

Dispersion systems as carriers of active substances

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Doctoral Thesis Summary



Tomas Bata University in Zlín

Faculty of Technology

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Disperzní systémy jako nosiče aktivních látek

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ABSTRAKT

Předložená doktorská práce je zaměřena na formulaci, přípravu a charakterizaci vhodných disperzních systémů, které mají schopnost účinně enkapsulovat, uchovávat a uvolňovat účinné látky využívané v kosmetickém, farmaceutickém či potravinářském průmyslu.

Dizertační práce se skládá ze dvou částí. Teoretická část práce je věnována stručnému popisu procesu enkapsulace a nejčastěji využívaných enkapsulačních systémů, s důrazem na problematiku systémů emulzních. Další kapitola se zabývá enkapsulací lipofilních účinných látek. Hlavní část práce tvoří problematika emulzí stabilizovaných pomocí částic. Pozornost je věnována zejména nanočásticím celulózy. Součástí je také krátké srovnání vlastností emulzních systémů připravených v rámci experimentální práce. Závěrečná část přináší přehled experimentálních metod použitých k charakterizaci zmíněných systémů.

Druhá část práce předkládá výsledky získané během doktorského studia formou krátkého shrnutí jednotlivých publikovaných článků. Výzkumné práce v plném znění jsou k dispozici v samém závěru dizertační práce.

ABSTRACT

The doctoral thesis is focused on the formulation, preparation and characterization of the dispersion systems with the ability to carry, effectively encapsulate and release active substances in the cosmetics, pharmaceutical or food industry.

The thesis is divided into two main sections. In the theoretical part, a brief description of encapsulation process and most common encapsulation systems is provided, with emphasis on the emulsion-based systems. The next chapter deals with challenges associated with encapsulation of lipophilic active ingredients. The center theme of the thesis is focused on particle-stabilized emulsions, primarily on those stabilized by nanocellulose particles. Following this, a comparison of properties of prepared systems is presented. Finally, the last section provides an overview of laboratory techniques used for characterization of discussed systems.

The second part of the thesis presents results conducted during the doctoral study in form of short summaries on four research papers. The full-length versions of the papers are available at the end of the publication.

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THEORETICAL BACKGROUND

1. INTRODUCTION TO ENCAPSULATION

Many bioactive compounds (such as flavours, drugs, enzymes, cells, or other materials) cannot be simply incorporated into products in their regular form, and must first be encapsulated within some kind of suitable system before they can be introduced into the final product. Encapsulation is a common practice in the fields of pharmacy, cosmetics and food industry to improve the properties of products. By encapsulation their quality can be enhanced in terms of time and local controlled release, storage stability, protection from chemical, physical, or biological degradation [1-3].

More specifically, encapsulation may be defined as a process in which active ingredients (solids, liquids or gases) are entrapped within a secondary material, which completely embeds them or where they are dispersed in and delivered in small capsules [3, 4]. Capsules may range from nanometre to several millimetres in size and have a multitude of different shapes, depending on the materials and methods used for their preparation. In particular, capsules having diameter in the nanometre range are referred to as nanocapsules, while those with diameter between 3 and 8 μm are called microparticles, microspheres or microcapsules. Capsules larger than 1000 μm are then called macroparticles [3].

The schematic diagram of a capsule is shown in Figure 1a. Generally, capsules consist of a core material, which is also referred to as the active ingredient, internal phase, payload or fill, and a coating also known as the wall, shell, carrier or membrane. The morphology of the internal structure of capsule depends largely on the selected materials and the encapsulation methods that are employed. Capsules can be grouped into two main types: reservoir (core & shell), matrix or their combination (Figure 1b) [3, 5, 6]. For example, micelles, microemulsions, emulsions nanoemulsions can be all classified as matrix type encapsulation systems. Reservoir encapsulation systems include for instance multilayer emulsions, multiple emulsions, liposomes, and polymeric capsules [4, 7].

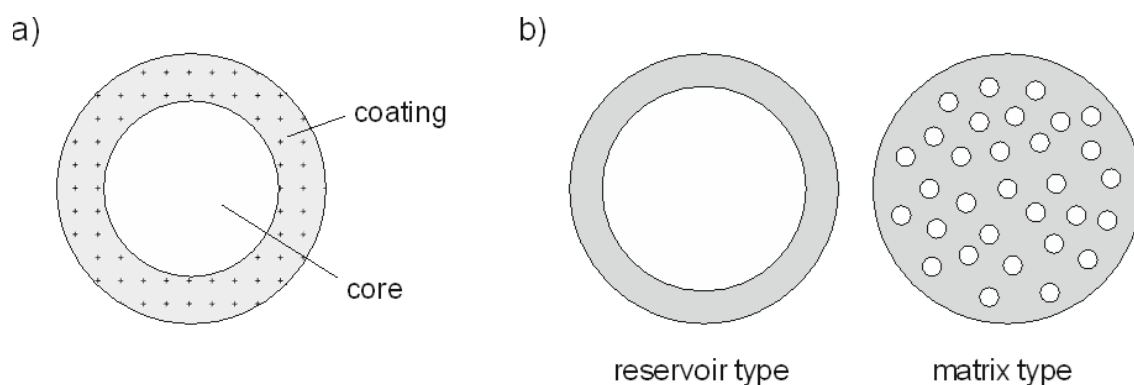


Figure 1(a) Scheme of a capsule. (b) Types of capsules [6].

In encapsulation, the role of coating material is crucial, as it determines the stability of capsule, the efficiency of encapsulation process, and the degree of protection for the core [3, 5, 6, 8]. Importantly, it must be also inert toward active ingredients and preferably nontoxic and biodegradable [3]. Other factors to be considered depend mainly on intended application of the final capsules and include the physicochemical properties of the coating material (solubility, molecular weight, and diffusibility), its film-forming and emulsifying properties, and cost. In practise, when the encapsulated active ingredient is intended for incorporation into foods, the wall materials need to be food grade [9]. In the cosmetic formulations for topical applications, the skin safety and compatibility of encapsulating materials play an important role [10]. The most-used coating materials gaining popularity across a variety of industries are polymers of both synthetic and natural origin. Synthetic polymers typically include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), acrylic polymers, while commonly used naturally occurring polymers are polysaccharides (gums, starches, celluloses, alginates, cyclodextrins, chitosan), and proteins (gelatine, casein, soy proteins, zein, rice protein) [9, 11-14].

1.1 Encapsulation systems

Due to the number of methods and techniques used for encapsulation, it is difficult to establish a uniform classification of encapsulation systems. In the literature, some authors classify them according to the physicochemical processes involved in encapsulation (i.e. physical, chemical, mechanical) [6], while other classifications are, for example, based on the techniques involved (i.e. atomization, spray coating, coextrusion, and emulsification) [15], or on the morphology of capsules (i.e. matrix type, reservoir type, their combination) [14]. Alternatively, they can also be classified according to the major components used in their fabrication as surfactant-based, emulsion-based, biopolymer-based, or hybrid [1]. Brief summary of encapsulation systems that use water as the dispersing medium is given below (Table 1).

Table 1 Selected examples of colloidal encapsulation systems [1].

Type of Delivery System
Surfactant-based systems
– Swollen micelles
– Microemulsions
Emulsion-based systems
– Liposomes
– Conventional emulsions
– Nanoemulsions
– Multiple emulsions
– Particle-stabilized emulsions
– Multilayer emulsions
– Solid lipid nanoparticles
Biopolymer-based systems
– Microclusters
– Hydrogel particles
– Coated hydrogel particles
– Biopolymer nanoparticles/microparticles
Hybrid systems
– Filled hydrogel particles
– Filled liposomes
– Multilayer liposomes
– Colloidosomes

1.1.1 Spontaneously-assembled surfactant-based systems

The main building components for surfactant-based encapsulation systems are surfactants and phospholipids. These surface-active molecules consist of a polar head group and a nonpolar tail group. Different surfactants vary according to the nature of their head and tail groups. The head group may differ in its size, polarity, charge (positive, neutral, negative), and chemical reactivity. The tail group may vary in the number, length, unsaturation, and branching of the nonpolar chains. The functionality of surface-active molecules depends on the nature of the head and tail groups, as well as surrounding environment. Therefore, it is possible to prepare surfactant-based delivery systems with a wide range of structures and functional properties by using different kinds of surface-active molecules. The main driving force for the formation of these system is usually self-assembly of the surfactants based on the hydrophobic effect, and so they are primarily formed spontaneously by simply mixing surfactant, oil, and water together. The main types of surfactant-based systems are micelles, microemulsions, and liposomes (Figure 2) [1, 16].

