Phase Inversion in n-undecane/water Emulsions

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Diploma thesis
2013

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Univerzita Tomáše Bati ve Zlíně
Fakulta technologická
Ústav technologie tuků, tenzidů a kosmetiky
akademický rok: 2012/2013

ZADÁNÍ DIPLOMOVÉ PRÁCE
(PROJEKTU, UMĚLECKÉHO DÍLA, UMĚLECKÉHO VÝKONU)

Jméno a příjmení:	**Bc. Jiří POHLODEK**
Osobní číslo:	**T11148**
Studijní program:	**N2901 Chemie a technologie potravin**
Studijní obor:	**Technologie a ekonomika výroby tuků, detergentů a kosmetiky**
Forma studia:	**prezenční**
Téma práce:	**Fázová inverze v emulzích n-undekan/voda**

Zásady pro vypracování:

I. Teoretická část

1. **V teoretické části vypracujte literární rešerši, ve které se věnujte emulzím a nanoemulzím, jejich typickým vlastnostem a stabilitě. Popište jejich přípravu a zaměřte se na metodu fázové inverze. Uveďte postupy vhodné pro stanovení teploty fázové inverze u neionických surfaktantů.**

II. Praktická část

1. **V praktické části se věnujte nalezení teploty fázové inverze vybraných neionických surfaktantů a jejich směsí konduktometrickou metodou. Získaná data využijte při přípravě nanoemulzí O/V v systému n-undekan/voda metodou PIT. Postup prakticky ověřte stanovením velikosti částic připravených nanoemulzí pomocí Fotonové korelační spektroskopie. Získané výsledky zpracujte, přehledně uspořádejte a diskutujte.**

Rozsah diplomové práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná/elektronická**

Seznam odborné literatury:

[1] HIEMENZ, P. C., RAJAGOPALAN, R. Principles of Colloid and Surface Chemistry. 3rd Edition, Boca Raton FL, Taylor and Francis, 1997. 650 p. ISBN 0-8247-9397-8.
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Vedoucí diplomové práce:	**doc. Ing. Věra Kašpárková, CSc.** Ústav technologie tuků, tenzidů a kosmetiky
Datum zadání diplomové práce:	**11. února 2013**
Termín odevzdání diplomové práce:	**22. května 2013**

Ve Zlíně dne 11. února 2013

L.S.

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ABSTRAKT

Diplomová práce se zabývá přípravou nanoemulzí s využitím metody fázové inverze PIT a sledováním vlivu jednotlivých faktorů na jejich stabilitu. V práci byla konduktometricky stanovena charakteristická teplota fázové inverse (PIT). Zjištěné hodnoty PIT byly využity pro přípravu nanoemulzí typu O/V. Emulze byly připraveny se surfaktanty o HLB v rozsahu 9.7 – 11.5 s použitím kombinace Brij 30 + Brij 56 a Brij 30 + Brij 98. V připravených nanoemulzích byla měřena velikost částic pomocí fotonové korelační spektroskopie a sledována jejich stabilita při teplotách 4 °C, 25 °C a 35 °C. Průběžně byla prováděna vizuální kontrola rozpadu nanoemulzí a měření velikosti částic. Práce prokázala přímou souvislost mezi velikostí částic a složením emulze, tedy obsahem a typem surfaktantu. Rovněž byl prokázán vliv množství surfaktantu, poměru olej/voda a teploty skladování na stabilitu emulze.

Klíčová slova: Nanoemulze, Surfaktant, Nizko-energetická metoda, Emulgace, Teplota fázové inverze, fázové chování

ABSTRACT

The thesis deals with the preparation of nanoemulsions using the phase inversion method (PIT) and with the observation of the effect of individual factors on their stability. In the thesis, the characteristic phase inversion temperatures (PIT) were determined using conductivity measurements. The obtained PIT values were applied for the preparation of O/W nanoemulsions. The nanoemulsions were prepared within 9.7 – 11.5 HLB range, by using the combination of Brij 30 + Brij 56 and Brij 30 + Brij 98 surfactants. The particle size of prepared nanoemulsions was measured by photon correlation spectroscopy and their stability was determined at storage temperatures of 4 °C, 25 °C and 35 °C. The on-going visual inspection and particle size measurements were carried during the process. The results proved the direct correlations between particle size and emulsion composition in terms of amount and type of surfactants. The effect of surfactant concentration, oil-to-water ratio and storage temperature on the stability of the nanoemulsion was proven.

Keywords: Nanoemulsion, Surfactant, Low-energy method, Emulsification, Phase Inversion Temperature, Phase Behavior.

ACKNOWLEDGEMENTS

I would like to express my gratitude to doc. Ing. Věra Kašpárková, CSc. whose help, stimulating suggestions and encouragement helped me all the time of research and writing of this thesis.

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

CONTENTS

INTRODUCTION

Emulsions have experienced rapidly increasing practical applications in last decades. They became an integral part of chemical, cosmetic, agricultural, pharmaceutical, food, and other industries. With increasing use of emulsions in various sectors, there is no wonder that they are gaining growing interests among common people as well as among scientists. The research is mainly focused on the possibility of the future use of emulsions, on their methods of preparation, as well on their characteristic properties.

Nanoemulsions became one of the very important representatives of the emulsions. Due to their specific properties, the nanoemulsions are noting an increasing usage in many areas. They are transparent or translucent to the naked eye even at high droplet volume fraction and possess stability against sedimentation and creaming. Despite their metastability, nanoemulsions can persist over many months or years due to the presence of a stabilizing surfactant that inhibits the coalescence of the droplets.

The theoretical part of the thesis is aimed at emulsions. It describes their classification, possibilities of their preparation as well as their breakdown and destabilization. Later on, the thesis is aimed at nanoemulsions, and it investigates their characteristics, possibilities of their preparation, and their stabilization. The major part of the thesis will outline the preparation of nanoemulsions by using phase inversion method. Finally, practical applications of nanoemulsions are mentioned.

The experimental part of the thesis deals with the determination of phase inverse temperature (PIT) that is suitable for the preparation of nanoemulsions. The PIT was determined by conductometric measurements and the results were processed statistically. The gathered PIT values were used in preparation of the nanoemulsions using phase inversion method, while using different types of non-ionic surfactant. The influence of various factors on stability of emulsions was observed. The stability of the nanoemulsions, as well as the change in their particle size was followed visually and monitored using photon correlation spectroscopy.

Conclusions regarding the impact of surfactant type and concentration, O/W ratio, time and storage temperature on the stability of the nanoemulsions are drawn and provided.

I. THEORY

1 EMULSIONS

The term emulsion refers to a system where one fluid is dispersed in another and the fluids are not miscible in each other. Emulsions are thermodynamically or kinetically stable. Sometimes they are also defined as heterogeneous dispersions of liquid in the liquid environment. In other words, they are part of a more general class of two-phase systems, where one liquid (the dispersed phase) is dispersed in the other (the continuous phase).

The formation of emulsions requires similar conditions as for the formation of other lyophobic sols. The fluids, which together form the emulsion, must be mutually immiscible, or only partly miscible. To separate the phases, the dispersed liquid is stabilized by the inter-facial film of surfactant molecules capable of preventing the droplet coalescence. The droplet size (0.1 - 10 mm) in emulsions often exceeds colloidal dimensions. Conversely, films between drops can be of colloidal dimensions [1].

The physiochemical properties of an emulsion are defined by its structure, type, concentration as well as by the interactions between its components. [1] The droplet interactions are responsible for the rheology, stability, appearance, and other characteristics of the emulsion. If the temperature, the pH or the surfactant concentration change, the properties of the emulsion can change immensely. [2] Therefore, the important characteristics of an emulsion are governed by: the present surfactant, the type of emulsion and its stability and the equipment used for its production.

Nowadays, the emulsions belong to one of the most intensively studied systems and are widely used in many industrial sectors such as pharmaceutical, food, cosmetics industry, in manufacturing and processing of rubber, macromolecular compounds, or in the manufacture of lubricants and paints. In some industries, emulsion may be unwelcome phenomenon, for example during oil refining, as they make the separation of liquid phases more difficult and easily increase the viscosity of the system.

1.1 Classification of Emulsions

Structure of emulsion is influenced by the volume ratio of the dispersed phase, the type of surfactant, and its concentration.

The most important characteristic of the surfactant is its solubility (in both phases) or in the case of the solid stabilizers, the wetting behavior. Emulsions can be classified according to different criteria provided below.

1.1.1 Classification according to the polarity of dispersed and continuous phase

1.1.1.1 Direct Emulsion

It is known as the O/W (oil in water) emulsion, in which a polar liquid, usually water or an aqueous solution, is the dispersion medium. Oil phase (dispersed phase) is of nonpolar character, regardless of whether or not it has an oily consistency.

1.1.1.2 Inverted Emulsion

Conversely, where water or aqueous solutions are dispersed in a less polar oil phase, the system is assigned as water-in-oil (W/O) emulsion.

The type of emulsion can be determined using different procedures listed below:

- according to the electrical conductivity (conductivity is governed by the dispersion medium and O/W emulsion are much more conductive than the W/O emulsion);

- according to their ability to dissolve polar or non-polar dyes;
- according to the ability to mix with polar or nonpolar solvent (emulsion may be diluted with a liquid which is mixed with the dispersion medium);
- by observing under UV light (fluoresce of oil phase);
- according to the wettability (emulsion O/W wets the hydrophilic surface, emulsion W/O not and vice versa).

Basically, it is possible to prepare emulsions of O/O (oil in oil), however, this possibility is limited by the miscibility of organic liquids and also by the fact that there are only a few substances, which are the surfactants at the interface of two organic liquids, and can thus act as stabilizers of emulsions.

1.1.2 Classification of Emulsions according to the concentration of dispersed phase

1.1.2.1 *Diluted Emulsions*

In diluted emulsions, dispersed phase occupies up to 2 % of the total emulsion volume. The diameter of droplets is typically 100 nm, thus close to the size of colloidal particles.

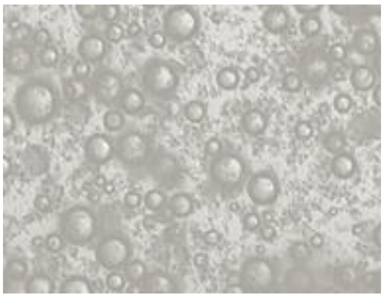

Figure 1: Diluted Emulsion. [3]

1.1.2.2 *Concentrated Emulsions*

In concentrated emulsions, the dispersed phase is formed by undeformed spherical droplets. In mono-disperse systems their concentration can be up to 74 % of total emulsion volume. This corresponds to the tightest geometric arrangement of the spherical particles.

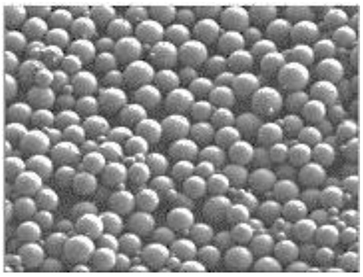

Figure 2: Concentrated Emulsion. [4]

1.1.2.3 *Polydisperse Emulsions*

Typically, emulsions are polydisperse systems, which means that the diameter of the droplets varies from droplet to droplet. Polydisperse emulsions can be characterized by small droplets that fill in spaces between the large droplets. These emulsions can be prepared even more concentrated.

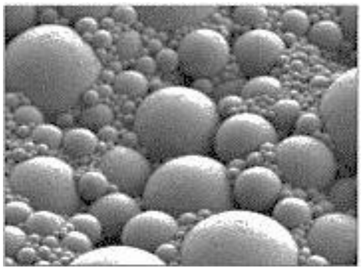

Figure 3: Polydispersed Emulsion. [4]

1.1.2.4 *Gel Emulsions*

Gel emulsions refer to highly concentrated emulsions, where the particles of the dispersed phase are located so close that they are mutually deformed. They shape in polyhedrons, which are separated by the thin surfactant films of colloidal dimensions.

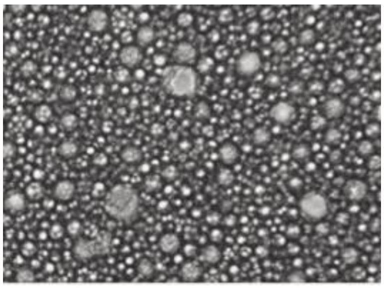

Figure 4: Gel Emulsion. [5]

1.1.2.5 *Multiple emulsions*

Multiple emulsions are a special type of water-in-oil-in-water emulsions (W/O/W). They consist of dispersed oily globules containing smaller aqueous droplets. Generally, multiple emulsions are prepared with two surfactants of opposite solubility. To produce a W/O/W emulsion, a low hydrophilic-lipophilic balance (HLB) (<10) surfactant is first dissolved in oil. Consequently, water is added and a W/O emulsion is formed. The system is then emulsified again in an aqueous solution with surfactant of a high HLB number (>10) to produce a W/O/W double emulsion. Both surfactants mix at the water/oil interfaces, and the stability of the films is governed by the composition of the binary surfactant mixture [6].

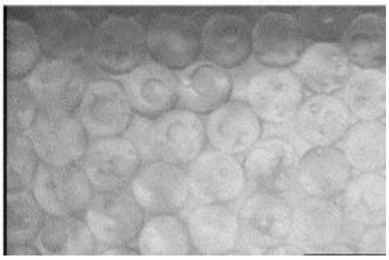

Figure 5: Multiple W/O/W Emulsion. [7]

1.2 Properties of Emulsions

The color and appearance of emulsions depend on the concentration, particle size of the dispersed phase, and the refractive index of the two liquid phases involved. The emulsions are generally turbid, concentrated emulsions are opaque. Only in case of identical or very similar refractive indexes of the two phases, the emulsions are translucent.

Properties of dilute emulsions are similar to those of dilute sols. Particles of O/W type emulsions carry the negative electric charge, appear electrophoretic mobility, and can coagulate those electrolytes whose coagulation ability increases with cations valence.

Sedimentation of the dispersed phase depends on the density ratio of the two fluids. Dependent on the density, the downward or upward sedimentations may occur, characterized by the presence of the concentrated disperse phase on the bottom and the surface of the emulsion, respectively. Highly concentrated gel or emulsion is not a result of the tight deposit of particles capable of sedimentation, and exhibit some of the same mechanical properties as a gel (e.g. can be cut with a knife).

The viscosity of diluted emulsions is often governed by Einstein's equation and diluted emulsions behave as newtonian liquid. The viscosity of concentrated emulsions depends much more on the ratio of the volumes of the two liquid phases than on the viscosity of the dispersion medium, and also the viscosity of emulsifier is applied here. These systems usually exhibit "non-newtonian" behavior.

1.3 Emulsion break-down processes

There are four basic ways of emulsions instability. Each of these ways leads to the loss of homogeneity in oil droplets distribution.

1.3.1 De-emulsification

De-emulsification is a process in which an emulsion is permanently broken down into its constituents. It can be induced by different techniques provided below:

- Chemical de-emulsification, i.e. change the HLB;
- Add an emulsifier of opposite type;
- Add agent of opposite charge;
- Freeze-thaw cycles;
- Add electrolyte. Change the pH;
- Raise temperature;
- Apply electric field;
- Filter through fritted glass or fibers;
- Centrifugation.

The various break-down processes are known and are described n te following part of the thesis.

1.3.2 Flocculation

The hydrophilic-hydrophobic interactions result in attractive forces between dispersed droplets. Flocculation refers to a process by which individual droplets cluster together into flocks without rupture of the stabilizing layer at the interface. The flocks act as larger drops. Therefore the oil drops are no longer evenly distributed through the water. The flocks may then easily float to the top or settle to the bottom of the liquid phase.

