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PhD dissertation

Investigation on domestic fruits seed oils in personal care emulsion systems

by

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ABSTRACT

The use of fruit seed oils in personal care products is of significance to both their function and image. Poland is an important processor of fruit products within the EU, and thus has a large availability of seeds from domestic fruits, which are normally considered to be a waste material. Unfortunately, current literature is scarce of the suitability of these oils for topical use in the form of cosmetic emulsions. Published data on these oils is generally limited to their use in the food industry.

The aim of this dissertation was to determine the viability of domestic fruit seeds, such as apple, blackberries, blackcurrants, plum and strawberries as sources of unsaturated fatty acids (PUFAs) in personal care emulsions. To that end the seed oils were tested for their fatty acids composition. Consequently, the fruit seed oils were used as an additive in the oily phase components in oil-in-water (O/W) systems and nanostructured lipid carriers (NLCs). The latter as a delivery and protection system for the PUFAs

The O/W systems stability, rheological properties and the sensory analysis were tested. The best emulsion obtained during the formulation optimization process had the composition 4% and 5% of seed oil with an o:w phase ratio 20:80. The best stability had the emulsions with ratio polar:nonpolar lipid 60:40 respectively.

In fact, the bioactive components are useful only if they are able to penetrate the skin unchanged. Therefore, an alternate way to deliver naturally occurring PUFAs was presented. Thermodynamic (DSC) and structural techniques (^1H NMR) were applied in order to characterize the obtained systems in terms of seed oil incorporation into the NLC, and oxidative stability tests were used to confirm the protective quality of the systems. During the formulation optimization process the most stable nanosuspension with the best seed oil incorporation was a mixture of 4% nonionic emulsifiers, 88% water and 6% lipids with a ratio of 6:2, wax:oil. The oxidative stability tests showed that the NLC was an effective method of protection of the PUFAs.



Most of the research within this dissertation was conducted at the Gdańsk University of Technology in Department of Colloid and Lipid Science (formerly: Department of Fats and Detergents Technology).

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LIST OF ABBREVIATIONS

AA	-	arachidonic fatty acid
ALA	-	α -linolenic fatty acid
AOE	-	nonionic emulsifier, mixture of ethoxylated fatty alcohols C ₁₆₋₁₈
CER	-	Ceramide
CHOL	-	Cholesterol
CMC	-	critical micelle concentration
DAG	-	Diacylglycerols
DGLA	-	dihomo-gamma-linolenic fatty acid
DHA	-	docosahexaenoic fatty acid
DSC	-	differential scanning calorimeter
EFA	-	essential fatty acids
EPA	-	eicosapentaenoic fatty acid
ETA	-	eicosatetraenoic fatty acid
FFA	-	free fatty acid
GLA	-	gamma-linolenic fatty acid
HLB	-	hydrophilic-lipophilic balance
IL	-	intercellular lipids
IV	-	Iodine value (IV)
LA	-	linoleic acid
m.p.	-	melting point
MUFA	-	mono unsaturated fatty acid
NLC	-	nanostructured lipid carrier
O/W	-	oil-in-water emulsion
OL	-	occlusive layer
PCS	-	photon correlation spectroscopy
Pdi	-	Polydispersity
PUFA	-	polyunsaturated fatty acid
PV	-	peroxide value (PV)
RI	-	refractive index
SC	-	stratum corneum
SD	-	standard deviation
SDA	-	stearidonic fatty acid
SFA	-	saturated fatty acid
SLN	-	solid lipid nanoparticles
SV	-	saponification value (SV)
TAG	-	Triacylglycerols
TEWL	-	transepidermal water loss
UV	-	ultra violet radiation
W/O	-	water-in-oil emulsion
ζ	-	zeta potential
λ	-	electromagnetic wavelength



Introduction

This thesis will discuss the viability of seed oils derived from the food processing industry by-products for a variety of cosmetic emulsions. The domestic fruit seeds¹, such as apple, blackberries, blackcurrants, plums, and strawberries are an important source of unsaturated fatty acids, which are a crucial component of topical cosmetic products. This thesis will investigate the formulations, emulsion stability, and various modifications [1] to improve these fruit seed oils emulsions.

1.1 Components of vegetable lipids

The natural lipids consist of different compounds with diversified chemical structure. The main group are acylglycerols. The content of triacylglycerols (TAGs) in oils and fats reaches about 95 - 98%. Substantial content of specific acyl groups connected to glycerol (*i.e.* the chain length of the acyl group and the degree of saturation) determines the form and properties of the lipid. Other compounds such as diacylglycerols (DAGs, about 0.5%), free fatty acids (FFAs, about 0.1%) are also present [2]. The amounts of additional compounds such as phospholipids, free and esterified sterols (about 0.3%), tocopherols (about 0.1%), triterpene alcohols, and coloring matters like carotenes or chlorophylls are variable. However, these minor components are also important. They affect the oxidative stability of the oil, as well as the dermatological properties (*i.e.* tocopherols). Oils also contain some amounts of hydrophilic compounds (*i.e.* hydrophilic phenols or hydratable phosphatides). The phospholipids or some sterols show emulsification properties, so they may act as co-emulsifier in dispersed systems. The content of saponifiable matter is variable. The composition of the oil may differ according to the method of obtaining and processing them. The most popular modification of oils is refining. It is a multi-step treatment (degumming, neutralization, bleaching, and deodorization) designed to purify the oil from phosphatides, FFA, color substances, undesired odoriferous and/or flavoring. Additionally, also metals, residual soap (from neutralization), pesticides, herbicides, polycyclic aromatic hydrocarbons

¹ For the sake of readability all tested oils will be referred to as “seed oils”, although in some cases the correct form should be ‘kernel oil’ or ‘pit oil’.

are removed from oil. The process can also be carried out so as to remove only certain components in a predetermined amount (*i.e.* incomplete). If the terms of cultivation, harvesting and storage of plants are in accordance with the recommendations, the oil does not have to be refined and the amount of the minor compounds is balanced and does not threaten the quality of the oil and of its users.

According to literature, seeds account for 4 – 12 wt % of the fruit, and contain 10 – 28 wt % of oil [3-5].

The oils are used as the main active ingredient in many personal care products, as their beneficial effect on skin is well known, and will be described in the following sections.

1.2 Skin and transdermal penetration

The skin is the largest organ of human body. It consists of three main layers: subcutaneous tissue, dermis, and the uppermost epidermis. The area of interest in this dissertation is limited to the epidermis, as it is the main area of cosmetic activity. The construction and functioning of the epidermis is exceptional and vital to the health of humans.

The epidermis is a constantly renewing multilayered epithelium. In Figure 1 a detailed layered structure is presented. Closest to the dermis is the *stratum basale*. It is a continuous layer, which is with some exceptions one-cell thick. The cells are cuboidal and have large nuclei. These form the first step of cells growth in the epidermis [6]. Above these is the *stratum spinosum*. It is composed of several layers of polygonal cells. This is where keratinization² process begins. The desmosomes³ and gap junctions are formed, which join the cells together and facilitate intercellular communication. In this layer lamellar bodies occur, which are the source of polar lipids, free sterols, phospholipids and enzymes. These lipids form an intercellular lipid (IL) structure. The *stratum granulosum* is the next layer, which is two to five cells thick. The nuclei contain grains of keratohialin, a protein that plays a key role in keratin fibers formation. The outermost layer of the epidermis is the SC, where

² Keratinization is a process of cytodifferentiation. The epidermal cells proceed from stratum basale to stratum corneum.

³ Desmosome is a cell structure that enables the mutual adherence of the skin cells

cells (corneocytes) have no longer nuclei and cytoplasmic organelles. The cells become flattened. Filaggrin (protein) is responsible for keratin filament aggregation, and is essential for epidermal homeostasis regulation. In SC, the filaggrin monomers become incorporated in the so called cellular envelope, which is responsible for the skin barrier function [7]. The component that join the envelope with the IL is the CER 1, of which linoleic acid (LA) is the main component. The filaggrin is degraded into derivatives of hygroscopic molecules of low molecular weight, such as urea, pyrrolidone carboxylic acid (1,2), glutamic acid, and other amino acids, which form natural moisturizing factor (NMF) [6, 8, 9].

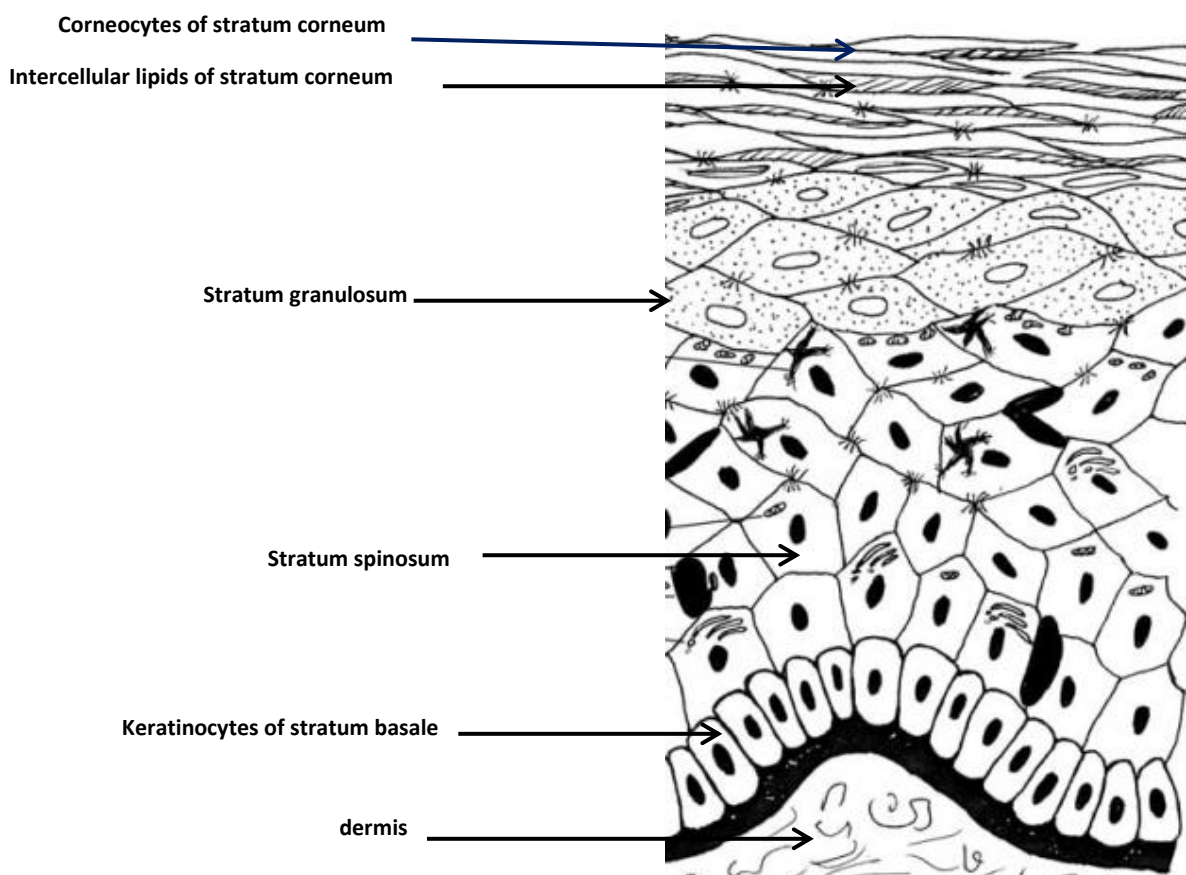


Figure 1 Cross section of the epidermis [10] - clear differences in the construction of the epidermis. Change in the appearance and characteristics of the cells can be observed



The process of cells differentiation is also called keratinization, because the amount of keratin inside the cells increases with cell maturation. The process of cell renewal takes about 28 days.

The skin is an effective link between an environment and the body. It reacts to physical and chemical stimuli received from inside and outside the body. It is also a permeable barrier, which protects but also enables selective penetration of chemical components into its structure. The personal care chemicals can penetrate the skin in three possible routes: via hair follicles, via sweat ducts, and through the epidermis. The last can occur through cells and intercellular area, or only through the intercellular area, which is longer but more advantageous due to its uniformity. This transdermal penetration phenomenon is complex and dependent on many factors which limits the efficacy of topical formulations. The human skin is composed of cell layers, which differ from each other in structure and properties. This means in practice that the active substance, which penetrates, must overcome a relatively small distance (the epidermis is 0.2 to 0.8 mm thick) within very diverse conditions [11]. The most important is the difference in total water content between the cells of SC (10%) and the stratum basale (over 70%). This is caused by the osmotic gradient [12], of which the natural consequence is the transepidermal water loss (TEWL). The water loss is at least 0.5 mg of lipids per hour/cm², about 300 g/day) [13]. This phenomenon keeps homeostasis up by regulation numerous bio-chemical reactions occurring in the skin like proteolysis of desmosomes [14, 15].

The cells are surrounded by IL. This is a lipid bilayer that consists of phospholipids, ceramides (CER), FAs, cholesterol (CHOL) and its esters (cholesterol-3-sulphate), and sterol esters. These IL occur in various forms such as crystalline, semi-crystalline, gel, and liquid. It is well organized and acts as a cells adhesive. The skin penetration enhancers may disrupt the ILs organization (as shown in Figure 2), by fluidization, polarity alteration, phase separation or lipid extraction. As long term side effect of this, is an increase in the TEWL. That is why the most preferable cosmetic compounds have a small molar mass (<600 Da), good lipid affinity, and a high partition coefficient [16], which results in a relatively quick transition through the SC. The skin surface is protected by the dead cells of SC but also by the sebum. This is a thin layer of lipids produced by the sebaceous glands and secreted on the skin surface. The sebum consist of triglycerides, wax esters, and squalene [17]. Together



with sweat they form a so called hydrolipid coat, which is a naturally occurring emulsion. Sweat is the aqueous phase of the emulsion. The characteristic features of the sebum is the acidic pH which makes skin surface a hostile environment for bacteria and fungi. The sebum protects the skin from penetration of undesirable chemical substances. Thus, to some extent, the task of cosmetics is to overcome the skins natural protection capabilities to enhance its function.

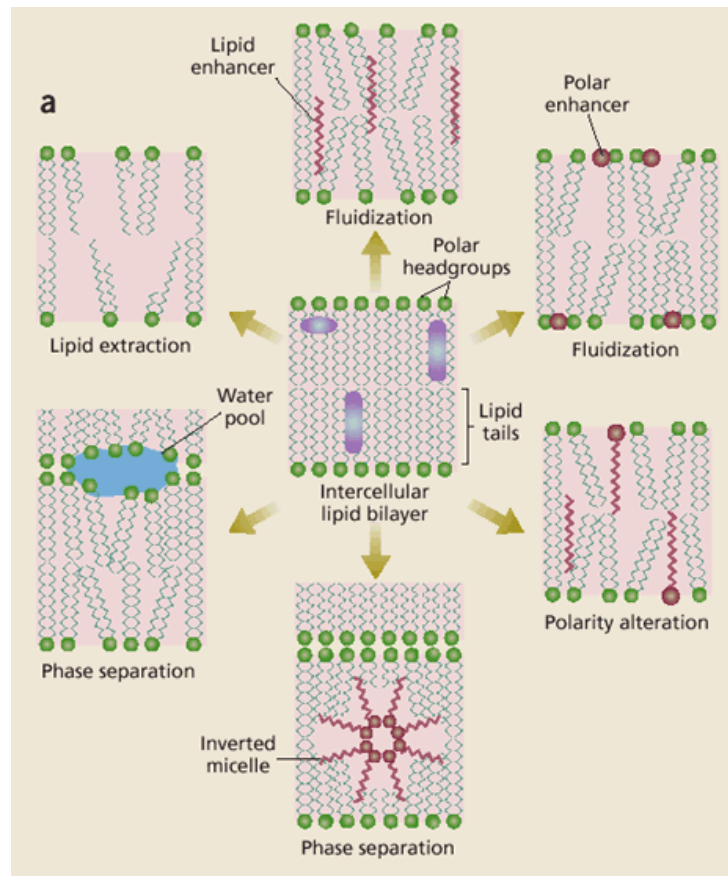


Figure 2 The ways of IL modification by penetration enhancers in SC [18]. Any disturbances in the IL chemical composition of lipids contribute to TEWL increase with all the consequences.

The driving force for an active substance to penetrate the skin is its concentration gradient. While it is important, the concentration gradient does not insure absolute penetration [19]. The reason for that is the structure of the SC. Its biphasic composition of hydrophilic cells and lipophilic intercellular space cause a greater possibility of penetration for lipophilic compounds with low polarity [20], even though the area is incomparably smaller, *i.e.* <1 %. The corneocytes area is estimated to be 99.9% in SC [18]. The penetration ability of chemicals depends on their physical and chemical properties (stability, polarity,

hydrophilicity, lipophilicity, and molecular weight). Equally important is the size of the particles. The best solution is therefore a combination of small sized dispersed phase containing bioactive substance and its appropriate carrier. Only this can help the bioactive component to reach its destination in original (stable) form. This goal may be reached by penetration enhancers [21] and so called delivery systems. Such systems include cyclodextrins [22] and liposomes [23] or emulsion systems such as microemulsions [24], nanocapsules [25, 26], nanoemulsions [27] and lipid nanoparticles [28]. Commonly known and used in the cosmetic industry are liposomes. However, they have a number of disadvantages. The main and most important is that they cause a decrease in the viscosity of the crystal structure of ILs. Moreover, they are sensitive to shear stress that normally occurs during the application of the cosmetic product.

Microemulsions are not widely used on the skin due to the high content of emulsifiers. There is a high risk that the excess of emulsifier will emulsify the ILs, which can cause dryness of the skin, resulting in irritation. Lipid nanoparticles is one possible delivery system due to the specific structure of the matrix and its ability to incorporate active substances into the spaces between the FA chains. These will be discussed in later chapters.

1.3 The role of vegetable oils in personal care products

The use of lipids in cosmetic products is wide. They are present in every physicochemical form of cosmetic products, among which the most popular are various emulsion systems. The method of action and characteristics of lipids depend primarily on their chemical structure. Their composite activity is due to the fact that they are mixtures of various chemical compounds. The individual components of lipids may have extraordinarily different properties. They can be used as surfactants, emulsifiers, lubricants, plasticizers and solvents [29].

From a dermatological viewpoint, the emollients⁴, which include the cosmetic lipid components are divided into two groups: containing biochemical active substances and those whose activity is limited to the occlusive layer (OL) formation. The OL is formed on the skin surface after the cosmetic application. It moisturizes the upper skin layer through TEWL

⁴ Lipophilic moisturizers, which reduce TEWL, and are often designed to soften the skin



reduction. This is crucial due to a number of aspects, the most obvious of which is dryness of the skin. It is also noteworthy that a lower water content in the skin inhibits the function of enzymes⁵. It must also be emphasized that TEWL reduction is a physical process assisted by SFAs and PUFAs [30], and therefore, is partly seed oils dependent.

PUFAs, due to their chemical affinity are able to complement structures of the IL of the SC [31, 32]. These bio-active components of plant lipids are comprised mostly of PUFAs, and thus include essential fatty acids (EFAs). PUFAs play an important role in the correct functioning of an organism, including the skin physiology [33]. As skin is a peripheral organ and thus, if insufficient quantities of PUFAs are supplied, the skin is often deficient in these components. This is aggravated by the often limited supply of PUFAs in food. Their deficiency induces characteristic changes in skin such as extensive TEWL, which manifests dryness, or keratinization disorders. These often lead to an excessive epidermis desquamation⁶ and hyperproliferation⁷ [34, 35]. In addition, PUFA deficiency of the skin will lead to an “ectodermal defect”, which is caused by an impaired production of ceramides and lipids in the skin, resulting in excessive drying and skin irritation or even dermatitis (skin inflammation). The symptoms of acute inflammation are pain, heat, redness and swelling. It is a response of the organism to remove the injurious factor and to initiate the healing process. The inflammation is influenced by the presence or absence of EFAs (also commonly known as “omega acids”). These EFAs were named due to their double bonds position: n-6 (ω -6) and n-3 (ω -3) [36]. α -linolenic acid (ALA, 18:3n-3) is the parent compound of the (ω -3) fatty acids (FAs) and linoleic acid (LA, 18:2n-6) is the parent compound of the (ω -6) FAs. These two EFAs have a major impact on the proper skin functioning [37, 38], since are progressively converted into various bio-active substances. One of these derivatives, icosanoids are synthesized from LA and ALA as a precursor. The icosanoids derived from ω -6 are much more pro inflammatory as compared to the derivatives of ω -3. These signaling molecules are synthesized as required, *i.e.* immediately after mechanical trauma of the cell [39]. In addition, LA and its derivatives protect the skin against the side effects of ultraviolet radiation (UV). UV-B suppresses the immune system and induces an inflammatory response

⁵ By lowering the level of hydration of the enzymes, and their access to substrate molecules, and altering their tertiary structure

⁶ Skin peeling which is unnoticeably when normal and intensified when result from disease or injury

⁷ Abnormally accelerated process of physiological cell renewal of the epidermis



by disruption of prostaglandin and leukotriene synthesis [40, 41]. The photo protection mechanisms in the skin relies on a balance between inflammatory, immune and antioxidant systems, so as melanin production. Increased TEWL, inflammation processes and keratinization disorders lead to skin dryness, which additionally intensifies these processes. Moreover, LA can be incorporated in the lamellar bilayers structure, and also is an essential part of ceramides synthesis which takes place in the epidermis [42]. This is crucial for proper skin functioning. The ILs organization was not fully known and understood until 2012. Iwai *et al.* [43] as first described it as stacked bilayers of fully extended CERs with cholesterol (CHOL) molecules associated with the CER sphingoid moiety. The intercellular lipids of the SC is presented in Figure 3.

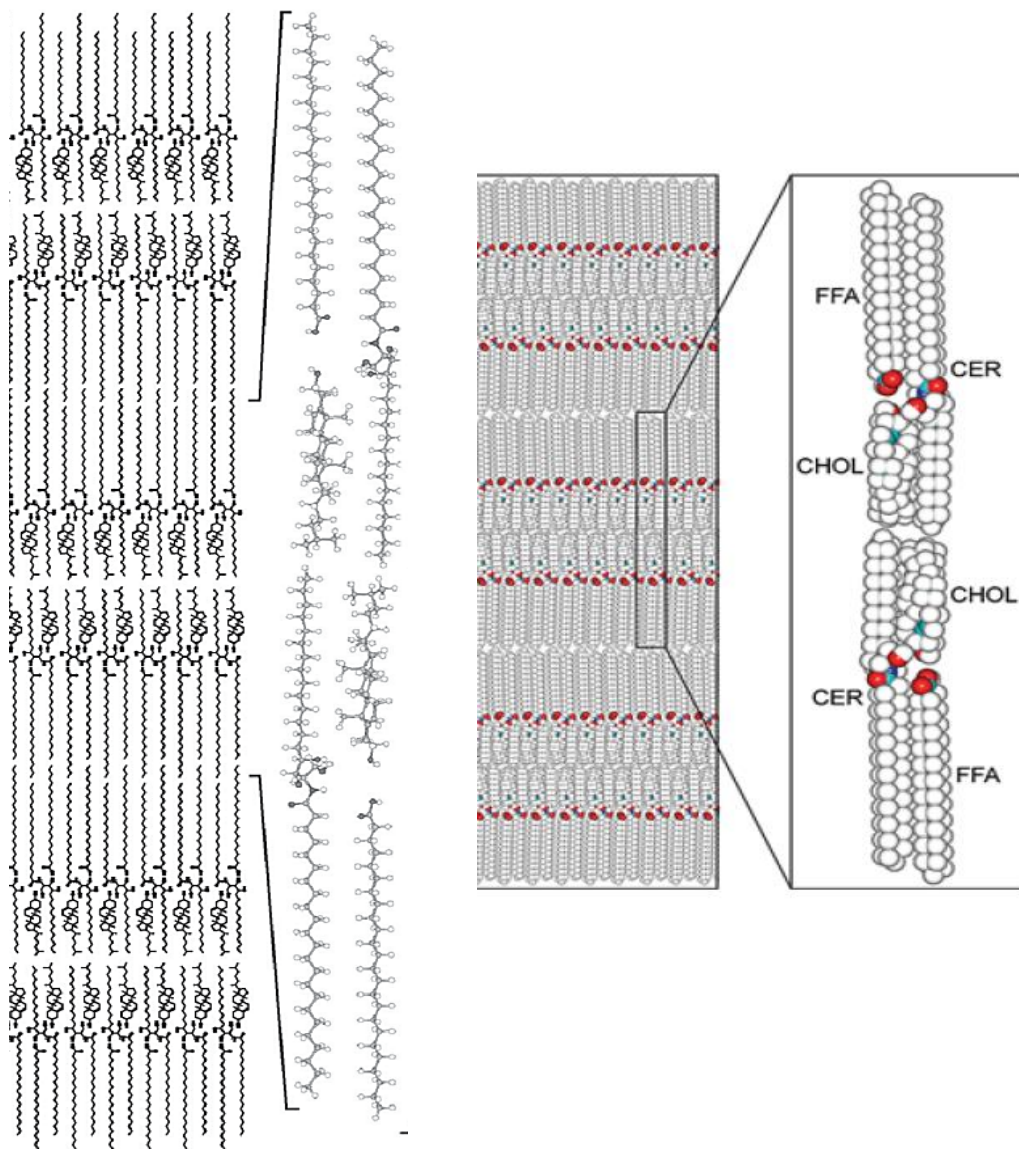


Figure 3 The intercellular lipid structure tetracosanylphytosphingosine (C24:0) in fully extended conformation with cholesterol associated with the CER sphingoid part, and FFA (lignoceric acid, C24:0) associated with the CER FA part [43, 44].

The intercellular lipid structure is condensed and ductile and thus make the SC a barrier with low permeability [43, 45]. That indicates that stay-on type personal care products should contain compounds forming part of SC, thereby the IL structure can be supplemented without disrupting it after application of penetration enhancers.

Since the CERs and FAs play a key role in the organization of the bilayer structure and barrier function of the SC, they should be the key bioactive components of the cosmetic

formulations. The other possibility is to let the skin to synthesize the CER by itself. Natural oils can supplement skin in FA also via external use [46]. It may also occur by topical use of the emulsion systems. The mechanism is presented in Figure 4.