1.1.2 Emulsion-based systems

The key building components for emulsion-based systems are oil droplets, which may differ in their size, composition, physical state, interfacial characteristics, and structure [16]. Differently from microemulsions and other spontaneously-produced systems, they are required energy input to be formed. Emulsion-based systems are usually fabricated by mixing or blending emulsifier, oil, and water phase together [17]. The principal emulsion-based delivery systems are emulsions, nanoemulsions, particle-stabilized emulsions (Pickering emulsions) and solid lipid nanoparticles (SLNs), but these systems can be used as building materials to construct more complex structures, such as multilayer emulsions, colloidosomes, microclusters, and filled hydrogel particles [1, 18, 19].

1.1.3 Biopolymer-based systems

The key building materials for biopolymer-based delivery systems are biopolymers, such as proteins and polysaccharides. These systems can be fabricated using a variety of different preparation methods depending on the biopolymers involved, and the desired functionality. The most common biopolymer-based delivery systems are molecular complexes and hydrogel particles. Similarly, to emulsion based systems, they can also be used for development of more complex structures, such as filled hydrogel particles [1, 20].

1.1.4 Hybrid systems

The fundamental building components used in surfactant-, emulsion-, and biopolymer-based delivery systems can themselves be used to create structured encapsulation systems with novel or improved functional properties. In general, three main approaches could be used for preparation of more complex structured encapsulation systems: coating, embedding, and clustering. For example, by employment of electrostatic deposition additional biopolymer coatings could be form around charged particles in liposomes, emulsions, SLNs, multiple emulsions, or hydrogel particles. The embedding strategy can be used to trap microemulsions, emulsions, nanoemulsions, SLNs, or multiple emulsions within hydrogel particles. The clustering approach can be used to develop the colloidosomes or microclusters from nanoemulsions, emulsions, multiple emulsions, SLNs, or other types of particles [1, 4, 21].

2. ENCAPSULATION OF LIPOPHILIC ACTIVE INGREDIENTS

Lipophilic active ingredients (LAI) usually refers to diverse class of biological compounds with a wide range of activities, including antimicrobial, antioxidant, anti-inflammatory, health-improving and skin-conditioning properties of which the common and defining feature is their poor solubility in water [22-25]. This broad definition covers a number of different compound groups with various functionalities in the product formulations, such as triglycerides of animal, fish, or plant origin, fatty acids, phospholipids, carotenoids, phytosterols, oil-soluble vitamins, and essential oils with their individual constituents (Table 2) [26, 27]. Selection of the active ingredient therefore depends on the final application of the formulation/product. For example, the food industry has been interested in lipophilic components with health benefits, which can be used in the nutraceuticals and functional food [28, 29]. In the cosmetic industry, the trend continues to shift lipophilic substances from being used in common, daily-care products towards being part of newly-developed active ingredients providing stronger benefits to their targets whether it is the skin, hair, nail, mucous membrane, or teeth [30]. Today, besides the common application in cosmetics such as vehicles (bases), lipophilic ingredients have been used for special purposes in specific products for skin care and treatment or hair care. For example, some products are marketed as having anti-wrinkle and skin enhancement effects [31].

Table 2 Major classes of the most frequent lipophilic active ingredients used in cosmetics, pharmaceutical and food products and their selected examples [1, 10, 31-35].

Lipophilic Active Ingredient	Example
Triacylglycerols (particularly oils and fats rich in essential fatty acids and other bioactive compounds)	Plant oils: almond, apricot, avocado, macadamia, olive, soybean, sesame Fish oils: cod liver, salmon, tuna Plant fats: cocoa, coconut, palm, shea butter
Essential oils and their components	Oils: lemon, cinnamon, clove, thyme, tea tree, rosemary, pine, orange Components: limonene, carvacrol, citral, eugenol, thymol, cinnamaldehyde, linalool, linalyl acetate, eucalyptol
Fatty Acids	oleic (C18:1), conjugated linoleic acid (C18:2), α -linolenic acid (C18:3), eicosapentaenoic acid (C20:5), docosahexaenoic acid (C22:6)
Oil-soluble vitamins	A, D, E
Tocopherols	α -tocopherol
Carotenoids	lycopene, β -carotene, lutein
Phytosterols	stigmasterol, β -sitosterol

2.1 Encapsulation systems for lipophilic ingredients

A wide range of systems have been developed to encapsulate lipophilic ingredients (Figure 2). Each of them have their own specific advantages and disadvantages in terms of protection of active substance, delivery, cost, ease of use, biodegradability and biocompatibility [36]. Development of suitable systems can lead to improvement of the stability, water dispersibility, and efficacy of lipophilic ingredients in products, by maintaining/preservation of their biological properties [36, 37].

Among these strategies, emulsion-based systems are considered to be the most-common forms used for encapsulation of lipophilic actives. The thesis is focused on some of them, namely conventional emulsions (macroemulsions), nanoemulsions and mainly on emulsions stabilized with solid particles.

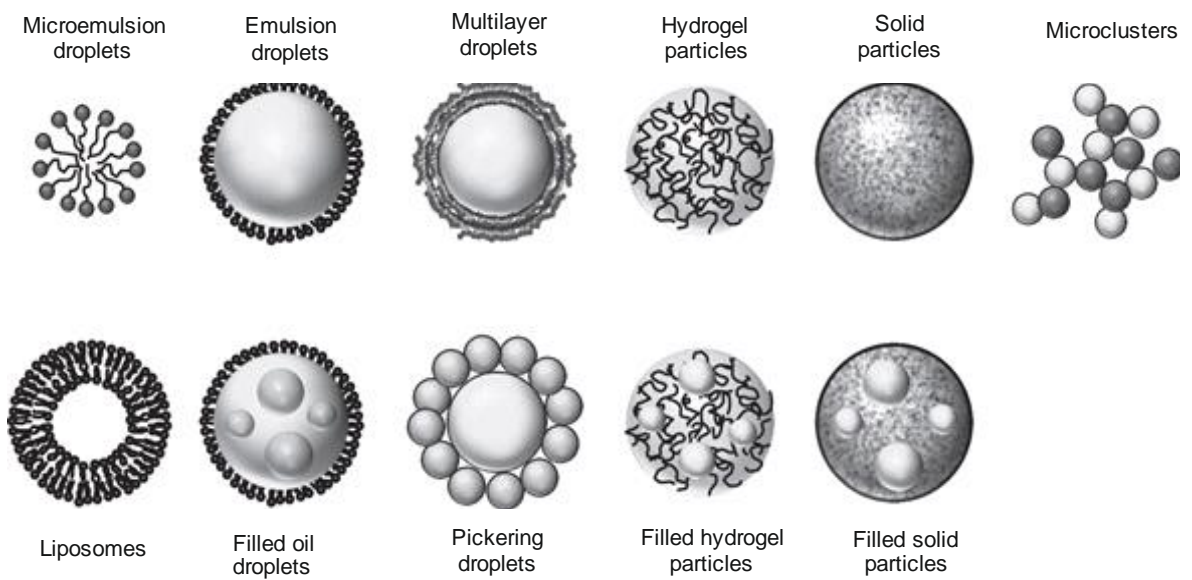


Figure 2 Examples of colloidal systems that can be used to encapsulate lipophilic bioactive agents [1].