1.3.3 Coalescence

Flocculation could lead to coalescence. Instability in a disperse system occurs when attractive forces between molecules in the individual phases exceed attractive forces between molecules in the opposite phases [8], the droplets merge and form a larger droplet, so the average droplet size increases over time.

1.3.4 Creaming

Most oils possess lower density than water and oil droplets will therefore float to the top. However, the drops will not necessarily coalesce. [9] Creaming is the principal process by which the disperse phase separates from an emulsion and is typically the precursor to coalescence

1.3.5 Sedimentation

The phenomenon opposite to creaming is called sedimentation. During sedimentation, oil droplets concentrate, due to higher density, in the bottom of the vessel. The settling (or creaming) rate for disperse phases can be estimated from the Stokes' equation:

$$v_s = \frac{d^2 \Delta\rho g}{18\eta_c}$$

d	Droplet diameter
g	Acceleration due to gravity
$\Delta\rho$	Density difference between dispersed and continuous phase
ηc	Continuous phase viscosity
v_S	Stokes velocity

Breaking

Due to combination of creaming and coalescence, the oil phase can completely separate from the water phase and settle out at the top of the emulsion in a single, continuous layer.

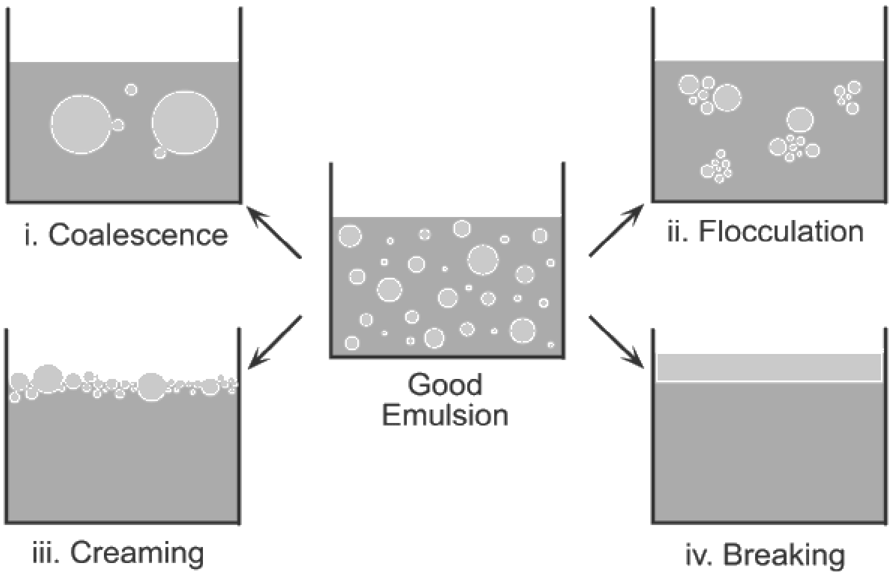

Figure 6: The destabilization of emulsions. [10]

Figure 6 shows that flocculation and creaming leave the fine oil droplets intact while making them less distributed throughout the continuous water phase. These two processes can be reversed by putting in a small amount of energy (for example shaking or moderate stirring). Brownian motion, within the water phase, can provide enough energy to keep exceptionally small droplets agitated and hence creaming is less likely to happen with fine emulsions. [10]

1.3.6 Ostwald ripening

Ostwald ripening is the process causing large droplets growing at the expense of small ones. As larger droplets have a lower surface to volume ratio than the smaller ones, Ostwald ripening occurs with a reduction in interfacial energy and therefore is energetically favourable. This is the main breaking-down process for nanoemulsions. In order to avoid this process, the emulsion droplets should be as monodisperse as possible.

1.4 Stability of Emulsions

The stability of emulsions is one of the most important indicators of their use. It is mainly influenced by the type and amount of surfactant, ratio of disperse phase and disperse medium, the emulsion type, viscosity of the external phase and the method of emulsion preparation.

Similarly to all lyophobic colloids and disperse systems, emulsions are also unstable. To ensure sufficient shelf life, the emulsions have to be stabilized. One of the possibilities is their stabilization through the electric double layer, which comes into consideration only for diluted emulsions where collisions are relatively rare. Solubility of cations and anions differs in the aqueous and oil phase. Anions are generally more soluble in the oil, cations in the aqueous phase. That is why the droplets in O/W emulsions tend to have a negative charge.

Generally, the greater the thickness of the bi-layer and the higher charge of the droplets, the more effectively the repulsive forces between the droplets prevent the coalescence. The effect of the electrolyte concentration on these two variables is, however, counter-going, the charge of a droplet increases with increasing electrolyte concentration, whereas the bi-layer thickness decreases with increasing electrolyte concentration.

1.4.1 Surfactant

Another possibility how to stabilize emulsions is use of surfactants. The term surfactant is in fact abbreviation for “surface active agents” and describes molecules affecting the surface energy of the oil/water interface. The surfactant molecule possesses amphiphilic character, which means that it contains both hydrophobic (non-polar) and hydrophilic (polar) groups. This is shown in Figure 7.

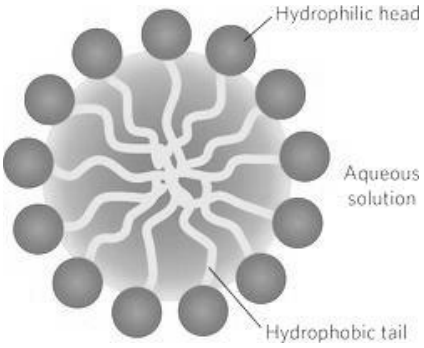

Figure 7: The action of surfactants on the oil/water interface. [11]

Surfactants increase the stability of emulsions as well as make them substantially easier to prepare, as they can act as a ‘bridge’ between the oil droplets and the water, forming thus protective layer on the surface of the droplet, which reduces the surface energy of the interface and the amount of energy needed to form new oil/water surfaces.[10]

In other words, surfactant forms two mono-molecule layers that surround a slab of water. It is dispersed in water and adsorbed at the interface between air and water, or at the interface between oil and water, where water is mixed with oil. The water phase also contains free surfactant molecules that flow along with the water. Insoluble hydrophobic groups may extend from the water phase into the air or into the oil phase, while the water-soluble head group remains in the water phase. This layer prevents the coalescence of two droplets in case of thermal or sedimentation collision.

The surfactant must meet the following requirements:

- Accumulate at the interface of the two phases;
- Create a cohesive, elastic film on the interface not adhering to films formed around other droplets.

The surfactant prevents re-aggregation of the oil droplets, by binding to the oil-water interface. It prevents contact of oil droplets and the emulsion is thus more stable than in its absence.

The right choice of surfactant is an important factor for the preparation of emulsions. To the most frequently used surfactants belong:

- Micellar colloids (soaps, detergents);
- Molecular colloids (polysaccharides, proteins, polyvinyl alcohol);
- Solid, insoluble particles.

The stability of emulsion formed using a suitable surfactant is characterized by size of emulsion droplets (particles). The size and size distribution of particles are important characteristics providing the information needed for production, quality inspection and determination of emulsion stability over time.

Bancroft's Rule (1912)

The type of emulsion, i.e. oil in water or water in oil, is governed by the type of surfactant used. For emulsion formation, Banckroft rule applies saying that "The phase, in which the surfactant is most soluble, constitutes the continuous phase of the emulsion. This is shown in Figure 8 and 9.

Figure 8: O/W emulsion. [12]

The long tail on the surfactant is to represent the long-range interaction of a hydrophilic molecule through water, which is presented in Figure 9.

Figure 9: W/O emulsion. [12]

2 NANOEMULSIONS

It is very important to distinguish between emulsions, nanoemulsions and microemulsions since it influences the methods used to prepare them, the strategies used to stabilize them, and the approaches used to design their application. Nanoemulsions, in literature also called mini-emulsions, ultrafine emulsion, and sub-microne emulsion, have several physical properties that differentiate them from the other emulsion types. [13]

Microemulsions and nanoemulsions are distinctly different types of colloidal dispersions. Emulsions with droplet size in range of 50 – 200 nm are often referred to as nano-emulsions. [14] They are transparent or translucent to the naked eye even at high droplet volume fraction and possess stability against sedimentation and creaming. Emulsions of a size up to 500 nm are milky rather than transparent.

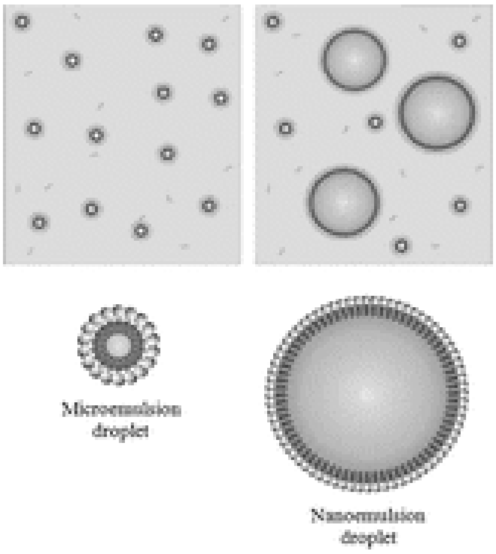

Figure 10: Microemulsion and Nanoemulsion Comparism. [15]

Compared to the microemulsions with a size ranging from 5–50 nm that are thermodynamically stable, nanoemulsions are only kinetically stable. Despite their metastability, nanoemulsions can persist over many months or years due to the presence of a stabilizing surfactant that inhibits the coalescence of the droplets. [15]

Depending on the composition and correspondinly to classical emulsions, three types of nanoemulsion are most likely to be formed. Oil-in-water type nanoemulsions, wherein oil droplets are dispersed in the continous water phase, have been subject of research for a long period of time and have been assessed in details contrary to water-in-oil nanoemulsions, the research of which is rather young. In third type, bi-continuos nanoemulsion, microdomains of oil and water are dispersed within the system. However, all three types of nanomulsions are of big scientific interest, which is reflected by the numerous publication, fundamental studies, patents and practical applications in chemical, pharmaceutical, cosmetic, and food industry.

Table 1: Comparison of emulsion types.

	Size [nm]	Thermodynamic stability	Appearance	Energy under preparation	Kinetic stability
Emulsions	500 or more	Unstable -results in phase separation	Milky white	Large energy input	Great stability
Nano emulsions	50-200	Stable-without phase separation	Transparent/ opaque	Energy input	Unstable
Micro emulsions	5-50	Stable	Clear	No energy input	Stable

2.1 Nanoemulsion formation

As nanoemulsions cannot be formed spontaneously, energy input, usually from the chemical components or some mechanical apparatus, is required. For preparation of nanoemulsions with the droplet size between 50 – 200 nm, three methods may be applied:

- Use of high pressure homogenizers (supported by using an appropriate surfactant);
- Use of low energy emulsification method at constant temperature (EIP method – Emulsion Inversion Point);
- Application of the phase inversion temperature concept (PIT).

2.1.1 Use of high energy emulsification methods

The formation of nanoemulsions by the so-called dispersion or high energy emulsification methods is generally accomplished by stirring, high pressure homogenizers and ultrasonic generators. It has been shown that the smallest size is achieved by using the device supplying the available energy in the shortest time and having the most homogeneous flow. [16] High pressure homogenizers are the most widely used emulsifying machines to prepare nanoemulsions. The conventional high pressure homogenizers work in the pressure range between 50 and 100 MPa. Pressure as high as 350 MPa has been achieved in a recently developed device, which was used for preparation of nanoemulsion. [17] However pressure during nanoemulsion production has to be adapted to the system under preparation. For example oil-in water nanoemulsion with methylcellulose as emulsifier could only be stabilized by selecting a homogenization pressure lower than 150 MPa because of a very strong elongation flow produced at higher pressures that promoted the irreversible degradation of low chain molecules.

The method of ultrasonic emulsification is efficient in reducing droplets size but it is only suitable for small batches [16]. A recent study on the preparation of polymerizable nano-emulsions [18] has shown that the efficiency of the dispersion process is highly dependent on the sonication time at different amplitudes. In general, the more hydrophobic the monomer is, the longer the sonication time is required. The process of ultrasonic emulsification is generally inefficient in nanoemulsion preparing, as the production of small droplets of submicrone size requires application of high energy. However, there are other methods which doesn´t require such a high energy input. The calculations below show that the mechanical energy required for emulsification exceeds the interfacial energy by several orders of magnitude.

Example:

To produce an emulsion with dispersed phase volume fraction of $\varphi=0,1$ with particle size of d = 0.6 μm using a surfactant that gives interfacial tension of $\gamma=10$ m.Nm^{-1}, the net increase in surface free energy is $A_\gamma = 6\varphi\gamma/d = 10^4$ J.m^{-3}, i.e. an efficiency of the process is 0.1%. The rest of the energy (99,9%) is transferred to heat. [19].

The intensity of the process or the effectiveness in formation of small droplets is often controlled by the net power density ($\varepsilon(t)$) defined as

$$p= \varepsilon(t).\ dt$$

where

t is the time during which the emulsification occurs
p is homogenizer pressure.

Droplet breaking will occur only at high ε values, which means that the energy dispersed at low ε levels is ineffectively wasted. Batch processes are generally less efficient than continuous processes. Using a stirrer in a large vessel, most of the energy applied at low intensity is dispersed as heat. [19, 20]

Several procedures may be applied to improve the efficacy of emulsification when producing nanoemulsions:

- Optimization of the stirring efficiency by increasing ε and decreasing time of emulsification. The emulsion is preferably prepared at high volume fraction of the disperse phase (φ) and diluted afterwards.
- Addition of more surfactant, which leads to formation a lower interfacial tension γ.
- Using of such surfactant mixture that results in more reduction in γ of the individual components. If possible, it is more efficient to disperse the surfactant in the dispersed phase rather that in the continuous phase, this often leads to smaller droplet size.
- It may be useful to emulsify in steps of increasing intensity, particularly with emulsions having highly viscose disperse phase.

2.1.2 Use of Low Energy Emulsification Methods

Low-energy methods use the internal chemical energy of the system to perform emulsification. When changes in the surfactant spontaneous curvature are produced during the emulsification process, they are usually called phase inversion methods. Phase inversion in emulsions can be of two types: catastrophic phase inversion (CPI) and transitional phase inversion (TPI). TPI enables the preparation of nanoemulsions and can be divided into two groups: 1) Phase Inversion Temperature method (PIT) and 2) Emulsion Inversion Point method (EIP). In these cases, emulsification is triggered by a change in temperature and composition, respectively.

2.1.3 Phase Inversion Temperature (PIT) Principle

The PIT method, belongs to the procedures frequently used in industry. For application of the PIT principle, the transitional inversion method is used, which has been demonstrated by Shinoda and coworkers [21,22] when using non-ionic surfactans of the ethoxylate type. These surfactants are highly dependent on temperature, becoming lipophilic with increasing temperature due to the dehydration of the polyethyleneoxide chain. [23] When an oil-in-water emulsion prepared using a non-ionic surfactant of the ethoxylate type is heated, then at a critical temperature (the phase interversion temperature - PIT), the emulsion inverts to a water-in-oil emulsion. At the PIT, the droplet size reaches a minimum and the interfacial tension reaches a minimum as well. However, the small droplets are unstable and they coalesce very rapidly. By rapid cooling of the emulsion that is prepared at a temperature near the PIT, very stable and small emulsion droplets could be produced. Parameter that has to be taken into account for the successful preparation of small nanoemulsions droplets is the rate of cooling. If the cooling is not fast enough, coalescence predominates and polydisperse coarse emulsions are formed instead of nanoemulsions.

