



Designing novel colloidal materials from polyphenol-polymer complexes

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Abstract

This thesis explores the utilization of physico-chemical interactions between natural polyphenols and biocompatible polymers to design novel materials and complex colloidal dispersions and emulsions. These usually unwanted interactions and the resulting insoluble complexes are important in the creation of structures that could potentially be used in the design of novel foodstuffs. The theme of this thesis is therefore the interactions of polyphenols with polymers and emulsion stability and properties. The results are as follows.

Chapter 3 presents a study on the interaction of polymer-polyphenol and the fabrication of new colloidal particles using the self-association of polymer-polyphenol complexes. The phase diagram of the physical appearance of mixtures of polyvinylpyrrolidone (PVP) with catechins and tannic acid has been explored and we have characterized the different structures by using a combination of light microscopy, UV-Vis and IR spectroscopy and dynamic light scattering techniques. The molar ratio between polymers and polyphenols has been found to play an important role in defining the structure of the complexes and their stability. By further optimizing the reaction conditions it is possible to obtain various structures resulting in colloids, microgels, and macroscopically gelled interfaces, which find applications in emulsion stabilization as explored in the following chapters.

In Chapter 4 emulsification techniques and consideration on emulsion stability and formulation are investigated. Novel biodegradable surfactant free emulsions stabilized by non-covalently associated micro-gel particles made from PVP and TA have been successfully prepared. The strong interaction between PVP and TA, driven by hydrophobic interactions and hydrogen bonding between phenolic and pyrrolidinone rings, leads to formation of microgels during the emulsification process. The molar ratio between TA and PVP was found to play an important role in determining the mechanism of emulsion stabilisation. At low molar ratio a shell of PVP-TA microgels is formed and over time wrinkles have been observed on these shells, while at high molar ratio $[TA]/[PVP]$ we observe Pickering emulsions stabilised by microgel particles and the continuous phase is a dispersion of microgel particles. The structure and stability of the

systems have been investigated by light scattering, confocal and light transmission microscopy. The presence of TA leads to halochromism of the emulsions at high pH and the absence of surfactants makes these emulsions particularly desirable in terms of biodegradability.

Chapter 5 focuses on the rheological properties of emulsions stabilised by gel particles and on the relationship between oil volume fraction and rheological behaviour. The rheological properties of emulsions and complex fluids are of pivotal importance in relation to their technical applications. These properties depend strongly on the emulsions composition, oil-phase volume fraction, microscopic droplet structure, and interfacial interactions. Our rheological tests showed that PVP-TA particles stabilised emulsions behave like flocculated dispersions and polymeric gels confirming the qualitative observation on emulsion gelation observed in the experiments carried out in the previous chapter.

Chapter 6 introduces a new technique, microfluidics. Microfluidics devices were designed and built with the purpose of making monodisperse emulsions stabilized by PVP-TA complexes. The emulsions produced with microfluidics devices were found to be monodisperse rather than the polydisperse ones resulting from bulk emulsification processes. The basic theory behind the effect of microfluidic scaling affects the hydrodynamics is outlined. Experimental details on the design process and on how to use photo-lithography and soft-lithography to build microfluidics devices are given. Finally we have demonstrated how our microfluidics devices can be successfully used to make monodisperse emulsions stabilised by PVP-TA and double emulsions (oil in water in oil emulsions).

Declaration

This thesis is submitted for the degree of Doctor of Philosophy in Physical and Theoretical Chemistry at the University of Oxford. No part of this thesis has been accepted or is currently being submitted for any degree, diploma, certificate or other qualification in this University or elsewhere. This thesis is wholly my own work, except where indicated.

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Chapter 1

Introduction

This thesis presents work on designing novel colloidal materials from polyphenol-polymer complexes. In this chapter we first introduce the terms colloids (Section 1.1), Hydrogen bond (Section 1.2), Hydrophobic interactions (Section 1.3), polyvinylpyrrolidone (Section 1.4), polyphenols (Section 1.5) and emulsions (Section 1.6). Finally, this chapter provides an outline of the thesis (1.7)

1.1 Colloid

The term colloid refers to any substance consisting of particles, substantially larger than atoms or ordinary molecules having at least one dimension in the size range of 1 nm to 1 μm . Colloidal systems may exist as dispersions of one substance in another, such as emulsions or smoke particles in air, or as single materials, such as thin films and fibres, or the membrane of a biological cell¹. Colloidal particles are usually dispersed in a (molecular) solvent and are small enough to exhibit thermal or Brownian motion. Brownian motion was observed for the first time in pollen grains in water by the botanist Robert Brown in 1827 [1]. This irregular motion is the result of numerous collisions between the colloidal particles and the solvent molecules. Since all colloids exhibit Brownian motion, they are sometimes referred to as "Brownian particles". The lower limit for the size range of Brownian particles has been fixed at 1 nm because it has to satisfy the requirements that the solvent molecules interact with the colloidal particles only in an averaged

¹Encyclopdia Britannica, Inc., 1 Jun. 2016. <http://academic.eb.com/levels/collegiate/article/24793>.

way. In other words, many solvent molecules are supposed to interact at the same time with the surface of a single colloidal particle and this is possible only if the particle is at least ten times the linear dimension of a solvent molecules, therefore circa 1 nm. The upper limit to the size range of colloidal particle is set at $1\mu\text{m}$. This upper size limit fulfils the requirement that the particles must show measurable thermal motion on the length scale of a typical experiment. If the particle is too big, its displacement is irrelevant compared to its size; therefore the particle does not show Brownian motion. Moreover, the gravitational force becomes relevant and the particle would sediment.

To explain phenomena found in the colloidal domain the DLVO theory is of paramount importance. DLVO stands for Derjaguin/Landau and Verwey/Overbeek who independently described the theory that explains quantitatively the aggregation of colloidal particles in aqueous dispersions and describes the force between charged surfaces interacting through a liquid medium [2]. It considers potential curves, to be a superposition of an attractive potential due to van der Waals forces between two particles and a repulsive potential due to the interaction of the double layers surrounding the particles [3]. Van der Waals forces are almost always present, and they result from interactions of induced fluctuating dipoles of atoms and molecules. In the simplest situation, this interaction can be modelled as:

$$U_{vdW}(D) = -\frac{A_{12}}{12\pi D^2} \quad (1.1)$$

where D is the distance between two interacting bodies (eg: a molecules and a surface) and A_{12} is the Hamaker Constant:

$$A_{12} = \pi^2 C \rho_1 \rho_2 \quad (1.2)$$

where ρ_1 and ρ_2 are the number of atoms per unit volume in two interacting bodies and C is the coefficient of the particle-particle pair interaction [4].

The double layer interaction can be approximated as:

$$U_{dl}(D) = \frac{2\sigma^2}{\epsilon\epsilon_0 k} e^{-kD} \quad (1.3)$$

where σ is the surface charge density per unit area, ϵ_0 is the permittivity of vacuum, ϵ is the dielectric constant of the solvent, and k is the inverse Debye length, which is proportional to

the square root of the ionic strength. It is interesting to note that in this description the solvent only enters in double layer potential through the dielectric constant ϵ and the ionic strength.

Combining the van der Waals potential with the double layer potential gives the DLVO potential and the resulting curve is shown in figure 1.1.

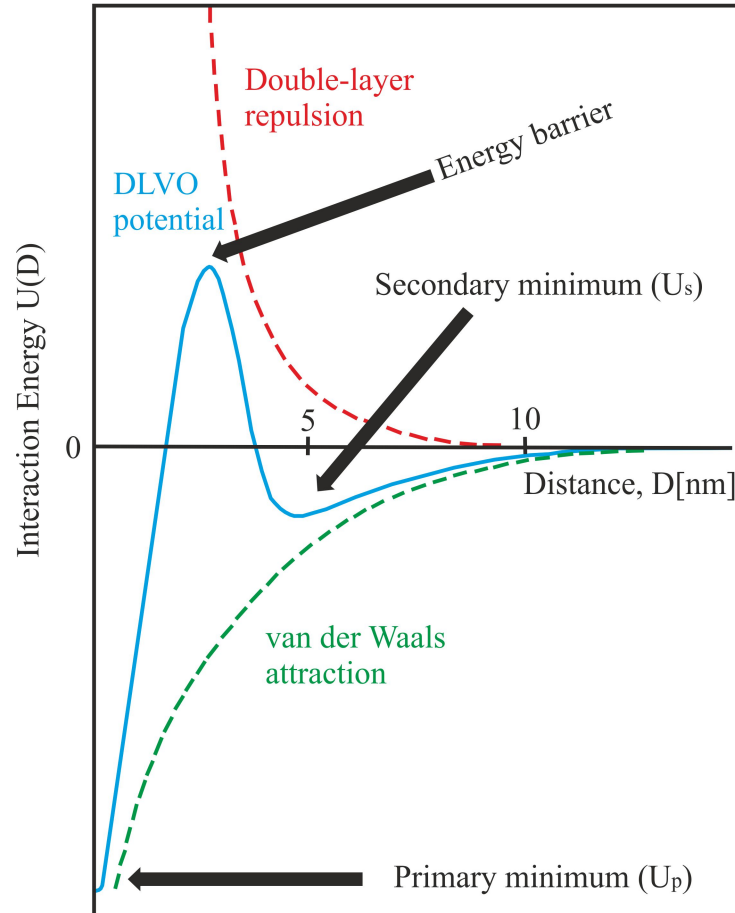


Figure 1.1: DLVO potential (redraw from [4])

For small double-layer potentials, the van der Waals potential prevails for all distances and particles will be attracted when they approach each other and the corresponding colloidal systems will flocculate. For sufficiently large values of the double-layer potential, as in figure 1.1, the repulsion due to the interaction of the double layers appears to dominate for intermediate distances and the curves show a more or less pronounced maximum due to a the balance between the double layer repulsion and the van der Waals attraction. The resulting potential barrier, if sufficiently high with respect to $k_B T$, will prevent agglomeration of the particles and the colloidal system will be stable. For very small distances, and for comparatively large distances the van der Waals potential becomes dominant. For short-range forces, the van der Waals attraction wins

out giving a primary minimum (see figure 1.1) in the potential. Particles in this minimum have coagulated and are irreversibly bound. At large distances we have a weak minimum, secondary minimum, that will be of importance if its depth is larger than $k_B T$. The secondary minimum is given by the fact that the van der Waals attraction is longer ranged than the double layer repulsion. In this minimum, particles may flocculate if its depth is greater than $k_B T$, but they can be easily redistributed into solution by agitation. From eq. 1.3, we see that increasing the salt concentration (and therefore the ionic strength) or decreasing the surface charge will reduce the double layer repulsion and destabilise the suspension of particles [4]. DLVO theory considers the idealised interaction between solid colloidal particles and does not take into account hydrophobic interactions or the formation of hydrogen bonds which we will discuss in the next sections.

Colloids can be subdivided in four classes:

1. rigid entities (e. g. spherical particles, rigid rods, etc...);
2. very large flexible molecules such as macromolecules (polymer solutions, large proteins in solution) and very long viruses (e.g. fd-virus);
3. assembly of small molecules which are in thermodynamic equilibrium with their environment, (e.g. a micro-emulsion);
4. emulsions.

In this thesis we will focus on colloids that are very large flexible molecules, such as macromolecules (polymer solutions) and in particular on the water soluble polymer polyvinylpyrrolidone (1.4) and PVP-polyphenol complexes (Chapter 3) and on emulsions stabilized by colloidal particles (Chapters 4, 5 and 6).

1.2 Hydrogen bond

A hydrogen bond is a non covalent interaction that may form when a hydrogen atom is covalently bound to a highly electronegative atom such as fluorine, oxygen or nitrogen. These covalent bonds are polar and therefore the hydrogen atom has a partial positive charge δ^+ . Although the charge is only partial, due to the hydrogen atom small size, the charge density is strong and

able to attract a proton acceptor atom, such as for example oxygen, nitrogen and fluorine. In other words the electronegative atom covalently attached to the hydrogen atom is the proton donor while the electronegative atom non-covalently linked to the hydrogen atom is the proton acceptor. Proton acceptors are atoms that have at least one lone pair of electrons like nitrogen, or two lone pairs like oxygen, or three like fluorine. The energy of this interaction is between 10 to 60 KJ/mol which is lower than the energy involved in covalent and ionic bonds (typically between 400 and 1000 KJ/mole) but higher than van der Waals interactions [5]. The hydrogen bond has a decisive influence on the structure and properties of substances. For example, the higher boiling and melting point of water and other hydrides such as NH_3 and HF , which are anomalously high compared to those of heavier hydrides of the same Groups (V, VII and VII of the Periodic table) and considering their low molecular weight, can be explained by the presence of extended H-bonds in these substances. A single molecule of water can form four H-bonds with four different molecules of water acting as both, proton acceptor with the two lone pair on the oxygen atom, and proton donor as schematically shown in figure 1.2. The hydrogen bond is non-covalent but directional and plays an important role in the crystal structure of many substances such as ice, crystalline hydrogen fluoride, and ammonio azide (NH_4N_3) to cite a few [5].

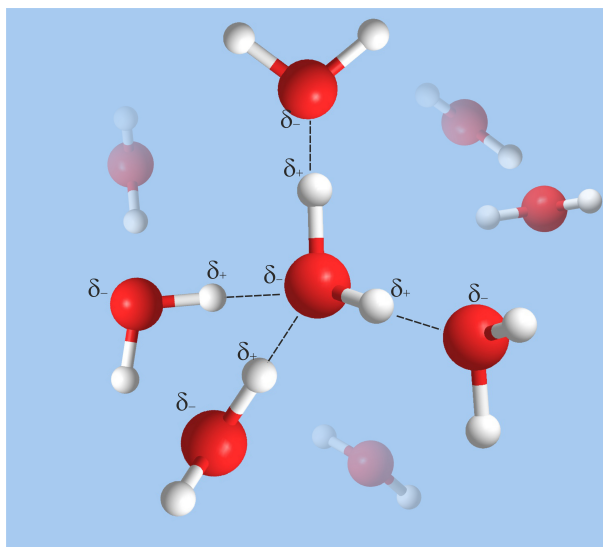


Figure 1.2: Model of hydrogen bonds linking water molecules

In colloid science, hydrogen bonding has been largely explored as the driving force for layer by layer (LbL) film formation and many synthetic and natural polymer chains are strengthened by hydrogen bonds [6–8]. The hydrogen bond is also of paramount importance in biology. For

instance, the secondary and tertiary structures of proteins are determined by intra-molecular hydrogen bonds $R-N-H \cdots O-R$ between the amine hydrogen and carbonyl oxygen atoms in the protein main chain (peptide backbone). Moreover, DNA structure, that controls the synthesis of proteins in living organisms and stores their genetic information, is dominated by H-bonding. Its structure involves two poly-nucleotide chains (with a phosphate-deoxyribose backbone) forming a double helix connected by multiple H-bonds between the pyrimidine residue (thymine or cytosine) and purine residue (adenine or guanine) as shown in figure 1.3.

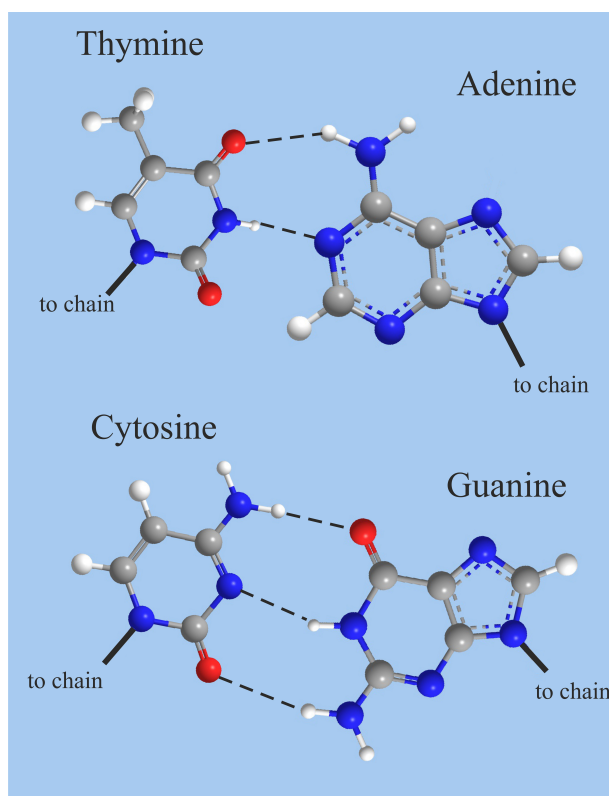


Figure 1.3: Model of hydrogen bonds in DNA chains between the pyrimidine residue (thymine or cytosine) and purine residue (adenine or guanine).

Another common H-bond is found in alcohol and carboxylic acids. In their liquid state they are generally dimers with a double hydrogen bond $R-O-H \cdots O-R$ and have an energy typically between 10 and 30 KJ/mole [5] [9]. This is the same kind of hydrogen bond that can also be found between polyphenols (see Section 1.5) and polyvinylpyrrolidone (see Section 1.4) discussed in this thesis. An example of such a H-bond is shown in figure 1.4.

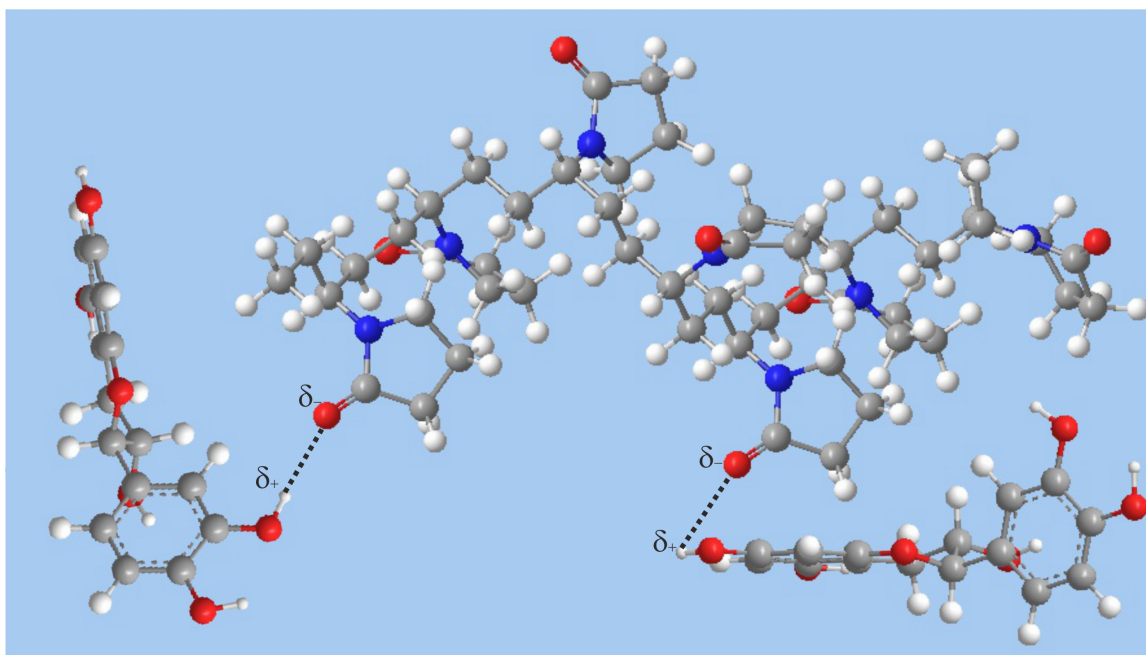


Figure 1.4: Model of hydrogen bonds between catechins and heptameric structure of PVP.

1.3 Hydrophobic interactions

Hydrophobic interactions are at the base of many important physico-chemical phenomena such as the stabilization of protein structures, the formation of biological membranes, and the effect of surfactants on surface tension. These are all partly based on hydrophobic interactions [10]. Complex macromolecules, such as proteins, contain hydrophobic and hydrophilic regions and in aqueous environment they tend to fold and aggregate in order to minimize the energy of the system by for example forming worm like micelles [11].

From a physico-chemical point of view, this phenomenon can be explained as an entropic effect originating from the disruption of hydrogen bonds between water molecules (in their dynamic liquid state) by the nonpolar chain which is incapable of forming hydrogen bonds with water. Under these conditions, the hydrogen bonds are reoriented tangentially to the hydrophobic surface leading to the formation of a water solvation shell around the nonpolar surface. The water molecules have restricted mobility which translate in a significant loss in translational and rotational entropy, however, the enthalpic component of the Gibbs free energy ($\Delta G = \Delta H - T\Delta S$) is favourable because water-water hydrogen bonds in the solvation shell are strengthened by their restricted mobility. Never the less the entropic effect is dominant [10] because nonpolar molecules reduce the surface area exposed to water minimizing their

surface tension by aggregating together. Several authors [9,12] have observed the occurrence of hydrophobic interactions between proteins and polyphenols (see Section 1.5) and in particular it has been reported that proline residues are hydrophobic surfaces because they are flat and rigid and therefore can interact with other planar hydrophobic surfaces such as phenolic aromatic rings [13]. Similarly, polyvinylpyrrolidone (see Section 1.4) has many pyrrolidinone rings that are good hydrophobic binding sites able to interact with phenol rings. An example of such interaction is shown in figure 1.5.

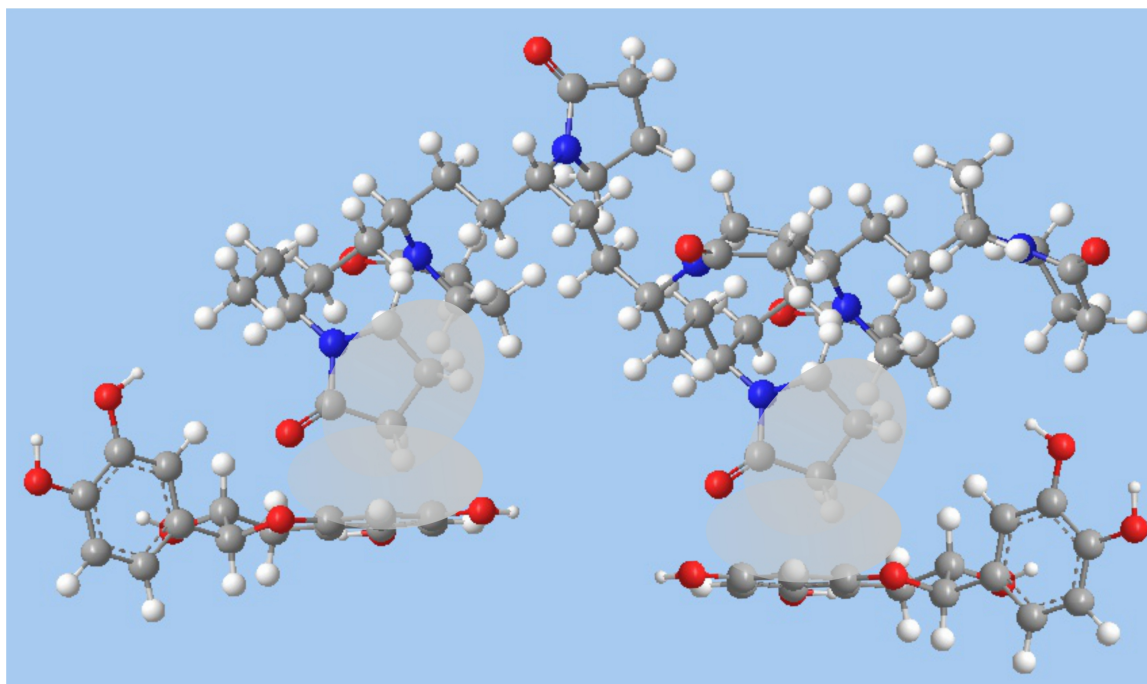


Figure 1.5: Model of hydrophobic interactions between catechin molecules and heptameric structure of PVP. The gray regions are involved in the hydrophobic interactions.

1.4 Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a synthetic water soluble biocompatible polymer which has important pharmaceutical and numerous technical applications [14]. It is widely used in the food industry, health and personal care (H&P) and pharmaceutical industry. It is physiologically compatible, non-toxic, essentially chemically inert temperature-resistant, pH-stable, non-ionic, and colourless. It is one of the most widely used excipients in the pharma-industry [15] and it has a wide range of technical applications that span from coating agent to the fabrication of nanotubes and nanowires [16]. PVP uses are numerous and various in nature; it finds appli-

cations in photographic products, dyeing and ink industries, detergents, adhesives, paints and coatings, paper auxiliary and others. PVP has a good biocompatibility, and therefore has many biomedical applications such as a carrier for drug delivery. It used to be employed as a blood plasma extender [17].

The process for its production was patented by BASF in 1939. Vinylpyrrolidone is obtained by reacting acetylene with pyrrolidone and then polymerized either in bulk, in solution or in suspension [18]. The cross-link form of PVP is polyvinyl polypyrrolidone (PVPP). The main difference between PVP and PVPP is their solubility in water: the former is soluble while the latter is insoluble in water. PVPP is widely used in the food industry to clarify fruit juices, beer and wine [12,19] and binding assay for polyphenols have been developed using PVPP [20]. Moreover PVPP and PVP find applications in the chromatographic analysis of polyphenols and in the removal of tannin (included tannic acid) from plant extracts [21].

PVP has similar properties to those of a protein [22]. It contains a proton accepting carbonyl group which are responsible for formation of hydrogen bonds with hydroxyl donor. This is very useful for the purpose of our research since it allows us to work with a model system, which can be potentially easily translated into a food system but does not pose the same technical challenge of working with proteins. More details on PVP will be given in Chapters 3 and 4.

1.5 Polyphenols

Polyphenols are a class of organic chemicals characterized by the presence of a large number of phenol substructures, the number and characteristics of which underlie the unique physical, chemical, and biological properties of particular members of the class [20]. The term polyphenol derives from the ancient Greek word *πολύς* (polus, meaning many, much) and the word phenol which refers to the chemical structure/substructure formed by an aromatic ring, phenyl group, bounded to an hydroxyl (-OH).

Polyphenols are secondary metabolites of plants and they possess a great structural diversity. They are ubiquitous and they constitute one of the most numerous group of plant metabolites and are an integral part of both human and animal diets [23]. Secondary metabo-

lites of plants are molecules that are not essential to the growth, development and reproduction of the plant, but they play an important role in plant defence, inter-specific competition and in facilitating reproduction processes. Examples of secondary metabolites among others are pigments, mycotoxin, flavourings.

From the point of view of the human consumption of polyphenols, extensive research has linked polyphenols and cancer prevention [24–28]. Moreover, research has also shown that polyphenols have a role in combatting Alzheimers disease by selectively dissociating toxic amyloid beta oligomers into soluble peptides at substoichiometric concentrations [29]. Recent studies have furthermore shown the role of polyphenols in the prevention of obesity and obesity-related chronic diseases [30,31] and that they have beneficial effects on plasma HDL-cholesterol concentrations in the plasma [32].

From a chemical point of view, polyphenols are an extremely large family of natural molecules containing at least 8000 structures [23]. They can be classified into several classes. The two main groups of polyphenols are flavonoids and tannins.

Flavonoids is the most important class of polyphenols. They are well known antioxidants [33] and depending on their chemical structure, some authors divide them into 13 different subclasses [34]. In this thesis we will be concerned with one particular class: catechins. Catechins [35,36] is a collective name for galloylated flavanol monomers such as catechin, epicatechin (EC), epicatechin gallate (ECG), and epigallo catechin gallate (EGCG) [37]. In chapter 3 a more detailed description and chemical structure of catechins will be given. Catechin, epicatechin, galocatechin are the monomeric constituents of the condensed tannins. Tannins have higher molecular weights than flavonoids and are highly hydroxylated molecules. Like flavonoids they can form insoluble complexes with carbohydrates and proteins. Tannins are usually subdivided into two groups: hydrolyzable and condensed tannins. Condensed tannins are highly polymerized molecules while hydrolyzable tannins are smaller and easily hydrolysed with acid, alkali, and hot water and by enzymatic action. The products of this hydrolysis are polyhydric alcohol (Sugar alcohol) and phenylcarboxylic acid. The best-known hydrolyzable tannin is tannic acid which is a gallotannin consisting of a pentagalloyl glucose molecule. Further details and its molecular structure are given in chapter 3, and 4.

Polyphenols interact with proteins and glucides mainly via hydrogen bonding [6, 13, 38,

39]. Through the same mechanisms polyphenols interact also with synthetic polymer such as PVP, PVPP. In this case the hydrogen bonds forms between the carbonyl group of the vinylpyrrolidone and the OH group of phenyl group [40]. Many new and interesting applications using the hydrogen-bonded interactions of natural polyphenols with PVP such as non-ionic layer-by-layer assembly for multifunctional cytoprotective materials and for the fabrication of responsive microcapsule reactors have been reported [8,41].

1.6 Emulsions

An emulsion can be defined as a dispersion of one immiscible liquid in another. They are heterogeneous opaque systems of two immiscible liquid phases, in which one is the continuous phase and the other phase is dispersed as droplets of microscopic or colloidal size. Emulsions are thermodynamically unstable systems and they are kinetically stabilized [42]. A schematic representation of the thermodynamic and kinetic stability of emulsions is presented in figure 1.6. To make an emulsion it is necessary to spend energy, e.g. mechanic agitation, to disperse one liquid into the other. In this way spherical droplets are formed. The geometry is imposed by the fact that the system tries to minimise the interfacial tension. A way to do so is to minimise the surface area and the smallest possible surface area to volume ratio is given by the sphere.