3. PARTICLE-STABILIZED EMULSIONS

Emulsions find applications in a numerous industrial processes and commercial products where immiscible liquid phases coexist. Conventionally, emulsions are stabilized by the surfactants or amphiphilic polymers, which adsorb at the oil-water interface and prevent the droplet coalescence due to electrostatic and steric repulsive forces (Figure 3. Left). However, it has been showed that in many cases, they are not biologically compatible and are environmentally harmful. For example, some surfactants cause tissue irritation and even cell damage. These negative effects limit the use of surfactant-based products, especially in cosmetic, pharmaceutical, and biomedical applications [38]. Recently, there has been trend towards surfactant-free emulsions, driven by both legal and consumer requirements. On this basis, emulsions stabilized solely by fine solid particles instead of surfactants, have gained more interest of research and product development throughout various industries [39-41]. These emulsions are known as Pickering emulsions (Figure 3. Right), named after Spencer Umfreville Pickering [42], who reported first scientific study on particle stabilized emulsions. This classic work demonstrated clearly that fine solid particles are capable to remain at an oil–water interface and stabilize emulsion droplets. The presence of particles (usually nano- or microscale) at the interface affects not only the way of preparation, but also the properties of Pickering emulsions. Compared to classical emulsions stabilized by surfactants, particle-stabilized emulsions exhibit superior stability, low toxicity, and in particular cases also stimuli-responsiveness [43]. A wide variety of solid particles can be used in Pickering emulsions, including organic particles (such as cellulose and its derivatives, starch, and zein) or inorganic particles (e.g., silica, titanium dioxide, clay). Recently, thanks to the rapid development of material science, numerous alternative particles can be produced, which further enriches and expands the application of Pickering emulsions into different fields [43, 44]. Pickering stabilization is applied, for example, in emulsions and foams in food, pharmaceutical and cosmetic products.

On the other side, Pickering-type stabilization plays an adverse role in biological waste water purification or oil recovery, in which undesired foams and emulsions are stabilized by bacteria or minerals present in the system [45, 46].

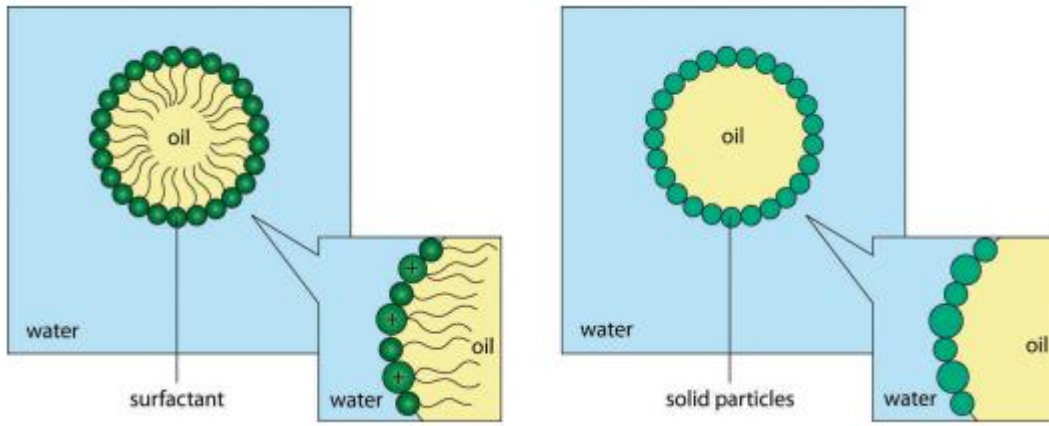


Figure 3 Surfactant-based emulsion (left) and a Pickering emulsion (right) [46].

3.1 Pickering emulsion theory

The Pickering stabilization mechanism is different from that of observed on conventional emulsifiers (surfactants and biopolymers). In principal, the stabilization of emulsions by particles is attributed to their partial dual wettability, resulting in the spontaneous accumulation of dispersed particles at the oil-water interface [47, 48]. This layer of closely packed particles surrounding the droplet provides so-called steric hindrance, which acts as a mechanical barrier and protects the emulsion droplets against coalescence. The assembly of particles at the oil-water interface is allowed by large reduction of total free energy, ΔG , occurring when a particle adsorb at the interface. This can be expressed by equation (1)

$$\Delta G = \pi\gamma_{ow}r_p^2(1 - \cos\theta_{ow})^2 \quad (1)$$

where r_p is the particle radius, γ_{ow} the interfacial tension at oil-water interfaces and θ the three-phase contact angle measured into the water phase [49]. This energy of attachment of particles to interfaces reaches a maximum at contact angle of 90° and for example, a particle with radius of $0.5 \mu\text{m}$ at a hydrocarbon/water interface ($\gamma_{ow} \approx 50 \text{ mN m}^{-1}$) will have $\Delta G \approx 10^6 k_B T$ (where k_B is Boltzmann's constant and T is the absolute temperature). Under these conditions, once the particles are attached to the oil/water interface, it is practically impossible to replace them from the interface. Therefore, the particles are considered to be irreversibly adsorbed, which provides an efficient barrier to droplet coalescence [50, 51]. In comparison, the surfactant molecules ($\approx 0.5 \text{ nm}$ in radius) adsorb and desorb on a relatively faster timescale, and they could be easily detached ($\Delta G \approx 5 k_B T$) and not be effective as stabilisers [49, 52]. Also it should be noted that the adsorption of particles does not lead to reduction in the interfacial tension [53].

As it has been mentioned previously, droplet stability in Pickering emulsions is mainly attributed to the steric hindrance provided by a surrounding particulate layer (Figure 4a). However, the presence of other configurations of stabilizing

particles, such as the sharing of adsorbed particles by two droplets, so-called bridging (Figure 4b), or a formation of a three-dimensional particle network between emulsion droplets, may contribute to the stabilization of droplets as well (Figure 4c) [47, 54]. It has been also shown that particles of non-spherical shape, e.g. polymer-based fibres, amorphous clumps or rod-like crystals, can act as stabilizers of Pickering emulsions (Figure 4d) [55].

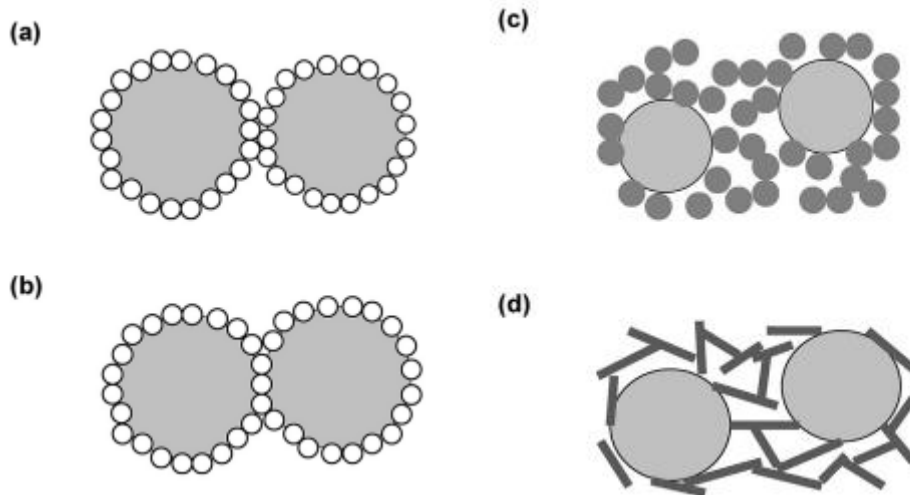


Figure 4 Possible configurations of particles in Pickering emulsions: a) particle bilayer; b) bridging layer; c) 3-D network of interconnected particles; d) stabilization by non-spherical (rod-like) polydisperse particles [55].

3.2 Factors influencing the stability of Pickering emulsions

From Eq. 1 it is evident that the stability of Pickering emulsions is influenced by the properties of the particles adsorbed at the interface. To prepare stable Pickering emulsions, it is necessary to use solid particle of an appropriate size, wettability, and concentration [50, 54]. Other factors involved in stabilization of the emulsions include the oil/water ratio, particle interactions, the ratio of hydrophilic to hydrophobic particles in mixtures, the presence of any other emulsifiers, and pH or ionic strength of the aqueous phase [56]. The mutual particle interactions plays also an important role, as it was already mentioned above [50].