A clear demonstration of the phase inversion that occurs on heating an emulsion is presented in a study dealing with the phase behavior of emulsions as a function of temperature. This is illustrated in Figure 11, which shows schematically what happens when the temperature is increased [24, 25]. Arrow in the picture presents increasing temperature.

At low temperature over the Winsor I region oil-water macroemulsions can be formed and are quite stable (Figure 11 a, b). When increasing the temperature, the oil-water emulsion stability decreases and macroemulsion finally resolves when the system reaches the Winsor III phase region (both oil-in-water and water-in-oil emulsions are unstable, Figure 11 c). At higher temperature, over the Winsor II region, water-oil emulsions became stable (Figure 11 d, e).

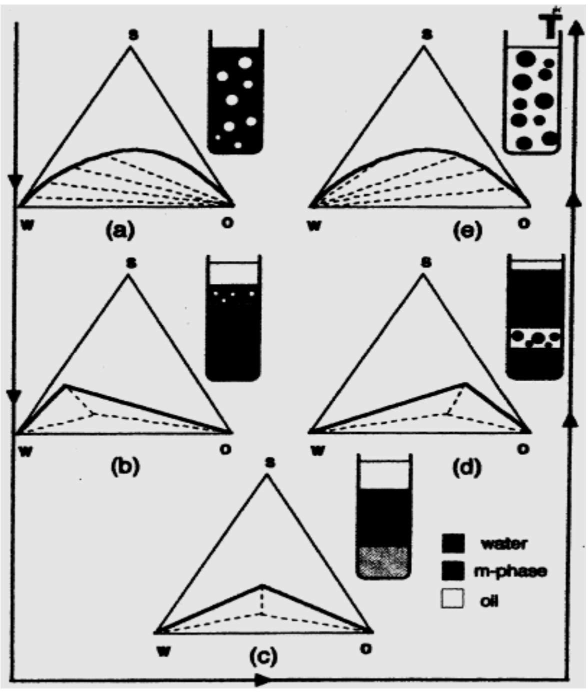

Figure 11: The PIT concept. [26]

2.1.4 **Emulsion Inversion Point method (EIP)**

Transitional Inversion can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures with different HLB values. This is illustrated in Figure 12 which shows the average droplet diameter and rate constant for attaining steady size as a function of the HLB number.

By adding for example water into oil-surfactant mixtures, water droplets are first formed in a continuous oil phase. When increasing the water volume, the spontaneous curvature of the surfactant changes from stabilizing a W/O emulsion to an O/W emulsion. At the EIP, the affinity of the surfactant toward oil and water phase is balanced and a nanoemulsion forms. The interfacial tension is minimal and the system goes through a point of zero spontaneous curvature [27].

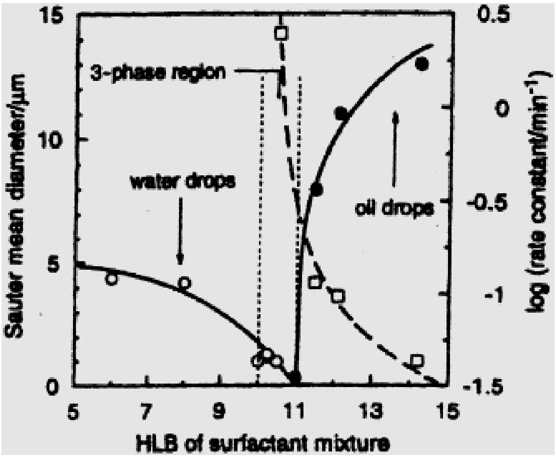

Figure 12: Emulsion droplet diameters (circles) and rate constant for attaining steady size (squares) as function of HLB-Cyclohexane/Nonylphenol Ethoxylate. [28]

A study of the phase behaviour of water-oil-surfactant systems showed that EIP can be carried out using three different procedures [29], as schematically shown in Fig 13.

- Stepwise addition of oil to a water surfactant mixture;
- Stepwise addition of water to a solution of the surfactant and oil;
- Mixing all the components in the final composition and pre-equilibrating the samples prior to emulsification.

In these studies, the system water/Brij 30 (polyoxyethlene lauryl ether with an average of 4 mol of ethylene oxide)/ decane was chosen as a model to obtain oil-in-water emulsions. The results showed that nano-emulsions with droplet sizes of 50 nm were formed only when water was added to mixtures of surfactant and oil (method b). [29]

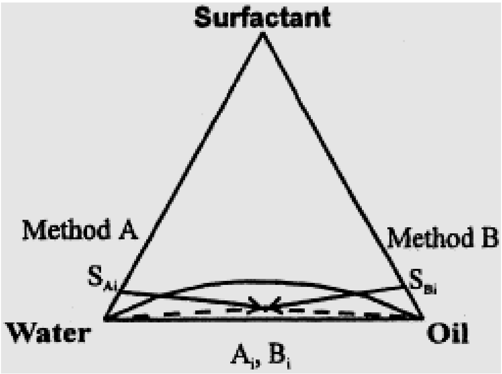

Figure 13: Schematic representation of the experimental path in two emulsification methods: Method A, addition of decane to water surfactant mixture, method B, addition of water to decane/Brij 30 solutions. [30]

2.2 Correlations of emulsion composition/properties with PIT

As the diploma thesis is dealing with nanoemulsion properties and preparation, this procedure is discussed in more details.

2.2.1 Determination of Phase Inversion temperature.

There are several ways how to determine PIT. One of the possibilities is usage of temperature-scanning conductivity, which is one of the most common ways of PIT determination. This procedure requires addition of small amount of electrolyte, usually NaCl into water phase. Another possibility is to determine PIT by microscopy measurements or through the monitoring of turbidity, including observation with the naked eye.

2.2.2 HLB Temperature

Figure 16 shows the graph of conductivity changes with temperature for emulsion with 20 % hexadecane and different concentrations of surfactant ($C_{12}E_4$). Regardless the concentration of surfactants, the conductivity of the emulsion slightly increases with the increase of temperature at the beginning of the process. Once it reaches the maximum, it decreases suddenly. With tested surfactant concentrations of 3, 3.5, 4, and 5 wt %, the further temperature increase leads to a rapid reduction in conductivity. The HLB temperature/phase inversion temperature represented the average value between the maximum and the minimum values of conductivity.

Regarding 6, 7, and 8 wt % surfactant concentrations, the decrease of conductivity after reaching the maximum was not continuous and another apparent maximum was seen. The PIT value was gathered as the average between the highest and the lowest conductivity values (i.e. omitting the intermediate evident maximum).

The deficiency of continuity in conductivity curves for the highest surfactant concentration leaded to the formation of liquid crystalline phases. As it is apparent for a similar system, at such high surfactant concentration (above 5 wt %) the transition from O/W to W/O systems passes through the formation of two types of lamellar phases. [31]

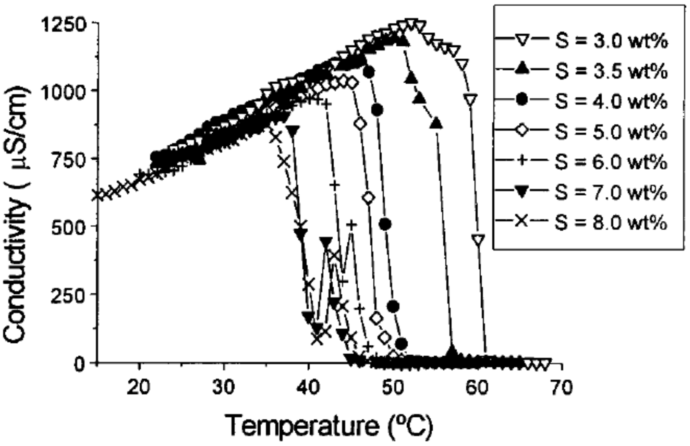

Figure 14: Dependence of conductivity of emulsion on temperature. [31]

2.2.3 Correlation between HLB and PIT

The phase inversion temperature of an emulsion is the temperature at which the hydrophilic and lipophilic character of the nonionic surfactant balances, and the HLB of the surfactant changes with temperature. Hence, a correlation between the PIT (HLB temperature) and the HLB value is expected. [32] The relation is shown in Fig. 15.

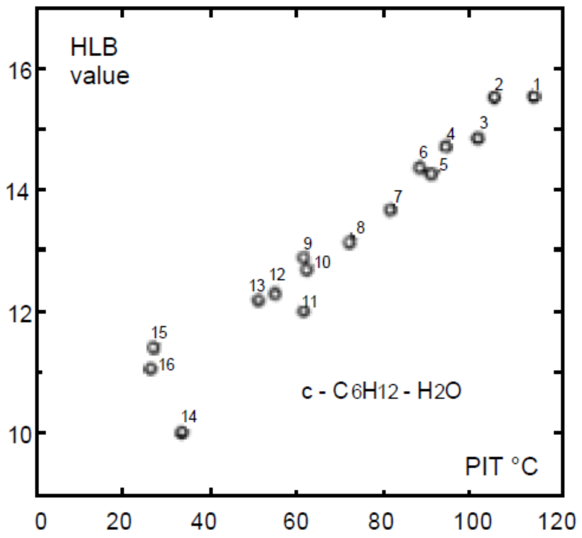

Figure 15: The correlation between the HLB values of nonionic surfactants and the phase inversion temperatures in c-C6H12-H2O emulsions stabilized with the surfactants. (3 wt %):

1. Tween 40;
2, i-$R_9C_6H_4O(CH_2CH_2O)_{17\,7}H$;
3. Tween 60,
4. i-$R_9C_6H_4O(CH_2CH_2O)_{14\,0}H$;
5. i-$R_{12}C_6H_4O(CH_2CH_2O)_{15}H$;
6, $R_{12}O(CH_2CH_2O)_{10\,8}H$;
7, i-$R_8C_6H_4O(CH_2CH_2O)_{10}H$;
8. i-$R_9C_6H_4O(CH_2CH_2O)_{9\,7}H$;
9, i-$R_8C_6H_4O(CH_2CH_2O)_{8\,5}H$;
10, i-$R_{12}C_6H_4O(CH_2CH_2O)_{9\,7}H$;
11, $R_{12}O(CH_2CH_2O)_{6\,3}H$;
12, i- $R_{12}C_6H_4O(CH_2CH_2O)_{9\,4}H$;
13, i-$R_9C_6H_4O(CH_2CH_2O)_{7\,4}H$;
14, i-$R_{12}O(CH_2CH_2O)_{4\,2}H$;
15, i-$R_8C_6H_4O(CH_2CH_2O)_6H$;
16, i-$R_9C_6H_4O(CH_2CH_2O)_{6\,2}H$. [32]

In Figure 16 it is shown, that the PIT also changes with the types of oil used in emulsion. This figure shows example of correlation between HLB and PIT in different types of O/W emulsions containing different oils.

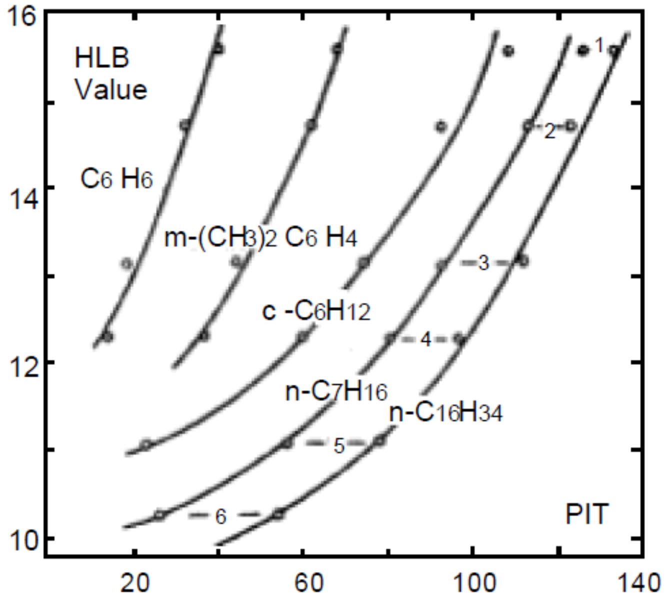

Figure 16: The correlation between the HLB values and the PITs in various oil-water (1 :1 emulsions stabilized with nonionic surfactants. (1.5 % per system).

1, $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{17\ 7}H$;	*4,* $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{7\ 4}H$;
2, $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{14\ 0}H$;	*5,* $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{6\ 2}H$;
3, $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{9\ 6}H$;	*6,* $i\text{-}R_9C_6H_4O(CH_2CH_2O)_{5\ 3}H$. *[32]*

2.2.4 Correlation between PIT and oil type

The Figure 17 shows the result of research conducted by Jiajia Rao and David Mc Clements.[33] They measured and compared the PIT temperature of emulsions with a different oil chain length by conductivity measurement.

The PIT of the mixed systems increased as the alkyl chain length of the hydrocarbon oils increased: 25.0, 29.5, 38.5, and 45.5 °C for decane, dodecane, tetradecane, and hexadecane, respectively. This result is in agreement with earlier studies of the effects of hydrocarbon type on the PIT performed with various nonionic surfactants. This effect can be attributed to the ability of oil molecules to penetrate between surfactant tails, thereby altering the optimum molecular geometry of the surfactant layer at the oil/water interface. Short chain hydrocarbon oils are able to penetrate more easily between the surfactant tails and thereby favor a curvature that is closer to planar than long-chain hydrocarbon oils. Emulsions containing short-chain hydrocarbons would therefore have to be heated to a lower temperature before the surfactant curvature tended toward unity and, hence, would have a lower PIT. [33]

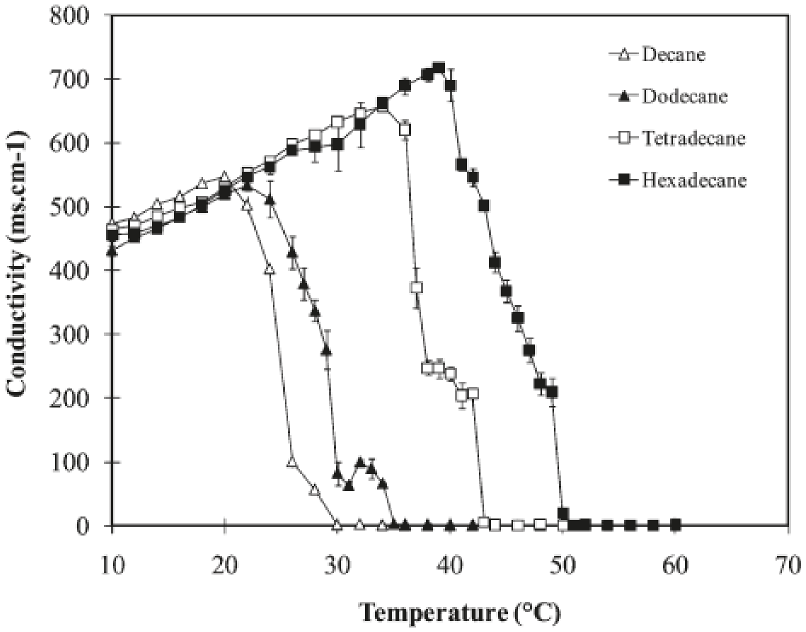

Figure 17: Temperature dependence of the conductivity of hydrocarbon (20 wt %), nonionic surfactant (6 wt % Brij 30), and water phase (10 mM NaCl) systems. [33]

2.3 Nanoemulsion application

Nanoemulsions have become the centerpiece of the interests of many researchers. Compared to the conventional emulsions, nanoemulsions with their high kinetic stability, small droplet size, and optical (semi)transparency have a considerable advantage, so they are often used in many technological applications. Provided that nanoemulsions are undergoing breakdown with time, only few studies on specific application of nanoemulsions were reported during the last decade. Although several studies show that nanoemulsions are able to be stable for several months and even years, it is obvious that the Ostwald ripening results in the growth of droplets of the internal phase, which limits the number of practical applications of nanoemulsions. This deficiency may be limited by using the nanoemulsions only as a basis for the production of other nanomaterials such as nanometer size latexes, polymeric nanoballs, solid lipid nanoparticles, etc. Another practical application of nanoemulsions is believed to be in pharmaceutical industry, for example for the encapsulation of drugs and their targeted delivery to the human body. There are also publications on the possible use in the agrochemical and oil industry, the manufacture of printer ink, etc.

