Stabilization of dispersion systems by polymeric emulsifiers

Ing. Lucie Urbánková, Ph.D.

Doctoral Thesis Summary

Tomas Bata University in Zlín

Tomas Bata University in Zlín
Faculty of Technology

Doctoral thesis summary

Stabilization of dispersion systems by polymeric emulsifiers

Stabilizace disperzních systémů polymerními emulgátory

Author: **Ing. Lucie Urbánková, Ph.D.**

Study programme: P2808 Chemistry and material technology

Study course: 2808V006 Technology of macromolecular compounds

Supervisor: doc. Ing. Věra Kašpárková, CSc.

Reviewers: doc. Ing. Zdeňka Kolská, Ph.D.
prof. Mgr. Marek Koutný, Ph.D.

Zlín, June 2020

Published by **Tomas Bata University in Zlín** in the Edition **Doctoral Thesis Summary.**
The publication was issued in the year 2020.

Key words in Czech: *biopolymer, stabilizace, protein, polysacharid, nanocelulóza, interakce, emulze*.

Key words: *biopolymer, stabilization, protein, polysaccharide, nanocellulose, interaction, emulsion.*

Full text of the doctoral thesis is available in the Library of TBU in Zlín.

ISBN 978-80-7454-925-0

ACKNOWLEDGMENT

First and foremost, I would like to express my deepest gratefulness to my supervisor, doc. Ing. Věra Kašpárková, CSc., for her excellent guidance, care, patience, sharing insightful suggestions, as well for her support during my Ph.D. study.

I would like to also thank Dr. Romain Bordes, for allowing me to work in his group at the Chalmers University of Technology, providing me an inspirational work environment and the possibility of expanding valuable scientific knowledge.

My great thanks go to the members and the technicians of the Fat, Surfactants and Cosmetics Technology Department for a friendly working environment and their help during my doctoral study.

Special thanks also belong to my family, especially to my husband for his support, patience and endless love. Thanks belong also to my sister, for her help with the thesis. Finally, great thanks go to my parents for their endless and loving support during my studies.

Finally, I would like to thank the Centre of Polymer systems for collaboration on this thesis. The doctoral work was supported by the internal grant agency of TBU in Zlín by following projects: IGA/FT/2016/006, IGA/CPS/2017/001, IGA/CPS/2018/001 and IGA/CPS/2019/004. This work was also supported by the Czech Science Foundation (19-16861S). The financial support granted to my research work by the funding provider is addressed and acknowledge in the respective places in published papers.

CONTENT

ABSTRACT **5**

ABSTRAKT **6**

1. INTRODUCTION TO DISPERSION SYSTEMS **7**

2. EMULSION SYSTEMS **8**

2.1 Emulsion stabilization 9

3. BIOPOLYMERS AS EMULSIFIERS **10**

3.1 Proteins in the stabilization of emulsions 10

3.1.1 Caseins 10

3.2 Polysaccharides in emulsion stabilization 11

4. PARTICLES AS EMULSION STABILIZERS **12**

4.1 Types of particles 12

4.2 Nanocellulose particles 13

5. INTERACTIONS OF BIOPOLYMERS AND PARTICLES AT OIL-WATER INTERFACE **14**

5.1 Nanocelluloses and their interactions with biopolymers at the oil-water interface 15

6. AIMS OF WORK **16**

7. EXPERIMENTAL PART **17**

7.1 Materials, Sample preparation and Experimental methods 18

7.2 Results and discussion 20

8. THE THESIS CONTRIBUTION TO SCIENCE AND PRACTICE **38**

BIBLIOGRAPHY **39**

LIST OF ABBREVIATIONS **46**

LIST OF UNITS **47**

LIST OF SYMBOLS **47**

LIST OF FIGURES **48**

LIST OF TABLES **49**

LIST OF PUBLICATION **50**

CURRICULUM VITAE **52**

ABSTRACT

Biopolymers and biopolymer-based particles are a natural alternative to replace potentially toxic synthetic surfactants stabilizing the oil-water interface in emulsions. These more friendly species allow for the preparation of biocompatible, surfactant-free emulsions for pharmaceutical and cosmetic uses. However, the application of bio-based emulsifiers may, in some cases, be insufficient to prepare stable emulsions. Under these conditions, the ability of some natural emulsifiers to form and stabilize emulsions in synergy with other biopolymers or particles can be advantageously utilized. Therefore, the thesis is at first focused on stabilization of emulsions by single emulsifying protein, sodium caseinate, and in the next step on the investigation of the interactions between the biopolymer and particles at the phase interface. More specifically, the emulsions stabilized by mixtures of sodium caseinate and cellulose nanocrystals are investigated to understand processes of adsorption, complexation or layer-by-layer formation taking place at the oil-water interfaces. The knowledge gathered in the thesis enables to control the emulsion properties *via* variations in the composition of their stabilizing layer. The practical application of such stabilized emulsions is further verified through the preparation of emulsion-based oleogels, which can serve for delivery of bioactive lipophilic substances.

Keywords: biopolymer, stabilization, protein, polysaccharide, nanocellulose, interaction, emulsion.

ABSTRAKT

Biopolymery a biopolymerní částice jsou jednou z alternativ pro nahrazení potenciálně toxických syntetických surfaktantů stabilizujících rozhraní olej-voda v emulzích. Tyto mírnější druhy stabilizátorů umožňují připravit biokompatibilní emulze, vhodné pro farmaceutické a kosmetické využití, bez přítomnosti syntetických surfaktantů. Použití těchto stabilizátorů však může být v některých případech nedostačující, protože nejsou schopny vytvořit stabilní emulze. V takových případech pak může být výhodná schopnost některých přírodních emulgátorů vytvořit a stabilizovat emulze v synergii s jinými biopolymery anebo částicemi. Tato práce je proto nejprve zaměřena na problematiku stabilizace emulzí pouze proteinem, kaseinátem sodným, a v dalším kroku na studium interakcí mezi biopolymerem a částicemi na fázovém rozhraní. Konkrétně jsou studovány emulze stabilizované směsí kaseinátu sodného a nanokrystalické celulózy, se snahou porozumět stabilizaci emulzí prostřednictvím adsorpce, komplexace nebo vrstvení kaseinátu a nanocelulózy na rozhraní olej-voda. Znalosti získané v práci tak umožní kontrolovat vlastnosti emulzí pomocí změn ve složení stabilizující vrstvy na povrchu kapek. Praktické použití studovaných emulzních systémů je zaměřeno na přípravu oleogelů, které mohou sloužit jako nosiče bioaktivních lipofilních látek.

Klíčová slova: biopolymer, stabilizace, protein, polysacharid, nanocelulóza, interakce, emulze.

1. INTRODUCTION TO DISPERSION SYSTEMS

Dispersion systems are two-phase systems with one of the phases (dispersed phase) dispersed in the second, continuous phase. The continuous phase can be gas, liquid or solid in which particles, droplets or bubbles (with at least one dimension in the range 1–1000 nm) are dispersed. The basic types of colloidal dispersions can be classified into aerosols (liquid/particles in gas), foams (gas in liquid), suspensions (solid particles in liquid) and emulsions (liquid in liquid). However, in practice, the systems may be more complex, and dispersions with all three types of dispersed phases occur (Cosgrove 2005).

Recently, these systems are widely applied in various disciplines, products and industrial processes (Schramm 2006), and the scientific interest is paid to their formation, properties and stability. The thesis, therefore, focuses on the topics related to dispersion systems, more specifically to emulsions, mechanisms of their stabilization (steric and electrostatic), and emulsions stabilized with biopolymers and particles. In addition, the investigation of interactions of biopolymers and particles at the oil-water interface brings a new approach in the understanding of emulsion stabilization, as the synergy between stabilizing agents leads to their adsorption, complexation and/or layer-by-layer deposition at the oil-water interface. Moreover, the composition of the surface layer formed around the droplets can control the emulsion properties, as it is discussed below in more detail.

2. EMULSION SYSTEMS

Emulsions are, besides others, used in many industrial fields as delivery vehicles for aqueous- or oil-based actives. They can be, for instance, applied in the pharmaceuticals where they serve as drug delivery systems for parental, oral, and topical routes. Emulsions are colloids consisting of mixtures of at least one liquid dispersed in another in the form of fine droplets. Moreover, the liquids used in emulsions should be immiscible (Schramm 2006; Bouyer, et al. 2012; McClements, D. J., et al. 2007; McClements, David Julian 2015).

The main role of emulsions is to encapsulate hydrophilic or lipophilic active molecules inside the dispersed phase, which ensures their protection against environmental stress and degradation and allows for their controlled delivery. Emulsions may also reduce drug toxicity, enhance its penetration through the skin or behave as detoxifying systems to entrap toxic molecules in the dispersed phase (Schramm 2006; Bouyer, et al. 2012; McClements, D. J., et al. 2007; McClements, David Julian 2015).

Commonly, most of the emulsions are not thermodynamically stable due to the positive free energy associated with the contact of oil and water phases. The positive free energy originates from the interaction of oil and water molecules, and can mainly be attributed to the fact that water molecules are capable of forming strong hydrogen bonds with neighbouring water molecules, but not with molecules of oil. This thermodynamic instability is the main challenge when working with emulsions, as they are prone to destabilization and phase separation. The increase of thermodynamic stability can be accomplished by the addition of emulsifiers[1] (emulsifying/stabilizing agents, surfactants) with surface activity and/or thickening properties, which allow for emulsion formation and stabilization. Emulsifiers are surface-active molecules that rapidly adsorb onto the oil-water interface during the emulsification, creating thus a protective barrier that prevents droplets from coming close together and aggregating. Emulsions are commonly stabilized by synthetic surfactants, nevertheless, their use can increase toxicity and induce various adverse effects, such as skin irritations in case of topically applied emulsions. Substituting synthetic surfactants with more friendly natural molecules (biopolymers) and solid particles have attracted a lot of attention, as they enable to prepare biocompatible, surfactant-free emulsions (Schramm 2006; Bouyer, et al. 2012; Dickinson 2017; McClements, David Julian 2009). The application of natural polymers, such as proteins and/or polysaccharides as emulsifiers and stabilizers, has been a successful approach for

[1] *For purpose of this thesis, the terms as surfactant, emulsifier and stabilizer are used in followed meaning: emulsifier – general term, emulsifying and stabilizing agents; surfactant – small amphiphilic molecule; stabilizer – for Pickering emulsions.*

the formulation of emulsions in the food industry. This knowledge has, however, led to a better understanding of a new generation of emulsions for pharmaceutical and cosmetic applications (Bouyer, et al. 2012).

2.1 Emulsion stabilization

The main role of the emulsifier is to adsorb at the surface of the freshly formed droplets and thus prevent them from coalescing with their neighbours and form larger droplets again. Emulsifiers commonly comprise both hydrophobic and hydrophilic components that become integrated at the oil-water interface to lower interfacial tension (Dickinson 2009; Lam, R. S. H. and Nickerson 2013).

To stabilize emulsions, emulsifying and stabilizing agents are commonly used. Emulsifiers may be in the form of low molecular weight synthetic surfactants, e.g. sucrose esters, polyglycerol esters or natural molecules, or be comprised of solid particles or larger macromolecules, such as proteins and polysaccharides (McClements, David Julian and Gumus 2016).

Stabilization of emulsions can be accomplished using the following ways. The first mechanism lies in an action of classical amphiphilic surfactants (Fig 2.1a), such as sodium dodecyl sulphate. These molecules decrease interfacial tension and increase emulsion stability but can be irritating and potentially toxic towards the environment. The second way comprises the use of polymers, such as proteins (Fig. 2.1b) and polysaccharides (Fig. 2.1c). In addition to their ability to lower interfacial tension, polymers induce steric or electrostatic interactions, changes in the interface viscosity or elasticity, or changes in the bulk viscosity of the system. Finally, the third possible way of stabilization is related to action of small non-surfactant colloidal solids (Fig. 2.1d) that adsorb at interface and form a physical barrier between droplets, thereby delaying or preventing coalescence (Bouyer, et al. 2012; McClements, David Julian 2009; Lam, R. S. H. and Nickerson 2013).

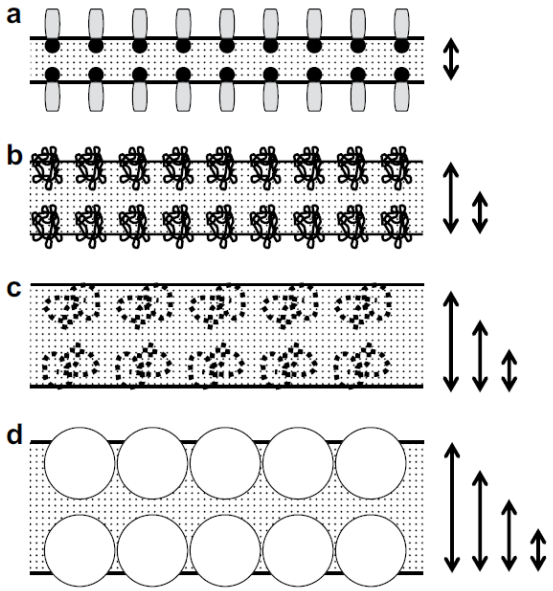

Fig. 2.1 Possible ways of stabilization of emulsions by a) surfactants, b) proteins, c) polysaccharides and d) particles (Dickinson 2009).

3. BIOPOLYMERS AS EMULSIFIERS

Besides synthetic polymers, also biopolymers, such as proteins and polysaccharides can serve as natural emulsifiers/stabilizers with the ability to improve the texture and stability of emulsions. The interfacial activity of many biopolymers results from their composition, as they can contain both hydrophilic and lipophilic regions distributed along their backbones. Similarly, many proteins can act as emulsifiers thanks to their ability to adsorb at the oil-water interface. These amphiphilic proteins comprise both polar and non-polar groups, which are oriented towards the water and oil phases, respectively. On the other hand, polysaccharides work mostly as thickening agents with the ability to increase the viscosity of the continuous phase by forming an extended network, thus improving the emulsion stability. Contrary to proteins, only a few polysaccharide derivatives exhibit surface activity and are able to adsorb at the oil-water interface (Bouyer, et al. 2012; Friberg, et al. 2003).

3.1 Proteins in the stabilization of emulsions

Commonly used proteins suitable for the emulsion formation include, for example, whey protein, casein, ovalbumin, soy and bovine serum proteins (Lam, R. S. H. and Nickerson 2013). The interfacial layers formed by the proteins are usually relatively thin and electrically charged; therefore the major mechanism of stabilization is electrostatic repulsion. Thanks to their amphiphilic structure, proteins are able to adsorb at the oil-water interface, stabilize emulsion droplets by reducing the interfacial tension and slow down droplet coalescence by forming protective layers. Unlike low-molecular-weight emulsifiers that diffuse rapidly to the interface, proteins tend to be bulkier and diffuse at a much slower rate. Once at the interface, some level of partial denaturation is often needed to expose buried hydrophobic amino acids to the surface. Proteins then re-align themselves to a position with their surface hydrophobic amino acids inside the oil and hydrophilic acids being positioned in the aqueous phase (McClements, David Julian 2009; Lam, R. S. H. and Nickerson 2013). Moreover, at the interface strong viscoelastic protein films, which resist mechanical stresses, can be developed providing droplets electrostatic and steric stabilization (Tcholakova, et al. 2006). The ability of proteins to generate repulsive interactions (steric and electrostatic) between oil droplets and to form an interfacial membrane resistant to rupture also influences the long-term stability of these systems (Bouyer, et al. 2012; Lam, R. S. H. and Nickerson 2013; Rodríguez Patino and Pilosof 2011; McClements, David Julian 2004).

3.1.1 Caseins

Caseins are the main protein components of mammalian milk. They mainly consist of a partially aggregated mixture of four individual flexible caseins (α_{S1}, α_{S2}, β and κ). Two of the caseins (α_{S1} and β) are efficient emulsifying agents capable of decreasing the interfacial tension during emulsification and protecting

newly formed droplets against flocculation and coalescence by a combination of electrostatic and steric repulsion. In emulsion formulations, soluble sodium caseinate (CAS) is commonly used. CAS-stabilized emulsions destabilize by a flocculation mechanism in the presence of calcium salts. Calcium ions strongly bind to negatively charged phosphoserine groups of casein, which reduces the net negative charge (Bouyer, et al. 2012).

In o/w emulsions, CAS has been often used as a surface stabilizing and encapsulating agent (Drusch, et al. 2012; Dickinson 1999). The barrier formed by CAS at the oil-water interface is essential for protecting the encapsulated bioactive agents against oxidation and provides also an effective shield against flocculation and coalescence due to the mentioned combination of electrostatic and steric repulsion. For example, CAS used for encapsulation provided good protection of fish oil against oxidation through a physical barrier effect (Day, et al. 2007; Dickinson and Davies 1999; Livney 2010; Nielsen and Jacobsen 2009).