3.2.1 Particle wettability (contact angle)

In order to adsorb at the interface, particles are required to be wetted by both liquid phases of emulsion. This dual particle wettability is the key feature of Pickering stabilization [49]. According to the empirical Finkle's rule [57], the type of emulsion formed, i.e. oil-in-water (o/w) or water-in-oil (w/o) depends on the preferential wettability of the particles in both liquids. In general, the less-wetting liquid becomes the dispersed phase of the emulsion. Similarly to the hydrophilic-lipophilic balance (HLB) number used to describe the preferential wettability of surfactants, the parameter determining particle wettability and a location of the

particle at an interface between oil and water is the contact angle [44, 48, 49, 58]. The contact angle also controls the type of emulsion formed, as can be seen in Figure 5.

By theory, the contact angle measured at the water phase, θ_w , is given by the Young's equation (2)

$$\cos\theta_w = \frac{\gamma_{so} - \gamma_{sw}}{\gamma_{ow}} \quad (2)$$

where γ_{so} , γ_{sw} , and γ_{ow} are the solid–oil, solid–water, and oil–water interfacial energies, respectively [59]. In practice, it has been shown that for hydrophilic particles with contact angles $\theta < 90^\circ$ oil-in-water emulsions are formed, whereas hydrophobic particles with contact angle $\theta > 90^\circ$ lead to water-in-oil emulsions [58, 60]. However, the particles very hydrophilic ($0^\circ \leq \theta \leq 20^\circ$) or very hydrophobic ($160^\circ \leq \theta \leq 180^\circ$) would become completely wetted by either the water or oil phase, which leads to the instability at the interface. In this case, the particles become completely dispersed in a single phase, and no stable emulsions can be produced [58-60]. In contrast, the contact angle equal to 90° corresponds to the point of phase inversion, where particles are equally wetted by both phases and the type of emulsion will be governed by the properties of particles as well as properties of liquids forming the emulsion phases [48, 61]. Examples of predominantly hydrophilic particles favouring o/w emulsions cover both particles of inorganic origin, such as metal oxides (unmodified silica, TiO_2 , and CuO) [62- 65], and organic-based materials, including polysaccharides (cellulose, chitin nanocrystals, and starch nanocrystals) [66-68]. In case of w/o emulsions, more hydrophobic particles such as polystyrene [69], Fe_3O_4 [65] or crystalline monoacylglycerols [70] have been employed. In addition, the hydrophobicity of some particles, such as silica or cellulose nanocrystals, can be tuned by varying the particle coating, surface modification, or the degree of substitution by functional groups [46, 71-73].

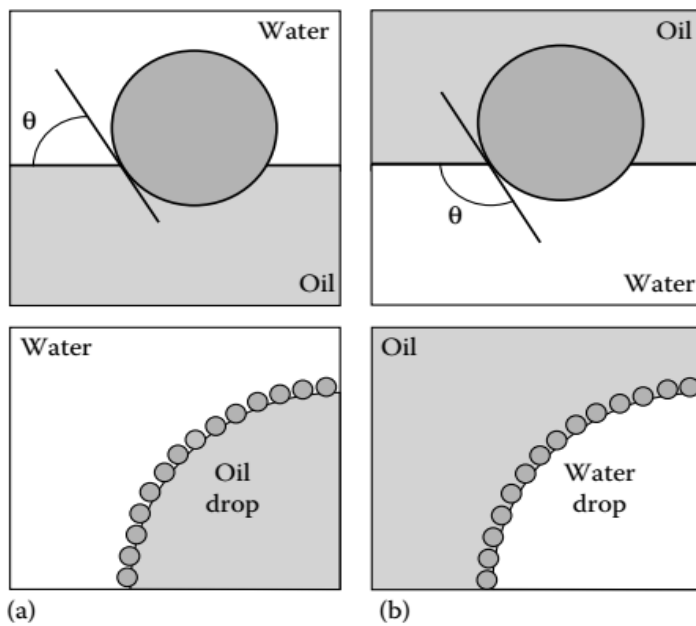


Figure 5 Changes in wettability of solid particle at the oil–water interface and the type of emulsion formed at different contact angles: (a) $\theta < 90^\circ$ favouring o/w emulsions, whereas (b) $\theta > 90^\circ$ favouring w/o [74].

3.2.2 Particle size

Stabilization energy of the particle assembly at the interface increases in proportion to the square of particle size r (Eq. 1). This indicates that the larger particles will provide better emulsion stability. For particles above a certain size (≈ 10 nm), the decrease in total free energy under absorption ΔG is much larger than the thermal energy, which leads to an irreversible adsorption of large particles at the interface. On the other hand, very small particles (< 1 nm) are attached to the interface by a ΔG comparable to the thermal energy. As a result, they can easily be replaced from the interface, and they may not be efficient as stabilizers [46, 48, 75].

3.2.3 Particle concentration

Particle concentration is also an important factor influencing emulsion stability. In general, the increase in particle concentration (at fixed content of disperse phase) leads to reduction of droplet size, and also improves surface coverage, which imparts to the emulsion additional stability [43, 48]. This trend has however a limit, at which one fraction of particles acts as stabilizer, whereas another fraction would remain non-adsorbed in one of the phases. At this point further decrease in droplet size can only be achieved by improving the emulsification conditions [48, 54, 76]. It has been showed that high particle concentrations do not necessarily lead to a full coverage of the droplet by particles, as in some emulsions stable droplets were observed even without this dense coverage [54, 55].

3.3 Formation of Pickering emulsions

The preparation of a Pickering emulsion involves the dispersion of particles into the continuous phase of an emulsion, following by distribution of the particles, based on their partial wettability, in each of the two immiscible phases [38]. Similarly to classical surfactant-stabilized emulsions, Pickering emulsions are formed by mixing a water and oil phase together in presence of emulsifier, in this case solid particles. As mentioned earlier, the high energy required to remove adsorbed particles from the o/w interface results in the superior stability of Pickering emulsions compared with conventional emulsions. On the other hand, in most cases, in order to prepare a stable Pickering emulsion, the high-energy methods, such as homogenization or sonication, need to be applied to overcome this energy barrier [43]. Various types of Pickering emulsions across different industries have been well-documented in literature, including multiply Pickering emulsions [44, 48].

4. NANOCELLULOSE-STABILIZED EMULSIONS

4.1 Cellulose

Cellulose is the most abundant naturally occurring biopolymer, with about 10^{10} – 10^{11} tons produced per year globally [77]. As a renewable, biodegradable, and also non-toxic material, which simultaneously combine low price and excellent mechanical properties, cellulose and its derivatives have played an important role in the development of novel environmentally friendly and biocompatible products [78, 79].

4.1.1 Source materials

Cellulose serves as the main building block of the cell wall structure of higher (vascular) plants, and is often combined with other biopolymers, such as lignin and hemicelluloses [80]. Cellulose can be isolated from various sources. The main sources of cellulose are plants, including wood, bast fibres (flax, hemp), seed fibres (cotton), grasses (bagasse, bamboo) and their agricultural residues. Alternative cellulose feedstocks are marine animals (tunicate), algae, fungi, invertebrates, and bacteria [81-83]. Among the sources, cotton and bast fibres are the purest materials, containing approximately 90 and 70–80% w/w cellulose, respectively. In comparison, the major industrial source of cellulose, wood consists of only 40–50% w/w cellulose [84]. In case of nonconventional sources, the cellulose content in aquatic species is reported to be in the range of 10–40% w/w, and the yield produced by biotechnological process employing bacteria may reach at maximum 0.6 g/g of glucose per day [78].