The majority of publications on nanoemulsion applications deal with the preparation of polymeric nanoparticles using a monomer as the disperse phase (the so-called miniemulsion polymerization method). Contrary to emulsion and microemulsion polymerization, in nanoemulsion polymerization, droplet nucleation is reported to be the dominant mechanism, making possible the preservation of size and composition of each droplet during the formation of latex particles. Many studies showed that nanoemulsion systems have a great potential in practical use in the future.

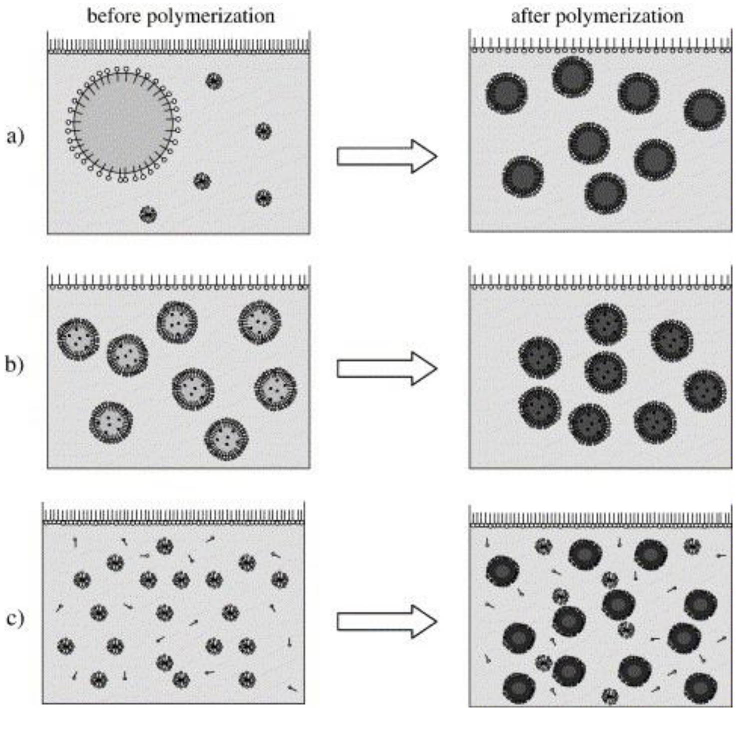

Figure 18: Schematic representation of heterophase polymerization process a) Emulsion polymerization, b) Nanoemulsion polymerization, c)Microemulsion polymerization. [13]

Consequently, nanoemulsion droplets can be considered as small nanoreactors. Surfmers (surfactants containing a polymerizable group) are used in nanoemulsion polymerization to stabilize, protect and functionalize the polymer particles. In that context, Guyot et al. [34] reported nanoemulsion polymerization of methylmethacrylate or styrene in the presence of acrylic surfmers which gave the latexes with good stability. However, a non-polymerizable surfactant was required to achieve stable nanoemulsions. Recently, Pich et al. [35] have obtained fluoro-containing nanoparticles by nanoemulsion polymerization of different monomers in the presence of a fluorinated monomer-surfmer. In addition to stabilizing the monomer nanodroplets and polymer nanoparticles, this surfmer also provided surface functionalization with fluoro-groups.

The production of low-viscosity high solid content latexes is another important application of nanoemulsion polymerization. Do Amaral and Asua [36] prepared them using acrylic monomers and showed that particle size is controlled by droplet size of the nanoemulsion, which is dependent on the type and concentration of surfactant.

2.3.1 The use of nanoemulsions for controlled delivery of medicines in human body

Nanoemulsions are successfully used for controlled drug delivery in human body and are, therefore, attracting increasing attention of many researchers. O/W nanoemulsions have found applications in cancer therapy, vaccines, HIV treatment, antiviral drugs and disinfectants, including those against Ebola virus. It was repeatedly emphasized that penetration of drugs entrapped in nanoemulsions is much higher compared with macroemulsions, micellar solutions, gels, suspensions and others. Nanoemulsions alone, as well as various nanoemulsion-based structures, as for example nanospheres and nanocapsules, can be used for controlled delivery of medicines in human body.

2.3.2 The use of nanoemulsions in cosmetic industry

Recently, the cosmetic products using the O/W nanoemulsions with the organic phase experienced enormous interest. Formulations containing mixture of oils with different molecular masses, which provides an optimal effect on human skin, are mainly in focus.

An increase in the fraction of high-molecular-mass oils results in increasing stability of O/W nanoemulsions against the Ostwald ripening. On the other side, the high-molecular-mass oils are worse absorbed and leave oily films on the skin surface. Emulsions containing low-molecular-mass oils penetrate faster into the surface layers of skin, however, its stability against the Ostwald ripening decreases. Acceptably stable nanoemulsions can be obtained by mixing the oils with a certain ratio of the low- and high-molecular-mass components.

Resulting from the above mentioned facts, the kinetic stabilization of nanoemulsions against the Ostwald ripening is due to different solubility of such oils in the aqueous phase. It was proposed to utilize nanoemulsions in various creams, lotions, gels, remedies for hair care, etc. Some of the compositions and methods of preparation of nanoemulsions, e.g. containing vitamins A, D, C, E, phospholipids, as well as nanoemulsions applied for the epidermis restoration are patented.

2.3.3 The use of nanoemulsions in food industry

Food nanotechnology and nanoemulsions is one of the fastest evolving field with many advantages over conventional food emulsions. Sub-micron droplets prolong emulsion physical stability, provide faster flavor release, enhance mouth feel, and the droplets are small enough they scatter light weakly and appear transparent, which allows delivery of oil soluble micronutrients and bioactives in an imperceptible way. [31] Nanoemulsions are increasingly used for encapsulation of bioactive compounds and act as transport vehicles in probiotic foods and other functional foods.

2.3.4 The use of nanoemulsions in agricultural industry

In terms of agricultural applications, nanoemulsions can be used for hydrophilic and hydrophobic pesticides, but are largely being developed for those that are poorly water soluble. For example, pyrethroids have been successfully formulated as lipid nanoemulsion, hindering the need for the organic solvents such as benzene and toluene normally required for its formulation. In one case, the negative effects of such pesticides on aquatic life were studied and found to be reduced compared with conventional formulations, while not affecting efficacy.

The main advantages of using nanoemulsions are therefore: 1) solubilization of hydrophobic pesticides, 2) no precipitation or creaming, 3) increased stability, and 4) improved uptake. [37]

3 AIM OF THE THESIS

In the experimental part of the thesis, the temperature of the phase inversion (PIT) of selected non-ionic surfactants and their mixtures will be determined using conductometric method. The obtained data will be used for O/W nanoemulsions preparation. Nanoemulsions consisting of n-undecan, water and Brij surfactants, with different HLB values will be prepared. The PIT process will be practically verified by measurements of the particle size of prepared emulsions using photon-correlation spectroscopy. Stability of prepared nanoemulsions stored at 4 °C, 25 °C and 35 °C will be determined and discussed.

II. **ANALYSIS**

4 MATERIALS AND INSTRUMENTS

4.1 Materials

Distilled H_2O, filtered H_2O

Sodium chloride NaCl, Sigma-Aldrich

Brij 30, $C_{212}H_{25}$ $(OCH_2CH_2)_n$,OH, n~4, HLB 9,7; Sigma-Aldrich

Brij 56, $C_{16}H_{33}(OCH_2CH_2)_nOH$, n~10, HLB 12,9; Sigma-Aldrich

Brij 98, $C_{18}H_{35}(OCH_2CH_2)_nOH$, n~20, HLB 15,3; Sigma-Aldrich

Igepal 520, $(C_2H_4O)_n \cdot C_{15}H_{24}O$, n~5 , HLB 10; Sigma-Aldrich

Igepal 720, $(C_2H_4O)_n \cdot C_{14}H_{22}O$, n~12.5, HLB 14; Sigma-Aldrich

n-Undecane $CH_3(CH_2)_9CH_3$, Sigma-Aldrich

4.2 Instruments

Common laboratory glass

Analytical balances

Magnetic stirrer

Double-wall glass reactor

Circulating water bath thermostats, IsoTemp- H5P and Thermo Haake- C10

Homogenizers, Heidolph- RZR 2052 Control and Heidolph- RZR 2020

Conductometer, Ino Lab-cond 730

Laboratory oven

Photon correlation spectrophotometer, ZetaNano ZS, Malvern

4.3 Apparatus

The apparatus for measuring of conductivity consisted of conductometer, magnetic stirrer, circulating tempered water bath and double-wall glass reactor (Figure 19). For nanoemulsion preparation, two homogenizers, two circulating tempered water baths and ice bath were employed.

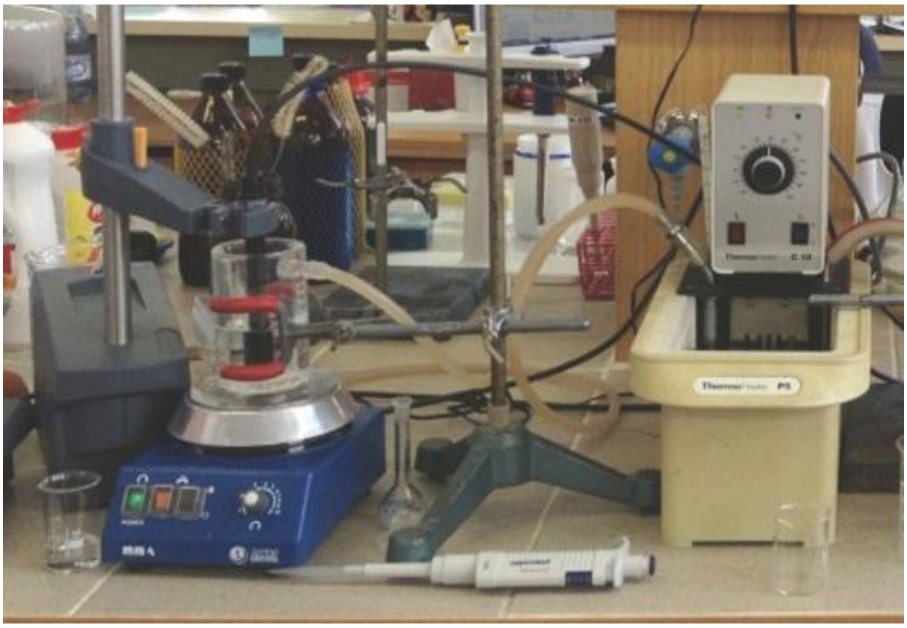

Figure 19: *Apparatus for the PIT measurement.*

5 METHODS

5.1 PIT determination by conductivity measurement

After preliminary tests which are mentioned in the discussion, following method for PIT determination was chosen. Emulsions with an oil–to–water weight ratio of 20/80 were prepared by simple mixing of all components and the conductivity of the resulting emulsions was measured as a function of a temperature.

The required amounts of surfactants and n-undecane were weight out. The surfactants were dissolved in water phase containing 0.1 M NaCl. If necessary, they were heated under stirring. Then, the oil phase was added. The system was homogenized at laboratory temperature (23±2 °C). Homogenization took place for 5 minutes at 750 rpm. After five minutes the rate was increased and the mixture was homogenized at 1050 rpm for 15 minutes. Finally, the prepared emulsion was cooled down to 20 °C and conductivity was measured.

The conductivity measurements were performed in a double-wall reactor connected to thermostated circulating water bath. During the continuous temperature increasing, the conductivity values were recorded in the temperature intervals of one degree of Celsius. The emulsion was stirred continuously with a magnetic stirrer during measurement. When the conductivity reached 0 µS the system was cooled down and conductivity measurement was repeated. As the correlation between HLB a PIT exists, the temperature interval used for PIT determination was dependent on the surfactant type used in respective emulsion.

5.1.1 Principle of the PIT conductivity measurement

Determination of the PIT by this method is based on the ability of the continuous phase of O/W emulsion, added NaCl in low concentration, to conduct electricity. On the other hand, the invert emulsion W/O does not conduct the electricity, as continuous phase is oil, showing conductivity approaching 0 µS. As the O/W emulsion is heated, it inverts to emulsion W/O resulting in the rapid change of conductivity. PIT temperature is in the middle of the maximum and zero conductivity values. In the diploma thesis, the PIT was determined from the second derivative of dependence between conductivity and temperature as a point of intersection with x axis.

5.2 Preparation of the nanoemulsions

There were prepared several types of emulsions differing in HLB of surfactant used. The HLB values of 9.7, 10.1, 10.5, 11 and 11.5 and surfactant concentration of 3 % or 5 % were applied. Oil-to-water ratio (O/W) of 5/95, 10/90, 20/80 and 30/70 were chosen.

For each emulsion containing 5 % of surfactant, two modifications were prepared. In order to obtain desired HLB value, two different combinations of surfactant were employed:

- Brij 30 with Brij 56;
- Brij 30 with Brij 98.

The nanoemulsions were prepared by phase inversion emulsification method. The required amount of water, surfactant and n-undecane were weight out. Firstly the surfactants with desired HLB were mixed together and homogenized. If necessary, they were heated under stirring. Water added surfactant mixture was heated above the PIT in the beaker. Separately, n-undecane was also heated above the PIT and oil phase was gradually mixed with water under stirring. At first, homogenization was performed for 5 minutes at 750 rpm, then the speed increased and the mixture was homogenized at 1050 rpm for 15 minutes. The prepared emulsion was rapidly cooled by immersion in an ice bath under continuous homogenization at 1050 rpm for another 10 minutes.

After preparation, each emulsion was divided into three parts in order to test its stability. One part was stored at laboratory conditions at 23±2 °C, one was placed at fridge with temperature of 4 °C and one was stored at laboratory oven at elevated temperature of 35 °C.

