SUMMARY

Milk proteins and polysaccharides are ingredients used in food products and pharmaceutical products. The main motivation of this research was to better understand and control the interactions of ingredients within food products. This thesis aimed to investigate the behavior of protein / polysaccharide mixtures under mildly acid conditions, as in food products. Then, usually the protein is positively charged and the polysaccharide negatively charged and can form an electrostatic complex.

In Chapter 1, an introduction to the behavior of protein / polysaccharide mixtures is given. Emphasis is put on aggregative phase separation and complex coacervation in particular. Complex coacervation is the term used for the liquid / liquid phase separation arising from the formation of a coacervate layer composed of highly concentrated protein / polysaccharide complexes. Few theoretical descriptions were developed from the 1930’s until today and an overview of the current status of research is given. Complex coacervates and protein / polysaccharide complexes in general find application in the field of purification of macromolecules. Also coacervates are applied as new food ingredients like fat replacers and meat analogues, or as new biomaterials in medicine (e.g. wound dressing, protheses). The most important industrial application is the use of complex coacervates in microencapsulation, where the liquid coacervate is used as a coating around sensitive materials. Traditionally complex coacervation of gelatine and gum arabic is mainly used industrially. Nowadays, there is, however, a need to replace gelatine for health and religious reasons, and that is the reason why whey proteins (WP) were chosen as the proteins of interest in this thesis. WP (mainly â-lactoglobulin – iso electric point pI = 5.2) is a protein present in milk. In the first part of the thesis three different polysaccharides were used in combination with WP, i.e. gum Arabic (GA), the exopolysaccharide EPS B40 (EPS B40) and carrageenan (CG). These polysaccharides carry different charged groups; GA bears carboxyl groups, EPS B40, phosphate groups, and CG sulfate groups. The physicochemical conditions for the formation of an electrostatic complex were investigated and compared for each WP/polysaccharide system (Chapters 2-4). The following chapters (Chapters 5-7) deal exclusively with the WP/GA system. An attempt is made to understand the structure of
the WP/GA coacervate which is poorly understood so far. A direct application of this system was tested by encapsulating oil with WP/GA complex coacervates (Chapter 8).

In **Chapter 2**, it was shown that WP/GA mixtures form an electrostatic complex in a specific pH range. By slowly acidifying the mixture with glucono-α-lactone (GDL), various pH boundaries were determined. Soluble WP/GA complexes were formed at pH\(_c\), close to the iso electric point (pI) of the α-lactoglobulin (α-lg). On lowering even further the pH, macroscopic phase separation (complex coacervation) took place at a pH designated as pH\(_{O1}\). Finally, at pH\(_{O2}\), complexation was suppressed because GA tends to neutrality. It was also shown that α-lg was the main complex forming protein (as compared to α-lactalbumin). In the region of soluble complexes (between pH\(_c\) and pH\(_{O1}\)), and at low ionic strength, the GA molecule shrunk when WP interacted with the molecule, due to a reduction of the intramolecular repulsion. Increasing ionic strength in the system led to a shift of the pH boundaries to more acidic pH values, which was summarized in a state diagram and could be understood from a newly developed theory. Finally, a phase diagram was made, which showed the influence of the total biopolymer concentration (Cp) on the stability of the complexes. The resulting phase diagram has a similar shape as the phase diagram derived in the Overbeek and Voorn theory for Cp < 12%. However, a ‘metastable’ region appeared at high Cp.

In **Chapter 3**, the exopolysaccharide EPS B40 was used instead of GA and the behavior of WP/EPS B40 complexes was studied as a function of pH, ionic strength and protein to polysaccharide (Pr:Ps) ratio. EPS B40 is a natural thickener in yoghurt-like products and carries phosphate groups. Here again, soluble complexes were formed at pH\(_c\), and phase separation (precipitation) took place at pH\(_{O}\). The strength of the interaction was strongly pH- and salt- dependent. Light scattering and viscosity measurements showed that at low salt concentration the compaction of EPS B40 was induced by the interaction of WP with the polysaccharide, leading to a reduction of the hydrodynamic radius of the EPS B40 molecule and an increase of the molar mass. Varying Pr:Ps ratio showed that phase separation was a consequence of charge neutralization of the complex and that the apparent stoichiometry of the complexes depended on the order of mixing the compounds. In time, rearrangement of the WP/EPS B40 complexes occurred to form fully neutralized complexes and free EPS B40 (cooperative binding).

In **Chapter 4**, the interaction between WP and a sulfated polysaccharide, *i.e.* a non gelling carrageenan (CG), was studied as a function of pH, ionic strength, temperature,
and Pr:Ps ratio. The pH boundaries pH\(_c\) and pH\(_o\) were also determined. Below pH\(_o\) precipitation occurred. The values of pH\(_c\) and pH\(_o\) were salt dependent; the presence of 45 mM of NaCl was favorable for the complex formation by screening the residual negative charges of CG. In the presence of CaCl\(_2\), WP/CG complexes could be formed up to pH 8, which was well above the pI of the WP, highlighting the involvement of calcium bridges. The pH boundaries pH\(_c\) and pH\(_o\) were slightly affected by temperature changes. Saturation of the CG seemed to occur at Pr:Ps = 30:1. At lower Pr:Ps ratio, when WP and CG were mixed at low pH, the pH of the mixture increased. The WP/CG complexes entrapped protons as a result of the residual negative charge on the CG.