The emulsifying capacity of CAS depends on its concentration, the pH of the aqueous phase as well as on the type of oil being encapsulated (Amine, et al. 2014; Perrechil and Cunha 2010; Hebishy, et al. 2017). The properties and stability of emulsions are also affected by mutual interactions of emulsion components. These interactions can change the structure of the protein at the interface and thus modify emulsion structure and its behaviour (Huck-Iriart, et al. 2011).

3.2 Polysaccharides in emulsion stabilization

Polysaccharides are natural polymers consisting of one or more types of monosaccharides linked together by glycosidic bonds. They are known for water-holding and thickening properties originating from their hydrophilic character and high molecular weight. Polysaccharides are usually not good emulsifiers because they mainly comprise hydrophilic monosaccharides and are, therefore, not particularly surface-active and do not adsorb at oil-water interfaces. These non-adsorbing polysaccharides enhance the emulsion stability by gelling or modifying the viscosity of the aqueous continuous phase, which slows down droplet movement. On the other side, polysaccharides such as gum arabic, modified starch or cellulose derivatives contain non-polar and polar groups and are, therefore, amphiphilic molecules that can adsorb at interfaces. They display surface/interfacial activity and are classified as adsorbing polysaccharides. They can stabilize emulsions by two mechanisms 1) by adsorption at the surfaces of oil droplets and 2) by preventing droplet flocculation and coalescence through electrostatic and/or steric repulsive forces. The surface activity of these polymers results mostly from the presence of a protein fraction in their structure or the combination of hydrophobic and hydrophilic groups along the polysaccharide backbone (cellulose derivatives) (Bouyer, et al. 2012; McClements, David Julian and Gumus 2016).

4. PARTICLES AS EMULSION STABILIZERS

The phenomenon whereby solid particles protect emulsion droplets against coalescence by interfacial action is called Pickering stabilization. Pickering emulsions, type of emulsion stabilized by solid particles located at the oil-water interface, have been discovered a century ago while being extensively studied in recent decades (Dickinson 2017; Yang, et al. 2017; Lam, S., et al. 2014).

4.1 Types of particles

It has been demonstrated by many kinds of research that numerous types of inorganic particles including silica, clay, and hydroxyapatite, as well as a range of organic particles (chitosan, cyclodextrin, etc.), can effectively serve as Pickering stabilizers (Yang, et al. 2017).

At first, the intense research into particle-stabilized systems has focused on using inorganic particles such as silicas. Nevertheless, inorganic particles are limited in their relevance to applications requiring biocompatibility and biodegradability. For this reason, the particles with a biological origin, such as biopolymer particles (micro- and nanoparticles of proteins; starch nanocrystals, particle of chitin or cellulose), are more appropriate stabilizers (Dickinson 2017; Lam, S., et al. 2014; Tavernier, et al. 2016; Timgren, et al. 2011; Yusoff and Murray 2011). As illustrated in Fig. 4.1, emulsions can be stabilized using different types of particles with biological origin. The adsorption of particles at the interface is influenced by their size and bulk concentration (Dickinson 2017; Lam, S., et al. 2014; Joye and McClements 2014; Matalanis, et al. 2011). It was also documented that certain types of microorganisms (Fig. 4.1 a, b) could serve as stabilizers, thanks to their surface properties (surface charge, functional groups, and special structures).

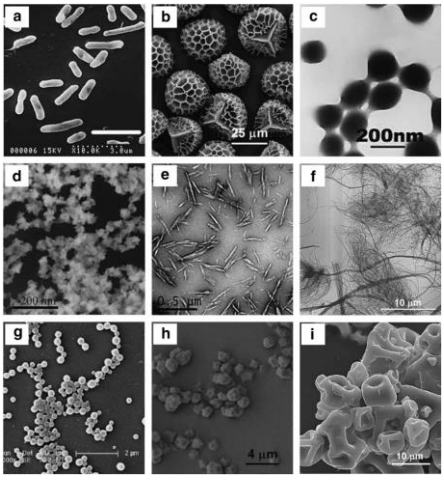

Fig. 4.1 Examples of bio-derived particles of various origins applicable to Pickering stabilization: a) E. coli bacteria; b) moss spore; c) ethyl cellulose nanoparticles; d) chitosan nanoparticles; e) cellulose nanocrystals from cotton; f) silylated cellulose fibres; g) acetylated starch phthalic ester nanospheres; h) quinoa starch granules; i) spray-dried soy protein particles (Lam, S., et al. 2014).

4.2 Nanocellulose particles

In recent years, nanocelluloses have attracted a lot of interests as promising stabilizers for Pickering emulsion particularly in cosmetics, pharmaceutics and food industries.

The fibre of cellulose contains both crystalline and amorphous domains. The amorphous regions of cellulose can be cleaved to produce microcrystalline cellulose (MCC). Using mechanical, chemical or a combination of mechanical, chemical or enzymatic processes, two main types of nanocellulose can be yielded from MCC. The longer semi-crystalline elementary fibrils, cellulose nanofibres, are the first type with the length in several microns comprising both crystalline and amorphous regions. Using acidic hydrolysis, the amorphous regions of fibrils can be hydrolysed and thus cellulose nanocrystals (CNC) are obtained.

The CNC is the smallest building block of the cellulose fibres with a length between 70 nm up to a few microns and a width between 5 and 20 nm. As a result, CNC appears in the form of rigid, rod-like nanoparticles. These features have important consequences for the interfacial behaviour of the CNC. The exact role of CNC particles in the stabilization of emulsions is still a subject of intensive research (Capron, et al. 2017).

In general, cellulose particles of various sizes and aspect ratios have demonstrated their efficiency to stabilize emulsions. Several emulsion tests have been reported with various types of nanocelluloses, cellulose microfibres and cellulose nanofibres (Oza and Frank 1986; Andresen and Stenius 2007; Xhanari, et al. 2011; Mikulcova, et al. 2016), cellulose nanocrystals (Kalashnikova, et al. 2013; Kalashnikova, et al. 2012; Cherhal, et al. 2015; Cherhal, et al. 2016) and bacterial nanocellulose (Ougiya, et al. 1997; Kalashnikova, et al. 2013; Kalashnikova, et al. 2011; Lee, et al. 2014). The results of these studies point out that the aspect ratio governs both the droplet coverage and resulting droplet size (influencing thus the long term stability of the system). When long semi-crystalline fibres are used, the droplet size tends to be somewhat bigger, and the fibres may protrude from the oil-water interface in the continuous phase. Thus a strong network preventing coalescence is formed (Capron, et al. 2017).

5. INTERACTIONS OF BIOPOLYMERS AND PARTICLES AT OIL-WATER INTERFACE

Application of known emulsifiers in the formation of emulsions may, in some cases, be limited by their insufficient functional properties. These are for example related to their questionable stability towards the variation of pH, presence of salts, heating, dehydration, freezing and/or chilling. Therefore, the knowledge of shortcomings related to properties of emulsifiers has triggered research targeted at finding alternative methods for improving emulsion stability by developing novel emulsification strategies (Guzey and McClements 2006). The ability of some of the natural emulsifiers to form and stabilize emulsions in synergy with other polymers or biopolymers can be given as an example. Here protein-protein or protein-polysaccharide combinations can be mentioned. The different polymers can be used in combinations by applying a number of different approaches, such as co-adsorption, complexation and layer-by-layer (l-b-l) method Fig. 5.1 (McClements, David Julian and Gumus 2016).

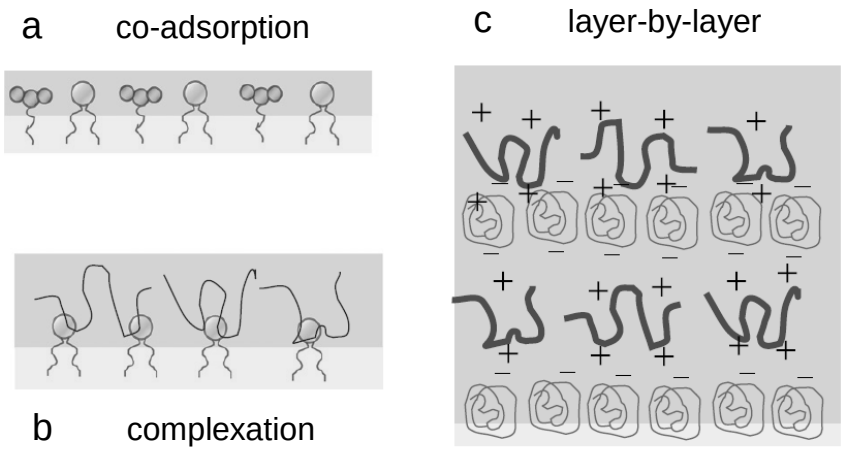

Fig. 5.1 Schematic representation of different types of mixed interfacial layers that can be formed at oil droplet surfaces to stabilize emulsions: a) co-adsorption; b) complexation; c) layer-by-layer (McClements, David Julian and Gumus 2016).

In case two types of stabilizing agents are mixed and used for emulsification, the oil-water interface is preferentially covered by one having a higher surface activity. When two emulsifiers are both adsorbed to the oil droplet surfaces as individual molecules, this process is called co-adsorption (Fig. 5.1a). The resulting interface may consist of a homogeneous mixture of two different emulsifiers, or it may contain regions rich in one emulsifier and depleted in another. The overall composition of the interface will depend on the relative affinity of the two emulsifiers for the oil-water interface, as well as their relative concentrations (McClements, David Julian and Gumus 2016).

On the other hand, if two components interact together through physical or non-physical interactions (such as electrostatic, hydrogen bonding, hydrophobic forces, and covalent bonding), the complex is formed (as shown in Fig. 5.1b)

(Dickinson 2011). This complexation can occur before or after emulsification (McClements, David Julian and Gumus 2016).

During the last procedure, l-b-l method (Fig. 5.1c), an emulsion is prepared by homogenizing oil, water, and emulsifier (bearing a charge) together. The emulsifier-coated droplets with an electrical charge are then mixed with a solution containing polymers (or particles) having an opposite charge. This causes their adsorption onto the droplet surfaces through electrostatic attraction. The electrostatic deposition process can be several times repeated to form a series of layers of opposite charges around the droplets. These steps can improve stability and functional performance of emulsions (McClements, David Julian and Gumus 2016; Guzey and McClements 2006).

If the polymers are used for stabilization of droplets, the interactions between their layers depend on many factors including biopolymer characteristics (size, molecular weight, conformation, mixing ratio, biopolymer type, distribution of reactive sites), solvent properties (pH, ionic strengths, and temperature), total biopolymer concentration and emulsion preparation method (Neirynck, et al. 2004; Tippetts and Martini 2012).

5.1 Nanocelluloses and their interactions with biopolymers at the oil-water interface

So far, only a few studies have been, however, focused on the use of combinations of CNC particles with other types of biopolymers. Modifications of CNC particles with polymers, such as whey protein, bovine serum albumin, hydroxyethylcellulose, methylcellulose or fusion proteins occur due to their electrostatic interactions, covalent binding or other types of interactions between particles and polymers. Electrostatic interactions of CNC and whey protein at the oil-water interface were studied in the works of Sarkar, et al. (Sarkar, et al. 2017; Sarkar, et al. 2018). As CNCs are negatively charged and whey protein carries a positive charge, emulsion droplets were primarily covered with whey protein and CNC then formed the second layer. An analogous study published by Liu, et al. (2018*)* reported on high internal phase Pickering emulsions stabilized using bovine serum albumin and oppositely charged CNCs (Liu, F., et al. 2018). Also, Hu, et al. (2016) studied emulsions stabilized with CNC and water-soluble polysaccharides (Hu, et al. 2016; Hu, et al. 2015), namely surface-active hydroxyethylcellulose and methylcellulose, which were able to adsorb onto CNCs. The study aimed to prepare emulsions that could be dried and re-dispersed again. As demonstrated by Varjonen and Paukkonen, cellulose nanofibres can be covalently modified by using fusion proteins with hydrophobin, a cysteine-rich protein. The combination of protein and cellulose nanofibres leads to a synergic improvement in the formation and stability of o/w emulsions, resulting in systems stable for several months (Varjonen, et al. 2011; Paukkonen, et al. 2017).

6. AIMS OF WORK

The thesis is mainly devoted to the description and application of selected biopolymers (proteins and polysaccharides) as emulsifying and stabilizing agents for bioactive lipophilic substances, and investigation of properties and applications of such stabilized emulsions. During the study, the interest has been mainly focused on particle-stabilized Pickering emulsions. However, this interest has further developed to the investigation of interactions of the biopolymers and particles at the oil-water interface with an effort to find practical aspects and applications of these systems. Therefore, the aims of this work coming out of these presumptions can be summarised as follows:

- To investigate the influence of the preparation procedure and formulation on the behaviour of protein-stabilized emulsions.
    - Determination of the emulsifying properties of sodium caseinate in o/w emulsions containing bioactive triacylglycerol oils.

- To formulate emulsions stabilized by a combination of protein and cellulose nanoparticles.
    - Investigation of interactions between polymers (protein) and particles (cellulose) at air-water and oil-water interfaces, fundamental study of their emulsifying properties.

- To further develop and investigate practical applications of protein/particle-stabilized systems.
    - Preparation and characterization of emulsion-based oleogels with potential applications as carriers of lipophilic active ingredients.

7. EXPERIMENTAL PART

For the sake of clarity, sections Materials, Methods, Result and discussion together with Summary of individual goals of the thesis are divided into:

a) **Study on CAS-stabilized emulsions:** the study was dealing with determining the emulsifying properties of sodium caseinate and long-term stability of CAS-stabilized emulsions containing tamanu and black cumin seed oils, which were prepared by high-shear homogenization (Ultra-turrax) and sonication. The main aim of the study was to characterize CAS as an emulsifying agent and compare the emulsions containing mentioned oils formed by the two methods of preparation. This study was conducted to look closer into the protein-stabilized emulsions.

b) **CNC/CAS stabilized emulsions – interactions at the oil-water interface:** the study was aimed at understanding how the interactions between two bio-based emulsifiers can influence the formation and stability of oil-in-water emulsions (o/w) with hexadecane. Interactions between CNC and CAS were studied in bulk, at air-water and oil-water interfaces. Emulsions were prepared through different routes of the addition of the emulsifiers and at two different pH, 3 and 7.

c) **Oleogels based on CNC and CAS:** the topics studied the practical application of the emulsion systems described in point b). The effort was exerted to replace hexadecane by oil with practical relevance (olive oil), to compare these two systems and to find an optimal formulation for preparation emulsion-based gels, which will be suitable for carrying lipophilic active ingredients.

7.1 Materials, Sample preparation and Experimental methods

All oils and chemicals used in the thesis are summarized below.

Non-traditional vegetable oils from *Calophyllum inophyllum* (Tamanu oil, TA) and *Nigella sativa* (Black cumin oil, BC) were obtained from *Nobilis Tilia* (Czech Republic). Hexadecane was sourced from Sigma-Aldrich (Germany). Extra virgin olive oil was purchased from a local store.

Casein sodium salt from bovine milk (CAS) was provided from Sigma-Aldrich. Cellulose nanocrystal (CNC) powder prepared *via* the sulphuric acid route was purchased from CelluForce (Canada).

All other chemicals used, polyethyleneimine (PEI), calcium chloride ($CaCl_2$), sodium chloride (NaCl), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Sigma Aldrich and Merck (Germany) and were of analytical grade. In all studies, Milli-Q water was employed.

The **caseinate-stabilized o/w emulsions** were formulated with 1, 2, 5, 7.5, 10 or 12 wt% CAS. The CAS dispersions were prepared by dispersing the powder in Milli-Q water under gentle stirring at room temperature for 4 hours. Emulsifications were carried out by mixing each of the above CAS dispersions and oil phase (5, 10, 20 and 30 wt%; TA or BC oil) at 25 °C with two different methods: 1) a rotor-stator homogenization with an Ultra-Turrax model T25 (IKA, Germany) for 12 min at 13,400 rpm and 2) an ultrasonic homogenization (UP400S, Hielscher, Germany) with 400 W, 24 kHz, for 1 min, operated with 100% output. The antimicrobial activities of oils (TA and BC) and CAS-stabilized emulsions were evaluated using eight bacterial strains obtained from the Czech Collection of Microorganisms (CCM, Czech Republic). The gram-positive (*Micrococcus luteus* CCM 732, *Staphylococcus aureus subps. aureus* CCM 3953, *Bacillus cereus* CCM 2010, *Enterococcus faecalis* CCM 2665) and gram-negative (*Escherichia coli* CCM 3954, *Pseudomonas aeruginosa* CCM 3955, and *Salmonella enterica subsp. enterica ser. Enteritidis* CCM 4420, *Serratia marcescens subsp. marcescence* CCM 303) strains were employed in the test.