5.2.1 Examples of calculations

Calculation for preparing 0.1M NaCl:

$V_{NaCl} = 1dm^3$

$M_{NaCl}= 28\ g.mol^{-1}$

$m = c\ .\ V\ .\ M$

$m = 0{,}01\ .\ 1\ .\ 58 = \underline{0{,}58\ g}$ NaCl

Calculation for preparing 50 ml of emulsion W/O 80/20 with 5 % of surfactant:

5 % from 100 g of emulsion is 5 g of surfactant

5 % from 50 g of emulsion is 2.5 g of surfactant

95 g …………… 100%

$\underline{\text{x g …………….. 80\%}}$

x= $\underline{38\ g}$ H_2O

95 g ………… 100 %

$\underline{\text{x g …………... 20 \%}}$

x= $\underline{9{,}5\ g}$ $CH_3(CH_2)_9CH_3$

Calculation of amount of Brij 30 and Brij 98 required for emulsion O/W 80/20 with 5 % surfactant of HLB = 10.5:

$HLB_1 = 9{,}7$ (Brij 30)

$HLB_2 = 15{,}3$ (Brij 98)

$HLB = \sum HLB_{1.}\ x_1 + HLB_{2.}\ x_2$

$10{,}5 = 9{,}7\ .\ x_1 + 15{,}3\ .\ (1 - x_1)$

$10{,}5 = 9{,}7\ x_1 + 15{,}3 - 15{,}3\ x_1$

$x_1 = 0{,}857$

$x_2 = 0{,}143$

$m_{\text{Brij 30}} = 0{,}857 . 2{,}5 = \underline{2{,}1425\ g}$

$m_{\text{Brij 98}} = 0{,}143 . 2{,}5 = \underline{0{,}3676\ g}$

The amounts of surfactant needed for preparation of nanoemulsions with studied HLB values are summarized in Table 2.

Table 2: Concentrations of surfactant needed for nanoemulsion preparation

HLB of surfactant mixture	System I (5 % surfactant)		System II (5 % surfactant)	
	Brij 30 (HLB 9.7) m [g]	Brij 56 (HLB 12.9) m [g]	Brij 30 (HLB 9.7) m [g]	Brij 98 (HLB 15.3) m [g]
9.7	2.5	0	2.5	0
10.1	2.19	0.31	2.32	0.18
10.5	1.85	0.62	2.14	0. 36
11.0	1.48	1.02	1.92	0.58
11.5	1.1	1.4	1.69	0.81

5.3 Measuring of particle size and stability of nanoemulsions

After preparation, the z-average diameter of emulsion droplets and their polydispersity index (PDI) were measured by photon correlation spectrophotometry (PCS). For the spectroscopy measurements, standard polystyrene cells were used. Spectrometer wasn`t able to detect data in concentrated nanoemulsions, therefore, before each measurement the emulsion was diluted. For dilution, 1 ml of filtered water and 3µl of nanoemulsion were mixed. The measurement was performed not only on freshly prepared nanoemulsions but also on the samples stored at different temperatures. These nanoemulsions were also regularly observed by visual inspection. Later on, the data were inspected and compared, in order to established correlations between the stability of the nanoemulsions and their composition.

5.3.1 Principle of photon correlation spectroscopy

PCS measures Brownian motion and relates it to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. When the monochromatic and coherent light with known frequency from the light source hits the particle, the light scatters in many directions. Due to the Brownian motion, there are time dependent changes in light intensity of scattering particles. These fluctuations of light intensity contain the information about the size of particles. With respect to Brownian motion and under same conditions, the small particles have a higher average velocity than larger ones. Also the shift in frequency of light is different. The smaller particles are causing greater shift in frequency than the larger ones. The velocity of the Brownian motion is defined by a quantity known as the translational diffusion coefficient (*D*). The size of a particle *d* is calculated from the translational diffusion coefficient using the Stokes- Einstein equation

$$d(H) = kT/3\pi\eta D,$$

where

d(H)	hydrodynamic diameter
D	translational diffusion coefficient
k	Boltzmann’s constant
T	absolute temperature
η	viscosity

The z-average diameter calculated from PCS measurements is the best value to report and is defined as the “intensity averaged particle diameter”. Another parameter derived from PCS measurements is polydispersity index (PDI). It is dimensionless and scaled such that values smaller than 0.05 are rare. PDI values higher than 0.7 indicate that the sample has a broad distribution and is not suitable for the PCS technique.

The PCS is used for measuring particle size, stability and of emulsions, polymers, micelles, proteins or nanoparticles.

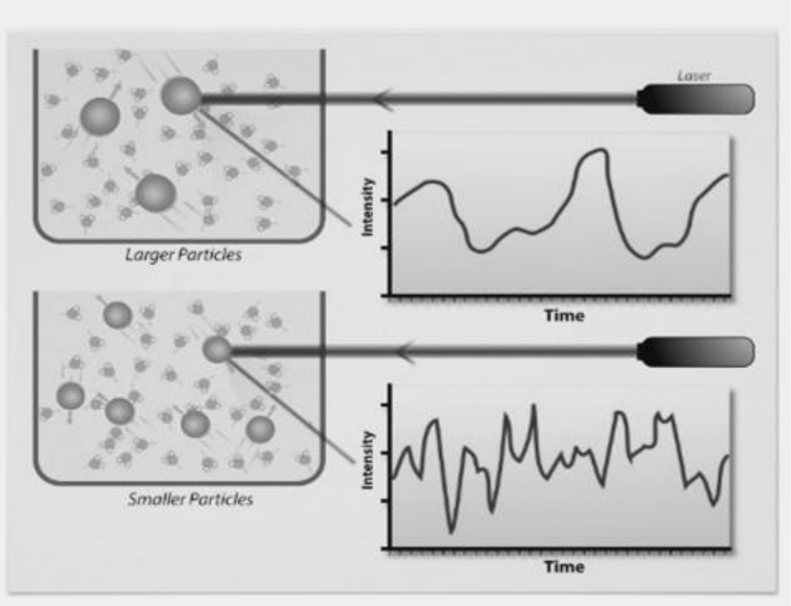

Figure 20: Illustration of the dynamic light scattering principle. [38]

5.3.2 **PIT determination by PCS measurement**

The PCS instrument has the capability of automated measurement of particle size and scattering intensity as a function of temperature. Built-in temperature control enables analyses over the range of 2 to 90 °C. This useful option enables following of different processes in samples. PIT determination using PCS is based the monitoring of changes, which occur during transition of O/W to W/O emulsions, prepared with non-ionic ethoxylated surfactants. When passing the PIT temperature, O/W emulsion converts to W/O emulsion. It results in sudden change in intensity of scattered light, and z-average particle diameter, respectively, which can be measured by PCS.

For PIT measurements, 3 – 10 μl emulsion were diluted in 1ml filtered water and mixed in cuvette. The cuvette was placed in instrument and temperature trend measurement was set up to cover defined temperature limits with expected PIT temperature. Examples of temperature intervals used for emulsions with 5 % surfactant are given in Table 3. Increment of 1 °C was used and equilibration time at each temperature of 120 s was employed. Values of scattering intensity and z-average diameter were recorded as a function temperature. The determination of PIT was based on the localization of the position of a breaking point in the temperature dependence of z-average particle diameter. Concretely, PIT was calculated as the point of intersection of two linear functions with mutually different slopes, one recorded prior and the second after PIT transition. This procedure for PIT determination is shown in the Figure 21.

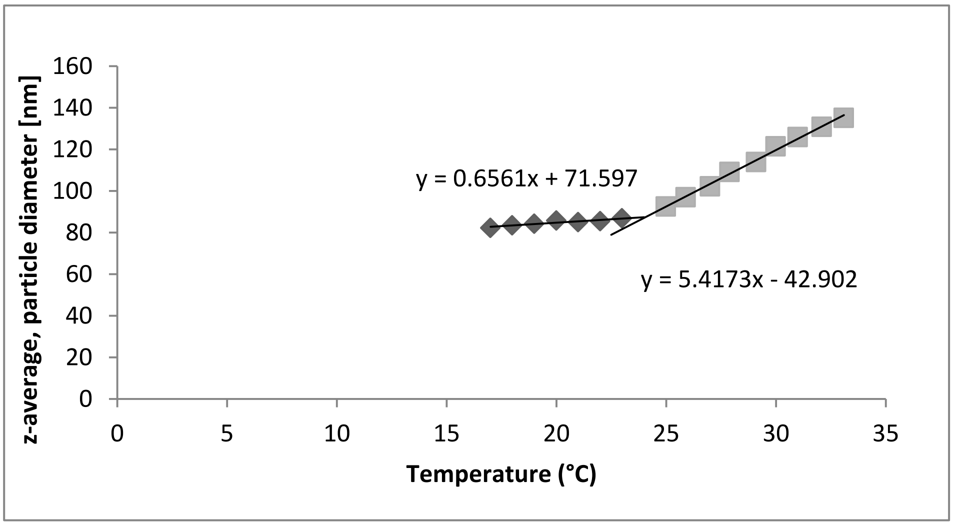

Figure 21: Determination of PIT from PCS by intersection of two linear functions.

Table 3: Example of temperature interval used for determination of PIT from PCS measurements (Brij 30 + Brij 56, 5 % surfactant).

HLB	Temperature interval [°C]
9.7	17 – 40
10.5	35 – 55
11.0	45 – 65
11.5	55 – 70

6 RESULTS AND DISCUSSION

6.1 Determination of PIT by conductivity measurement

At the beginning, it was necessary to test the validity of PIT temperature determination process. Several different procedures were used until the suitable method for emulsion preparation for PIT measurements was established.

Experiment No. 1:

The emulsion with HLB 9.7 was prepared at temperature of 70 °C using Brij 30 surfactant. The emulsion was homogenized by mixing the water and oil in ratio of O/W 80:20, in the presence of 5 % of surfactant. Prior homogenization, surfactant was dissolved in water phase. Homogenization was performed with 750 rpm for 5 minutes. Later, the emulsion was homogenized with increased rate of 1500 rpm for 15 minutes. This procedure resulted in preparation of a mixed emulsion. As the conductivity of the freshly prepared emulsion was approaching zero, it was impossible to detect the PIT.

Experiment No.2:

The process was the same as described in Experiment No.1; however, the emulsion was prepared at laboratory, not increased temperature. This resulted in invert emulsion.

Experiment No.3:

The emulsion was prepared at laboratory temperature; however, the mixing process was changed. Once the surfactant was dissolved in water phase, oil phase was gradually added under mixing. Though the emulsion was successfully prepared, it was not stable enough during the conductivity measurements.

Experiment No. 4:

The W/O ratio was changed to 95:5. Otherwise, the procedure as described for O/W 20:80 was employed. This emulsion broke down soon after preparation.

Experiment No. 5:

In this experiment, the surfactant Brij 30 was replaced by Brij 56. O/W ratio of 20/80 and 5 % surfactant content was again used. The PIT temperature was not detected during the experiment, as by continuous heating of emulsion up to 90 °C, no rapid change in conductivity was observed.

Experiment No. 6:

Finally, the procedure for measurement of PIT temperature was established, which is outlined in part 6.1 of the thesis. The process was exactly the same as in Experiment 3, however, the conductivity measurements were conducted after cooling the emulsion to 17 °C in order to lower temperature sufficiently below phase transition of the used surfactant, Brij 30. Using this procedure, all emulsions with O/W ratio of 20/80, 3 % or 5 % surfactant content were analyzed and their PIT temperatures were determined.

The PIT temperatures of all studied emulsions can be found in Table 4.

Table 4: PIT values in [°C] determined using conductivity measurements for two different surfartant mixtures (Brij 30 + Brij 56, Brij 30 + Brij 98) and concentrations 3 % and 5 %.

HLB	Brij 30 + Brij 56 5 %	Brij 30 + Brij 98 5 %	Brij 30 + Brij 56 3 %
9.7	24.5	24.5	44.7
10.1	36.4	32.9	48.7
10.5	44.6	43.7	61.8
11.0	57.5	60.9	67.4
11.5	68.4	76.6	78.3

Figures 22-24 shows the variation of conductivity with temperature, as well as dependence of PIT on HLB of surfactant used. The three Figures depict results for emulsions prepared with mixture of Brij 30 + Brij 56, 3 % and 5 % surfactant content and for the Brij 30 + Brij 98 with 5 % surfactant. At each graph, the O/W 20/80 emulsions with different HLB (9,7; 10,1; 10,5; 11; 11,5) are presented. At all HLB values and concentrations of surfactants, each of the curves illustrates the same dependence between conductivity and temperature. With increasing temperature, the conductivity of the emulsions slowly decreased (or remained unchanged) and then suddenly fell down to zero conductivity value.

With an increase of surfactant HLB, the conductivity curves moved towards higher temperatures, so it is seen that the PIT value is affected by the HLB of the surfactant in emulsion.

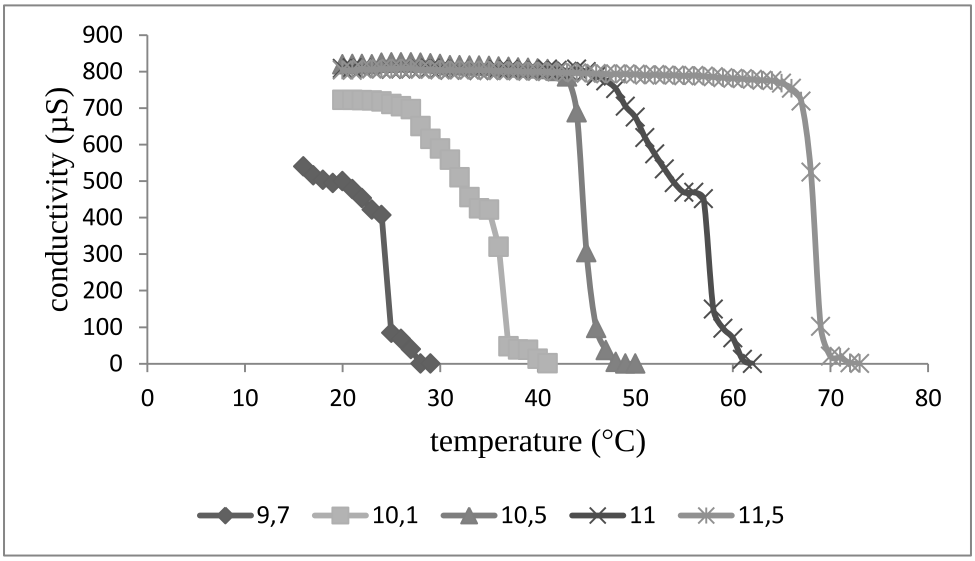

Figure 22: Dependence of conductivity of emulsions with different HLB on temperature. Emulsions contains 5 % of Brij 30 + Brij 56.

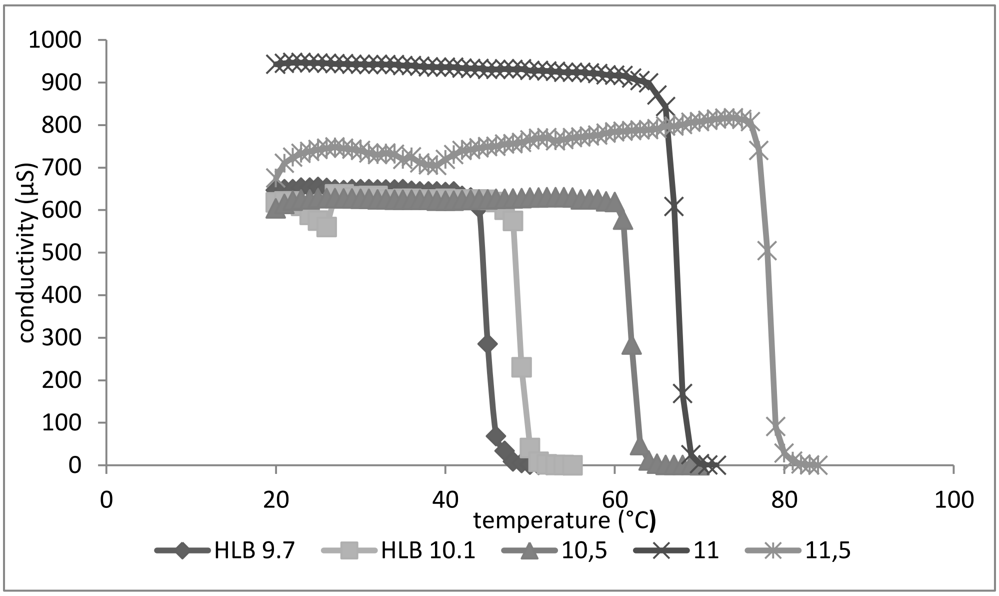

Figure 23: Dependence of conductivity of emulsions with different HLB on temperature. Emulsions contains 3 % of Brij 30 + Brij 56.