A further way to minimise the surface tension is by adding surfactants [43] or surface active particles which can adsorb strongly at the liquid-liquid interface of immiscible fluid. Surfactants are amphiphilic molecules formed by a hydrophilic head and a hydrophobic tail. The heads are soluble in polar solvents while the tails are soluble in apolar solvents therefore surfactant molecules are adsorbed at the interface as schematically shown in figure 1.6 forming micelles. Therefore surfactants lower the interfacial energy between two immiscible liquids thermodynamically. On the other hand, particles are able to stabilise interfaces kinetically. Emulsions stabilized by particles are called Pickering emulsions, named after the British chemist Pickering who described these systems in 1907 [44]. In this case the particles form a layer around the droplets to prevent coalescence as shown in figure 1.6. The mechanisms behind particle stabilization is influence by particle size, hydrophobicity and shape [45]. Generally, an emulsion can be oil in water (o/w) or water in oil (w/o) and this depends on a number of variables. A general rule to predict if an emulsion will be o/w or w/o is the Bancroft rule [46]. The Bancroft

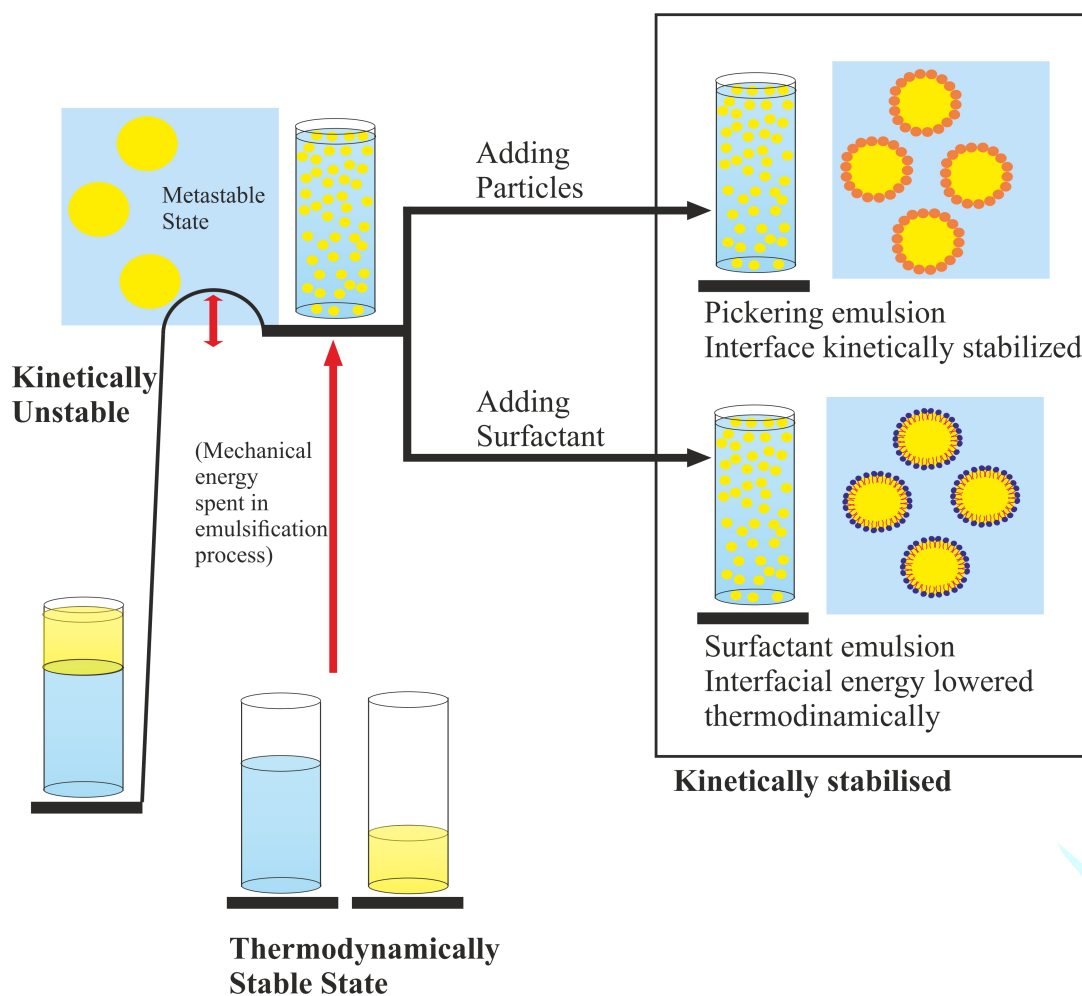


Figure 1.6: Emulsion Stability: Kinetic versus Thermodynamic Stability

rule states: the phase in which an emulsifier is more soluble makes up the continuous phase so in order to have a stable emulsion the surfactant must be soluble in the continuous phase [47]. A similar rule for emulsion stabilized by particles instead of surfactant is the Finkle rule that states: hydrophilic colloids will tend to make water the dispersing phase while hydrophobic colloids will tend to make water the dispersed phase [48]. What the Bancroft and Finkle rules state is that what makes an emulsion oil-in-water or water-in-oil is not the relative percentages of oil or water, but which phase the emulsifier is more soluble in or preferentially dispersed in case of particles. So even if the emulsion is 60% oil and 40% water, if the emulsifier is more soluble in water (or in the case of particles preferentially dispersed) it will create an oil-in-water system. These rules are empiric, but can be rationalised by considering the interfacial tension at the oil-surfactant and water-surfactant interfaces [49]. They are very useful rules of thumb for most systems. Nevertheless, some authors report anti-Bancroft and anti-Finkle behaviour

for particular systems such as microgels stabilized emulsions [50–52].

1.6.1 Emulsions instabilities

Emulsions present 4 types of instability that can lead to de-emulsification:

1. Flocculation;
2. Creaming, also referred to as gravitational separation;
3. Disproportionation, also referred to as Ostwald ripening;
4. Coalescence.

Flocculation is generally defined as the aggregation of droplets to give clusters, in which all droplets maintain their own integrity. It is the result of a weak net attraction that arises through various mechanisms such as DLVO interaction combined with Brownian motion or sedimentation.

Creaming, as sedimentation in case of water in oil emulsions, is governed by the Stokes equation:

$$V = \frac{2r^2(\rho_2 - \rho_1)g}{9\eta_1} \quad (1.4)$$

Where V is the creaming (or settling) velocity, r is the droplet radius, ρ_2 is the density of the droplet, ρ_1 is the density of the continuous phase, η_1 is the viscosity of the continuous phase and g the gravitational acceleration.

From this equation we can postulate methods for slowing down creaming:

1. Reduce the density difference, $\Delta\rho$;
2. Reduce droplet size, r ;
3. Increase the continuous phase viscosity, η_1

Creaming can be measured by the “creaming index” which is calculated as:

$$CI = \frac{100 \times H_L}{H_E} \quad (1.5)$$

where H_L is the height of the lower phase or serum and H_E is the height of the original emulsion measured via visual observation with long-term storage tests. In figure 1.7 the schematic of a creaming emulsion is shown.

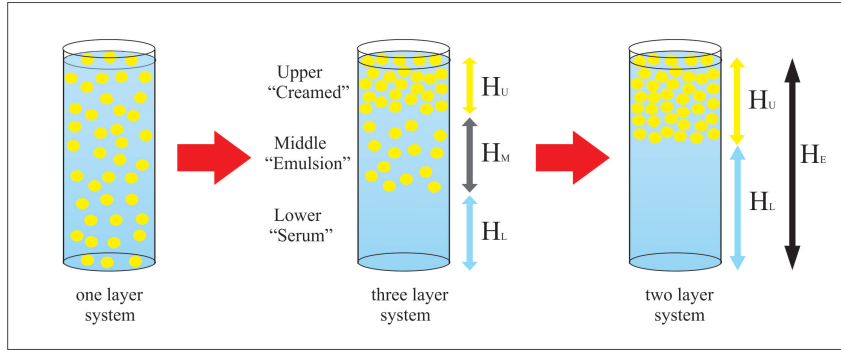


Figure 1.7: Measuring creaming stability via visual observation with a long-term storage tests.

The creaming index provides information about the extent of droplet aggregation and emulsion stability. Creaming is not an actual breaking of the emulsion, but a separation of it into two emulsions, one richer in the dispersed phase than the other. In some cases creaming can lead to coalescence, which leads to the complete break up of the emulsion into bulk oil and water phase. In other cases the system reaches a kinetically trapped state.

Ostwald ripening originates from the diffusion of the dispersed phase from smaller to larger droplets [53]. If the molecules of the dispersed phase have some solubility in the continuous phase, the transport through molecular diffusion of material from the small toward the large drops is allowed. This is the case for emulsions containing oils with small molecules, especially those with high polarity and polarizability such as, for example hexane, benzene, xylene, and PDMS. The process is driven by the pressure differential between small and large droplets which is governed by the Laplace equation

$$\Delta P = 2\gamma/r, \quad (1.6)$$

where ΔP is the Laplace pressure, γ is the surface tension and r is the droplet radius. Many authors have reported that particles are able to completely arrest the Ostwald ripening in

foams and emulsions [54–56]. This is of particular importance for our research since we have chosen to stabilise emulsions with particles instead of surfactants.

Finally, coalescence is the process in which two or more droplets irreversibly merge and occurs when droplets collide [57]. The kinetics of droplet coalescence is governed by the collision frequency and the coalescence probability, which are described by:

$$f_{coal} = f_{collisions}p_{coal}, \quad (1.7)$$

where f_{coal} is the coalescence frequency, $f_{collision}$ is the collision frequency and p_{coal} is the coalescence probability [58]. The viscosity of the continuous phase, the volume fraction of the dispersed phase, the net attractive forces and the rate of creaming are all factors that influence the coalescence frequency. Coalescence is an irreversible process that leads to the break down of emulsions. Flocculation and creaming can be reversed by the input of much less energy than the one required in the original emulsification process while Ostwald ripening and coalescence lead to the breaking up of the emulsion into bulk oil and water phases. Flocculation and creaming can be a precursor to coalescence and therefore de-emulsification.

1.7 Outline of the thesis

In this thesis we study the interaction and self-assembly properties of polyphenols (catechins and tannic acid) with PVP. The overall aim is to utilise physico-chemical interactions between natural polyphenols and polymers to design colloidal complexes that can then be used to stabilise emulsions. In Chapter 2 we will present the experimental methods and techniques we have employed in this work. In Chapter 3 we will focus on polymer-polyphenol interactions and how we can obtain soluble complexes and gel colloidal particles. We will follow with a study on emulsions stabilised solely by gel colloidal particles (Chapter 4) and in Chapter 5 we will study the rheological properties of PVP-TA gel particles stabilised emulsions. Finally, in Chapter 6 we will give an overview on mono-disperse microgels stabilised emulsions manufactured with microfluidics.

Chapter 2

Experimental methods and techniques

2.1 Introduction

This thesis is concerned with the investigation of polymer phenol interactions to make particles able to stabilise emulsions. We have therefore used a wealth of techniques to characterise particles, molecular complexes, and emulsions. Such techniques are detailed in this chapter. We have also used microfluidic techniques to produce monodisperse emulsions stabilized solely by polymer polyphenol particles, and this will be fully addressed in Chapter 6. In Section 2.2 we give an overview on microscopy techniques. In Section 2.3 we give a brief summary on rheology, where we will discuss a selection of mathematical models relevant to our research work followed by an overview on the specific rheometer we have used. This is followed by Section 2.4 in which a short outline on dynamic light scattering and the instrument we have used to perform analyses is given. Finally a full description of the instrument we used to prepare emulsions is given in Section 2.5

2.2 Microscopy

Microscopy is an important technical tool for the investigation of the microscopic world. It is used in many areas of scientific research to observe and monitor microscopically sized specimens that are not observable with the unaided human eye [59]. The first microscopes were built at the end of the sixteenth century and allowed the first observation of cells and bacteria by Robert Hook who first used the word cell in 1665 in his “Micrographia”. Bacteria were first observed in the 18th century by Antony van Leeuwenhoek [60] who is widely regarded as the father of microscopy [61]. While the second had as principal interest the observation of microscopic life [62], Hook was concerned with the observation and description of larger objects in order to understand their behaviour and function of their microscopic structure [63]. The development of microscopy in terms of design and manufacture is parallel and critical to the development and advancement in many fields of the natural sciences such as botany, biology, microbiology, materials science and colloidal science and it has indeed shaped them [64]. Nowadays the field of microscopy has advanced and expanded into three main branches:

- Optical microscopy

- Scanning probe microscopy
- Electron microscopy

Optical microscopy includes conventional light microscopy, fluorescence microscopy, confocal/multiphoton microscopy and stimulated emission depletion microscopy [65,66].

Scanning probe microscopy refers to techniques such as: Scanning tunneling microscopy (STM), Atomic force microscopy (AFM), and Near-field scanning optical microscopy [67].

Electron microscopy techniques include: scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Scanning transmission electron microscopy (STEM) and focus ion beam microscopy (FIB).

In this thesis we have used optical microscopy, bright-field and confocal, and scanning electron microscopy, SEM as imaging techniques. The basic theory and description of these techniques and instruments is given in the following sections.

2.2.1 Light transmission microscopy

To monitor our microfluidics experiments (Chapter 5) we have used an Olympus CKX41 inverted microscope, light source 6 V, 30 W halogen lamp, lamp socket (U-LS30-3-5). This is a digital microscope that allows live image transmission to a computer screen by aid of a digital microscope camera mounted on the trinocular port. Objectives with magnification 4x, 10x and 40x have been used. In an inverted microscope the objective is positioned under the specimen leaving more space for sample manipulation. This set is ideal for microfluidic experiments where multiple tubes are connected to the microfluidic devices. A full description of the microfluidic devices we have fabricated and used in this thesis is given in Section 6.3.1.

2.2.2 Laser Scanning Confocal Microscopy

Minsky's patent for the first confocal microscope was issued in 1961 [68]. The idea of building a confocal microscope was driven by the need to visualize nerve cells and in particular the way they are connected in the human nervous system [68]. His first attempts to produce clear images of these specimens were via conventional optical microscopy, but the particular challenge of this

specimen was due to the scattering of light from above and below the focal plane that contributed to a general loss of contrast. This was due to the fact that in optically dense samples, such as biological specimens and colloidal systems [69], the focal image point is clouded by aberrant rays of scattered light deflected by points of the specimen that are not the point observed. The original idea of Minsky was to remove all rays not initially aimed at the focal point. The essential technical innovations of Minsky's invention were [70]:

1. Single-point illumination and formation of the image by "point by point" examination;
2. The optical information at each point was obtained by double focusing;
3. A pinhole aperture to reject out-of-focus light before it reaches the detector;
4. Stage moving system to perform the scanning

In figure 2.1 a general simplified schematic diagram of a confocal microscope light path is shown. Light from a very bright source is focused by an objective lens onto a point in the specimen via a beam-splitter (dichroic mirror). The light from the sample is then focused onto a detector throughout the beam-splitter and a pinhole. The pinhole is placed before the detector at the conjugate focal plane to the imaged point to ensure that any light originating from points outside the focal point is blocked before reaching the detector.

Single-point illumination is able to measure only one point at a time. The speed of the scanning process is given by the sum of the time intervals it takes to collect enough light to measure each image point. To reduce the scanning time a brighter light can be used. In Minsky's prototype a carbon arc, the brightest source available in the 1950s, was used and later replaced by a zirconium arc for practicality reasons [68]. Subsequent improvements utilise a laser as light source and laser scanning rather than translating the sample has become the norm. These two improvements enabled confocal microscopy to become a powerful tool for many scientific fields, especially for studying biological and colloidal systems [64, 71]. Scanning confocal microscopy is far more practical today because computers can easily transform and enhance the images.

The commercial confocal system used in this thesis is a Zeiss Exciter LSM 5 laser scanning confocal microscope (see figure 2.2). This contains a 543 nm HeNe laser source and is fitted with a 63x oil immersion objective, with a numerical aperture (NA) of the objective, of 1.4. The laser light coupled into the microscope is focused by the objective. The light reflected or

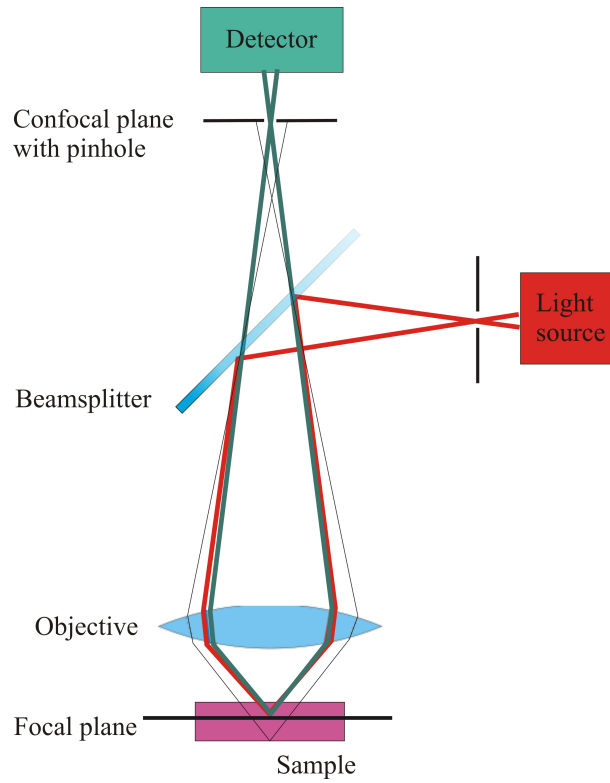


Figure 2.1: General schematic of light confocal microscopy

emitted by the sample surface passes the objective and is collected by a tube lens. In this way, the laser beam converges at a second focal point, which is optically conjugate to the first. A pinhole arranged in this confocal plane ensures that only light from the focal plane reaches the detector, whereas light reflected or emitted by regions above or below it does not. A 2D image is then produced by scanning the sample point by point. Changing the focal depth enables 3D images to be produced.

2.2.3 Scanning Electron Microscopy: SEM

Electron Microscopes were invented in 1931 by Max Knoll and Ernst Ruska. This kind of microscope passes the optical limitations of the light. In scanning electron microscopy the sample, or specimen, is scanned by a focused electron beam usually generated by an electron gun fitted with a tungsten filament cathode. The secondary and back scattered electrons are collected by a detector and used to form the image [60]. In this thesis we have used scanning electron microscope JEOL model is JSM-6010LV.



Figure 2.2: Confocal microscope

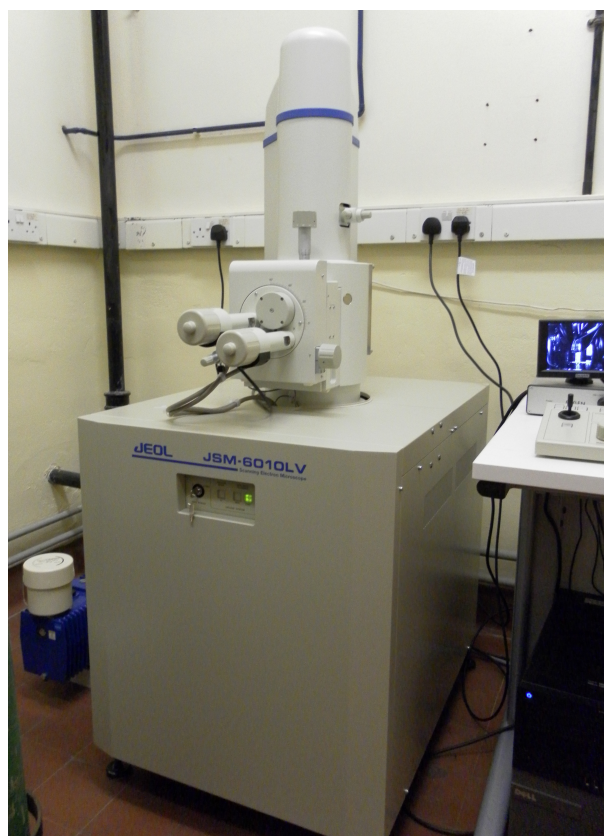


Figure 2.3: Scanning Electron Microscopy JEOL model is JSM-6010LV

2.3 Rheology

Rheology is the branch of mechanics that studies the deformation and flow of matter¹. The term was coined by Professor Eugene Bingham in 1928 and derives from the Greek word $\rho\epsilon\omega$ (rheo) meaning flow. The year after Eugene Bingham, Wolfgang Ostwald, Ludwig Prandtl and Markus Reiner founded the Society of Rheology. The subject of rheology itself is of course much older than its name: already in the 1687 Sir Isaac Newton in his "Principia" gives his definition of viscosity as: "The resistance arising from the want of lubricity in the parts of a fluid, is proportional to the velocity with which the parts of the fluid are separated from each other" [72]. In modern terms this definition is better expressed as: the shear stress is proportional to the shear rate and the constant of proportionality, for a given temperature and pressure, is the shear viscosity of the fluid. This is true only for Newtonian fluids which is a small class of low molecular weight liquids in a finite range of shear rate (or stress). Water is an example of a low viscosity Newtonian fluid. For all Newtonian fluids the shear viscosity varies with temperature but not with shear rate or stress.

Rheology is more often concerned with non-Newtonian fluids which are materials for which the viscosity is a function of the shear rate (or stress). The development of colloid chemistry and polymer science played a pivotal role in the advancement of rheology [73]. Examples of these materials are suspensions such as paint, inks, drilling fluids, adhesives and polymer melts, emulsions such as creams, gels, and many foodstuffs e.g. mayonnaise and ketchup. Rheological properties can define many sensorial attributes in several materials such as foodstuffs and personal health care products e.g. body lotions, shampoo, and hair conditioner. The majority of rheological tests involve applying a specific force to a material and measuring the resulting flow and/or deformation. Classical theory such as Hooke's law for an ideal spring can describe ideal elastic behaviour. Likewise, Newtonian fluid-mechanics can describe viscous behaviour for an ideal liquid. Rheology is the science that describes those materials that show intermediate behaviour or in other words viscoelastic materials. It lies at the interface of engineering, physics, material science and chemistry. At this point it is useful to define some fundamental rheological parameters in the frame of reference of a general parallel plate geometry [74]:

¹Definition retrieved from the Society of Rheology website <http://www.rheology.org> on the 4th of September 2013

- Strain, or shear strain γ , is the displacement within a body when a force is exerted on it. Mathematically it is defined as:

$$\gamma = \frac{dx}{h} = [\frac{m}{m}], \quad (2.1)$$

where dx is the deformation and h the distance between the plates.

- Shear rate, $\dot{\gamma}$ (gamma dot), is the rate of change of strain in the thickness of the sample and it is given by:

$$\dot{\gamma} = \frac{dv}{dh}. \quad (2.2)$$

In a laminar flow the velocity differential between adjacent layers of like thickness is constant, the differential can therefore be approximated as:

$$\dot{\gamma} = \frac{v}{h} = [\frac{m/sec}{m} = sec^{-1}]. \quad (2.3)$$

- Shear Stress σ is the force per unit area which acts on the liquid.

$$\sigma = \frac{F}{A} = [\frac{N}{m^2} = Pa]. \quad (2.4)$$

For some experiments (such as oscillatory tests) the stress varies sinusoidally, and can be represented as:

$$\sigma(t) = \gamma_0[G'(\omega)\sin(\omega t) + G''(\omega)\cos(\omega t)], \quad (2.5)$$

where ω is the frequency of oscillation expressed in radians per second, G' is the storage modulus and G'' is the loss modulus. This second relationship, 2.5 is true only within the linear viscoelastic regime (LVR).

- Dynamic Viscosity, η , is a measure of a fluid resistance to flow. Mathematically it is defined as:

$$\eta = \frac{\sigma}{\dot{\gamma}} = [\frac{Pa}{s^{-1}} = Pasec] \quad (2.6)$$

- Kinematic Viscosity, ν is typically measured by a capillary viscometer and is related to the dynamic viscosity through the density of the material:

$$\nu = \frac{\eta}{\rho}. \quad (2.7)$$

- Elastic modulus, G , is the constant of proportionality between the applied stress and the resulting strain in a simple shear deformation. In the simplest case it can be defined as:

$$G = \frac{\sigma}{\gamma}. \quad (2.8)$$

- Storage modulus G' : is the part of the shear stress that is in phase with the shear strain under sinusoidal conditions of small amplitude oscillatory shearing (see equation 2.5). It represents the storage of elastic energy.
- Loss modulus G'' is the part of the shear stress that is in phase with the shear rate under sinusoidal conditions of small amplitude oscillatory shearing and it represents the viscous dissipation.

2.3.1 Mathematical Rheology Models

To make any quantitative comparison of materials using rheology, it is necessary to fit the experimental data to a mathematical model. In literature [75, 76] there is an extensive list of mathematical models that could be used. For the purpose of this thesis we will discuss only those models relevant to interpretation of rheological data of emulsions and dispersions. First we will discuss such models for flow experiments and then for oscillatory tests.

Rheology mathematical models for flow tests

A flow test is a procedure in which a specified stress is applied to a sample and the sample's deformation, as a function of time, is simultaneously measured. The aim of a flow test is to determine the viscosities of a sample at different shear rates and plot flow curves.

Flow models are the most basic forms of data processing in rheology. Most flow models belong in viscometry because the elastic behaviour of the material is not considered. Essentially we need a flow equation relating viscosity to rate of shear for non-Newtonian fluids. A widely accepted model to fit flow curves is the Cross model [77]:

$$\frac{\eta - \eta_{\infty}}{\eta_0 - \eta_{\infty}} = \frac{1}{1 + (K\dot{\gamma})^m}, \quad (2.9)$$

where K is the relaxation time and m is the shear thinning index.

The Cross equation gives an accurate fit of experimental data over a wide range of shear rates, involves a minimum number of independent constants which have a real physical significance and are readily evaluated. If we accept the concept of limiting viscosities η_0 (zero shear viscosity) and η_∞ (infinite shear viscosity) we require two further constants to define the path between these limits, which are the relaxation time and the shear thinning index.

At the zero shear stresses, predictions about the appearance of a sample can be made, as well as its stability as sedimentation, levelling and other similar processes can occur under very small gravitational forces.

For the majority of structured liquids at high shear rates $\eta_0 \gg \eta_\infty$ and $K\dot{\gamma} \gg 1$ therefore the Cross model simplifies to the Sisko equation [76].

$$\eta = \eta_\infty + \frac{\eta_0}{k\dot{\gamma}^m} \quad (2.10)$$

The Sisko equation is very good in describing the flow behaviour of most emulsions and suspensions and structured liquids in general. In Chapter 5 we will compare these two models in fitting our experimental data.

Rheology mathematical models for oscillatory tests

Oscillatory tests are performed by applying a sine-wave-shaped input of stress or strain, and electronically resolving the resulting sinusoidal strain or stress output into a “solid-like response” in phase with the input, and a “liquid-like” response which is out of phase with the input. The solid-like response is described by the storage modulus G' (or elastic modulus) and the liquid-like response is related to G'' , loss modulus (or viscous modulus). An example of G' and G'' for structured liquids is reported in figure 2.4 which is the viscoelastic spectrum for non-Newtonian fluids [76].

In the viscous or terminal region, figure 2.4, G'' is dominant. In theory all materials have this region but at such low frequencies that it may not be possible to detect it. In this region G'' increases linearly with the frequency, while G' is quadratic. The relaxation time, λ_{max} , is equal to:

$$\lambda = \frac{G'}{G''\omega} \quad (2.11)$$

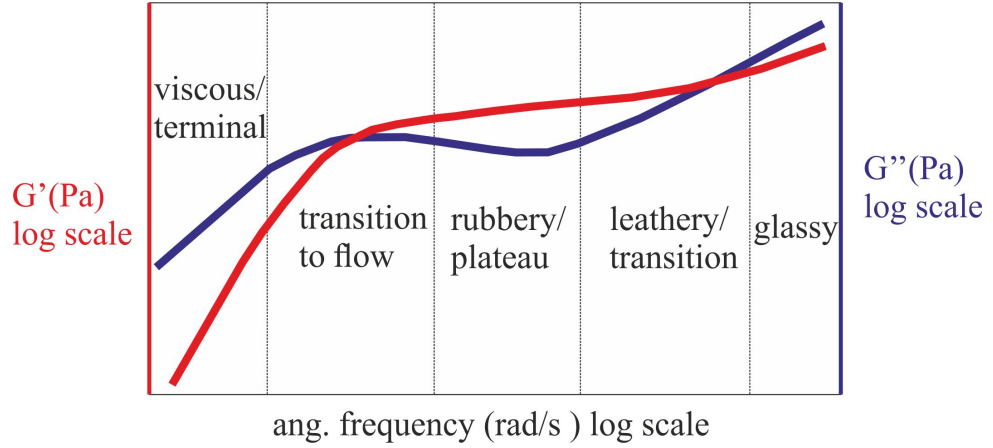


Figure 2.4: Regions of the viscoelastic spectrum of non-Newtonian liquids [76].