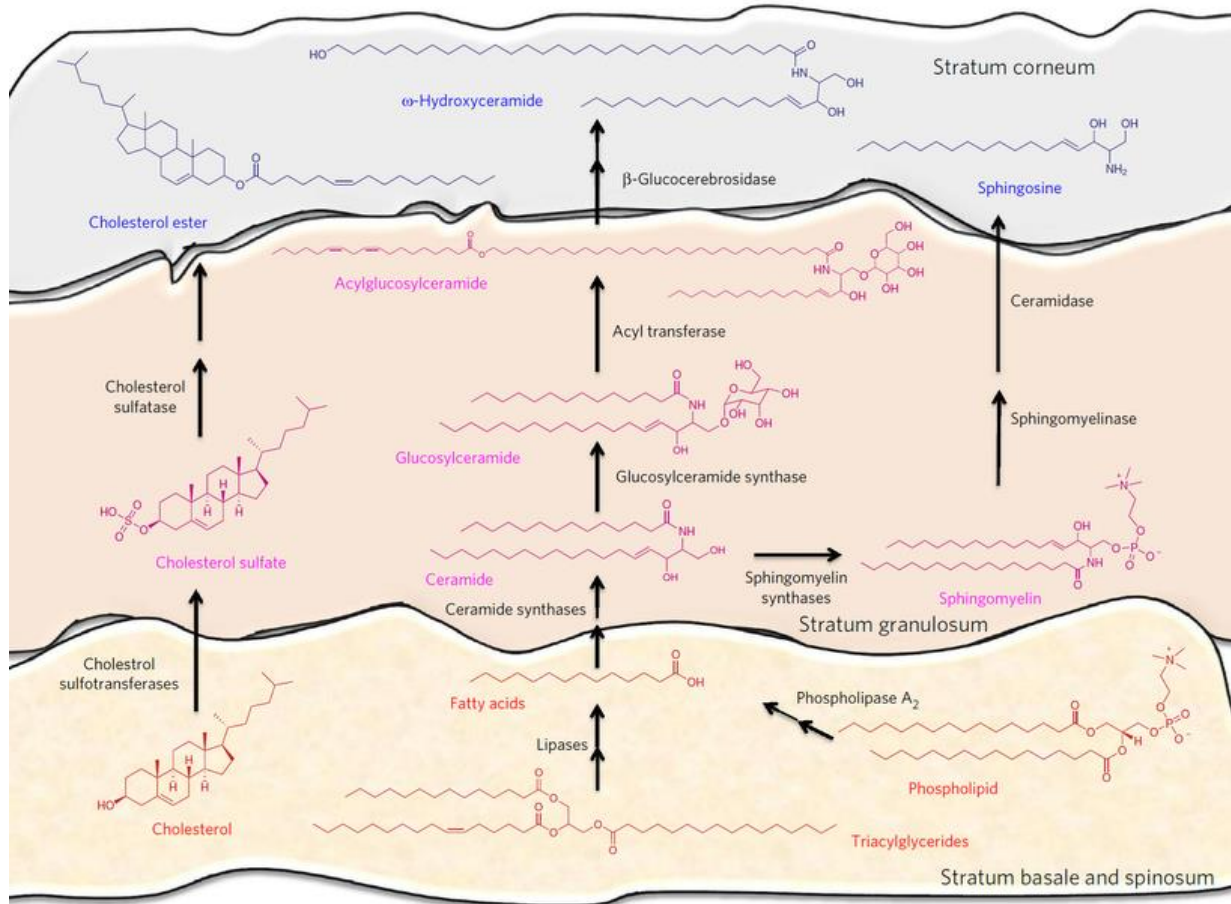


Figure 4 Lipids and enzymes that are secreted at the interface between layers are further modified to form long chains of ceramides [46]

It is therefore of primary interest of this work to design such emulsions to facilitate and complement the skin. To that end, the possibility of supplementing the UFAs, from a variety of sources has been investigated. The supplementation can concern also the PUFAs, since the ALAs main advantage is anti-allergenic activity by the allergic mediators decrease [47]. The skin allergy leads to disruption of the intercellular lipid composition of the SC. On the other hand, lipid disorders in SC enhance allergy symptoms. The mutual relationship of causes and effects suggests the cosmetic emulsion should contain FAs.

1.4 Domestic fruit (seed) oils in comparison to exotic fruit (seed) oils

Oils from distant corners of the world are still gaining popularity. The exotic seed oils are desirable components of all cosmetics. It is noted that some oils are occasionally more fashionable than others for a given time. The advertisers praise the “magnificent” properties of an oil. This is especially true for exotic oils, where the word “exotic” usually allows the consumer to feel that the oil is unique and exclusive. Their effect on the skin, nevertheless, in most cases it is nothing more than marketing and product imaging, which is enhanced by additives of an exotic nature.

Analyzing available data, on popular seed oils it appears that our domestic seed oils are the same or even better if taking under consideration the amount of its valuable components. The components, which to a large extent determine the quality of oil are UFAs of the C18, *i.e.* C18:1, C 18:2 and C 18:3 [38]. The comparison of most popular seed oils due to its origin is presented in Table 1.

Table 1. Most popular seed oils used in personal care products in comparison with tested seed oils [47-56]

Oil common name	Plant Latin name	Fatty acid quantity % in total seed oil				Origin Most cultivated crop
		C18:0	C18:1	C18:2 (LA)	C18:3 (ALA)	
Moringa	<i>(Moringapterygosperra)</i>	6.0	71.8	0.6	0.1	Africa
Marula	<i>(Sclerocaryabirrea)</i>	7.4	71.3	6.7	<0.5	Africa
Andiroba	<i>(Carapaguaianensis)</i>	5.0-12.0	45.0-55.0	5.0 15.0	– 0.0-1.0	Brazil
Argan	<i>(Arganiasponosa)</i>	4.3-7.2	43.0-50.0	29.0-37.0	4.3 – 7.2	Morocco
Cloudberry	<i>(Rubuschamaemorus)</i>		13.0-19.0	40.0-52.0	27.0-38.0	Northern parts/countries (Canada, Russia, Scandinavian counties)
Grape	<i>(Vitisvinifera)</i>	3.0-6.0	12.0-27.0	60.0-76.0	0.0-0.5	
Hemp	<i>(Cannabis sativa)</i>	2.0-4.0	8.0-15.0	50.0-60.0	15.0-25.0	Asia
Perilla	<i>(Perillafrutescens)</i>	1.0-3.0	12.0-22.0	13.0-20.0	0.0-1.0	East Asia
Meadowfoam	<i>(Limnanthes alba)</i>	0.0-3.0	8	51	20	California
Jjoba	<i>(Simmondsiachinensis)</i>	0.0-0.14	5.0-15.0	<0.5	<0.5	Central America
Amarantus	<i>(Amaranthuscruentus)</i>	1.0-4.0	16.0-25.0	41.0-61.0	<0.5	Central America
Blackcurrant	<i>(Ribesnigrum)</i>	1.0-3.0	10.0-16.0	43.0-49.0	12.0-16.0	Europe, Asia
Blackberry	<i>(RubusFruticosus)</i>	1.0-4.0	15.0-19.0	50.0-65.0	14.0-20.0	Europe
Raspberry	<i>(RubusIdeaus)</i>	0.0-3.0	12.0-16.0	48.0-60.0	20.0-30.0	Europe, Asia
Strawberry	<i>(Fragariaananassa)</i>	1.0-4.0	14.0-19.0	38.0-48.0	30.0-39.0	Europe, Asia
Plum	<i>(Prunusdomestica)</i>	0.0-3.0	60.0-80.0	15.0-25.0	0.0-0.5	Europe, Asia
Apple	<i>(Pyrusmalus)</i>	1.0-3.0	28.0-34.0	52.0-64.0	0.5-2.0	Europe, Asia

The seed oils from domestic fruits are a rich source of UFAs. The blackcurrant-, blackberry-, raspberry- and apple seed oil are characterized by an exceptionally high content

of LA. The plum and apple seed oils are rich in oleic FA (up to 80% and 34% respectively). The comparably high amount of this FA contain argan, cloudberry, grape, hemp and amarantus oil. The significant content of ALA is present in raspberry and strawberry seed oil (up to 30% and up to 39% respectively). Lower amounts of ALA (but still significant) is present in berries seed oils (up to 20%). The comparably high amount of this fatty acid content contain only cloudberry and hemp oils. Taking into account the data presented in Table 1, it can be seen that the native seed oils can be considered as a valuable component in pharmaceutical and cosmetic industry. Their wide spread use in the cosmetic industry is only restricted by marketing.

1.5 Fruit seeds as a waste by-product

The demand for natural plant ingredients is still increasing in all industries, in particular the cosmetic industry [57]. Public attention is not just on the use of plant materials, but rather the reutilization of waste plant materials which is consistent with the principles of green chemistry [58]. The scale of the problem related to the amount of waste material is even greater because Poland is the biggest fruit processor of apples, blackcurrants, raspberries and strawberries in this part of the world [59]. For this reason, there are hundreds of tons of seeds that are by-products of food processing. Studies have shown (Chapter 1.4) that cold pressed seed oil contain a significant amount of bioactive components such as PUFAs and tocopherols [60, 61] which are crucial for proper skin functioning. This additionally justifies the use of waste seeds as raw materials for the oil production. This is even more profitable if these oils should be extracted by cold pressing⁸. This method is simple, inexpensive and ecological. It is admittedly less efficient than pressing after heating the raw material (seeds), but the quality of the cold pressed oil is higher [62] and thus more suitable for topical application.

The cold pressed domestic fruit seed oils should be therefore used as components of the personal care products formulation.

⁸ Cold pressing is a mechanical method for extracting oil from raw materials, which are squeezed under high pressure in a single step.



1.6 Formulation process

The formulation process is applied when either a new raw material or new technology is incorporated into the cosmetic product. In this dissertation the viability of cold pressed domestic fruit seed oils obtained from waste material as new potential cosmetic ingredients is investigated. In this case, the first step of the formulation process is the determination of the general product quality features (physical and chemical characterization) in order to anticipate its influence on the skin, and most suitable type of personal care product in which it will be incorporated. It is crucial to state the initial assumptions and select the basic ingredients that could fulfill the intended goals of the product. The optimization should be a deliberate action carried out with only basic ingredients in order to ease further changes and to have no interferers that may occur while using dozens of ingredients at time. For the formulating of an emulsion system the components of individual phases, the emulsifier or emulsifier mixtures with suitable HLB should be planned, in addition the mixing parameters are chosen. The latter directly affect the particle size. The optimization process concern the qualitative and quantitative selection of individual components, phases and their volume ratios. One parameter or component is altered in each iteration or change. After every change the system stability should be tested. The stability test is the starting point for further steps of optimization. After selection of the best formulation, other components may be added, for example thickening agents. The schematic steps of formulation optimization process is presented in Figure 5.

During further procedures microstructure, stability, rheological behavior and sensory analysis is tested. The final goal is a prototype (“base”) that can be ready to use or prepared for further optimization in order to design a scale-up manufacturing process. All the rheological behavior profiles parameters have to be taken under consideration for proper condition adjustment of technical mixers. Thus the flow behavior is crucial also in terms of power consumption, blending speed and time, final viscosity, and so in post treatment time, *i.e.* while pouring into the containers. The latter is of great importance also in terms of application.

The formulation optimization process is complex and is based on consequent testing in order to obtain the system with best parameters.



This thesis will follow this formulation process. Initially, the PUFA content for the fruit seed will be analyzed, then simple O/W emulsions will be prepared, and analyzed. Eventually sensory analysis will be conducted and more advanced formulations will be used to protect the PUFAs.

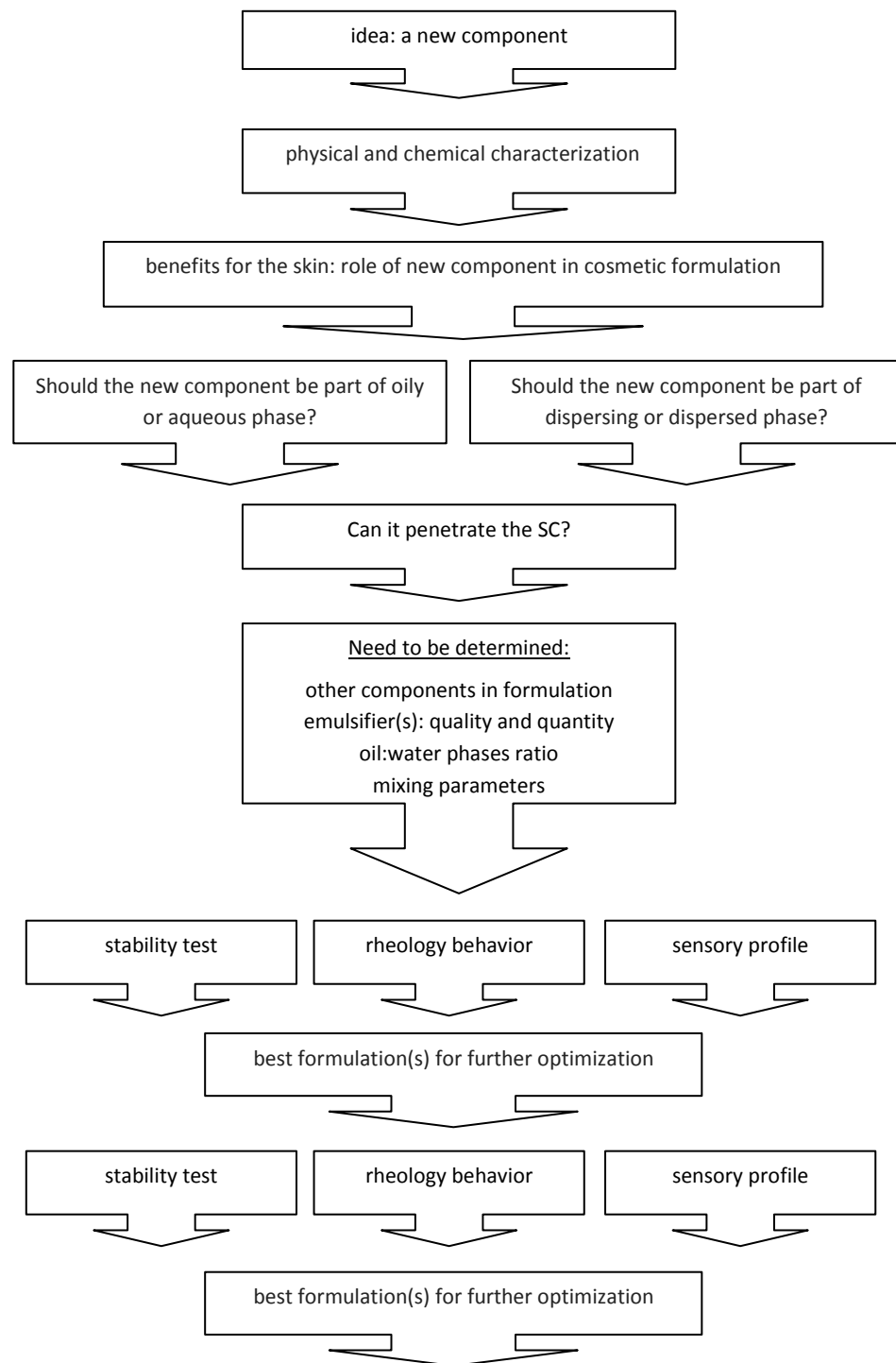


Figure 5 Schematic steps of formulation optimization process

2 Aims of the research

It was aimed that the research will be a contribution to the efficient use of native fruits' seeds and kernels, which are waste by-products in the food processing industry. The fruits investigated are: apples, strawberries, raspberries, blackberries, blackcurrants and plums. The scope of this work covers the physicochemical analysis of domestic fruit seed oils and their viability as an oily phase in oil-in-water emulsion systems. The simple emulsions and nanostructured lipid carriers were chosen as representatives of personal care emulsions. It was assumed that the obtained systems form a model product that can be modified depending on the purpose and needs of the cosmetic or pharmaceutical industry. Therefore, systems contain only the required number of ingredients most commonly used in these industries. They contain neither the excipients⁹, such as those extending microbial stability, nor thickeners, coloring agents, pH regulators and perfumes.

Preparation of various kinds and types of emulsion systems requires the use of different techniques. However, using similar or the same components allows one to compare the obtained systems. The comparative tests are provided in terms of their physical and chemical stability, rheological behavior and sensory analysis. Experiments were conducted in order to help to determine the influence of the seed oil on systems stability, rheological and sensory properties.

The primary aim of the research was chemical and physicochemical analysis of the selected seed oils. It was necessary to determine their appropriateness of use in cosmetic formulations. Due to the fact that these oils contain components that are sensitive to external factors, it was decided to use them as ingredients in dispersed (internal) phase of the systems. Otherwise it would be necessary to apply additional stabilizing components. Nowadays the pharmaceutical and cosmetic industries are focusing on these specific directions of research, whose aim is the reduction of the additional compounds such as emulsifiers, fragrances, UV- and bacteriostatic. The reason is to minimize the potential skin irritation. For the same reason also the amount of biologically active substances is reduced,

⁹ These are additional components used in formulation, such as binders, bulking agents, fillers, diluents, *etc.*

since not the amount of bioactive substance but the way it is incorporated into the system determines its real effect.

Given the current state of knowledge about cosmetic preparations it was necessary to verify the applicability of the oils in lipid nanoparticles, which are modern emulsion system derived from a simple oil-in-water emulsion.

3 Organization of the thesis

In this thesis different types of emulsion formulations were developed to assess the suitability of selected fruit seed oils as emulsion system components. The dissertation also includes results of chemical characteristic of the oils. The emulsions were developed intended with respect to dermal use. The thesis categorizes several experimental works, as discrete chapters (Chapter 5, 6 and 7), with individual methodology, results and conclusion sections. This was done to aid understanding of each separate process and aspect of the formulation process. The thesis consists of nine chapters, which are organized as follows:

The first chapter is an introduction to the thesis. It contains description of the main components of vegetable oils and its role in personal care products. In this part also the issue of possible use of seeds, which are a waste product in the fruit processing industry has been raised.

In the second chapter the aims of the research are outlined.

In the third chapter a structure of the dissertation is presented.

Chapter four contains a literature review. It is an introduction to the experimental part. The essential issues has been presented in the area of emulsion construction and its basic ingredients, the physicochemical and chemical stability, and so the rheological behavior of the systems. In this section the issues related to the sensory analysis are presented.

Chapter five presents characterization of the selected fruit seed oils that in the further parts of the thesis were the subject of research.

Chapter six presents the results of the studies of selected seed oils as lipid phase components of traditional and popular oil-in-water emulsion.

Chapter seven relates to the new technology. It contains the results of new form of oil-in-water emulsion system which is lipid nanoparticles. These form of emulsion can be treated as a final product or as a component of other emulsion to create a multiple system.

Chapter eight and nine present the summary of the research, the final conclusions and the outlook for further studies.

The schematic structure of the thesis is presented in Figure 6.

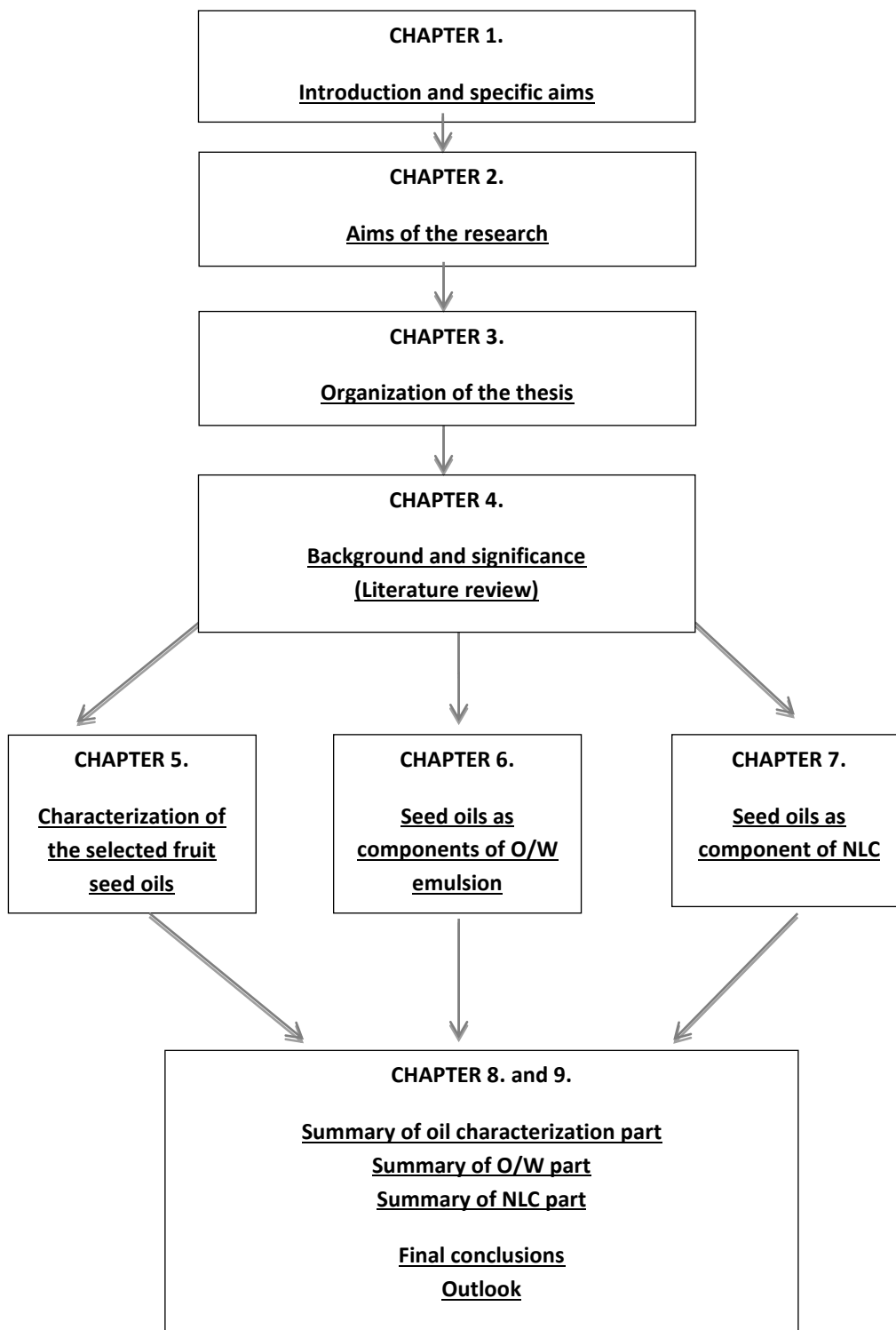


Figure 6 Diagram of thesis construction

4 Background and significance (Literature review)

4.1 Organization, types and classes of emulsion systems

An emulsion system is a special kind of a colloidal system where one phase is dispersed in the other. The main components of such a system are at least two immiscible phases [63]. Their contact results in an interfacial tension increase, which is proportional to the area of the contact. Also the free energy of the system and the entropy increases in proportion to the interface. Such strong interactions prevent the creation of an emulsion. Therefore, the emulsion, with a certain exception, is heterogeneous and thermodynamically unstable. Two immiscible phases will create an emulsion briefly after vigorous stirring. To facilitate the emulsification process and to sustain the result for longer, a surfactant (surface active agent) is needed, which organizes itself on the interphase decreasing the tension. Interfacial area of the emulsion reaches several square meters per gram. The differences are due to the internal phase droplet size. The greater the degree of dispersion, the greater the contact surface phases. There are several types of emulsions based on the dispersed droplet size: macroemulsions (> 400 nm), miniemulsion (100 - 400 nm), microemulsions (5 - 10 nm or 100 - 140 nm) and nanoemulsions/micelles (<5 nm). There is no rigid division, and the exact nomenclature is variable depending on the authors of the papers, or books [64-67]. Nanoemulsions are however not strictly speaking an emulsion, since the oily phase is comprised of the alkyl chains of the surfactants. In addition, any of the above emulsions may be classified as "nanoemulsions" since they all occur in the nm range. The presence of two phases does not imply the presence of only two components. Each phase may contain many components with the same affinity. Ingredients are divided in two phases: "aqueous" and "oily". Therefore, there are oil-in-water (O/W), where droplets of oily phase are dispersed in the aqueous phase, and water-in-oil (W/O), where droplets of aqueous phase are dispersed in oily phase. The emulsions are therefore classified due to the nature and organization of the phases in the system (Figure 7).



As used herein, the term "oil" or "oil phase" is broadly used to describe an oil or an oil plus oil-soluble components desired in the final oil-in-water emulsion. Similarly, the term "water" or "water phase" is used in an analogous broad sense.

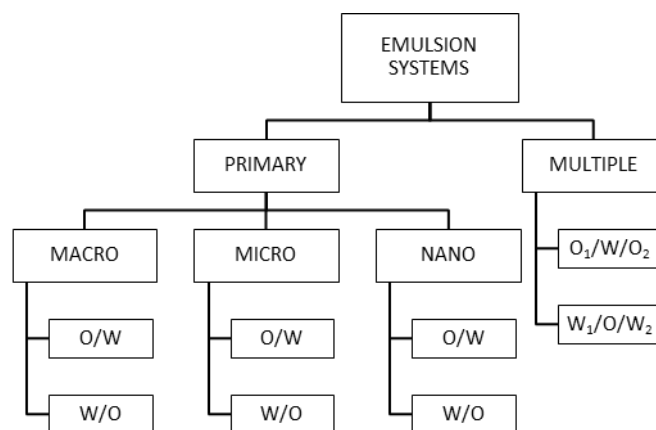


Figure 7. Types and classes of emulsion systems. The systems are divided by type of dispersion (organization of the phases) and the particle size of the internal phase.

To a certain degree of accuracy, the difference in particle size of the emulsion can be determined by observation of the emulsion. The differences in shades are the result of differences in the absorption and reflection of electromagnetic waves of the visible spectrum (400 - 780 nm). Among the emulsion systems microemulsion can always be easily identified. The diameter of the dispersed phase droplets is smaller than the wavelength of visible light, which causes the system to be transparent [68].

Analyzing literature, it can be assumed that the primary emulsions are the starting point for further modification (Figure 7). Frequently discovery of new emulsions are unintentional. An example is the first written mention of multiple emulsions. Seifriz in 1925 described the accidental result of his research as a emulsion systems “with three or four components” [69, 70]. His discovery of multiple emulsions initiated a number of studies. Multiple emulsions are considered to be systems with relatively low stability and poor reproducibility of preparation [71, 72]. The method of multiple systems analysis has not changed significantly since that time, in contrast to the preparation methods. Notwithstanding the multiple emulsions continue to arouse interest. Often referred to as “emulsions of emulsions” [73] as a dispersed phase (inner) forms a primary emulsion, and



the dispersion phase (external) is an aqueous or oil phase, depending on the system type. In this system two types of emulsions (O/W and W/O) coexist [74].

Accidental discoveries, intentional improvement of the already known systems and technological advances have resulted in a multitude of types and kinds of emulsions. In the 90s of the last century, the use of a fat with a high melting point (m.p.) (solid at room temperature) as a single component of the oil phase has resulted in solid lipid nanoparticles (SLN) formation [75-77]. A simple modification has created a number of opportunities to produce new bio-actives delivery systems. This discovery also led to a significant decline in interest in micro- and multiple emulsions. The first due to the relatively high content of surfactants, the latter - due to the difficulties in process repeatability. The new emulsions combine the advantages of these systems (small droplet diameter and the protection of the lipophilic active substance) and do not have their disadvantages. The diameter of the dispersed phase droplets of lipid nanoparticles ranges from 40 to 1000 nm [78].

4.2 Emulsifying agents

The emulsifiers belong to a group of surfactants. These are the chemical compounds whose structure has two parts having opposite properties: nonpolar or weak polar part (hydrocarbon origin), and polar part (both kinds: which can create ions, and other that cannot) [79]. This determines their crucial property, which is their ability to reduce surface or interphase tension. Depending on their chemical structure they can be cationic, anionic, amphoteric (zwitterionic), or nonionic. In a given environment (air-water or oil-air) (hydrophilic or lipophilic) surfactant molecules are adsorbed at the interface according to their affinity. After complete saturation of the border they begin to organize inside in form of micelles. This point is called the critical micelle concentration (CMC), and is the characteristic value for each surfactant concentration at a given temperature [80]. Beyond the CMC two kinds of micelles can be formed due to the chemical affinity of molecule and the external environment. One is where the non-polar alkyl groups are turned inwards (in hydrophilic solution) or inverted micelles, where the non-polar alkyl groups are faced outwards (in hydrophobic solution) [81]. Further exceeding the surfactant concentration



cause the changes in the systems energy. This results from surface tension, conductivity, and viscosity of the environment.

Due to the affinity, some chemical compounds may be incorporated in the space within the micelles. This is the solubilization phenomena and is frequent used in cosmetic industry (micelle solutions). In this case the interface is not formed, which is characteristic for the emulsion systems. In emulsion systems the surfactant is the most important factor that determines and helps to predict the type of emulsion that will be formed. In 1949 Griffin presented his pioneer idea about hydrophilic-lipophilic balance (HLB), which refers to nonionic emulsifiers [82]. The HLB is a parameter that shows the ratio of the hydrophilic part to the hydrophobic part in an emulsifier compound or particle. This method is still used today, however is applicable to pure emulsifier and does not take under consideration factors like: temperature, oil type, co-surfactant concentration, presence of electrolyte, and synergism of the nonionic surfactants mixtures [83] which affect the emulsifier properties and cause changes of the surfactant monolayer curvature. This can change, in some extent, the preferred dispersion type that will be formed. Moreover, the emulsification method, phases volume ratio, and phases viscosity also influence the type of dispersion. That is why the HLB is rather a hint for choosing a suitable emulsifier rather than a main tool in emulsion formula optimization, especially that the required HLB (HLB of the emulsifier adjusted to given oily phase of the emulsion) still has to be determined experimentally [84].