4.1.2 Chemical composition and structure

Regardless of its source, the cellulose can be characterized as a linear homopolysaccharide composed of anhydrous *D*-glucose molecules (so called AGU units) linked together by β -1,4-glycosidic bonds. The smallest repeating unit in the polymer, cellobiose, consists of two AGU [80, 84, 85]. The average chain length of cellulose, indicated by the number of AGU unites, is usually expressed as degree of polymerization (DP). The DP of cellulose varies depending on the source material and is of about 10 000 in native wood ($M \approx 2\,000\,000\text{ g mol}^{-1}$), as well as on the isolation method. In processes connected with isolation of cellulose from wood the DP further reduces to about 300–3000 ($M \approx 50\,000\text{--}500\,000\text{ g mol}^{-1}$) [78, 80, 84]. Each AGU has six carbon atoms with three hydroxyl groups (at C2, C3, and C6 atoms), which gives cellulose molecule a high degree of functionality. Due to the molecular structure, cellulose possesses advantageous properties, such as hydrophilicity, insolubility in water and most organic solvents, degradability and chirality [84, 86, 87].

Native cellulose does not occur as an isolated molecule but is found in the form of fibrils (Figure 6). Cellulose is synthesized in the cells in the form of individual molecules that crosslink with each other and produce fibrillary structures during

biosynthesis [88]. The basic structural units, known as elementary fibrils or nanofibers, are made of 36 individual cellulose molecules with diameter of about 5 nm. These entities aggregate into microfibrils, which are up to 20 nm in diameter and a few micrometers in length, where the cellulose chains are arranged in crystalline (highly ordered) and amorphous (disordered) regions. These microfibrils further aggregate into larger entities called macrofibrils and cellulose fibers, which are visible under a light microscope [80, 89-92].

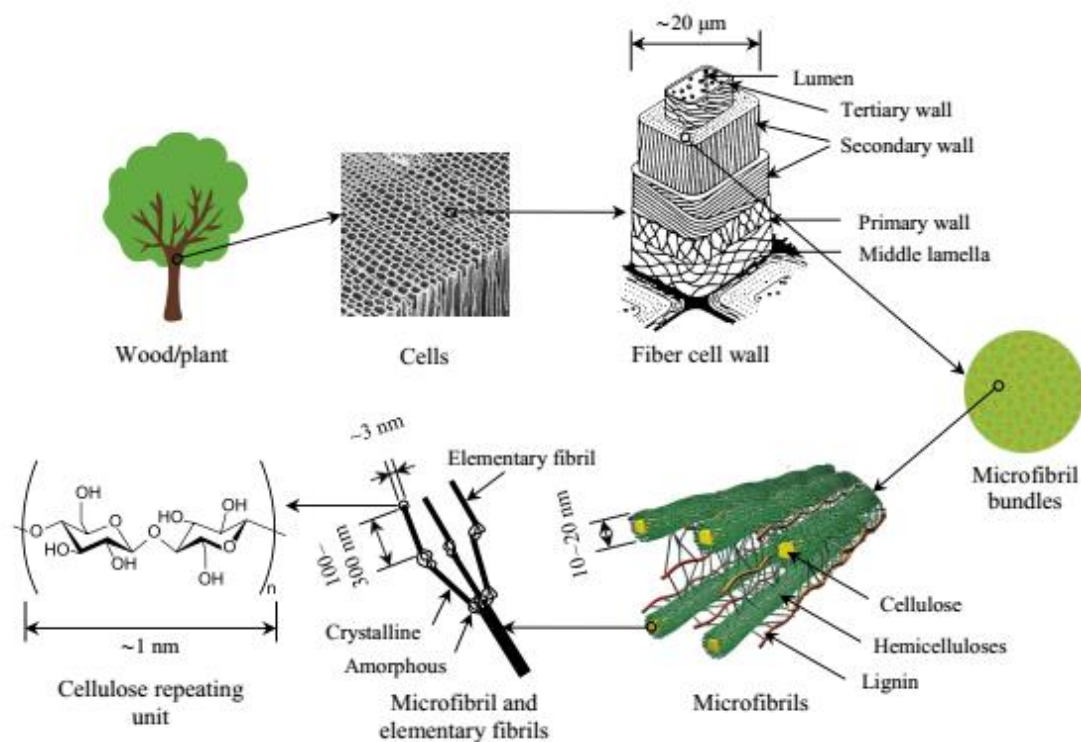


Figure 6 Schematic diagram of the structure and composition of the wood/plant [86].

4.2 Nanocellulose

Cellulose can also be used as a source for nanoscale cellulose materials. The individual cellulose elements with a diameter in nanometre range (< 100 nm) are generally referred to as nanocellulose. Based on the treatment conditions, which affect the dimensions, composition, and properties, the nanocellulose may be classified in three main categories: (i) cellulose nanocrystals (CNCs) also called whiskers; (ii) nanofibrillated cellulose (NFC) alternatively named microfibrillated cellulose (MFC), or cellulose nano-/microfibrils and (iii) bacterial nanocellulose (BNC) or microbial cellulose. However, it should be noted that standard terminology and nomenclature for different types of cellulose nanomaterials has not yet been adopted [86, 93, 94]. Summary of nanocellulose types with average dimensions is presented in Table 3.

In general, CNCs and MFC are produced by top-down methods involving enzymatic/chemical/physical disintegration of cellulose fibers to the nanosized

material. In contrast, BNC is fabricated *via* bottom-up approach from low molecular weight sugars by aerobic bacteria. Irrespective of the particles type, nanocellulose exhibits hydrophilic character, relatively large specific surface area, broad potential of surface chemical modification, and excellent mechanical strength [86, 94]. These properties make the nanocellulose promising source for the various applications such as novel composites, bioactive filters and membranes, food packaging, drug delivery, stimuli-responsive materials, biomedical applications and many others [79, 81, 94].

Table 3 Dimensions of nanocellulose particles from published studies [85].

Cellulose Type	Diameter (nm)	Length (nm)	Aspect Ratio
Microfibrillated cellulose	10–40	> 10 000	> 1 000
Cellulose nanocrystals	2–20	100–600	10–100
Bacterial cellulose	5–6	1 000–9 000	160–1 800

4.2.1 Microfibrillated cellulose

Microfibrillated cellulose (MFC) is produced from purified wood-based fibres using a variety of mechanical processes including high-pressure homogenization, high-intensity ultrasonic treatments, and microfluidization [88]. These treatments generate high shearing forces resulting in a strongly entangled and disordered networks of nanofibrils, having both crystalline and amorphous domains [78, 84, 88]. Usually, before performing the final mechanical separation to MFC, the raw materials are pre-treated mechanically, chemically, and/or enzymatically in order to reduce energy consumption in the process [95].

Newly produced MFC forms an aqueous gel (2–7% w/w) possessing pseudoplastic and thixotropic properties [96]. Depending on the processing conditions, cellulose fibres can be further disintegrated to nanofibrils with the lateral dimensions from ~ 3 nm, which represents elementary fibrils, to tens of nanometres, corresponding to microfibrils [86, 97]. Typically, MFC has a diameter of 10–40 nm and a length of few micrometres with an aspect ratio (length to diameter ratio, L:D) of nearly 1 000 [85].

4.2.2 Cellulose nanocrystals

Cellulose nanocrystals (CNCs) are highly crystalline, rod-shaped particles with nanoscale dimensions. CNCs are commonly obtained through hydrolysis of cellulose-containing materials, which selectively dissolves the amorphous cellulose regions, while leaving the less accessible crystalline parts unaffected [98, 99]. The first report on colloidal cellulose nanocrystals was published by Rånby [100] who treated wood and cotton with concentrated sulfuric acid. Acid hydrolysis by using concentrated mineral acids such as sulfuric and hydrochloric acids is the most common preparation method of CNCs [99]. More recently, CNCs have been obtained by more energy-efficient production, whereby cellulose

materials were oxidized with ammonium persulfate [101] or 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl radical (TEMPO) [102]. The size of the crystallites depends on the hydrolysis conditions and the cellulose source. In general, a milder hydrolysis and/or a more crystalline source material result in larger particles [98]. Selection of hydrolysis/oxidation treatment also determines the surface charge of resulting CNCs. While hydrolysis of cellulose with hydrochloric acid gives uncharged nanocrystals, CNCs produced by sulfuric acid and ammonium persulfate routes are charged negatively, with charge originating from surface bearing sulphate half-esters ($-\text{OSO}_3^-$) and carboxyl ($-\text{COO}^-$) groups, respectively. Suspensions of uncharged CNCs tend to flocculate or gel at low concentrations ($< 0.5\%$ w/v) due to strong interactions between particles. On the contrary, charged CNCs are readily dispersed in water and form stable colloidal dispersions (2–10% w/w) as a result of electrostatic repulsion between particles [84, 94, 98, 103]. However, increasing ionic strength of medium leads to their aggregation caused by shielding of the charges [104, 105].