Moving to higher frequency regions, the next region is the “transition-to-flow”. The name refers to the transition seen from higher to lower frequency. At higher frequency the elastic behaviour dominates and G' is bigger than G'' . The crossover frequency for G' and G'' , for the Maxwell model, that we will discuss shortly, is given by the inverse of the relaxation time λ . In the rubbery plateau region (see figure 2.4) the elastic behaviour dominates and G'' is lower than G' . The storage modulus increases with frequency, while the loss modulus decreases towards a minimum before rising again. The lower the slope of G' as a function of the frequency, the deeper is the G'' valley. In this region the loss modulus can be approximated for a linear viscoelastic system by [76]:

$$G'' \approx \frac{\pi G'}{2} \frac{d \ln G'}{d \ln \omega} \quad (2.12)$$

where the second term is the local slope of the logarithmic plot of G' versus ω . Moving to higher frequency, we observe the leathery or higher transition crossover region. In this region the value of the loss modulus rises faster than the storage modulus and when $G' = G''$ another crossover point is found and another characteristic time can be obtained. This is due to high-frequency relaxation and dissipation mechanisms. The final region of the viscoelastic spectrum of non-Newtonian fluids is the glassy region where G'' is once again dominant. As already mentioned, due to time scales and instrumental limits, e.g. available frequency range, for most materials only two regions of the viscoelastic spectrum are seen. This depends on the relaxation time: if $\omega \lambda_{max} \sim 1$ the viscous and transition to flow regions are seen; if $\omega \lambda_{max} > 1$ only the plateau and leathery/higher transition are seen; if $\omega \lambda_{max} < 1$ the loss modulus is always dominant.

To fit the viscoelastic spectrum and describe viscoelasticity, mechanical models combining springs and dash-pots are generally used [76]. A spring is the representation of an elastic element that obeys Hooke's law for which the deformation of the spring is proportional to the applied force. Therefore the elastic modulus is the constant of proportionality for a simple shear deformation (γ) and the shear stress $\sigma = G\gamma$. In an analogue way linear viscous response can be modelled using a dash-pot composed by a plunger moving through a viscous Newtonian liquid which is mathematically described by the equation $\sigma = \eta\dot{\gamma}$, where the viscosity is the constant of proportionality between the rate of deformation of matter and the applied stress.

The spring therefore models energy-storing mechanisms such as a polymer segment being stretched, while the dash-pot models energy dissipation due to the movement of any entity through a liquid continuous phase [78]. Springs and dash-pots can be combined to model viscoelastic materials. Viscoelastic solids are better modelled by connecting elements in parallel, Kelvin-Voigt model, while viscous elastic liquids are better described by connecting these two elements in series into the Maxwell model (see figure 2.6). In other terms the viscoelastic spectrum can be fitted by the Maxwell model at low frequency, i.e. up to the rubbery plateau, and by the Kelvin-Voigt at high frequency.

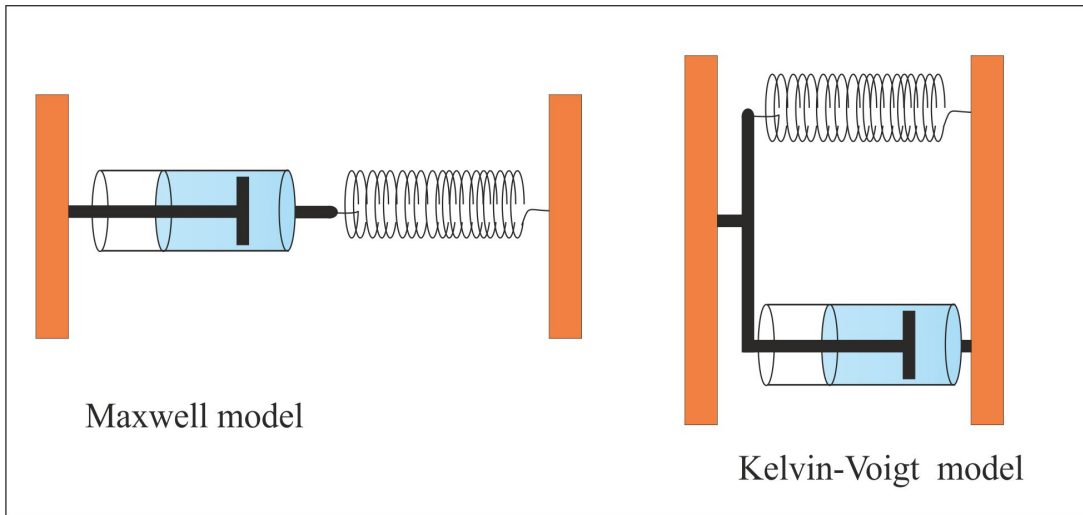


Figure 2.5:

Figure 2.6: Mechanical analogues of Maxwell and Kelvin-Voigt models

For a single Maxwell element, the storage and loss moduli are given by [76]:

$$G' = \frac{\eta\lambda\omega^2}{1 + (\omega\lambda)^2}, \quad (2.13)$$

$$G'' = \frac{\eta\omega}{1 + (\omega\lambda)^2}, \quad (2.14)$$

where λ is the relaxation time.

A further model of the Maxwell type written as a combination of a dash-pot and a Maxwell element is the Jeffreys model (or Oldroyd B), see figure 2.8 for the mechanical analogue.

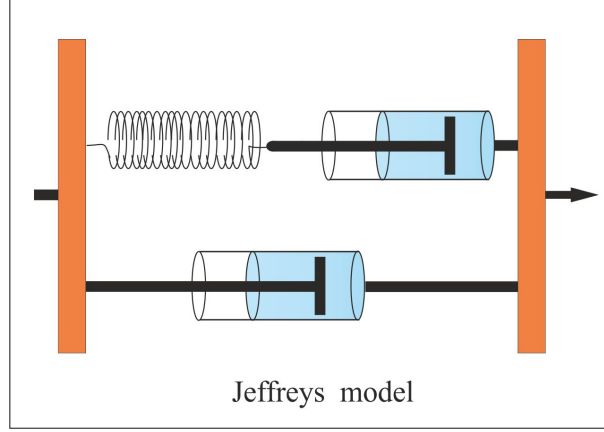


Figure 2.7:

Figure 2.8: Mechanical analogous of Jeffreys model

The Jeffreys model is ideal to describe the rheological behaviour of dispersions of deformable particles, for example emulsions. For Jeffreys model the storage and loss moduli are given by [79]:

$$G' = \eta_0\omega \frac{(\lambda_1 - \lambda_2)\omega^2}{1 + \lambda_1^2\omega^2} \quad (2.15)$$

$$G'' = \eta_0\omega \frac{1 + \lambda_1\lambda_2\omega^2}{1 + \lambda_1^2\omega^2} \quad (2.16)$$

The rubbery plateau region is best fitted by the power law model. This model provides the best fit for flocculated dispersions and polymeric gels [74]. It is not a physically realistic model for a non-Newtonian fluid but it approximates the case in which a series of Maxwell elements is replaced by a continuous function [76]. In this case the storage and loss moduli can be fitted by the following relationship:

$$G' = k \coth(n\pi/2) \omega^n \quad (2.17)$$

$$G'' = k\omega^n \quad (2.18)$$

where n is flow behaviour index (which is dimensionless) and k is the flow consistency index.

In this section all the relevant mathematical models used in this thesis have been discussed. In the next section we move to describe the instrument we have used to perform our rheological measurements.

2.3.2 Rheometer TA AR-G2

The TA AR-G2 is a stress controlled, combined motor and transducer (CMT) rheometer. The instrument consists of a main unit (see figure 2.9) and a separate control box containing the electronics. The main unit comprise of a head, a base and a column. The head is attached to a ball-slide, which is mounted within the column. This configuration allows control of the vertical position of the head. The head contains the drag cup motor, the magnetic thrust bearing that supports the rotating spindle and an optical encoder that determines its angular position and velocity. The optical encoder consists of a non contacting light source and photocell, arranged on both sides of a transparent disc mounted on the rheometer spindle. On the edge of the disc there is a diffraction grating photographically etched as radial lines. The diffraction pattern formed by the light when the spindle rotates is detected by a photocell and the resulting signal produces high resolution angular position data and successive readings of the angular position gives the angular velocity.

The base contains the normal force transducer, the Peltier plate temperature control system and the lower stage. The removable upper stage, or geometry, is attached to the instrument's rotating spindle. To perform any measurement the sample is loaded between the lower stage and the geometry. We chose to use parallel plates as the measuring system because, since we have mainly measured the rheological properties of emulsions, this is the best geometry for our purpose. In this geometry by design the stator is a flat Peltier plate that allows temperature control during the experiment, while the rotor is a flat aluminium plate. The gap between the two plates can be varied but it is preferable to set it at 1% of the plate diameter to minimize parallel errors. On the AR-G2 rheometer the thrust disc that allow non contact bearings that allow low friction and therefore better data acquisition, is levitated magnetically.

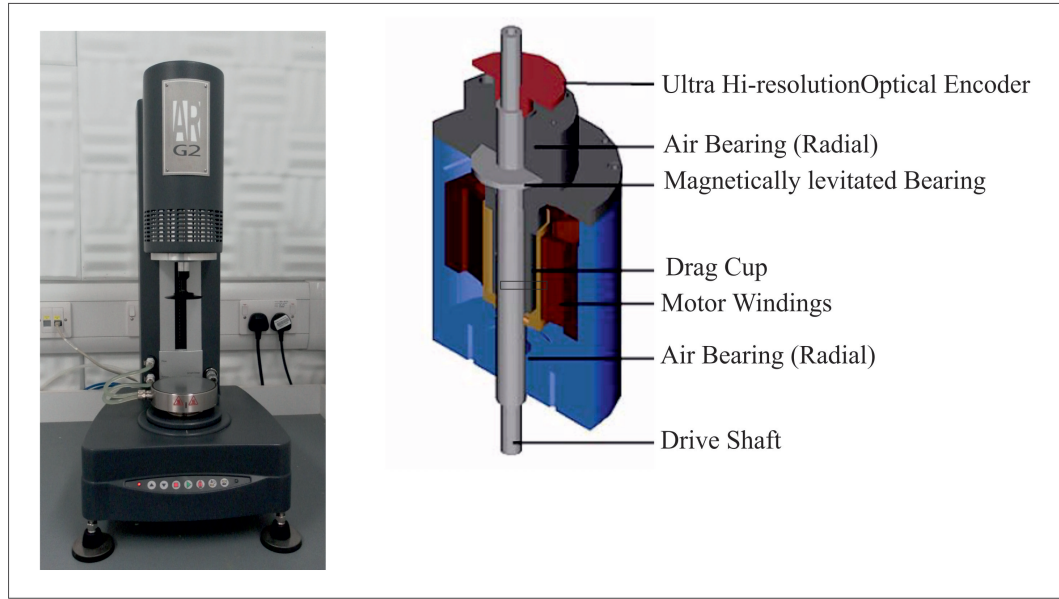


Figure 2.9: TA instruments AR-G2 Rheometer

2.4 Dynamic light scattering DLS

Dynamic light scattering is a technique for measuring the size of particles in the sub micron region by measuring the fluctuation of the intensity of the scattered light caused by the position changes of the scattering centres due to Brownian Motion [80]. The speed at which the particles are diffusing due to Brownian motion is measured by the rate at which the intensity of the scattered light fluctuates when detected using a suitable optical arrangement.

In figure 2.10 we report the schematic of a typical DLS set-up. In this set-up the sample is contained in a fixed holder and it is illuminated by a coherent monochromatic light source, typically a laser.

The incident light is then scattered by the sample in multiple directions. Each small volume hit by the laser light in a sample scatters light, thus the intensity measured will be the sum of all such contributions. The detector, at an angle θ , collects the emerging scattered photons, and a digital correlator performs the real-time computation of the photon correlation functions by working at a fixed range of simultaneous lag times between 0.125 s and several hours.

The second order autocorrelation curve, also defined as the intensity auto-correlation func-

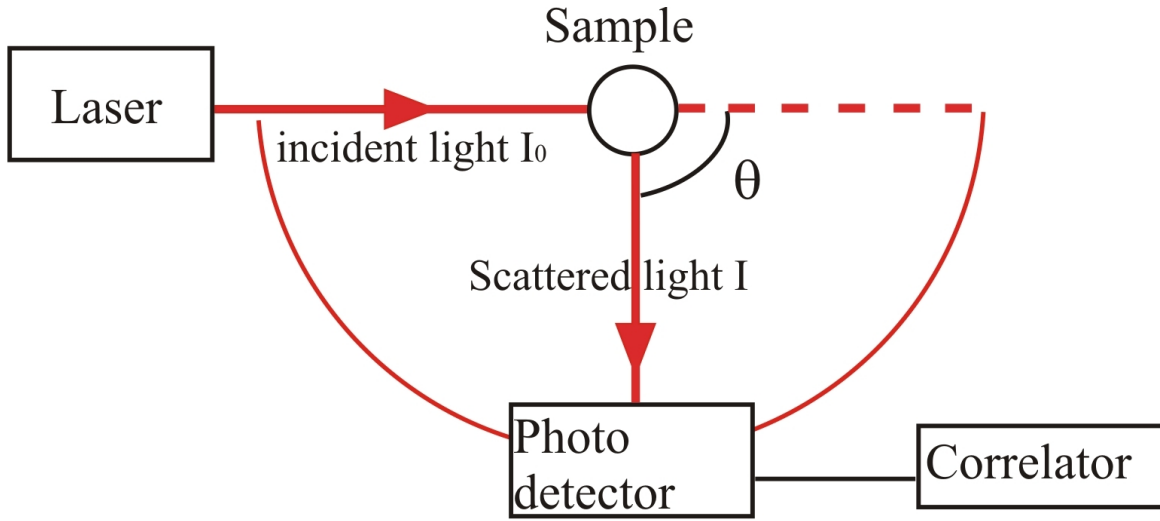


Figure 2.10: Scheme of a typical DLS set-up

tion (IACF) [1], is generated from the intensity trace as follows:

$$g_I(q, \tau) = \frac{\langle I(t)I(t + \tau) \rangle}{\langle I(\tau) \rangle^2}, \quad (2.19)$$

where $g_I(q, \tau)$ is the intensity autocorrelation function at a particular wave vector, q , and delay time, τ and I is the intensity of the incident light.

The wave vector q is defined as:

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (2.20)$$

Where λ is the wave length of the incident light beam, n the refractive index of the solvent and θ is the angle between the incident light beam and the detected scattered light beam. The second-order autocorrelation function plot is continuously updated by the correlator during the measurements. The Siegert equation relates the intensity autocorrelation function with the electric field autocorrelation function (EFAF) as follows [80]:

$$g_I(q, \tau) = 1 + \beta [g_E(q, \tau)]^2, \quad (2.21)$$

where β is an instrumental constant that depends on the geometry and alignment of the laser beam in the light scattering set-up.

The information on the particles sizes can be found in two ways:

1. by fitting a single exponential to the correlation function to obtain the mean size of the intensity weighted distribution (which is the z-average diameter) and estimate the width of the distribution with the polydispersity index;
2. by fitting an integral type model function to the correlation function, using a constrained regularization method in the software called CONTIN 2DP.

The first method is called Cumulants analysis, and it is defined in ISO 13321 part 8. It states that the electric field autocorrelation function $g_E(q, \tau)$, can be fitted to:

$$g_E(q, \tau) = \exp(-\Gamma\tau) \left(1 + \frac{\mu_2}{2!} \tau^2 - \frac{\mu_3}{3!} \tau^3 \dots \right). \quad (2.22)$$

The average decay rate Γ is related to the diffusion coefficient by:

$$\Gamma = q^2 D. \quad (2.23)$$

Therefore by using the Stokes-Einstein equation (2.24) the radius of an ideal spherical particle that diffuses like the observed particles can be calculated under the assumption that the scattering particles behave as hard spheres in the dilute solution and within the Rayleigh-Debye theory [80].

$$D = \frac{k_B T}{6\pi\eta r}. \quad (2.24)$$

The second term of the cumulants expansion gives the polydispersity index, PDI which, as already mentioned, is an estimate of the width of the distribution.

$$PDI = \frac{\mu^2}{\Gamma^2}. \quad (2.25)$$

The CONTIN method is mathematically more complex and provides the “intensities particle size distribution” that can be plotted as histogram. If the distribution presents a substantial tail or more than one peak, it is advisable convert the intensity size distribution to a volume distribution particles using Mie theory [81]. This second particle distribution gives a more realistic view of the importance of the tail or a second peak present.

2.4.1 ALV/ CGS Compact Goniometer System

To perform DLS measurements we have used the ALV/CGS Compact Goniometer System. This instrument is designed to perform both Dynamic and Static light scattering measurements. The correlation function is continuously plotted during the measurement and the hydrodynamic radius is calculated in the approximation of a spherical particle that moves in the same way of the observed particles.

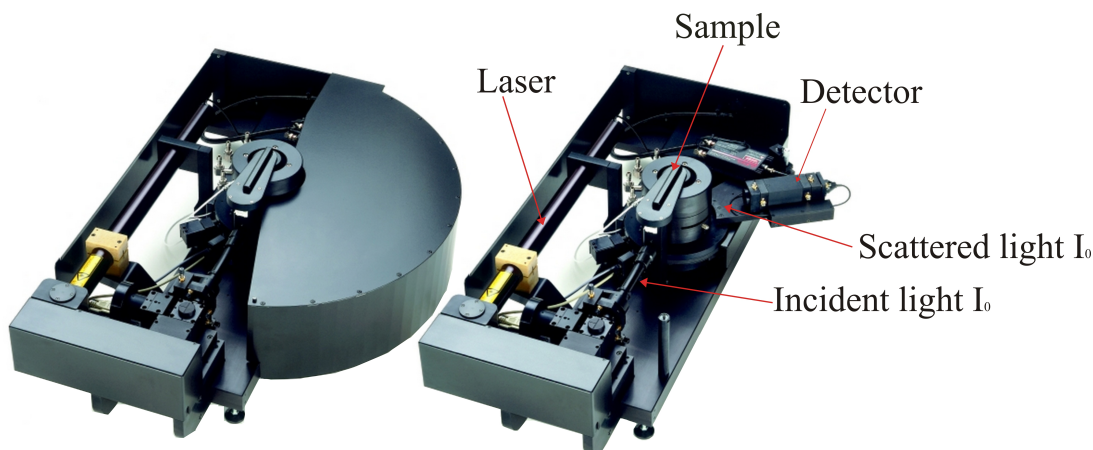


Figure 2.11: ALV/ CGS Compact Goniometer System

In figure 2.11 the picture of the ALV/ CGS Compact Goniometer System is shown. The light source is a 22 mW HeNe laser, (class 3B) with a wavelength of 632.8 nm. (The laser beam tubing and the main cover, of the ALV /CGS-3, allow to operate it as a Class 1 Laser.) The sample is loaded in a quartz cell and positioned in the sample holder. The measuring system comprises of a quartz vat filled with toluene, which is the index matching fluid. The detector is mounted on an arm that can be moved at different angles. The signal collected by the detector is transmitted to the Multiple Tau Digital Correlator that performs real-time computation of the photon correlation functions. The ALV5000 software performs the data analysis and it is able to do the Cumulant and CONTIN fits.

2.5 High-performance dispersing instrument

As explained in Chapter 1, section 1.6, to prepare an emulsion it is necessary to spend mechanical energy to disperse one liquid phase into the continuous phase. For this purpose we have used a high-performance dispersing instrument: T 25 digital ULTRA-TURRAX by IKA. This instrument has a 700/500 W (input/output) electric motor that enables a wide speed range from 3000 to 25,000 rpm with a speed deviation of 1%. A LED digital speed display and a rotating knob allow the user to set and monitor the actual speed. Our instrument mounted stainless steel dispersing element S 25 N - 18 G. In figure 2.12 A, a picture of the instrument (motor mounting the dispersing element) is shown. The dispersing tool is formed by a stainless steel (AISI 316L) stator and internal rotor. Due to the high radial speed, the phase to be dispersed is drawn axially into the gap between the rotor and the stator as shown in figure 2.12 C. The high speed and small gap between them produce extremely strong shear forces able to make a good dispersion. The smallest droplet sizes that this system can reach for emulsions is 5 to 20 μm and its operational range is from 10 to 1.500 mL and it can work at immersion depth from 40 to 165 mm.

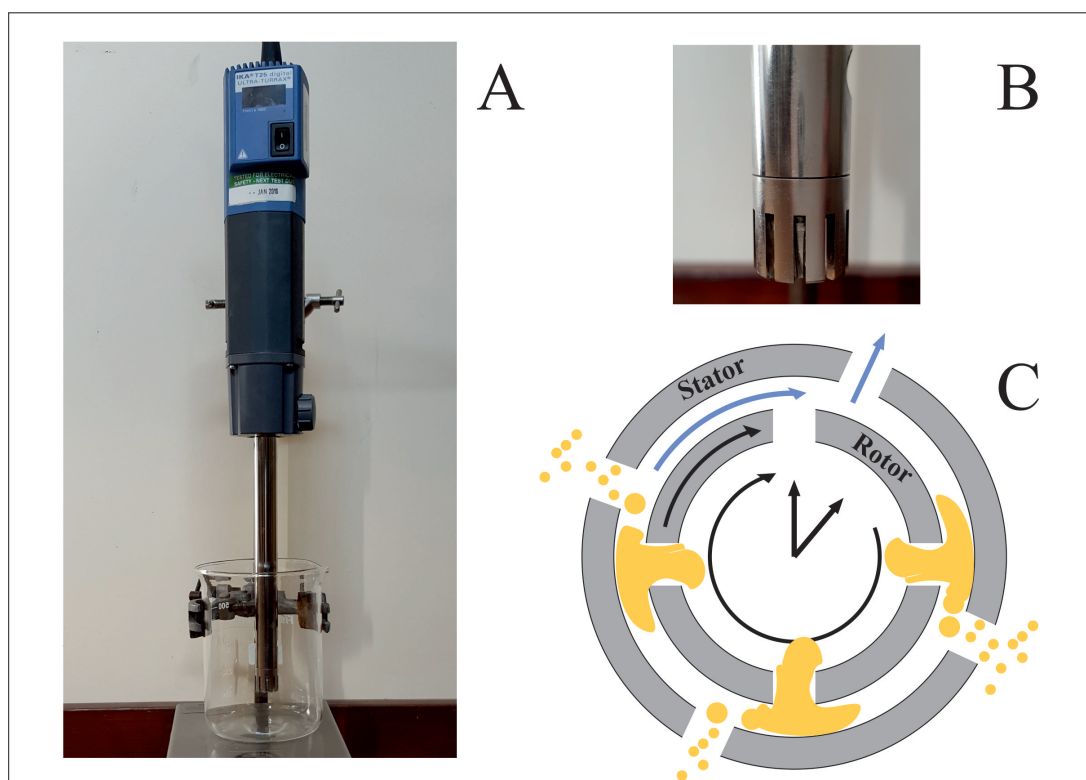


Figure 2.12: A: Picture of the high-performance dispersing instrument, T 25 digital ULTRA-TURRAX; B: Enlarged detail of the rotor/stator head of the dispersing element S 25 N - 18 G; C: Diagram of the cross-section of the rotor/stator head

Chapter 3

Polymer-polyphenols interactions: from soluble complexes to gel colloidal particles

3.1 Introduction

Natural polyphenols associate strongly with proteins and polysaccharides giving insoluble complexes responsible for turbidity and sedimentation in foodstuff such as beer, wine and plant derived beverages [82]. This normally unwanted effect is sometimes prevented by adding insoluble polymers, for instance polyvinyl polypyrrolidone (PVPP), which is an insoluble cross-linked polymer of polyvinylpyrrolidone (PVP). PVPP is able to absorb polyphenols such that they can be subsequently removed from the system. PVPP, like PVP, has a strong affinity for polyphenols forming complexes through a range of interactions such as, primarily, hydrogen bonding between CO-N groups of the polymer and OH phenol groups of polyphenols, van der Waals interactions and hydrophobic interactions between phenolic and pyrrolidone rings [12]. These interactions and the analogous interactions between polyphenols and proteins have been widely studied in a variety of systems [83, 84]. Polyphenols lead to problems in terms of stability of foodstuff products and palatability due to the interaction with proteins. This is responsible for both haze formation in beverages and astringency, the latter due to the ability to precipitate saliva proteins [85–88]. On the other hand polyphenols can play an interesting role in foam stabilization [37] and most importantly they have been shown to have numerous biochemical and physiological benefits, including antioxidant, anti-microbial, deodorant [89], thermogenic activities [90] and several studies have been done to demonstrate their role in cancer prevention [25–27, 91].

Here, we exploit these normally unwanted effects to synthesize new colloidal particles, which are stable in water, with a one-step assembly, environmentally friendly, non-toxic and economically effective method. Among the potential applications for these novel systems we envisage new delivery systems and the ability to stabilize emulsions. For this purpose we investigate the following systems: PVP-catechins and PVP-tannic acid particles.

Catechins are a class of natural polyphenols which belong to the subclass of flavonoids which have a general skeletal structure C6-C3-C6 [92]. Members of the catechin family are: catechin, epicatechin, epigallocatechin and epigallocatechin gallate. Green tea (*Camellia Sinensis*) is particularly rich in these catechins and therefore green tea extract is a good source of them. The catechin structure is formed by two benzene rings, the first one from the left, is a resorcinol moiety (2,4-dihydroxybenzene) while the second is a catechol moiety (3,4-dihydroxybenzene).

The third ring, in the middle, is a dihydropyran heterocycle. This moiety has two chiral centers carbons 2 and 3, therefore four diastereoisomers are theoretically possible. The two isomers in trans configuration are called catechin and the two in cis configuration are called epicatechin. Naturally occurring catechin is typically (+)-catechin and epicatechin is typically (-)-epicatechin [38,93]. Epigallocatechin differ from the epicatechin in the second benzene ring which is a gallo moiety (3,4,5-trihydroxybene) and epigallocatechin gallate is the ester of epigallocatechin and gallic acid. Catechin molecular structures are shown in figure 3.1, A to D and their 3D structures are shown in figure 3.2.

Tannic acid (TA) belongs to the family of condensed tannins, a subclass of polyphenols, and has a higher molecular weight than catechins. It is a polydentate ligand and has the ability to form multiple hydrogen bonds due to the presence of numerous terminal hydroxyl groups [8]. TA is formed by five digalloyl ester groups esterified to a glucose core [6]. Its structural formula is shown in figure 3.1, F and its 3D structure is shown in figure 3.3, A. TA is a weak polyacid, $pK_a \sim 8.5$, and its phenolic groups act as excellent H donors to form strong hydrogen bonds with compounds containing carbonyl groups which are stable at neutral pH values [6].

Polyvinylpyrrolidone (PVP) is a water-soluble synthetic polymer, which has important pharmaceutical [15, 94] and numerous technical applications [95–98]. The particular ability of PVP to bind phenolic compounds finds applications in chromatographic analysis of these compounds and in the removal of tannins from plant extract [21]. Moreover, PVP provides a useful model system for understanding how polyphenols interact with the proline-rich proteins, which are well known to strongly bind polyphenols [99]. PVP is polar, water soluble, and easily available. It is used in the food industry as stabilizer, thickeners agent E1201, and in personal care. Its molecular structure is shown in figure 3.1, E and the 3D structure of its monomeric precursor, N-Vinylpyrrolidone, and an example of a heptameric structure are shown in figure 3.3 B and C.

Molecular simulations have shown that the formation of PVP-polyphenol complexes is due to two types of interaction: hydrophobic interaction between the phenolic and pyrrolidinone rings and H bond between the hydroxyl functions and the CO-N moieties of the polymer [12]. In figure 3.4 a schematic 3D structure of catechin bounded by hydrophobic interactions and H-bonds to an heptameric PVP chain is shown.