4.3 Emulsion and its effect on the skin

The effectiveness of cosmetics product *i.e.* emulsions is based on two main functions: moisturizing and biological effect. The first is based on reducing the TEWL using a so called incomplete occlusive layer [30]. It is formed after cosmetic application and evaporation of water and volatile compounds. The skin hydration and occlusive layer are of primary interest in the cosmetic industry. Unfortunately, the thin residue of cosmetic on the skin is exposed to external factors *i.e.* high temperature, oxygen and UV radiation. This may cause the free radicals formation and thus easily damage the double bonds in the acylglycerols [85]. In addition, with the presence of water, hydrolysis may occur. FFA are separated from the triacylglycerols (TAGs), causing a decrease in pH [86]. There is an interaction between the

reduced pH and inflammatory mediators, which lead to skin irritation and inflammation¹⁰ [86-88] due to skin tissue mediators excitation [89]. Even though, the cosmetic is well protected in the container, after application it is endangered. In many cases the structure of bioactive components are being destroyed on the skin surface. A result of which the bioactive component may be deactivated in a best case scenario, or in a worst case scenario it will lead to skin irritation.

Decades ago the aim was to use the maximum permissible concentration of the bioactive substance in the cosmetic formulation. This resulted in skin irritation. Nowadays it is known that the delivery system is much more important, as it enables to control the release in the skin. This allows for small doses of bioactive component to be applied, which may release slowly into the skin. That is particularly crucial in case of EFAs such as LA, arachidonic acid (AA), and docozahexaenoic acid (DHA) since they may accumulate in tissues. It is also important in case of components with usage restriction [90].

The EFAs are comprised of LA and ALA are classified as ω 6 and ω 3. It means that first double bond is at the sixth or third carbon atom from the end of the carbon chain. Docozapentaenoic acid (DPA) and docozahexaenoic acid (DHA) FAs are final products of ω 6 and ω 3 FAs conversion. Both regulate formation of the inflammation process. Both are converted in the body [91]. The path of conversion is presented in Figure 8.

	ω 6 FA	ω 3 FA	
	↓	↓	
LA	C18:2n-6	C18:3n-3	ALA
	↓	↓	
GLA	C18:3n-6	C18:4n-3	SDA
	↓	↓	
DGLA	C20:3n-6	C20:4n-3	ETA
	↓	↓	
AA	C20:4n-6	C20:5n-3	EPA
	↓	↓	
	↓	↓	
DPA	C22:5n-6	C22:6n-3	DHA

Figure 8 EFAs path of changes occurring in the organism. Elongation (Increase in chain length by adding two carbon atoms) and desaturation (three new double bonds formation)

¹⁰ Acute or chronic response of the tissue to triggering stimuli such as: UV radiation, physical irritants (injury), chemical irritants (detergents, acids), and allergens.



Both ω -6 and ω -3 FAs are important structural components of cell membranes, serve as precursors to bioactive lipid mediators, and provide a source of energy. Long-chained ω -3 PUFA in particular exert anti-inflammatory effects. Both dietary intake, topical supplementation and endogenous metabolism influence whole-body status of EFA.

Taking into account the above, the PUFAs should be incorporated in kind of vehicle to provide a slow release to the skin. This is why the bioactive chemicals are not applied direct on the skin surface, but used as emulsion component.

4.4 Stability of the emulsion systems

A crucial emulsion feature that indicates its usefulness is its stability. The interfacial tension increase is the main reason of the instability of the system. The migration of the dispersed droplets favors larger aggregates. Keeping the lowest possible interfacial tension determines the stability of the emulsion. The emulsifier (or mixture of emulsifiers) should form a flexible film at the interface. A decrease in stability of emulsion system stems from the fundamentals of physical chemistry and thermodynamics of dispersed systems.

In dispersed systems a series of forces operate. The interfacial region is characterized by specific physicochemical properties that are different from two bulk phases.

A large value of surface free energy causes a decrease of total surface area of the dispersed particles unless the interfacial tension is reduced to keep the system more stable. For that a droplet curvature should be taken into account. The curvature is considered to be close to planar when the droplet size of >10 nm. A curved interface influences the Laplace pressure (P), which is the pressure difference between the inside and outside of a curved surface. The smaller the droplet of dispersed phase, the bigger the pressure inside of the droplet. That is why nanoemulsions may contain a smaller (than usual for larger emulsion sizes) amount of preservatives, because the pressure is high enough to create bacteria unfriendly environment, which is crucial for water-in-oil systems.

In emulsion systems van der Waals attraction take place. There are three types of these force that takes place between atoms or molecules. There are dipole-dipole

interaction (also known as Keesom's), dipole-induced dipole (also known as Debye's) and induced dipole-induced dipole (also known as London's) [92]. The Keesom's and Debye's forces are vectors with large attraction, but different orientation and therefore the forces are cancelled [93]. London's interactions are crucial in emulsion systems. It arises from charge fluctuation. The energy between two atoms or molecules is short range. It is inversely proportional to the sixth power of the separation distance between atom or molecule. On a macroscopic scale, the London's interaction can be added resulting in stronger attraction. The interaction decrease very rapidly with increasing distance separating the atoms. To counteract the van der Waals attraction, two phenomena take place. The first is an electrostatic (Coulombic) repulsion between two similar charged molecules, and the second is steric repulsion due to the nature (size and branching among others) of the emulsifier. The electrostatic repulsion occurs by the adsorption of an ionic surfactant. The surface potential decreases linearly to Stern or zeta potential, and then exponentially with increasing distance. When charged droplets approach each other, the double layer starts to overlap. The maximum value is when droplets become very close and decreases to zero outside the double layer. The repulsion phenomena occurs when droplets separation becomes less than twice the double layer extension. The double layer extension decreases with increase of electrolyte concentration. Thus, the repulsion decreases with increase of electrolyte concentration. This phenomenon and van der Waals theory results in the DLVO theory. The DLVO theory is developed independently by Derjaguin, Landau [94], and, Verwey, Overbeek [95], and it is based on the London-van der Waals attraction and repulsion forces between two close spheres. It explains the emulsion systems stability as a state of balance of two opposing forces: electrostatic and van der Waals. The DLVO theory is therefore an explanation of droplets tendency to agglomerate or stay discrete. The steric repulsion occurs when nonionic surfactant (or polymer) is used.

There are few processes leading to emulsion system breakdown [96]. The processes are more likely to take place simultaneously rather than sequentially. Therefore, it is necessary to observe the system stability at all stages of formula optimization. Thus the stability is a first parameter on the further studies on emulsion systems.



4.4.1 Creaming and sedimentation

When Brownian motion is exceeded, a concentration gradient builds up with larger droplets moving faster to the top (creaming), when droplets density < medium density, or to the bottom sedimentation, when droplets density > medium density. Creaming and sedimentation result from gravitational or centrifugal forces. The creamed or sedimented emulsion can be easily re-dispersed by shaking [97]. The example of creamed emulsion is presented in Figure 9.



Figure 9 Example of creamed emulsion obtained during formulation optimization process (photo by Krasodomska)

4.4.2 Flocculation

It refers to the droplets aggregation into larger units, without any changes in size. Groups of droplets are formed. This phenomena is the result of DLVO theory. It occurs when the repulsion forces of the droplets are insufficient. Flocculation occurs as strong or weak. Flocculation is found in O/W systems [98].

4.4.3 Ostwald ripening

Due to the curvature effect, smaller droplet have larger solubility than larger ones. Especially in systems of significant degree of polydispersity, a phenomenon occurs of equalization of the pressure inside the droplets. The smaller droplets ceases to exist in droplet form and are deposited on the larger ones. Small crystals or colloid particles dissolve, and redeposit onto larger droplets [99].

4.4.4 Coalescence

It refers to the process of thinning and disruption of the dispersed phase droplets. The phenomenon involves the fusion of two or more droplets into a larger one. The limit of the coalescence is phase separation, which is a final and obvious symptom of emulsion destabilization.

4.4.5 Phase inversion

It refers to the process of exchange between the inner and outer phase. In many cases during the transformation a multiple emulsion is temporary formed. The main causes of this phenomena are too high concentrations of the internal phase and insufficient amount, or the incorrect type of surfactant. It needs to be highlighted that this phenomenon can also be caused intentionally since this is one of the methods of the emulsion preparation [65].

All the discussed possible steps of destabilizing process that may occur in emulsion are presented schematically in Figure 10 [100].

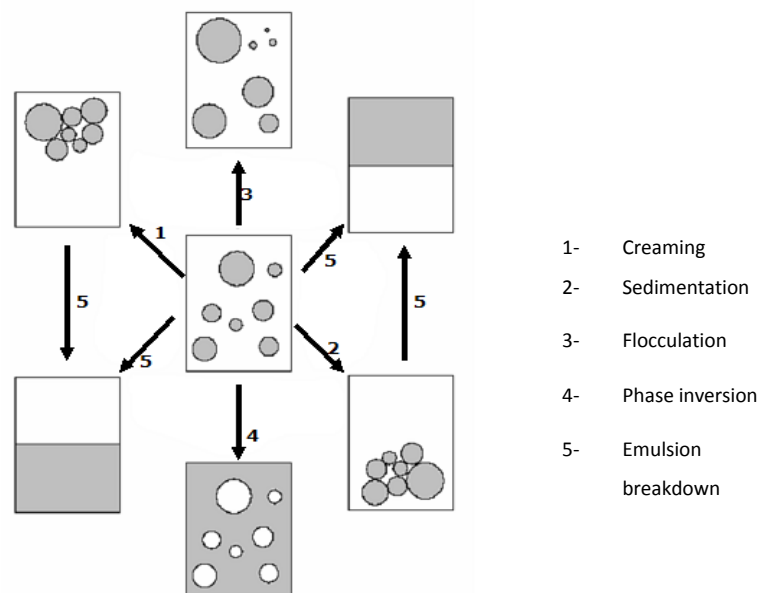


Figure 10 Scheme of emulsion instability symptoms [100]

In the formulation process, samples that exhibited any of the above, are discarded, and a better formulation was sought. Thus, during the formulation optimization process, only systems with the best stability are subjected to further testing.

4.5 Rheological behavior of emulsion systems

Rheology is a tool to control and predict certain phenomena. Knowledge of issues and ways to deform solids and liquids not only allows, in the case of cosmetics, to adapt the product to the needs of the market, but most of all allows to examine the quality (stability, strength) of components, intermediate and final products. Rheology enables to predict the deformation caused by the action of the force applied in the technological process, as well as force that acts during the product application. From a rheological viewpoint, every emulsion is a fluid with its own specific viscosity. The viscosity is resistance of fluid to flow; it is measured by the ratio of shear stress (applied force) to shear rate (movement), and is described as the ratio of shear stress to shear rate ($\eta = \tau / \dot{\gamma}$) [101]. Application of every cosmetic product involves shear stress, which can affect the viscosity [102]. Fluids, for which the ratio is independent of the shear rate are called Newtonian fluids, and the other with a dependent shear rate are non-Newtonian. The viscosity of Newtonian fluids is a constant value (at a given temperature and pressure), regardless of the applied force shear. An example of a Newtonian fluid is water. Emulsions are non-Newtonian fluids. They are divided in two groups: rheologically stable, and rheologically unstable. To the first group includes pseudo plastic fluids. Their characteristic feature is dilution caused by shear. At a constant temperature the viscosity decreases with increasing shear rate. Another pseudo plastic fluid are dilatant fluids. At a constant temperature their viscosity increases with increasing shear rate. This phenomenon is known as translated dilation, or the expansion of the volume with the shear forces. Packed particles under the influence of shear forces change their position in such a way as to increase the distance between them. This increases the friction force between particles. Rheologically stable non-Newtonian fluids can have a limiting shear stress, after which a rapid decrease in viscosity when a force is applied, when it is said that the liquid begins to flow. The other group includes rheologically unstable systems, *i.e.* thixotropic fluids. The shear-thinning occurs, and while reducing shear stress, a slow return

to the initial viscosity is observed. For antithixotropic fluid shear forces cause an increase of the viscosity.

When the flow curve obtained with increasing shear rate does not coincide with the curve obtained when decreasing shear rate, a hysteresis loop is formed. Its shape and surface can be considered as thixotropy measure and is useful in system characterization. Most common O/W cosmetic emulsions are pseudoplastic or thixotropic in nature.

The rheological profile describes the flow properties providing important information on how a cosmetic acts during (and after) the application [103]. It helps to predict its stability [104] and is to some extent associated with the sensory acceptance [105, 106]. This is all the more necessary in the personal care products that the cosmetics are applied with shear stresses in the range of 10^2 - 10^4 s^{-1} , which can affect the system structure. This shear stress is equivalent of light rubbing and spreading on the skin surface. If the product is applied with too much force, phase may separate for example. The skin-feel during application, *i.e.* during shear stress, is very important sensory parameter that is evaluated subconsciously and influence the consumer's choice.

4.6 Sensory analysis

The development of new cosmetic emulsions in scientific literature is often restricted only to stability tests, especially if long term stability is the final aim of the formula optimization process. However, many other parameters should be considered to describe the product, if its purpose is eventual placement on the market. Even the product with the best dermatological properties and highest stability will not be used, if it is difficult to spread or leaves a sticky layer on the skin. Therefore, sensory analysis plays an important but often underappreciated role.

It is often thought that sensory analysis is connected more with marketing than with actual scientific research and development. It is a common misconception, that sensory analysis is a measurement tool of consumer preferences, which should be performed to adjust scent or color to make it more attractive and thereby increase its sale [107, 108]. This



is true, if we think only about those two parameters, as these are often adjusted by additional additives in cosmetic formulation.

However, sensory analysis should be perceived not only as determinant of consumer choice behavior, but also as information about viscosity, which is complementary to the rheological profile. From a sensorial perspective, viscosity is described by a number of parameters such as grip, slipperiness, consistency, cushion effect and spreadability. Those parameters can provide much more information about its behavior on the skin, which is crucial in the personal care industry. Lukic *et al.* in studies on rheological and textural parameters of W/O emulsions highlight that sensory analysis complement the rheological profile, and that “modified sensory studies could be useful for fast in-line screening along with instrumental characterization” [109]. Gilbert *et al.* [110, 111] highlight rheology and textural profile as full information about the personal care emulsions.

Sensory analysis has originated from food analysis, and has since then found application in many other areas. Since the beginning of the twentieth century, it is often used in quality control of processed materials and end-products [112]. In this regard, sensory analysis was developed in its original field, *i.e.* meat [113], dairy [114, 115], confectionery [116], bakery [117], soft drinks [118, 119], and alcohol in particular the wine industry [120-122]. With the latter, it constitutes an integral part, on the basis of which a distinct profession is formed, namely the sommelier. In each of these cases it was demonstrated that the sensory analysis reflects to some extent the chemical composition of the tested product. The possibility of identifying the presence of certain chemical compounds based on the senses is therefore undeniable. Therefore, it is surprising that this tool is not fully utilized in cosmetic industry from the very beginning of the product design and formulation process. The sensory analysis procedure is still in its infancy when targeting cosmetic emulsions.

The available scientific reports do not show clear evidence of the possibility of replacement of sensory analysis with rheological data. Despite the fact that the correlation between rheological profiles and sensory analysis is obvious, both do not give the same information. Moreover, the results do not overlap and their dependency cannot be formulated in the form of a mathematical formula. This implies, that the rheological analysis is insufficient to characterize the emulsion in parameters strictly connected to the viscosity



i.e. texture perception. Gilbert *et al.* [111] recognize the need for a complete emulsion characterization with the sensory profile. They suggested instrumental studies. Wiechers [123] proved that the results of skin feel parameters, which are the base of sensory analysis, differ from their measurable equivalent obtained on the device.

By sensory analysis some emulsion instability symptoms, or other unfavorable changes such as rancidity can be detected.

4.7 Lipid oxidation

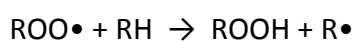
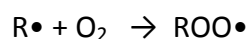
Lipid compounds, due to the chemical structure (unsaturation), are easily changed by external influences. The changes relate to the structure and properties of the lipids, which is highly undesirable. The changes concern color, smell, and flavor. It may lead to the emulsion system destabilization. Even though the PUFAs are recommended in skin care products, they cause a common industry challenge. The oxidation phenomenon shortens the shelf-life of such lipid compounds and products containing them. Even before the results of the oxidation process become observable, the primary products of this reaction may accelerate the photooxidation of lipid compounds on the skin surface. This may cause skin irritation leading to tissue inflammation. The oxidation processes are therefore important as they may occur in cosmetic products, on the skin surface and in the skin. The latter is concerned to be the major cause of the skin aging [124].

The FA alkyl chain is susceptible to oxidation both at double bonds and adjacent allylic carbons. The primary oxidation products are allylic hydroperoxides $-CH=CHCH(OOH)-$ from unsaturated center, in which double bond(s) may be shifted or undergone isomerization from *cis* to *trans* configuration. These products are unstable and can decompose to different secondary oxidation products. Reactions of hydroperoxides include rearrangement to products of similar molecular weight, dimerization to give higher molecular weight, and fission to shorter chain compounds (aldehydes and acids). Lipid oxidation process involves oxygen in its triplet or singlet form. There are many reactions that occur during the oxidation process. The overall reaction can be promoted by heat, light, metals, several initiators and can be inhibited by antioxidants. The process can only be inhibited, but not prevented, even if antioxidants are used[125].

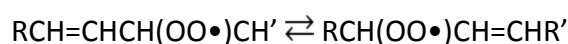
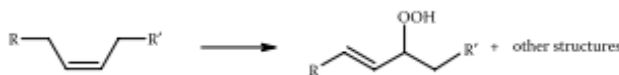


An example described here is the reaction of an oxygen molecule reacting with olefinic lipids. The oxygen exists in two forms: the common ground state triplet form $^3\text{O}_2$ (a diradical form), and the much more reactive form of excited singlet form $^1\text{O}_2$. The triplet form reacts at allylic positions of UFAs chains. The singlet form is electrophilic and reacts at an olefinic carbon atom. Both of them give allylic hydroperoxides, which vary due to the amount and structure.

Autoxidation is a radical chain reaction consisting of three stages. The initiation stage is schematically described as: $\text{RH} + \text{I}\cdot \rightarrow \text{R}\cdot + \text{IH}$ where $\text{I}\cdot$ is an initiator. The lipid alkyl radical formation so as delocalization of the odd electron is a consequence of double bond migration in the primary hydroperoxide products. The reaction of the alkyl radical $\text{R}\cdot$ of UFA with oxygen give peroxy radicals $\text{ROO}\cdot$. It proceeds rapidly and is followed by a hydrogen transfer reaction with other labile hydrogen in UFA which is rate determining step, propagating the chain [126]:



The reaction of oxygen addition, which is reversible, leads to β -scission or β -fragmentation of the C-O bond of the peroxy radicals. This influence the stereochemistry of the hydroperoxides. The peroxy radical can also rearrange with inversion of the double bond configuration from *cis* to *trans* [127]:



cis

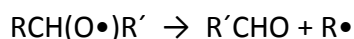
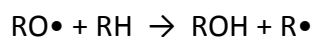
trans

Cyclic peroxide and hydroperoxide structures may be formed if β -olefinic system is present in the radical. It occurs only in the autoxidation process of species with three or

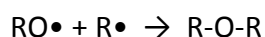
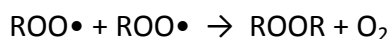


more double bonds. In photo-oxidation, they can be formed from structures containing two double bonds, *i.e.* linoleate.

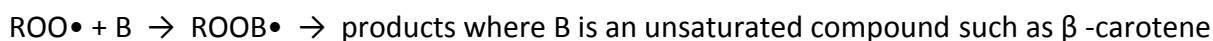
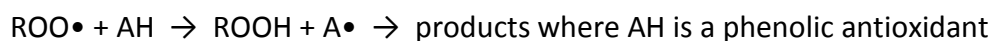
The last stage is the termination. Hydroperoxide is subject to homolysis, which leads to peroxy or alkoxy radicals, and this also can react. Thus, the following reactions are possible:



The formation of alcohols eliminates reactive species, but unsaturated aldehydes may be unstable. Reaction of alkyl, alkoxy, and peroxy radicals leads to termination with dimer or dimer-like products:



Termination involving an antioxidant occurs as follows:



The reaction with ($\text{R}\bullet$) affects autoxidation at its initiation stage, which is the most desirable. When unsaturated compounds are contained in emulsions, oxidation differs in detail from the above process. Also, the rates of oxidation in natural mixtures, such as vegetable oils, can differ from pure compounds due to the effects prooxidants and antioxidants present in the natural lipids.

The initiators of free radical oxidation can be the hydroperoxides, which is formed in process of photooxidation [128].

Photooxidation is a much faster process than the described autoxidation above. The reactivity is dependent on the degree of unsaturation. Photooxidation is promoted by the presence of sensitizers such as chlorophyll which causes the formation of $^1\text{O}_2$ from $^3\text{O}_2$ [129].



(ascorbic acid) are used, which can regenerate used antioxidant, and quenchers of singlet oxygen like β -carotene [136].

Considering the oxidative changes in the UFA, the oils containing PUFAs should not be applied directly on the skin surface as skin care treatment. These kind of sensitive and unstable components should be incorporated in internal phase of dispersed systems.

5 Characterization of the selected fruit seed oils

The results of this chapter have previously been peer-reviewed and published in Colloids and Surfaces A: Physicochemical and Engineering Aspects [137].

The use of fruit seed oils in personal care products is of significance to both their function and image. Poland is an important processor of fruit products within the EU, and thus has a large availability of seeds, which are normally considered to be a waste material or feed for livestock. Recently, attention has been drawn to the possibility of using recyclable materials from fruit processing. As emphasized by Helbig *et al.* in 2008 [138] and Van Hoed *et al.* in 2009 [139], seed oil, obtained as a by-product from fruit processing, gained commercial interest only recently. The enormous potential of this by-product is often highlighted, as a rich source of bioactive lipid components, but also as a source of fiber, protein, minerals or vitamin C. Mandal [140] and Yu *et al.* [141] claim in accordance that the seed oils should be considered as value-added source of edible oil or as a supplement.

The research aim of this chapter is to qualify and quantify the components of selected domestic fruit seed oils. The data are obtained experimentally. The most important was to determine the fatty acid composition, the characteristic features and the presence of unsaponifiable matter.

The fact that the oils can be obtained from waste material by cold pressing, is consistent with the at least four (of twelve) assumptions of Green chemistry [142, 143].

1. No solvents are used.
2. No high temperature is needed.
3. Seeds are renewable feedstock which is agricultural waste.
4. The residues after the production process do not accumulate in the environment.

5.1 Materials

Since the materials of plant origin used for the test are not composed of individual chemical substance, their composition may differ according to the manufacturer. All the cold



pressed seed oils used in tests are in compliance with the requirements in the current Cosmetic Directives 67/548 EEC et 1999/45/CE. The fruit seeds were supplied by GreenField sp. z o.o. (Poland). The seed oil were purchased from The Kerfoot Group (UK).

5.1.1 Fruit seed oils

All the oil were described as in compliance with the requirements in the current Cosmetic Directives 67/548 EEC et 1999/45/CE.

5.1.1.1 Apple seed oil, INCI¹¹: *Pyrus malus* seed oil

Malus domestica belongs to the *Rosacea* family. It is one of the most widely cultivated and the most widely known fruit tree. The tree originated in Central Asia. There are more than 7500 known cultivars of apples. Different cultivars vary in taste and use, including wide possibility of processing, fresh eating and alcohol production (cider). According to FAO data About 70 million tons of apples were grown worldwide in 2010. Poland is on third place in world classification, first in EU [145].

The apple seeds contain small amounts of amygdalin, a toxic glycoside initially isolated from the peach seeds. It is a water soluble sugar and cyanide compound known as a cyanogenic glycoside [146].

5.1.1.2 Blackcurrant seed oil, INCI: *Ribes nigrum* seed oil

The blackcurrant (*Ribes nigrum*) is a woody shrub from the *Grossulariaceae* family. It is originated in Europe and Asia. Blackcurrants can be eaten raw but are usually processed. Poland is the world greatest producer and processor of blackcurrant fruits [147].

5.1.1.3 Blackberry seed oil, INCI: *Rubus Fruticosus* seed oil

The blackberry is a fruit produced by many species in the *Rosaceae* family. In the botanical sense of the word, the fruit is not a berry. It is an aggregate fruit composed of small drupelets (kind of seeds or stone fruit) [148].

¹¹ INCI is International Nomenclature of Cosmetic Ingredients. It was established over 45 years ago by the Personal Care Products Council. The names are used for listing ingredients on cosmetic product labels. It is a combination of Latin and English. Since only Latin expressions are commonly italicized in text, there are therefore not. [144] Groot, A.C. and J.W. Weijland, *Conversion of common names of cosmetic allergens to the INCI nomenclature*. Contact Dermatitis, 1997. **37**(4): p. 145-150.

5.1.1.4 Plum kernel oil, INCI: Prunus domestica kernel oil

A plum is a drupe fruit from the *Rosacea* family. The subgenus is distinguished from other subgenera like peaches and cherries. The fruit has one stone (pit). Plum has about 40 species.

5.1.1.5 Raspberry seed oil, INCI: Rubus Ideaus seed oil

The red-fruited species native to Europe and northern Asia. The fruit is not a berry, but an aggregate fruit consisting of numerous drupelets around a central core. Poland is second world producer of raspberries, first in EU [145].

5.1.1.6 Strawberry seed oil, INCI: Fragaria ananassa seed oil

Technically, the strawberry is an aggregate accessory fruit, meaning that the fleshy part is derived not from the plant's ovaries but from the receptacle that holds the ovaries. Each apparent "seed" (achene) on the outside of the fruit is actually one of the ovaries of the flower, with a seed inside it. The seeds comprise only about 1% of total fresh weight of a strawberry, they contribute a lot of valuable compounds. Kashubian strawberry (Truskawka kaszubska or Kaszëbskô malëna) is the first Polish fruit commercially protected under EU law. They are produced in the Kashubian (Eastern Pomerania region of northwestern Poland). Poland is ninth world producer of strawberries, first in EU [145].

The presented seed oils are commercially available and are considered to have positive effects such as lowering the low-density cholesterol and thus reduce risks of heart diseases [141]. Polyphenols have potentially beneficial effects on health including antiviral, antimicrobial, and antioxidant activity. These properties are usually due to tocopherols, phytosterols and phenolics [149]. Most of the fruit seed oils originate from China (and interestingly not EU), and the oils are extracted with solvents or by supercritical CO₂.

5.2 Experimental approach

In the experimental approach the domestic fruit seeds, which are food processing industry by-product, were tested for their chemical composition.

5.3 Determination of the lipid content in the seeds

To determine the lipid phase content in fruit seeds, the Soxhlet extractor was used. The crushed seeds were washed with the organic solvent, which was evaporated afterwards using Buchi R-114 evaporator, Switzerland. The result is shown as mean values of the lipid fraction (%) obtained in one cycle (4 hours) of extraction process (net content of the four thimbles).

5.4 Determination of fatty acid composition in the studied oils

Fatty acid composition was determined by GC using a capillary column DB-23 (JLW Scientific, 30m x 0,25 mm, 0,25 μ m) at 180°C. FA were separated using BF₃-methanol mixture as the methylating agent according to ISO 12966:2011 [150]. The analysis was performed according to ISO 5508:1999 [150, 151]. The results of the analysis are presented in the tabulated form.

5.5 Chromatographic analysis of the unsaponifiables

The unsaponifiables (petroleum ether extraction) were subjected to thin layer chromatography (TLC). The stationary phase were the aluminum plates coated with silica gel (Silica gel 60 F254 0.2 mm, length. 20 cm and width. 20 cm, Merck). The mobile phase was chloroform (CHEMPUR bp at 61 ° C, density of 1.48 g / cm³). As standard samples squalane, squalene, sitosterol and β -sitosterol (Buchs), cholesterol (Co-op Chemists) and derivatives of vitamin A and E: retinyl palmitate and tocopherol acetate (BASF) were used. The chloroform solutions of unsaponifiables and reference substances were prepared in a chloroform solution at a concentration of 5%. Chloroform was a developing system. An image developed with sulfuric acid (H₂SO₄) an aqueous 50% solution. The resulting color patches identified as the compounds (eg. squalane) or groups of compounds (eg. tocopherols).