To investigate the **CNC/CAS interaction at the oil-water interface**, stock dispersions of CAS (2 wt%) were prepared by stirring CAS powder in Milli-Q water for 4 h at room temperature. Similarly, CNC dispersions (2 wt%) were prepared by initial stirring for minimum 12 h followed by sonication with Vibracell Sonicator (Sonics and Materials Inc., USA) at 40% output during three cycles with a duration of 1 min to remove aggregates. Oil-in-water emulsions with 20 wt% concentration of hexadecane oil and total particle content of 0.2 wt% were prepared by sonication (Vibracell Sonicator, Sonics and Materials Inc., USA) at 20% output. The preparation of emulsions followed three different routes (Fig. 7.1): 1) route R1: the CNC and CAS dispersions (each 0.2 wt%) were premixed in aqueous phase in 1:1 ratio, hexadecane (20 wt%) was added to CNC/CAS mixture and the system was sonicated for 1 min. 2) route R2: primary emulsion containing 40 wt% of oil and 0.2 wt% of CAS was prepared by sonication (1 min), then the CNC dispersion (0.2 wt.%) was added to aqueous phase to get final

emulsion containing 20 wt% oil and 0.2 wt% particles in total, and sonicated shortly (20 s). 3) route R3: primary emulsion containing 40 wt% of oil and 0.2 wt% of CNC was prepared by sonication (1 min), then the CAS dispersion (0.2 wt.%) was added to aqueous phase to get final emulsion containing 20 wt% oil and 0.2 wt% particles in total and sonicated shortly (20 s). All the emulsions were prepared with either NaCl (5 mM) or $CaCl_2$ (0.5 mM) as background electrolyte.

For preparation of **oleogels based on CNC and CAS**, stock dispersions of CAS (2 wt%) and CNC (2 wt%) were prepared by the same way as stated above, nevertheless, CNC (2 wt%) was sonicated by UP400S (Hielscher, Germany) (60% output, three cycles, 1 min) to remove aggregates. Oil-in-water emulsions with an oil concentration of 20 wt% and total particle content of 0.2 wt% and 0.3 wt% were prepared by sonication at 60% output. The oil phase was composed of olive oil or hexadecane and the preparation followed the three above described routes R1, R2 and R3. After emulsification, the samples were centrifuged at 6,000 rpm for 3 min, the upper emulsion layer was separated from supernatant, transferred to cylindrical mold (diameter of 12 mm) placed on the Petri dish, and dried for 48 hours at ambient temperature. All the emulsions were prepared with either NaCl (5 mM) or $CaCl_2$ (0.5 mM) as background electrolyte.

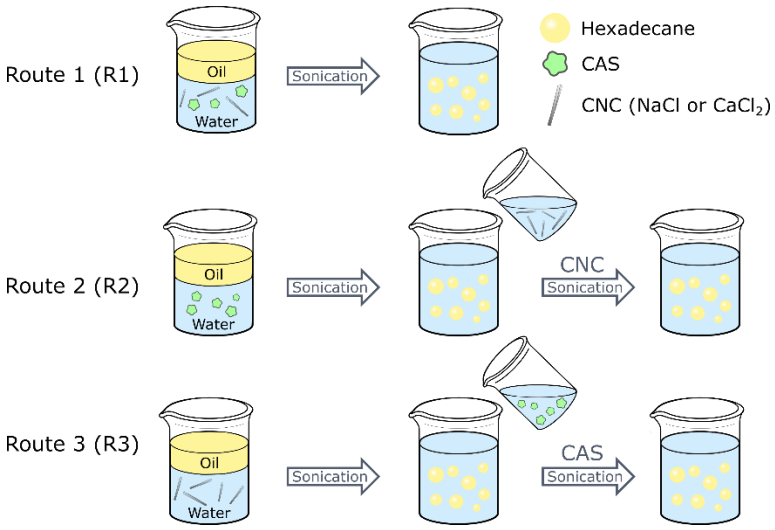

Fig. 7.1 The routes of emulsion preparation (Pinďáková, et al. 2019).

The CAS and CNC dispersions were characterized by several methods, such as dynamic light scattering (DLS), atomic force microscopy (AFM), quartz crystal microbalance with dissipation (QCM-D), and their interfacial tensions were determined by the pendant drop technique. Emulsions were characterized by the size and distribution of the emulsion droplets using laser diffraction, the encapsulation efficacy (*EE*) of emulsions were determined. The emulsion droplets were observed by optical microscopy. The stability of emulsions was assessed by measuring zeta potential and visual observation (expressed as the creaming index). Antimicrobial activities of TA and BC oils and CAS-stabilized emulsions

were studied using disc diffusion assay. The oleogels were characterized by the loss of water and released oil after drying. The characterization of the gels was conducted using rotation rheometer with parallel plate configuration. The re-dispersibility of oleogels in water were evaluated, successfully re-dispersed oleogels in the form of emulsions were observed by optical microscopy.

All the analyses were conducted at least in triplicates, with the Dean-Dixon method being utilized to calculate the means and standard deviations. The Student T-test was applied to detect any statistical differences between the samples (Statistica, StatSoft, Inc., Palo Alto, CA, USA) at an antimicrobial activity. The P (probability) value of ≤ 0.05 was considered to be statistically significant.

7.2 Results and discussion

The presented doctoral thesis is focused on the preparation of emulsions stabilized by biopolymers or bio-based particle, especially sodium caseinate (CAS) and cellulose nanocrystals (CNC). The understanding of the deposition mechanism of CNC and CAS at the oil-water interface is important for the control of emulsion properties and the practical application of emulsions in the pharmacy and cosmetic industries.

CAS-stabilized emulsions

The study was conducted with the aim 1) to investigate the behaviour of CAS under emulsification of triacylglycerol-based oils, in this case rarely studied tamanu and black cumin oils showing pharmaceutical and nutritional effects, and 2) to prepare o/w emulsions, which can serve as carrier systems with therapeutic and physiological benefits for humans. The study was also aimed at finding the optimum emulsion formulation in terms of the concentrations of both protein and oils, and at establishing a suitable emulsification procedure when using two commonly available methods, high-shear homogenization (Ultra-Turrax) and sonication.

Concerning the emulsion properties, the influence of processing parameters (Ultra-Turrax *vs* sonicator) and compositions (a type of oil, o/w ratio, concentration of CAS) must be taken into account. The most important findings are discussed below briefly.

A key parameter, which has a crucial effect on emulsion stability, is the size of emulsion droplets. Light diffraction analyses showed that emulsion droplet size ($D_{(4,3)}$) was influenced by all studied variables, especially by the method of preparation (Ultra-Turrax *vs* sonicator) and concentration of stabilizing CAS (1 to 12 wt%); and to a lesser extent by type and content of encapsulated oils.

Emulsification with Ultra-Turrax (UT) led to coarse emulsions with bigger droplet sizes, and their properties were notably affected by composition, such as oil and CAS contents (Fig. 7.2 a, c). On the other hand, the homogenization with the sonicator (US) yielded fine emulsions (Fig. 7.2 b, d). Although droplets after sonication were notably smaller than droplets treated with UT, some differences

between emulsions containing BC and TA oils were observed. These can be explained by character, composition and physical properties of the oils, which influence emulsifying efficiency and hence droplets size of emulsions.

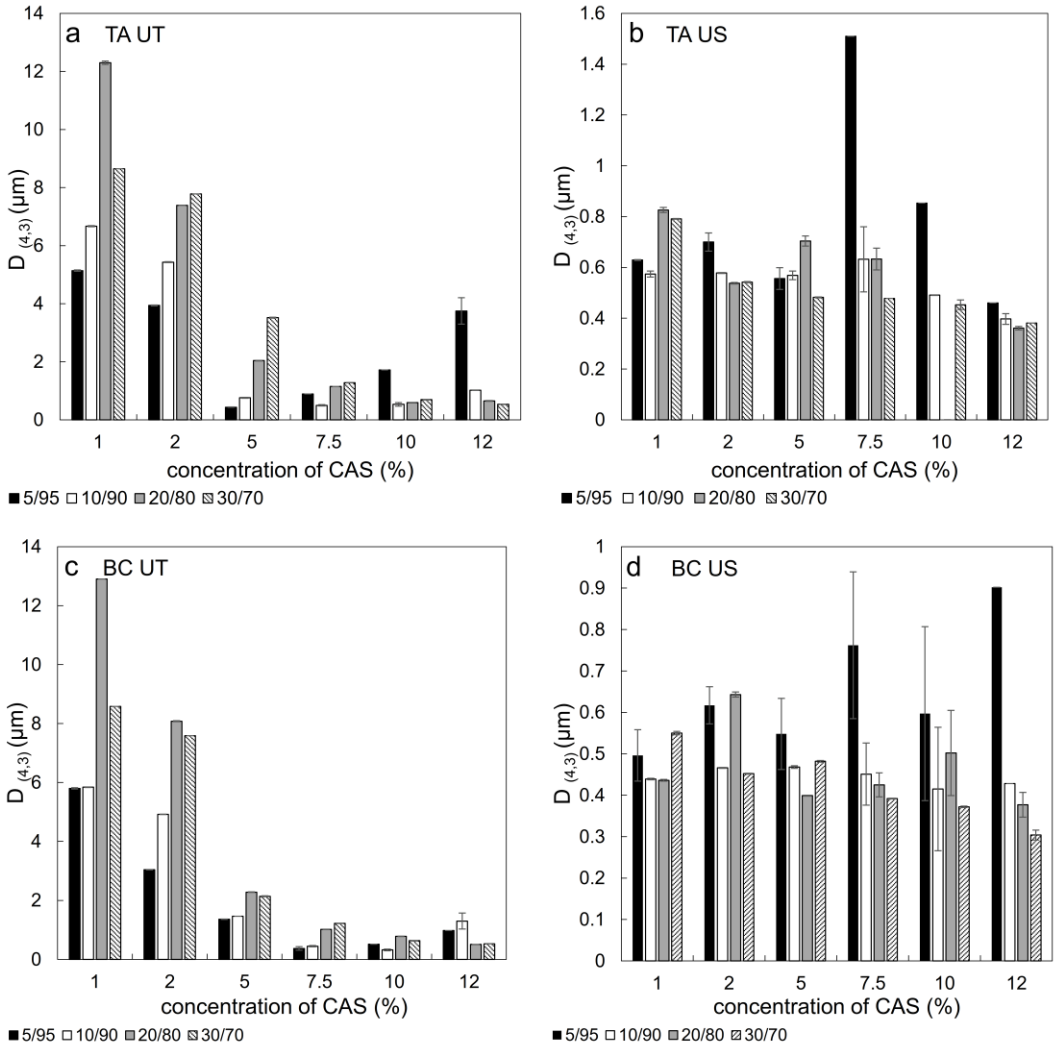

Fig. 7.2 Influence of CAS concentration and o/w ratio on volume-weighted diameter of emulsion droplets ($D_{(3,4)}$) of a) TA oil-emulsions prepared by Ultra-Turrax (UT); b) TA oil-emulsions prepared by sonication (US), c) BC oil-emulsions prepared by UT, and d) BC oil-emulsion prepared by US.

A stability-indicating parameter of dispersion systems is zeta (ξ) potential. Immediately after preparation, the ξ potential ranged from –53 to –41 mV for emulsions with BC oil (prepared with US) and from – 60 to – 46 mV for those prepared with UT. TA emulsions behaved similarly with potential values varying from – 55 to –41 mV and – 59 to –44 mV for US and UT emulsions, respectively. In current study, ξ potential measurements were conducted on emulsions with non-adjusted pH (TA oil emulsions pH = 6.44 ± 0.03, BC oil emulsions pH = 6.45 ± 0.04).

In the protein-stabilized emulsion droplets, the four main destabilization routes can occur (creaming, coalescence, flocculation and Ostwald ripening). Kinetic

stability, described by creaming index *CI*, provides indirect information on the droplet flocculation and destabilization processes occurring in an emulsion. Not all emulsions prepared in the presented study showed kinetic stability, even immediately after preparation. Some of them formed a bottom serum layer and a small cream phase on the top. On the other hand, samples mainly with high CAS content exhibited good stability against creaming (Fig. 7.3). In addition, the extent of destabilization depended on the content of the oil. Nevertheless, the formation of stable emulsions can be considered to be a synergic effect of the US processing resulting in small droplets with narrower distribution and a sufficient amount of CAS.

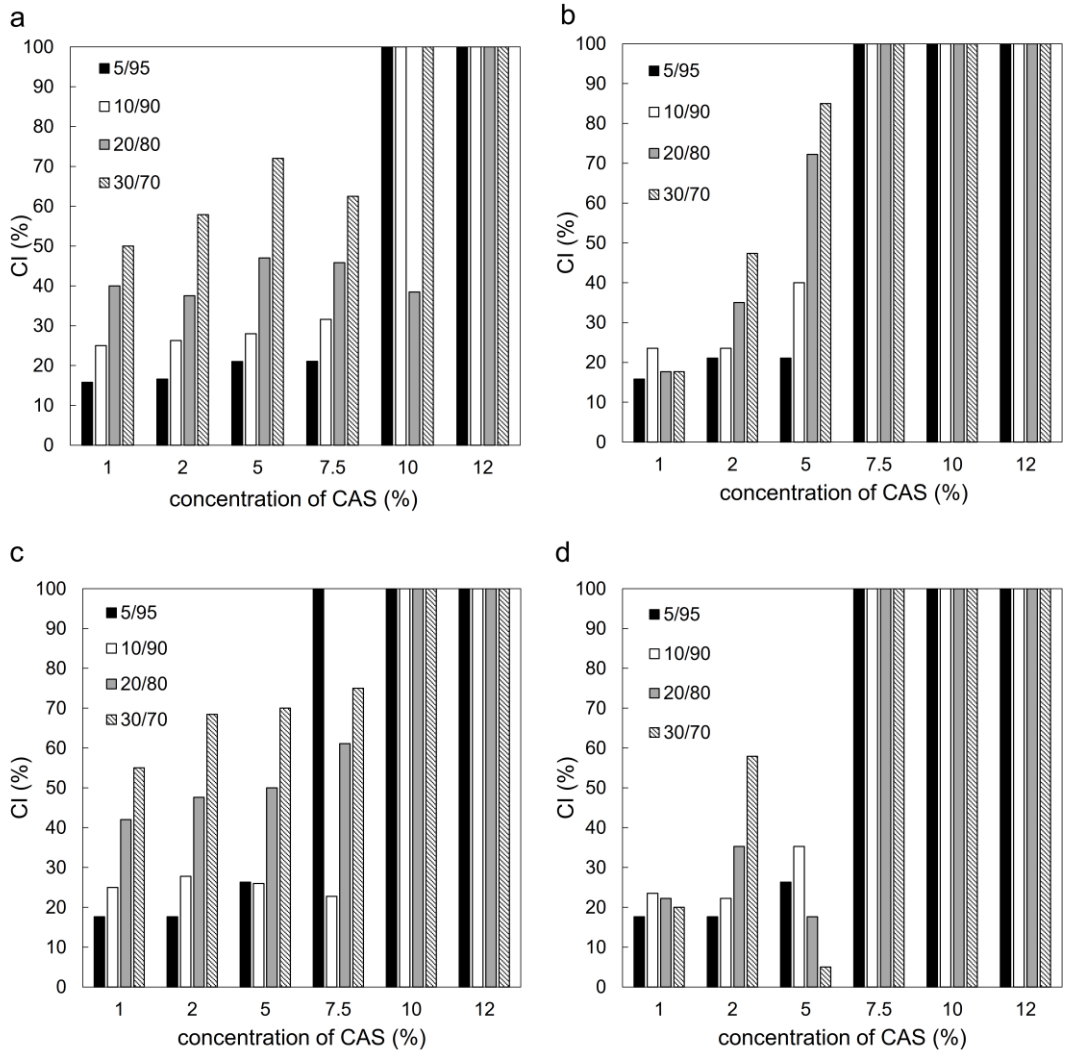

Fig. 7.3 Comparison of creaming index (CI; CI ~ 100 % is for stable emulsion) determined on freshly prepared emulsions as affected by processing method and composition of emulsions a) BC-emulsions prepared with Ultra-Turrax (UT), b) BC-emulsions prepared with sonication (US), c) TA-emulsion prepared with UT, and d) TA-emulsion with US.

In the evaluation of emulsion stability, long-term stability is also an important parameter and was considered in this study. Not surprisingly, the highest stability exhibited emulsions containing the highest CAS content (12 wt%). For example,

BC emulsions with 30 wt% oil prepared by both procedures remained unchanged for 7 days of storage with *CI* being of 100 %. Stability studies also supported the fact that US treatment, which provided smaller droplets with narrower distribution assured production of emulsions with limited creaming.