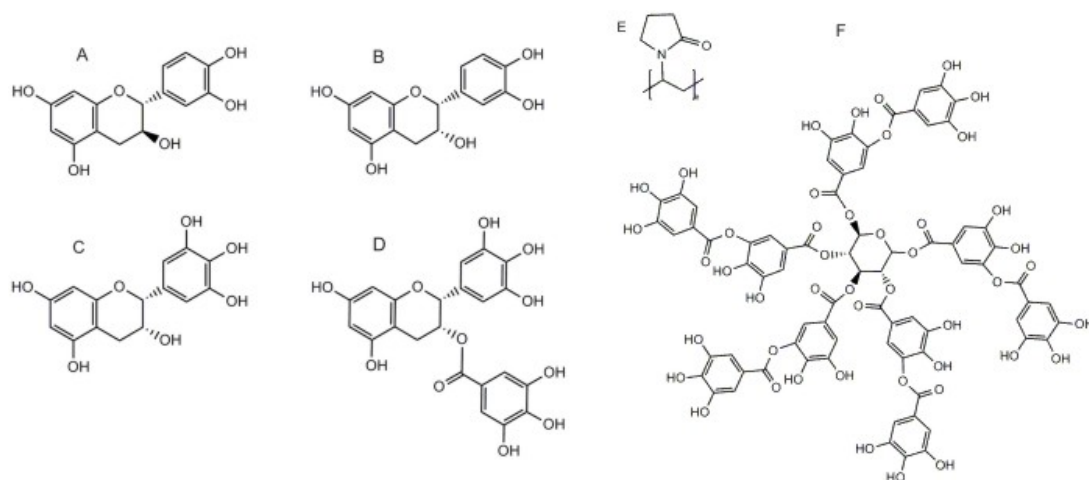


Figure 3.1: Chemical structures of: A: Catechin. B: Epicatechin (EC). C: Epigallocatechin (EGC) D: Epigallocatechin gallate (EGCG). E: polyvinylpyrrolidone (PVP monomer). F: tannic acid (TA)

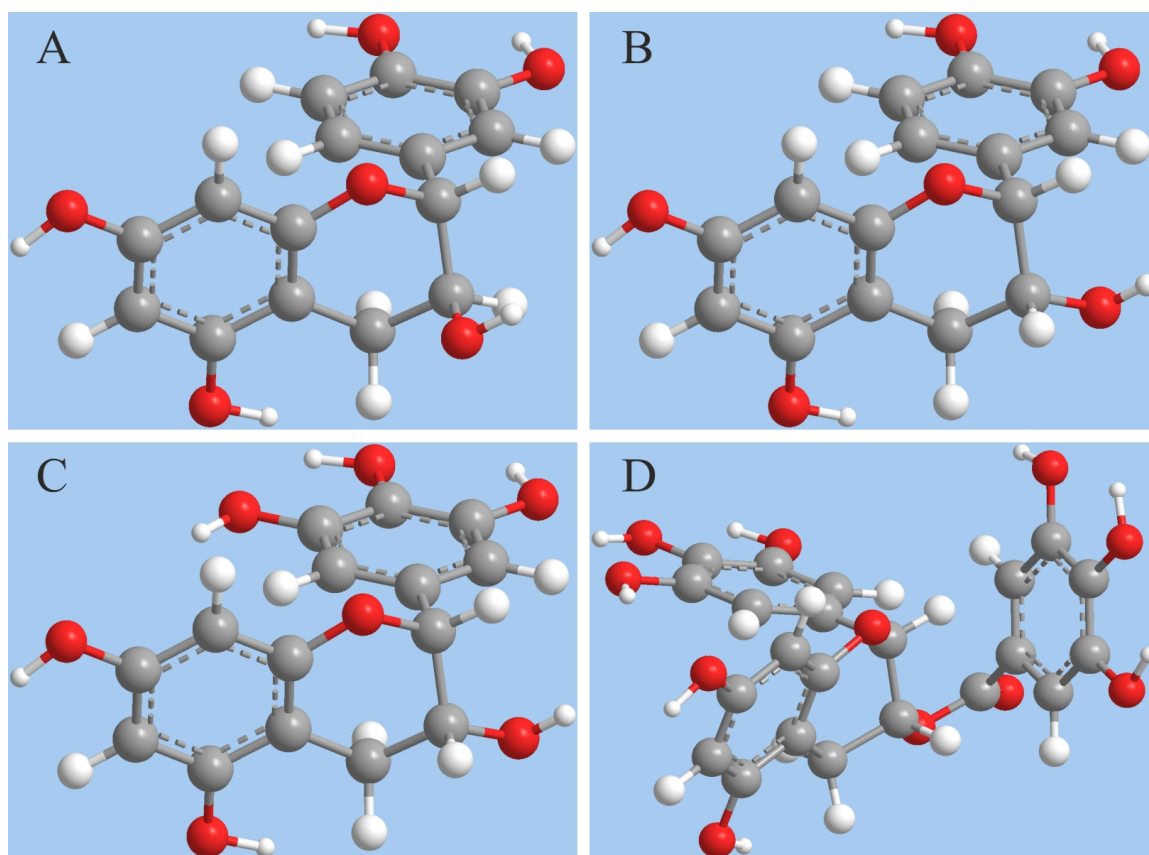


Figure 3.2: 3D structures of A: Catechin. B: Epicatechin (EC). C: Epigallocatechin (EGC). D: Epigallocatechin gallate (EGCG)

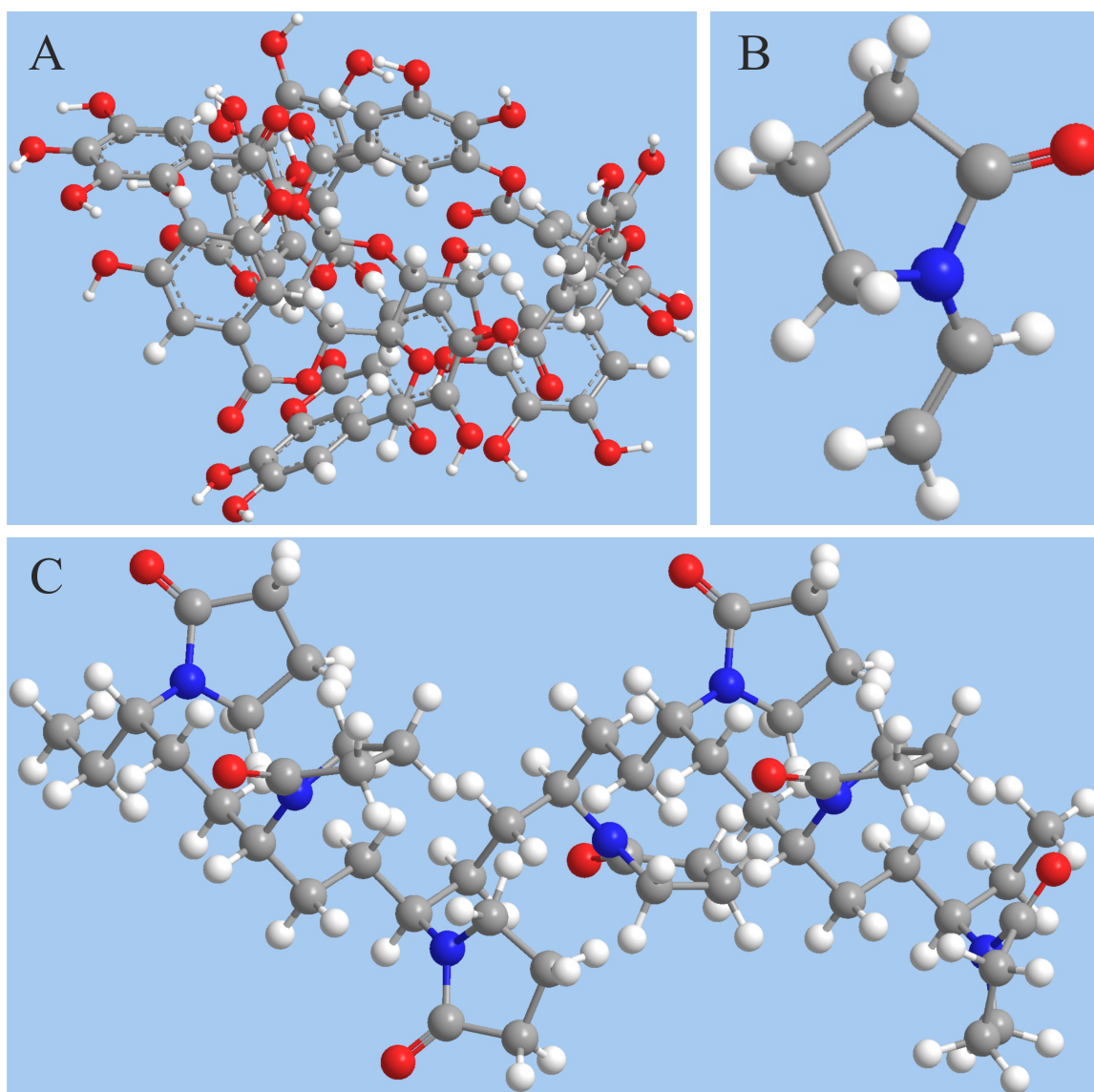


Figure 3.3: 3D structures of A: Tannic Acid (TA); B: N-Vinylpyrrolidone(precursor to polyvinylpyrrolidone); C: Polyvinylpyrrolidone (PVP) (heptameric structure)

In this chapter, we demonstrate that it is possible to synthesize new colloidal particles using polyphenol polymer complexes in water. We prove this for several systems, for both classes of polyphenols. Then we describe how we have constructed the relative phase diagram for the two systems PVP-Catechins and PVP-Tannic Acid and we will discuss the surprising behaviour at high concentrations of polymer and the non-trivial role that the molar ratio plays. In the next sections we will discuss the materials and methods (Section 3.2), the results we have obtained for the PVP-catechins (Section 3.3.1) and PVP-TA (Section 3.3.2) systems, and we our conclusions.

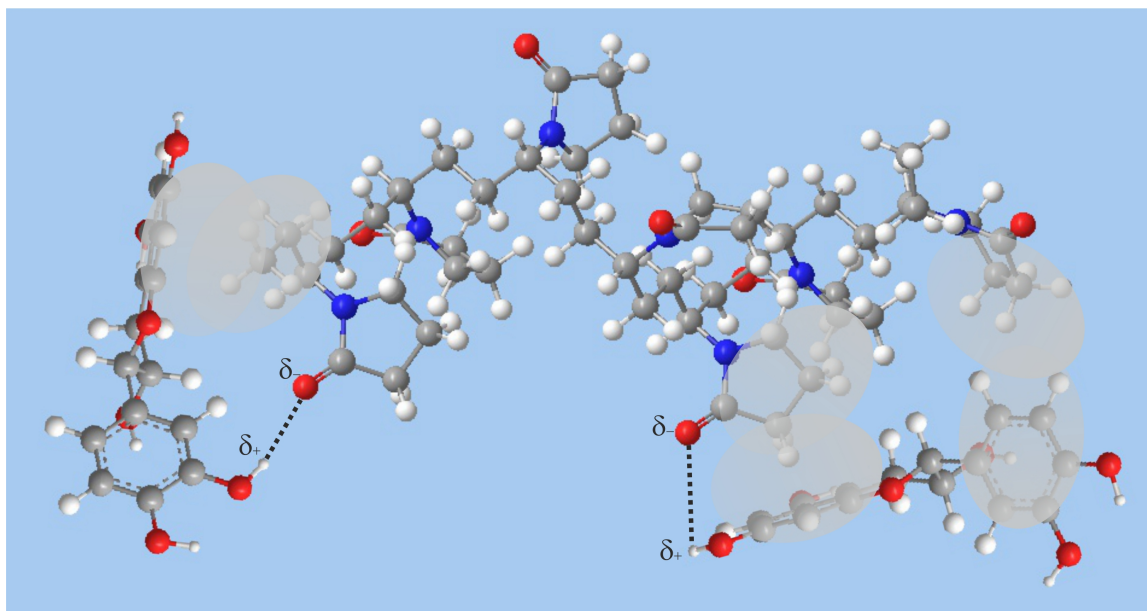


Figure 3.4: Model of hydrophobic interactions and H-bond between catechin molecules and heptameric structure of PVP. The gray regions are involved in the hydrophobic interactions and H-bonds are represented by the dot lines and the partial charges on the H and O atoms.

3.2 Materials and Methods

PVP40 (molecular weight 40000 Dalton equal to circa 360 monomeric units) was purchased from Sigma-Aldrich. No further purification was needed. Catechins in the form of green tea extract Sunphenon 90LB, which is a commercially available mixture of highly purified polyphenols, was purchased from Tayo Kagako, at given composition: polyphenols >80%, catechins >80%, EGCG >40%. Tannic acid was purchased from Sigma-Aldrich. All solutions were prepared using deionised water.

3.2.1 Preparation of stock solutions

Polyvinylpyrrolidone PVP 40, green tea extract 90LB and tannic acid stock solutions were prepared by dissolving them in deionised water and mixing with a Teflon magnetic stirrer. Stock solutions were stored in the fridge at 4 °C. For some experiments the solutions were freshly prepared and used within a day to avoid self polymerization of the polyphenols [100]. We prepared four solutions of PVP40 at these concentrations: 1%(0.25 mM), 1.5% (0.38 mM), 3% (0.75 mM), and 5% (1.25 mM). We also prepared solutions of tea extract 90LB at 1% w/v (42 mM) and solutions of TA at 1% w/v (5.88 mM). A weighted average molecular weight of

237 g/mol was calculated for the generic polyphenol, “catechin”, contained in the tea extract. The molecular weight of tannic acid is 1701 g/mol.

3.2.2 Dynamic light scattering

Dynamic light scattering measurements were performed with a ALV/LSE-5003 multiple Tau digital correlator equipped with laser (JDS Uniphase) wave length 632.8 nm. Measurements were carried out at 5 different angles, 30°, 60°, 90°, 120° and 150°. The DLS measurements were repeated three times for each angle and each time the instrument performed 5 runs. The dispersions were diluted with filtered DI water in order to minimize multiple scattering and dust contamination. The mean apparent hydrodynamic diameter, the polydispersity index and the volume average particle size were calculated using the ALV 5000 Software.

3.2.3 UV spectroscopy and pH measurements

The pH were monitored for all the experiment using a portable electronic pH-meter, VWR pH 100. UV-Vis spectroscopy measurements were carried out with UV-Vis spectrometer Evolution 60 Thermo Scientific single diode to monitor the residual unbounded polyphenols concentration. UV-Vis spectra were collected from 250 nm to 750 nm.

3.2.4 FTIR measurements

The vibrational spectra of dried samples were measured by mean of ATR-FITR (Attenuated total reflectance-Fourier transform infrared spectroscopy) spectrophotometer Thermo scientific Nicolet 6700, ZnSe crystal cell, beam splitter KBr. With this technique we have investigated the evolution in the vibrational spectra following the formation of the different complexes.

3.3 Results and discussion

We studied the interactions between PVP and catechins and PVP and TA by quick addition of an ever-increasing volume of catechin solutions, or TA solutions, to PVP solutions and vigorously shaking the vials. We prepared several different samples, varying the concentration of the reactants in a systematic way: four different starting point concentrations of PVP for the PVP-catechin systems and 3 for the PVP-TA system. We plotted each system as two different phase diagrams, one for each type of polyphenol, by plotting the concentration of polyphenols against the concentration of PVP (see Fig. 3.11). An overview of the molar concentrations for every system prepared is reported in tables 3.1 for PVP-catechin and 3.2 for PVP-TA.

Sample	Catechins [mM]	PVP A [mM]	PVP B [mM]	PVP C [mM]	PVP D [mM]
1	3.83	1.14	0.68	0.34	0.23
2	7.02	1.04	0.63	0.31	0.21
3	9.72	0.96	0.58	0.29	0.19
4	12.04	0.89	0.54	0.27	0.18
5	14.05	0.83	0.50	0.25	0.17
6	15.80	0.78	0.47	0.23	0.16
7	17.35	0.74	0.44	0.22	0.14
8	18.73	0.69	0.42	0.21	0.14
9	19.96	0.66	0.39	0.20	0.13
10	21.07	0.63	0.38	0.19	0.13

Table 3.1: Molar concentration of catechins and PVP in each sample prepared for each set of experiments. PVP A, was made with a PVP40 solution at 5% (PVP40-5%), PVP B, was made with a PVP40 solution at 3% (PVP40-3%), PVP C, was made with a PVP40 solution at 1.5% (PVP40-1.5%), PVP D, was made with a PVP solution at 1% (PVP40-1%)

In the next sections we will first present and discuss the results for the PVP-catechins system (3.3.1); then we will discuss this for the PVP-TA (3.3.2) system and subsequently we will draw conclusions.

3.3.1 PVP-catechin systems

The catechins were used as a mixture of catechin, epicatechin, epigallocatechin and epigallocatechin gallate because we were interested in investigate their natural occurring form. Since PVP does not have any chiral centers, its long flexible chain can freely rotate therefore we assume that its interaction with the diastereoisomers catechin (isomer in trans configuration)

Sample	TA [mM]	PVP E [mM]	PVP F [mM]	PVP G [mM]
1	0.53	1.14	0.45	0.23
2	0.98	1.04	0.42	0.21
3	1.36	0.96	0.38	0.19
4	1.68	0.89	0.36	0.18
5	1.96	0.83	0.33	0.17
6	2.20	0.78	0.31	0.16
7	2.42	0.74	0.29	0.15
8	2.61	0.69	0.27	0.14
9	2.78	0.66	0.26	0.13
10	2.93	0.63	0.25	0.13

Table 3.2: Molar concentration of Tannic acid and PVP in each sample prepared. Each set denoted with the same letter were prepared adding increasing volume of TA solution 1%. PVP E, was made starting with a PVP40 solution at 5% (PVP40-5%), PVP F, was made with a PVP40 solution at 2% (PVP40-2%), PVP G, was made with the PVP40 solution at 1% (PVP40-1%)

and epicatechin (isomer in cis configuration) would not be greatly affected by stereo-specificity. Epigallocatechin differ from the epicatechin in the second benzene ring which is a gallo moiety (3,4,5-trihydroxybene) therefore it has an extra hydroxyl group able to form an hydrogen bond with PVP. Epigallocatechin gallate (EGCG) is the ester of epigallocatechin and gallic acid. It has therefore an extra phenyl group, which as already mentioned is an hydrophobic surface prone to hydrophobic interactions with the pyrrolidinone rings and three hydroxyl groups able to form multiple H-bonds. The main difference between EGCG and the other catechins is the CO group which is a proton acceptor in the formation of H-bond with a proton donor such as a hydroxyl groups of another polyphenol molecule.

The particles and the complexes prepared were characterized by means of optical microscopy, UV-Vis spectroscopy, IR spectroscopy and dynamic light scattering. In figure 3.5 the visual appearance of the systems prepared is shown: on the left side there is the observation at the time of preparation and on the right the same sets after two months of conservation in the fridge. Set A, at the top of the picture, has the highest concentration of PVP and set D, the one at the bottom, has the lowest concentration. The concentration of catechin increases left to right and it is the same for every set down each column. For most of the systems in sets D and C we have abundant particle formation. We have imaged the biggest particles with transmission microscopy. An example of these are shown in figure 3.6.

The most interesting aspect of this experiments is that in spite of the higher concentration

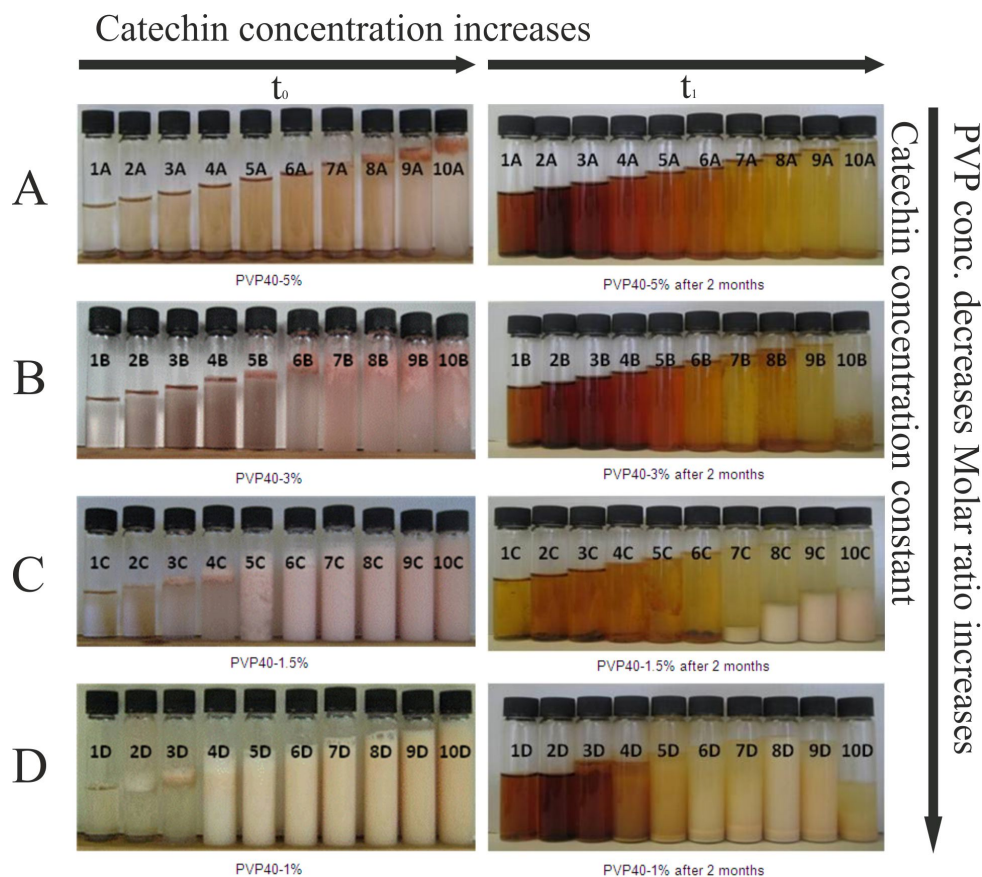


Figure 3.5: Overview of the four sets of experiments to make colloidal particles with PVP catechin complexes. On the left side, samples directly after preparation and on the right the samples after two months. The concentration of catechins in each set increase from the first vial in the set to the tenth from 3.83 mM to 21.07 mM, while the concentration of PVP decrease in each set as shown in table 3.1

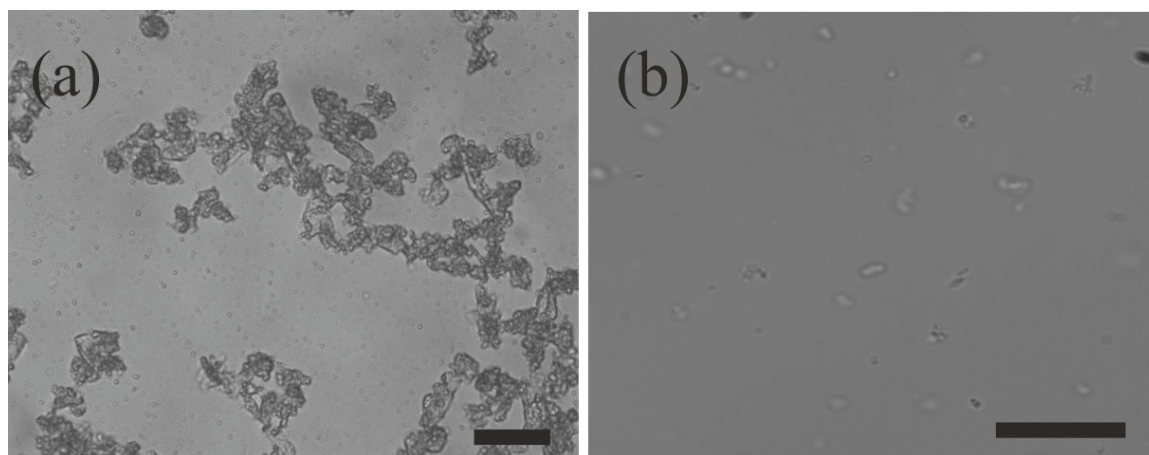


Figure 3.6: Microscopy images of PVP-catechin complexes prepared by direct precipitation. (a) fractal structures and smaller colloidal particles. Obj 40X scale bar 10 μm . (b) colloidal particles. Obj 63X oil immersion. Scale bar 10 μm .

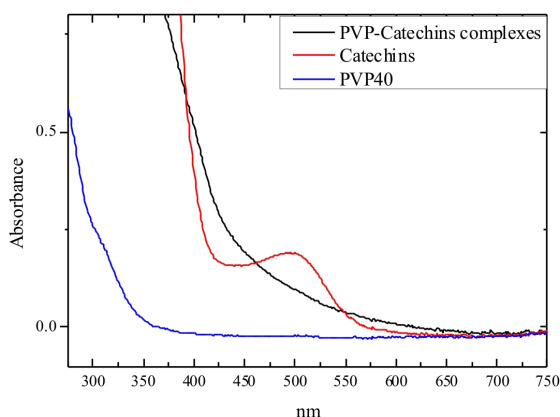


Figure 3.7: Example of a UV-Vis spectrum. The red line is the spectrum of the catechins solution (12mM) with its characteristic shoulder of absorption at 500 nm; the black line is the spectrum of sample 4B (Catechins 12.04 mM, PVP 0.54 mM molar ratio 22.59); the blue line is the spectrum of PVP40 0.54 mM solution.

of PVP for sets A and B, the majority of the systems in these two sets are clear and transparent and, surprisingly, we do not observe any particles by transmission light microscopy.

The UV-Vis spectra of these transparent solutions, see figure 3.7, present a wide absorption peak, but they do not present the characteristic shoulder of absorption at 500 nm that is present in the UV-Vis spectrum of the catechin solutions. From a colorimetric point of view, the catechin solutions are orange, opposite to the pink colour of the clear solution of complexes. We can therefore assume that in these transparent systems catechins are bounded to the PVP and that we have soluble PVP-catechin complexes that do not precipitate and present a different UV-Vis absorbance compared with the free PVP and catechins (fig: 3.7).

Looking at the ensemble of the sets of experiments and analysing the general behaviour, we note that in each set (A, B, C, D), we observe the formation of an extended polymer network for some vials (e.g. vials 3D and 10A). This is particularly easy to see in the last three vials in set A, which are still pink, clear and transparent, but show a jelly like macroscopic agglomerate at the water air interface due to the formation of a polymer network cross-linked by the catechins [37, 101].

The IR spectra of one of these macroscopic agglomerates is shown in figure 3.8, red line (3D), together with the IR spectra of a dried solution of soluble complexes, black line (1D), and the spectra of some dried particles, blue line (10D). The broad peak at 3250 cm^{-1} is due

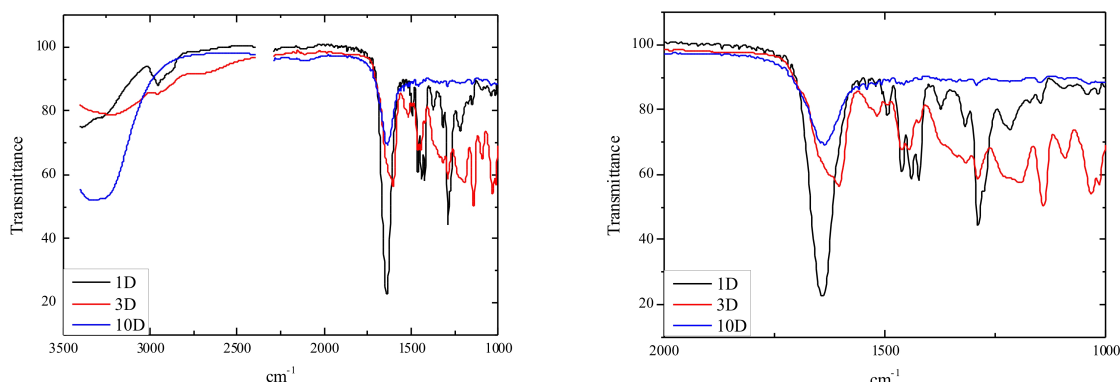


Figure 3.8: FTIR spectra of PVP-catechins complexes 10D molar ratio 170, particles; 3D molar ratio 50, macroscopic agglomerated, 1D molar ratio 17, soluble complexes.

to the absorption by the stretching mode of the phenolic -O-H bound and it is broader than expected for a phenolic bond, because of the hydrogen bond between the -OH of the catechins and the -C=O of the pyrrolidone. The difference in intensity between the three spectra is due predominately to the different concentration of catechins in the three samples. The spectrum of sample 1D, which is the one with only soluble complexes, presents a peak at 3000 cm^{-1} which is in the typical position of stretching vibrations of hydrogen bonds [102]. The peak at 1650 cm^{-1} is correlated to the stretching of the -C=O of the pyrrolidone. This peak is less intense for sample 10D which is due to the fact that the double bond is delocalised to form the H bond with the O-H of the polyphenols. The peaks below 1500 cm^{-1} are the “finger print” of the molecules and are due to the vibrational mode of the molecule in its ensemble.

The systems for which we have formation of particles are polydisperse, but it is easy to separate colloidal particles of similar size thorough centrifugation or sedimentation. DLS measurements of the smallest particles have been carried out. We have recorded an average size of $110 \pm 0.5\text{ nm}$ for these particles and, the polydispersity index (μ^2/Γ^2) evaluated from the cumulant fit of the correlation function recorded with DLS, was in the range of 0.4/0.5 for all the systems. By fitting an integral type model function to the correlation function, using a “constrained regularization method” CONTIN 2DP using the ALV 5000, software we have analysed the size distribution function. In figure 3.9 we shown an example of the volume average particle size distribution. The figure shows a normal distribution cantered at 250nm with a range from about 70nm to 600nm.