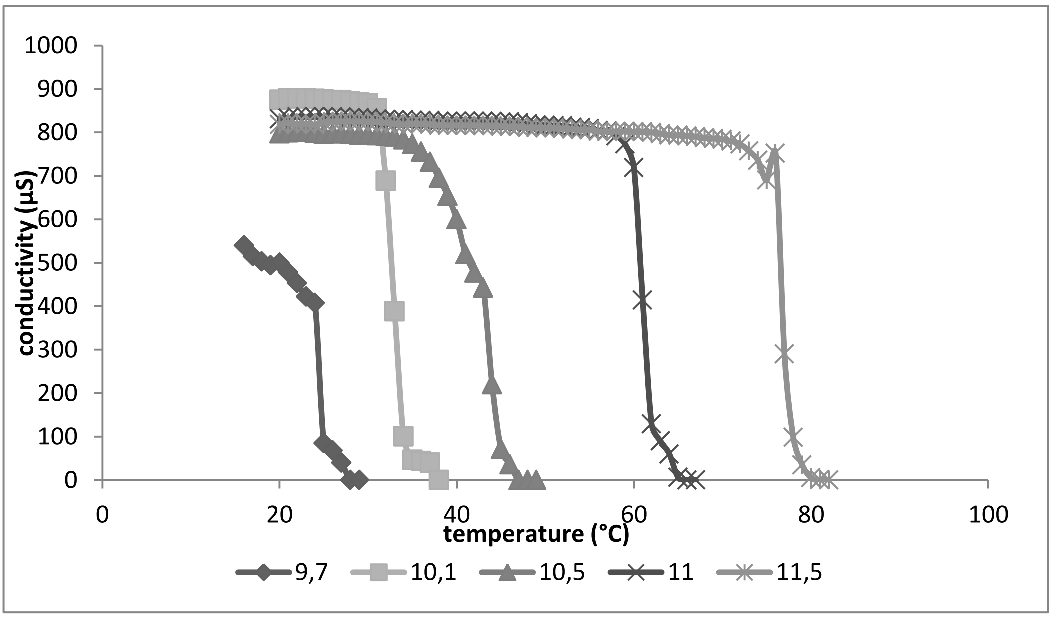

Figure 24: Dependence of conductivity of emulsions with different HLB on temperature. Emulsions contain 5 % of Brij 30 + Brij 98.

After preparation of emulsions using the Brijs, the different types of surfactant were also tested for emulsion preparation with the aim to determine PIT temperature. The emulsions with HLB 10.5 and 11 were prepared by using the mixture of surfactants Igepal 520 and Igepal 720. For those emulsions, the same procedure was used as for the preparation of emulsions with Brij surfactants. The PIT was also measured by conductivity with increasing temperature. However, PIT value of those emulsions, within the temperature range from 20 to 95 °C, was not determined, as there wasn’t any visible drop on the conductivity curve. Due to technical and physical reasons (water in circulating bath) it was impossible to increase the temperature, so the experiment was terminated.

6.1.1 Influence of HLB on PIT

The summary results of PIT dependence on HLB are presented in Figures 25 and 26. Here, already discussed fact can be seen, namely that the PIT is influenced by HLB value used for emulsion preparation. It is clearly evident that with the increasing HLB, the PIT increases as well. From the measured results it is obvious that the correlation between PIT and HLB is almost linear. The minor deviations from linearity are caused only by inaccuracy during the measurement process. The graphical expression of linear dependency comes out from the measurement of the two replicate, reproducible results.

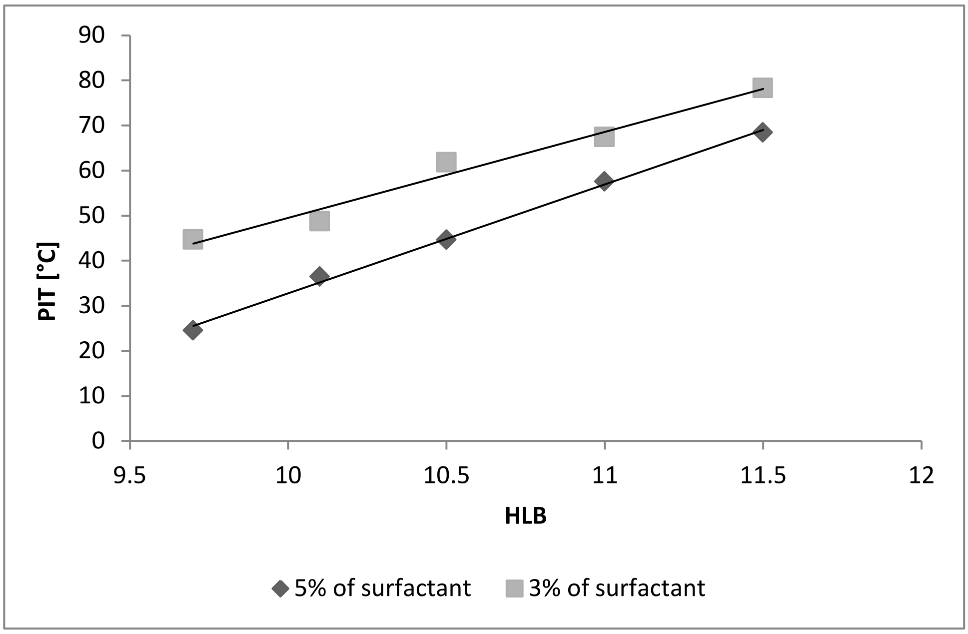

Figure 25: Dependence of PIT on HLB for emulsions O/W 20/80 containing 5 % and 3 % of surfactants Brij 30 + Brij 56.

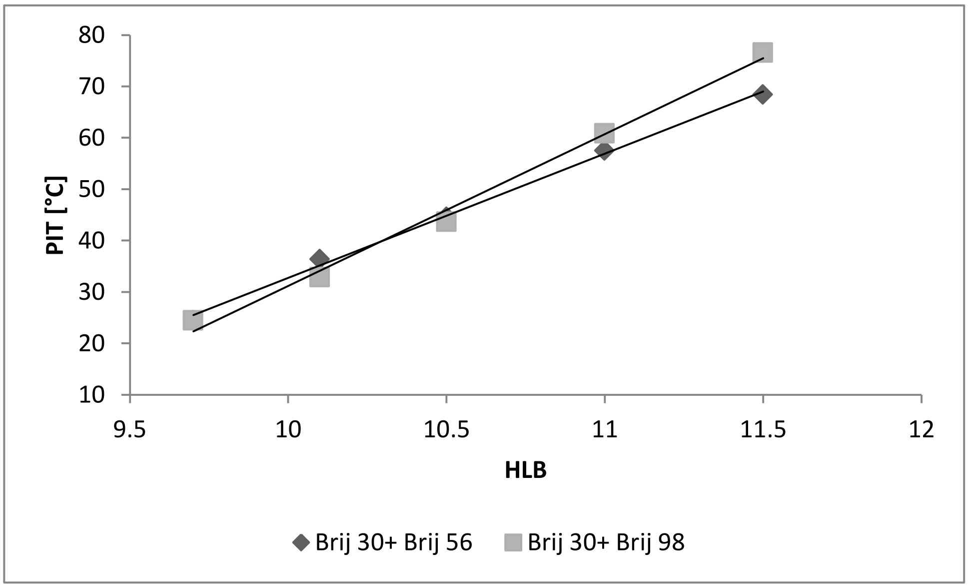

Figure 26: Dependence of PIT on HLB for emulsions O/W 20/80 prepared with 5 % of surfactant Brij 30 + Brij 98, and emulsion prepared with 5% of surfactant Brij 30 + Brij 56.

6.1.2 Influence of Surfactant Concentration on PIT

The PIT is also influenced by the concentration of surfactant in emulsion. From the Figure 25, this influence is clearly visible. By using the 5 % surfactant, the lower values of PIT are recorder when compared to use of 3 % surfactant, irrespective of HLB.

6.1.3 Influence of the Brij Type on PIT

From the measured results, the unambiguous influence of Brij type on the PIT value was not proved. Although the values of PIT in emulsions obtained by using Brij 30 + Brij 98 were slightly higher when compared with PIT obtained by using Brij 30 + Brij 56, the differences were, in most cases, minimal. However, from the careful inspection of the data it seems that the difference in PIT values, measured in both surfactant systems, increases with growing HLB. This might be correlated with increasing content of Brij 98 with higher HLB of 15,3 in the surfactant mixture. For more precise values it would be necessary to repeat the measurements and evaluate data from larger series of repeated analyses.

6.2 **PIT Determination by PCS Temperature Trend**

Using PCS, PIT temperature was determined not only for emulsions with O/W ratio of 20/80, as it was performed in case of conductivity, but also for other studied O/W ratios of 5/95, 10/90 and 30/70. However, due to limited time available for the thesis, this study was performed only for emulsions with HLB 9,7 and 10,5 containing 5 % surfactant. Results are presented in Figure 27 and show that PIT temperature is lower in emulsions with higher oil content. From the Figure it is obvious that this is valid for both studied HLB values.

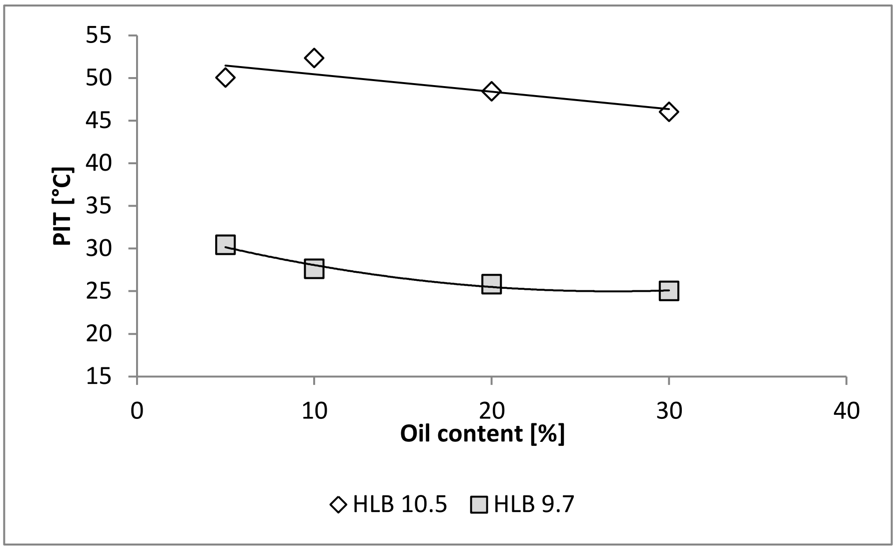

Figure 27: Dependence of PIT on oil content [%] in emulsion. Comparison of emulsion of HLB 10,5 and emulsion of HLB 9,7.

In the next step, the comparison between PIT temperature determined using conductivity and PCS for emulsions with O/W 20/80 was performed. This is illustrated in Figure 28 which demonstrates that reasonable correlation between both procedures exists. However, as it was mentioned above, the procedure for PIT determination using PCS requires additional experimental work.

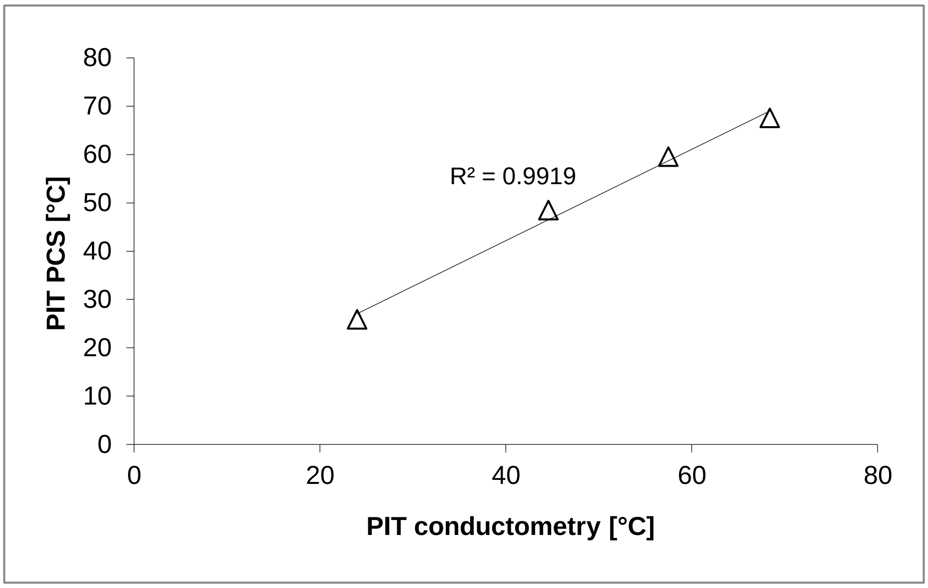

Figure 28: Comparison between PIT temperature determined using conductivity and PCS for emulsions with O/W 20/80.

6.3 Size of Particles

From the literature it is known that particle sizes in nanoemulsions are influenced by different variables. This is discussed in the following part of the thesis. All emulsions were prepared using PIT procedure, with 3 % and 5 % surfactant mixture of Brij 30 + Brij 56.

6.3.1 Dependence of Particle Size on Oil Content and HLB

The size of particles in nanoemulsions is influenced by oil content. In Figures 29 and 30, the z-average particle diameter can be seen for the tested HLB as a function of O/W ratio. These dependencies show that the certain trend across the particle sizes recorded at different HLB exists. Among emulsions with 5 % surfactant, the largest particle sizes can be found for the emulsions with O/W ratio of 5/95 and 30/70. Conversely, smaller particles were observed in emulsions with O/W 10/90 and 20/80. At HLB 9,7 and 10,1, the emulsions with the largest particles were prepared with O/W ration of 5/95. Emulsions with HLB of 10,5; 11 and 11,5 showed the biggest size of particles when O/W ration was of 30/70.

Similar trend can be observed for emulsions with 3 % of surfactant. The emulsions with the largest size of particles were 30/70 emulsions. Here, the sizes of particles were markedly bigger in contrast to the emulsions with the lower oil concentrations.

Generally, it can be seen that particle sizes of all prepared emulsions were laying in the size range below 1μm and emulsions with 5 % surfactant were all below 200 nm in size. The smallest particle sizes were observed for emulsions with O/W 10/90, irrespective of HLB, which were in the range from 32 to 43 nm. Of these samples, HLB 11 and O/W 20/80 provided emulsion with the smallest particle size of 32 nm.