To summary this study, the main target was to gather deeper knowledge on the behaviour of biopolymer, sodium caseinate, under the emulsification of bioactive triacylglycerol-based oils (tamanu and black cumin oil). For this purpose, oil-in-water emulsions stabilized with sodium caseinate were prepared by ultrasound treatment or high shear homogenization using Ultra-Turrax. It was found out that the ability to form stable emulsions of small, initial particle size was primarily controlled by the used method of preparation together with the concentration of stabilizing caseinate and, to a lesser extent, by the type and amount of used oils. Sonication was a more efficient emulsification procedure and afforded emulsions with small particle sizes throughout the entire used concentration ranges of oil and caseinate. In comparison with sonicated emulsions, the properties of emulsions prepared with Ultra-Turrax depended, to a higher extent, on their composition and the emulsions were more prone to destabilization during their storage. Finally, it was proved that both oils and their selected emulsions were efficient in suppressing the growth of gram-positive bacterial strains (*S. aureus and B. cereus*). Obviously, the results of the study served in the following experimental work and pointed to an appropriate emulsification procedure, namely sonication which will be selected for the preparation of other emulsion systems.

Results of the study were summarized and published in *Caseinate-Stabilized Emulsions of Black Cumin and Tamanu Oils: Preparation, Characterization and Antibacterial Activity" by Urbánková L., et al. Polymers 2019, 11(12), 1951; https://doi.org/10.3390/polym11121951*.

CNC/CAS interactions at the oil-water interface

The second study is focused on the interactions between sodium caseinate (CAS) and cellulose nanocrystals (CNC), and on their role in the stabilization of oil-in-water (o/w) emulsions. At first, the interactions of CNC and CAS dispersed in the aqueous environments were investigated. In the next step, the interfacial behaviour at the air-water and oil-water interface was studied. This study was also complemented by investigating the adsorption profile of CAS on the model surface of CNC by quartz crystal microbalance. Finally, the emulsions were prepared *via* three routes, where the interactions between species were rationalized as a function of the order of addition of CAS and CNC during emulsification.

Before the interactions of CNC and CAS were investigated, both types of particles were studied separately at different pH and ionic strengths using AFM and dynamic light scattering. The morphology of the CNC particles visualized by the AFM images was of rod-like shape with a length ranging from 80 to 220 nm and a diameter of 4 nm (Fig. 7.4a), similar to those reported by Mikulcová (Mikulcova, et al. 2018). The hydrodynamic diameter of CNC was also measured as a function of pH using DLS and was about 60 nm with good stability within a broad pH range (Fig. 7.5a). The colloidal stability of the CNC was lost when the pH of the dispersion decreased below 2.5 as a result of the decrease of the charge density.

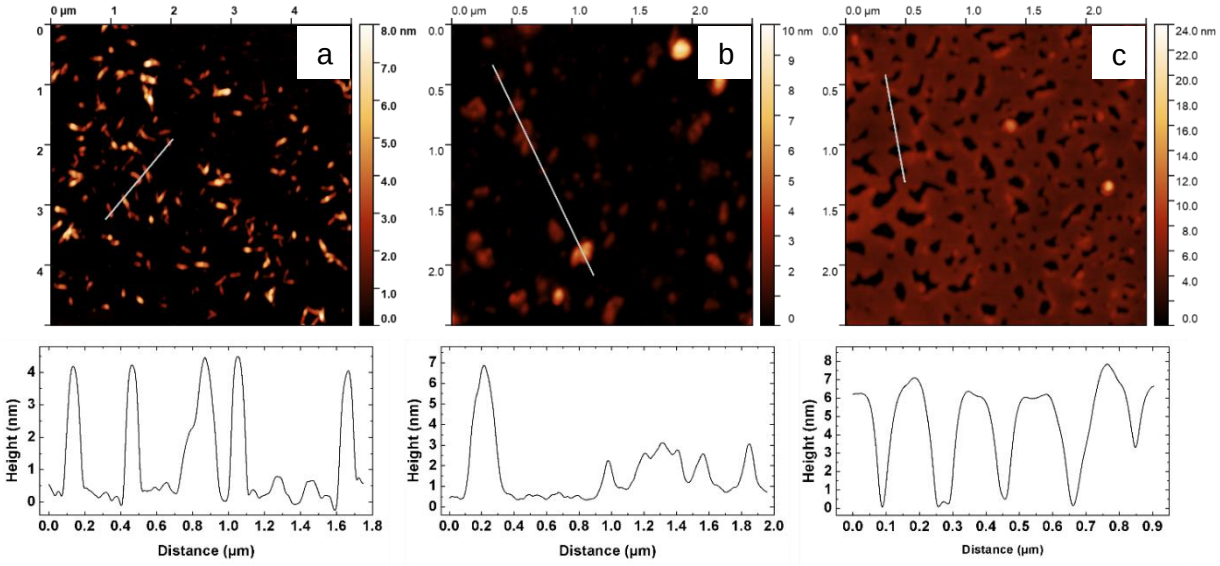

Fig. 7.4 AFM micrographs and corresponding height profiles (below) of dried aqueous dispersions of a) CNC, and CAS at b) pH 7 and c) pH 3.

The behaviour of CAS was more complex and large differences could be noticed when changing the pH, as judged by the AFM pictures Fig. 7.4b and 7.4c. At pH 7, globular aggregates with lateral dimensions of 40 to 150 nm can be seen, resulting from the assembly of smaller CAS units. When the pH was decreased to 3, the AFM images showed a film-like structure. The mean particle size of CAS as a function of pH is presented in Fig. 7.5b. While the size of CAS particles

varied only slightly below pH 3.5 and above pH 5, aggregation occurred between pH 4 and 5, i.e. at pH close to the isoelectric point of CAS (Liu, Y. and Guo 2008; Fox and Brodkorb 2008; Post, et al. 2012; Gorji, et al. 2015).

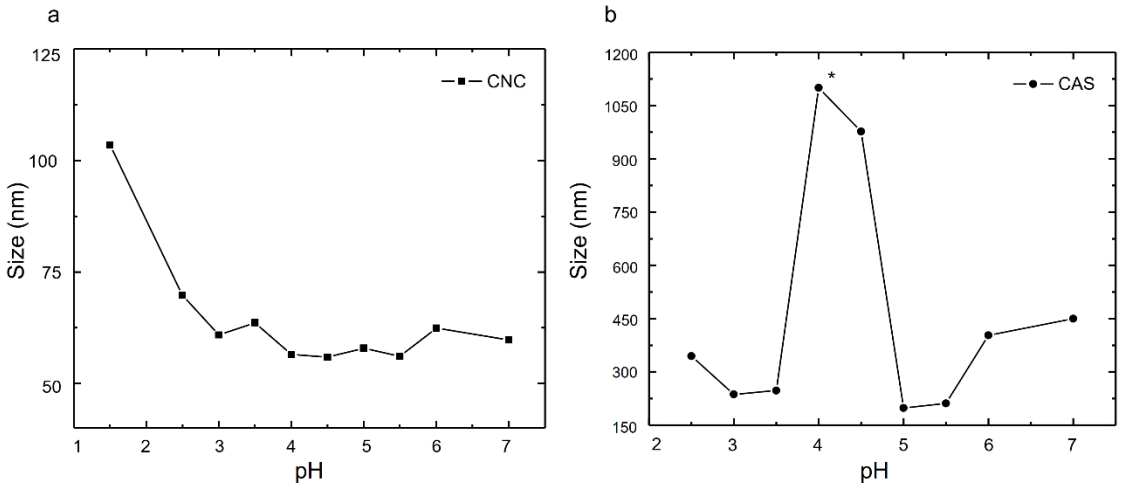

Fig. 7.5 Size of a) CNC and b) CAS as a function of pH (: at pH 4, sedimentation occurred).*

As NaCl and $CaCl_2$ are often used to boost the emulsifying properties of CNC by reducing the electrostatic repulsion (Mikulcova, et al. 2016; Mikulcova, et al. 2018; Capron and Cathala 2013), the size development of CAS and CNC with the change of salt concentration was determined by DLS. The strong effect of salt on the aggregation of CAS and CNC, and the role of the valence of the ions on the aggregation was confirmed. Ca^{2+} caused a rapid increase in the size of CNC at low concentration (0.25 mM) and a similar effect was observed for CAS with the formation of large aggregates (casein binds Ca^{2+} and thus reduces the electrostatic repulsion between particles) (Thomar, et al. 2014; Ye and Singh 2001). In comparison with $CaCl_2$, higher concentrations were needed for NaCl to trigger the loss of CAS stability (> 10 mM). The next part of this study investigated the behaviour of CNC/CAS complexes at interfaces, and more specifically their function in the stabilization of the oil-water interface. Therefore, the adsorption measurements at the solid surface using quartz crystal microbalance with dissipation monitoring (QCM-D), and surface and interfacial tension measurements at the hexadecane-water interface using the pendant drop technique were conducted (pH 7 and 3).

To study the effect of pH on the successive adsorption of CNC and CAS, QCM-D crystals coated with a silicon dioxide layer were modified with polyethyleneimine as anchoring film followed by the deposition of a thin layer of CNC. Fig. 7.6 shows the mass uptake of the QCM-D crystals at pH 7 and 3, left and right respectively. For both pHs, the sequence of injection was the same. First, the CNC covered crystals were exposed to water adjusted at the studied pH. CAS suspension was then flowed into the measurement chamber, until a steady state was reached, followed by a rinsing step. This led to the adsorption of a layer of CAS that was finally exposed to a CNC suspension prior to a final rinsing step.

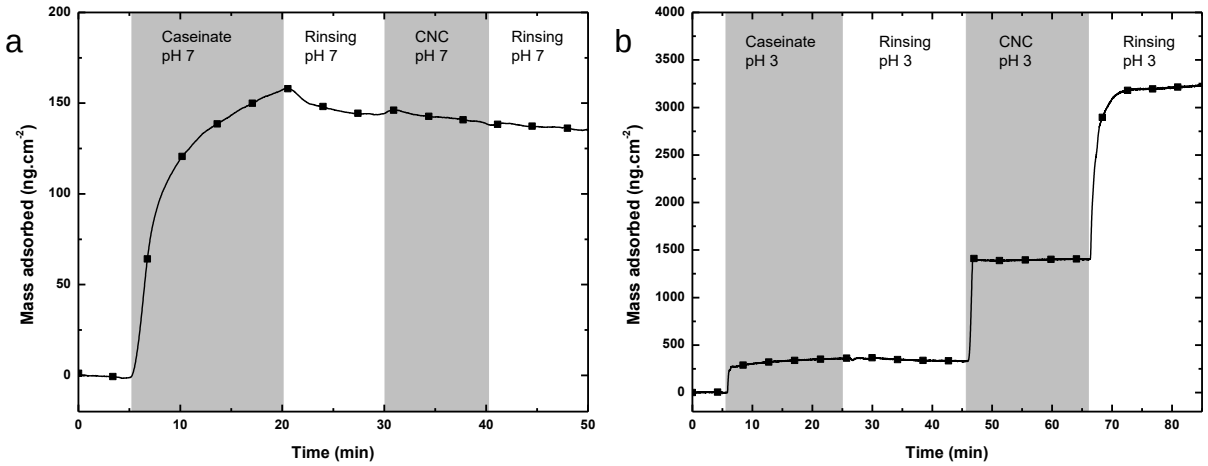

Fig. 7.6 Adsorption of CAS on CNC surface at pH = 7 (a) and pH = 3 (b) as determined by QCM-D.

At pH 7, CAS adsorbed readily onto the CNC-coated surface, most likely through hydrophobic interactions, as CAS presents an amphiphilic character. With the rinsing step a small portion of CAS desorbed, and the mass remained constant at around 150 ng.cm^{-2}. As expected, CNC did not adsorb under this pH condition as both CNC and CAS bear the same charge.

At pH 3, the positive charge of CAS drove the adsorption and a plateau at around 360 ng.cm^{-2} was reached. The following rinsing step, at the same pH, did not induce any significant desorption. The overall positive charge of the CAS layer enabled very strong adsorption (1390 ng.cm^{-2}) upon the injection of CNC in the next step. The increase in dissipation continued during the last rinsing step along with large mass uptake, most likely due to the reorganization of the CNC/CAS multilayer.

Surface and interfacial activity of CAS and CNC were measured using the pendant drop technique, at pH 7 and 3. First, the activity of the individual components, that is CAS and CNC, was determined, and then their mixtures CNC were tested, to evaluate their capacity to compete for the interface. These measurements constitute the basis of the rationalization of the emulsification experiments.

Surface activity of CNC and CAS solutions was determined at pH 7 and the results are shown in Fig. 7.7. The absence of surface activity for the cellulose nanoparticles was confirmed, as previously reported (Hu, et al. 2015). On the other hand, proteins are known for their surface activity, emulsifying and foaming properties (Abascal and Gracia-Fadrique 2009) and it was confirmed that CAS exhibited good surface activity and was able to decrease surface tension already at a very low concentration (Fig. 7.7a).

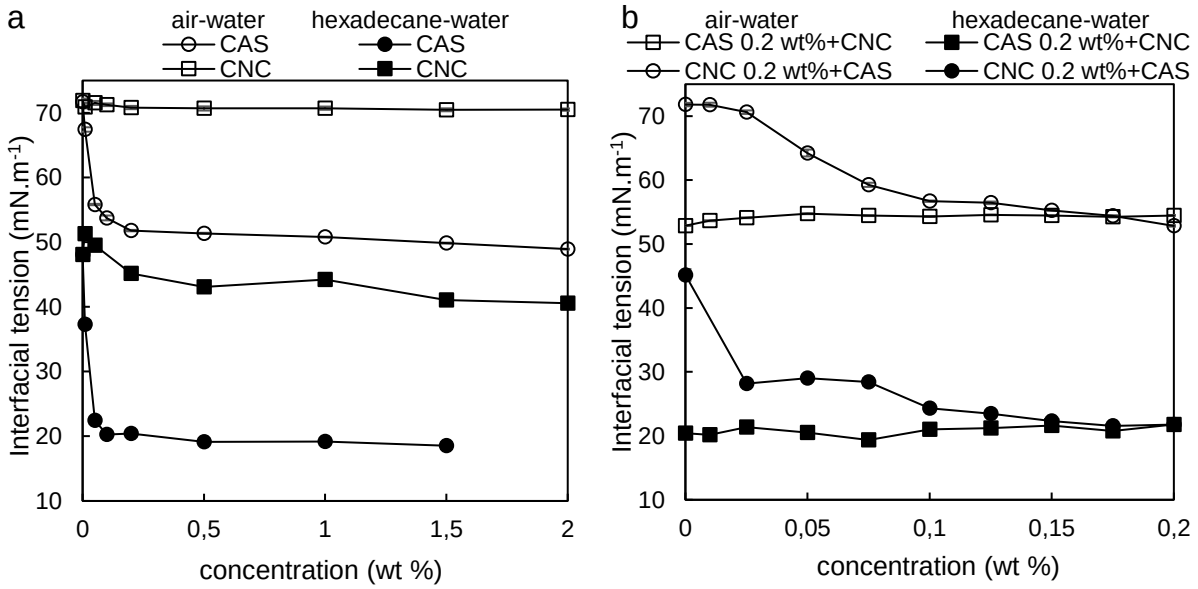

Fig. 7.7 Surface tension at pH 7 of CNC and CAS dispersions a) and their mixtures b) at air-water (empty symbol) and hexadecane-water interface (plain symbol). The error was below 1 and 2 % for the measurements at the air/water and oil/water interface, respectively.

The trend was, however, different for the hexadecane-water interface at pH 7. Here, CNC particles were able to slightly reduce the interfacial tension of the hexadecane-water interface due to their intermediate wettability since hexadecane is less hydrophobic than air (Fig. 7.7a). Surface-activity of CAS at the hexadecane-water interface was also confirmed.

The values of surface and interfacial tension were then determined for the mixed systems where CAS (or CNC) concentrations were kept at 0.2 wt% while CNC (or CAS) were added (Fig. 7.7b). As one could anticipate, increasing concentration of CNC in CAS dispersion (0.2 wt%) did not cause any significant changes in the interfacial tension as a result of repulsive interactions between CAS and CNC, combined with the lack of surface activity of CNC. On the other hand, when CAS was added to a CNC dispersion, the surface tension decreased gradually with increasing concentration of CAS in CNC dispersion. This clearly demonstrated that the most surface-active compound was the one ruling the interfacial behaviour, in the case where there was no strong attractive interaction between the two compounds.

The corresponding measurements were conducted at pH 3 (Fig. 7.8) and showed that the lowering of pH did not influence the activity of CAS at the air-water interface nor the hexadecane-water interface.