5.6 Common parameters used in the vegetable lipids specification

Acid value (AV) of tested seed oils was carried out by potentiometric titration using TitroLine alpha plus titrometer (SI Analytics GmbH) according to the standard ISO 660: 1998. The analytical result is given in mg KOH needed to neutralize free fatty acids contained in 1 g of a test oil (mg KOH / g). The acid number is a measure of the amount of free fatty acids in the oil sample, therefore determines the degree of hydrolysis of fats in time.

Saponification value (SV) represents the number of milligrams of potassium hydroxide required to saponify 1g of oil sample. The test was performed by titration method according to the standard ISO 3657: 1994. The analytical result is given in mg of KOH needed to neutralize free fatty acids and to saponify the acylglycerols contained in 100 g of tested oil sample (mg KOH / g).

Iodine value (IV) was used to determine the level of unsaturation in acyl groups, which occurs in the form of double bonds, which react with iodine compounds. The analytical result is given in g of halogen (expressed as iodine), which joins the double bonds of fatty acids in 100 g of oil (g I₂ / 100 g). The lower the IV, the less C=C bonds are present in the oil sample. The test was performed by iodometric titration method according to standard ISO 3961:1996.

Peroxide value (PV) was performed by titration according to the standard ISO 3960: 1996. This value indicates how many ml of standard solution of sodium thiosulfate (Na₂S₂O₃) is needed for the iodine titration, which was liberated from potassium iodide (KI) by the action of peroxide in 1 g of the oil. The PV is defined as the amount of mmol of peroxide oxygen per 1 kg of tested oil. It gives the information about the severity of the tested oil degradation.

Based on the obtained results, **ester value** (EV=SV-AV) and **percentage amount of FFAs** were calculated.

5.7 Physical properties of the tested oils

Refractive index (RI) measurements were carried out in accordance with PN-60 / A-86920 standard using the Abbe refractometer. The test method utilizes the phenomenon of refraction of light (electromagnetic radiation) through the medium of lower density to a



medium with higher density. Reading the scale coefficients were performed at room temperature (25 °C). The result is specific and characterizes the oil, so it helps to check the purity of the oil in the comparative evaluation.

Density was carried out by using pycnometer. This is a comparative method utilizing the mass and density at a given temperature. To determine the density, a following equation was used (1):

$$\rho_c = \rho_w \frac{m_{PC} - m_P}{m_{PW} - m_P} \quad (1)$$

m_P - mass of dry and empty pycnometer

m_{PW} - mass of standard liquid that fills the pycnometer (water)

m_{PC} - mass of tested oil that fills the pycnometer

ρ_w - density of water at 25°C (997,07 kg/m³)

5.8 Results

The domestic fruit seed oils, as a subject of studies, were tested in a number of ways, not only as a compound, but also as a product. The amount of lipid fraction in seeds were investigated. The efficiency of the extraction process (Table 2) the oil from seeds was checked using the Soxhlet extractor.

Table 2 The amount of lipid fraction in domestic fruit seeds, expressed in percentage value

Parameter	Seed oil			
	Raspberry	Strawberry	Blackberry	Blackcurrant
Seed weight (g)	51.19	54.20	50.52	57.94
Oil weight (g)	9.64	8.38	7.39	5.30
Lipid fraction (%)	18.83	15.45	14.62	9.15
Unsaponifiable fraction (%)	0.32	0.34	0.36	1.2

This method was performed only to investigate the amount of oil in the seeds. Due to the chemical composition of the oils, and their destiny, it is recommended rather to use other method, *i.e.* cold pressing. Since in that method neither organic solvents nor heat is used to extract oil from the seeds. The residual remaining after that process is cake or flour that can be also utilized [152].

Based on the obtained data, it was stated, that the percentage amount of the lipid fraction in tested fruit seeds is in the range of 9 (for blackcurrant seed oil), to 19 (for raspberry seed oil). That means that from one tone of the seeds at least 100 kg up to 190 kg of oil can be recouped, which makes a significant amount.

5.8.1 Seed oils composition

The seed oil composition was tested on oils samples obtained by cold pressing method. The composition of the acyl groups of esters in oils is shown in Table 3. The transesterification was performed due to analyze the methyl esters on gas chromatography.

As it can be seen from the FA composition (Table 3) the tested oils differ in terms of degree of unsaturation. The highest concentration of PUFA was detected in blackberries and blackcurrant seed oils. It was found that the LA and ALA are the main components of the tested oils. These measurements are comparable to literature [139, 149, 153]. The FA composition may be slightly different due to the fruit origin, but despite this, a high PUFA content remained in all cases.

Table 3 FAs composition in tested cold pressed fruit seed oils based on gas chromatography analysis

Fatty acids		FA composition in extracted fruits seed oils (%)			
		Raspberry	Strawberry	Blackberry	Blackcurrant
Saturated	C12 : 0	-	-	trace	<0.5
	C14 : 0	trace	trace	trace	<0.5
	C16 : 0	4.8	4.6	4.4	7.6
	C17 : 0	trace	trace	trace	trace
	C18 : 0	1.7	1.5	2.6	1.6
	C20 : 0	0.9	0.9	0.9	<0.5
	C22 : 0	<0.5	<0.5	<0.5	<0.5
Monounsaturated	C16 : 1	<0.5	<0.5	trace	<0.5
	C17 : 1	-	trace	-	-
	C18 : 1	16.7	15.9	16.8	12.2
	C20 : 1	<0.5	<0.5	<0.5	1.0
	C22 : 1	<0.5	<0.5	<0.5	<0.5
Polyunsaturated	C18 : 2	47.6	48.0	60.0	47.3
	C18:3 α	27.3	27.9	14.1	12.9
	C18:3 γ	trace	<0.5	trace	13.2
	C18 : 4	-	-	-	2.4
	C20 : 2	-	trace	trace	<0.5

The result of all characteristic values were analyzed based on titration methods. The overall results are presented in table 4.

Table 4 The results of qualitative analysis of characteristic values based on standardized titration methods on cold pressed domestic fruit seed oils

Value	Seed oil				Unit
	Raspberry	Strawberry	Blackberry	Blackcurrant	
AV	2.3	1.8	2.0	1.4	mg KOH/g
SV	184.3	187.7	186.3	186.0	mg KOH/g
FFA	1.14	0.90	1.00	0.73	%
EV	182	185.9	184.3	184.6	-
IV	97	100	99	95	g I ₂ /100g
PV	31.85	52.4	52.7	260.1	mmol O ₂ /kg

The acid values are lower than 2.3mg KOH/g in all cases of the oils investigated. All tested oils contain low level of FFA , which also suggests low levels of hydrolytic and lipolytic activities in the samples. The SV is in the range of 184.3 – 186.3 mgKOH/g. The IV for all the seed oils are in the range of 95-100 g I₂/100g. Thus these oils have no siccative properties, and thus include as non-drying oils (when IV<115).

The RI results of the seed oils at 25°C are as follows: raspberry (1.4804), strawberry (1.4794), blackberry (1.4784), blackcurrant (1.4794).

The density of the seed oils expressed in kg/m³ are as follows: raspberry (926.03), strawberry (923.71), blackberry (902.56), blackcurrant (858.23).

5.9 Conclusions

The results of chemical analysis showed an increase of PV values. The results supplied by the manufacturer showed significantly lower PV values. The differences result from the storage time (*i.e.* 12 months) of the raw material in room temperature.

The qualitative composition confirms that the ingredients can be used both in the food and personal care industry. As an example, Glampedaki and Duschk propose to use diluted wine as the aqueous phase and grape seed oil as the oil phase in O/W system for cosmetic emulsions [154].

In this chapter the suitability of using a waste product was shown. In this case domestic fruit seed oil may be substituted for popular exotic seed oils [155]. Fruit seeds, such as apple, blackberries, blackcurrants and strawberries can be used as sources of UFAs in personal care products.

6 Seed oils as components of the O/W emulsion

The results of this chapter have previously been published and peer-reviewed in Colloids and Surfaces A: Physicochemical and Engineering Aspects [137].

6.1 Introduction

In the previous chapter the composition of the domestic fruit seed oils were determined. The novelty being that the oils are cold pressed and are derived from waste material of fruit processing. Based on the obtained results, the oils were proved to be a valuable source of PUFAs, and thus can be component of personal care products. In this chapter the viability of oils, obtained from domestic fruit seed of apples, blackberries, blackcurrants and strawberries as sources of UFAs in emulsions for personal care products is presented. O/W emulsion was chosen as the most popular physicochemical form of personal care products. In this kind of system the seed oils will be components of internal phase. That will provide to some extent protection of the PUFAs, because the oils will not be directly exposed to oxygen and UV radiation.

6.2 Materials

The materials used in the test was divided due to the phases.

6.2.1 Oily phase

6.2.1.1 Fruit seed oils

Cold pressed seed oils used in formulations are presented in Chapter 5.1.1. These compounds were kindly provided by Mega Sort Sp. z o. o., Poland.

6.2.1.2 Other lipid compounds

6.2.1.2.1 Paraffin oil, INCI: Paraffinum liquidum

Crude oil derivative, with viscosity of 866 kg/m^3 (at 15°C) and dynamic viscosity of 185 mPas (at 20°C) was used as a plasticizer and emollient with no bioactive components. Its trade name is ONDINA 934, Poland

6.2.1.2.2 Beeswax, INCI: Cera alba.

This wax is a mixture of lipid compounds. Its main components are palmitate, palmitooleate and oleate esters of long-chain (C30–32) aliphatic alcohols, with the ratio of triacontanyl palmitate to cerotic acid 6:1. The bees wax has relatively high m.p. (62-64 °C) and good skin tolerance. It was purchased from Pharma Cosmetic, Poland.

6.2.1.2.3 Wheat germ oil, INCI: Wheat germ oil

This oil was (kindly provided by AAK Sweden) chosen as complementary compounds. It was chosen as a natural component containing a mixture of TAGs.

Since the oily phase of cosmetic emulsions always contain a mixture, those additional lipids were chosen to resemble commercially available products without reducing the possibility of comparison of the systems with different seed oils.

6.2.1.3 Emulsifiers

A modified acylglycerol emulsifier (mMAG) was used to stabilize the O/W systems. The modification is the addition of a FA to MAG, and thereby forming DAG or TAG. The preparation and properties of mMAG have previously been described by Szeląg and Pauzder [156], Szeląg *et al.* [157] and Macierzanka *et al.* [158-160]. The composition of mMAG is presented in Figure 11. HLB values of the emulsifier (according to Griffin method [161]) were 7.8–9.4. The composition of the emulsion formulation was adjusted according to the HLB value.



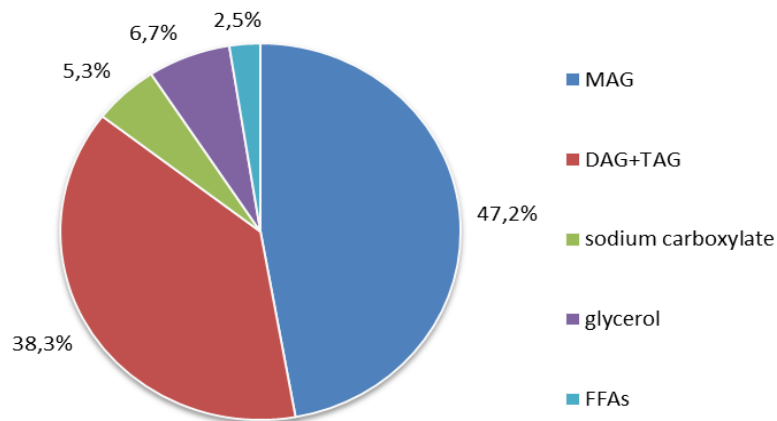


Figure 11 mMAG specification

6.2.1.4 Water phase

Water (INCI: Aqua) water twice-distilled under laboratory conditions.

6.3 Experimental approach

In the experimental approach a formulation optimization process was carried out. The best chosen emulsion system was tested in order to stability, rheological behavior and sensory analysis.

6.3.1 Emulsion preparation

The formulations of the emulsion systems were simplified (five ingredients instead of dozens) in accordance to formulation optimization process, and in order to better highlight the impact of the various individual fruit seed oils. Typical phase ratios for O/W emulsions were tested. Based on experimental studies, 10% of mMAG was found to be sufficient to stabilize an O/W emulsion. In the Table 5 is presented a framework of emulsion composition for further optimization process.



Table 5 Framework of emulsion composition for preliminary studies

O:W phase ratio	Emulsion composition		
	Oily phase	Emulsifier	Water phase
40:60	30	10	60
30:70	20	10	70
20:80	10	10	80

Systems were prepared in lab scale quantities. The O/W emulsions were prepared by a standardized method in a glass, thermostatic emulsor equipped with a mechanical stirrer with a paddle agitator. The oil phase (paraffin oil, beeswax, fruit seed oil, and mMAG) and water phase (distilled water) were heated to 80°C and mixed under continuous stirring (500 rpm), and allowed to cool under stirring. Each formulation was prepared in triplicate.

6.3.2 Emulsion stability test

Emulsion stability was determined using a Turbiscan Lab Expert (Formulacion, France) to detect first signs of instability e.g. creaming [162]. The undiluted emulsions were placed into cylindrical glass tubes. The obtained BS data were then elaborated as BS profiles by the Turbiscan EasySoft converter. The method is based on detection the amount of light (a pulsed near infrared LED at a wavelength of 880) that is vertically transmitted through the sample and the amount of light that is back scattered from the particles. Two different synchronous optical sensors received the light of entire height (both transmitted and backscattered) at an angle of 180° and 45°. The analysis of stability was carried out as a variation of back-scattering (BS) profiles in function of time. These were performed regularly for several weeks in order to observe any changes in stability. The absence of preservatives in the formulation did not allow us to determine long term stability (>two months). The measurements were made in triplicate for each of the three replicates of one dispersion, thus resulting in nine measurements per dispersion. The averaged value is presented.

6.3.3 Rheological behavior

Rheological tests are not only a measurement of the systems stability against shear forces, but it also provides important information about the behavior of the system during skin application [103]. A rotational viscometer with a coaxial cylinder (Rheotest2, RHEOTEST Medingen GmbH, Germany) was used. The rheological studies were performed 24 hours after the emulsion preparation. The sample of emulsion was placed in the space between the two cylinders. Samples were equilibrated to 25, 35, 45, or 55°C. The inner cylinder was spinning with determined speed, the other one was stationary. This method of the measurement mimicked the best the cosmetic emulsion application process. and the shear stress was determined at shear rates $\dot{\gamma}$ (s^{-1}) from 3 to 1312 s^{-1} and from 1312 to 3 s^{-1} in one single measurement to obtain hysteresis loops [163] The loops give crucial information if the system is able to back to the starting viscosity. Different test conditions *i.e.* higher shear stress and higher temperatures were applied in order to check the systems stability. The given results are the average value of three measurements.

6.3.4 Sensory analysis

The sensory analysis was performed 24 hours after the emulsion preparation. Tests were performed to assess the descriptive and qualitative parameters according to available standards [164]. A sensory evaluation of the visio-tactile properties of the experimental emulsions was realized with help of 13 panelists, who were trained to evaluate the samples prior, according to Parente *et al* [165]. The samples used in the training were all commercially available emulsions, so that no bias could be created. The experiments were conducted as a single-blind method. All samples were tested during the same session. The conditions, *i.e.* lighting and temperature were constant for all panelist to assure similar sensory evaluation during the course of the experiment.

Qualitative sensory analysis (objective) parameters, expressions and their specific meaning [164], are presented in Tables 6 and 7. The procedure to the sensory analysis is presented in Table 8. The parameters were evaluated using a seven point bipolar scale (-3 with “zero point” to +3) with a middle point called *just about right* (JAR point). One end



point is labeled as *much too little*, followed by *too little*, *little* and the other as *much*, *too much*, *much too much*. The JAR point is the point at which the panelist feels that the sample is optimal, and thus acts as a control, as each panelist has his or her personal perception since the valuation should be provided as objective as possible. For two parameters (homogeneity and smoothness) other scale was prepared, because none of them can be evaluated as *too much*. For them unipolar scale (from 1 to 7) was developed. The description was as follows: *much too little*, *too little*, *little*, *sufficient*, *rather much*, *much*, *perfect*.

Table 6 The parameters used in sensory analysis describing the tested emulsions. All the parameters are also evaluated on the subconscious level during the first contact with the cosmetic emulsion. The test mimics the real way of emulsion application.

Appearance	Pick-up	Rub-out	Skinfeel after application	Skinfeel 20 minutes after application
Color	Consistency	Consistency	Tackiness	Tackiness
Consistency	Cushion effect	Tackiness	Amount of residue	Amount of residue
	Slipperiness	Amount of residue	Oiliness/ Greasiness	Oiliness/ Greasiness
	Tackiness	Oiliness/Greasiness		
	Amount of residue	Spreadability		

The given results are the average values of 13 measurements for each parameter. Error is given as the standard deviation. The sensory analysis consists of three stages. In the first, appearance, adhesion, consistency, cushion effect are tested as parameters describing the systems viscosity. In the second stage the skin-feel immediately after emulsion application is tested, which includes: homogeneity, spreadability, absorbency, stickiness, oiliness, and smoothness. In the third stage some of the parameters (stickiness, oiliness, and smoothness) are repeated in order to test the skinfeel after 20 minutes, *i.e.* cosmetic residue. Since the skin feel changes with time, mostly due to water evaporation and temperature adjustment of the sample, two evaluations are necessary to conduct (just after application, and 20 minutes after application). That gives the opportunity to evaluate the cosmetic terms of skin-feel.

Table 7. Explanation of the expressions and parameters used in sensory analysis

Sensory expression	Definition
Panel	Group of assessors selected to take part in a sensory test
Panelist	Group of assessors participating in the sensory test
Point scale	Numerical form of a category scale, which has established intervals and a starting and end point. Here we have a 7-point scale. with 0 as the JAR “just about right” point
Product profile	Combination of the intensity of various characteristic attributes of a sample
Scoring	Coded samples are evaluated by the panelist who records his reactions on a descriptive graduated scale. These scores are given as numerical values
Sensory parameters of tested emulsion	Definition
Appearance	Characteristics that encompass all visually perceptible sensory impressions of a food. Examples include shape, surface, structure, color, luster, clarity, cloudiness, and opalescence.
Intensity	Expression of attributes
Grip / Slipperiness	The possibility of scooping emulsion with a spatula and observe the emulsion behavior. If it slows down or stay at place
Consistency	The possibility of scooping emulsion with a fingertip and observe the emulsion behavior. If the emulsion spreads or if creates a characteristic cone
Cushion effect	Amount of emulsion perceptible between the fingers (forefinger and thumb) while rubbing them against each other
Spreadability	Ease of spreading the emulsion on the skin
Smoothness	Degree of skin smoothness on the skin surface in comparison to the other forearm
Homogeneity	A good quality emulsion should seems homogenic with smooth surface with no visible air bubbles
Absorbency	Skinfeel after about 5-20 minutes after emulsion application
Tackiness	The degree of leaving sticky layer on the skin surface after emulsion application. It is evaluated right after application and after some time. <i>i.e.</i> 20 minutes
Oiliness/Greasiness	The degree of leaving oily/greasy layer on the skin surface after emulsion application. It is evaluated right after application and after some time. <i>i.e.</i> 20 minutes
Scent	Total (positive) olfactory impression gained from breathing through the nose and from expiratory olfaction

Table 8 Description of the sensory analysis procedure for personal care emulsion

Procedure of sensory analysis	
Stage 1	Description of emulsions characteristics.
Adhesion	Take some amount of emulsion with fingertip. Emulsion with good adhesion can easily be taken and does not flow off.
Consistency	Dip a finger in the emulsion at an angle of approx. 60 ° and quickly pull out. The emulsion creates characteristic cone on the fingertip and does not spill.
Cushion effect	About 0.5 ml of emulsion should be placed between forefinger and the thumb, followed by rubbing against each other to determine a perceptible amount of cream.
Stage 2	Apply 0.5 ml emulsion on cleansed left forearm and spread it on the skin with four fingers of right hand (7 circular movements). Then the following parameters should be assessed immediately after emulsion application.
Homogeneity	The smoothness and uniformity of the emulsion surface in a beaker should be assessed. While applying on the skin, the presence of lumps and air bubbles must be assessed.
Spreadability	Degree of emulsion resist while applying.
Absorbency	Time need to perceptible absorption of the emulsion.
Stickiness	Press a cleansed hand to the forearm skin surface and assess its stickiness.
Oiliness	Press a cleansed hand to the forearm skin surface and assess its oiliness.
Smoothness	Press a cleansed hand to the forearm skin surface and assess its smoothness (in comparison to the other forearm).
Stage 3	The skin-feel 20 minutes after application

6.4 Results

6.4.1 Formulation optimization process

It was assessed that acceptable good stability is when the BS is not lower than 60%, and does not decrease more than 5% points during the period of test. The preliminary studies showed that the best stability was found in emulsions with an o:w phase ratio of 20:80. The further investigation showed that good results were obtained also by phase ratio of 19:81 and 22:78 (Figure 12, Table 9). However, all the systems remained stable during the test. In this period (80 days) BS values decreased insignificantly (maximally 4% points). In a more detailed analysis the oil phase may be further divided into polar and nonpolar lipids. The beeswax, wheat germ and fruit seed oils are more polar lipids, whereas paraffin wax is non-polar.

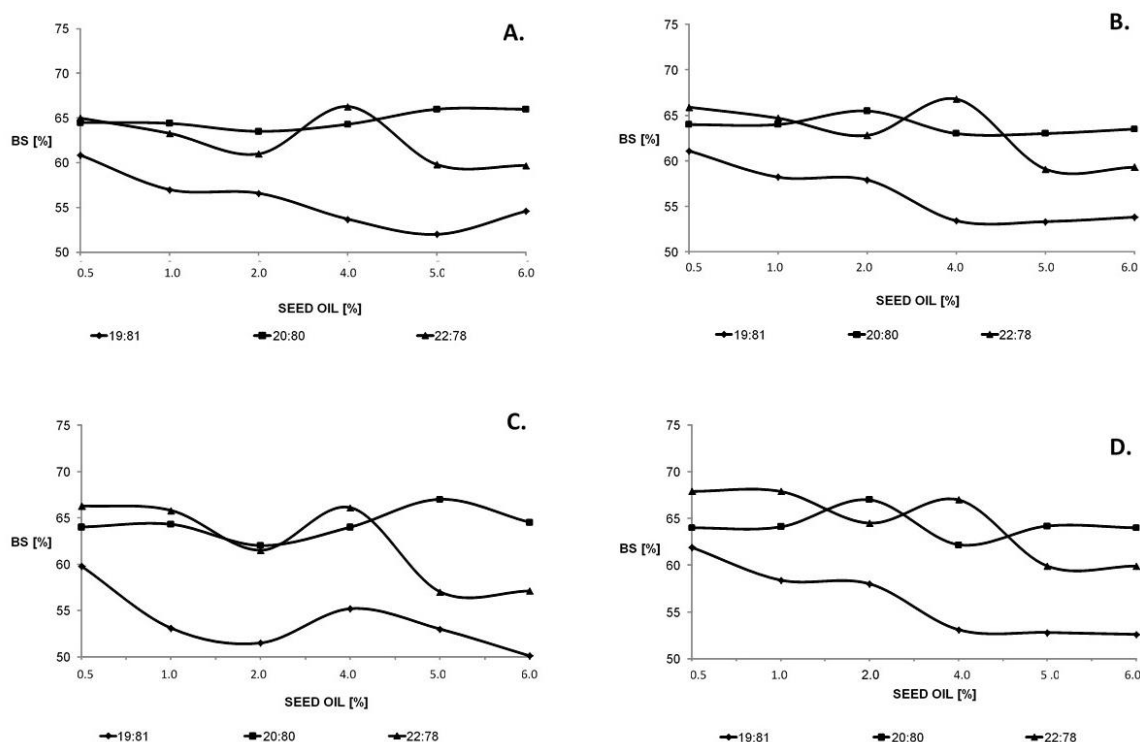


Figure 12 Stability of the obtained systems with different phase ratio contained fruit seed oils A. Raspberry, B. Strawberry, C. Apple and D. Blackcurrant

Several samples were prepared with varying polar:nonpolar lipid ratios as shown in the supporting information Table 9. The best emulsion obtained during the formulation optimization process had the composition 4% and 5% of seed oil with an o:w phase ratio was 20:80. The best stability had the emulsions with lipid ratio polar:nonpolar 60:40 respectively. Despite the fact that there were only few components of the formulation, the majority of them consist of multiple components, *i.e.* emulsifier and oily phase each made up of a variety of chemical components, as is often the case with natural components. Generally, a low share of internal oil phase, and multicomponent formulation contributed to good stability.

Table 9 Composition of emulsion systems prepared for formulation optimization. The stability of the samples was determined by measuring BS. Acceptable good stability when BS>60%.

O:W phase ratio	Composition (%)						Stability of emulsions containing different fruit seed oils expressed in % of backscattered light			
	Seed oil	Wheat germ oil	Paraffin oil	Bees wax	mMAG	Water	Raspberry	Strawberry	Apple	Blackcurrant
19:81	0.5	2.5	4	2	10	81	60.85	61.1	59.8	61.9
	1	2	4	2	10	81	57	58.2	53.1	58.4
	2	2	3	2	10	81	56.6	57.9	51.5	58
	4	1	2	2	10	81	53.7	53.4	55.2	53.1
	5	0	2	2	10	81	52	53.3	53	52.8
	6	1	2	2	0	10	81	54.6	53.8	50.1
20:80	0.5	3.5	4	2	10	80	64.5	64	64	64
	1	2.5	4.5	2	10	80	64.4	64	64.3	64.1
	2	2	4	2	10	80	63.5	65.5	62	67
	4	0	4	2	10	80	64.3	63	64	62.2
	5	0	4	1	10	80	66	63	67	64.2
	6	2	2	0	10	80	66	63.5	64.5	64
22:78	0.5	4.5	5	2	10	78	65	65.9	66.3	67.9
	1	3.5	5.5	2	10	78	63.3	64.7	65.8	67.9
	2	4	4	2	10	78	61	62.8	61.5	64.5
	4	2	4	2	10	78	66.3	66.8	66.1	67
	5	2	3	2	10	78	59.8	59.1	57	59.9
	6	2	2	2	10	78	59.7	59.3	57.1	59.9

6.4.2 Rheology

Based on the obtained data it was stated that the formulations with fruit seed oils and mMAG gave stable emulsions. The most stable systems were selected for rheometric measurements. The emulsions were compared in terms of their flow properties. Figure 13

shows the rheograms (shear stress vs. shear rate) of the all emulsion systems at different temperatures (25, 35, 45 and 55°C).