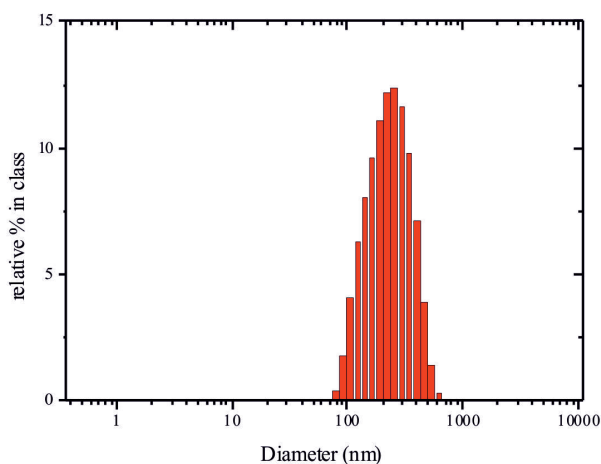


Figure 3.9: Volume average particles size distribution of catechins-PVP sample 8D, high molar ratio. The figure shows a normal distribution centred at 250nm with a range from about 70nm to 600nm

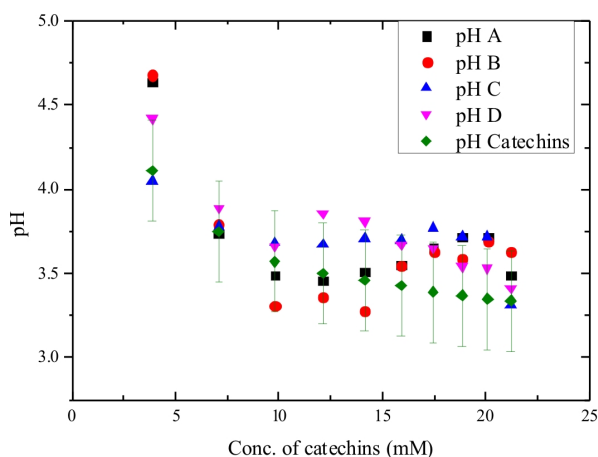


Figure 3.10: Variation of pH in each set of PVP-catechin systems and control set of catechins in deionised water at the same concentration of each vials.

Over time, discolouration due to oxidation [103] occurs for every system studied. For the systems in vials 1-6 A, 1-4 B, 1-2 C, 1D the colour changes from pink to brown/orange and after two months no significant changes are noticeable. For set C, which was prepared from a PVP solution at 1.5%, after a month we observe a clear phase separation due to the sedimentation of the bigger particles which compact over time while in set D, that was prepared from a PVP solution at 1% and therefore has the lowest PVP concentration in the range studied, the dispersions are more stable over time. The measurements of pH are reported in figure 3.10 but they do not give any significant indication on a correlation between pH and behaviour for these

systems. With the exception of the first vials in every set which have the lowest concentration of catechins, the pH values do not vary significantly (the average pH is 3.4 ± 0.2 with a standard deviation within the experimental error of the measurements), and therefore the solution of catechins seems self buffered in this range.

Sample	Catechins [mM]	D	C	B	A
10	21.07	169.44	112.96	56.48	33.89
9	19.96	152.504	101.66	50.83	30.50
8	18.73	135.55	90.37	45.18	27.11
7	17.35	118.619	79.07	39.54	23.72
6	15.80	101.66	67.78	33.89	20.33
5	14.05	84.72	56.48	28.24	16.94
4	12.04	67.78	45.18	22.59	13.56
3	9.72	50.83	33.89	16.95	10.17
2	7.02	33.89	22.59	11.30	6.78
1	3.83	16.94	11.30	5.65	3.39

Table 3.3: Molar ratio catechin/PVP. The different colours represent different behaviour of the systems. Green area clear, transparent system; yellow area formation of jelly macro structure; Orange area colloids in coexistence with a jelly agglomerate; red area formation of particles.

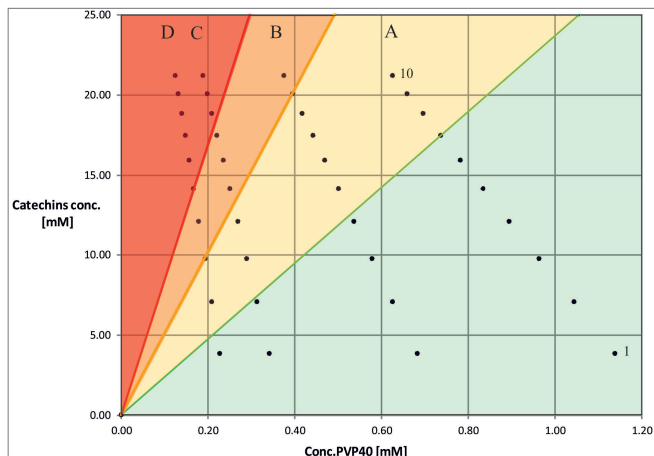


Figure 3.11: Phase diagram for PVP-catechin systems. The green area represents the region of existence of catechin-PVP soluble complexes, for which the system is clear and transparent. The yellow area is the region of macro agglomerated and soluble complexes. The orange area indicates macro agglomerated and colloidal dispersions. The red area indicates colloidal particles and small fractal structure formation.

As already mentioned, the most interesting phenomenon observed for these experiments is that in sets prepared at lower concentration of PVP there is more precipitation of particles for the same concentration of catechins. For instance, in set D, which was prepared starting from a solution of PVP40 1%, we have precipitation of complexes already at a catechin concentration of 12 mM (vial 4D in figure 3.5), while for set A, which is at higher PVP concentration, for the

same catechin concentration (vial 4A in figure 3.5), there is no precipitation of complexes or turbidity. This perhaps counter-intuitive behaviour is related to the molar ratio which plays an important and more complex role than one would expect.

A correlation between the structures formed in the experiments and the molar ratio, shown in table 3.3, has been found. We have identified three molar ratios, for which we have observed a change in the behaviour of the PVP-catechin systems and we have used them to plot three lines on the phase diagram (Fig. 3.11 on the left). These lines delimit four different areas of the phase diagram that we have highlighted in green, yellow orange and red. In the following Sections we will discuss each area moving in the phase diagram in the direction of decreasing the PVP concentration and increasing the catechin concentration and therefore increasing the molar ratio catechin/PVP, in other words from the green area to the red one.

Green area

Below molar ratio 24, soluble PVP-catechin complexes are observed in the range of concentration considered. For these molar ratios catechins bind to PVP chains without compromising their solubility. The complexes are probably single molecules of PVP with many molecules of catechin bounded by the hydrophobic interactions of the aromatic rings to the pyrrolidone ring [85]. As already mentioned, the IR spectra of these samples present a peak at 3000 cm^{-1} , which confirms the presence of hydrogen bonds in the PVP molecules [102, 104]. We can speculate that the amount of catechin present is not sufficient to form a hydrophobic layer around the long and flexible PVP chain and therefore the complexes are still soluble because the PVP chain retain its solubility in water [85]. This hypothetical mechanism is shown in figure 3.12.

Yellow area

In the yellow area of the phase diagram, the molar ratio is between 24 and 50, and we observe the formation of a big agglomerate at the water air interface. In this case, the ratio between catechins and PVP is higher than in the green area and we speculate that inter-molecular cross linking occurs as reported by other researchers [88]. The hypothetical mechanism is shown in figure 3.13 in which we assume that each heptameric structure belong to a different PVP chain (of ~ 360 monomeric units). The solutions below the polymer networks present the same UV

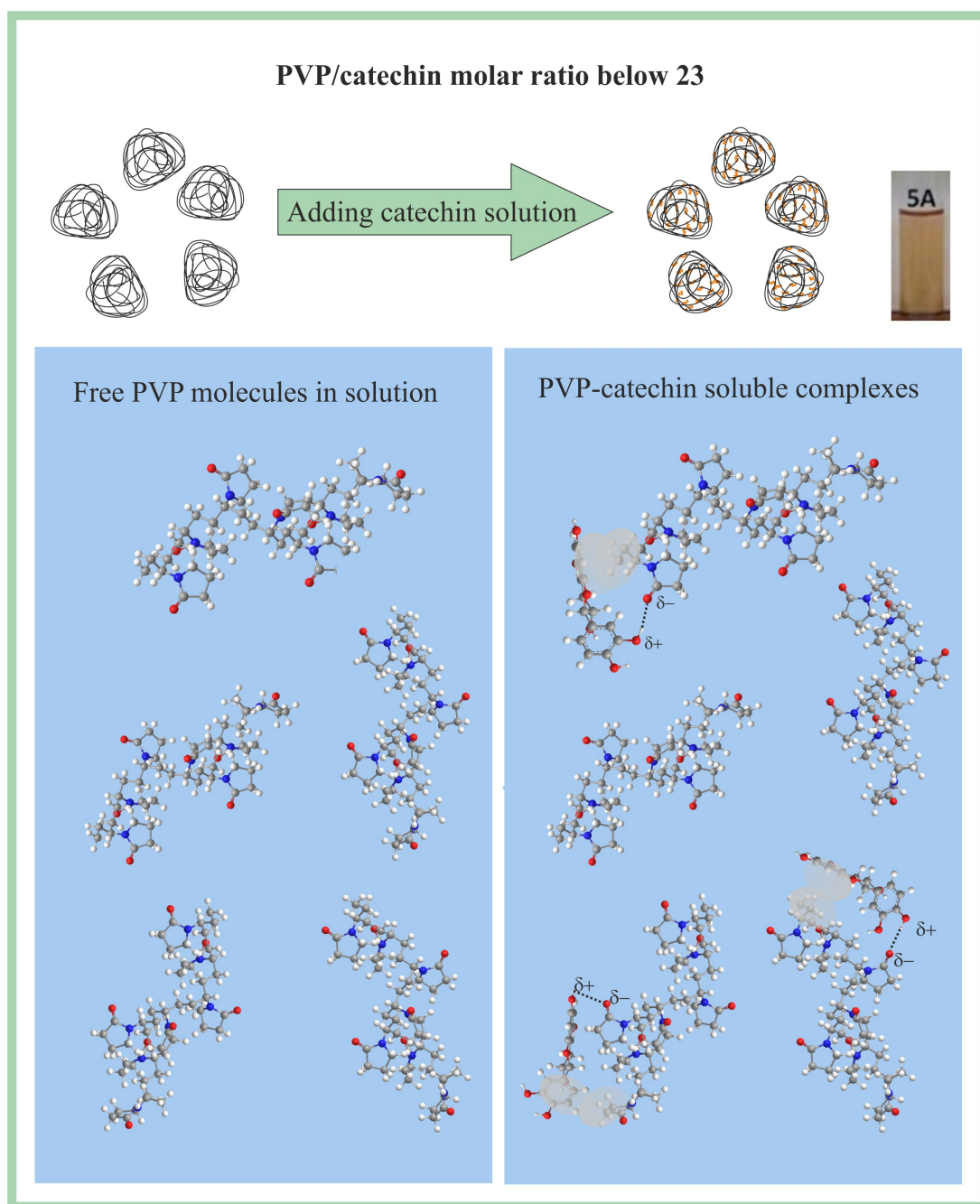


Figure 3.12: Diagram explaining the mechanism of the complex formation: top the left is schematic representation of PVP molecules in water solution, the green arrow represent the addition of catechin solution, on the top right, the schematic representation of catechin molecules (in orange) and the PVP chains complexes. Below, on the left, 3D structures of heptameric PVP chains and on the right a 3D model of hydrophobic interactions and H-bond between catechin molecules and heptameric structure of PVP. The gray regions are those involved in the hydrophobic interactions and H-bonds are represented by the dot lines and the partial charges on the H and O atoms.

spectrum as the one reported for the green area therefore we assume that soluble complexes are still in solution and only a part of the PVP-catechin complexes are cross linked into these big

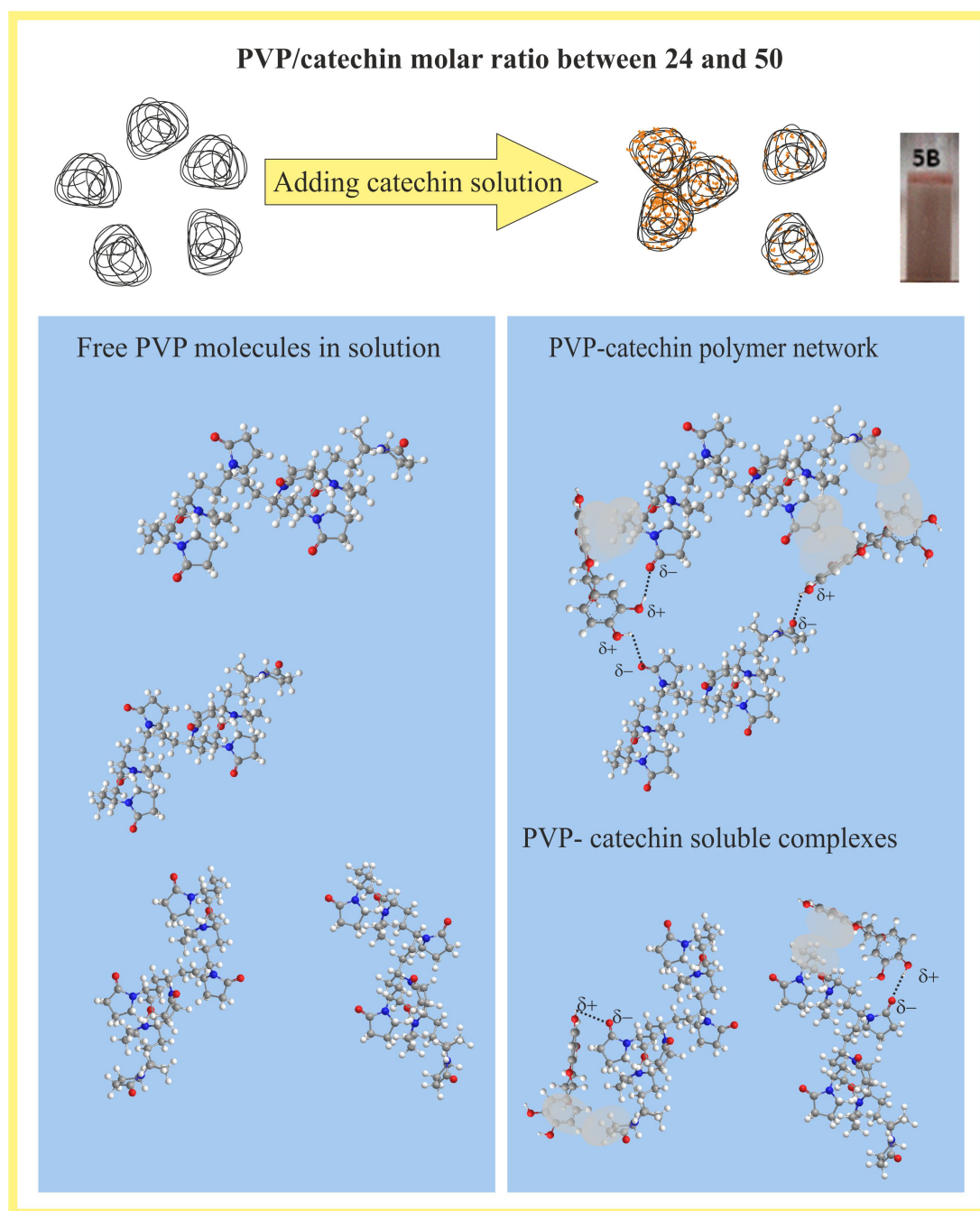


Figure 3.13: Diagram explaining the mechanism of the complex formation: top the left is schematic representation of PVP molecules in water solution, the yellow arrow represent the addition of catechin solution, on the top right, the schematic representation of catechin molecules (in orange) and the PVP chains complexes. Below, on the left, 3D structures of heptameric PVP chains and on the right a 3D model of hydrophobic interactions and H-bond between catechin molecules and heptameric structure of PVP. The gray regions are those involved in the hydrophobic interactions and H-bonds are represented by the dot lines and the partial charges on the H and O atoms.

agglomerates. Moreover, we observe that the polymer network is a surface active complex that sits at the air water interface.

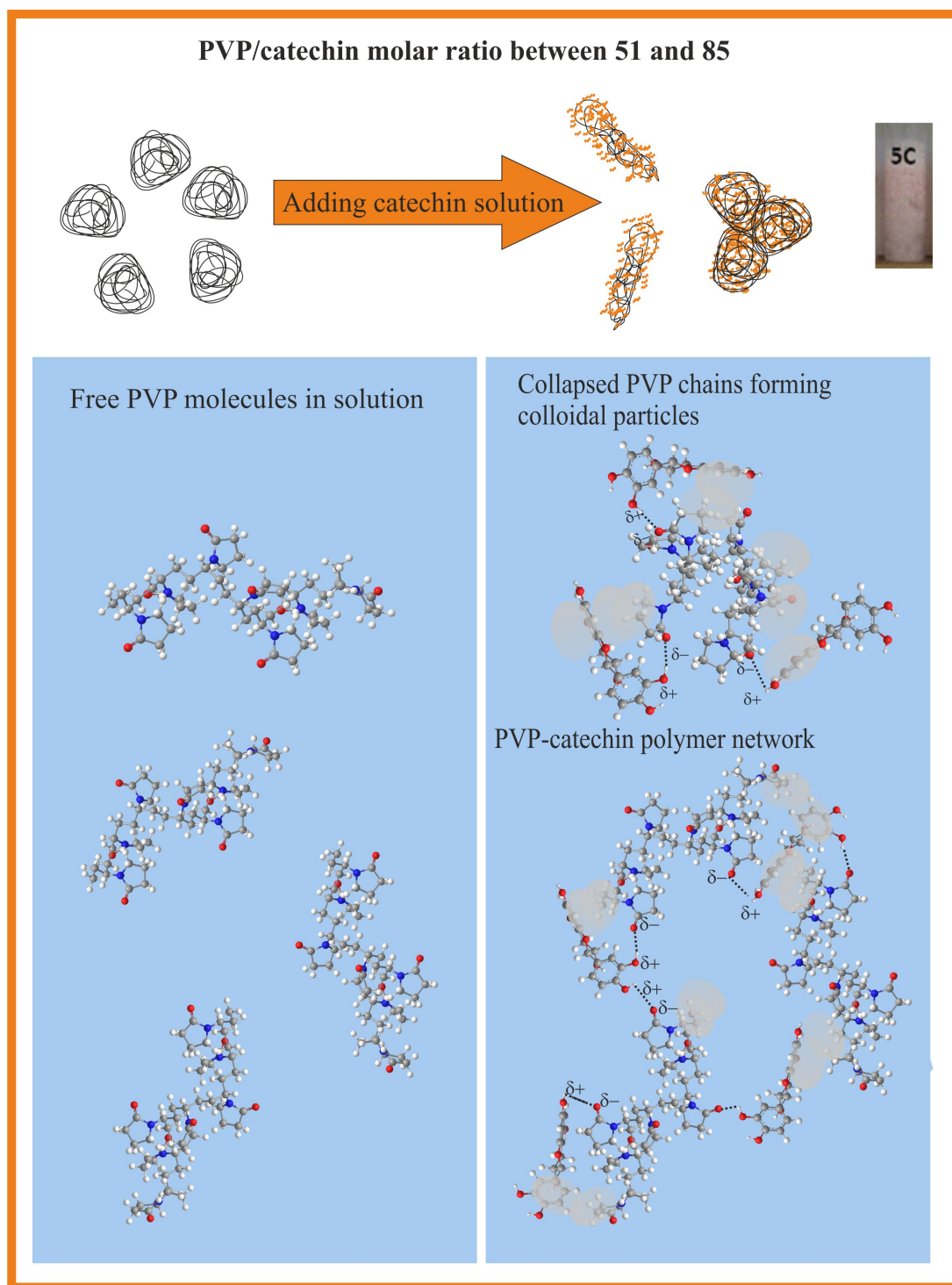


Figure 3.14: Diagram explaining the mechanism of the complex formation: top the left is schematic representation of PVP molecules in water solution, the orange arrow represent the addition of catechin solution, on the top right, the schematic representation of catechin molecules (in orange) and the PVP chains complexes. Below, on the left, 3D structures of heptameric PVP chains and on the right a 3D model of hydrophobic interactions and H-bond between catechin molecules and heptameric structure of PVP. The gray regions are those involved in the hydrophobic interactions and H-bonds are represented by the dot lines and the partial charges on the H and O atoms.

Orange area

In the orange area of the phase diagram, the molar ratio is between 51 and 85, and in addition to the polymer network, we observe turbidity in the solutions. This is due to the formation of colloidal particles via intra-molecular cross linking [21] which collapses the flexible PVP chain into un-soluble complexes. This mechanism is schematically reported in figure 3.14.

Red area

In the red area of the phase diagram, the molar ratio is above 85 and we do not have the formation of a big agglomerate as described for the yellow and orange areas but we observe the formation of many fractal particles that we are able to image with transmission light microscopy (see figure 3.6). Some of these tend to sediment over time but for molar ratio catechins/PVP above 135 no sedimentation is observed. We speculate that for these systems, the concentration of catechin is such that all the PVP chains collapse due to intra-molecular cross linking with several catechin molecules with the mechanism schematically reported in figure 3.15.

These complexes tends to aggregate giving a bimodal distribution of particle sizes from colloiddally size particles to bigger particles that, in some cases, sediment. This is the area of the phase diagram for which the synthesis of particles is possible and the best stability for these dispersions has been found to be at a molar ratio above 135, for which the dispersion presents very mildest sedimentation even after several months. As already mentioned in section 3.2, the catechins used in these experiments are a mixture of catechin, epicatechin, epigallocatechin and epigallocatechin gallate (EGCG). Since PVP does not have any chiral center, and its chain is flexible, the different catechin diastereomers interact with PVP regardless their chirality. On the other hand, EGCG tends to favour molecular bridging because it has three sites involved in hydrophobic interactions [38]. We can thus speculate that we are in the presence of at least two competitive processes: intermolecular bridging between the PVP molecules and the EGCG, which promotes the formation of the polymer network, as occurring in the yellow and orange areas of the phase diagram and the interaction of catechin, epicatechin, epigallocatechin with PVP, which favour the formation of smaller colloidal particles [105] once they reach the appropriate molar ratio (i.e. red area of the phase diagram). It is known that complexation between polyphenols and proline-rich proteins involve both hydrophobic interactions and hy-

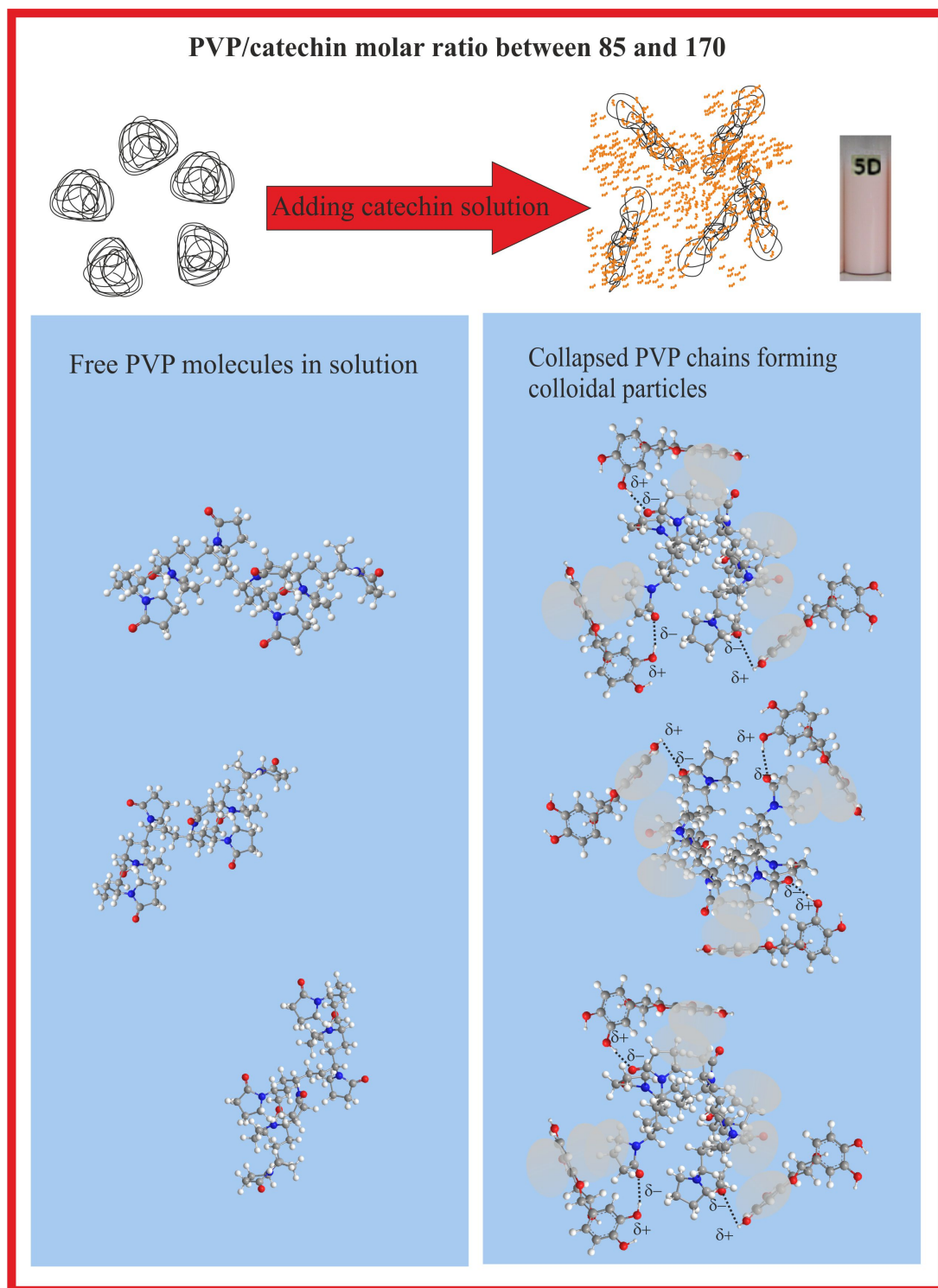


Figure 3.15: Diagram explaining the mechanism of the complex formation: top the left is schematic representation of PVP molecules in water solution, the red arrow represent the addition of catechin solution, on the top right, the schematic representation of catechin molecules (in orange) and the PVP chains complexes. Below, on the left, 3D structures of heptameric PVP chains and on the right a 3D model of hydrophobic interactions and H-bond between catechin molecules and heptameric structure of PVP. The gray regions are those involved in the hydrophobic interactions and H-bonds are represented by the dot lines and the partial charges on the H and O atoms.

drogen bonding since on one hand, hydrophobic interactions (which, as described by [4], are mainly an entropic effect), that are relatively long-range, depend on the respective solubility of both the protein and the polyphenol moiety in the solvent. On the other hand, the formation of hydrogen bonds is considered to occur mainly between the hydrogen atom of the polyphenol acidic hydroxyl groups and the peptide bond H-acceptor sites. This short range phenomenon tends to stabilize the complex formed.

When the catechin concentration is much less than the PVP concentration, we have soluble complexes but for molar ratio above 24 the polyphenol molecules have a tendency to bridge several polymer chains, resulting in a polymer network. However, many soluble PVP-catechin complex are in solution, which is consistent with UV-Vis spectra. When the catechin concentration increases (molar ratios above 51), they can bind and saturate PVP chains without bridging, resulting in smaller particles and for even higher molar ratios (above 85) the saturation is such that no polymer network is observed.

3.3.2 PVP-Tannic acid particles

Following an analogous strategy to the one used to study the PVP-catechin systems, we studied the PVP-TA system and successfully synthesised particles of PVP-TA complexes. In this experiment, we have observed the formation of soluble complexes and particles by increasing TA/PVP molar ratio but no polymer network at the air water interface has been observed. The phase diagram (figure 3.16) for the PVP-TA system shows therefore only two regions. The data of the molar ratios are shown in table 3.4.

In figure 3.17 we report the visual appearance of the systems prepared by adding increasing volume of TA solutions at 1% w/v to three PVP solutions at different initial concentration (see table 3.2). Set E is the one at higher concentration of PVP while set G is the one at lower concentration. As already observed for the PVP-catechin systems, the system at lower concentration of PVP is the one that gives particles formation for smaller volume of TA solution added.

The particles and complexes prepared have been characterized by means of optical microscopy, IR spectroscopy and dynamic light scattering. In figure 3.18 a light transmission microscopy image is shown. We observe small particles and bigger particles that have a flake

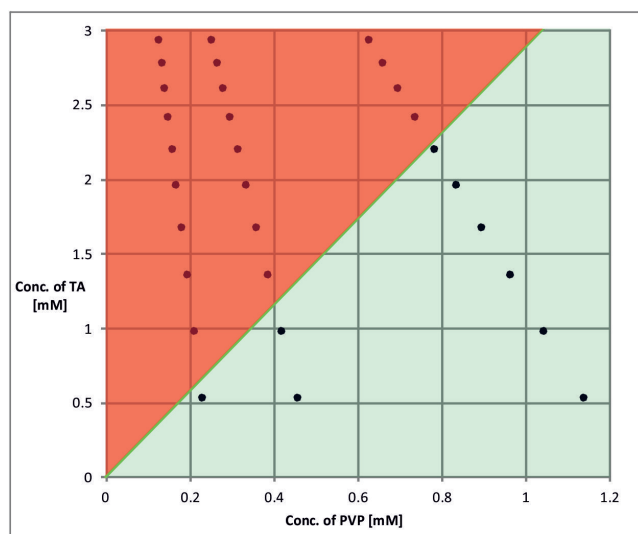


Figure 3.16: Phase diagram of PVP-TA system. The green area represents TA-PVP soluble complexes, for which the system is clear, limpid and transparent. The red area is the region in which the complexes form colloidal particles and small fractal structures.