Morphology and dimensions of CNCs depend mainly on the source of cellulose. Typically, nanocrystals with diameters of 2–20 nm and lengths of 100–600 nm are produced [85]. Compare to MFC, CNCs exhibits lower aspect ratio ranging between 10 and 100.

4.3 Application of nanocellulose for emulsion stabilization

Various grades of nanocellulose particles, including MFC [106], CNCs [107, 108] and BNC [66, 109], have been successfully used as particulate stabilizer offering sustainable and potentially low-cost replacement of standard surfactants used for preparation of emulsions [110]. In comparison with other types of particulate stabilizers, nanocellulose have shown superior properties when it comes to biocompatibility, biodegradability, low density, thermo-mechanical behaviour and costs [90, 111].

Due to nanocellulose hydrophilic nature, o/w emulsions are preferentially formed [66, 106]. In order to produce w/o emulsions, suitable surface modifications need to be introduced. These modifications involve for example silylation [73], polymer grafting, [112] or esterification [113]. In this context, the contact angle between a particle and a given liquid–liquid interface is also of great importance and can successfully be controlled by the degree and type of particle functionality. By this way, the emulsions with tailor-made properties for specific applications can be prepared. For example, using multifunctional proteins leads to a self-assembly properties of NFC [114] and grafting of CNCs with poly(*N*-isopropylacrylamide) or poly[2-(dimethylamino)ethyl methacrylate] affords temperature-sensitive or pH-responsive properties to emulsions [38]. Recently, preparation of multiple emulsions, particularly, oil-in-water-in-oil have also been reported by using both native and hydrophobized NFC and CNCs [71]. In addition to ability of cellulose particles to stabilize different types of emulsions, they are

also reported to afford systems with reasonably good stability during storage, including those highly resistant to coalescence [115].

Among all three reported cellulose types, the behaviour of CNC particles as emulsion stabilisers is better described in literature and this cellulose type is also more frequently used for preparation of Pickering emulsions. Previous works, for example, clearly documented that unmodified CNCs can efficiently stabilize emulsions and perform better when having a low surface charge density, or when the surface charge is screened by the presence of salts in dispersion medium [116]. Furthermore, lower concentrations of CNCs are needed for stabilization of emulsion droplets when nanocrystals with higher aspect ratios are used. On the contrary, MFC-based emulsions have received less attention and it was reported that, due to its very high aspect ratio, MFC forms in emulsions strongly entangled and disordered networks. This stabilizing network is more evident at higher MFC concentrations and it contributes to overall stability of the emulsions [106, 117]. Regarding bacterial cellulose, its stabilizing properties are not yet fully explored [109], however BNC obtained by hydrochloric acid hydrolysis of bacterial cellulose were successfully used for stabilization of hexadecane/water interface [66].

Until now, the research on the Pickering emulsions have primarily been focused on model systems containing well-defined oils, such as hydrocarbons, as dispersed phase. Most of the studies worked with emulsions having a low content of oil phase, being in the range of 10–30% w/w. Trials to incorporate larger amounts of oil, high internal phase emulsions (HIPEs) with oil fractions higher than 90% w/w stabilized by less than 0.1% w/w CNCs have been recently reported [116]. Recently, several works have also been devoted to emulsion formulations containing oils derived from plant sources. For example, in study by Mikulcová et al. (2016) [107], CNC and MFC particles were used to stabilize emulsions loaded with up to 40% w/w limonene, cinnamaldehyde, or eugenol with more than 95% encapsulation efficiency. In another work, Wen et al. (2014) [108] reported on carboxylated CNCs prepared from corn-cob cellulose by oxidation with ammonium persulfate, which were then used to prepare d-limonene emulsions. An observed reduction in stability with respect to creaming at low pH or high salt concentration was attributed to an electrostatic screening effect. Currently, CNCs obtained by sulfuric acid hydrolysis treatment of asparagus stems showed a satisfying stabilization for food grade palm oil emulsions [118]. In regard to stabilization properties of MFC, the particles isolated from mangosteen rind were used to prepare stable soybean oil-in-water emulsions at neutral pH [106].

In principle, previous studies have shown that various cellulosic particles derived from different sources, are capable of acting as stabilizing agents of emulsion systems. It was established that these particles adsorb at the oil-water interface, thus providing a steric barrier against droplet coalescence *via* the Pickering mechanism. Moreover, some works reported on contribution to the

stabilization from an interconnected network of fibrils present is dispersion phase of emulsion [106, 117]. Despite the significant research progress on Pickering emulsions stabilized by cellulosic particles, more investigations are needed to be done for a better understanding of the surface properties, adsorption and aggregation of particles at the oil-water interface.

5. EVALUATION OF THE STUDIED EMULSION SYSTEMS

In this work, two main types of emulsion-based systems, conventional and Pickering emulsions, have been prepared and studied. This part of the thesis will provide their brief and critical evaluation.

For a long time, conventional emulsions have dominated across the industries due to their easy use, relatively low costs and controlled preparation. However, in recent years, emulsions stabilized solely by solid particles, have attracted more attention. Micro- or nano-sized particles, as the main components of the Pickering emulsion, affect not only the preparation, but also the potential applications and properties of resulting formulations.

During research conducted within this doctoral thesis, surfactant-based and particle-based emulsions, each one consisting of antimicrobial oil of plant origin, were successfully prepared [107, 119]. Owing to different mechanism of stabilization, as described previously, these systems differ in various aspects such as type of stabilizing agent (surfactants *vs* particles) and its concentration needed for successful droplet stabilization, emulsifying properties, emulsification techniques needed for their preparation, encapsulated volume of oil phase, size of emulsion droplets, long-term stability, intended application etc. Some of these key features are discussed in the context of experimental results from the doctoral study.

To begin with, selection of suitable emulsifying agent required to produce stable classical emulsions is based on HLB system, whereas the crucial parameter for preparation of Pickering emulsions is the particle contact angle [49, 62]. In our work, classical o/w emulsions were formulated by employing pairs of non-ionic surfactants (Span, Tween) at HLB values ranging from 6 to 10, in order to find an optimum HLB for best performances in emulsification process and stability studies. In case of the study conducted on the emulsions stabilized by particles, naturally hydrophilic cellulose particles with water contact angle of approximately 50° favouring formation of o/w emulsions were selected [21]. The overall results revealed that type of the stabilizing agent, whether they are emulsifiers or particles, and its concentration play an important role in both conventional and Pickering emulsions. In particular, for classical emulsions the most efficient emulsification was observed at 10% w/w concentration of surfactants. By comparison, only relatively small particle concentrations were required to successfully stabilize Pickering emulsions. It has been shown that up to 40% w/w oil can be safely encapsulated by using of only 0.5% w/w of cellulose particles. This contrasted with classical emulsions, where at least 5% w/w of surfactants concentration was required for stabilization of 5% w/w oil content. Though the antimicrobial oils used in both types of emulsions were of different origin, which will influence performance of resulting emulsions, this comparison indicates that particle stabilized emulsions might be of advantage with respect to

reduction of presence and amount of surfactants in cosmetic products, which are known for their irritation potential.