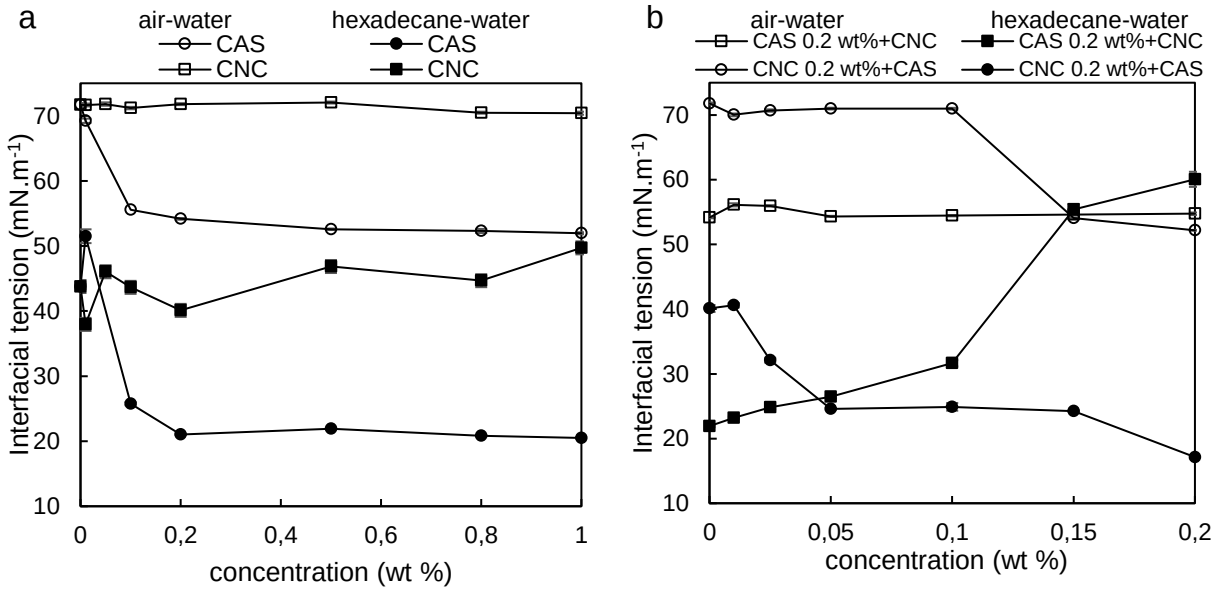

Fig. 7.8 Surface tension at pH 3 of CNC and CAS dispersions (a) and their mixtures (b) at air-water (empty symbol) and hexadecane-water (plain symbol) interface (pH 3). The error was below 1% and 2% for the measurements at the air/water and oil/water interface, respectively.

Interfacial tension of the mixed systems CAS/CNC and CNC/CAS as a function of increasing concentrations of CNC or CAS (Fig. 7.8b) did not exhibit major differences between behaviour at pH 3 and 7, except for the hexadecane-water interface. In this case, the interfacial tension rapidly increased with the introduction of CNC in the CAS dispersion because CAS was probably displaced from the interface through interaction with CNC.

The final part of the study was focused on the preparation of emulsions and their stabilization by the interaction of CNC and CAS at the oil-water interface. Three different routes for the preparation of emulsions were investigated and are presented in Fig. 7.1. The emulsions composed of 20 wt% hexadecane and a total particle concentration of 0.2 wt% were prepared by different routes of emulsification. To investigate the possibility of inducing attractive interaction between CNC and CAS, the pH was adjusted in different ways, and results from emulsions prepared with aqueous phases at pH 7, at pH 3, and from emulsions prepared at pH 7 and then adjusted to pH 3.

Before investigating the competitive behaviour of CAS and CNC for the oil-water interface in an emulsification process, their individual performance was tested. The size distributions of primary emulsions (PEs) stabilized with only one type of particles are shown in Fig. 7.9. Here, it is important to mention the weak emulsifying CAS at a concentration of 0.2 wt% with about 70 % of emulsified oil.

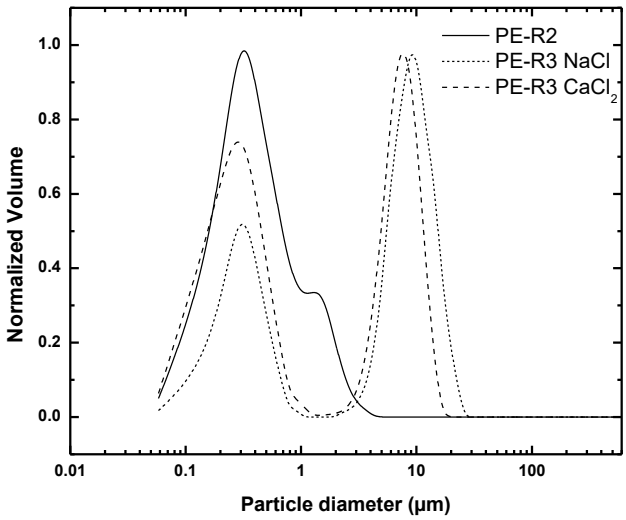

Fig. 7.9 Size distribution curves of the primary emulsions PE-R2, PE-R3 $CaCl_2$, PE-R3 NaCl at pH 7.

The competitive behaviour of CAS and CNC at the hexadecane-water interface in emulsions were studied by different routes of preparation. The first route **R1**, where the CAS/CNC mixture was used for emulsification, showed poor emulsifying capacity with 25 % of free oil. In the presence of NaCl (R1-NaCl), the size of the emulsion droplets was in-between those measured for droplets stabilized with CNC and CAS alone. Nevertheless, the emulsion with $CaCl_2$ (R1-$CaCl_2$) had a multimodal distribution with a primary droplet size of 4 µm and large flocks of about 50 µm (Fig. 7.10a). This tendency to flocculate, caused by aggregation of CAS in the presence of Ca^{2+}, was confirmed by optical microscopy (Fig. 7.11). In this route, competition between CNC and CAS might occur, however, due to its higher surface activity CAS probably dominate (Sarker, et al. 1999).

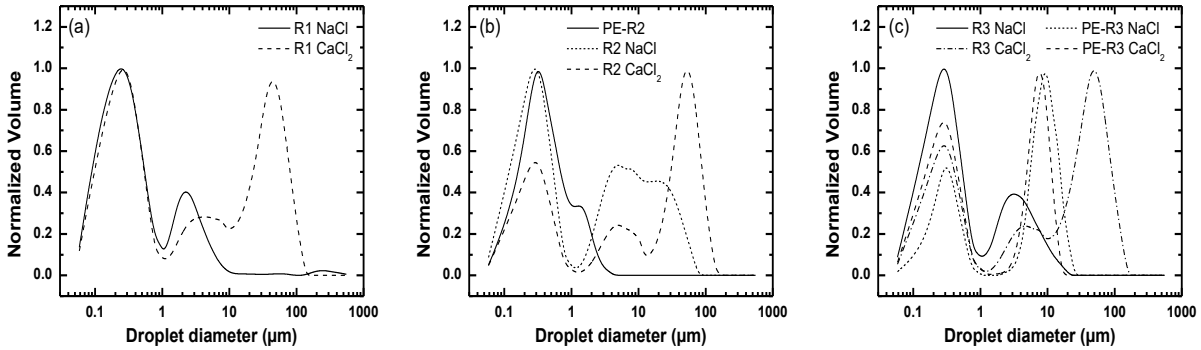

Fig. 7.10 Distribution curves of the emulsions prepared via route a) R1, b) R2 and c) R3 at pH 7. PE-R2 and PE-R3 stand for primary CAS and CNC emulsion, respectively.

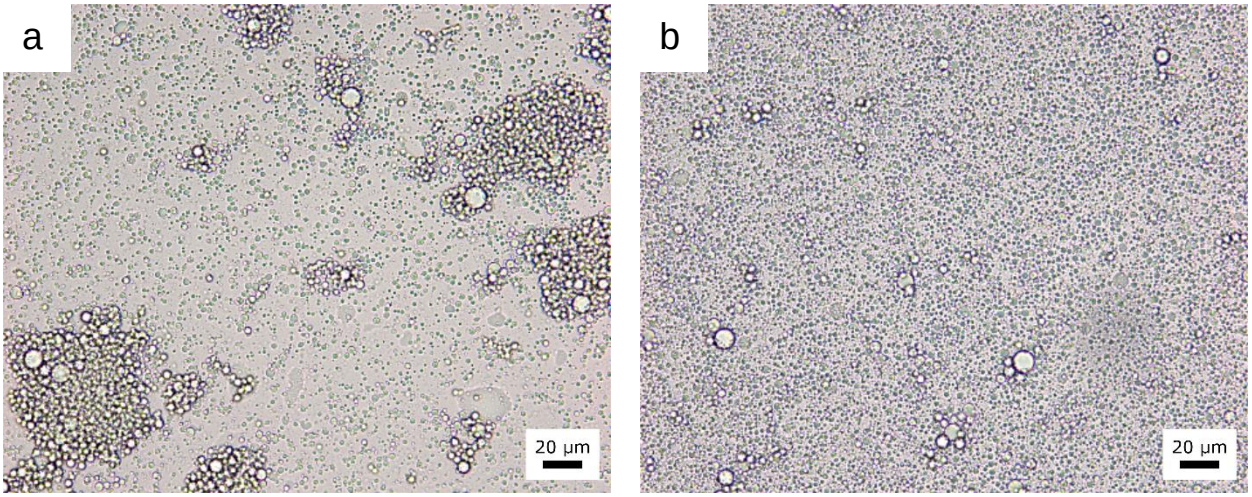

Fig. 7.11 Optical micrograph of emulsions prepared by route R1 $CaCl_2$ a), R1 NaCl b), at pH 7.

The addition of CNC and $CaCl_2$ to a CAS stabilized primary emulsion (route 2, **R2**) caused not an only aggregation of CAS and flocculation of the droplets (Fig. 7.12), but it also caused increasing of the encapsulation efficacy as the amount of free oil after emulsification decreased. Distribution curves (Fig. 7.10b) were also changed and the shoulder of the primary emulsion shifted towards larger droplet sizes together with the appearance of flocks. The smaller droplets were most likely stabilized primarily by CAS and the subsequent addition of CNC further improved coverage of droplet interface and improved stability of emulsion droplets. It can be also suggested that CNC particles probably stabilized larger emulsion droplets seen on the image, which were formed from the remained free oil.

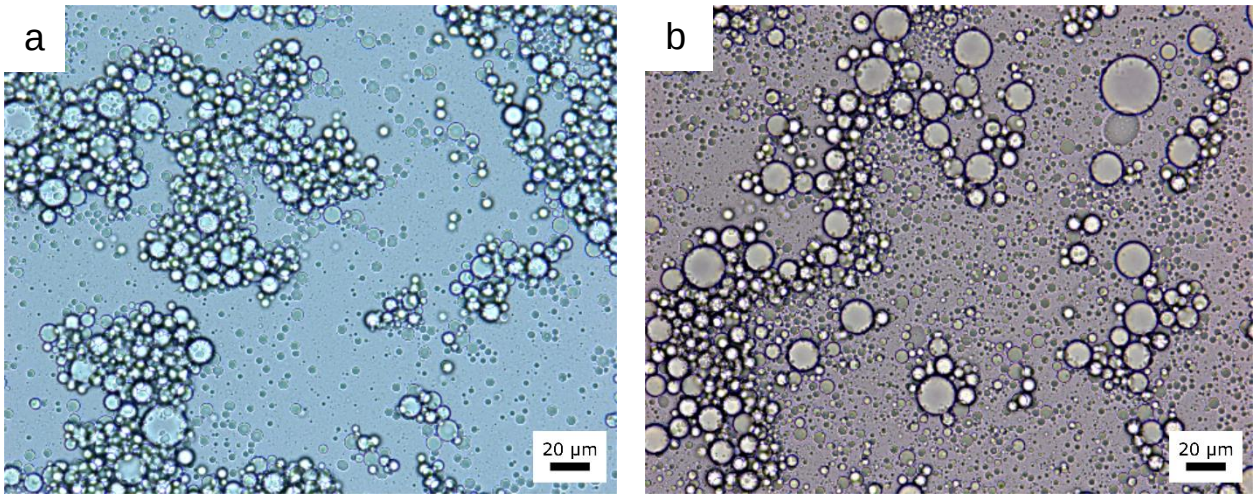

Fig. 7.12 Optical micrograph of emulsions prepared by route R2 at pH 7: a) $CaCl_2$, b) NaCl.

The emulsifying capacity of the route **R3** was high with the absence of free oil, and this was observed already for the primary emulsions. The primary emulsion, in the presence of NaCl or $CaCl_2$, showed droplets of relatively large sizes. The subsequent addition of CAS significantly decreased the droplet size (Fig. 7.13),

hence demonstrating the synergy between CNC and CAS. The partial depletion of CNC originally present at the oil-water interface and its replacement with the more surface CAS is not too probable as particles once adsorbed at the interface have very high energy of desorption (Binks 2007; Giermanska-Kahn, et al. 2005).

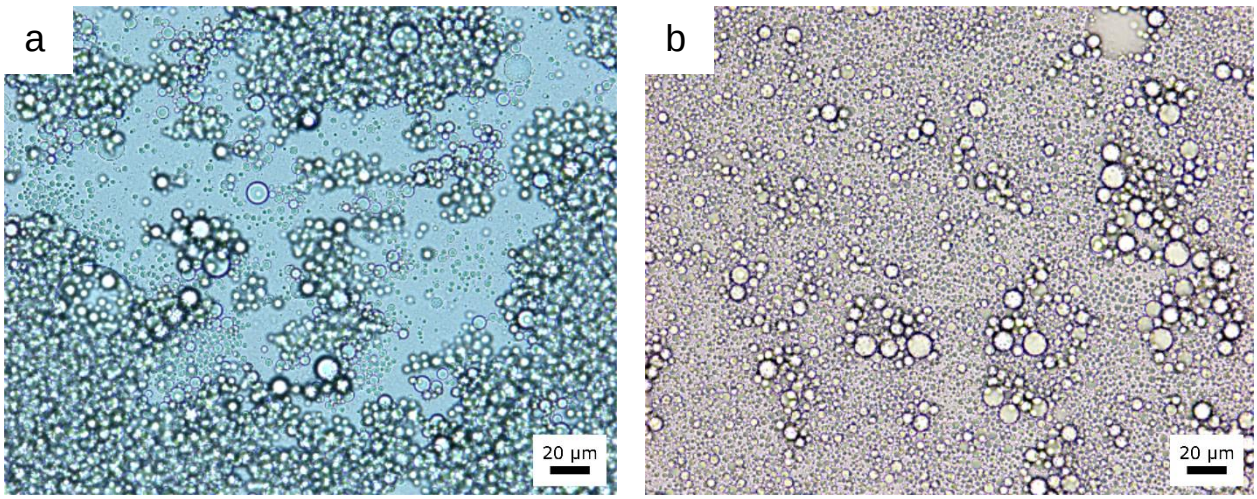

Fig. 7.13 Optical micrograph of emulsions prepared by route R3 at pH 7: a) $CaCl_2$, b) NaCl.

The situation when conducting the emulsification at pH 3 was significantly more complex as there was a net attractive electrostatic interaction between CNC and CAS. Typically, at pH 3, CNC and CAS tended to aggregate, as shown earlier, and the main droplet size centered around 10–12 µm was present (Fig. 7.14). The presence of NaCl or $CaCl_2$ had a minor influence.

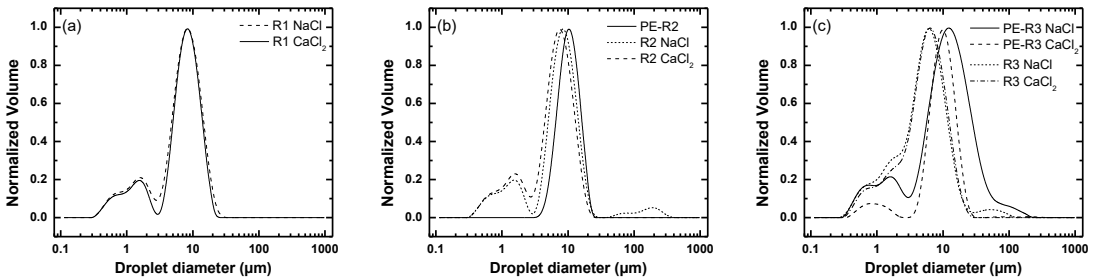

Fig. 7.14 Distribution curves of the emulsions prepared via route a) R1, b) R2 and c) R3 at pH 3. PE-R2 and PE-R3 stand for primary CAS and CNC emulsion, respectively.

As an alternative way to prepare emulsions at pH 3, the effect of decreasing the pH of emulsions prepared at pH 7 was tested. Under these conditions, CNC and CAS had no attractive interaction during the emulsification step, while the decrease in pH of emulsions was meant to gradually induce attraction between CAS and CNC.

To conclude, the study demonstrates the importance of interactions between a protein, sodium caseinate (CAS) and particles, cellulose nanocrystals (CNC), when used in a competitive way for emulsification. The studied emulsions were prepared through different routes of the addition of the protein and the particles,

at pH 7 and 3, at which CNC and CAS had repulsive and attractive interactions, respectively.