In Chapters 2-4, it appeared that complex coacervation occurred only in the case of WP/GA system. For WP/EPS B40 and WP/CG, precipitation took place. Comparing the results, it seemed fair to conclude that the intensity of the WP / polysaccharide interaction correlated with the zeta potential of the polysaccharide: CG > EPS B40 > GA, which paralleled the stoichiometry of the complexes. The interaction between WP and CG was much stronger than for the other two polysaccharides and pH\(_c\) (pH\(_c\) = 5.5) was also higher than the pH\(_c\) of EPS (pH\(_c\) = 5.3) and gum arabic (pH\(_c\) = 5.2). Nevertheless, the influence of parameters like ionic strength, Pr:Ps ratio, and pH was qualitatively similar for all the systems studied. In general, an understanding of the biopolymer interactions enables the control and adjustment of the properties of food dispersions.

The second part of the thesis focused on the WP/GA coacervate phase and its characteristics. In Chapter 5, the influence of previously studied parameters (pH, ionic strength, Pr:Ps) on the velocity of phase separation was studied. The composition of the coacervate phase in water content and biopolymer concentration was also determined and the internal structure of the coacervate was analyzed by small angle X-ray scattering (SAXS). At a defined Pr:Ps, an optimum pH (pH\(_{opt}\)) was found, at which the strength of interaction, the kinetics of phase separation, the volume of coacervate phase, and the concentration in biopolymer were maximum. SAXS measurements also revealed that at pH\(_{opt}\) the coacervate phase was dense and structured. A specific correlation length was measured at Q = 0.7 nm\(^{-1}\) which was attributed to the structure factor of the WP. The stronger the electrostatic interaction was (i.e. the closer to pH\(_{opt}\)), the more pronounced the peak was. At small Q, a peak due to the presence of the charged GA was visible. Increasing the WP/GA electrostatic interaction led to a reduction of the peak intensity because of a greater neutralization of the GA molecule.
When salt was added to the system, the coacervate phase became less concentrated, less homogeneous and less structured because of the screening of the electrostatic interactions.

In Chapter 6 the viscoelastic properties of the WP/GA coacervate phase was investigated as a function of pH. The coacervate phase was much more viscous than elastic in the pH range 3.0 – 4.5. In the pH range between 3.0 and 4.5, the coacervate did not have a strong shear thinning behavior at low shear rates (below 20 s\(^{-1}\)). At a shear rate of 30 s\(^{-1}\), for pH\(_{\text{opt}}\), a sharp viscosity decrease occurred. Hysteresis (thexotropy) upon increasing and decreasing shear rate could be measured around pH\(_{\text{opt}}\). It was shown that hysteresis was due to a slow structure buildup of the coacervates and in time, the equilibrium coacervate structure could be fully recovered. By decoupling the effect of biopolymer concentration and electrostatic interactions, it appeared that the highly viscous behavior was mainly due to the strong electrostatic interactions.

In Chapter 7, the diffusivity of WP and GA was measured in their coacervate phase by means of nuclear magnetic resonance (NMR), fluorescence recovery after photobleaching (FRAP), and diffusing wave spectroscopy (DWS). Independently of the technique used, the results showed that the diffusion of WP and GA within the coacervate phase was slowest at pH\(_{\text{opt}}\) because of strongest electrostatic interactions. Furthermore, FRAP results showed that WP molecules diffused 10 times faster than GA molecules, which proved that WP and GA moved independently in the coacervate phase. Finally, DWS measurements revealed that the coacervate phase rearranged in time (days to weeks) leading to a loss of the turbidity of the coacervate phase and a decrease of the diffusion coefficient.

The last chapter of this thesis (Chapter 8) presents a direct application of the WP/GA system studied in Chapters 2, 5, 6, 7. The WP/GA coacervates were used for encapsulation of flavors and oils. A smooth biopolymer shell around the oil droplets was achieved at pH\(_{\text{opt}}\) with a payload (i.e. amount of oil in the capsules) up to 80%. The emulsions made of oil droplets encapsulated with WP/GA coacervates were highly unstable and their creaming rate was maximal at pH\(_{\text{opt}}\). Around pH\(_{\text{opt}}\) the zetapotential was close to zero because of the presence of a neutral coacervate phase at the oil / water interface. Finally, encapsulated lemon oil droplets were incorporated into Gouda cheese and the flavor release was monitored by mass spectrometry (MS Nose). The results described in Chapter 8 were in good agreement with previous results reported
in Chapters 2, 5, 6, 7. This indicates that the efficiency of the WP/GA coacervates as a biopolymer coating can be predicted from their properties in the bulk solution. At pH$_{\text{opt}}$ the coacervates are the most viscous and the most homogeneous and it leads to the formation of the best capsules. The results show how a fundamental understanding of the WP/GA interactions and of the WP/GA coacervate structure could lead to the prediction of the characteristics of the encapsulated product.