Sample	TA [mM]	G	F	E
10	2.93	23.51	11.76	4.70
9	2.78	21.16	10.58	4.23
8	2.61	18.81	9.40	3.76
7	2.42	16.46	8.23	3.29
6	2.20	14.11	7.05	2.82
5	1.96	11.76	5.88	2.35
4	1.68	9.40	4.70	1.88
3	1.36	7.05	3.53	1.41
2	0.98	4.70	2.35	0.94
1	0.53	2.35	1.18	0.47

Table 3.4: Molar ratio catechins/PVP. The different colours represent different behaviour of the systems. Green area limpid, transparent system; yellow area formation of jelly macro structure; red area formation of particles.

like structure.

The IR spectra of dry soluble complexes and particles are reported in figure 3.19. The black line is the spectrum of a dried solution of soluble complexes (3E), and the red line is the spectrum of some dried particles from vial 9E. The broad peak at 3250 cm^{-1} is due to the absorption by the stretching mode of the phenolic -O-H bound and it is broader than expected for a phenolic bond, because of the hydrogen bond between the -OH of the catechins and the -C=O of the pyrrolidone. The spectrum of sample 3E, which is the one with only soluble complexes, presents a peak at 3000 cm^{-1} which is a typical position of stretching vibrations of hydrogen bonds [102].

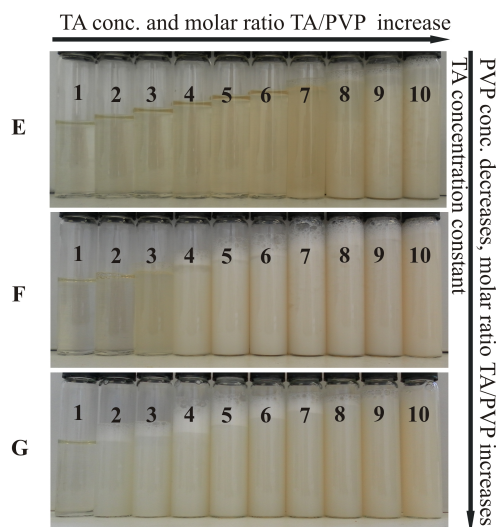
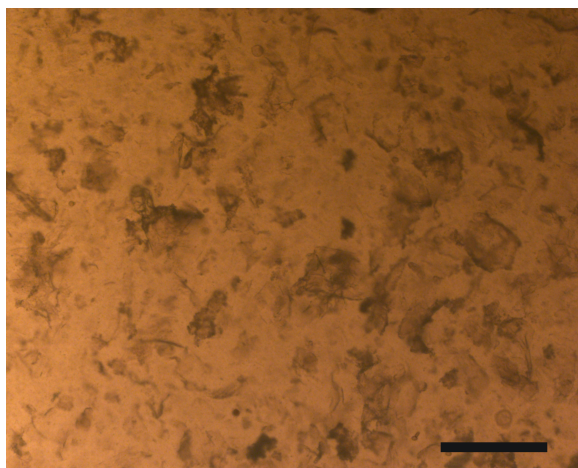


Figure 3.17: Overview of the three sets of experiments to synthesize colloidal particles with PVP-TA complexes. The concentration of TA increases in each set from left to right and the concentration of PVP decreases from the top to the bottom as shown in table 3.2.



(a) TA particles

Figure 3.18: Microscopy image of PVP-TA complexes prepared by direct precipitation. Obj 40X, scale bar 10 μm .

The peak at 1650 cm^{-1} is correlated to the stretching of the -C=O of the pyrrolidone. This peak is less intense for sample 9E which is due to the fact that the double bond is delocalised to form the H bond with the O-H of the polyphenols. The peaks below 1500 cm^{-1} are the "finger print" of the molecules and are due to the vibrational mode of the molecule in its ensemble.

DLS measurements show a decrease in the particle sizes when increasing the PVP concentration at constant TA concentration. In figure 3.20 we report the particle size distribution for samples 6E, 6F and 6G. The average sizes calculated with the cumulant fit method are respec-

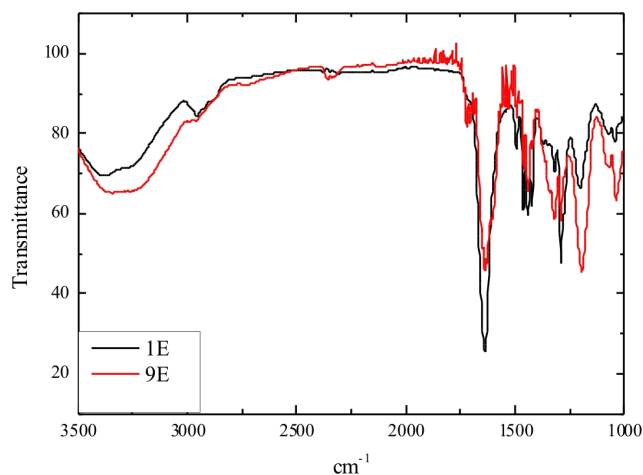
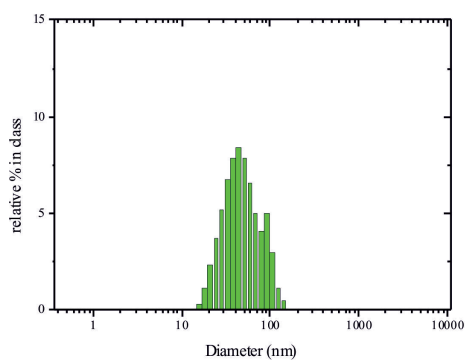
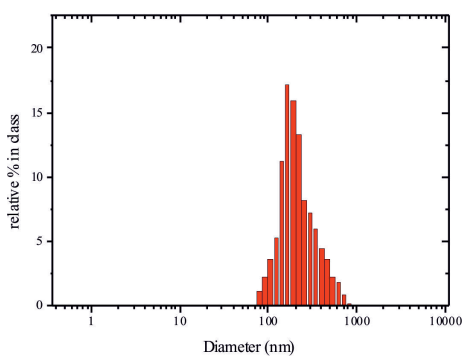


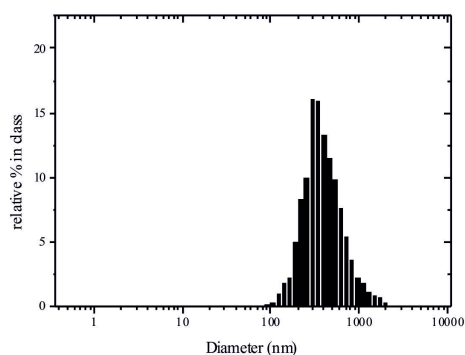
Figure 3.19: FTIR spectra of PVP-TA complexes 3E molar ratio 2.3; 9E molar ratio 21.



(a) DLS-6E



(b) DLS-6F



(c) DLS-6G

Figure 3.20: Volume average particle size distribution of samples 6E, 6F, 6G.

tively 40 nm in 6E, 111 nm in 6F and 135 nm for 6G. Despite TA being a polydentate ligand, we have not observed the formation of any extended polymer network at the air water interface

as in the case of catechins in the range of concentration investigated, consequently, only one critical molar ratio has been identified for the PVP-TA system and the relative line has been plotted on the PVP-TA phase diagram reported in figure 3.11.

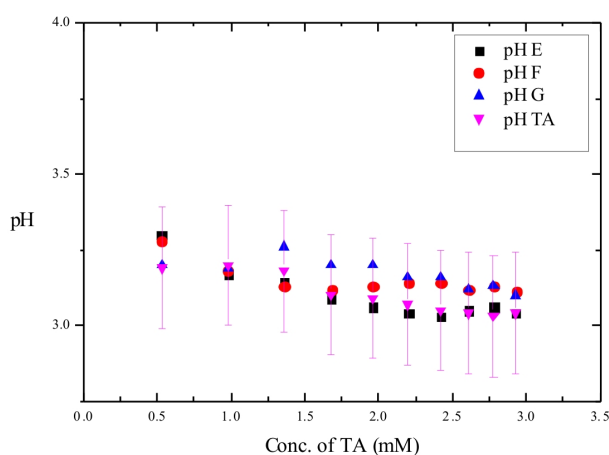


Figure 3.21: Plot of pH measurements for each set of PVP-TA systems and TA control set solution in deionised water.

The pH measurements show that the PVP-TA system is self-buffered as well by the TA and no direct link between phase behaviour and pH has been observed. The average pH of the systems is $\text{pH}=3.1\pm0.1$ and the measurements are plotted in figure 3.21.

Over time the particles in systems at molar ratio between 3.29 to 4.23 (3F and 7,8,9E) sediment and compact in a jelly agglomerate at the bottom of the vials. For system 3F (molar ratio 3.53) and 7E (molar ratio 3.29) some of the particles grow on the vials wall as showed in figure 3.22. This is an indication of a surface active process that leads to the formation of a polymer network as seen for the catechin system but in this case the process takes weeks instead of occurring almost instantaneously.

For vials at molar ratio 4.70 the colloidal dispersions is stable and do not present any instability due to sedimentation or aggregation but for molar ratios above 5.88 we have observed some sedimentation but no formation of jelly agglomerate.

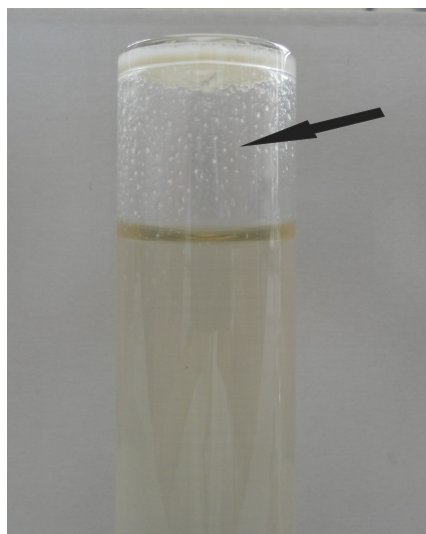


Figure 3.22: Gelled aggregated sediment and sticky gelled particles on glass vial. The vial has been inverted to make easier the observation on the gelled sticky sediment.

3.4 Conclusions

In this chapter we have shown that it is possible to synthesise colloidal particles from PVP-catechins and PVP-TA complexes. We have investigated a range of concentrations and molar ratios for both systems in order to achieve the best colloidal dispersion in term of particle size distribution and stability against sedimentation. For the PVP-catechin systems the most suitable conditions are at molar ratios above 135 and at PVP concentrations of 0.14 mM, which has been experimentally demonstrated for the last three experimental points in set D. The formation of the complexes is due to two types of interaction: hydrophobic interaction between the phenolic and pyrrolidinone rings and H bonds between the hydroxyl functions and the CO-N linkages. For molar ratio below 23 we have observed the formation of soluble complexes. Above that point the catechin molecules adopt conformations that are more favourable for stacking and intermolecular bridging therefore we have observed the formation of polymer network which appear to be surface active. When the molar ratio increases above 51, catechin molecules can bind and saturate PVP chains without bridging, resulting in smaller particles coexisting with a polymer network. When the molar ratio increases further above 85 catechin molecules bind and saturate PVP chains without bridging, resulting in smaller and much less polydisperse objects. The system scattering intensity increases proportionally with the molar ratio and so its stability against sedimentation.

For the PVP-TA system the best colloidal dispersion in term of stability against sedimentation is between molar ratio 3.29 and 4.70, PVP concentration 0.21 mM, experimental points 7-10E, 3-4F and 2G. This system did not shown the complexity and richness in behaviour that we have observed for the PVP-catechin system. Nevertheless, over time for intermediate molar ratios the particles sediment and compact into a jelly agglomerate at the bottom of the vials and some systems shown some of the particles growing on the vials wall. This is an indication of a surface active process that leads to the formation of a polymer network as seen for the catechin system but in this case the process takes weeks instead of occurring almost instantaneously.

In both cases we found that for more concentrated PVP solutions, a higher amount of polyphenols is necessary to make insoluble complexes suitable for particle formation. This is due to the fact that in both cases a critical molar ratio has to be reached to have a sufficient number of polyphenols molecules to cover of the PVP molecules and form colloidal size particles. Only in the case of the PVP-catechin systems we observed the formation of a polymer network before observing turbidity in the sample. This more complex behaviour probably stems from the fact that we have used green tea extract which is a mixture of catechin, epicatechin and epigallocatechin gallate, therefore we envisage some sort of competitive reaction among these different polyphenols molecules and the intermolecular complexation.

3.5 Acknowledgements

This work is supported by BBSRC (Biotechnology and Biological Sciences Research Council) industrial case studentship and Unilever. I would like to thank Dr Simon Fairclough (University of Oxford, ICL) for providing the facility for the infra red measurements.

Chapter 4

Surfactant free emulsions stabilised by non-covalently associated microgels

4.1 Introduction

Emulsions are ubiquitous in everyday life. Their importance and applications span from personal care products to food stuff and from building materials to agricultural products [43]. Typically, emulsions are stabilised by the addition of surface active molecules or particles, which lower the interfacial tension of the two immiscible phases and provide steric or electrosteric stabilization [42]. Emulsions stabilized by particles are known as Pickering emulsions [7, 51]. These emulsions are usually stabilized by inorganic particles, most commonly silica particles, which may or may not be functionalized. In this kind of emulsions, the oil globules are prevented from coalescing by being enveloped in a thin film consisting of particles which are smaller than the globules themselves [44]. In recent years more complex particles like Janus particles [48, 100] and “colloidal surfactants” [106] have been designed to stabilize emulsions. The need for very stable emulsions has led to the development of various encapsulation processes that lead to formation of a complete shell around the oil droplet (i.e. core-shell morphology [52]). One of the widely explored approaches for encapsulation of oil droplets is using polymeric shells [107] or complex coarcervation [108, 109], but recently new approaches using H-bond driven interactions between non-charged molecules have been reported [101, 110].

Here we will show that it is possible to stabilize emulsions solely by using PVP-TA micro-gel particles that form due to the strong non-covalent interaction (H-bond driven and hydrophobic interactions) between the tannic acid (TA) and polyvinylpyrrolidone (PVP). As already explained in the previous chapter, these interactions are hydrogen bonding of the hydroxyl moieties of TA with the amide groups of the PVP and hydrophobic interactions between phenolic moieties of TA and pyrrolidone rings of PVP [12]. In Chapter 3 we have already discussed in detail the PVP-TA complex and particle formation and the role of the molar ratio in a homogeneous system, the water phase. In this chapter we will investigate how the emulsification conditions affect the particle formation (section 4.3.1), the effect of varying the oil volume fraction on the emulsion stability (section 4.3.2) and, we will discuss how the $[TA]/[PVP]$ molar ratio affects the emulsion stabilization mechanism that can be an encapsulation of the oil droplet in a proper polymeric shell or Pickering stabilization (section 4.3.3). Finally we will draw some considerations on the effect of the pH on the emulsion stability (section 4.3.4).

4.2 Materials and Methods

PVP40 (molecular weight 40000 u), soy-bean oil and TA were purchased from Sigma-Aldrich. No further purification was needed. Deionised water was used for all the experiments. Solutions of TA and PVP were prepared in deionised water. Emulsions were prepared by dispersing soy-bean oil in a solution of TA at a specific concentration (see tables 4.1, 4.2, 4.3) using dispersing tool T 25 digital ULTRA-TURRAX by IKA at 19,000 rpm for two minutes. This high speed was chosen to make a narrow size distribution of oil droplets [111]. The design of the dispersing tool is such to avoid air incorporation in the emulsion. To this dispersion a solution of PVP was immediately added while stirring with magnetic stirrer to homogenize the system. The use of the dispersing tool in this second stage was avoided in order not to grind the forming micro-gel structures. Using this method we have prepared different emulsions and ran two main sets of experiments:

1. Variation of the oil volume fraction at constant concentration of TA and PVP in the total volume of the emulsion, see table 4.1,
2. Variation of the molar ratio, $[TA]/[PVP]$, at constant oil volume fraction. For this experiment we will discuss two sets of experiments run at the same oil volume fraction and molar ratios but at a different overall concentration of TA and PVP as given in table 4.2 and table 4.3;

Sample	Oil ϕ	TA % w/v	PVP % w/v	TA [mM]	PVP [mM]	TA/PVP <i>molarratio</i>
TA002	0.05	0.14	0.2	0.87	0.05	12
TA003	0.1	0.14	0.2	0.91	0.05	12
TA004	0.2	0.14	0.2	1.02	0.06	12
TA005	0.3	0.14	0.2	1.18	0.07	12

Table 4.1: Experiment varying the oil volume fraction at constant concentration of TA and PVP in the total volume of the emulsion.

In the following paragraphs we will describe the techniques and methods we have used to characterise our emulsions.

Sample	Oil ϕ	TA % w/v	PVP % w/v	TA [mM]	PVP [mM]	TA/PVP <i>molar ratio</i>
TA006	0.33	0.50	1.00	2.91	0.25	12
TA007	0.33	1.00	1.00	5.91	0.25	24
TA008	0.33	1.50	1.00	8.82	0.25	35
TA009	0.33	2.00	1.00	11.81	0.25	47

Table 4.2: Concentrations and molar ratio of experiments varying the molar ratio between PVP and TA at constant oil volume fraction $\phi=0.33$.

Sample	Oil ϕ	TA % w/v	PVP % w/v	TA [mM]	PVP [mM]	TA/PVP <i>molar ratio</i>
TA010	0.33	0.63	1.25	3.67	0.31	12
TA011	0.33	1.25	1.25	7.32	0.31	24
TA012	0.33	1.88	1.25	11.02	0.31	35

Table 4.3: Concentrations and molar ratio of experiments varying the molar ratio between PVP and TA at constant oil volume fraction $\phi=0.33$. In this set the overall concentration of TA and PVP is 25% higher than in set reported in table 4.2.

4.2.1 Emulsion stability and Creaming index

The stability of the emulsions has been evaluated by direct visual observation and the calculation of the creaming index. To perform these tests we used glass vials 20 mm diameter and 60 mm high sealed with a screwed cup to avoid evaporation from the emulsions. The total emulsion height (H_E) and serum layer height (H_L lower layer) were measured. The creaming index (CI(%)) was calculated as reported by Sun [112] as:

$$CI(\%) = 100 \frac{H_L}{H_E} \quad (4.1)$$

A detailed description of emulsion instability and creaming index calculation was given in chapter 1 section 1.6.1.

4.2.2 Light transmission and confocal microscopy imaging

For the microscopy imaging of the emulsions we have used a Zeiss Exciter LSM 5 Confocal Laser Scanning Microscope (CLSM) which is able to perform both confocal and light transmission microscopy imaging. Details on this instrument and techniques were given in chapter 2.

The microscope was mounted on a rolling table to allow observation in the vertical and horizontal planes. The observation in the vertical plane is particular interesting to monitor creaming in the emulsions. To work in fluorescence mode we used different fluorochrome dyes, Nile red, fluorescein and Rhodamine-B. Nile red is a lipophilic stain (IUPAC name 9-diethylamino-5-benzo[α]phenoxazinone) ideal for dyeing the oil phase of emulsions. It is widely used for the detection of intracellular lipid droplets by fluorescence microscopy and flow cytometry [113]. Nile red can work for yellow fluorescence (excitation, 450-500 nm; emission, 528 nm) and red fluorescence (excitation, 515-530 nm; emission, 525-605 nm). Fluorescein (IUPAC name 3',6'-dihydroxyspiro[2-benzofuran-3,9'-xanthene]-1-one) is one of the most common fluorescent reagents for biological research [41]. It has high absorptivity, excellent fluorescence and its excitation wavelength is 488-494 nm with an emission maximum of 512-518 nm. [114]. It is commonly used in diagnostic (ophthalmology and optometry tests) and for biological labels [24]. Rhodamine-B (IUPAC name [9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride) is a water-soluble dye. It has an excitation wavelength of 540 nm and emission wavelength 625nm. It is commonly used in fluorescence microscopy for colloidal science [115–117] and in biotechnology applications [118].

4.2.3 Light Scattering SLS

The droplet size distribution of the emulsions was measured with a Malvern Mastersize2000 particle size analyser. This instrument measures the intensity of the light scattered from a laser beam passing through the sample. The sample is loaded in the automated sample dispersion units that ensure that the sample is delivered to the measurement area at the correct concentration and in a suitable, stable state of dispersion. The particle size distribution is calculated from the pattern of the scattered light by the automated software.

4.3 Result and discussion

4.3.1 Emulsification process and particle formation

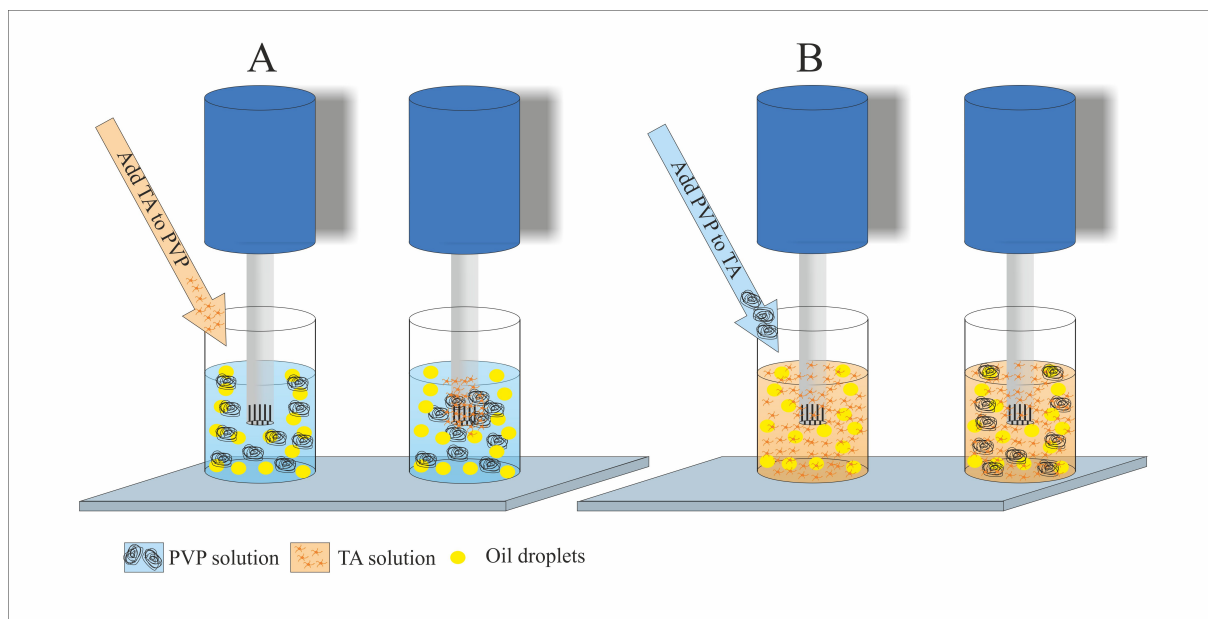


Figure 4.1: Schematic of PVP-TA complex formation during emulsification process: (A) adding TA solution to the oil dispersion in PVP solution give the formation of a big sticky agglomerated on the dispersing tool; (B) adding PVP solution to the oil dispersion in TA solution give the formation of complexes of appropriate sizes to perform interface stabilization of the droplets.

In chapter 3 we have followed the protocol of adding a solution of TA to a solution of PVP at different concentrations. When this was done in a three component system, adding a solution of TA to the dispersion of oil in a PVP solution, we observed the formation of big agglomerates of PVP-TA complexes that stuck to the dispersing tool. This was unexpected and inconvenient since these agglomerates were not suitable for emulsion stabilization because they were too big compared to the oil droplets and therefore they were unable to stabilize the interface. Their sudden formation was in contrast with what we have observed in chapter 3, where a gelled aggregate sediment, see figure 3.22, was observed only at a much longer time scale than the emulsification process. Once the method of preparation has been changed by adding a PVP solution to an oil dispersion in TA solution, we did not observe the formation of any big agglomerate stuck on the dispersing tool. We speculate that the sudden formation of this big sticky agglomerate when we add TA to the oil dispersion in PVP solution is due to the chemical property of TA and the material of the dispersing tool. As already mentioned in

previous chapters, TA is a polydentate ligand, and therefore has the ability to bond to metal. Metals have catalytic properties. The dispersing tool is made of stainless steel and any TA molecule added is driven towards it. TA molecules hence bind to the metal and to other PVP molecules cross-linking numerous PVP molecules onto the dispersing tool and therefore forms an insoluble network that stick onto it as schematically shown in figure 4.1 (A), i. e. this fast reaction can be thought of as being catalysed by the dispersing tool.

By changing the method of preparation by adding PVP solution to the oil dispersion in TA solution, the PVP molecules are complexed by TA at a very high molar ratio ($[TA]/[PVP]$) which, as already discussed, prevents multiple cross linking and the consequent formation of a big agglomerate onto the stainless steel dispersing tool. Under these conditions particles able to sit at the oil droplet interface are formed. In figure 4.1 (B) we show this mechanism. We further optimized the emulsification procedure, as already mentioned in the “materials and method section”, by using a Teflon coated magnetic stirrer to homogenize the system in order to avoid grinding the forming microgel structures. For some molar ratios this allowed the formation of shells of complexes around the droplets as we will discuss in sections 4.3.3.

4.3.2 Effect of varying the oil volume fraction on emulsion stability

Emulsion of different oil volume fractions were prepared keeping the concentration of TA and PVP constant in the total volume of the emulsions. We prepared the emulsions as explained in the “Materials and method” section. Emulsions at ϕ_{oil} 0.05, 0.10, 0.20, 0.30 were prepared. We noticed that increasing the oil volume fraction from 0.05 to $\phi=0.30$ gave a fairly good result with respect to sedimentation of the PVP-TA particles and creaming of the emulsion. As the oil volume fraction increases the emulsions are better stabilized against creaming as shown in figure 4.2. In the following sections we will discuss how the variation of the ϕ_{oil} affects the stability, the creaming index and the microscopic morphology

Emulsion stability and creaming index

The creaming index was evaluated by a long-term storage test in refrigerator at 4°C. We followed the moving boundary between the serum and the cream for 11 days and we did a final reading after 3 months from the preparation of the emulsions. The vials used for the creaming stability

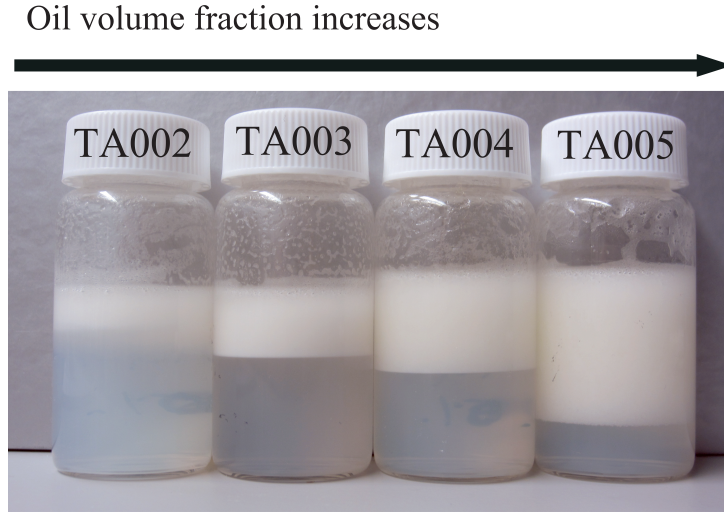


Figure 4.2: Emulsions prepared at constant concentration of TA and PVP in the total volume of the emulsion. The oil volume fraction (ϕ_{oil}) varies from 0.05, 0.10, 0.20, 0.30 left to right.

ϕ	H_E mm	H_{Lt_0} mm	H_{Lt_f} mm	$CI(\%)_{t_0}$	$CI(\%)_{t_f}$
0.05	40	28	37	70	92.5
0.10	40	20	35	50	87.5
0.20	40	15	31	37.5	77.5
0.30	40	7	25	17.5	62.5

Table 4.4: Creaming index of emulsions measured after 24h from preparation, t_0 , and after 6 months from preparation, t_f . H_E is the total height of the emulsion in the container, H_{Lt_0} and H_{Lt_f} are the heights of the serum layer at t_0 and t_f .

test are shown in figure 4.2. In the picture the oil volume fraction (ϕ_{oil}) increases from 0.05 to 0.10, 0.20, 0.30 left to right. To calculate the creaming index we have used the formula given in chapter 1 section 1.6.1 and the results are reported in table 4.4 and plotted in figure 4.3. All emulsions separated into a top cream layer and a serum layer at the bottom. After less than 5 days all emulsions reached a gelling point that arrested the creaming process. The upper layer turned into a gelled thick cream and the oil droplets were prevented from moving any further. Emulsion ϕ_{oil} 0.05 was the least stable against creaming with $CI(\%)=90$ after 3 months. It also showed moderate coalescence. Emulsion at ϕ_{oil} 0.30 was the best in term of stability and no coalescence was observed at any stage. This emulsion was also better in term of creaming but after 5 days its $CI(\%)$ had increased to 62.5 which is relatively high.