The approach revealed that the order of addition controlled the adsorption of dominating species at the oil-water interface, as well as the overall behaviour and properties of the emulsions. At pH 7, the emulsifications went as expected and the properties of final emulsions resulted from a trade-off between the higher surface activity of CAS and the irreversibility of adsorption of CNC. At pH 3, the attractive interactions between CNC and CAS led to a different situation. When initially combined, CAS and CNC aggregated, and the aggregates performed better as a droplet stabilizer than the individual components. The properties of the continuous phase remained unchanged. When added in step-by-step fashion, gelling of the emulsion occurred, the extent of which could be controlled through the order of addition during the emulsification process. The results of the study pointed to the importance of controlling the balance between interfacial behaviour, which can be achieved by cleverly addressing the order of addition of multiple stabilizers, and the regulation of the interactions between the stabilizers.

The results of this study were published in *"Pinďáková, L., et al. Role of protein-cellulose nanocrystal interactions in the stabilization of emulsion by: Journal of Colloid and Interface Science. 557 (2019) 196–206"* and served as the background for the following study aimed at the development and characterization of emulsion-based gels/oleogels.

Oleogels based on CNC and CAS

In this study, emulsion-based gels were prepared from emulsions stabilized by CAS and CNC with respect to the order of their addition to the emulsion system, and their adsorption at the oil-water interface. Here, the experience gathered in the previously described study was employed. Model o/w emulsions were prepared with hexadecane (HD) and then, the HD was substituted with olive oil (OO), which can be used as a suitable carrier for bioactive lipophilic substances. The preparation of emulsions followed three ways presented in chapter 7.1. To prepare emulsion-based oleogels, the emulsions were dried at ambient temperature, and then thoroughly characterized using relevant methods, including the amount of released oil during drying, microscopy and rheology measurement.

The individual performance of CAS and CNC particles alone at olive oil-water and hexadecane-water interface was tested before studying the competitive behaviour of CAS and CNC in mixtures for both oil-water interfaces in an emulsification process. The droplet size distributions revealed similar behaviour of primary emulsions PE-R2 and PE-R3 prepared with 0.2 and 0.3 wt% of CAS and CNC, respectively. All curves are multimodal, the difference between the PE-R2 and PE-R3 is caused by the difference in encapsulation efficacy of CAS and CNC.

To characterize emulsions prepared from mixtures of CAS/CNC in more details, their droplet sizes $D_{(4,3)}$ and distribution curves were recorded. The main

difference in $D_{(4,3)}$ values was observed between emulsions with HD and OO, and the emulsification route played a lower role. The OO formed larger droplets than HD, which is clearly documented by comparison of distribution curves of emulsions prepared with 0.3 wt% stabilizers and NaCl (Fig. 7.15). The difference is probably caused by the character of the oil phases with various physicochemical properties or structure of their molecules.

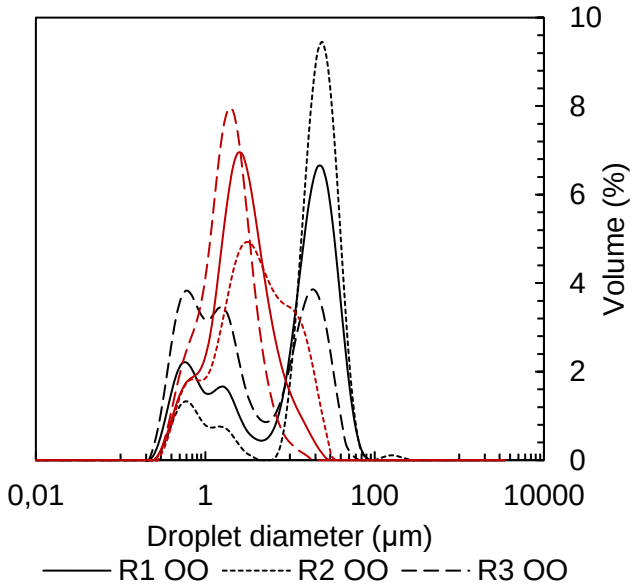

Fig. 7.15 Comparison of distributions for emulsions prepared with olive oil and hexadecane (0.3 wt%, NaCl) prepared via three different routes of CAS and CNC addition (R1, R2, R3).

The second parameter, which significantly influenced the droplet size and stability of emulsions, was the total concentration of stabilizing particles (CNC) and protein (CAS). Generally, the emulsions formulated with total higher amount of stabilizers (0.3 wt%) formed smaller droplets than samples prepared with 0.2 wt% of CNC/CAS due to higher amount of emulsifier available to stabilize smaller droplets with higher surface area (Bai, et al. 2019). This was confirmed by observation of emulsions by optical microscopy (Fig. 7.16).

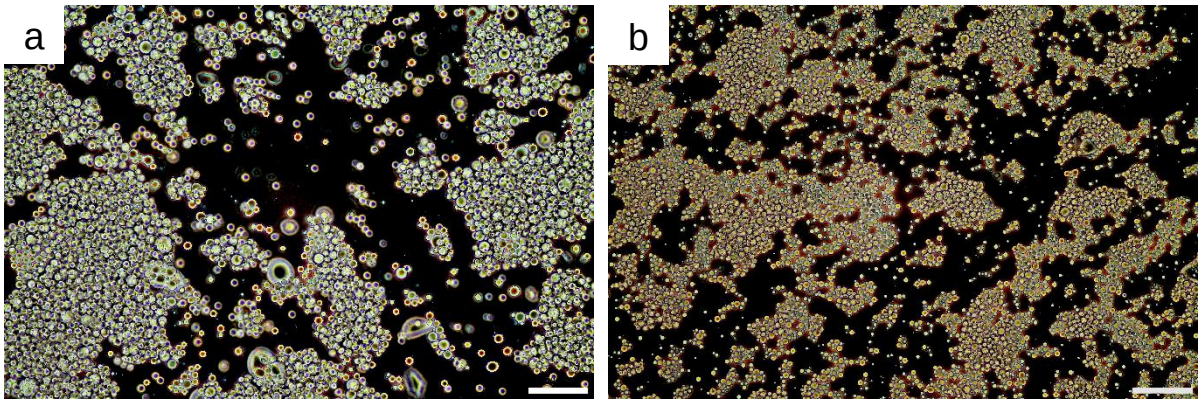

Fig. 7.16 Microscopy images of HD-emulsions prepared via R1 and $CaCl_2$ as background salt; a) 0.2 wt% CNC/CAS; b) 0.3 wt% CNC/CAS.

The last and the most important studied parameter with influence on droplet size and distribution was the effect of the preparation route with respect to the order of CAS and CNC addition. This variable was studied to understand the relationship among emulsion droplet size, structure and arrangement of the oil-water interface, and composition of inter-droplet space, with regard to properties of oleogels. The summary of droplet sizes expressed as $D_{(4,3)}$ is given in Tab. 7.1. This study extended knowledge gathered during the previous study conducted only with hexadecane and one concentration of stabilizing CAS/CNC. The mechanism of stabilization of the oil-water interface thus prepared emulsions is described in the previous study and was published by Pinďáková (2019) (Pinďáková, et al. 2019).

Tab. 7.1 Average sizes of emulsion droplets (± SD) prepared by route R1, R2 and R3 containing hexadecane or olive oil stabilized with 0.2 or 0.3 wt%. stabilizer and different background electrolytes.

$D_{(4,3)}$ ± SD (μm)											
0.2 wt% NaCl			0.3 wt% NaCl			0.2 wt% $CaCl_2$			0.3 wt% $CaCl_2$		
Hexadecane oil											
R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
8.3 ± 2.0	12.0 ± 0.3	4.3 ± 0.1	3.8 ± 0.1	6.2 ± 0.1	2.4 ± 0.0	16.3 ± 0.7	16.9 ± 0.3	14.1 ± 0.9	7.4 ± 0.0	5.9 ± 0.3	5.6 ± 0.2
Olive oil											
0.2 wt% NaCl			0.3 wt% NaCl			0.2 wt% $CaCl_2$			0.3 wt% $CaCl_2$		
R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**
15.2 ± 0.4	33.5 ± 3.8	20.0 ± 2.0	17.9 ± 1.7	23.9 ± 2.9	8.8 ± 1.0	25.0 ± 0.0	11.3 ± 0.0	35.8 ± 2.2	24.7 ± 1.0	3.6 ± 0.2	6.3 ± 0.1

The emulsions were used for the preparation of oleogels. The recent interest in oleogels is obviously connected to their broad range of applications (food processing, drug delivery and cosmetics) (Jiang, et al. 2018; Patel, et al. 2015). The presented study mainly focuses on the preparation of oleogels *via* drying of emulsions stabilized by a relatively low amount of CNC/CAS; moreover, the study evaluates emulsions with respect to the route of preparation and their performance under oleogel formation. Examples of the dried oleogels are shown in Fig. 7.17. The obtained oleogels with HD oil were transparent, solid and compact, whereas oleogels prepared from emulsions containing OO were yellowish (thanks to the colour of the oil) and also transparent. The structure of OO-oleogels was less compact and solid than that of HD-oleogels. This is likely

due to the different properties of the oils used and varying droplet sizes of emulsions. It can be suggested that generally smaller droplets of HD-emulsions are able to get better and tighter arrangements during drying.

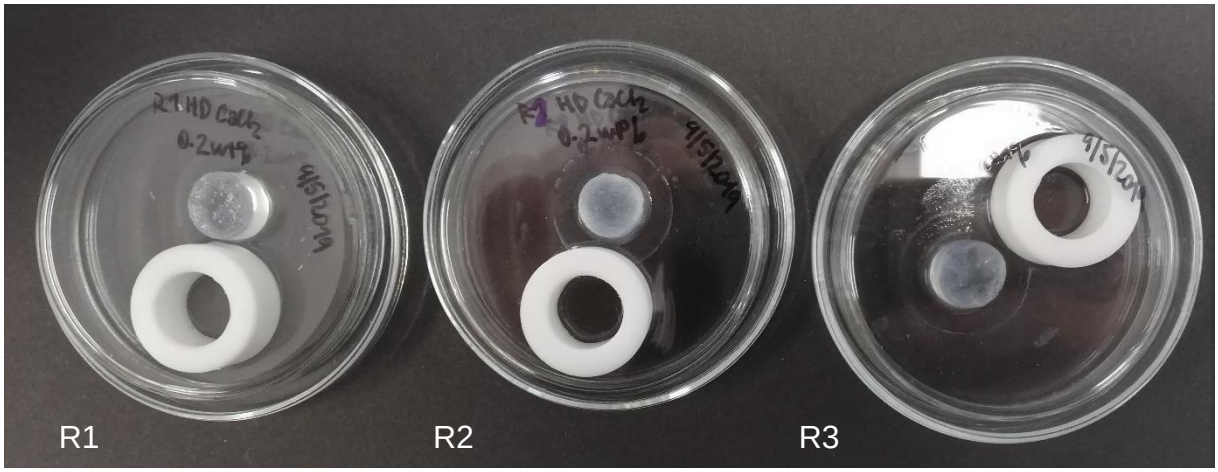

Fig. 7.17 Picture of dried oleogels prepared from hexadecane with 0.2 wt% stabilizer and $CaCl_2$.

During the evaporation of water under oleogel formation, all formulations kept the shape and were stable, nevertheless; a fraction of oil was released during drying, which was measured and compared. The amount of oil released from studied formulations is given in Fig. 7.18. Here, a closer correlation between the amount of oil released from hexadecane oleogels and the route of their preparation is demonstrated. The best oleogels with the lowest amount of released oil were prepared using the route R3, which conforms well to the performance of starting emulsions in terms of droplet size. For olive oil oleogels, the variability among the preparation routes is, with respect to oil liberation, smaller.

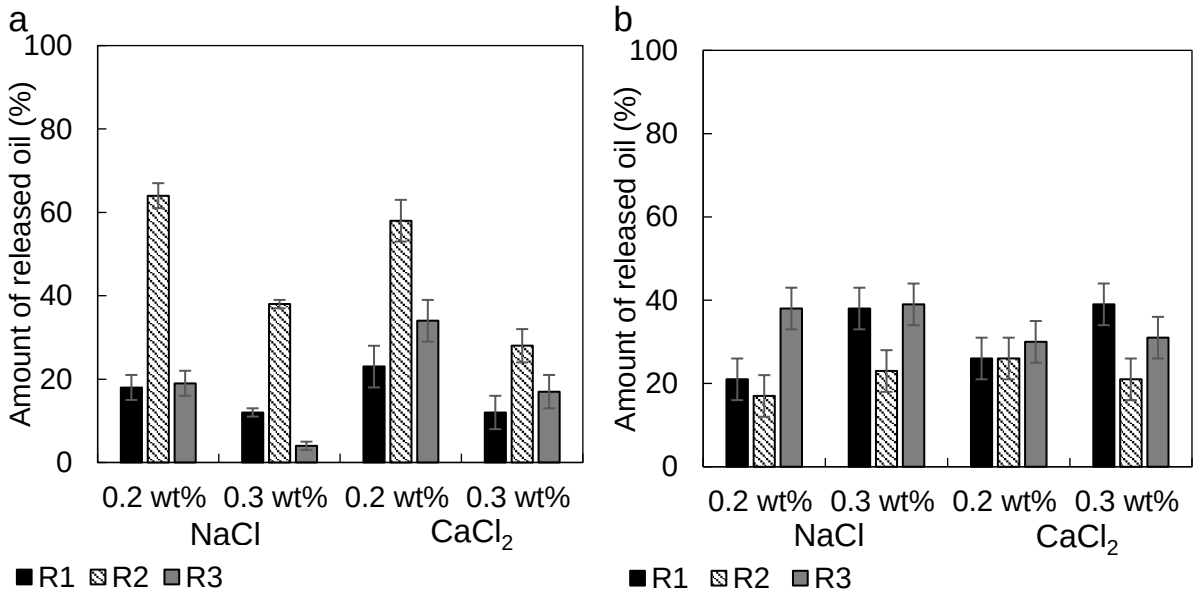

Fig. 7.18 Amount of oil released during drying of oleogels prepared with emulsifications routes R1, R2, R3 containing a) hexadecane b) olive oil.

The dried oleogels were then characterized by a rheometer to gain insights into their viscoelastic properties. Both the storage (*G'*) and loss (*G''*) moduli, as well as loss factor (*tan(δ)*), were determined.

The *G'* and *tan(δ)* dependencies on angular frequency for HD-oleogels are shown on Fig. 7.19. The trend among values of *G'* and *tan(δ)* is common for all formulation of oleogels and values of *G'* are ranked as follows *G''* R3 ≥ *G''* R1 > *G''* R2, except for oleogels with 0.2 wt% of CNC/CAS and NaCl as background salt. The higher values of *G'* indicated better elastic properties for oleogels prepared *via* routes R1 and R3.

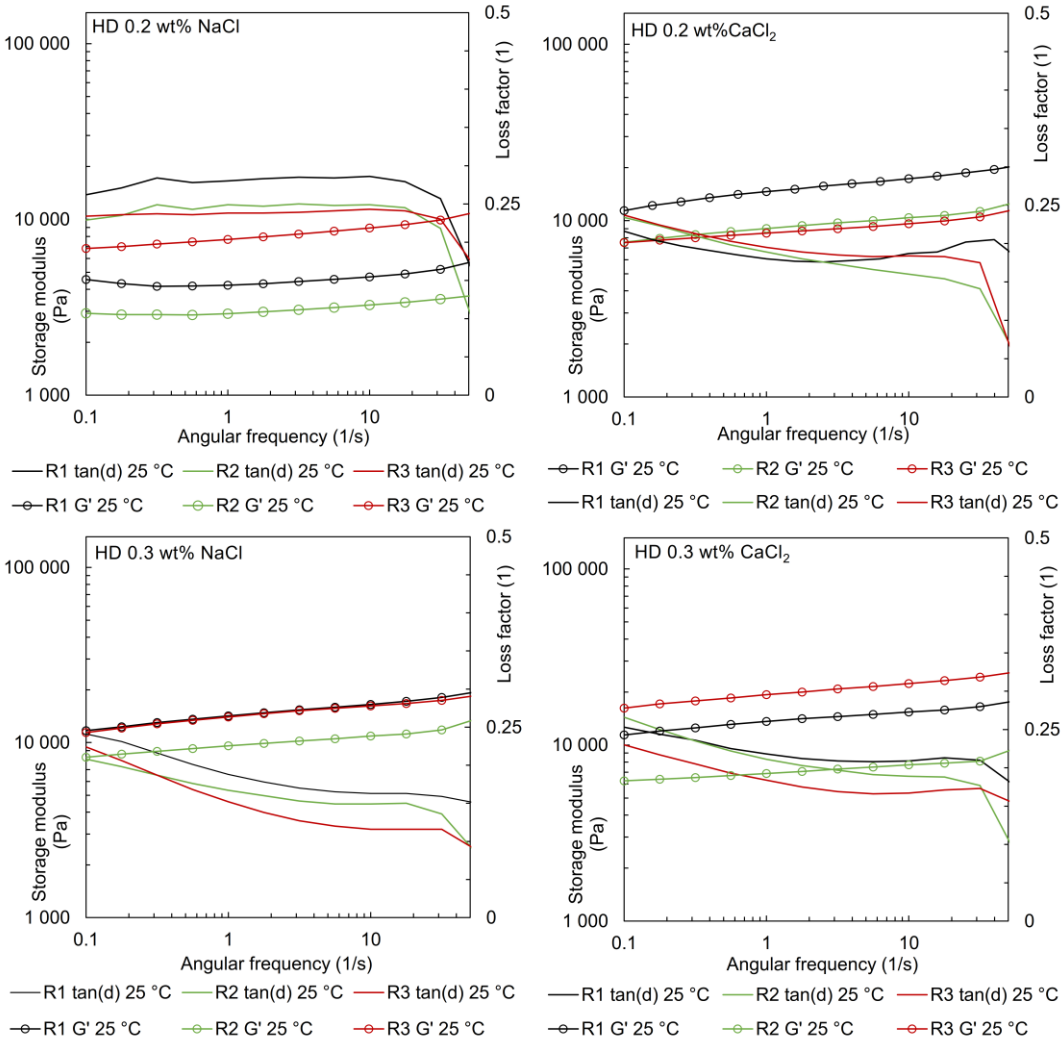

Fig. 7.19 Dynamic storage moduli (G') and loss factor (tan(δ)) responses of oleogels prepared from hexadecane emulsions.

