen, um ein wirksames Produkt zu erhalten, ist die Entwicklung von Formulierungen, die die Abgabe von Wirkstoffen an die richtige Stelle in der Haut ermöglichen.

Das Buch ist in mehrere Kapitel unterteilt. Kapitel I widmet sich der Struktur einer Haut. Die menschliche Haut ist die Hauptschutzbarriere, die das Eindringen von exogenen Verbindungen in den Körper begrenzt. Bei der Entwicklung neuer transdermaler Formulierungen ist es wichtig, die Fähigkeit von Wirkstoffen, in die Haut einzudringen, vorherzusagen. In den folgenden Abschnitten des Kapitels I werden wichtige Aspekte des Eindringens von Wirkstoffen in die Haut erörtert. Anschließend werden Transportwege durch die Epidermis und Faktoren vorgestellt, die den Permeationsprozess beeinflussen. Die letzte Einheit des Kapitels I befasst sich mit ausgewählten in-vitro-Methoden, mit denen die Durchlässigkeit der Wirkstoffe vom Produkt auf die Haut beurteilt werden kann. Diese sind ein hilfreiches Instrument für das Design neuer Formulierungen.

Kapitel II der Monographie enthält Informationen zu Nanoemulsionen, ihrer Zusammensetzung, thermodynamischen Stabilität und Herstellungsverfahren. Darüber hinaus wurden Methoden zum Testen physikalisch-chemischer Eigenschaften der Nanosysteme und deren Anwendungen in der kosmetischen und pharmazeutischen Industrie beschrieben.

In Kapitel III kann der Leser sein Wissen über multiple Emulsionen als physikalisch-chemische Formen erweitern, mit denen die Freisetzung von Wirkstoffen in die Haut gesteuert werden kann. Ähnlich wie in Kapitel II wurden in den folgenden Unterabschnitten physikalisch-chemische Eigenschaften, Methoden zur Gewinnung und Charakterisierung der verschiedenen Systeme erörtert.

Der letzte Teil der Monographie, Kapitel IV, befasst sich mit Submikron-Vehikeln der Wirkstoffe, die in kosmetischen und pharmazeutischen Produkten verwendet werden. Verschiedene Arten von Nanopartikeln aus der Gruppe der anorganischen, polymeren und lipiden (SLN und NLC) Nanoträger wurden diskutiert. Unter anderem werden auch Liposomen als wirksame Form der Wirkstoffabgabe durch die Haut vielfach diskutiert. Ähnlich wie in den vorhergehenden Abschnitten sind in Kapitel IV die vollständigen Informationen zu den Methoden zur Gewinnung, Prüfung der physikalisch-chemischen Eigenschaften und zum Aufbringen der Submikron-Träger für biologische Wirkstoffe beschrieben.

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