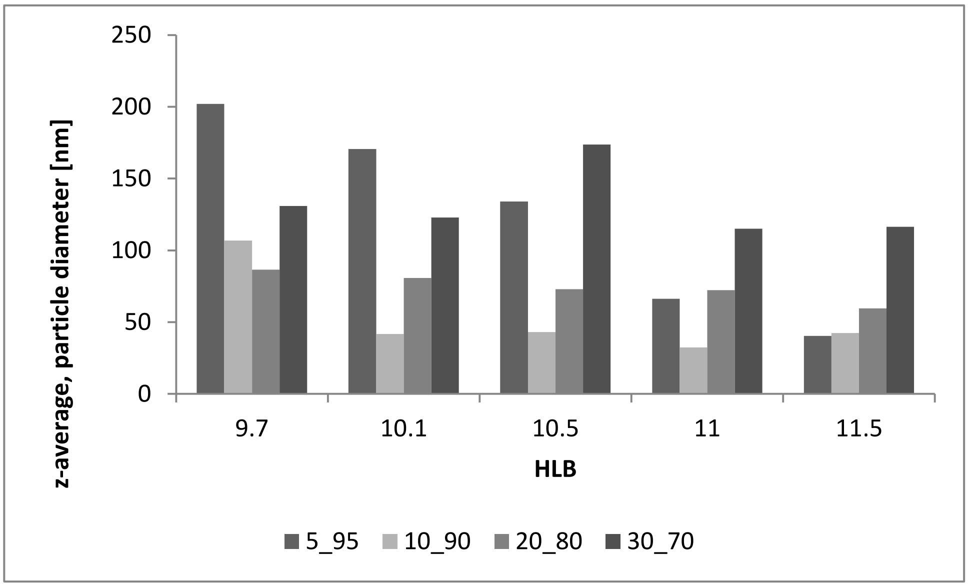

Figure 29: Dependence of particle size on O/W ratio recorded for emulsions with 5 % of surfactant Brij 30 + Brij 56.

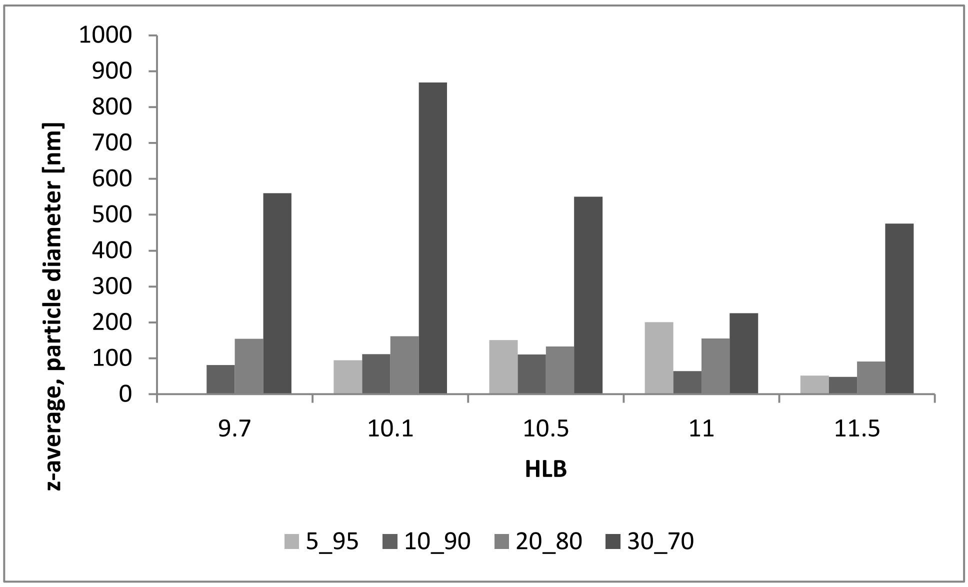

Figure 30: Dependence of particle size on O/W ration for emulsion with 3 % of surfactant Brij 30 + Brij 56.

6.3.2 Dependence of PDI on Oil Content and HLB

As can be seen in Figures 31 and 32, the oil content in emulsions influences also the polydispersity index (PDI value). The broadest particle size distribution was observed in emulsions with O/W 5/95. These emulsions show much higher PDI values when compared to other studied emulsion types.

For emulsions with 5 % surfactant, only minor influence of HLB on PDI can be seen, if the O/W ratio of 5/95 is not taken into consideration. For HLB ranging from 9.7 to 11.5, the PDI tends to decrease with increasing oil content. In most cases, PDI is of about 0.2, which means reasonably narrow particle size distribution.

As can be seen in Figure 32, the high values of PDI occur also in emulsions with O/W 5/95 and 3 % of surfactant. In addition, high PDI is also observed for O/W 30/70 and HLB 10.1. However, when these results are compared with the results on the emulsions with 5 % of surfactant, the data did not prove any clear correlation between HLB and the PDI.

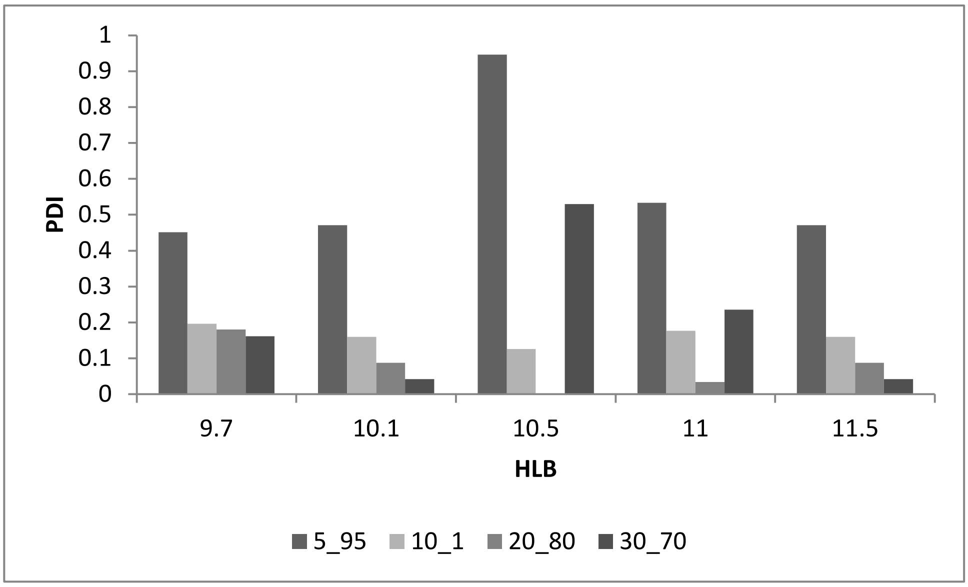

Figure 31: PDI dependence on O/W ratio and HLB, emulsions with of 5 % of surfactant.

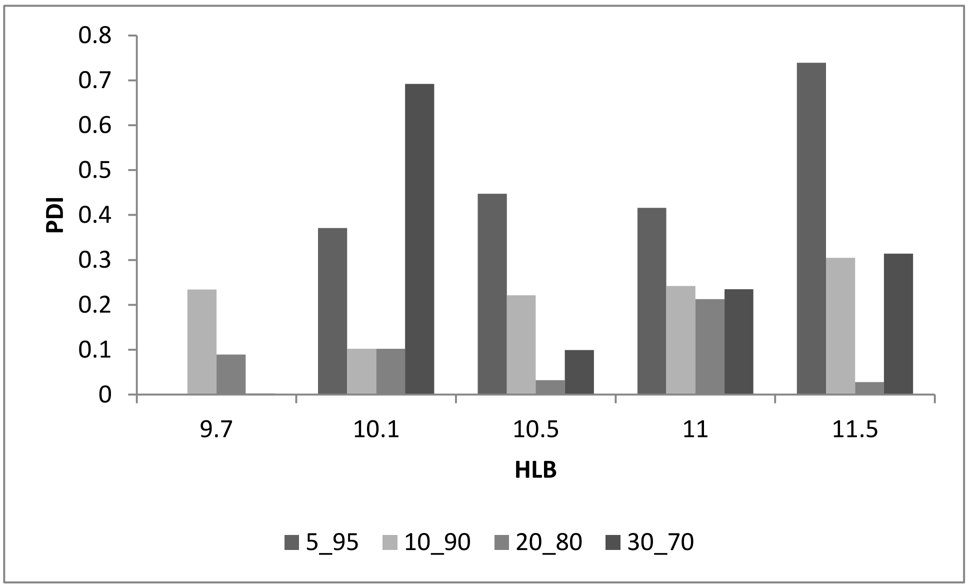

Figure 32: PDI dependence on O/W ratio and HLB, emulsions with 3 % of surfactant

6.3.3 Comparison of nanoemulsions prepared with PIT and EIP Techniques

In the following part of the thesis, particle sizes of nanoemulsions produced by PIT method are compared with nanoemulsions prepared using EIP method. These nanoemulsions were prepared with mixture of Brij 30 + Brij 98, using 3 % or 5 % surfactants andO/W ratio from 5/95 to 30/70. Data taken for comparison have not been published, yet and are used with kind permission of Petra Ševčíková.

Briefly described, EIP emulsifications were carried out using RZR 2020 stirrer, a glass vessel and a burette. Depending on the required HLB, low– and high– HLB surfactants were pre-dissolved in the calculated ratios in the oil and water phase, respectively. The surfactant containing water phase was added drop-wise at the rate of 1ml/min to the surfactant containing oil phase under continuous stirring. During emulsifications, the stirring rate was controlled at 1050 rpm and all experiments were run at the room temperature (25 °C).

The measured results of the particle size of emulsions prepared with the different percentage of oil phase and with various HLB can be seen in Figures 33 and 34. Figure 33 shows the emulsions with 5 % of surfactant and Figure 34 the emulsions with 3 % of surfactant.

For emulsions prepared by EIP, it was seen that emulsions prepared with HLB 10 (5 % surfactant) contained notably bigger particles compared to emulsions prepared with HLB 10,5 and 11. Another increase of particle size can be again observed for HLB 11,5 especially for oil content above 20 %. Regarding PIT emulsion, correlation between particle sizes and HLB was also observed, however it was not so notable, as for EIP emulsions.

Similarly to emulsions prepared by PIT, the influence of O/W ratio on particle size can be observed in EIP emulsions. However, the dependencies show different trend. Regarding the emulsions prepared by EIP, the smallest particles sizes are observed with O/W 5/95, what is exactly the opposite to the emulsions prepared by PIT procedure. Later then, with the increasing amount of oil in EIP emulsions, the particle size increases, as it can be seen in Figures 33 and 34. The PIT technique provided the smallest particle sizes in emulsions with O/W 10/90 with bigger particles for both higher and lower oil content.

Due to their instability, the EIP emulsions prepared with HLB 10 (5 % of surfactant) behave differently compared with those prepared with surfactants of higher HLB. Moreover not all studied O/W ratio were measured, as emulsions prepared with oil content below 15 % at this HLB were extremely unstable. Such a high instability at low HLB was not observed for PIT emulsions with 5 % surfactant.

Regarding particle sizes, PIT process (5 % surfactant) provided particles, which all were smaller than 200 nm. Contrary to this observation, EIP emulsions across the O/W ratios used gave particles smaller than 200 nm only at HLB 10.5 and 11.

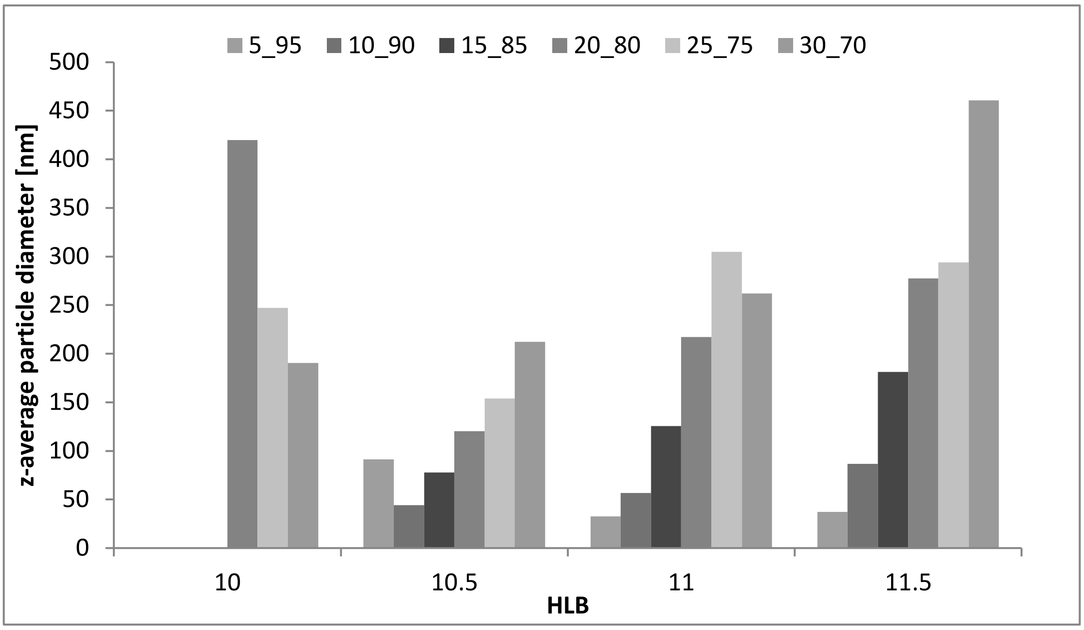

Figure 33: Dependence of particle diameter recorded for emulsion prepared by EIP method with Brij 30 + Brij 56, 5 % surfactant (unpublished data of Petra Ševčíková)

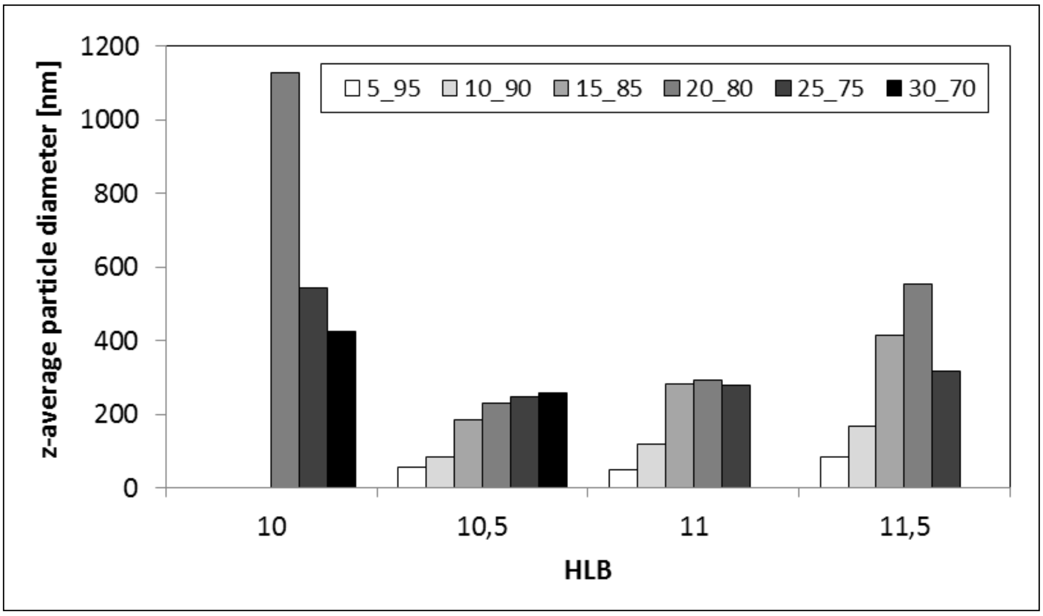

Figure 34: Dependence of particle diameter recorded for emulsion prepared by EIP method with Brij 30 + Brij 56, 5 % surfactant (unpublished data of Petra Ševčíková)

Comparison of PIT and EIP methods illustrates that even though both procedures employ the phase transition mechanism to produce small emulsion particles, due to different nature of transition (EIP – composition, PIT – temperature), the impact of different variables (HLB, O/W ratio) on resulting nanoemulsions is different.