The effects of CNC/CAS concentration on *G'* values recorded for olive oil-based oleogels slightly correlate with corresponding results recorded for hexadecane oleogels. The trend of the *G'' vs* frequency dependencies for gels prepared by the route R3 is also similar to HD-oleogels and their values of *G'* were also higher than those recorded for oleogels formulated using R2 and R1 routes. This again indicated higher elasticity for this formulation.

In general, the viscoelastic behaviour of the studied oleogels is influenced by a sum of various factors, including the droplet sizes in the starting emulsions, the route of emulsion preparation and also by the type of background salt. These findings support hypotheses about the differences in arrangements of CNC and CAS at the oil-water interface and composition of the inter-droplet space described in our previous work (Pinďáková, et al. 2019). The characteristics of the interfacial stabilization layers (the route of preparation) really significantly affect viscoelastic properties of the oleogels and the different viscoelastic properties of samples prepared by the route R1, R2 and R3 are caused by all the above mentioned variables.

The impact of droplet size of emulsions, which is primarily influenced by the route of preparation and concentration of CNC/CAS on the viscoelastic behaviour of oleogels, can be explained as follows: small droplets have a better arrangement in oleogel structure because they are tightly packed during drying with smaller inter-droplet space. Thus the elastic portion of modulus can prevail. On the other side, bigger droplets are packed "loosely" with larger space among droplets, which can be filled with free oil, free particles and aggregates of CAS (in the case $CaCl_2$ is present). The latter factors can, therefore, lead to the higher viscosity of the gels.

As sizes of emulsion droplets are influenced by the composition of stabilizing layers at the oil-water interface (this is controlled by the route of preparation), the structure/composition of phase interfaces and inter-droplet spaces significantly affected the viscoelastic properties of prepared oleogels.

To summary, the study further develops the knowledge on the interactions between a protein, sodium caseinate (CAS) and particles, cellulose nanocrystals (CNC) at the oil-water interface described in the previous chapter, and uses this knowledge in preparation of oleogels. Correspondingly to the previous study, this approach demonstrated that the order of stabilizer addition controlled not the only composition of the surface layer and inter-droplet space in emulsions but also the overall behaviour of the oleogels. The practical performance of the gels depended on their viscoelastic properties and the studied oleogels can serve as a gel form of liquid oils or as carriers of bioactive substances. The paper summarizing the result of the study is in preparation.

8. THE THESIS CONTRIBUTION TO SCIENCE AND PRACTICE

The topic of stabilization of dispersion systems with polymeric emulsifiers, solved within the thesis, remains still not fully explored. This thesis deals not only with emulsion stabilization using the polymeric emulsifiers represented by proteins but also with the application of particulate stabilizers derived from biopolymers. Moreover, the thesis investigates combinations of different emulsifiers and stabilizers, which can offer a new approach to the formulation of emulsions.

Therefore, the most important contributions of the doctoral thesis to science and practice can be summarized in the following achievements. The first study provided a better understanding of emulsification procedures (sonication *vs.* high share homogenization) conducted in presence of single emulsifier (protein sodium caseinate, CAS) with respect to the protection of encapsulated, triacylglycerol-based, antibacterial oils. Moreover, the work confirmed that the antibacterial action of the oils was retained after emulsification, which is an interesting possibility for the application of such systems. The output gathered within this part of the thesis can be used under the preparation of cosmetic formulations containing bioactive oils (such as tamanu or black cumin) stabilized by natural biopolymer caseinate.

The next study on emulsions stabilized by a combination of protein and cellulose nanoparticles provided deeper insight into understanding the interactions between CAS and particles (cellulose nanocrystals, CNC) at air-water and oil-water interfaces. The pioneering work related to model emulsions stabilized with CNC/CAS brings a closer look into the mechanism of adsorption of CNC and CAS at interfaces. The work done contributed to the better knowledge within the theory of emulsion stabilization and understanding of the polymer-particle stabilized systems.

During the last study, emulsion-based oleogels were investigated. The results of the work revealed that dominating species at the interface (CNC or CAS) and type of the oil used affected the viscoelastic properties of oleogels and subsequently their behaviour related to possible practical application. From the more elastic oleogels containing lipophilic substances, these can be slowly released by (bio)degradation of the gel structure. On the other hand, the more viscous oleogels can be suitable for the better spreading of cosmetic ingredients on the skin. Such oleogels can find widespread applications as carriers of lipophilic active ingredients.

BIBLIOGRAPHY

Abascal, D. M. and Gracia-Fadrique, J. Surface tension and foam stability of commercial calcium and sodium caseinates. *Food Hydrocolloids.* 2009, vol. 23, no. 7, p. 1848-1852. doi: 10.1016/j.foodhyd.2009.02.012.

Amine, C. et al. Investigation of emulsifying properties and emulsion stability of plant and milk proteins using interfacial tension and interfacial elasticity. *Food Hydrocolloids.* 2014, vol. 39, p. 180-186. doi: 10.1016/j.foodhyd.2014.01.001.

Andresen, M. and Stenius, P. Water-in-oil emulsions stabilized by hydrophobized microfibrillated cellulose. *Journal of Dispersion Science and Technology.* 2007, vol. 28, no. 6, p. 837-844. doi: 10.1080/01932690701341827.

Bai, L. et al. Oil-in-water Pickering emulsions via microfluidization with cellulose nanocrystals: 1. Formation and stability. *Food Hydrocolloids.* 2019, vol. 96, p. 699-708. doi: 10.1016/j.foodhyd.2019.04.038.

Binks, B. P. Colloidal particles at liquid interfaces. *Physical Chemistry Chemical Physics.* 2007, vol. 9, no. 48, p. 6298-6299. doi: 10.1039/b716587k.

Bouyer, E. et al. Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field?. *International Journal of Pharmaceutics.* 2012, vol. 436, no. 1-2, p. 359-378. doi: 10.1016/j.ijpharm.2012.06.052.

Capron, I. and Cathala, B. Surfactant-Free High Internal Phase Emulsions Stabilized by Cellulose Nanocrystals. *Biomacromolecules.* 2013, vol. 14, no. 2, p. 291-296. doi: 10.1021/bm301871k.

Capron, I., Rojas, O. J. and Bordes, R. Behavior of nanocelluloses at interfaces. *Current Opinion in Colloid & Interface Science.* 2017, vol. 29, p. 83-95. doi: 10.1016/j.cocis.2017.04.001.

Cherhal, F., Cousin, F. and Capron, I. Structural Description of the Interface of Pickering Emulsions Stabilized by Cellulose Nanocrystals. *Biomacromolecules.* 2016, vol. 17, no. 2, p. 496-502. doi: 10.1021/acs.biomac.5b01413.

Cherhal, F., Cousin, F. and Capron, I. Influence of Charge Density and Ionic Strength on the Aggregation Process of Cellulose Nanocrystals in Aqueous Suspension, as Revealed by Small-Angle Neutron Scattering. *Langmuir.* 2015, vol. 31, no. 20, p. 5596-5602. doi: 10.1021/acs.langmuir.5b00851.

Cosgrove, T. *Colloid Science: Principles, Methods and Applications*2nd edition.Wiley - Blackwell, 2005. 394 p.

Day, L. et al. Characterisation of fish oil emulsions stabilised by sodium caseinate. *Food Chemistry*. 2007, vol. 105, no. 2, p. 469-479. doi: 10.1016/j.foodchem.2007.04.013.

Dickinson, E. Biopolymer-based particles as stabilizing agents for emulsions and foams. *Food Hydrocolloids.* 2017, vol. 68, p. 219-231. doi: 10.1016/j.foodhyd.2016.06.024.

Dickinson, E. Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids.* 2011, vol. 25, no. 8, p. 1966-1983. doi: 10.1016/j.foodhyd.2010.12.001.

Dickinson, E. Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids.* 2009, vol. 23, no. 6, p. 1473-1482. doi: 10.1016/j.foodhyd.2008.08.005.

Dickinson, E. Caseins in emulsions: interfacial properties and interactions. *International Dairy Journal.* 1999, vol. 9, no. 3, p. 305-312. doi: 10.1016/S0958-6946(99)00079-5.

Dickinson, E. and Davies, E. Influence of ionic calcium on stability of sodium caseinate emulsions. *Colloids and Surfaces B: Biointerfaces.* 1999, vol. 12, no. 3, p. 203-212. doi: 10.1016/S0927-7765(98)00075-7.

Drusch, S. et al. New insights into the microencapsulation properties of sodium caseinate and hydrolyzed casein. *Food Hydrocolloids.* 2012, vol. 27, no. 2, p. 332-338. doi: 10.1016/j.foodhyd.2011.10.001.

Fox, P. F. and Brodkorb, A. The casein micelle: Historical aspects, current concepts and significance. *International Dairy Journal.* 2008, vol. 18, no. 7, p. 677-684. doi: 10.1016/j.idairyj.2008.03.002.

Friberg, S., Larsson, K. and Sjoblom, J. *Food Emulsions*. 4th.CRC Press, 2003.

Giermanska-Kahn, J. et al. Particle-stabilized emulsions comprised of solid droplets. *Langmuir.* 2005, vol. 21, no. 10, p. 4316-4323. doi: 10.1021/la0501177.

Gorji, S. G., Gorji, E. G. and Mohammadifar, M. A. Effect of pH on turbidity, size, viscosity and the shape of sodium caseinate aggregates with light scattering and rheometry. *Journal of Food Science and Technology-Mysore.* 2015, vol. 52, no. 3, p. 1820-1824. doi: 10.1007/s13197-013-1144-2.

Guzey, D. and McClements, D. J. Formation, stability and properties of multilayer emulsions for application in the food industry. *Advances in Colloid and Interface Science.* 2006, vol. 128, p. 227-248. doi: 10.1016/j.cis.2006.11.021.

Hebishy, E. et al. Ultra high-pressure homogenized emulsions stabilized by sodium caseinate: Effects of protein concentration and pressure on emulsions structure and stability. *Lwt-Food Science and Technology.* 2017, vol. 76, p. 57-66. doi: 10.1016/j.lwt.2016.10.045.

Hu, Z. et al. Dried and Redispersible Cellulose Nanocrystal Pickering Emulsions. *Acs Macro Letters.* 2016, vol. 5, no. 2, p. 185-189. doi: 10.1021/acsmacrolett.5b00919.

Hu, Z. et al. Synergistic Stabilization of Emulsions and Emulsion Gels with Water-Soluble Polymers and Cellulose Nanocrystals. *Acs Sustainable Chemistry & Engineering.* 2015, vol. 3, no. 5, p. 1023-1031. doi: 10.1021/acssuschemeng.5b00194.

Huck-Iriart, C. et al. Structures and stability of lipid emulsions formulated with sodium caseinate. *Current Opinion in Colloid & Interface Science.* 2011, vol. 16, no. 5, p. 412-420. doi: 10.1016/j.cocis.2011.06.003.

Jiang, Y. et al. Cellulose-rich oleogels prepared with an emulsion-templated approach. *Food Hydrocolloids.* 2018, vol. 77, p. 460-464. doi: 10.1016/j.foodhyd.2017.10.023.

Joye, I. J. and McClements, D. J. Biopolymer-based nanoparticles and microparticles: Fabrication, characterization, and application. *Current Opinion in Colloid & Interface Science.* 2014, vol. 19, no. 5, p. 417-427. doi: 10.1016/j.cocis.2014.07.002.

Kalashnikova, I. et al. Cellulosic nanorods of various aspect ratios for oil in water Pickering emulsions. *Soft Matter.* 2013, vol. 9, no. 3, p. 952-959. doi: 10.1039/c2sm26472b.

Kalashnikova, I. et al. Modulation of Cellulose Nanocrystals Amphiphilic Properties to Stabilize Oil/Water Interface. *Biomacromolecules.* 2012, vol. 13, no. 1, p. 267-275. doi: 10.1021/bm201599j.

Kalashnikova, I. et al. New Pickering Emulsions Stabilized by Bacterial Cellulose Nanocrystals. *Langmuir.* 2011, vol. 27, no. 12, p. 7471-7479. doi: 10.1021/la200971f.

Lam, R. S. H. and Nickerson, M. T. Food proteins: A review on their emulsifying properties using a structure–function approach. *Food Chemistry*. 2013, vol. 141, no. 2, p. 975-984. doi: 10.1016/j.foodchem.2013.04.038.

Lam, S., Velikov, K. P. and Velev, O. D. Pickering stabilization of foams and emulsions with particles of biological origin. *Current Opinion in Colloid & Interface Science.* 2014, vol. 19, no. 5, p. 490-500. doi: 10.1016/j.cocis.2014.07.003.

Lee, K. et al. Phase Behavior of Medium and High Internal Phase Water-in-Oil Emulsions Stabilized Solely by Hydrophobized Bacterial Cellulose Nanofibrils. *Langmuir*. 2014, vol. 30, no. 2, p. 452-460. doi: 10.1021/la4032514.

Liu, F. et al. Pickering high internal phase emulsions stabilized by protein-covered cellulose nanocrystals. *Food Hydrocolloids.* 2018, vol. 82, p. 96-105. doi: 10.1016/j.foodhyd.2018.03.047.

Liu, Y. and Guo, R. pH-dependent structures and properties of casein micelles. *Biophysical Chemistry*. 2008, vol. 136, no. 2-3, p. 67-73. doi: 10.1016/j.bpc.2008.03.012.

Livney, Y. D. Milk proteins as vehicles for bioactives. *Current Opinion in Colloid & Interface Science.* 2010, vol. 15, no. 1-2, p. 73-83. doi: 10.1016/j.cocis.2009.11.002.

Matalanis, A., Jones, O. G. and McClements, D. J. Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. *Food Hydrocolloids.* 2011, vol. 25, no. 8, p. 1865-1880. doi: 10.1016/j.foodhyd.2011.04.014.

McClements, D. J., Decker, E. A. and Weiss, J. Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science.* 2007, vol. 72, no. 8, p. R109-R124. doi: 10.1111/j.1750-3841.2007.00507.x.

McClements, D. J. *Food Emulsions: Principles, Practices, and Techniques*. Third Edition.CRC Press, 2015. 690 p.

McClements, D. J. *Modern biopolymer science*. Stefan KASAPIS, Ian T. NORTON a Johan B. UBBINK. San Diego: Academic Press. 2009. CHAPTER 4 - Biopolymers in Food Emulsions. 129-166 s. ISBN 9780123741950.

McClements, D. J. Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science.* 2004, vol. 9, no. 5, p. 305-313. doi: 10.1016/j.cocis.2004.09.003.

McClements, D. J. and Gumus, C. E. Natural emulsifiers — Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and Interface Science.* 2016, vol. 234, p. 3-26. doi: 10.1016/j.cis.2016.03.002.

Mikulcova, V., Bordes, R. and Kasparkova, V. On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles. *Food Hydrocolloids.* 2016, vol. 61, p. 780-792. doi: 10.1016/j.foodhyd.2016.06.031.

Mikulcova, V. et al. Pickering oil-in-water emulsions stabilized by carboxylated cellulose nanocrystals - Effect of the pH. *Food Hydrocolloids.* 2018, vol. 80, p. 60-67. doi: 10.1016/j.foodhyd.2018.01.034.

Neirynck, N. et al. Improved emulsion stabilizing properties of whey protein isolate by conjugation with pectins. *Food Hydrocolloids.* 2004, vol. 18, no. 6, p. 949-957. doi: 10.1016/j.foodhyd.2004.03.004.

Nielsen, N. S. and Jacobsen, C. Methods for reducing lipid oxidation in fish-oil-enriched energy bars. *International Journal of Food Science and Technology.* 2009, vol. 44, no. 8, p. 1536-1546. doi: 10.1111/j.1365-2621.2008.01786.x.