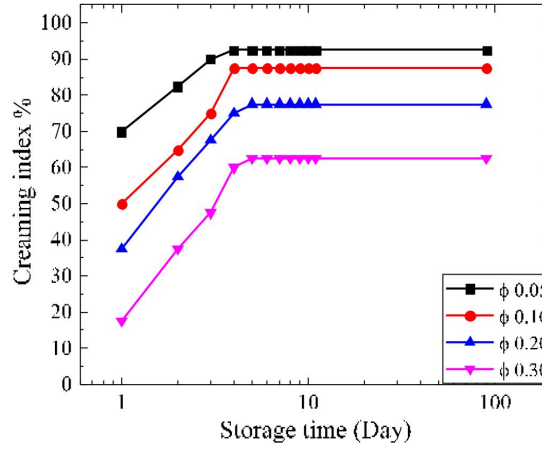


Figure 4.3: Plot of creaming index for long storage test of emulsion at ϕ_{oil} 0.05, 0.10, 0.20, 0.30.

Microscopy observations

As already mentioned, emulsions at low oil volume fraction showed moderate coalescence. In figure 4.4, D, F, G we show the top layer of the cream for emulsions at ϕ_{oil} 0.05. The majority of the droplets in this layer show coalescence. Through time the cream ages and gels and coalescence slows down but does not stop. In figure 4.4 A, B, C, stable droplets in the bulk of the cream are shown.

In figure 4.5 a selection of images from emulsions at ϕ_{oil} 0.10 is shown. In figure 4.5 A we can see the upper layer of the cream where some of the droplets are deformed. This indicates some form of coalescence but no coalescence was observed on the macroscopic scale.

Emulsions at higher oil volume fraction did not show any coalescence. Light transmission microscopy images of emulsions at oil volume fraction ϕ_{oil} 0.20 and, 0.30 are reported in figures 4.6, 4.7.

As previously mentioned, emulsions at higher oil volume fraction were relatively more stable against creaming than those at lower oil volume fraction, although they had the same overall concentration of TA-PVP complexes. The better stability of these emulsions can be explained by the fact that the lower water fraction leads to a higher concentration of TA-PVP complexes for emulsions at higher oil volume fractions. In other words, if the concentration of TA and PVP is constant in the total volume of the emulsion, the continuous phase is richer in

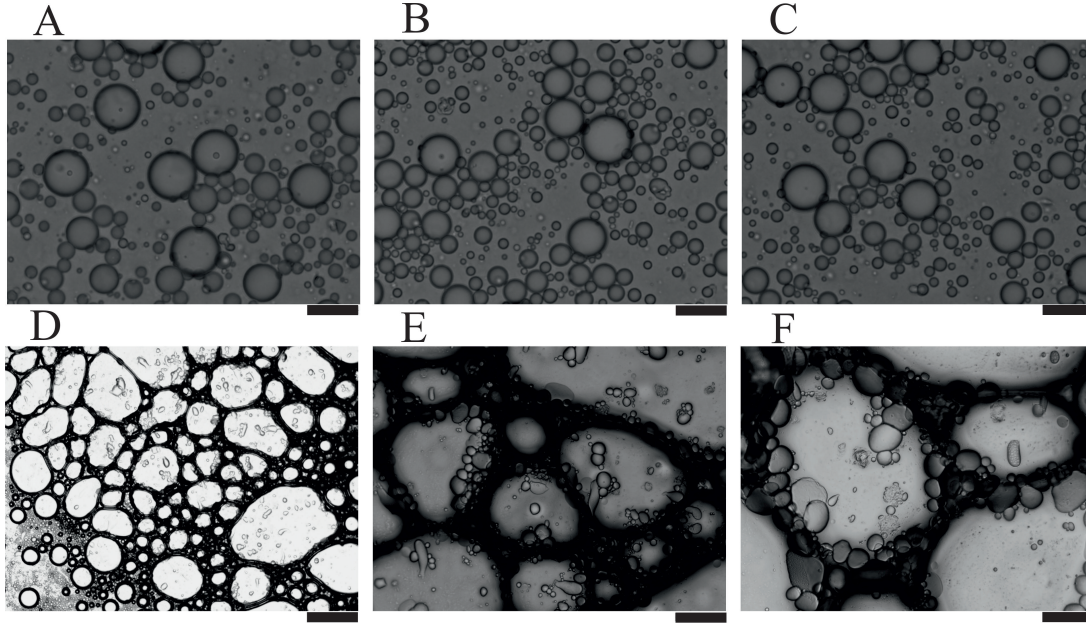


Figure 4.4: Light microscopy images of emulsion at oil volume fraction $\phi = 0.05$. Scale bars A, B, C = 20 μm , D = 400 μm ; E = 200 μm ; F = 100 μm ; . Picture A, B, C show the emulsion phase. Pictures D, E, F show coalescence that occurs in samples at this oil volume fraction after preparation that occurs in the upper layer of the emulsion.

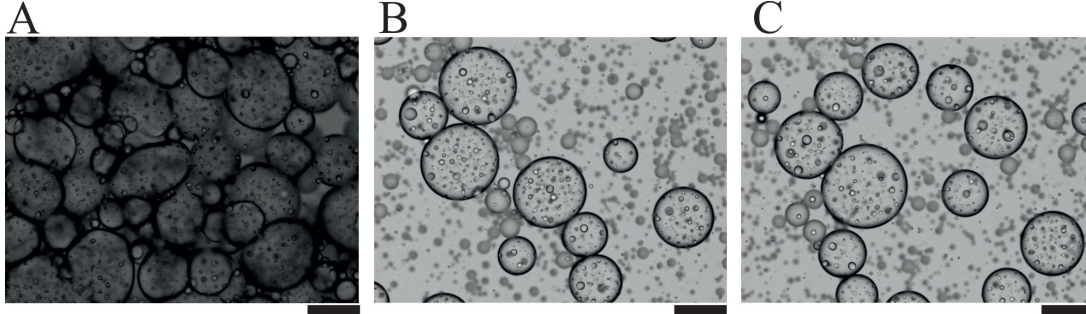


Figure 4.5: Light microscopy images of emulsion at oil volume fraction $\phi = 0.10$. Scale bars A = 20 μm ; B = 20 μm ; C = 20 μm .

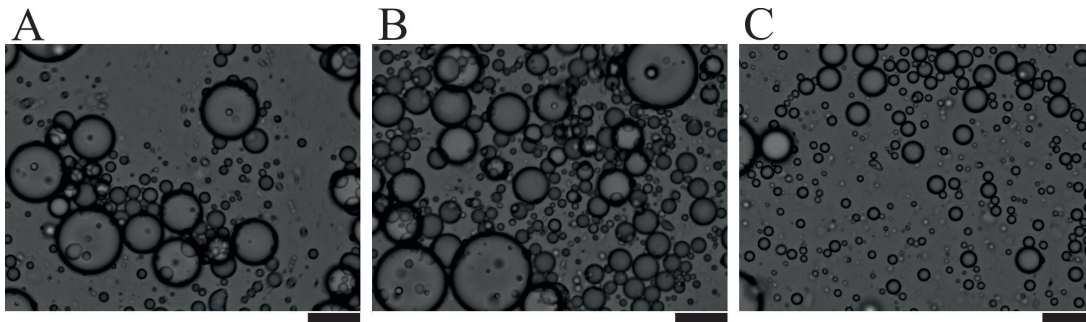


Figure 4.6: Light microscopy images of emulsion at oil volume fraction $\phi = 0.20$. Scale bars A = 20 μm ; B = 20 μm ; C = 20 μm .

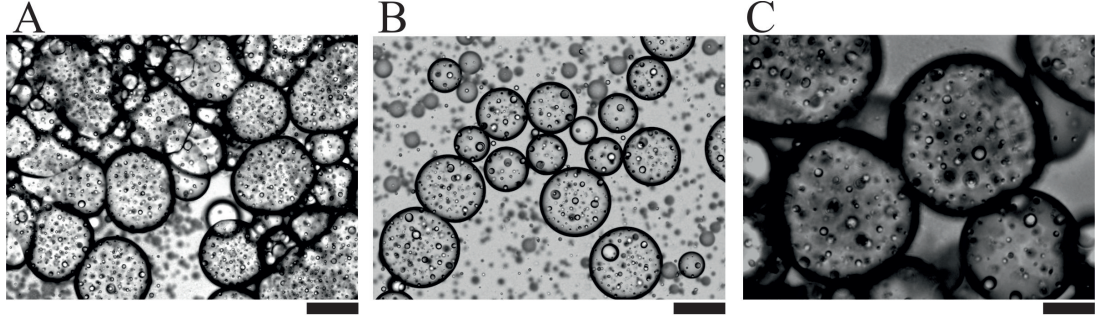
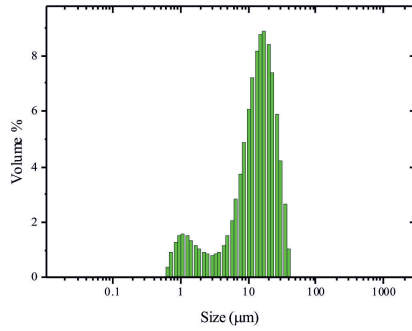


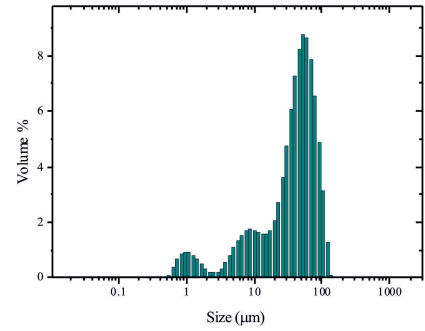
Figure 4.7: Light microscopy images of emulsion at oil volume fraction $\phi = 0.30$. Scale bars A= 20 μm ; B= 50 μm ; C= 50 μm .

complexes for higher oil volume fractions and a better stability against coalescence is therefore achieved.

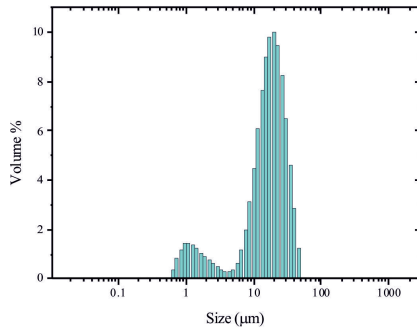
Light scattering results



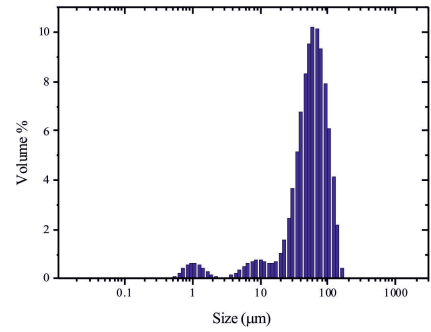
(a) Volume average particles size distribution for emulsion at oil volume fraction $\phi = 0.05$



(b) Volume average particles size distribution for emulsion at oil volume fraction $\phi = 0.10$



(c) Volume average particles size distribution for emulsion at oil volume fraction $\phi = 0.20$



(d) Volume average particles size distribution for emulsion at oil volume fraction $\phi = 0.30$

Static light scattering (SLS) measurements have been performed using the Malvern Master-size2000 particle size analyser. The volume average particle size distributions for these emulsions

are shown in figure 4.3.2. All emulsions are poly-disperse, the volume average particle size distributions show either 2 or 3 peaks. The first peak is centred at $1/1.5\mu\text{m}$ and is determined by the TA-PVP particles, and the second and third are determined by the droplets. The volume average droplet size distribution for some samples shows a bimodal distribution of the droplets because the velocity of the dispersing tool during the preparation of such emulsions was increased throughout the process. These data are important for understanding the light transmission microscopy images reported in figure 4.5 and 4.7. From only the observation of the image it is not possible to understand if the smaller droplets are inside or outside the bigger droplets. The SLS data confirm that the smaller droplets are not inside the bigger ones.

4.3.3 Emulsions behaviour at different the molar ratios

Variation of the molar ratio $[\text{TA}]/[\text{PVP}]$ keeping the oil volume fraction and the PVP concentration constant at $\phi_{oil}=0.33$, was done by adding PVP solution to oil dispersions in TA solutions at different initial concentrations of TA, see table 4.2. We first prepared a set of four emulsions staining the soy-bean oil with Nile red dye before emulsification. A second set was prepared with the same procedure but unstained. Samples taken from this second set were later stained with either fluorescein or Rodamine-B. The overall concentration of TA and PVP was increased by 25%. Since no difference between the emulsions prepared at molar ratio $[\text{TA}]/[\text{PVP}]$ 35 and 47 was observed, as we will discuss in the following section, we chose to repeat only the first 3 point of the first set. The overall concentration of TA and PVP was increased with the aim of improving emulsion stability. The choice of dyes will be explained in the second subsection.

Observation on stability and creaming index

All the emulsions prepared are stable against coalescence. This was verified by a long-term storage test at 4°C for 3 months. A certain level of creaming was observed but all the emulsions reached an equilibrium between the cream phase and the lower water phase (serum) a couple of hours after preparation. After that no further evolution in the equilibrium emulsion/serum was observed for any emulsions because the cream gelled. Analogous behaviour was observed and discussed for the emulsions prepared by varying the oil volume fraction. This phenomenon was faster in this case because the concentration of the PVP-TA complexes is higher. The creaming

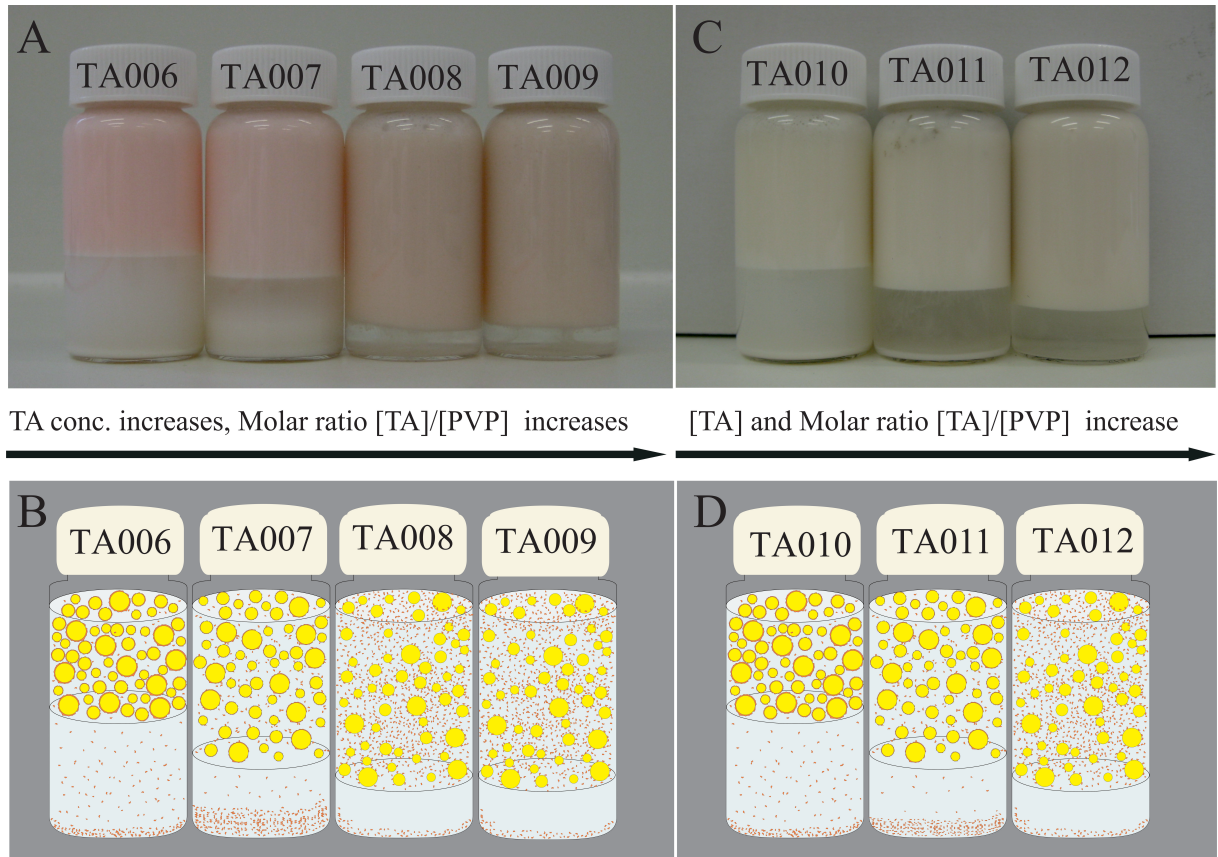


Figure 4.8: (A) Emulsions prepared at different $[TA]/[PVP]$ molar ratio stained with Nile red dye. The molar ratio increase from left to right, the oil fraction is constant at $\phi_{oil} = 0.33$. Molar ratio for each sample are: TA006=12, TA007=23, TA008=35, TA009=47. (B) Schematic of the emulsions prepared at different $[TA]/[PVP]$ molar ratio: in yellow the oil droplets and in orange the PVP-TA complexes. (C) Emulsions prepared at different $[TA]/[PVP]$ molar ratio unstained. The molar ratio increase from left to right, the oil fraction is constant at $\phi_{oil} = 0.33$. Molar ratio TA010=12; TA011=23; TA012=35; (D) Schematic of the emulsions prepared at different $[TA]/[PVP]$ molar ratio: in yellow the oil droplets and in orange the PVP-TA complexes.

index data are reported in table 4.5.

sample	molar ratio [TA]/[PVP]	H_E mm	H_L mm	CI(%)
TA006	12	50	20	40
TA007	23	50	15	30
TA008	35	50	5	10
TA009	47	50	5	10
TA010	12	50	20	40
TA011	23	50	15	30
TA012	35	50	5	10

Table 4.5: Creaming index measured after 2h from preparation, H_E is the total height of the emulsion in the container, H_L is the height of the serum layer.

The variation of the molar ratio $[TA]/[PVP]$ affects the level of creaming and appearance of the emulsions. As the molar ratio $[TA]/[PVP]$ increases the creaming index decreases. Emulsions at molar ratio $[TA]/[PVP]$ 35 and 47 have the same and best creaming index. The creaming is therefore inversely proportional to the $[TA]/[PVP]$ molar ratio up to 35 and then become constant. No difference in the creaming index was measured between the “Nile red dyed set” and the “unstained set”. Using the confocal microscope in the configuration that allows observation in the vertical plane, we examined the creaming process at microscopic level. The experiments were carried out in a home-made cell constructed from the bottom of a glass vial which had been cut in half across its width, filled with Rhodamin-B stained emulsion and glued onto a coverslip. This system is airtight and the emulsions were therefore prevented from dry off during the observation. In figures 4.9 A we show an example of the home made cell from the coverslip side and in B a diagram showing how we moved the confocal stage to collect images from different areas of the sample.

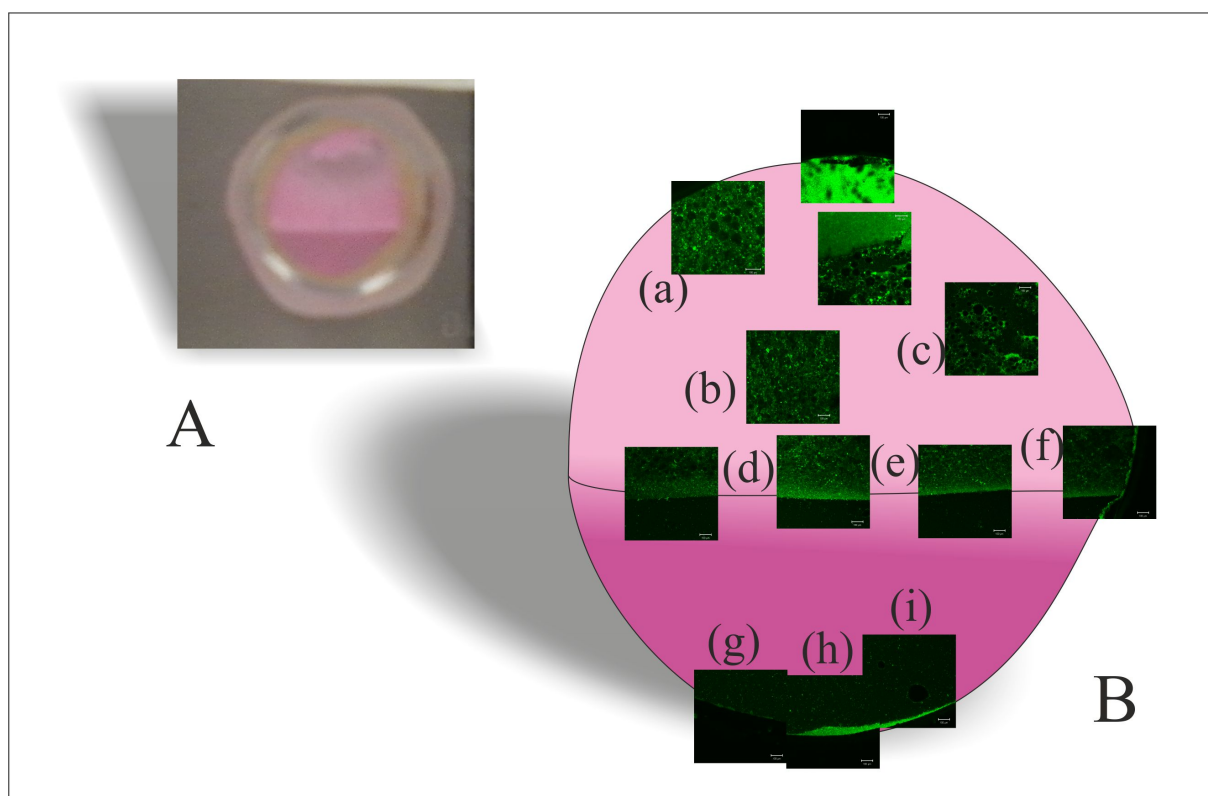


Figure 4.9: A: Home made cell loaded with emulsion stained with Rhodamine-B. B: Diagram representing the optical cell and confocal images taken in different areas of the sample.

In figure 4.10 a selection of confocal pictures taken in different areas of the sample is shown. In figures 4.10 (a), (b) and (c) the cream phase rich in oil droplets is shown. In figures 4.10 (d),

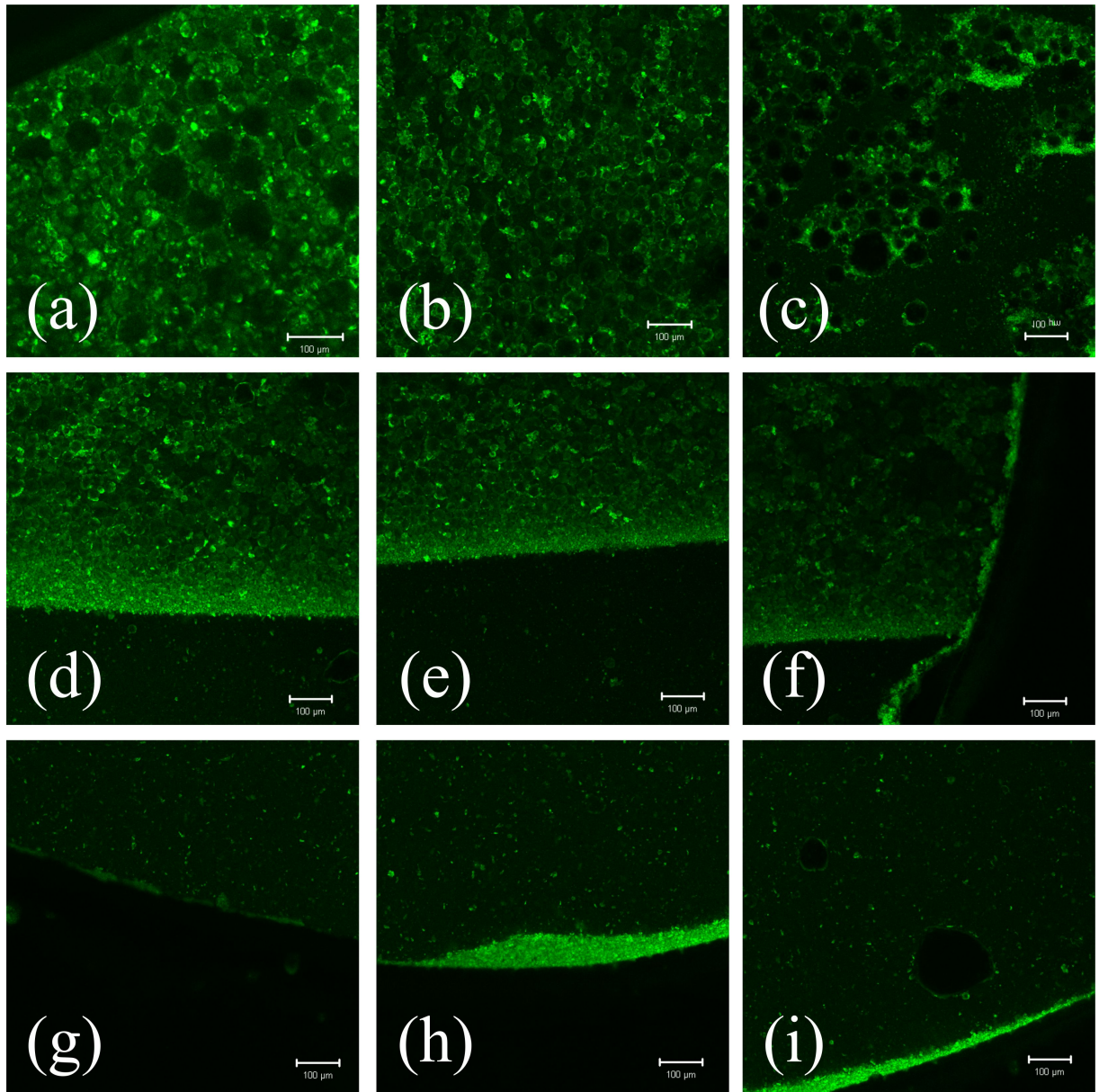


Figure 4.10: Observation of one emulsion in the vertical plane, scale bar 100 μ m

(e) and (f) the interface between the serum and the cream phase is clearly visible. We notice a concentration gradient for the PVP-TA particles decreasing from the interface moving up into the cream phase. In figures 4.10 (g), (h) and (i), the serum phase and some particle precipitate are visible. In Pickering emulsions, the precipitate consists of any insoluble substance which is wetted more easily by water than by oil [44, 48]. In our case this is composed of PVP-TA particles which are more wettable by the water phase. In figure 4.8 B and D we show a schematic representation of how the emulsions separate into cream rich in oil droplets and serum which is a dispersion of PVP-TA complexes. More information on the oil droplet shapes, coating and

stabilization mechanisms are given in the next section.

Light transmission and confocal microscopy observations

Information on the micro-structure of the emulsions have been gathered by confocal and light transmission microscopy imaging. In figure 4.11 we show an example of the emulsions prepared with the soy-bean oil dyed with Nile red.

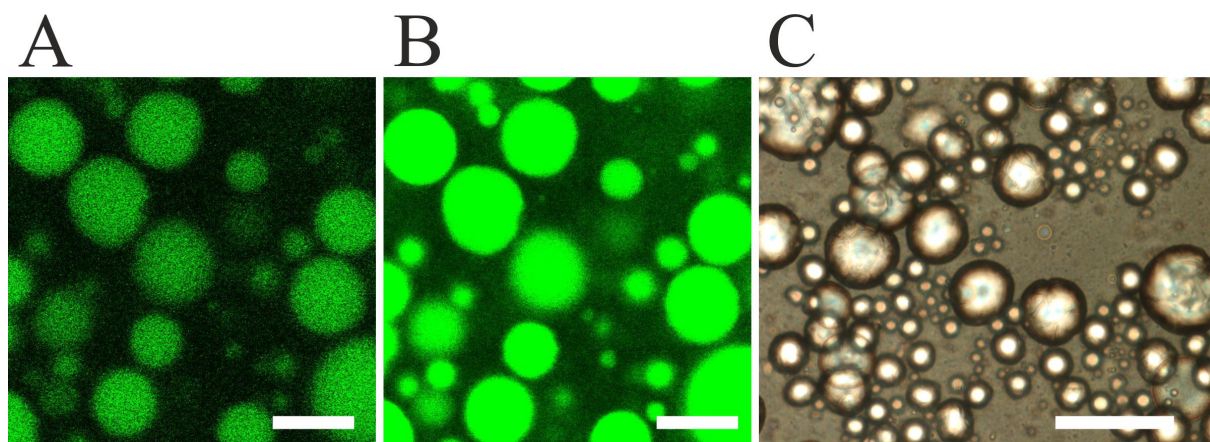


Figure 4.11: A and B Confocal image of TA006 (molar ratio 12) at different focal planes scale bar 20 μm . ; C Light transmission microscopy image of sample TA006 scale bars 20 μm .