7 STABILITY OF EMUSIONS

7.1 Effect of Storage Temperature on Emulsion Stability

After production, the emulsions were stored in different conditions, namely at 35 °C, 25 °C and 4 °C.

Definitely, the shortest shelf-life of the emulsions was seen after storage at 35 °C and the stability of emulsions improved with decreasing storage temperature. Regarding HLB, the shortest shelf-life of emulsions was observed for emulsions with HLB 9.7, which broke down almost immediately, when stored at increased and laboratory temperature. This instability is obvious from the fact that the ambient temperature (23±2 °C) is in close vicinity of the PIT temperature of the emulsions. The higher temperature causes the increase in movement of particles, which consequently leads to a higher probability of their collisions inside the emulsion. This phenomenon of thermal motion of the particles affects the disintegration of emulsion. Another factor that affects the stability is the storage temperature approaching to the PIT. The closer the temperature is to PIT, the less stable is the emulsion. The stability of emulsions (in days) can be seen in Table 5.

Table 5: Stability of undecane-in-water nanoemulsions stored at different temperatures.

HLB	Temperature [°C]	Stability in days			
		O/W 5/95	O/W 10/90	O/W 20/80	O/W 30/70
9.7	4	6	13	10	12
	25	2	6	6	9
	35	0	0	0	0
10.1	4	1	3	3	8
	25	1	1	2	6
	35	1	1	1	5
10.5	4	2	2	8	16
	25	2	8	6	12
	35	2	2	2	8
11.0	4	1	10	10	10
	25	1	9	2	10
	35	2	2	2	1
11.5	4	1	8	16	8
	25	1	2	9	2
	35	1	2	7	6

7.2 Effect of Oil Content on the Stability of Emulsions

The emulsions with O/W 5/95 were the first the subject of destabilization. Some of them failed to be prepared on first trial, so they had to be prepared repeatedly. The preparation of 5/95 emulsion with 3 % of surfactant failed even after repeated trials. The most stable were the emulsions of 30/70 composition. This conclusion is based on visual observation of emulsion. Unstable emulsions were those, at which creaming occurred.

7.3 Effect of Surfactant Concentration on the Stability of Emulsion

When comparing the emulsion with 3 % and 5 % of surfactant, the emulsion with 3 % of surfactant shows the unambiguously shorter shelf-life irrespective of composition and storage temperature. It can, therefore, be postulated the conclusion that the concentration of surfactant notably influences the stability of the emulsions.

7.4 Effect of HLB on the Stability of Emulsion

The emulsions with different values of HLB and with the same composition (O/W ratio), have the different stability. However, the direct correlation between length of stability and the HLB values was not determined. The emulsions with HLB 10.1 were the less stable. Conversely, emulsions with HLB of 10.5 were the most stable.

From the observation of all prepared emulsion, it can be concluded that the most stable emulsion was the one prepared by using 5 % of surfactants Brij 30 + Brij 56 with HLB of 10,5 and O/W 30/70. This emulsion was stable for 16 days when stored at temperature of 4 °C. At temperature of 25 °C, it remained stable for 12 days and stability of 8 days was detected even in more extreme conditions, which means temperature of 35 °C.

7.5 Effect of Storage Conditions on the Emulsion Particle Size

For all storage temperatures and also for all types of emulsions, the changes in particle size were detected within time. The longer the time from the emulsion preparation, the bigger the particle sizes were observed. This dependence is shown in the Figure 35 and illustrates that the particle sizes increase with storage time almost linearly.

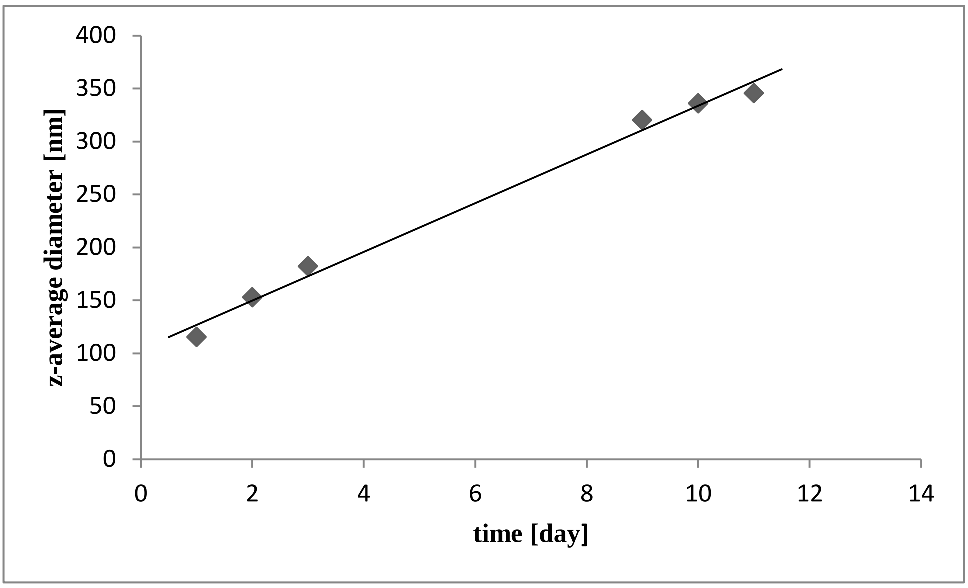

Figure 35: *Dependence of particle size on time for emulsion: HLB 11, O/W 30/70 with 5% of surfactant stored at 4 °C.*

In the Figure 36, changes of particle size distributions measured in different days after emulsion preparation are provided. The composition of the emulsion is as follows: O/W 20/80, HLB of 11 and 5 % of Brij 30 + Brij 56 surfactants. The distribution curves demonstrate that polydispersity of the samples was nearly the same, but the size of the particles changed. With increasing storage time, the mean particle size increased, as can be seen from the shift of distribution curves.

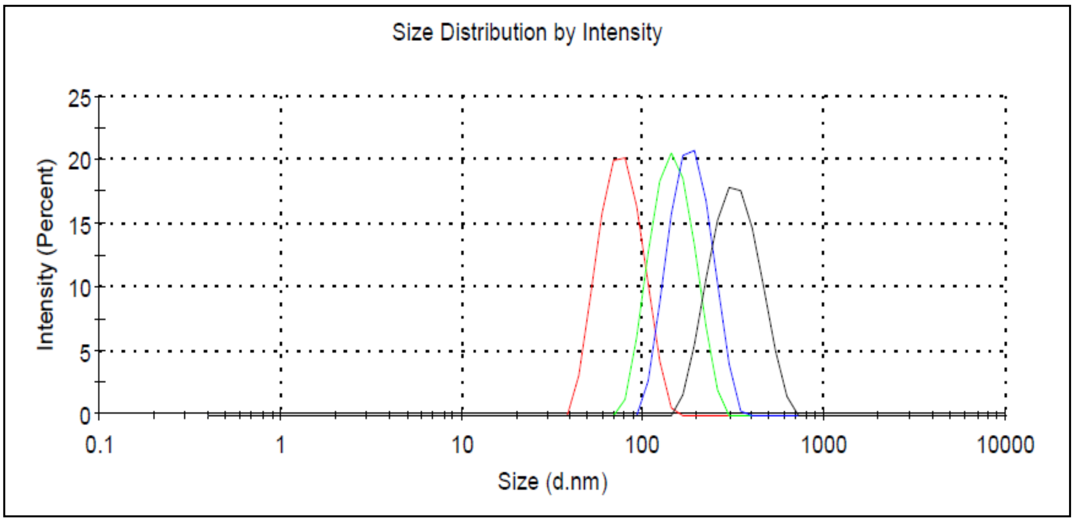

Figure 36: Dependence of the particle size distribution on storage time: Red line: day of preparation, green line: 1 day , blue line: day 5, black line: day 7(storage temperature 4 °C).

The changes in particle size are strongly influenced by storage temperature. Higher temperatures cause a significant increase in particle size within time. When observing the same type of emulsion stored under different temperature conditions, it is evident that emulsion stored under temperature of 4 °C are subjected to a slower growth in particle size, oppositely to those that are stored in the laboratory conditions, or under temperature of 35 °C. This fact is clearly seen in the Figure 37, where the increase in particle size of emulsion (O/W 20/80, 5 % surfactant, HLB 9.7) within time is illustrated for samples stored at two different temperatures of 4 °C and 25 °C.

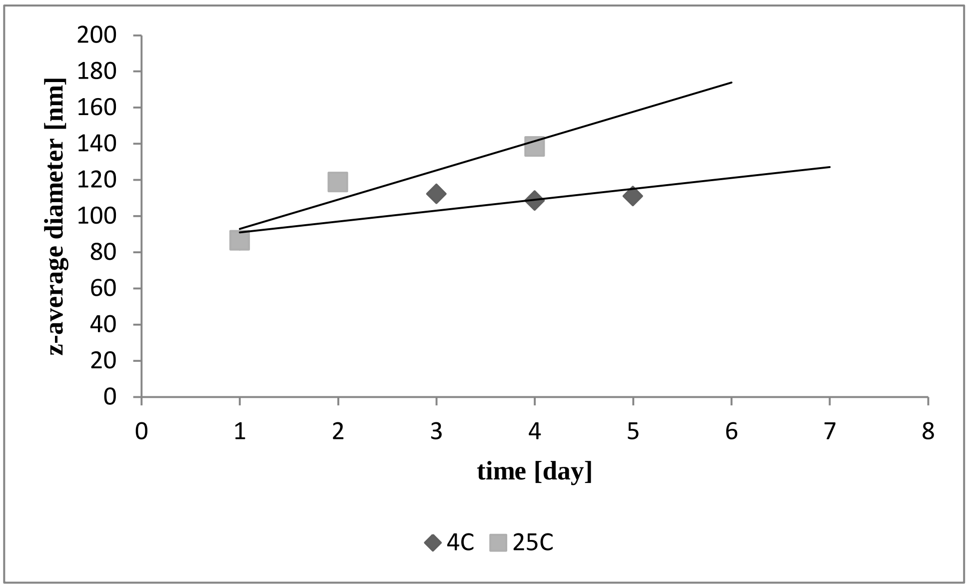

Figure 37: Comparison of strorage temperature dependence on particle size change within time. Emulsion with HLB 9,7; O/W 20/80 with 5 % of surfactant.

In the Figure 38, the comparison of emulsion with O/W 20/80, HLB of 11 and 5 % of Brij 30 + Brij 56 stored at different temperatures is given. From the Figure it is evident, that rise of storage temperature increases the particle size, which influenced the stability of the emulsions. It can be also seen that temperature of 35 °C caused not only particle size increase but also broadening of the particle size distribution, which another factor is contributing to emulsion break-down.

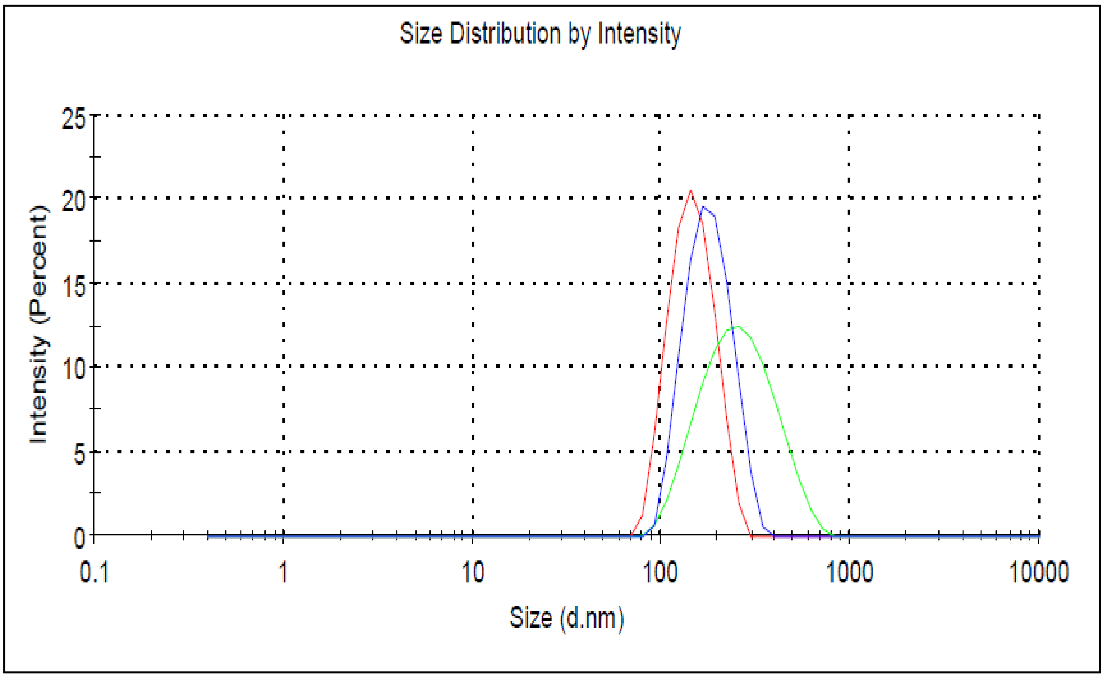

Figure 38: Comparison of particle size distributions of emulsions stored at different temperatures. Red line: emulsion stored at 4 °C, blue line: emulsion stored at 25 °C, green line: emulsion stored at 35 °C.

CONCLUSION

Nanoemulsions are dispersion systems with the potential to be transparent and very stable. In order to show these properties, the nanoemulsion particles must not only be in the nano-size range but also have a size distribution as narrow as possible. The master thesis is focused on the production of such nanoemulsions, using low-energy emulsification method, PIT.

Prior to emulsifications, phase inversion temperatures (PIT) of studied nanoemulsions with surfactants of different HLB were determined, using conductivity measurements. During conductivity measurements, all curves showed a characteristic course with a rapid decrease of conductivity, when temperature approached PIT. The measurements resulted in conclusion that there is a linear dependence between PIT and HLB.

PIT was also influenced by surfactant concentration. At emulsions with lower surfactant concentration, the higher values of PIT can be expected. Therefore, it is evident that during the preparation of emulsions using PIT, the samples with the lower surfactant concentrations need larger energy input to be heated near the PIT and subsequently to be rapidly cooled down to produce small particles.

Based on the results and discussion provided in the thesis it can be concluded that properties of nanoemulsions are dependent on composition in terms of surfactant HLB, its concentration and O/W ratio. All prepared samples can be classified as the nanoemulsions, as their particle sizes were lower than 200 nm. Exception here was emulsion prepared with O/W 30/70 and 3 % of surfactant, showing particles ranging from 200 to 900 nm.

The stability of emulsions was directly connected to the particle size and distribution and to the concentration of the surfactant. Emulsions with lower concentrations of surfactant were less stable than those with its higher concentrations.

The stability of emulsion was also notably influenced by storage temperature. It was proved that under lower temperatures (4 °C), the samples had a better stability within time. This corresponds with the observed fact showing that the growth in the particle size is influenced by the storage temperature.

The presented experiments were essential to understand the conditions for successful emulsification of the chosen system with the selected low-energy technique. The thesis can be, hence, considered as the fundamental study leading to further applications of the PIT technique in the preparation of more functional formulations, applicable for example in cosmetics and food industry.

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LIST OF ABBREVIATIONS

Fig.	Figure
Tab.	Table
NM	Nanoemulsion
PIT	Phase inversion temperature
HLB	Hydrophylic-lipophilic balance

LIST OF FIGURES

LIST OF TABLES