Ougiya, H. et al. Emulsion-stabilizing effect of bacterial cellulose. *Bioscience Biotechnology and Biochemistry.* 1997, vol. 61, no. 9, p. 1541-1545. doi: 10.1271/bbb.61.1541.

Oza, K. P. and Frank, S. G. Microcrystalline Cellulose Stabilized Emulsions. *Journal of Dispersion Science and Technology.* 1986, vol. 7, no. 5, p. 543-561. doi: 10.1080/01932698608943478.

Patel, A. R. et al. Biopolymer-Based Structuring of Liquid Oil into Soft Solids and Oleogels Using Water-Continuous Emulsions as Templates. *Langmuir*. 2015, vol. 31, no. 7, p. 2065-2073. doi: 10.1021/la502829u.

Paukkonen, H. et al. Hydrophobin-nanofibrillated cellulose stabilized emulsions for encapsulation and release of BCS class II drugs. *European Journal of Pharmaceutical Sciences.* 2017, vol. 100, p. 238-248. doi: 10.1016/j.ejps.2017.01.029.

Perrechil, F. A. and Cunha, R. L. Oil-in-water emulsions stabilized by sodium caseinate: Influence of pH, high-pressure homogenization and locust bean gum addition. *Journal of Food Engineering.* 2010, vol. 97, no. 4, p. 441-448. doi: 10.1016/j.jfoodeng.2009.10.041.

Pinďáková, L., Kašpárková, V. and Bordes, R. Role of protein-cellulose nanocrystal interactions in the stabilization of emulsion. *Journal of Colloid and Interface Science.* 2019, vol. 557, p. 196-206. doi: 10.1016/j.jcis.2019.09.002.

Post, A. E. et al. Effect of temperature and pH on the solubility of caseins: Environmental influences on the dissociation of alpha(S)- and beta-casein. *Journal of Dairy Science.* 2012, vol. 95, no. 4, p. 1603-1616. doi: 10.3168/jds.2011-4641.

Rodríguez Patino, J. M. and Pilosof, A. M. R. Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids.* 2011, vol. 25, no. 8, p. 1925-1937. doi: 10.1016/j.foodhyd.2011.02.023.

Sarkar, A. et al. Composite whey protein-cellulose nanocrystals at oil-water interface: Towards delaying lipid digestion. *Food Hydrocolloids.* 2018, vol. 77, p. 436-444. doi: 10.1016/j.foodhyd.2017.10.020.

Sarkar, A. et al. Modulating in vitro gastric digestion of emulsions using composite whey protein-cellulose nanocrystal interfaces. *Colloids and Surfaces B-Biointerfaces.* 2017, vol. 158, p. 137-146. doi: 10.1016/j.colsurfb.2017.06.037.

Sarker, D. K., Axelos, M. and Popineau, Y. Methylcellulose-induced stability changes in protein-based emulsions. *Colloids and Surfaces B-Biointerfaces.* 1999, vol. 12, no. 3-6, p. 147-160. doi: 10.1016/S0927-7765(98)00071-X.

Schramm, L. L. *Emulsions, Foams, and Suspensions: Fundamentals and Applications*. Wiley, 2006. 463 p.

Tavernier, I. et al. Food-grade particles for emulsion stabilization. *Trends in Food Science & Technology.* 2016, vol. 50, p. 159-174. doi: 10.1016/j.tifs.2016.01.023.

Tcholakova, S. et al. Coalescence stability of emulsions containing globular milk proteins. *Advances in Colloid and Interface Science.* 2006, vol. 123, p. 259-293. doi: 10.1016/j.cis.2006.05.021.

Thomar, P. et al. The influence of adding monovalent salt on the rheology of concentrated sodium caseinate suspensions and the solubility of calcium caseinate. *International Dairy Journal.* 2014, vol. 37, no. 1, p. 48-54. doi: 10.1016/j.idairyj.2014.02.007.

Timgren, A. et al. Starch particles for food based Pickering emulsions. *11th International Congress on Engineering and Food (Icef11).* 2011, vol. 1, p. 95-103. doi: 10.1016/j.profoo.2011.09.016.

Tippetts, M. and Martini, S. Influence of iota-Carrageenan, Pectin, and Gelatin on the Physicochemical Properties and Stability of Milk Protein-Stabilized Emulsions. *Journal of Food Science.* 2012, vol. 77, no. 2, p. C253-C260. doi: 10.1111/j.1750-3841.2011.02576.x.

Varjonen, S. et al. Self-assembly of cellulose nanofibrils by genetically engineered fusion proteins. *Soft Matter.* 2011, vol. 7, no. 6, p. 2402-2411. doi: 10.1039/c0sm01114b.

Xhanari, K., Syverud, K. and Stenius, P. Emulsions Stabilized by Microfibrillated Cellulose: The Effect of Hydrophobization, Concentration and O/W Ratio. *Journal of Dispersion Science and Technology.* 2011, vol. 32, no. 3, p. 447-452. doi: 10.1080/01932691003658942.

Yang, Y. et al. An Overview of Pickering Emulsions: Solid-Particle Materials, Classification, Morphology, and Applications. *Frontiers in Pharmacology.* 2017, vol. 8, p. 287. doi: 10.3389/fphar.2017.00287.

Ye, A. Q. and Singh, H. Interfacial composition and stability of sodium caseinate emulsions as influenced by calcium ions. *Food Hydrocolloids.* 2001, vol. 15, no. 2, p. 195-207. doi: 10.1016/S0268-005X(00)00065-5.

Yusoff, A. and Murray, B. S. Modified starch granules as particle-stabilizers of oil-in-water emulsions. *Food Hydrocolloids.* 2011, vol. 25, no. 1, p. 42-55. doi: 10.1016/j.foodhyd.2010.05.004.

LIST OF ABBREVIATIONS

AFM	Atomic force microscopy
BC	Black cumin oil
CAS	Casein/sodium caseinete
$CaCl_2$	Calcium chloride
CI	Creaming index
CNC	Cellulose nanocrystals
DLS	Dynamic light scattering
EE	Encapsulation efficacy
HCl	Hydrochloric acid
HD	Hexadecane
L-b-l	Layer-by-layer
MCC	Microcrystalline cellulose
NaCl	Sodium chloride
NaOH	Sodium hydroxide
OO	Olive oil
o/w	Oil-in-water
PE	Primary emulsion
PEI	Polyethyleneimine
QCM-D	Quartz crystal microbalance with dissipation monitoring
TA	Tamanu oil
US	Sonication
UT	Ultra-turrax

LIST OF UNITS

°C	Degree Celsius
h	Hour
kHz	Kilohertz
M	Molarity (mole per liter)
min	Minute
mM	Millimole per liter
mm	Millimeter
mPa.s	Millipascal second
mV	Millivolt
nm	Nanometer
$ng.cm^{-2}$	Nanogram per square centrimeter
Pa	Pascal
rpm	Revolutions per minute
s	Second
W	Watt
wt%	Percentage by mass

LIST OF SYMBOLS

$D_{(4,3)}$	Volume mean diameter
G'	Storage modulus
G''	Loss modulus
pH	Potential of hydrogen
Θ °	Theta degree
tan(δ)	Loss factor
ζ	Zeta

LIST OF FIGURES

Fig. 2.1 Possible ways of stabilization of emulsions by a) surfactants, b) proteins, c) polysaccharides and d) particles (Dickinson 2009)......9

Fig. 4.1 Examples of bio-derived particles of various origins applicable to Pickering stabilization: a) E. coli bacteria; b) moss spore; c) ethyl cellulose nanoparticles; d) chitosan nanoparticles; e) cellulose nanocrystals from cotton; f) silylated cellulose fibres; g) acetylated starch phthalic ester nanospheres; h) quinoa starch granules; i) spray-dried soy protein particles (Lam, S., et al. 2014). 12

Fig. 5.1 Schematic representation of different types of mixed interfacial layers that can be formed at oil droplet surfaces to stabilize emulsions: a) co-adsorption; b) complexation; c) layer-by-layer (McClements, David Julian and Gumus 2016). 14

Fig. 7.1 The routes of emulsion preparation (Pinďáková, et al. 2019).....19

Fig. 7.2 Influence of CAS concentration and o/w ratio on volume-weighted diameter of emulsion droplets ($D_{(3,4)}$) of a) TA oil-emulsions prepared by Ultra-Turrax (UT); b) TA oil-emulsions prepared by sonication (US), c) BC oil-emulsions prepared by UT, and d) BC oil-emulsion prepared by US. 21

Fig. 7.3 Comparison of creaming index (CI; CI ~ 100 % is for stable emulsion) determined on freshly prepared emulsions as affected by processing method and composition of emulsions a) BC-emulsions prepared with Ultra-Turrax (UT), b) BC-emulsions prepared with sonication (US), c) TA-emulsion prepared with UT, and d) TA-emulsion with US......22

Fig. 7.4 AFM micrographs and corresponding height profiles (below) of dried aqueous dispersions of a) CNC, and CAS at b) pH 7 and c) pH 3. 24

Fig. 7.5 Size of a) CNC and b) CAS as a function of pH (: at pH 4, sedimentation occurred).*.....25

Fig. 7.6 Adsorption of CAS on CNC surface at pH = 7 (a) and pH = 3 (b) as determined by QCM-D. 26

Fig. 7.7 Surface tension at pH 7 of CNC and CAS dispersions a) and their mixtures b) at air-water (empty symbol) and hexadecane-water interface (plain symbol). The error was below 1 and 2 % for the measurements at the air/water and oil/water interface, respectively. 27

Fig. 7.8 Surface tension at pH 3 of CNC and CAS dispersions (a) and their mixtures (b) at air-water (empty symbol) and hexadecane-water (plain symbol) interface (pH 3). The error was below 1% and 2% for the measurements at the air/water and oil/water interface, respectively. 28

Fig. 7.9 Size distribution curves of the primary emulsions PE-R2, PE-R3 $CaCl_2$, PE-R3 NaCl at pH 7. 29

Fig. 7.10 Distribution curves of the emulsions prepared via route a) R1, b) R2 and c) R3 at pH 7. PE-R2 and PE-R3 stand for primary CAS and CNC emulsion, respectively. 29

Fig. 7.11 Optical micrograph of emulsions prepared by route R1 $CaCl_2$ a), R1 NaCl b), at pH 7. 30

Fig. 7.12 Optical micrograph of emulsions prepared by route R2 at pH 7: a) $CaCl_2$, b) NaCl. 30

Fig. 7.13 Optical micrograph of emulsions prepared by route R3 at pH 7: a) $CaCl_2$, b) NaCl. 31

Fig. 7.14 Distribution curves of the emulsions prepared via route a) R1, b) R2 and c) R3 at pH 3. PE-R2 and PE-R3 stand for primary CAS and CNC emulsion, respectively. 31

Fig. 7.15 Comparison of distributions for emulsions prepared with olive oil and hexadecane (0.3 wt%, NaCl) prepared via three different routes of CAS and CNC addition (R1, R2, R3). 33

Fig. 7.16 Microscopy images of HD-emulsions prepared via R1 and $CaCl_2$ as background salt; a) 0.2 wt% CNC/CAS; b) 0.3 wt% CNC/CAS. 33

Fig. 7.17 Picture of dried oleogels prepared from hexadecane with 0.2 wt% stabilizer and $CaCl_2$. 35

Fig. 7.18 Amount of oil released during drying of oleogels prepared with emulsifications routes R1, R2, R3 containing a) hexadecane b) olive oil. 35

Fig. 7.19 Dynamic storage moduli (G‘) and loss factor (tan(δ)) responses of oleogels prepared from hexadecane emulsions. 36

LIST OF TABLES

Tab. 7.1 Average sizes of emulsion droplets (± SD) prepared by route R1, R2 and R3 containing hexadecane or olive oil stabilized with 0.2 or 0.3 wt%. stabilizer and different background electrolytes. 34

LIST OF PUBLICATION

Publication related to the topic of the thesis

Publication I

Urbánková, L.; Kašpárková, V.; Egner, P.; Rudolf, O; Korábková, E. Caseinate-Stabilized Emulsions of Black Cumin and Tamanu Oils: Preparation, Characterization and Antibacterial Activity. *Polymers.* 2019, **11,** 1951**.**

Publication II

Pinďáková, L.; Kašpárková, V.; Bordes, R. Role of protein-cellulose nanocrystal interactions in the stabilization of emulsion. *Journal of colloid and interface science.* 2019, **557**, 196–206.

Other publications

Publication III

Pinďáková L., Kašpárková, V.; Kejlová, K.; Dvořáková, M.; Krsek, D.; Jírová, D.; Kašparová, L. Behaviour of silver nanoparticles in simulated saliva and gastrointestinal fluids. *International Journal of Pharmaceutics*. 2017, **527**, 12-20.

Publication IV

Kowalska, M.; Woźniak, M.; Kašpárková, V.; **Pinďáková, L.**; Żbikowska, A. Effect of ultrasonic processing on the particle size and distribution of the emulsion containing enzymatically interesterified fat. *Journal of Food Processing and Preservation*. 2018, **42**, e13692.

Publication V

Pavlačková, J.; Egner, P.; Polašková, J.; Hojerová, J.; **Pinďáková, L.**; Mokrejš, P.; Varaďová, V. Transdermal absorption of active substances from cosmetic vehicles. *Journal of Cosmetic Dermatology*. 2019; **18**, 1410-1415.

Publication VI

Egner, P.; Kašpárková, V.; Pavlačková, J.; Sedlaříková, J.; **Pinďáková, L.** Effect of process parameters and methylcellulose supplementation on the properties of n-undecane emulsions. *Journal of Dispersion Science and Technology*. 2017, **38**, 775-81.

CONFERENCE PROCEEDINGS

Pinďáková, L.; Kašpárková, V.; Egner, P.; Pučálková, L. Encapsulation of fish oil using sodium caseinate. *6th International Colloids Conference*, Berlin, June 19–22, 2016.

Pinďáková, L.; Kašpárková, V.; Egner, P. Preparation and characterization of emulsions stabilized by sodium caseinate-chitosan complex. *16th European Student Colloid Conference*, Florence, June 19–22, 2017.

Urbánková, L.; Kašpárková, V.; Bordes, R. Characterization and properties of gels based on emulsions stabilized by cellulose nanocrystals and caseinate. *33rd Conference of the European Colloid and Interface Society*, Leuven, September 8–13, 2019.

Kašpárková, V.; **Urbánková, L.**; Mikulcová, V.; Vlachynská, L.; Bordes, R. Pickering o/w emulsions: transdermal delivery of lipophilic actives. *33rd Conference of the European Colloid and Interface Society*, Leuven, September 8–13, 2019.

CURRICULUM VITAE

Name: Lucie Urbánková, roz. Pinďáková

Address: Vrbětice 141, 763 24 Vlachovice

Nationality: Czech

Email: urbankova@utb.cz

Tel.: +420 576 031 235

Employment history:

09/2019 – to now Assistant, Department of fats, surfactants and cosmetics

09/2018–08/2019 Laboratory technician, Department of fats, surfactants and cosmetics

Education:

11/2015 – to date Ph.D.,Chemistry and material technology
Faculty of technology, TBU in Zlín, Czech Republic
Topic: Stabilization of dispersion systems by polymeric emulsifiers

2013–2015 M.Sc., Chemistry and technology of fats, surfactants and cosmetics
Faculty of technology, TBU in Zlín, Czech Republic

2010–2013 B.Sc., Chemistry and technology of fats, surfactants and cosmetics
Faculty of technology, TBU in Zlín, Czech Republic

Research experience - projects:

2020 GA20-28732S Colloidal systems for topical formulations. Pickering emulsions and colloids on polymer basis.
(Member of the project team)

2019 IGA/CPS/2019/004 Biological properties of polymers
(Member of the project team)

2018 IGA/CPS/2018/001 Biological properties of polymers (Member of the project team)

2017 IGA/CPS/2017/001 Biological evaluation of polymers (Member of the project team)

2016 IGA/FT/2016/006 Particle-stabilized emulsions (Member of the project team)

Training:

10/2016 Training course Chromservis – Novinky v instrumentální technice

05/2018 Training course Malvern Panalytical – Průlomové technologie ve stanovení velikosti částic a zeta-potenciálu nanočástic

Training abroad:

02/2018–04/2018 Erasmus+ Programme internship: Chalmers University of Technology, Göteborg, Sweden

Lucie Urbánková

Stabilization of dispersion systems by polymeric emulsifiers

Stabilizace disperzních systémů polymerními emulgátory

Doctoral thesis summary

Published by: Tomas Bata University in Zlín,

nám. T. G. Masaryka 5555, 760 01 Zlín

Edition: published electronically

Typesetting by: Lucie Urbánková

This publication has not undergone any proofreading or editorial review.

Year of publication: 2020

First Edition

ISBN 978-80-7454-925-0