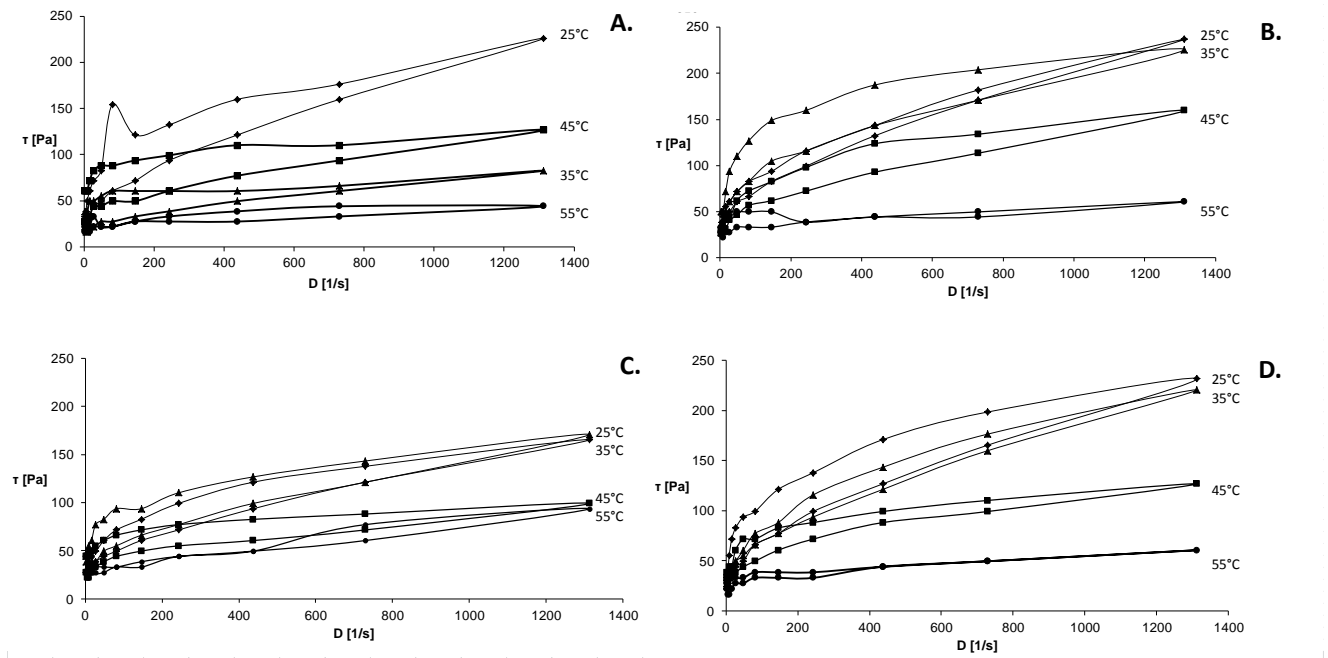


Figure 13 The rheograms (shear stress vs. shear rate plots) in form of hysteresis loops of the all emulsion systems at different temperatures. A. Raspberry, B. Strawberry, C. Apple and D. Blackcurrant

The shear stress curves do not form a straight line through the origin in all tested systems, therefore are not Newtonian fluids. Moreover, the flow curves obtained with increasing shear rate do not overlap with the curves of decreasing shear rate. The shape of formed hysteresis loops are characteristic for viscoelastic fluids [104, 163]. As expected, the surface area of the loops decreased significantly with increasing temperature in all tested systems, which is due to the reduced viscosity of the emulsion at higher temperatures. An increase in shear rate and temperature, decreases the viscosity significantly. This behavior is typical for most cosmetics emulsions, because it spreads better on the skin [96]. All investigated emulsions remained stable after the rheological measurement. This indicates a good stability of the system in adverse conditions, *i.e.* shear forces and higher temperature, even to 45°C, meaning it will remain stable even while rubbing into skin. In 55°C the emulsions were all broken. The data obtained from rheological measurements were shown

in the form of hysteresis flow curves and the viscosity dependence on shear rate for four temperatures. The temperatures (25-55°C) represent the normal range of use and application (higher temperatures due to friction).

In all cases, a decrease in viscosity with increasing shear rate was found, which indicates that the tested emulsions are pseudoplastic fluids. The shear stress increases with increasing shear rate, and then gradually decreases with decreasing shear rate. Moreover, a so called “memory of viscosity” phenomenon can be observed, which is typical sign of thixotropic behavior [166]. This thixotropic behavior is a favorable indication of ease of application of the cosmetic product [96]. Thinning while spreading on the skin followed by return to the original viscosity avoids the use of additional synthetic components e.g. cyclomethicones. An increase in shear rate and temperature decreases the viscosity significantly. This behavior is desirable for most cosmetics emulsions. The apple seed oil emulsion displayed a reduced sensitivity to temperature, as can be observed from the rheograms. This is thought to be due to the differing FA composition of the apple seeds, as it contains much more monounsaturated FA and a significantly lower amount of C18:3. The longer chained compounds have a high viscosity, and thus the stronger London-dispersive force which will keep the molecules attached even at high temperatures. Overall, all fruit seed emulsions were stable and are suitable for cosmetic application.

6.4.3 Sensory analysis

The order of testing (as indicated by the arrow in Figure 13) of the sensory parameters of seed oil emulsions was not accidental. It reproduced the natural way of its application. First the appearance was tested, then parameters from the following group: the pick-up, the rub-out, skin feel right after application and after water evaporation (absorption). Sensory analysis is complementary to information about viscosity obtained after rheological measurements. From the sensorial viewpoint, the viscosity is described by several parameters like grip/slipperiness, consistency, cushion effect and spreadability. These parameters are described in detail in Table 7. The numerical results with SD is shown in Table 10.

Table 10 Results of sensory analysis. Average of 13 measurements given by the panel is presented with the standard deviation (SD)

Parameter	O/W emulsion containing fruit seed oils							
	Raspberry		Strawberry		Apple		Blackcurrant	
	Average	SD	Average	SD	Average	SD	Average	SD
Grip/Slipperiness	1.46	0.78	1.15	0.8	1.08	0.64	1	0.82
Consistency	1	0.91	0.92	0.86	0.77	1.01	0.85	1.14
Cushion effect	0.54	0.88	0.46	0.66	-0.15	0.99	0.31	0.63
Spreadability	0.38	0.87	0.54	0.66	0.62	1.19	0.77	1.01
Absorbency	-1.15	1.07	-1.23	1.01	-1.15	1.21	-0.92	1.38
Tackiness	-0.15	1.07	-0.31	0.85	-0.15	0.99	-0.23	1.54
Tackiness after 20 min	-1.54	0.88	-1.08	0.86	-0.85	0.9	-1	0.82
Greasiness	-0.92	1.55	-1.15	0.8	-1.69	0.48	-1.38	0.96
Greasiness after 20 min	0.54	0.66	0.62	0.77	-0.15	1.41	-0.77	1.54
Scent	-1.54	1.13	-1.31	1.49	-2.43	0.53	-1.92	1.04

None of the described parameters has been evaluated as an extreme (*extremely strong*, or *extremely weak*). Although the consistency was perceived as *rather thick* for all the tested emulsions without any significant differences in SD (0.86-1.14), the cushion effect, showed some difference between the systems. The closest to the JAR point was the apple seed oil emulsion. Grip and consistency values were mostly similar, which conforms to the expectation as both are an indirect measure of viscosity. The raspberry emulsion seems to have the tendency to have stronger grip than others. The spreadability was assessed as JAR with tendency to *rather difficult*. Tackiness was seen as JAR with tendency to *rather weak*. There are significant difference in tackiness skinfeel right after the application and after water evaporation. The occlusive layer was felt as *rather weak*. The cushion effect and the greasiness of apple seed oil emulsion are noticeably closer to the JAR point than any other fruit seed oil. It seems that the differences between creams are more noticeable after water evaporation. The largest difference was between the apple and raspberry emulsions. Both of them are underestimated in terms of greasiness after absorption. Greasiness right after application was assessed as *too small*. After water evaporation, the occlusive layer components did not form a *too greasy* skinfeel. The panelists described the occlusive layer greasiness as JAR for raspberry and strawberry or *rather small* for apple and blackcurrant. In these parameters the biggest difference between the seed oils can be seen. In comparison

to other O/W emulsions, the absorbency was assessed as *rather slow*. This might be caused by differences in FA composition. Apple seed oil contains more SFA and less MUFA than other tested fruit seed oils. The biggest difference was observed between emulsion containing apple and raspberry seed oils in a few parameters, *i.e.* cushion effect, tackiness after absorption and greasiness (Figure 14).

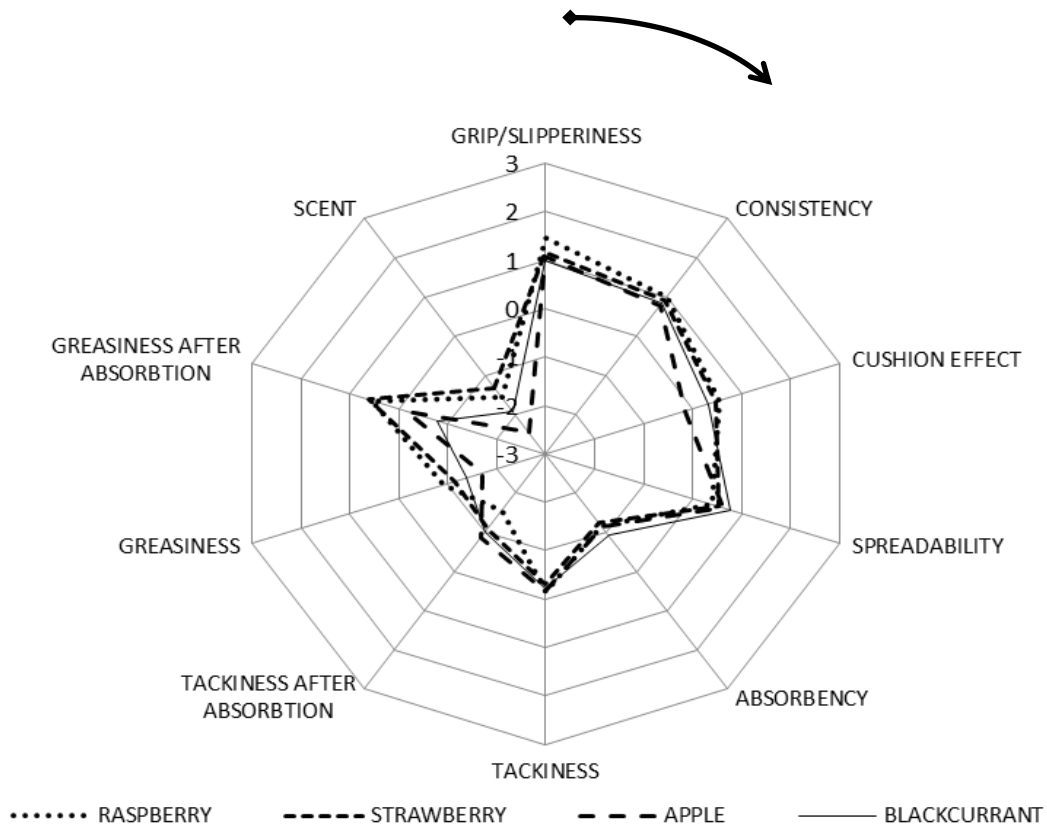


Figure 14. The average values of sensory test for seed oil emulsions, the arrow indicates the sequence of testing. Differences between the tested emulsions can be clearly observed on the plots. -3 is for much too little, 0 is for JAR, and +3 is for much too much

Thus generally all prepared emulsions from domestic fruit seed oils should find suitable application as cosmetic products with sufficiently high PUFA content. There is no parametric justification for only using exotic fruit seed products.

The only obviously negative parameter is scent which is clearly due to the fact that the emulsions were unscented. Many commercially used products are scented and thus the panelists expect a certain stronger scent.

The parameters that are influenced by the rheology of the sample (consistency, grip, and spreadability), show little variability (SD respectively 0.77-1.14, 0.64-0.81, and 0.66-1.19).

The greasiness is the parameter which has shown the greatest variability (SD 0.48-1.55). The lower was the viscosity of the tested emulsions, the better the sensory properties. The faster the system became fluid under the influence of the shear force, the better evaluated was in terms of sensory distribution and oily skin feel after application.

6.5 Conclusions

It was attempted to show the suitability of using a waste product, in this case domestic fruit seed oil as a substitute for popular exotic seed oils [155]. Fruit seeds, such as apple, blackberries, blackcurrants and strawberries can be used as sources of unsaturated fatty acids (UFAs) in personal care products.

It was successfully formulated cosmetic emulsions from these oils. The raspberries, strawberries, blackcurrants and apples cold pressed seed oils form a stable oil-in-water emulsion systems, even in a large range of concentrations, *i.e.* up to 6% by weight of the oil phase. The mMAG emulsifier was a very good stabilizer of the emulsion containing tested seed oils in a wide range of required HLB of the oil phase. Significant impact on the systems stability had phase ratio (oil:water). The best stability had emulsions in which the phase ratio oil:water was 20:80 and 19:81 respectively. Increasing the amount of the aqueous phase up to above 81% resulted in a partial destabilization of the emulsions. It was found that the analyzed fruit seed oil can be used as cosmetic emulsion components, even at relatively high concentration, *i.e.* approx. 10%. From dermatological reasons some restrictions on the use may appear due to high concentration of bioactive components, however this will require further investigation.

It appears that therefore fruit seed oils of domestic fruit have similar properties and thus their implementation or inclusion in cosmetic emulsions is only a question of marketing. With the success of marketing of organic and local products this has a realistic opportunity [167].



7 Viability of fruit seeds oils by nanostructured lipid carrier (NLC) nanosuspensions

The results of this chapter have previously been peer-reviewed and published in Journal of Colloid and Interface Science [168].

In previous chapter the viability of oils, obtained from domestic fruit seed as sources of UFAs in O/W emulsions was presented. The use of domestic fruits seed oils obtained from food processing waste material appeared to be a very good and promising solution in terms of ecology, economy, and dermatology. The following chapter presents development towards modern and realistically useful forms of personal care products. The O/W emulsions are present in cosmetic industry for ages. Over the years it was refined in order to obtain the best possible penetration into the skin of any bioactive compound introduced in the formulation, such as vitamins and PUFAs. During this time, and nowadays the O/W emulsion is still the most popular form and type of personal care product from so called “white cosmetics” (cosmetics except make-up products and perfumes). As a matter of fact, nothing indicates that this will change.

Guided by the principle that the bioactive components are useful only if they are able to penetrate the skin unchanged, the new systems presented in this chapter represent a new form of O/W emulsion that is a protective reservoir of biological active compounds that naturally occur in domestic fruit seed oils.

Even though PUFAs are essential to the correct functioning of the skin, their topical use in form of emulsions is not a case of “the more the better”. Presence of these ingredients in large quantities in the lipid matrix may cause changes in the intercellular lipid matrix composition, *i.e.* making it more fluid and thus more susceptible to washing out and hence increasing TEWL with all its consequences (details in chapter 4.3). Therefore, the PUFA components of seed oils should be applied in suitable, not more than enough amount with appropriate protection for the lipid matrix. The most popular protective methods are based on the addition of antioxidants, such as polyphenolic compounds [169, 170], or on the entrapment of PUFAs in clathrates to form a host-guest complex, such as cyclodextrins [171,



172]. All these methods increase manufacturing costs and, more importantly, need additional ingredients in the final formulation, giving no direct benefit to the skin.

Therefore, an alternate way to deliver PUFAs was needed. Over the years, emulsions, as a popular physicochemical form of personal care products, were refined in order to obtain the best possible penetration into the skin. Mühlen *et al.* [75] had used a fat with a high m.p., *i.e.* solid at room temperature, as a single component of the oil phase, which resulted in the formation of a starting O/W emulsion, when the temperature was higher than the m.p. of the fat, that converted into a suspension of solid lipid nanoparticles (SLN) after cooling [75]. This discovery opened the way to produce new bioactive delivery systems for ocular use by Boursis *et al.* [173], Kumar *et al.* [174], Fundaro *et al.* [175], and vascular injections by Blasi *et al.* [176]. These also led to a significant decline in the interest in micro- and multiple emulsions. The first, due to the high amount of surfactant required, the latter due to scarce reproducibility of the formulation. The SLN systems (more accurately they are nanosuspensions) combine the advantages of micro- and multiple emulsions (small droplet diameter and the protection of the lipophilic active substance), while being devoid of their disadvantages (difficult preparation reproducibility, high amount of surfactants) [177-179]. In early 2000s the SLN were modified by Jennings *et al.* by mixing solid with liquid lipids [180]. This modification significantly increased the possibility of drug incorporation [181]. The increased capacity is due to imperfections in the lipid matrix caused by the higher variety of alkyl chain lengths of the lipids [177, 182, 183]. That differences are showed on Figure 15. These new NLC [28] are almost solid at room or body temperature, with a mean particle diameter of 80–1000 nm [184], and thus allow for easy penetration into the skin. This is enhanced if the NLC is comprised of physiologically compatible lipids [185]. Moreover, NLCs enhance the chemical stability of compounds sensitive to light, oxidation and hydrolysis. All these factors give the NLCs a crucial advantage compared to other colloidal carriers, as they provide a method of controlled release, and, at the same time, an increase in chemical stability of the incorporated bioactive components [186].



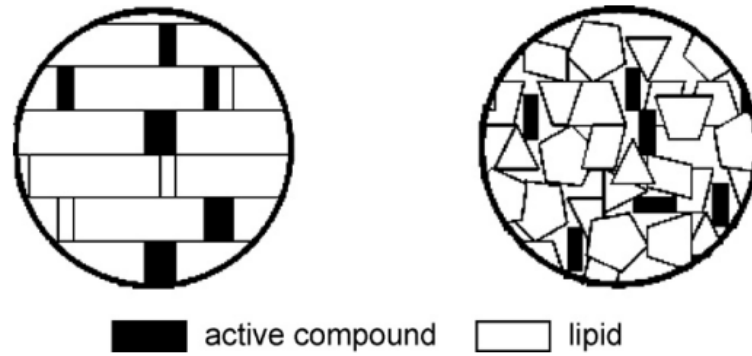


Figure 15. Difference in crystal lattice between SLN (left) and NLC (right) [78] which is crucial in biological active components incorporation

The NLCs became a very popular drug carrier for both internal (mostly parenteral [187] and oral [182, 188]), and topical use [183]. NLCs may be modified by either method of preparation (which usually results in various average size distributions of the NLCs), or by components (type of solid and liquid lipids, and specific inclusion of bioactive compounds). The modification by components is directly related to the NLC qualities and use. This applies especially to the solid lipid matrix. For example, when strong adhesiveness is required, carnauba wax (obtained from Brazilian palm leaves) is used as a thickener due to its m.p. > 80°C. This strong adhesiveness results in the formation of the occlusive layer, a protective barrier on the skin, which reduces the TEWL from the corneum surface. This is one of the most important mechanisms of the skin moisturizing process [189, 190]. Such properties are required in case of UV filters. Müller *et al.* [191] and Nikolić *et al.* [192] have used and enhanced these features by incorporation of UV blocking components into the lipid matrix. This resulted in new types of water resistant sun-screens [193]. The occlusive film also supports penetration of the active ingredients into the skin [194], especially for components that are sensitive to external factors, such as coenzyme Q₁₀ [195, 196] or ascorbyl palmitate. The latter incorporated in NLCs moisturized skin significantly better and could penetrate the skin significantly deeper [197].

Noteworthy is the fact that the tested cold pressed seed oils (from blackcurrant, raspberry, strawberry, and plum) contain high amounts of PUFAs, which are susceptible to degradation. Thus, it seems to be relevant and reasonable to incorporate them in a solid lipid matrix, which will result in doubled benefit. Since the fruit seed oils are diverse in terms

of their chemical composition, their inclusion as a component of the NLC matrix will cause crystal lattice defects [78]. This significantly increases the loading capacity of the NLC for bioactive components [78, 198]. Additionally, the NLC matrix protects the PUFAs and tocopherols against oxidation processes. Moreover, such entrapped bioactive components are released slowly to the skins surface. Therefore, the NLCs modified with fruit seed oils present a number of opportunities; firstly, they may be applied as a personal care product, in its own right, and secondly, they can serve as an improved matrix for incorporation of other bioactive compounds, due to their high loading capacity. Crucial is also the fact that due to the small size of NLCs particles they have larger surface area, which give greater adhesive properties

Therefore, the aim of the research presented in this chapter is to show that domestic cold pressed fruit seed oils obtained from waste materials can be applied as important NLC components, and by the incorporation into the NLCs have the advantage of being protected from oxidation.

7.1 Materials

The materials used for the experiments were divided in groups due to the function.

7.1.1 Oily phase

Five kinds of cold pressed fruit seed and kernel oils were used: blackcurrant (*Ribes nigrum*), blackberry (*Morus nigra*), raspberry (*Rubus idaeus*), strawberry (*Fragaria ananassa*) and plum (*Prunus domestica*). They were purchased from KerfootGroup (Northallerton, United Kingdom). Fatty acid composition was determined and shown in the previous paper [137]. These represent the five most commonly harvested fruit in Poland [199]. Beeswax also from the KerfootGroup was chosen as the NLC solid lipid matrix, due to its relatively high m.p. (62-64 °C, according to its origin), good skin tolerance and *in vivo* biodegradability [194, 200], and in addition it is a common cosmetic emulsion ingredient.

7.1.2 Emulsifiers

Two nonionic emulsifiers were chosen with two extreme HLB in order to form mixtures with accordingly adjusted HLBs to form stable systems [83, 201].

7.1.2.1 Lipophilic emulsifier

Myverol RX GMS 95P (MAG) is a molecular distilled emulsifier (HLB=3) and it was purchased from Kerry Bio-Science (Netherlands). It contains a mixture of glycerol palmitic (57.8%) stearic (37.3%) and myristic (1.3%) acid monoesters (MAG>95%).

7.1.2.2 Hydrophilic emulsifier

Chemal EO-20 (AOE), HLB=15) contains a mixture of ethoxylated fatty alcohols (palmitic and stearic) (average molecular formula $C_{16-18}EO_{20}$). It was purchased from CHEMCO (Sobowidz, Poland).

7.1.3 Water phase

7.1.3.1 Water

Water (INCI: Aqua) water distilled under laboratory conditions.

7.2 Experimental approach

In the experimental approach a formulation optimization process of NLC systems was carried out. The best chosen emulsion system was tested in order to its crucial features.

7.2.1 Lipid matrix formulation

The NLC matrix is composed of a mixture of solid (wax) and liquid (oil) lipids, which are in a solid state at room temperature. In order to determine the most suitable lipid matrix, different wax:oil mixtures in various ratios (8:0, 7:1, 6:2, 5:3 and 4:4) were prepared and tested for their thermal behavior. Calorimetric analysis was performed using differential scanning calorimeter (DSC). This method is used to obtain information about both physical and energetic properties of the lipid matrix [202], in this case the m.p. and presence of unincorporated oil [203]. In each case the mixtures of solid and liquid lipids in appropriate ratios were molten the same way as the nanoparticles were obtained. The vials with the lipid

mixtures were placed in a water bath at 75°C for 20 minutes. After that time the mixtures were allowed to cool to room temperature. DSC scans of the lipid mixtures were performed using DSC 131 Setaram (France) equipped with SETSOFT 2000. In each measurement 5–10 mg of lipid mixture samples were heated from 20° to 100°C at 5°C/min, under nitrogen.

7.2.2 NLC preparation

Nonionic emulsifiers AOE and mixtures of AOE and MAG were used. Hot homogenization was chosen as the simplest and easiest technique to apply in both laboratory and industrial scales. In addition, this method is suitable for sensitive compounds such as oils rich in PUFAs, since the compounds are exposed to high temperatures for relatively short times (20 minutes in this case) [28]. The nanosuspensions were prepared by melting the lipid phase (beeswax or beeswax with seed oil) and emulsifier agent (MAG or MAG with AOE) at approximately 15°C above the m.p. of the beeswax, *i.e.* 75°C. Water phase (distilled water) at a similar temperature was added to the lipid phase under stirring at 26000 rpm using an Ultra Turrax homogenizer (IKA® Werke GmbH & Co. Germany) for 10 minutes. The obtained O/W pre-emulsion was allowed to cool in order to allow the lipid to recrystallize and form the lipid nanoparticles. The best NLC was determined by varying the composition (emulsifier, wax, and oil) of a number of formulations.

7.2.3 Sample preparation

Each formulation was prepared twice and labelled (1A, 1B, 2A, 2B, and so on). Each one of these formulations was subdivided in two samples (1A₁, 1A₂, 1B₁, 1B₂,...), and each sample was measured three times. Thus, there were 12 results for each formulation obtained.

7.2.4 Characterization of the systems

Several techniques were employed to determine the SLN and NLC particles.



7.2.4.1 Photon correlation spectroscopy (PCS)

Photon correlation spectroscopy (PCS) is a technique used to determine the mean diameter of particle size and the width of the particle size distribution expressed as polydispersity index (Pdi). The measurements are based on the light scattering phenomena: the statistical intensity fluctuations (due to the Brownian movements) of the scattered light from the particles. The sample is tested after dilution and placed in a cuvette. The light scattered (at angle of 90°) is analyzed taking into account fluid viscosity and temperature. The diffusion rate of the particles depends on their size, so it can be calculated from the rate of fluctuation of the scattered light intensity. The small nanoparticles diffuse relatively fast, and the fluctuations in the scattered light are then rapid. Similarly, big particles they move much slowly, and therefore is the fluctuations in the scattered light are slow. The detected intensity signals are used to calculate the auto-correlation function $G(\tau)$, of which, a coefficient D is obtained. Base on that, the equivalent diffusional spherical diameter can be calculated using the Stokes-Einstein equation (2).

$$r = \frac{kT}{3\pi\rho D} \quad (2)$$

where ρ is the viscosity of the surrounding medium, k is Boltzmann's constant, T is the absolute temperature, D is a diffusion coefficient of a spherical particle .

Besides from the data received, the polydispersity is calculated. The Pdi is 0.0 when a monodisperse particles population are measured detected in sample. Pdi values of around 0.10-0.20 indicate a relatively narrow distribution. Values of 0.5 and higher are obtained in case of very broad distributions, *i.e.* when the sample contain high diversity of particles sizes. The measurement were carried out using Malvern Zetasizer nano ZS90 (Malvern Instruments Ltd, Worcestershire, UK).

7.2.4.2 Zeta potential (ZP)

The method is based on measurements of particles velocity after an electrical field is applied. On the particle a charge develops due to ionization of surface groups or adsorption

of ions. The surface charge generates a potential around the particle, which is at the highest near the surface and decays with distance.

The diluted sample is placed in an electric field, which causes movement with a given velocity (expressed in micrometers per second per volt per centimeter ($\mu\text{m/s}/(\text{V/cm})$). The spherical surface separating the moving particle, ions and solvent from the surroundings is called the surface of hydrodynamic shear. It is shown in Figure 16. The electrophoretic mobility is determined by the potential at this surface, which is the zeta potential (ζ). It is determined using the Helmholtz-Smoluchowski equation (3), which is applied to large particles in a diluted electrolytes solution:

$$\xi = \frac{v}{\eta \epsilon E} \quad (3)$$

where η is the viscosity of the dispersion medium and ϵ the permittivity of the environment

In an aqueous medium of low electrolytes concentration the potential does not change rapidly, and therefore, is usually equated with the Stern Potential at the Stern layer.

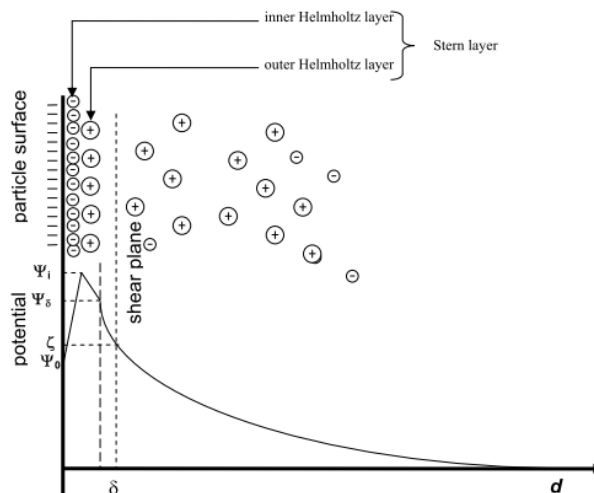


Figure 16 Schema of the electric double layer. ψ_0 is the Nernst potential, ψ_i is the potential of inner Helmholtz layer, ψ_δ is the Stern potential, δ is the thickness of the diffuse layer, ζ is the zeta potential at the surface of shear and d is the distance from the particle surface

The zeta potential help to determine the tendency of particles to flocculate. The measurement were carried out using Malvern Zetasizer nano ZS90 (Malvern Instruments Ltd, Worcestershire, UK).

7.2.4.3 Thermal analysis

With the help of DSC, physical and the energetic properties of a sample can be described by measurement of physical changes of a sample while heating or cooling. The DSC device consists of one oven that heat up the reference and the sample pans. During the test, differential heat flow and the sample temperature is monitored. Two important processed can be observed. The endothermic (heat-absorbing) processes like melting, boiling, sublimation; and exothermic, where energy is liberated, like crystallization. This method has many applications, such as materials identification and its quality analysis. DSC analysis has been used to determine the degree of lipid crystallinity determination. It allows the study of the melting and crystallization behavior of crystalline material like lipid nanoparticles. An accurately weighed amount of sample component was placed in 40 μl aluminum pans and analyzed. DSC scans have been performed from 25°C to 100°C at a

heating rate of 10°C/min, using an empty pan as reference. Melting points (melting peak maximum) were determined in order to estimate the possibility of the tested seed oil incorporation in the NLC matrix.