Regarding the long-term stability, particle-stabilized emulsions are considered to be of superior stability to classical emulsions, which has also been confirmed by our studies. Once prepared, Pickering emulsions showed superior stability irrespective of used type of cellulose particles and their concentrations. In case of conventional emulsions, storage stability period ranged from few days (10% w/w Tween 85/Span 85-based systems at HLB 7) to several months (10% w/w Tween 80/Span 80-based systems at HLB 9). Excellent long-term stability provided by Pickering emulsions is well-known and it is mainly attributed to the strong binding energies of the particle stabilizers with the interface, which arise from the relatively large size of solid particles (typically between 100 nm and 5 μm), when compared to surfactant molecules (0.4 to 1 nm) [10]. As demonstrated Binks et al. [49], surfactants adsorb at the interface less strongly than particles and produce emulsions with a reduced long-term stability.

Interestingly, conventional emulsifiers usually yielded smaller emulsion droplets compared to particles [9]. For example, in our studies, using Span–Tween systems resulted in droplet size ranging from 80 to 650 nm depending on the emulsification process. By comparison, the mean diameter of the droplets obtained when using cellulosic particles as stabilizer was of about 14–50 μm . This is, however, natural as stabilizing cellulose particles are notably bigger in their sizes in comparison with molecules of surfactants.

The other specific feature of the particles used as stabilizers is the fact that shape and size of particles influence the properties of the resulting emulsions [58]. For instance, in our study, emulsions prepared with smaller CNC particles afforded smaller droplets (14–34 μm) compared to those stabilized with larger MFC particles (27–51 μm). Additionally, the shape of the particles influenced the properties and performance of emulsions as well and contributed, for example, to antibacterial properties of the emulsions, especially at low content of antimicrobial oil. With this respect, CNC rod-shaped particles provided a more homogenous layer at the oil-water interface thus preventing oil leakage from the dispersed phase of emulsions into their continuous phase.

Another great advantage of using particles instead of surfactants is the possibility to modify the properties of particle surfaces and thus afford functionalities that are difficult to achieve in conventional emulsions. In this respect, stimuli-responsive systems, whose properties can be altered by external stimuli, such as temperature, pH, ionic strength, electric/magnetic fields, or light, has become increasingly important [120]. The interest in such responsive systems is reflected, for instance, in our systematic study on the pH-dependent behaviour of emulsions stabilized with carboxylated cellulose nanocrystals (cCNC) [121]. The employed cCNC was prepared *via* a one-step oxidation procedure of cellulose by APS, thus introducing carboxylic acid groups on the particles surface. By this way, a certain pH responsiveness to the colloidal suspension can be imparted,

which in turn has a key impact on colloidal behaviour of cCNC in environment with various pH values and ionic strengths.

In conclusion, the presented studies unambiguously documented that Pickering emulsions are promising systems, which may exhibit some unusual, advantageous features compared to classical emulsions. From an application perspective, biopolymer-based particles used as stabilizing agents are of particular interest. These particles are cheap, abundant in nature and less harmful alternative to conventional surfactants. For these reasons, the field of Pickering emulsions is recently receiving much attention both from the research and also industrial community. On the other way, emulsion systems stabilized with classical emulsifiers are still prevailingly used in practice and probably will never be fully replace by Pickering emulsions. Both emulsion types have their advantages and disadvantages and users should always critically evaluate the choice of the stabilizer, which is suitable for given application.

6. AIMS OF THE DOCTORAL STUDY AND THEIR FULFILMENT

The doctoral study “Dispersion systems as carriers of active substances” is focused on the formulation, preparation and characterization of the suitable particular systems with the ability to carry, effectively encapsulate and release active substances. The main goals of the work have been subdivided into three following areas covering different types of systems addressed:

6.1 Emulsions stabilized with surfactants

- a) Preparation of (nano)emulsion loaded with hemp seed oil by different emulsification methods (spontaneous emulsification vs high-intensity stirring) and comparison of the resulting emulsions in terms of their long-term stability and droplet size/distribution determined by photon correlation spectroscopy (PCS).
- b) Simultaneous monitoring of the effect of different physicochemical parameters, such as oil type (refined, unrefined), o/w ratio, HLB value, surfactant type and concentration on the parameters given above.
- c) Determination of antibacterial activity of the emulsions and hemp seeds oils used therein against common pathogenic bacteria.

This goal of the thesis was completed and results have been published in the paper Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions in the Molecules in 2017.

6.2 Emulsions stabilized with particles

- a) Preparation of cellulose nanocrystals (CNCs), intended for stabilization of emulsions, by using different procedures, concretely oxidation of microcrystalline cellulose (MCC) with ammonium persulfate or its hydrolysis with sulfuric acid. Comparison and characterization of prepared nanocrystals in terms of their morphology (AFM), particle size and zeta-potential (PCS) as well as particle charge density (titration, particle charge detector). Assessment of behaviour of the CNCs in aqueous suspensions in presence of salts or under different pH.
- b) Formulation of Pickering emulsions containing three different antimicrobial oils (cinnamaldehyde, eugenol and limonene) stabilized with CNCs prepared by hydrolysis route and commercial MFC. Evaluation of the effect of oil type, o/w ratio, type and concentration of cellulose particles on the physicochemical characteristics of the emulsions, including their stability during storage. Testing of

antibacterial activity of the emulsions against common pathogenic bacteria.

- c) Preparation of pH responsive triacylglycerol-in-water emulsions stabilized with carboxylated cellulose nanocrystals (cCNC) suited as carriers for lipophilic active ingredients in cosmetics and pharmacy. Physicochemical characterization of prepared emulsions by droplet size and zeta-potential analyses, phase behavior and determination of their pH responsiveness.
- d) Formation of model Pickering emulsions stabilized with binary mixtures of two different types of cellulose particles, namely MFC and CNCs, and their comparison with systems where each of the particle types was used individually. Examination of the influence of different physicochemical parameters, including type and concentration of cellulose particles, mass ratio MFC/CNCs in the mixtures, and o/w ratio on the size and distribution of emulsion droplets (laser diffraction), encapsulation efficacy and phase stability (optical microscopy).
- e) Introductory studies into biological properties of Pickering emulsions stabilized with CNCs or MFC and loaded with antibacterial oils (limonene, eugenol, cinnamaldehyde). Examination of transdermal absorption of these lipophilic active ingredients from MFC and CNCs emulsions by using the Franz diffusion cells on porcine skin permeation model. Examination of cytotoxicity of the emulsions on selected cell lines.

The tasks a) to c) were completed and resulted in two publications:

1) "On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles" published in the Food Hydrocolloids, 2016 and 2) "Pickering oil-in-water emulsions stabilized by carboxylated cellulose nanocrystals - effect of the pH" submitted to Carbohydrate Polymers.

In case of task d), results were obtained and publication is under preparation. Regarding the task e), work is in progress.

6.3 Emulsion-coated surfaces

- a) Preparation of surfaces coated with emulsion droplets by employing soft surface treatment, with the first layer formed by chitosan and the second one consisting of cCNC-stabilized emulsions containing essential oils.
- b) Real-time monitoring of adsorption processes by quartz crystal microbalance with dissipation monitoring (QCM-D). Characterization of prepared surfaces and determination of their antibacterial properties.

This goal of the thesis is still in progress.

LIST OF PAPERS

Publication I

MIKULCOVÁ, V., KAŠPÁRKOVÁ, V., HUMPOLÍČEK, P. and L. BUŇKOVÁ, Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions. *Molecules*, 2017. **22(5)**: p. 700.

Publication II

MIKULCOVÁ, V., BORDES, R. and V. KAŠPÁRKOVÁ, On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles. *Food Hydrocolloids*, 2016. **61**: p. 780-792.

Publication III

MIKULCOVÁ, V., BORDES, R., MINAŘÍK, A. and V. KAŠPÁRKOVÁ, Pickering oil-in-water emulsions stabilized by carboxylated cellulose nanocrystals - effect of the pH. *Submitted to Carbohydrate Polymers*.