All the systems were frozen and freeze-dried under reduced pressure using a LIO 5P freeze-dryer (5 Pascal, Italy) equipped with a vacuum pump RV12 (Edwards, England).

Turbidity is an optical property that scattered the light due to the presence of particles present in the samples. The turbidity increases with the size of the particles. The turbidity measurements were performed on UV/VIS Spectrophotometer (Lambda 40, Perkin Elmer, USA). The wavelength used to the investigation was evaluated empirically on 400 nm.

In addition, the pH was measured.

7.2.4.4 ^1H NMR

Nuclei have an angular momentum, and this angular momentum allows the nuclei to have magnetic properties. This intrinsic angular momentum is often referred to as spin. In some atoms (with even mass number like ^{12}C , ^{16}O) these spins are paired and cancel each other out resulting in no spin. However, many atoms (with an odd mass number, such as ^1H and ^{13}C) possess an overall spin.

If the ordered arrangement of spins is subjected to the magnetic field of the appropriate frequency the energy absorption will take place. The nuclei respond by precessing around the direction of the applied magnetic field. The spins of the lower energy level pass to the higher level, and thereby energy absorption occurs. The nuclei are in resonance with the applied radiation. The actual frequency at which an atom resonates is determined not only by the applied magnetic field, but also by minute differences in the magnetic microenvironment that each atom experiences within the molecule. This microenvironment is due to changes in the electron density of each nucleus. Thus, each chemically different carbon or hydrogen exhibits specific absorption. This allows to determine of how many different kinds of protons are present in the molecule. The strength



of the peak is proportional to the number of protons which come into resonance at that specific frequency.

For this thesis, the spectra of emulsion and its compounds were obtained using the high resolution proton nuclear magnetic resonance spectroscopy ($^1\text{H-NMR}$). The tests were performed at 400MHz (Bruker AC-400 Germany) instrument. The samples were prepared in deuterium compound *i.e.* deuterium oxide, in a thin-walled glass tube designed for the NMR test. This method gives the observation possibility of specific quantum mechanical magnetic properties of the atomic nucleus. It is helpful to assess the amount of seed oil possible to incorporate in solid lipid matrix. It will help to estimate what concentration of the seed oil is in excess and dispersed in continuous phase.

7.2.5 Oxidative stability

Preliminary studies carried out in accordance with the standard (BN-85 6140-01/04) did not give reproducible results. Therefore it was necessary to develop a new method for ensuring the test conditions in accordance with the made assumptions.

The standard method specifies for the light source to be parallel to the sample. In order to avoid heating of the sample (quartz lamp), it was placed at a distance of 100 cm from the lamp. Higher temperatures would result in high rates of reaction. Using this method the samples temperature increased, even at 100 cm, but the real disadvantage was that the light was incident at an acute angle, that is, it did not illuminate the sample evenly. It was also impossible to light a larger number of samples simultaneously. This precludes performing the test for a plurality of samples under the same conditions. Therefore a new method was required, which would not result in the samples temperature increasing, and for measuring the stability of many samples at once, in the same conditions. I therefore proposed a modification based on the type of the lamp, its setting and thus samples setting. The lamp was a water cooled tube UV lamp. The water cooling meant that the lamp could be placed closer to the sample. The geometry of the lamp (a long tube), meant that standing the lamp on a flat surface, samples could be arranged around the tube. The height of the tube meant that the radiation was at a more obtuse angle. The circular arrangement meant



that each sample was exposed to the same intensity of radiation. The newly developed method of oxidative stability test gave good reproducibility.

To determine the oxidative stability, all the samples of seed oils, SLN, NLC and O/W emulsions were placed in flat glass flasks (50 mm in diameter) and exposed to UV radiation ($\lambda=400\text{nm}$) using a UV-Vis Oriel lamp (1000 W). The distance from the lamp and the samples (100 mm) was the same for all samples. The exposure time was five hours. After UV exposure the PV was determined in triplicate by iodometric method (PN-EN ISO 3960:2012).

7.3 Results

7.3.1 SLN composition

Basic solid lipid nanoparticles (SLN) made of beeswax were designed to be the starting point for the development of the NLC (NLC = SLN + liquid oil) systems. The amount of water phase was empirically estimated in pre-test to be at least 80% w/w. The pre-formulations confirmed that the total lipid concentration in the dispersion systems was about 10% w/w, which corresponds to lipid concentrations of pharmaceutical interest [201]. The process parameters, involved in the preparation of the systems were optimized, including lipid and emulsifier (type and concentration), and lipid:emulsifier ratio. These preliminary studies showed that stable SLN could be obtained using a phase ratio of 10:90 (o:w), with an emulsifier HLB of about 12-14.

The obtained physically stable systems were in line with expectations in terms of size distribution, 264-393 nm, which is typical for lipid nanoparticles. No phase separation after 48 hours was observed. The mean diameter (Z-average), polydispersity index (Pdi), ζ potential and pH were measured at every step of the formulation optimization process. The results are shown as the mean value \pm SD in Table 11

Table 11 Process of emulsion formulation optimization showed step-by-step.: Emulsifying agent and lipid matrix adjustment. The shaded regions are the chosen formulations for further development.

Type of the lipid nanoparticles	Formulation number	Amounts of the components (%) wt.					Stabilization agents
		Beeswax	Seed oil	AOE	MAG	Water	
SLN formulation	1	8.0	-	2.0	-	90.0	AOE 2%
	2	8.0	-	4.0	-	88.0	AOE 4%
	3	8.0	-	3.5	0.5	88.0	AOE+MAG 4%
	4	8.0	-	1.75	0.25	90.0	AOE+MAG 2%
Lipid matrix composition beeswax: seed oil		8	:	0			
		7	:	1			
		6	:	2			
		5	:	3			
		4	:	4			
NLC based on 3rd and 4th SLN formulation	3	8.0	-	3.5	0.5	88.0	
	3A	6.0	2.0	3.5	0.5	88.0	AOE+MAG 4%
	4	8.0	-	1.75	0.25	90.0	
	4A	6.0	2.0	1.75	0.25	90.0	AOE+MAG 2%
3A chosen as best NLC formulation	3A	6.0	2.0	3.5	0.5	88.0	AOE+MAG 4%

The best results were achieved in systems containing a surfactant mixture: AOE+MAG 2% (formulation 4), and AOE+MAG 4% (formulation 3) [201]. Also the diluted systems with the mixture of emulsifiers seem to be visibly more homogeneous. The smallest mean size of the particles (formulation 2 and 3) has been obtained in systems containing 4% of emulsifying agent. The smallest particles obtained in basic systems were not smaller than 37 nm (formulation 2), 58 nm in formulation 1, 3, and 4. The smallest particles are much bigger than micelles, so it may be assumed that there is no excess of emulsifier.

7.3.2 Lipid matrix composition

To determine if the fruit seed oils were incorporated successfully into the beeswax matrix the thermal behavior of a number of wax:oil ratios (8:0, 7:1, 6:2, 5:3 and 4:4) were recorded, and the range of crystallinity was investigated (Table 12). It was assumed that the system comprising of equal ratio of lipids (4:4) is the theoretical borderline for incorporation capacity, since at that point more seed oil is present than solid lipid into which it should be incorporated. This would make the seed oil the dominant phase, and thus limit the ability of the solid phase to protect the seed oil.

Table 12 Melting points of the lipid mixtures of wax:oil ratios (8:0, 7:1, 6:2, 5:3 and 4:4)

Lipids ratio wax:oil _{w/w}	Melting points of the lipid mixtures (°C)				
	Blackcurrant	Blackberry	Raspberry	Strawberry	Plum
7:1	61.9	61.8	62.0	62.1	62.0
6:2	61.2	61.4	61.0	61.0	61.4
5:3	60.6	61.0	60.5	58.7	60.0
4:4	60.3	58.6	57.5	57.6	59.9

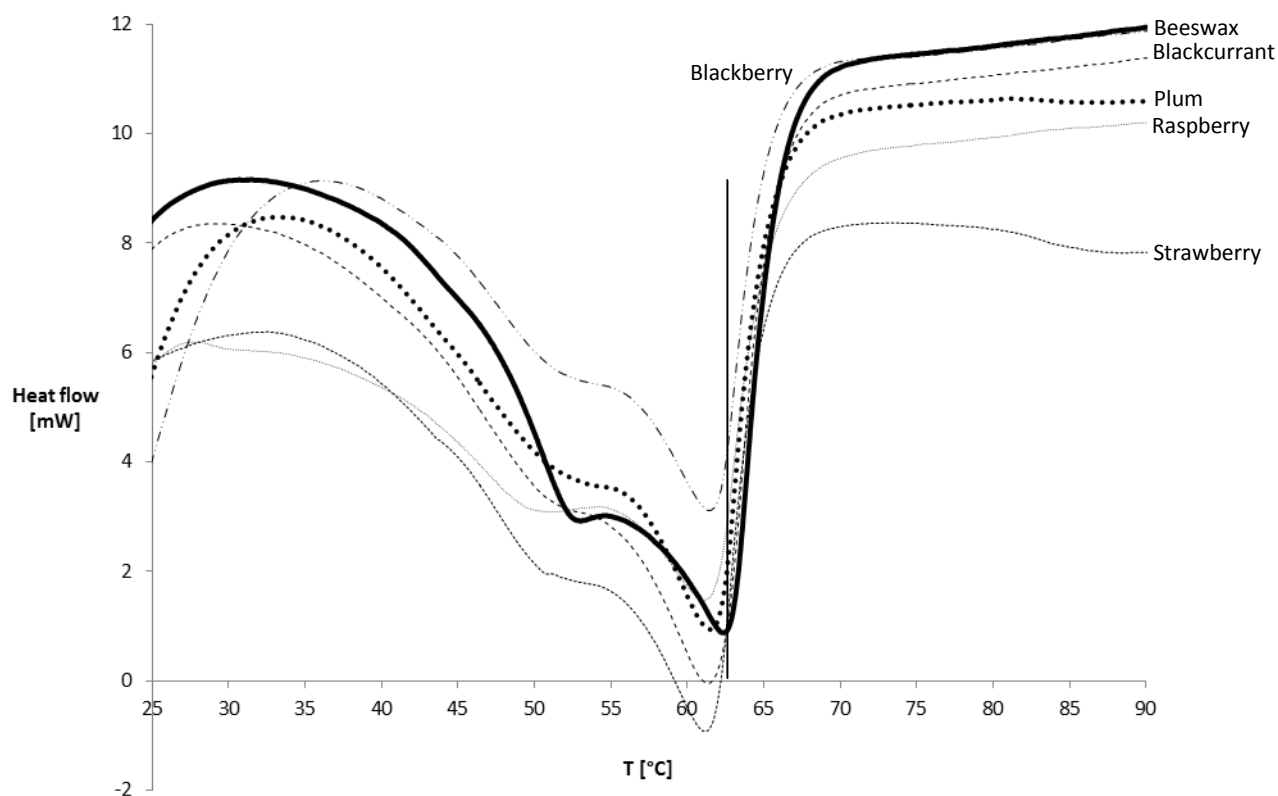


Figure 17 Comparison of the DSC thermograms of pure beeswax (solid line) with wax:oil (6:2) mixtures. The vertical line highlights the m.p. of pure beeswax which was measured here to be 63°C. As can be seen all the wax:oil mixtures display a lower m.p. due to the wax crystal lattice disruption by the oil

The thermogram of beeswax (Figure 16) showed that the sample is a single-phase material. The plot shows two peaks (52.5°C and 62.3°C) which represent the melting transitions of two different crystalline forms corresponding to the major components of beeswax, such as fatty esters and wax esters. Buchwald claimed that “these peaks probably represent the melting of distinct phases, each of which could be single or multicomponent in nature” [204]. The thermogram of beeswax (Figure 17) showed two peaks (52.5°C and 62.3°C) which represent the melting transitions of two different crystalline forms corresponding to the complexity of the bees wax [204]. The thermograms reflect the complexity of the chemical composition of the matrix, and highlight that the less ordered crystalline structure provides improved physical stability and lower expulsion of drug from

particles [205]. These thermograms shows that by increasing the amount of seed oil a depression in m.p. and melting enthalpies occurred. The shape of the first peak (at 52°C) has been flattened. This is the result of the disruption of the order of the lipid crystals. For less ordered crystals or amorphous compounds, the melting process does not require as much energy as compared to a pure compound in which a stronger lattice energy would need to be overcome.

The thermograms show that the peaks start to broaden in the samples containing a wax:oil ratio of 6:2 (at ratio 7:1 no changes were observed). This is an indication of significant changes in the crystal lattice, due to higher molecule mobility within the NLCs. Therefore, the 6:2 ratio was set as the optimum in terms of oil incorporation. Further addition of the seed oil (5:3 or 4:4) will lower further the crystallinity of the matrix and thus negatively affect the protective ability of NLCs. Wissing and Müller [1] showed a correlation between lower crystallinity and loss of occlusive function of the emulsion and thus TEWL of the skin. Therefore, in the systems I needed to maximize the amount of entrapped oil, but considering that the addition of the oil causes a loss in crystallinity.

7.3.3 Nanostructured lipid carrier composition

Stability measurements of the NLCs were made at 0, and 11 weeks. After 11 weeks clear distinctions between the formulations were visible. The results are presented in table 13.



Table 13 The systems stability change with time. Changes in emulsion systems are shown as comparison after system preparation (t=0), and after eleven weeks (Δt) as characterized by particle size, polydispersity index, zeta potential and pH. Formulation 3A shows the best stability.

Formulation symbol	Mean size (nm)		Pdi		ζ potential (mV)		pH	
	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks
3	274.5	+22.8	0.1977	-0.052	-34.1	+9.0	4.0	-0.2
3A	247.6	+13.3	0.1530	+0.083	-28.9	+2.5	4.1	+0.8
3B	258.1	+37.9	0.1982	+0.034	-32.2	+4.3	4.2	+0.8
4	337.0	+19.0	0.2540	-0.055	-38.0	+10.1	3.9	-0.3
4A	300.5	+69.5	0.4412	-0.317	-38.5	+9.7	4.1	+0.6
4B	355.0	+77.2	0.2734	-0.097	-33.9	+4.1	4.2	+0.8

From the DLS measurements, it can be observed that samples of the formulation 4 (2% of emulsifier) had a larger particle diameter and loss in polydispersity (Table 13), which indicates that the particles had agglomerated. The smallest changes can be observed in the formulation 3A.

Even though a ζ potential above 30 mV or below -30 mV is traditionally required for long term electrostatic stabilization [206], my emulsions were developed using nonionic emulsifiers and thus the magnitude of the ζ potential in the systems is sufficient to consider them as stable [207]. These impart stability to NLC dispersions by steric mechanisms, and thus stability may be followed by a change in ζ potential with time [208], especially when the other results indicate good stability as well. The best formulation in this case was 3A where we observed the smallest change in ζ potential. This formulation also had the lowest initial variation in size distribution and lowest observed turbidity, as compared to all other formulations. All other samples, with higher Pdi values, were turbid. Based on these results, the formulation 3A was chosen as the best carrier for the various seed oils.

7.3.4 Nanostructured lipid carriers containing different seed oils

In general, the addition of an oil to a solid lipid reduces its crystallinity due to decreased structure arrangement. However, the effect depends on the mixing ratio, but also on the composition of the liquid lipid. In fact, the results of the thermal characterization carried out on different NLC systems showed that changes of m.p. occurred not only in different lipid ratios, but also with different seed oils (among the same lipids ratios) (Figure 18).

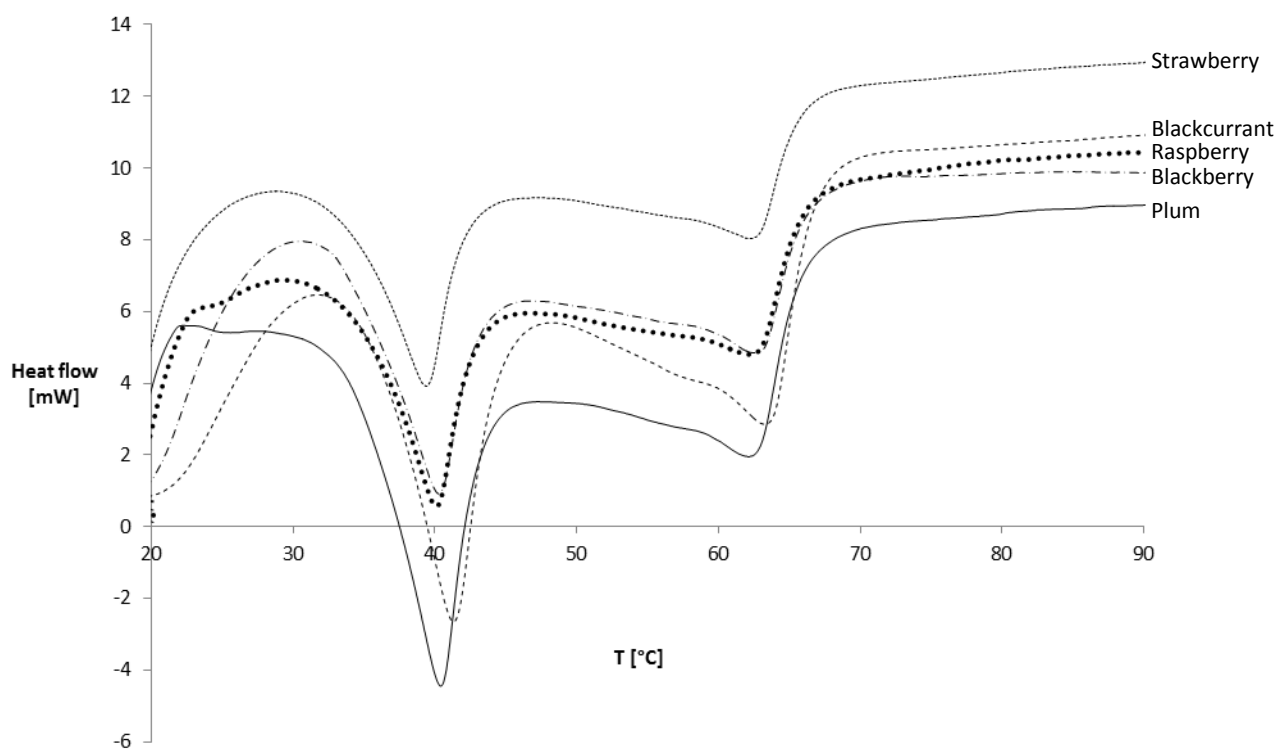


Figure 18 Thermograms of NLC emulsion systems based on formulation 3A, with different fruit seed oils. Difference observed are due to the FA composition of each seed oil, and strength of molecular interaction

The biggest difference was observed with plum and strawberry, which reflects the differences in FA composition of the oils used (Table 14). Plum has the most MUFAs C18:1 (about three times more than plum, and strawberry have the most PUFA C18:3 (in plum only traces was detected). The plum having the lowest amount of unsaturated MUFAs were able



to form regular repeating units within the lipid, whereas the PUFAs and their irregular positioning inhibit this process.

Table 14 Differences in NLC stability due to various seed oils. Changes in emulsion systems are shown as results comparison after system preparation (t=0) and eleven weeks (Δt) as characterized by particle size, polydispersity index, zeta potential and pH. Changes observed are due to seed oil FA composition. Interestingly in formulation 3A seed oil content is 2% of total phase.

3A Formulation containing fruit seed oil	Mean size (nm)		Pdi		ζ potential (mV)		pH	
	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks	t=0	$\Delta t=11$ weeks
Blackcurrant	247.6	+13.3	0.1530	+0.083	-28.9	+1.2	4.38	+0.4
Blackberry	228.6	+29.47	0.1341	+0.04	-27.9	+0.6	4.51	+0.5
Raspberry	221.0	+6.82	0.1407	-0.04	-28.1	+2.2	4.24	+0.7
Strawberry	248.5	-3.13	0.1710	0.00	-29.8	+2.0	4.32	+0.7
Plum	274.1	-14.49	0.2010	-0.02	-29.2	+0.5	4.34	+0.3

From Table 14 it can be observed that a broad range of seed oils were incorporated successfully into the NLC, with this given formulation. However, no significant differences in the NLC stability was observed with varying seed oils, as the seed oil is incorporated into the wax matrix, and thus has little or no effect on the stability of the NLC.

7.3.5 Characterization of nanostructured lipid carriers by ^1H NMR

In order to assess if the seed oil has been incorporated into the lipid matrix, ^1H NMR spectra were recorded. To make this comparison a spectrum of beeswax was compared to the spectrum of the NLC, as shown in Figure 19. It was assumed that if oil excess would occur, it would be dispersed in the continuous phase [209, 210] forming simple emulsion droplets and thus appear as additional peaks in the ^1H -NMR spectra. For comparison the following formulation were chosen 3, 3A, 3B, 4, 4A, and 4B. In addition, spectra of formulations containing no beeswax (only emulsifier, seed oil and water) were recorded. The latter systems represent samples in which an oil excess occurred, and consequently observe a dispersed phase with seed oil.

Analyzing the latter samples the signals of the hydrophilic emulsifier (AOE) and fatty acids are clearly visible at 1.2 ppm. When comparing emulsion to SLN it can be observed a decrease in peak intensities and at the same time a broadening of the same peaks. Moreover, it is likely that AOE arrange its hydrophilic part on the surface of the nanoparticles, whereas the fatty alcohol is immobilized toward the inner part of the nanoparticles (the signals due to the FAs are broad, it suggests that the FAs cannot move freely). The same phenomenon was observed by Casadei *et al.* [211], and Zimmermann *et al.* [212]. The seed oil is incorporated into the NLC when the PUFA peaks become broader and wider as shown by Jennings *et al.* [213]. The formulations containing both the beeswax and the oil show a progressive decrease of the mobility of the oil (broader signals) when the amount of beeswax is increased. It is observed in the range of 0.8 ppm – 2.1 ppm (the C-C bonds). The same phenomenon occurs for the C=C bonds at 5.2-5.4 ppm.

The fact that the seed oil peaks persist, even in the solid matrix may suggest that the lipid nanoparticles are not completely solid, and that some relaxation of the protons of the lipids can be observed even if the peaks are very broad. Moreover the intensity of the peaks due to the lipids increases when the amount of oil employed in the formulation is increased. It is likely that the structure of the nanoparticles is partially solid even when a very low amount of beeswax is used (ratio 6:2 beeswax:oil). Since the basic formulation 3 and 4 differ by the amount of emulsifier the spectra are the same as expected. Comparing 3A and 4A, the better incorporation was observed in 3A, which can be explain by smaller amount of liquid lipid and higher amount of emulsifier, so the oil is not repulsed from the lipid matrix. Comparing 3B and 4B, the better seed oil incorporation was observed in 4B, which contained more lipid and less emulsifier.



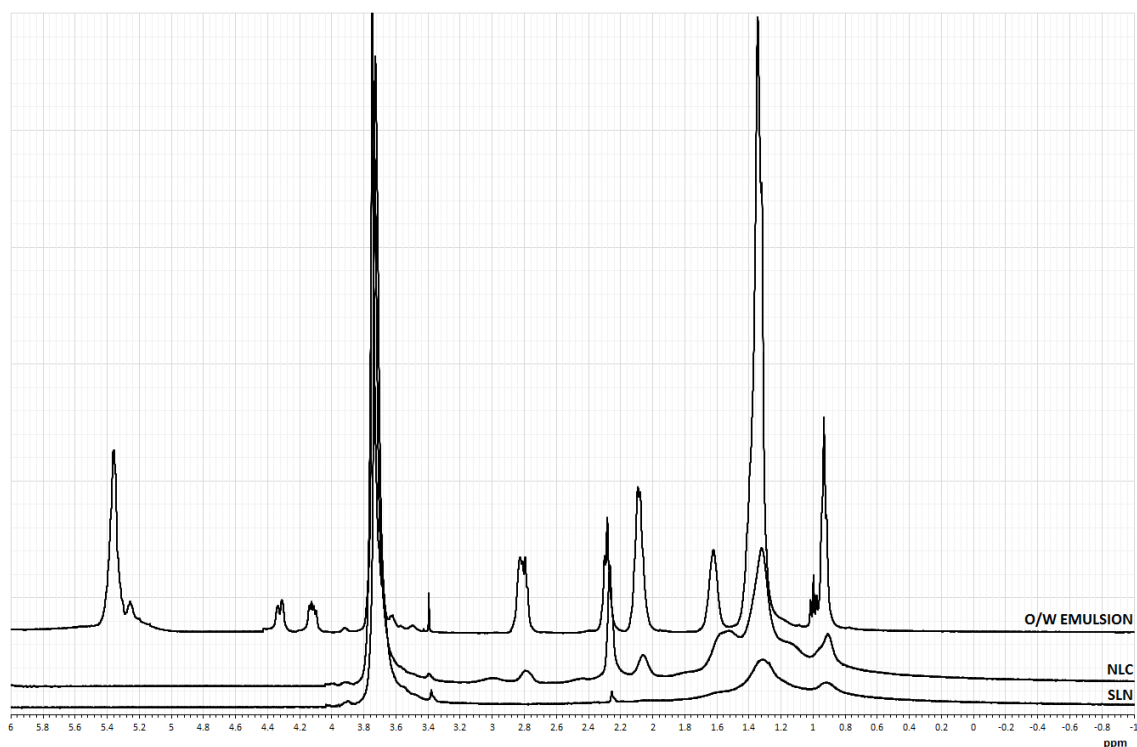


Figure 19 ^1H NMR spectra of O/W emulsions, SLN samples, and NLC (the NLC shown here has a wax:oil ratio of 6:2). The seed oil is evidently incorporated into the matrix structure, as can be seen by the disappearance of the peaks at 0.8-2.1 ppm

Based on data obtained it seems that it is possible to adjust the physical state of the nanoparticles simply by changing the ratio between beeswax and oil. This indicates the possibility to modulate the release rate of an active molecule encapsulated into the nanoparticles. This can also result in different textures, which is of great significance in its performance as a cosmetic emulsion. Altering the seed oil, from various fruit did not significantly alter the ^1H NMR spectra as shown in Figure 20.

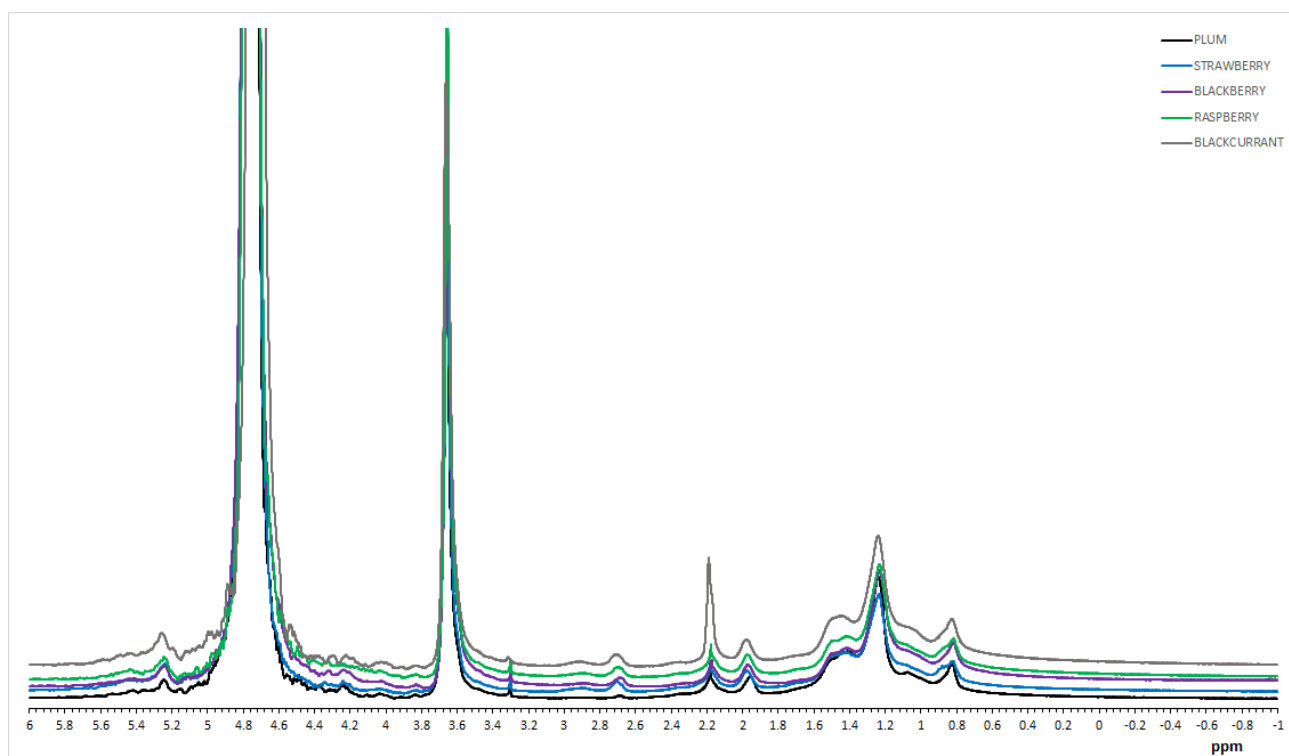


Figure 20 ^1H NMR of NLC nanosuspensions (formulation 3A) with varying fruit seed oils.

7.3.6 Oxidative stability of obtained systems

The PV of pure seed oils, O/W emulsions, SLNs and NLCs was measured before and after UV radiation. The results showed a difference in PV between pure seed oils and the oils incorporated in various emulsions. The blanks, *i.e.* the formulations without seed oil have low PV values, indicating that only the seed oil was oxidized under UV radiation. After UV exposure the NLC PVs were always lower than those of the O/W emulsions, showing that the NLC is an effective protection system. The detailed results are presented in the Table 15.



Table 15 PV values (mmol of O₂/kg) of the systems measured 24 hours after preparation, before UV radiation, and after 5 hours of UV radiation. Shaded regions highlight difference between O/W and NLC. The NLC offers effective protection against photodegradation of the seed oils.

Seed oil	Seed oil		O/W (no beeswax)		NLC	
	UV Exposure					
	Before	After 5h	Before	After 5h	Before	After 5h
Plum	1.50±0.02	36.79±1.09	0.57±0.06	6.51±0.11	0.59±0.03	4.88±0.60
Blackcurrant	7.75±0.05	101.74±2.09	3.05±0.06	12.13±0.12	0.86±0.003	10.58±0.53
Blackberry	9.78±0.03	252.77±3.03	2.47±0.52	15.19±0.24	1.54±0.19	12.09±0.86
Strawberry	10.69±0.003	137.91±0.92	2.33±0.07	11.42±0.71	0.55±0.01	5.21±0.66
Raspberry	1.53±0.04	111.94±6.27	2.88±0.11	18.31±0.22	1.00±0.01	16.57±0.23
Blank	-	-	0.21±0.05	0.88±0.01	0.11±0.02	0.75±0.01

7.3.7 Rheological behavior of the obtained systems

The obtained SLN systems were acting like Newtonian fluids. The addition of thickening agent seemed to be necessary. Sample 3A was prepared with different amounts (2, 3, 4, 5, and 6%) of thickening agent. The samples of 3A with 5% and 6% of thickening agent were chosen as typical for intended to be used on the skin. The percentage of thickening agent was chosen based on sensory analysis. From a sensorial viewpoint these samples were described as “jelly-like”. All the systems containing the thickening agent were thixotropic emulsions (Figure 21).

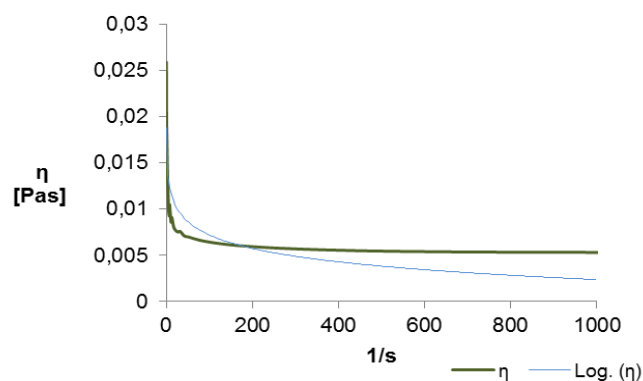


Figure 21 Viscosity vs. shear rate plot. The example of rheological behavior of the system containing 0.36% thickening agent

After addition of thickening agent the emulsion could be easily taken by a spatula and was stayed in position even after inversion of the spatula.

The addition of the thickening agent changes only its functional features so the consistence of the system can be modulated due to its application.

7.4 Conclusions

In this section I have attempted to show the suitability of using PUFA-rich oils as components of NLCs. I have used cold pressed fruit seed/kernel oils of common European fruits, such as plums, blackberries, blackcurrants, raspberries and strawberries as lipid matrix modifiers and bioactive components. In addition, the use of fruit seeds is environmentally sustainable, as they are considered a waste product. Nanosuspensions which had the best stability had a wax:oil ratio of 6:2. Increasing the amount of oil resulted in changes in the crystallinity of the wax, and thus lower inclusion. These results are comparable to Wissing *et al.* [1] I have successfully formulated stable NLC nanosuspensions for topical use, using these fruit seed oils.

Because of its low viscosity (0.025 Pas) the systems can be modified and adjusted depending on the application and needs.

In addition, it was shown that the NLC acts as an effective method of protection against oxidation by UV radiation. Therefore NLCs modified by common fruit seed oils should be



considered as an effective and environmentally beneficial method of supplementing the skin with LA [177]. NLCs have shown effective in the protection of bioactive components, and thus allow for the inclusion and design of environmentally friendly FAs.

8 Summary

In this thesis, I have attempted to show the viability of using domestic fruit seed oils in a number of cosmetic emulsions. I have shown that fruit seed oils are suitable, as sources of PUFAs, with surprisingly higher content of PUFAs than many other “exotic” fruit seed oils commonly advertised. The use of these domestic fruit seeds has the benefit of being doubly advantageous, in that first high PUFA oils may be cheaply used in cosmetic emulsions, and secondly waste material may be recycled and reused. The use of domestic fruit seed oils in emulsions is therefore a core aspect of the 12 rules of Green Chemistry. I have shown that specifically fruit seeds, such as apple, blackberries, blackcurrants and strawberries can be used as sources of UFAs in personal care products, especially in leave-on products. Using these oils, I have created stable emulsions, and have shown that cosmetic emulsions from these oils have successfully be formulated. Several other points should be noted:

- 1) The raspberries, strawberries, blackcurrants and apples cold pressed seed oils form a stable oil-in-water emulsion systems, even in a large range of concentrations, *i.e.* up to 6% by weight of the oil phase.
- 2) The mMAG emulsifier was good stabilizer of the emulsion containing tested seed oils in a wide range of required HLB of the oil phase.
- 3) The best stability had emulsions in which the phase ratio oil:water was 20:80 and 19:81 respectively. Increasing the amount of the aqueous phase up to above 81% resulted in a partial destabilization of the emulsions.
- 4) It was found that the analyzed fruit seed oil can be used as cosmetic emulsion components, even at relatively high concentration, *i.e.* approx. 10%. From dermatological reasons some restrictions on the use may appear due to high concentration of bioactive components, however this will require further investigation.
- 5) It appears that therefore seed oils of domestic fruits have similar properties to “exotic oils” and thus their implementation or inclusion in cosmetic emulsions is only



a question of marketing. With the success of marketing of organic and local products this has a realistic possibility.

To protect the PUFAs from oxidation, the cold pressed fruit seed oils of common European fruits, such as plums, blackberries, blackcurrants, raspberries and strawberries can be used as lipid matrix modifiers and bioactive components, specifically NLCs. Here it was found that the nanosuspensions with best wax:oil ratio was empirically tested to be 6:2. Increasing the amount of oil resulted in changes in the crystallinity of the wax, and thus lower inclusion. I have successfully formulated stable NLC nanosuspensions for topical use, using these common fruit seed oils. Because of its low viscosity (0.025 Pas) the systems can be modified and adjusted depending on the application and needs. In addition, I have shown that the NLC acts as an effective method of protection against oxidation by UV radiation. Therefore NLCs modified by common fruit seed oils should be considered as an effective and environmentally beneficial method of supplementing the skin with linoleic acid. NLCs have shown to be effective in the protection of bioactive components, and thus allow for the inclusion and design of environmentally friendly FAs. As part of this, I have developed a new modified method to determine the PV of emulsions.



9 Outlook

Taking into account the advantages of both, the tested oils and NLCs system, and based on obtained results it would be beneficial to conduct following further studies:

9.1 The domestic fruit seed oils should be applied in other, more specific systems, *i.e.* multiple emulsions:

- oil-in-NLC (O/NLC)

Internal phase can be a vehicle for lipophilic bioactive components, which can be incorporated due to the request and the specific skin needs: liposoluble vitamins (A, D, E, K) – or their ester derivatives if there are some law restrictions announced by Scientific Committee On Consumer Safety (SCCS).

- NLC-in-oil (NLC/O)

Internal phase containing the proper ratio of PUFAs, external phase containing more phospholipids and cholesterol to complete the intercellular lipid structure and improve the penetration of bioactive components. It would be immensely helpful to reduce skin dryness when induced by skin disease and/or intercellular lipid disorders, *i.e.* atopic dermatitis, psoriasis and contact dermatitis

These multiple emulsions have the advantages of the both O/W emulsion and NLC systems. The bioactive component incorporated in inner phase may be even more protected.

9.2 The all obtained systems should be tested in order to the actual influence of domestic fruit seed oils on the skin in form of various system types:

- release studies sustained release of bioactive compound after topical use to avoid skin irritation; control of the mechanism of the prolonged administration [214-217]
- Semipermeable membranes can be used for the studies [218-220]



- in vivo instrumental skin tests devices could be used for short term and long term influence on the skin. Some basic parameters should be tested such as TEWL, skin moisturization and its elasticity [221-223]

10 References

1. Wissing, S. and R. Müller, *The influence of the crystallinity of lipid nanoparticles on their occlusive properties*. International journal of pharmaceutics, 2002. **242**(1): p. 377-379.
2. Gunstone, F.D., J.L. Harwood, and A.J. Dijkstra, *The lipid handbook with CD-ROM*. 2007: CRC press.
3. Oomah, B.D., S. Ladet, D.V. Godfrey, J. Liang, and B. Girard, *Characteristics of raspberry (Rubus idaeus L.) seed oil*. Food chemistry, 2000. **69**(2): p. 187-193.
4. Huang, A.H., *Oil bodies and oleosins in seeds*. Annual review of plant biology, 1992. **43**(1): p. 177-200.
5. Bewley, J.D. and M. Black, *Seeds*, in *Seeds*. 1994, Springer. p. 1-33.
6. McGrath, J. and J. Uitto, *Anatomy and organization of human skin*. Rook's Textbook of Dermatology, Eighth Edition, 2010: p. 1-53.
7. Thyssen, J.P. and S. Kezic, *Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis*. J Allergy Clin Immunol, 2014.
8. Kezic, S., P.M. Kemperman, E.S. Koster, C.M. de Jongh, H.B. Thio, L.E. Campbell, A.D. Irvine, I.W. McLean, G.J. Puppels, and P.J. Caspers, *Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum*. Journal of Investigative Dermatology, 2008. **128**(8): p. 2117-2119.
9. Robinson, M., M. Visscher, A. LaRuffa, and R. Wickett, *Natural moisturizing factors (NMF) in the stratum corneum (SC). I. Effects of lipid extraction and soaking*. Journal of cosmetic science, 2010. **61**(1): p. 13.
10. Rhein, L.D., M. Schlossman, A. O'Lenick, and P. Somasundaran, *Surfactants in personal care products and decorative cosmetics*. Vol. 135. 2006: crc press.
11. Jiang, S., N. Ma, H. Li, and X. Zhang, *Effects of thermal properties and geometrical dimensions on skin burn injuries*. Burns, 2002. **28**(8): p. 713-717.
12. Cevc, G. and G. Blume, *Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force*. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1992. **1104**(1): p. 226-232.
13. Fluhr, J.W., P. Elsner, E. Berardesca, and H.I. Maibach, *Bioengineering of the skin: water and the stratum corneum*. 2004: CRC press.
14. Sato, J., M. Denda, J. Nakanishi, and J. Koyama, *Dry condition affects desquamation of stratum corneum in vivo*. Journal of dermatological science, 1998. **18**(3): p. 163-169.
15. Simon, M., N. Jonca, M. Guerrin, M. Haftek, D. Bernard, C. Caubet, T. Egelrud, R. Schmidt, and G. Serre, *Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation*. Journal of Biological Chemistry, 2001. **276**(23): p. 20292-20299.
16. Karande, P., A. Jain, and S. Mitragotri, *Discovery of transdermal penetration enhancers by high-throughput screening*. Nature biotechnology, 2004. **22**(2): p. 192-197.
17. Robosky, L.C., K. Wade, D. Woolson, J.D. Baker, M.L. Manning, D.A. Gage, and M.D. Reily, *Quantitative evaluation of sebum lipid components with nuclear magnetic resonance*. Journal of lipid research, 2008. **49**(3): p. 686-692.
18. Barry, B.W., *Breaching the skin's barrier to drugs*. Nature biotechnology, 2004. **22**(2): p. 165-167.
19. Zhai, H. and H.I. Maibach, *Occlusion vs. skin barrier function*. Skin Research and technology, 2002. **8**(1): p. 1-6.
20. Engelbrecht, T.N., A. Schroeter, T. Hauß, and R.H. Neubert, *Lipophilic penetration enhancers and their impact to the bilayer structure of stratum corneum lipid model membranes: neutron*

- diffraction studies based on the example oleic acid.* Biochimica et Biophysica Acta (BBA)- Biomembranes, 2011. **1808**(12): p. 2798-2806.
21. Trommer, H. and R. Neubert, *Overcoming the stratum corneum: the modulation of skin penetration.* Skin pharmacology and physiology, 2006. **19**(2): p. 106-121.
 22. Szente, L., L. Szejtli, J. Szeman, and L. Kato, *Fatty Acid-Cyclodextrin Complexes: Properties and Applications.* Journal of Inclusion Phenomena and Molecular Recognition in Chemistry, 1993. **16**: p. 16.
 23. Elgart, A., I. Cherniakov, Y. Aldouby, A.J. Domb, and A. Hoffman, *Lipospheres and pro-nano lipospheres for delivery of poorly water soluble compounds.* Chem Phys Lipids, 2012. **165**(4): p. 438-53.
 24. Naoui, W., M.A. Bolzinger, B. Fenet, J. Pelletier, J.P. Valour, R. Kalfat, and Y. Chevalier, *Microemulsion microstructure influences the skin delivery of an hydrophilic drug.* Pharm Res, 2011. **28**(7): p. 1683-95.
 25. Anton, N., J.P. Benoit, and P. Saulnier, *Design and production of nanoparticles formulated from nano-emulsion templates-a review.* J Control Release, 2008. **128**(3): p. 185-99.
 26. Kulkamp-Guerreiro, I.C., S.J. Berlitz, R.V. Contri, L.R. Alves, E.G. Henrique, V.R. Barreiros, and S.S. Guterres, *Influence of nanoencapsulation on the sensory properties of cosmetic formulations containing lipoic acid.* Int J Cosmet Sci, 2013. **35**(1): p. 105-11.
 27. Klang, V., N.B. Matsko, C. Valenta, and F. Hofer, *Electron microscopy of nanoemulsions: an essential tool for characterisation and stability assessment.* Micron, 2012. **43**(2-3): p. 85-103.
 28. Mueller, R.H., K. Maeder, and S. Gohla, *Solid Lipid Nanoparticles (SLN) for controlled drug delivery - a review of the state of the art.* European Journal of Pharmaceutics and Biopharmaceutics 2000. **2000**: p. 17.
 29. Hernandez, E., *Pharmaceutical and cosmetic use of lipids, in Bailey's Industrial Oil and Fat Products.* 2005.
 30. Zocchi, G., *Skin-Feel Agents, in Handbook of Cosmetic Science and Technology, A.O.B. M. Paye, H.I. Maibach Editor.* 2001, Marcel-Dekker Inc.: New York. p. 357-371.
 31. Van Smeden, J., M. Janssens, G. Gooris, and J. Bouwstra, *The important role of stratum corneum lipids for the cutaneous barrier function.* BBA-Mol. Cell. Biol. L., 2014. **1841**(3): p. 295-313.
 32. Mojumdar, E.H., R.W. Helder, G.S. Gooris, and J.A. Bouwstra, *Monounsaturated fatty acids reduce the barrier of stratum corneum lipid membranes by enhancing the formation of a hexagonal lateral packing.* Langmuir 2014. **30**(22): p. 6534-6543.
 33. Menon, G.K., G.W. Cleary, and M.E. Lane, *The structure and function of the stratum corneum.* Int. J. Pharm., 2012. **435**(1): p. 3-9.
 34. Foster, R.H., G. Hardy, and R.G. Alany, *Borage oil in the treatment of atopic dermatitis.* Nutrition, 2010. **26**(7-8): p. 708-18.
 35. Viola, P., F. Nobili, and M. Viola, *Handbook of Diet, Nutrition and the Skin Human Health Handbooks, Olive oil as a skin protector.* Handbook of Diet, Nutrition and the Skin Human Health Handbooks Vol. 2. 2012: Springer. 13.
 36. Jump, D.B., *The biochemistry of n-3 polyunsaturated fatty acids.* J. Biol. Chem., 2002. **277**(11): p. 8755-8.
 37. Goebel, A.S., U. Knie, C. Abels, J. Wohlrab, and R.H. Neubert, *Dermal targeting using colloidal carrier systems with linoleic acid.* Eur J Pharm Biopharm, 2010. **75**(2): p. 162-72.
 38. Ziboh, V.A. and R.S. Chapkin, *Biologic significance of polyunsaturated fatty acids in the skin.* Arch Dermatol, 1987. **123**(12): p. 1686a-1690.
 39. Noli, C., G. Carta, L. Cordeddu, M.P. Melis, E. Murru, and S. Banni, *Conjugated linoleic acid and black currant seed oil in the treatment of canine atopic dermatitis: a preliminary report.* Vet. J., 2007. **173**(2): p. 413-21.

40. Bouwstra, J.A., G. Gooris, F. Dubbelaar, A. Weerheim, A. Ijzerman, and M. Ponc, *Role of ceramide 1 in the molecular organization of the stratum corneum lipids*. Journal of lipid research, 1998. **39**(1): p. 186-196.
41. Uchida, Y. and W.M. Holleran, *Omega-O-acylceramide, a lipid essential for mammalian survival*. Journal of dermatological science, 2008. **51**(2): p. 77-87.
42. Mizutani, Y., S. Mitsutake, K. Tsuji, A. Kihara, and Y. Igarashi, *Ceramide biosynthesis in keratinocyte and its role in skin function*. Biochimie, 2009. **91**(6): p. 784-790.
43. Iwai, I., H. Han, L. den Hollander, S. Svensson, L.-G. Öfverstedt, J. Anwar, J. Brewer, M. Bloksgaard, A. Laloef, and D. Nosek, *The human skin barrier is organized as stacked bilayers of fully extended ceramides with cholesterol molecules associated with the ceramide sphingoid moiety*. Journal of Investigative Dermatology, 2012. **132**(9): p. 2215-2225.
44. Norlén, L., *Current understanding of skin barrier morphology*. Skin pharmacology and physiology, 2013. **26**(4-6): p. 213-216.
45. Norlén, L., I. Nicander, A. Lundsjö, T. Cronholm, and B. Forslind, *A new HPLC-based method for the quantitative analysis of inner stratum corneum lipids with special reference to the free fatty acid fraction*. Archives of dermatological research, 1998. **290**(9): p. 508-516.
46. Natarajan, V.T., P. Ganju, A. Ramkumar, R. Grover, and R.S. Gokhale, *Multifaceted pathways protect human skin from UV radiation*. Nature chemical biology, 2014. **10**(7).
47. Asif, M., *Health effects of omega-3,6,9 fatty acids: Perilla frutescens is a good example of plant oils*. Orient Pharm Exp Med, 2011. **11**(1): p. 51-59.
48. Kleiman, R., D.A. Ashley, and J.H. Brown, *Comparison of two seed oils used in cosmetics, moringa and marula*. Industrial Crops and Products, 2008. **28**(3): p. 361-364.
49. Technical Data Sheet, *Andiroba Oil Refined Organic Product Code: RF3160R*, Beraca
50. Cabrera-Vique, C., R. Marfil, R. Gimenez, and O. Martinez-Augustin, *Bioactive compounds and nutritional significance of virgin argan oil--an edible oil with potential as a functional food*. Nutr Rev, 2012. **70**(5): p. 266-79.
51. Thiem B., *Rubus chamaemorus L. – a boreal plant rich in biologically active metabolites: a review*, Biology Letters, 2004. **40**(1): p. 3-13
52. Bail, S., G. Stuebiger, S. Krist, H. Unterweger, and G. Buchbauer, *Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity*. Food Chemistry, 2008. **108**(3): p. 1122-1132.
53. Deferne, J.-L. and D.W. Pate, *Hemp seed oil: A source of valuable essential fatty acids*. J. Int. Hemp Assoc, 1996. **3**(1): p. 4-7.
54. Cahoon E., E. Marillia, K. Stecca, S. Hall, D. Taylor, and A. Kinney, *Production of Fatty Acid Components of Meadowfoam Oil in Somatic Soybean Embryos*, Plant Physiology, 2000. **124**: p. 243-251
55. Zaher F., O. El Kinawy, and D. El Haron, *Solvent extraction of jojoba oil from pre-pressed jojoba meal*, Grasas y Aceites, 2004. **55**(2): p. 129-134
56. Bruni, R., A. Medici, A. Guerrini, S. Scalia, F. Poli, M. Muzzoli, and G. Sacchetti, *Wild Amaranthus caudatus seed oil, a nutraceutical resource from Ecuadorian flora*. Journal of agricultural and food chemistry, 2001. **49**(11): p. 5455-5460.
57. Chemat, F., M.A. Vian, and G. Cravotto, *Green extraction of natural products: Concept and principles*. International journal of molecular sciences, 2012. **13**(7): p. 8615-8627.
58. Clark, J.H., *Green chemistry: challenges and opportunities*. Green Chemistry, 1999. **1**(1): p. 1-8.
59. *Główny Urząd Statystyczny*. Warszawa, lata, 2008. **2010**.
60. Schieber, A., F. Stintzing, and R. Carle, *By-products of plant food processing as a source of functional compounds—recent developments*. Trends in Food Science & Technology, 2001. **12**(11): p. 401-413.



61. Soong, Y.-Y. and P.J. Barlow, *Antioxidant activity and phenolic content of selected fruit seeds*. Food Chemistry, 2004. **88**(3): p. 411-417.
62. Wroniak, M., K. Krygier, and M. Kaczmarczyk, *Comparison of the quality of cold pressed and virgin rapeseed oils with industrially obtained oils*. Polish Journal of Food and Nutrition Sciences, 2008. **58**(1): p. 5.
63. McClements, D.J., *Food emulsions: principles, practices, and techniques*. 2015: CRC press.
64. Solans, C., P. Izquierdo, J. Nolla, N. Azemar, and M. Garcia-Celma, *Nano-emulsions*. Current opinion in colloid & interface science, 2005. **10**(3): p. 102-110.
65. Caldero, G., M.J. Garcia-Celma, and C. Solans, *Formation of polymeric nano-emulsions by a low-energy method and their use for nanoparticle preparation*. J Colloid Interface Sci, 2011. **353**(2): p. 406-11.
66. Tadros, T., P. Izquierdo, J. Esquena, and C. Solans, *Formation and stability of nano-emulsions*. Advances in Colloid and Interface Science, 2004. **108-109**: p. 303-318.
67. Porras, M., C. Solans, C. González, and J.M. Gutiérrez, *Properties of water-in-oil (W/O) nano-emulsions prepared by a low-energy emulsification method*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2008. **324**(1-3): p. 181-188.
68. Kumar, P. and K.L. Mittal, *Handbook of microemulsion science and technology*. 1999: CRC press.
69. Seifriz, W., *Studies in Emulsions. I-II*. The Journal of Physical Chemistry, 1925. **29**(5): p. 587-600.
70. Seifriz, W., *Studies in Emulsions. III-V*. The Journal of Physical Chemistry, 1925. **29**(6): p. 738-749.
71. Robbe-Tomine, L. and C. Hen-Ferrenbach, *Multiple Emulsions: Structure, Properties and Applications*. Editions De Sante, Paris, 1998: p. 141.
72. Aserin, A., *Multiple emulsion: technology and applications*. Vol. 1. 2008: John Wiley & Sons.
73. Pal, R., *On the viscoelastic behavior of multiple emulsions*. J Colloid Interface Sci, 2007. **313**(2): p. 751-6.
74. Schmidts, T., D. Dobler, P. Schlupp, C. Nissing, H. Garn, and F. Runkel, *Development of multiple W/O/W emulsions as dermal carrier system for oligonucleotides: effect of additives on emulsion stability*. Int J Pharm, 2010. **398**(1-2): p. 107-13.
75. Mühlen, A., C. Schwarz, W. Mehnert, and R. Müller, *Produktion von 'solid lipid nanoparticles'(SLN) für die kontrollierte wirkstoffapplikation*. Arch. Pharm, 1993. **326**: p. 752.
76. Gasco, M.R., *Method for producing solid lipid microspheres having a narrow size distribution*. 1993, Google Patents.
77. Schwarz, C., W. Mehnert, J. Lucks, and R. Müller, *Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization*. Journal of Controlled Release, 1994. **30**(1): p. 83-96.
78. Pardeike, J., A. Hommos, and R.H. Muller, *Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products*. Int J Pharm, 2009. **366**(1-2): p. 170-84.
79. Anastasiu, S., E. Jelescu, and Z. Grabowiecki, *Środki powierzchniowo czynne*. 1973: Wydawnictwa Naukowo-Techniczne.
80. A. Domínguez, A.F., N. González, E. Iglesias, L. Montenegro, *Determination of Critical Micelle Concentration of Some Surfactants by Three Techniques*. Journal of Chemical Education, 1997. **74**(10): p. 5.
81. Pletnev, M.Y., *Surfactants: chemistry, interfacial properties, applications: chemistry, interfacial properties, applications*. Vol. 13. 2001, Amsterdam, The Netherlands: Elsevier. 679.
82. Griffin, W.C., *Classification of surface-active agents by "HLB"*. J Soc Cosmetic Chemists 1946. **1** p. 311-326.