Publication IV

KEJLOVÁ, K., KAŠPÁRKOVÁ, V., KRSEK, D., JÍROVÁ, D., KOLÁŘOVÁ, H., DVOŘÁKOVÁ, M., TOMÁNKOVÁ, K. and V. MIKULCOVÁ, Characteristics of silver nanoparticles in vehicles for biological applications. *International Journal of Pharmaceutics*, 2015. **496(2)**: p. 878-885.

SUMMARY OF THE PAPERS

Paper I was focused on the formulation and characterization of hemp seed oil emulsions stabilized with pairs of non-ionic surfactants, prepared by low energy (emulsion inversion point) and high energy (high-intensity stirring) methods. The goal of this paper was to determine the influence of composition (HLB value, concentration of surfactants and content of encapsulated oil) and preparation procedure on properties of formulated emulsions, including their stability. In addition, the antibacterial potential of the hemp seed oil and prepared emulsions against common pathogens was evaluated.

It was found that the formation of stable emulsions of small, initial particle size was primarily dependent on the given method of preparation and the HLB value of the used surfactant pairs. In particular, the high-energy method led to efficient emulsification that afforded the emulsions with fine particles (151 ± 1 nm). Regarding the long-term stability, emulsions prepared at HLB 9 with 10% w/w concentration of surfactants performed best.

Testing of antibacterial properties of the oils using the disk diffusion and broth microdilution methods revealed the inhibition effects against *Micrococcus luteus* and *Staphylococcus aureus*, subsp. *aureus*. However, the emulsions formulated did not exhibit the antibacterial activity that had been anticipated.

In **Paper II**, Pickering emulsions containing antimicrobial oils (cinnamaldehyde, eugenol and limonene) stabilized with cellulose particles (CNCs and MFC) were successfully prepared and the effects of the oil type, o/w ratio, type and concentration of cellulose on the physicochemical characteristics of the emulsions, such as droplet size and phase behavior, were determined. The antibacterial activity of emulsions towards the most common gram positive and gram negative bacteria was investigated using agar diffusion and broth dilution methods.

The results showed that both CNC and MFC were capable to produce o/w Pickering emulsions with antimicrobial oil content as high as 40% w/w even at the lowest concentration of cellulose used 0.1% w/w. It was also found out that emulsion droplets stabilized with CNC particles were smaller than those stabilized with MFC. The prepared emulsions showed reasonably good stability during the 8-week storage in terms of changes in droplet size as well as occurrence of creaming or sedimentation.

Antibacterial testing revealed that the activity of emulsions was mainly influenced by the type and content of antibacterial oil loaded in emulsion droplets. The type of nanocellulose particles used for droplet stabilization showed, in this respect, only a minor contribution which was observed mainly at high concentrations of emulsions. In contrast, at low concentrations of emulsion, the effect of nanocellulose type appeared to be more important and MFC-stabilized emulsion exhibited better antibacterial activity.

Paper III was aimed at formulation of pH responsive triacylglycerol-in-water emulsions stabilized with carboxylated cellulose nanocrystals (cCNC). The effect of varying pH of dispersion phase (pH 2, 4, 7) on the particle characteristics and emulsion properties was studied and established. In the first step of the work, nanocrystals of carboxylated cellulose were prepared by oxidation of microcrystalline cellulose with ammonium persulfate and their physicochemical properties were determined, including behaviour in media with different pH and ionic strength, and visualization of particles by AFM.

In the next step, the emulsions were prepared. The results revealed, that using cCNC, stable Pickering triglyceride-in-water emulsions at pH of 2, 4 and 7 can be formulated. The size of emulsions droplets was influenced by oil and cCNC contents. Nevertheless, the most crucial parameter for emulsion formation was the pH value of the continuous phase and it was revealed that droplet size and stability of emulsions are governed just by the pH. At the same time, responsiveness of emulsions towards pH changes was not as dominant as expected, and pH variation did not trigger the release of oil from droplets. The strong adsorption of the cCNC onto the droplet surface, relatively polar triglyceride oil used in the droplets, and the limited ability to induce desorption of nanocrystals from oil surface could be possible explanation for this finding.

Paper IV

Main subject of this study was to assess behavior of silver nanoparticles (AgNPs) in various media used for testing of their biological properties. The behavior of AgNPs in terms of size and distribution was determined using two independent methods, photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM). The particles were subjected to contact with culture medium with/without serum (DMEM) and phosphate buffered saline (PBS).

Comparison of PCS and TEM analyses showed that both techniques provided qualitatively similar information with respect to characterization of the tested AgNPs, however the size of particles measured by PCS was bigger in absolute values. The study revealed that during exposure of AgNPs to the PBS and DMEM without serum, the agglomeration process occurred leading to the growth of the particles. The effect was due to the presence of ions in the dispersion medium. Interestingly, although the average size of the particles increased, width of the size distribution was not substantially changed during on-going agglomeration and only negligible distribution broadening was observed. Behavior of AgNPs in both serum free and serum added DMEM exhibited comparable behavior in terms of particle size distribution. However, the impact of serum proteins as such cannot be omitted. This study contributed to increased knowledge of AgNPs behavior in contact with physiologically relevant liquids. This can serve as a supporting information when using the AgNP in dispersion formulations to enhance their antibacterial properties.

CONTRIBUTION TO SCIENCE AND PRACTICE

The most important contributions of the doctoral thesis to science and practice can be summarized as follows:

- Better understanding of the preparation of emulsions by low energy (emulsion inversion point) and high energy (high-intensity stirring) methods; developing of an optimized formulation possessing good long-term stability.
 - *The outputs gathered within this part of the thesis can be used under preparation of cosmetic and food formulations containing triacylglycerol-based oils.*
- The mastering of the preparation of surfactant-free emulsions stabilized by solid particles intended for cosmetic or food applications.
- The development of novel antibacterial Pickering emulsions containing essential oils and stabilized by different cellulosic nanoparticles.
 - *The work related to Pickering emulsion brings the increased knowledge within theory and understanding of these systems and provides evidence of their practical applications in antibacterial protection.*
- The successful preparation of carboxylated cellulose nanocrystals containing –COOH groups on the surface by low-energy one-step procedure; clarification of their colloidal behaviour at various pH and ionic strength.
- The formulation of pH responsive triacylglycerol-in-water emulsions stabilized with cCNCs suited as carriers for lipophilic active ingredients in cosmetics and pharmacy.
 - *This part of the work notably contributed to description of encapsulation systems which are responsive to external stimuli, in this case pH. In practice, the pH responsiveness can be used for cosmetic, pharmaceutical and food applications.*

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LIST OF ABBREVIATIONS

AFM	atomic force microscopy
AgNPs	silver nanoparticles
AGU	anhydrous <i>D</i> -glucose units
APS	ammonium persulfate
BNC	bacterial nanocellulose
cCNCs	carboxylated cellulose nanocrystals
CI	creaming index
CNCs	cellulose nanocrystals
DLS	dynamic light scattering
DMEM	Dulbecco's Modified Eagle's medium
DP	degree of polymerization
EI	emulsion index
HIPES	high internal phase emulsions
HLB	hydrophilic-lipophilic balance
LAI	lipophilic active ingredients
LD	laser diffraction
MCC	microcrystalline cellulose
MFC	microfibrillated cellulose
OP	optical microscopy
PBS	phosphate buffered saline
PCS	photon correlation spectroscopy
PCD	particle charge density
PLA	poly(lactic acid)
PLGA	poly(lactic- <i>co</i> -glycolic acid)
QCM-D	quartz crystal microbalance with dissipation monitoring
SLNs	solid lipid nanoparticles
SPM	scanning probe microscopy
TEM	transmission electron microscopy
ZP	Zeta potential

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LIST OF PUBLICATIONS

Articles in journals with impact factor:

1. MIKULCOVÁ, V., R. BORDES, a V. KAŠPÁRKOVÁ, On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles. *Food Hydrocolloids*, 2016. **61**: p. 780-792.
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