83. Huibers, P.D. and D.O. Shah, *Evidence for synergism in nonionic surfactant mixtures: enhancement of solubilization in water-in-oil microemulsions*. Langmuir, 1997. **13**(21): p. 5762-5765.
84. Pasquali, R.C., M.P. Taurozzi, and C. Bregni, *Some considerations about the hydrophilic-lipophilic balance system*. Int J Pharm, 2008. **356**(1-2): p. 44-51.
85. Herrling, T., K. Jung, and J. Fuchs, *Measurements of UV-generated free radicals/reactive oxygen species (ROS) in skin*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2006. **63**(4): p. 840-845.
86. Schmid-Wendtner, M.-H. and H.C. Korting, *The pH of the skin surface and its impact on the barrier function*. Skin pharmacology and physiology, 2006. **19**(6): p. 296-302.
87. Stillman, M.A., H.I. Maibach, and A.R. Shallita, *Relative irritancy of free fatty acids of different chain length*. Contact dermatitis, 1975. **1**(2): p. 65-69.
88. Briganti, S. and M. Picardo, *Antioxidant activity, lipid peroxidation and skin diseases. What's new*. Journal of the European Academy of Dermatology and Venereology, 2003. **17**(6): p. 663-669.
89. Steen, K.H., A.E. Steen, and P.W. Reeh, *A dominant role of acid pH in inflammatory excitation and sensitization of nociceptors in rat skin, in vitro*. The Journal of neuroscience, 1995. **15**(5): p. 3982-3989.
90. Kurzępa, B., *Ustawa o kosmetykach z komentarzem*. 2009: Towarzystwo Naukowe Organizacji i Kierownictwa" Dom Organizatora".
91. Das, U.N., *Essential fatty acids: biochemistry and physiology*. Metabolic syndrome pathophysiology: the role of essential fatty acids, 2010: p. 181-200.
92. Tadros, T.F., *Emulsion science and technology: a general introduction*. Emulsion science and technology, 2009: p. 1-56.
93. Dzyaloshinskii E., E. Lifshitz, and L.P. Pitaevskii, *General Theory of Van Der Waals Forces*. Physics-Uspokhi, 1961. **4**(2): p. 153-176.
94. Derjaguin, B. and L. Landau, *Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes*. Acta physicochim. URSS, 1941. **14**(6): p. 633-662.
95. Verwey E.J.W., and J.T.G. Overbeek, *Theory of the stability of lyophobic colloids*. 1999: Courier Corporation.
96. Tadros, T., *Application of rheology for assessment and prediction of the long-term physical stability of emulsions*. Adv. Colloid Interface Sci., 2004. **108-109**: p. 227-58.
97. Robins M., A. Watson, and P. Wilde, *Emulsions - creaming and rheology*, Current Opinion in Colloid and Interface Science, 2002. **7**: p. 419-425
98. Palazolo G., D. Sorgentini, and J. Wagner, *Coalescence and flocculation in o/w emulsions of native and denatured whey soy proteins in comparison with soy protein isolates*, Food Hydrocolloids, 2005. **19**(3): p. 595-604
99. Voorhees, P.W., *Ostwald ripening of two-phase mixtures*. Annual Review of Materials Science, 1992. **22**(1): p. 197-215.
100. Sjoblom, J., *Encyclopedic handbook of emulsion technology*. 2001: CRC Press.
101. Tadros, T.F., *Fundamental principles of emulsion rheology and their applications*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 1994. **91**: p. 39-55.
102. Laba, D., *Rheological properties of cosmetics and toiletries*. 1993: CRC Press.
103. T. Moravkova, P.F., *The influence of emulsifier on rheological and sensory properties of cosmetic lotions*. Adv. Mater. Sci. Eng., 2013. **2013**: p. 7.
104. White, D.A., I.D. Fisk, J.R. Mitchell, B. Wolf, S.E. Hill, and D.A. Gray, *Sunflower-seed oil body emulsions: Rheology and stability assessment of a natural emulsion*. Food Hydrocoll., 2008. **22**(7): p. 1224-1232.

105. Liu, H., X. Xu, and S.D. Guo, *Rheological, texture and sensory properties of low-fat mayonnaise with different fat mimetics*. LWT-Food Science and Technology, 2007. **40**(6): p. 946-954.
106. Pokorny, J., J. Davidek, S. Dobiasova, P. Stern, and J. Čmolik, *Comparison of sensory analysis and rotational rheometry in the texture evaluation of margarines*. Journal of texture studies, 1984. **15**(4): p. 395-406.
107. Graham, J.A., *The psychology of fragrance*, in *Poucher's Perfumes, Cosmetics and Soaps*. 1993, Springer. p. 728-739.
108. Jones, G., *Beauty imagined: a history of the global beauty industry*. 2010: Oxford University Press.
109. Lukic, M., I. Jaksic, V. Krstonosic, N. Cekic, and S. Savic, *A combined approach in characterization of an effective W/O hand cream: the influence of emollient on textural, sensorial and in vivo skin performance*. International journal of cosmetic science, 2012. **34**(2): p. 140-149.
110. Gilbert, L., C. Picard, G. Savary, and M. Grisel, *Impact of Polymers on Texture Properties of Cosmetic Emulsions: A Methodological Approach*. Journal of Sensory Studies, 2012. **27**(5): p. 392-402.
111. Gilbert, L., C. Picard, G. Savary, and M. Grisel, *Rheological and textural characterization of cosmetic emulsions containing natural and synthetic polymers: relationships between both data*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013. **421**: p. 150-163.
112. Drake, M., S. Drake, F. Bodyfelt, S. Clark, and M. Costello, *History of Sensory Analysis*, in *The Sensory Evaluation of Dairy Products*, S. Clark, et al., Editors. 2009, Springer US. p. 1-6.
113. Schillign, M.W., *History, Background, and Objectives of Sensory Evaluation in Muscle Foods*, in *Handbook of Meat, Poultry and Seafood Quality*. 2007, Blackwell Publishing. p. 15-24.
114. Thompson, K.R., D.H. Chambers, and E. Chambers Iv, *Sensory Characteristics of Ice Cream Produced in the USA and Italy*. Journal of Sensory Studies, 2009. **24**(3): p. 396-414.
115. Childs, J.L. and M. Drake, *Consumer Perception of fat reduction in cheese*. Journal of Sensory Studies, 2009. **24**(6): p. 902-921.
116. McEwan, J.A., J.S. Colwill, and D.M.H. Thomson, *The Application of two free choice profile methods to investigate the sensory characteristics of chocolate*. Journal of Sensory Studies, 1989. **3**(4): p. 271-286.
117. Jensen, S., H. Oestdal, and A.K. Thybo, *Sensory profiling of changes in wheat and whole wheat bread during a prolonged period of storage*. Journal of Sensory Studies, 2010. **25**(2): p. 231-245.
118. Walker, S. and J. Prescott, *The influence of solution viscosity and different viscosifying agents on apple juice flavour*. Journal of Sensory Studies, 2000. **15**(3): p. 285-307.
119. Bower, J.A. and R. Boyd, *Effect of health concern and consumption patterns on measures of sweetness by hedonic and just about right scales*. Journal of Sensory Studies, 2003. **18**(3): p. 235-248.
120. Heymann, H., H. Hopfer, and D. Bershaw, *An Exploration of the Perception of Minerality in White Wines by Projective Mapping and Descriptive Analysis*. Journal of Sensory Studies, 2014. **29**(1): p. 1-13.
121. Runnebaum, R.C., R.B. Boulton, R.L. Powell, and H. Heymann, *Key constituents affecting wine body - exploratory study*. Journal of Sensory Studies, 2011. **26**(1): p. 62-70.
122. Kallithraka, S., J. Bakker, and M.N. Clifford, *Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and model solution*. Journal of Sensory Studies, 1997. **12**(1): p. 25-37.
123. Wiechers, J., *Comparing instrumental and sensory measurements of skin moisturization*. Cosmetics and toiletries, 1999. **114**(2): p. 29-34.

124. Fisher, G.J., *The pathophysiology of photoaging of the skin*. *Cutis*, 2005. **75**(2 Suppl): p. 5-8; discussion 8-9.
125. Luther, M., J. Parry, J. Moore, J. Meng, Y. Zhang, Z. Cheng, and L. Yu, *Inhibitory effect of Chardonnay and black raspberry seed extracts on lipid oxidation in fish oil and their radical scavenging and antimicrobial properties*. *Food Chemistry*, 2007. **104**(3): p. 1065-1073.
126. Bockisch, M., *Fats and oils handbook*. 1998: AOCS press.
127. Harwood, J.L., A.J. Dijkstra, and F.D. Gunstone, *The lipid handbook*. 2007: CRC Press.
128. Saldaña, M. and S. Martínez-Monteagudo, *Oxidative stability of fats and oils measured by differential scanning calorimetry for food and industrial applications*. 2013: INTECH Open Access Publisher.
129. Min, D. and J. Boff, *Chemistry and reaction of singlet oxygen in foods*. *Comprehensive reviews in food science and food safety*, 2002. **1**(2): p. 58-72.
130. Min, D.B. and J.M. Boff, *Lipid oxidation of edible oil*. *Food Science and Technology -New York-* Marcel Dekker, 2002: p. 335-364.
131. Knothe, G., *Some aspects of biodiesel oxidative stability*. *Fuel Processing Technology*, 2007. **88**(7): p. 669-677.
132. Carlsen, M.H., B.L. Halvorsen, K. Holte, S.K. Bøhn, S. Dragland, L. Sampson, C. Willey, H. Senoo, Y. Umezono, and C. Sanada, *The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide*. *Nutrition journal*, 2010. **9**(1): p. 1.
133. Lee, K.-g. and T. Shibamoto, *Determination of antioxidant potential of volatile extracts isolated from various herbs and spices*. *Journal of Agricultural and Food Chemistry*, 2002. **50**(17): p. 4947-4952.
134. Lee, J., N. Koo, and D. Min, *Reactive oxygen species, aging, and antioxidative nutraceuticals*. *Comprehensive reviews in food science and food safety*, 2004. **3**(1): p. 21-33.
135. Upton, P.G., K.T. Yamaguchi, S. Myers, T.P. Kidwell, and R.J. Anderson, *Effects of antioxidants and hyperbaric oxygen in ameliorating experimental doxorubicin skin toxicity in the rat*. *Cancer treatment reports*, 1986. **70**(4): p. 503-507.
136. Stahl, W. and H. Sies, *Antioxidant activity of carotenoids*. *Molecular aspects of medicine*, 2003. **24**(6): p. 345-351.
137. Krasodomska, O. and C. Jungnickel, *Viability of fruit seed oil O/W emulsions in personal care products*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2015. **481**: p. 468-475
138. Helbig, D., V. Böhm, A. Wagner, R. Schubert, and G. Jahreis, *Berry seed press residues and their valuable ingredients with special regard to black currant seed press residues*. *Food Chem.*, 2008. **111**(4): p. 1043-1049.
139. van Hoed, V., N. de Clercq, C. Echim, E. Andjelkovic, E. Leber, K. Dewetnick, and R. Vehre, *Berry seeds: a source of specialty oils with high content of bioactives and nutritional value*. *J. Food Lipids*, 2009. **16**: p. 17.
140. Mandal, M., *Effects of different extraction methods on the chemical properties of cranberry seed oils*. 2013, University of Wisconsin-Stout: University of Wisconsin-Stout, USA. p. 55.
141. Yu, X., Z. Li, F.R.v.d. Voort, and T. Yue, *Proximate composition of the apple seed and characterization of its oil*. *Int. J. Food Eng.*, 2007. **3**(5).
142. Poliakoff, M., J.M. Fitzpatrick, T.R. Farren, and P.T. Anastas, *Green chemistry: science and politics of change*. *Science*, 2002. **297**(5582): p. 807-810.
143. Anastas, P. and N. Eghbali, *Green chemistry: principles and practice*. *Chemical Society Reviews*, 2010. **39**(1): p. 301-312.
144. Groot, A.C. and J.W. Weijland, *Conversion of common names of cosmetic allergens to the INCI nomenclature*. *Contact Dermatitis*, 1997. **37**(4): p. 145-150.
145. Fao, F., *Statistical Yearbook 2013: World Food and Agriculture*. FAO Food Agric. Organization UN Rome Italy, 2013.

146. Bolarinwa, I.F., C. Orfila, and M.R. Morgan, *Determination of amygdalin in apple seeds, fresh apples and processed apple juices*. Food chemistry, 2015. **170**: p. 437-442.
147. International Blackcurrant Association IBA. *Blackcurrants growers*. 2013 [cited 2013].
148. Wang, S.Y. and H.-S. Lin, *Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage*. Journal of agricultural and food chemistry, 2000. **48**(2): p. 140-146.
149. Radočaj, O., V. Vujasinović, E. Dimić, and Z. Basić, *Blackberry (Rubus fruticosusL.) and raspberry (Rubus idaeusL.) seed oils extracted from dried press pomace after longterm frozen storage of berries can be used as functional food ingredients*. Eur. J. Lipid Sci. Technol., 2014: p. 1015-1024.
150. Kovacevic, A., S. Savic, G. Vuleta, R.H. Muller, and C.M. Keck, *Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): effects on size, physical stability and particle matrix structure*. Int J Pharm, 2011. **406**(1-2): p. 163-72.
151. *ISO 5508:1999 Animal and vegetable fats and oils. Analysis by gas chromatography of methyl esters of fatty acids*. 1999.
152. Ferreira, B.S., C.G. de Almeida, L.P. Faza, A. de Almeida, C.G. Diniz, V.L. da Silva, R.M. Grazul, and M. Le Hyaric, *Comparative properties of Amazonian oils obtained by different extraction methods*. Molecules, 2011. **16**(7): p. 5875-85.
153. Siuciurovici, A., N. Vukelici, L. Ignjatovic, I. Brcieski, and D. Jovanovici, *Physical-chemical characteristics and oxidative stability of oil obtained from lyophilized raspberry seed*. Eur. J. Lipid Sci. Technol., 2009. **111**(11): p. 1133-1141.
154. Glampedaki, P. and V. Dutschk, *Stability studies of cosmetic emulsions prepared from natural products such as wine, grape seed oil and mastic resin*. Coll. Surf., A, 2014. **460**: p. 306-311.
155. Caili, F., S. Huan, and L. Quanhong, *A review on pharmacological activities and utilization technologies of pumpkin*. Plant Food Hum Nutr, 2006. **61**(2): p. 70-77.
156. Szeląg, H. and B. Pauzder, *Rheological properties of emulsions stabilized by acylglycerol emulsifiers modified with sodium carboxylates*. Coll. Surf. A., 2003. **219**(1-3): p. 87-95.
157. Szelag, H. and A. Macierzanka, *Synthesis of modified acylglycerol emulsifiers in the presence of Na, K and Zn fatty acid carboxylates*. Tenside, surfactants, detergents, 2001. **38**(6): p. 377-382.
158. Szeląg, H. and W. Zwierzykowski, *Surface activity of acylglycerol emulsifiers modified with carboxylates*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2000. **164**(1): p. 63-69.
159. Szeląg, H. and B. Pauzder, *Rheological properties of emulsions stabilized by acylglycerol emulsifiers modified with sodium carboxylates*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2003. **219**(1): p. 87-95.
160. Macierzanka, A., H. Szeląg, P. Szumała, R. Pawłowicz, A.R. Mackie, and M.J. Ridout, *Effect of crystalline emulsifier composition on structural transformations of water-in-oil emulsions: Emulsification and quiescent conditions*. Coll. Surf. A, 2009. **334**(1-3): p. 40-52.
161. Griffin, W.C., *Classification of surface-active agents by HLB*. J Soc Cosmetic Chemists 1946. **1** p. 311-326.
162. Celia, C., E. Trapasso, D. Cosco, D. Paolino, and M. Fresta, *Turbiscan lab expert analysis of the stability of ethosomes and ultradeformable liposomes containing a bilayer fluidizing agent*. Coll. Surf B. Biointerfaces, 2009. **72**(1): p. 155-60.
163. Pal, R., *Rheology of simple and multiple emulsions*. Curr. Opin. Colloid Interface Sci., 2011. **16**(1): p. 41-60.
164. Dooley, L.M., K. Adhikari, and E. Chambers Iv, *A General Lexicon for Sensory Analysis of Texture and Appearance of Lip Products*. J. Sens. Stud., 2009. **24**(4): p. 581-600.
165. Parente, M.E., G. Ares, and A.V. Manzoni, *Application of Two Consumer Profiling Techniques to Cosmetic Emulsions*. J. Sens. Stud, 2010. **25**(5): p. 685-705.

166. Kanouni, M., H.L. Rosano, and N. Naouli, *Preparation of a stable double emulsion (W/O/W): role of the interfacial films on the stability of the system*. *Adv. Colloid Interface Sci.*, 2002. **99** (229): p. 26.
167. Yeon Kim, H. and J.-E. Chung, *Consumer purchase intention for organic personal care products*. *J Consum Mark*, 2011. **28**(1): p. 40-47.
168. Krasodomska, O., P. Paolicelli, S. Cesa, M.A. Casadei, and C. Jungnickel, *Protection and viability of fruit seeds oils by nanostructured lipid carrier (NLC) nanosuspensions*. *Journal of Colloid and Interface Science*, 2016. **479**: p. 25-33.
169. Negro, C., L. Tommasi, and A. Miceli, *Phenolic compounds and antioxidant activity from red grape marc extracts*. *Bioresource Technology*, 2003. **87**(1): p. 41-44.
170. Oksana, S., B. Marian, R. Mahendra, and S.H. Bo, *Plant phenolic compounds for food, pharmaceutical and cosmetics production*.
171. Duchêne, D., A. Bochot, S.-C. Yu, C. Pépin, and M. Seiller, *Cyclodextrins and emulsions*. *International Journal of Pharmaceutics*, 2003. **266**(1-2): p. 85-90.
172. Centini, M., M. Maggiore, M. Casolaro, M. Andreassi, R. Maffei Facino, and C. Anselmi, *Cyclodextrins as cosmetic delivery systems*. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 2007. **57**(1-4): p. 109-112.
173. Le Broulais, C., L. Treupel-Acar, C. Rhodes, P. Sado, and R. Leverage, *New ophthalmic drug delivery systems*. *Drug development and industrial pharmacy*, 1995. **21**(1): p. 19-59.
174. Kumar, S., B.O. Haglund, and K.J. Himmelstein, *In situ-forming gels for ophthalmic drug delivery*. *Journal of Ocular Pharmacology and Therapeutics*, 1994. **10**(1): p. 47-56.
175. Fundarò, A., R. Cavalli, A. Bargoni, D. Vighetto, G.P. Zara, and M.R. Gasco, *Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after iv administration to rats*. *Pharmacological Research*, 2000. **42**(4): p. 337-343.
176. Blasi, P., S. Giovagnoli, A. Schoubben, M. Ricci, and C. Rossi, *Solid lipid nanoparticles for targeted brain drug delivery*. *Advanced drug delivery reviews*, 2007. **59**(6): p. 454-477.
177. Muller, R.H., R.D. Petersen, A. Hommoss, and J. Pardeike, *Nanostructured lipid carriers (NLC) in cosmetic dermal products*. *Adv Drug Deliv Rev*, 2007. **59**(6): p. 522-30.
178. Garti, N., *Double emulsions—scope, limitations and new achievements*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 1997. **123**: p. 233-246.
179. Dluska, E. and A. Markowska, *One-step preparation method of multiple emulsions entrapping reactive agent in the liquid–liquid Couette–Taylor flow*. *Chemical Engineering and Processing: Process Intensification*, 2009. **48**(1): p. 438-445.
180. Jennings, V., A.F. Thünemann, and S.H. Gohla, *Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids*. *International Journal of Pharmaceutics*, 2000. **199**(2): p. 167-177.
181. Saupe, A., S.A. Wissing, A. Lenk, C. Schmidt, and R.H. Müller, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)—structural investigations on two different carrier systems*. *Bio-medical materials and engineering*, 2004. **15**(5): p. 393-402.
182. Severino, P., T. Andreani, A.S. Macedo, J.F. Fangueiro, M.H. Santana, A.M. Silva, and E.B. Souto, *Current State-of-Art and New Trends on Lipid Nanoparticles (SLN and NLC) for Oral Drug Delivery*. *J Drug Deliv*, 2012. **2012**: p. 750891.
183. Fang, J.Y., C.L. Fang, C.H. Liu, and Y.H. Su, *Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC)*. *Eur J Pharm Biopharm*, 2008. **70**(2): p. 633-40.
184. Hou, D., C. Xie, K. Huang, and C. Zhu, *The production and characteristics of solid lipid nanoparticles (SLNs)*. *Biomaterials*, 2003. **24**(10): p. 1781-1785.

185. Müller, R., S. Maaßen, H. Weyhers, F. Specht, and J. Lucks, *Cytotoxicity of magnetite-loaded polylactide, polylactide/glycolide particles and solid lipid nanoparticles*. International journal of pharmaceutics, 1996. **138**(1): p. 85-94.
186. Schafer-Korting, M., W. Mehnert, and H.C. Korting, *Lipid nanoparticles for improved topical application of drugs for skin diseases*. Adv Drug Deliv Rev, 2007. **59**(6): p. 427-43.
187. Joshi, M.D. and R.H. Muller, *Lipid nanoparticles for parenteral delivery of actives*. Eur J Pharm Biopharm, 2009. **71**(2): p. 161-72.
188. Muchow, M., P. Maincent, and R.H. Muller, *Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery*. Drug Dev Ind Pharm, 2008. **34**(12): p. 1394-405.
189. Rieger, M., *Skin, water and moisturization*. Cosmetics and toiletries, 1989. **104**(12): p. 41-51.
190. López-García, R. and A. Ganem-Rondero, *Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC): Occlusive Effect and Penetration Enhancement Ability*. Journal of Cosmetics, Dermatological Sciences and Applications, 2015. **5**(02): p. 62.
191. Müller, R., K. Mäder, and S. Wissing, *Mittel mit UV-Strahlung absorbierender und/oder reflektierender Wirkung zum Schutz vor gesundheitsschädlicher UV-Strahlung und Stärkung der natürlichen Hautbarriere*. vol. Deutsche Patentanmeldung, 2000(199): p. 32.
192. Nikolić, S., C. Keck, C. Anselmi, and R. Müller, *Skin photoprotection improvement: synergistic interaction between lipid nanoparticles and organic UV filters*. International journal of pharmaceutics, 2011. **414**(1): p. 276-284.
193. Chen, P.C., J.-W. Huang, and J. Pang, *An Investigation of Optimum NLC-Sunscreen Formulation Using Taguchi Analysis*. Journal of Nanomaterials, 2013. **2013**: p. 1-10.
194. Kheradmandnia, S., E. Vasheghani-Farahani, M. Nosrati, and F. Atyabi, *Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax*. Nanomedicine: Nanotechnology, Biology and Medicine, 2010. **6**(6): p. 753-759.
195. Pardeike, J., K. Schwabe, and R.H. Müller, *Influence of nanostructured lipid carriers (NLC) on the physical properties of the Cutanova Nanorepair Q10 cream and the in vivo skin hydration effect*. International journal of pharmaceutics, 2010. **396**(1): p. 166-173.
196. Hoppe, U., J. Bergemann, W. Diembeck, J. Ennen, S. Gohla, I. Harris, J. Jacob, J. Kielholz, W. Mei, and D. Pollet, *Coenzyme Q 10, a cutaneous antioxidant and energizer*. Biofactors-Oxford Then Amsterdam-, 1999. **9**: p. 371-378.
197. Üner, M., S. Wissing, G. Yener, and R. Müller, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for application of ascorbyl palmitate*. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2005. **60**(8): p. 577-582.
198. Hu, F.-Q., S.-P. Jiang, Y.-Z. Du, H. Yuan, Y.-Q. Ye, and S. Zeng, *Preparation and characteristics of monostearin nanostructured lipid carriers*. International journal of pharmaceutics, 2006. **314**(1): p. 83-89.
199. *Fruit market in Poland*. 2014, Agencja Rynku Rolnego. p. 1-28.
200. Al-Waili, N.S., *Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially controlled, single-blinded study*. Complementary therapies in medicine, 2003. **11**(4): p. 226-234.
201. Jores, K., W. Mehnert, M. Drechsler, H. Bunjes, C. Johann, and K. Mäder, *Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy*. Journal of Controlled Release, 2004. **95**(2): p. 217-227.
202. Golmohammadzadeh, S., M. Mokhtari, and M.R. Jaafari, *Preparation, characterization and evaluation of moisturizing and UV protecting effects of topical solid lipid nanoparticles*. Brazilian Journal of Pharmaceutical Sciences, 2012. **48**(4): p. 683-690.
203. Mehnert, W. and K. Mäder, *Solid lipid nanoparticles: production, characterization and applications*. Advanced drug delivery reviews, 2001. **47**(2): p. 165-196.

204. Buchwald, R., M.D. Breed, and A.R. Greenberg, *The thermal properties of beeswaxes: unexpected findings*. Journal of Experimental Biology, 2008. **211**(1): p. 121-127.
205. Souto, E. and R. Müller, *Cosmetic features and applications of lipid nanoparticles (SLN®, NLC®)*. International Journal of Cosmetic Science, 2008. **30**(3): p. 157-165.
206. Loo, C., M. Basri, R. Ismail, H. Lau, B. Tejo, M. Kanthimathi, H. Hassan, and Y. Choo, *Effect of compositions in nanostructured lipid carriers (NLC) on skin hydration and occlusion*. Int J Nanomedicine, 2013. **8**: p. 13-22.
207. Shah, R.M., G. Bryant, M. Taylor, D.S. Eldridge, E.A. Palombo, and I.H. Harding, *Structure of solid lipid nanoparticles produced by a microwave-assisted microemulsion technique*. RSC Advances, 2016. **6**(43): p. 36803-36810.
208. Kasongo, K.W., R. Shegokar, R.H. Muller, and R.B. Walker, *Formulation development and in vitro evaluation of didanosine-loaded nanostructured lipid carriers for the potential treatment of AIDS dementia complex*. Drug Dev Ind Pharm, 2011. **37**(4): p. 396-407.
209. Jain, K., S. Sood, and K. Gowthamarajan, *Optimization of artemether-loaded NLC for intranasal delivery using central composite design*. Drug delivery, 2014(0): p. 1-15.
210. Teeranachaideekul, V., P. Boonme, E.B. Souto, R.H. Müller, and V.B. Junyaprasert, *Influence of oil content on physicochemical properties and skin distribution of Nile red-loaded NLC*. Journal of Controlled Release, 2008. **128**(2): p. 134-141.
211. Casadei, M.A., F. Cerreto, S. Cesa, M. Giannuzzo, M. Feeney, C. Marianecchi, and P. Paolicelli, *Solid lipid nanoparticles incorporated in dextran hydrogels: a new drug delivery system for oral formulations*. International journal of pharmaceutics, 2006. **325**(1): p. 140-146.
212. Zimmermann, E., E. Souto, and R. Müller, *Physicochemical investigations on the structure of drug-free and drug-loaded solid lipid nanoparticles (SLNTM) by means of DSC and 1H NMR*. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 2005. **60**(7): p. 508-513.
213. Jennings, V., K. Mäder, and S.H. Gohla, *Solid lipid nanoparticles (SLN™) based on binary mixtures of liquid and solid lipids: a 1 H-NMR study*. International journal of pharmaceutics, 2000. **205**(1): p. 15-21.
214. Teeranachaideekul, V., E.B. Souto, R.H. Müller, and V.B. Junyaprasert, *Physicochemical characterization and in vitro release studies of ascorbyl palmitate-loaded semi-solid nanostructured lipid carriers (NLC gels)*. Journal of microencapsulation, 2008. **25**(2): p. 111-120.
215. Venkateswarlu, V. and K. Manjunath, *Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles*. J Control Release, 2004. **95**(3): p. 627-38.
216. Tiyaboonchai, W., W. Tungpradit, and P. Plianbangchang, *Formulation and characterization of curcuminoids loaded solid lipid nanoparticles*. Int J Pharm, 2007. **337**(1-2): p. 299-306.
217. Kumar, V.V., D. Chandrasekar, S. Ramakrishna, V. Kishan, Y.M. Rao, and P.V. Diwan, *Development and evaluation of nitrendipine loaded solid lipid nanoparticles: influence of wax and glyceride lipids on plasma pharmacokinetics*. Int J Pharm, 2007. **335**(1-2): p. 167-75.
218. Cortés-Rojas, D.F., C.R. Souza, and W.P. Oliveira, *Encapsulation of eugenol rich clove extract in solid lipid carriers*. Journal of Food Engineering, 2014. **127**: p. 34-42.
219. Neupane, Y.R., M. Sabir, N. Ahmad, M. Ali, and K. Kohli, *Lipid drug conjugate nanoparticle as a novel lipid nanocarrier for the oral delivery of decitabine: ex vivo gut permeation studies*. Nanotechnology, 2013. **24**(41): p. 415102.
220. Desai, P.R., P.P. Shah, P. Hayden, and M. Singh, *Investigation of follicular and non-follicular pathways for polyarginine and oleic acid-modified nanoparticles*. Pharmaceutical research, 2013. **30**(4): p. 1037-1049.
221. Heinrich, U., U. Koop, M.C. Leneveu-Duchemin, K. Osterrieder, S. Bielfeldt, C. Chkarnat, J. Degwert, D. Häntschel, S. Jaspers, and H.P. Nissen, *Multicentre comparison of skin hydration in terms of physical-, physiological-and product-dependent parameters by the capacitive*

- method (Corneometer CM 825)*. International journal of cosmetic science, 2003. **25**(1-2): p. 45-53.
222. Barel, A. and P. Clarys, *Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments: Evaporimeter® and Tewameter®*. Skin pharmacology and physiology, 1995. **8**(4): p. 186-195.
223. Ohshima, H., S. Kinoshita, M. Oyobikawa, M. Futagawa, H. Takiwaki, A. Ishiko, and H. Kanto, *Use of Cutometer area parameters in evaluating age-related changes in the skin elasticity of the cheek*. Skin Research and Technology, 2013. **19**(1): p. e238-e242.