The light transmission microscopy image shows a polydisperse array of droplets with interesting wrinkling shells. The confocal image of the fluorescently labelled droplets gives information on the shapes and sizes of the droplets but PVP-TA particles that are stabilizing the system are not visible under these conditions. To visualize the PVP-TA particles in fluorescence with the confocal microscope we have tried to stain the emulsions either with fluorescein or Rhodamine-B. Fluorescein was not able to stain the complexes and it was evenly distributing in the water phase therefore no relevant information could be gathered by using this dye for these particles. Rhodamine-B was able to selectively bind to the PVP-TA complexes giving clear information about their distribution and morphology. Unfortunately the Nile red and Rhodamine-B emission wavelengths are too close and Nile red stained emulsions were not suitable to be stained with this dye for lack of contrast between the oil droplets and the particles. Rhodamine-B worked very well with the unstained set of emulsions and it was found to bind selectively PVP-TA complexes. Information on the morphology and distribution of the complexes in the emulsion could therefore be obtained with this dye. We observed that for emulsions at molar ratio below 12 a shell of PVP-TA network is formed (see figure 4.12). Over time the

shell wrinkles as shown in figure 4.12 C. From the confocal images (see 4.12 A and B), the shell thickens appear not to be homogeneous and the continuous phase presents a dispersion of tiny PVP-TA particles.

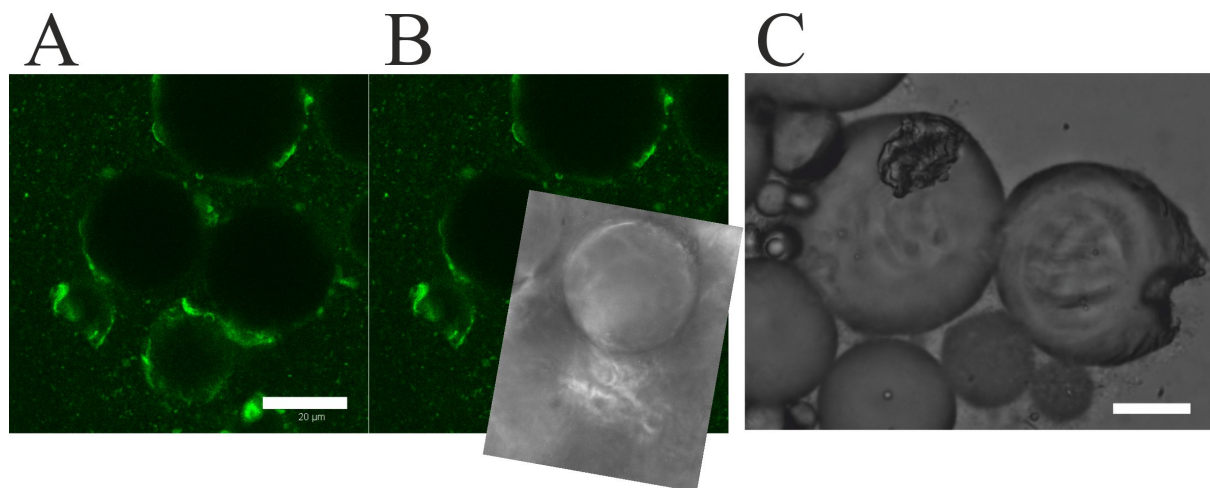


Figure 4.12: Selection of images of sample TA010 molar ratio 12: A and B are confocal images with overlapping light transmission microscopy image of a single droplet, scale bar 20 μm ; C light transmission microscopy image of droplets in wrinkled shell scale bars 10 μm

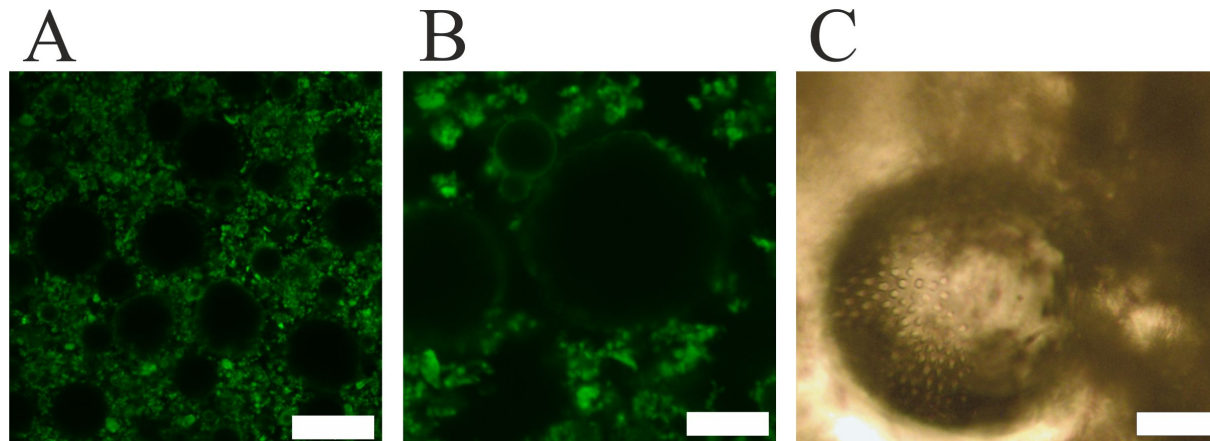


Figure 4.13: A and B Confocal image of emulsion TA011 (molar ratio 24), scale bars A= 20 μm ; B= 5 μm ; C Light transmission microscopy image of emulsion TA011 (molar ratio 24). C= 5 μm ;

For all the emulsions prepared at molar ratio higher than 24 the continuous phase looks like a distribution of PVP-TA particles that have aggregated into fractal structures (see figures 4.13 A, B, 4.14A, B and 4.15 A, B) and the droplets appear to be stabilized by discrete particles (see figure 4.14 B orthogonal section). Emulsions stabilized by TA-PVP complexes formed at molar ratios above 35 present better stability against creaming and flocculation but the confocal images show less well defined shells as reported in figure 4.13 B and C. We speculate that these emulsions

are therefore stabilized not only by the particle sitting at the inter-phase as in simple Pickering emulsions but also by steric effects of the complexes suspended in the surrounding water phase which promote kinetic stabilization of the system by reducing the collision frequency of the droplets [119].

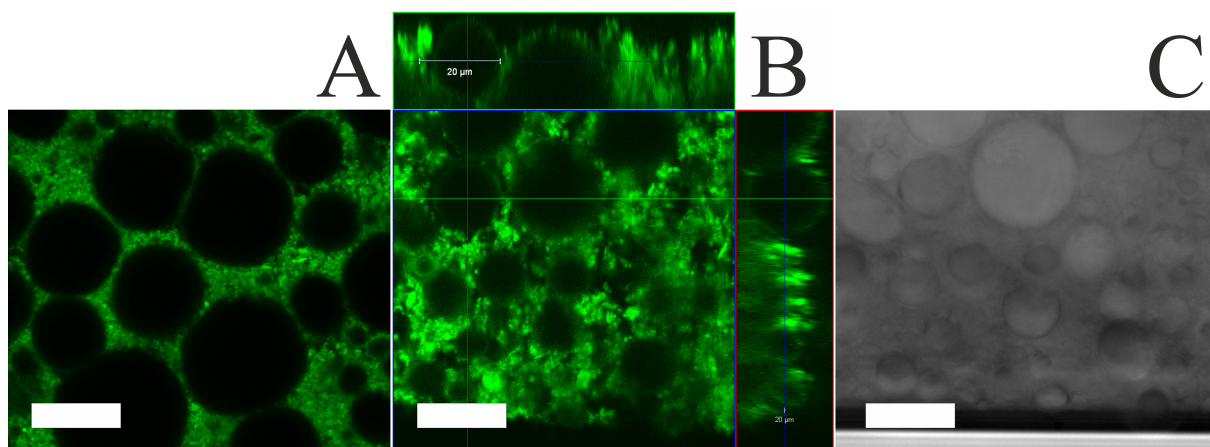


Figure 4.14: A: Confocal image of emulsion at molar ratio 35 sample TA012; B: confocal image orthogonal section of emulsion at molar ratio 35 sample TA012 using a square capillary (ID 0.5 mm) for the specimen; C: light transmission microscopy image of emulsion at molar ratio 35 sample TA012 using a square capillary (ID 0.5 mm) for the specimen which allowed to image the same area as in B. Scale bars 10 μm

With CSLM we acquired in-focus images from selected depths in the samples and 3D image of the emulsion is reconstructed in 4.15 B. In this image the steric effect is well visualized. In figure 4.15 C an extended network of PVP-TA complexes is clearly visible. We have already reported that over time the emulsion gels and this structure is the microscopic appearance of the gel. In chapter 3 we have already discussed the cross-linking of PVP-TA particles into a gel.

Confocal and light transmission microscopy images of the same spot were acquired using square capillary (ID 0.5 mm) for the specimen. This technique allow to load the samples relatively quickly and select only the cream and not the lower serum phase and the complexes sediments. Light transmission and confocal microscopy image of the same area are shown in figures 4.14 B and C.

4.3.4 Some considerations on the effect of pH on emulsion stability

Emulsions stabilized by PVP-TA particles have a pH between 4.5 and 7 depending on the amount of unbounded TA. Tannic acid is a weak acid with $\text{pK}_a=10$. Experiments run by drastically

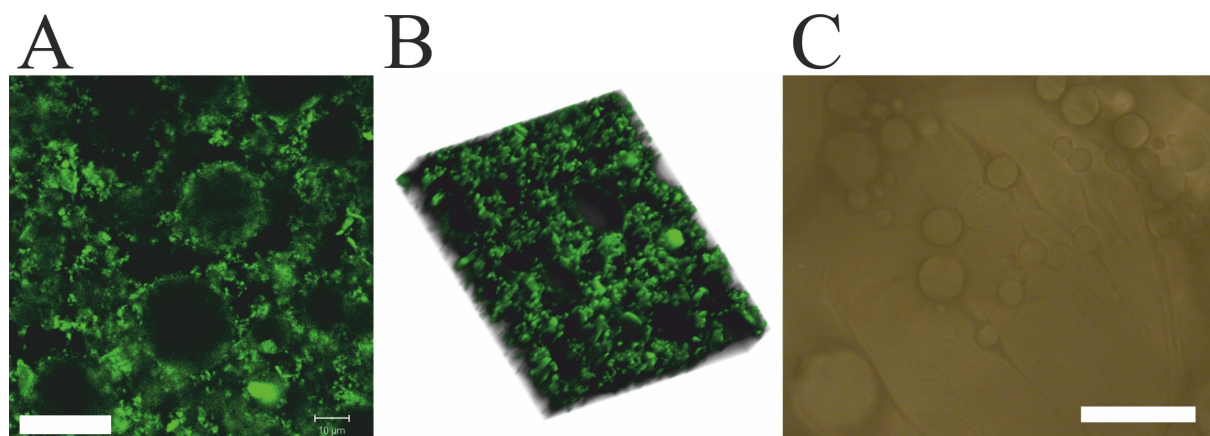


Figure 4.15: A: Confocal and light microscopy image of emulsion at molar ratio 35 sample TA012 scale bars 10 μm

lowering the pH of these emulsions by adding hydrochloric acid, showed that these emulsions are stable even at very low pH down to $\text{pH} \sim 1$. We observed that for those emulsions with turbid water phases, decreasing the pH leads to a clearer water phase. The observation with confocal microscopy confirms that no coalescence is present. Experiments by increasing the pH of the emulsion with KOH have shown that at pH 10 the emulsions change colour becoming yellow/orange, coalescence is triggered and ultimately phase separation is observed. The speed of this process is directly proportional to the pH. For emulsions brought up to pH 14 it is possible to re-emulsify the system to a stable emulsion by hand-shaking it. This is probably due to the fact that such strong basic conditions promote hydrolysis of some triglycerides of the soybean-oil promoting a saponification reaction for which surfactants able to stabilise the emulsion are produced. We have observed halochromism in the emulsion that is due to the fact that TA is halochromic. At pH above 10 TA loses a proton therefore a different LUMO level becomes accessible to an electronic transition.

4.4 Conclusion

In this chapter we have explore a simple method for the preparation of biodegradable surfactant free emulsions using PVP-TA complex non-covalently associated microgels. We observed that the stainless steel dispersing element catalysed the formation of an extended network of PVP-TA complexes during the emulsification process. This phenomenon was also seen during the preparation of the PVP-TA particles previously described in Chapter 3; however in this case

several weeks were required for the network of PVP-TA complexes to form. These agglomerates were unable to stabilize the emulsions because they were too big compared to the curvature radii of the oil droplets, therefore we modified the emulsification protocol in order to avoid their formation. The PVP solution was therefore added to the TA solution instead of adding TA to PVP solutions as done in the previous work (Chapter 3). An alternative approach to solve this problem could have been purchasing a new plastic dispersing element for the high-performance dispersing instrument; however the high cost of such tool and the consideration of potential hydrophobic interaction, with the tool itself, lead to the decision to utilise the revised protocol which worked successfully in solving the problem in the first instance. Experiments varying the oil volume fraction have shown that emulsions at higher oil volume fraction were relatively more stable against creaming than those at lower oil volume fraction. Although the overall concentration of TA-PVP complexes was the same, the better stability of these emulsions can be explained by the fact that the lower water fraction leads to a higher concentration of TA-PVP complexes for emulsions at higher oil volume fractions. In other words, since the concentration of TA and PVP was constant in the total volume of the emulsion, the continuous phase was richer in complexes for higher oil volume fractions and a better stability against coalescence was therefore achieved. Experiments varying the TA/PVP molar ratios showed that the complexes stabilised emulsions either through the formation of a continuous shell around the droplets or Pickering stabilization aided by particles steric effect in the continuous phase which reduced the collision frequency among droplets. The TA/PVP molar ratio has been identified as an important factor in determining the type of emulsion. At low molar ratio we observed the formation of coated droplets while at higher molar ratio the emulsion is stabilised by microgel particles dispersed in the continuous water phase. After less than a week all emulsions reached a gelling point that arrested any creaming process. This was due to the formation of a thick microgel network similar to the one observed in Chapter 3. All emulsions prepared showed good stability through time and at low pH. Drastic increase of the pH above 10 accelerates destabilization through coalescence. In this range of pH the emulsions were halochromic which is not surprising since TA is halochromic. The advantage in stabilizing an emulsion with these structures is that, having chosen to disperse food grade oil, the emulsion itself is completely biodegradable therefore these emulsions have the benefit of being environmental friendly [95].

Chapter 5

Rheology of PVP-TA gel particles stabilised emulsions

5.1 Introduction

The rheological properties of emulsions are of paramount importance for their technical applications and aesthetic qualities, therefore the ability to measure, adjust and, ideally, predict such properties is extremely important [120]. Consumers have various expectations of emulsion creaminess and consistency, which dictate their buying preferences. These sensory attributes can be related to quantitative physical measurements obtained on rheometers.

In qualitative terms, emulsions range from low viscosity milk-like Newtonian liquids, to thicker shear-thinning liquids and thick, cream-like materials with an apparent yield stress [121]. At the microscopic length scale, all emulsions have a continuous phase and a dispersed phase and are thermodynamically unstable systems. The dispersed phase is formed by droplets that are typically stabilised by surfactant adsorbed on their interfaces [122]. A short range repulsive interaction is therefore established between droplets and this prevents coalescence. In some cases emulsions can be stabilised by particles that form a layer around the droplets to prevent coalescence. Such emulsion are called Pickering emulsions, named after Pickering who described these systems in 1907 [44]. Here we present a rheological study of oil in water emulsions stabilised by a fractal-like gel structure [7]. The formation of these particles is performed with a single step method utilising solutions of polyvinylpyrrolidone (PVP) in water and tannic acid (TA). Tannic acid is a natural polyphenol that belongs to the group of hydrolysable tannins. It is a gallotannin consisting of a pentagalloyl glucose molecule esterified with five gallic acid units [23]. As polymer we have used PVP which is a biocompatible water soluble polymer. For details on TA, PVP and PVP-TA particles see Chapter 1 and 3.

5.2 Experimental methods

Particle stabilised emulsions have been prepared by dispersing different volumes of soy-bean oil in a freshly prepared suspension of PVP-TA particles dispersed in deionized water. The suspensions of PVP-TA particles were prepared by adding a solution of PVP 1% w/w to a solution of tannic acid 1% w/w in equal volumes using 20mL vials and vigorously shaking them with the plastic lid on. The procedure was repeated several times in order to prepare the necessary total volume of water phase for the emulsification process.

To disperse the soy-bean oil in the water phase we used dispersing tool IKA ULTRA-TURRAX T25 at 15,000 rpm. Each time the oil has been slowly added up to the needed volume. We have prepared emulsions at oil volume fractions, ϕ_{oil} , 0.30, 0.50, 0.60, 0.74, 0.82 keeping the concentration of particle in the water phase constant. All the reagents were purchased from Sigma and no further purification was performed.

5.2.1 Scanning electro microscopy imaging

PVP-TA particles were imaged with scanning electron microscopy, SEM. The preparation of the particle samples for imaging with SEM included drying a drop of PVP-TA particle dispersion onto a silicon wafer and gold coating it with a sputter coater, model SC7620 Quorum Technologies.

5.2.2 Creaming index

Creaming stability was followed over a long period (five months) to evaluate the relative stability of the emulsions. We poured the emulsions prepared in 20mm diameter, 60mm high glass vials sealed with a screw cap to prevent evaporation. The vials were kept at room temperature and the movement of any creaming boundary was tracked visually. The total emulsion height (H_E) and serum layer height (H_L lower layer) were measured. A creaming index (CI(%)) was calculated as reported by Sun [112] and as described in Chapter 1 Section 1.6.1.

$$CI(\%) = 100 \frac{H_L}{H_E}. \quad (5.1)$$

5.2.3 Rheological characterization

Rheology measurements were performed using a stress controlled rheometer, AR-G2 TA Instruments. Details on this instrument and rheology back ground were given in Chapter 2. Experiments were conducted with parallel plates of 60 mm in diameter. Theoretically the gap for this geometry can be any distance, but since any slight lack of parallelism between the plates results in a measurement error and this increases as the gap decreases, it is advisable to set the minimum distance at 1% of the geometry diameter, which in our case is $600\mu\text{m}$. The correct

filling of this geometry is with the sample forming a disc with its edge flush with the rim of the plate. We therefore loaded the samples by slightly overfilling and trimming. This technique is particularly useful especially for thick samples. In this geometry the shear rate varies across the plate from zero in the centre to a maximum at the rim. The rheometer software operates under the assumption that the sample is linearly viscoelastic or Newtonian and it measures the strain and shear rate at the rim. This geometry is also recommended for oscillatory experiments conducted within the linear viscoelastic regime. To evaluate the Linear Viscoelastic Region (LVR) we ran an Oscillatory Stress Sweep (OSS). After that we ran an Oscillatory Time Sweep (OTS) to determine if the materials properties were changing over time. Finally, to determine the storage modulus (G' elastic modulus) and the loss modulus (G'') we ran an Oscillatory Frequency Sweep (OFS). Following the recommendation of the manufacturer of the rheometer, we re-loaded a new sample for each test. To minimize evaporation of the water phase during the tests we used a solvent trap cover. We describe the experimental condition for each single test in the following paragraphs starting from the flow test that we performed to determine the viscosities.

Flow test

The aim of a flow test is to determine the viscosity of a sample at different shear rates and plot flow curves. By fitting the experimental data it is possible to calculate the zero shear viscosity (η_0) and the infinite shear viscosity (η_∞) which are needed to make comparisons between different materials. In this type of test a specific stress is applied to the sample and the strain as function of time, shear strain rate (or shear rate in short name), and the viscosities are measured. For the flow tests on our emulsion and particles in water dispersion we imposed the following conditions:

- Conditioning step:
 - Set temperature at 25 °C
 - Equilibration time 3 minutes
- Stepped Flow Step
 - Broad torque range from 1 to 10,000 μNm
 - Collect 5 points per decade in log mode

- Constant time 10 seconds
- Average last per second = 5 seconds.

We plotted the experimental data in overlapping rheological graphs of shear stress [Pa] vs. shear rate (reciprocal second [s^{-1}]) and viscosity [Pa·s] vs. shear rate [s^{-1}]. We fitted the viscosity curves using both the Sisko equation (2.10) and the Cross model (2.9). The Cross model is an empirical model which describes the whole theoretical flow curve. The former is very good at describing the flow behaviour of emulsions and suspensions in the practical everyday shear rate for these materials, 0.1 to 1000 s^{-1} [76], but not the whole flow curve. The results of these two different approaches will be discussed in the "Results and Discussion" Section 5.3.

Oscillatory Stress Sweep

The aim of the Oscillatory Stress Sweep (OSS) is to determine the Linear Viscoelastic Region (LVR). Within the LVR at a particular oscillation frequency the amplitude of the applied stress is directly proportional to the amplitude of the measured strain. This condition is relevant for model fitting purposes since the majority of mathematical models to fit oscillatory frequency experimental data are valid only under these conditions. In a general oscillatory test a sine-wave shaped input of stress is applied and then the resulting sinusoidal strain output is resolved into the elastic modulus G' (solid like response in phase with the input) and the viscous modulus G'' , that it is out of phase by a phase difference δ that varies throughout the test. To perform OSS tests we have imposed the following parameters:

- Conditioning step:
 - set temperature at 25 °C
 - Equilibration time 3 minutes
- Stress Sweep Stress:
 - We imposed a broad torque range from 1 to 10,000 μNm ;
 - Constant Frequency of oscillation set at 1 Hz;
 - Collected 10 points per decade in log mode;

The aim of the equilibration was to allow any structure to build up before data acquisition begins. After the data were collected we plotted G' and G'' against oscillatory stress σ [Pa] in an overlapping rheological graph. From these plots we can visually identify the LVR from which we can choose a suitable value of oscillatory stress for subsequent testing.

Oscillatory Time Sweep

To determine how the properties of the emulsions are changing over the time of testing we ran Oscillatory Time Sweep tests imposing the following conditions:

- First conditioning step
 - Set temperature at 25 °C
 - Equilibration time 3 minutes
- Second conditioning step
 - Pre-shear at a shear rate of 10 s⁻¹
 - Equilibration time 3 minutes
- Time Sweep step
 - Duration of experiment 15 minutes
 - Frequency 1Hz
 - Control variable oscillation shear stress chosen within the LVR determined with the oscillatory stress sweep
 - Sampling time every 5 seconds for 900 seconds

This test gives information about how the material properties are changing over time of testing, for example aggregation or sedimentation that may occur in the samples over time or the amount of time to form a stable structure. Only the elastic modulus G' is plotted since G'' is related to it by the phase difference δ .

Oscillatory Frequency Sweep

In an Oscillatory Frequency Sweep (OFS) a small amplitude sinusoidal stress is applied. The value of the stress is chosen within the LVR determined as explained above. The aim of this test is to determine the storage modulus, G' , and the loss module, G'' , which are a measure of how "solid-like" or "liquid-like" a viscoelastic material is. We have run OFS using the following parameters:

- Conditioning step 1 for thermal equilibration
 - Setted temperature at 25 °C
 - Equilibration time set at 3 minutes
- Conditioning step 2
 - Pre-shear
 - Equilibration
- Frequency sweep step
 - Frequency range= 0.1-10 Hz
 - 5 point per decade log mode
 - Controlled variable shear stress and strain within the LVR

The data generated are then plotted in overlapping rheological graphs of G' [Pa] and G'' [Pa] vs. angular frequency [rad/sec].

To fit these experimental data there are several options. Here we will mention the two that would probably be most suitable for fitting experimental data for emulsions: the Oldroyd (or Jeffreys) model that is best suited to dispersions of deformable particles, for example emulsions, and the power law, which usually provides the best fit for flocculated dispersions and polymeric gels. These models have been discussed in details in Chapter 2. We will discuss how these two models fit our experimental data in the "Result and discussion" section.

5.3 Results and discussion

5.3.1 Particles observation with SEM

The PVP-TA particles used to stabilise the emulsions were imaged with scanning electron microscopy, SEM, in which fractal-like gel structure can be observed (see figure 5.1). Note that we are visualizing the dry particle shapes and no information on the hydrated complexes can be gathered, except for the size of the dried particles. We observe a polydisperse sample with particle sizes ranging from 1 μm to 10 μm .

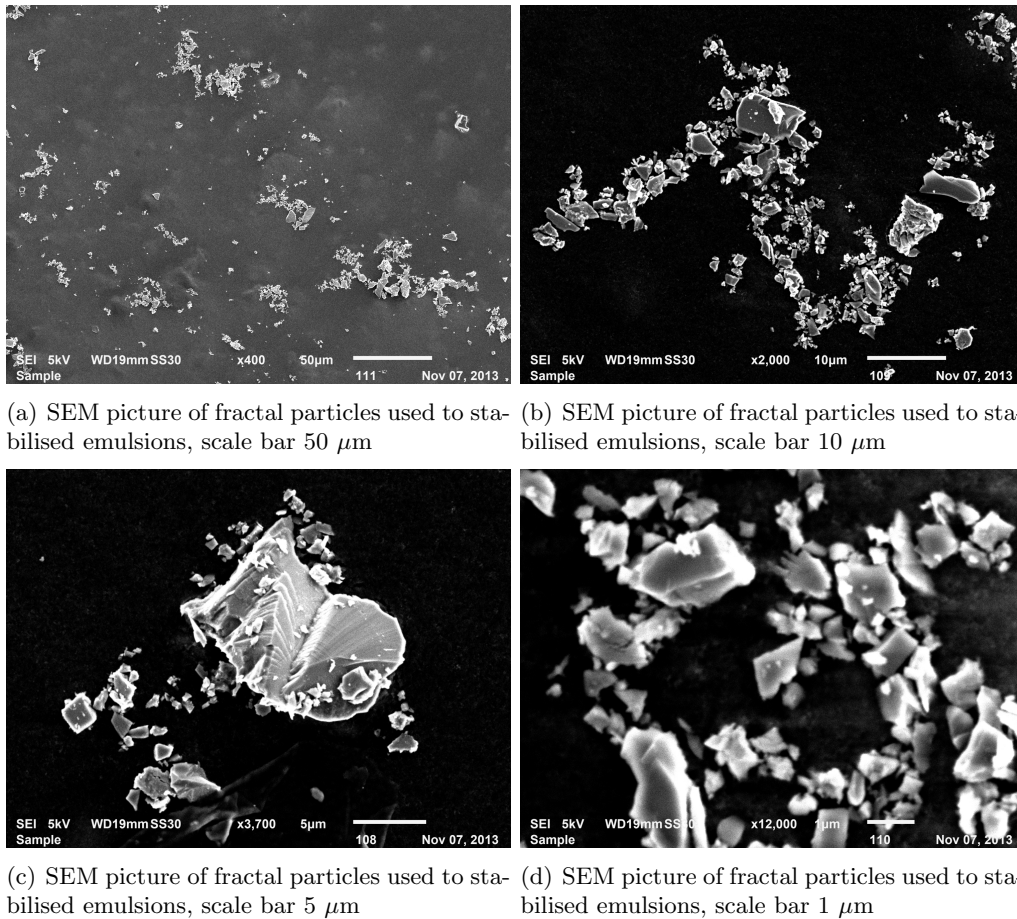


Figure 5.1: SEM images of PVP-TA dry gel particles.

Figure 5.1 shows that the particles are not homogeneously distributed. This is because these particles have a tendency to aggregate in the solution. The majority of particles are between 1 and 5 μm with a small number of bigger particles up to 10 μm . Some particles smaller than 1 μm are present. Many particles have a rhomboidal shape with well defined edges.

The bigger particles show more complex shapes.

5.3.2 Creaming stability

In figure 5.2 we show the picture of the vials prepared for the creaming stability test. The oil volume fraction (ϕ_{oil}) varies from 0.82 to 0.74, 0.60, 0.50, 0.30 left to right. Emulsions at ϕ_{oil} 0.30, 0.50 and 0.60 separated into a top cream layer and a bottom serum layer. The initial and end creaming indexes for each emulsion are reported in table 5.1 and the results of the long storage test are plotted in figure 5.3. Emulsion ϕ_{oil} 0.60 showed a good stability through time and a small $CI(\%)$ that did not increase with time. Emulsion ϕ_{oil} 0.50 was less stable than the former and the $CI(\%)$ increased steadily for 7 days until the system reached a gelling point that arrested the creaming process. The upper layer turned into a gelled thick cream and the oil droplets were then prevented from moving up through the continuous water gel phase (top phase). The gelled system opposes the buoyancy of the oil droplets which is the main cause of creaming. Emulsion ϕ_{oil} 0.30 was less stable against creaming and its already considerable $CI(\%)$ increased sharply in 5 days reaching 63.3. After the fifth day, the $CI(\%)$ stays stable. If we look at this data in terms of volume fraction, the cream, which is in this case the emulsion phase, is now occupying only the 36.7% of the total initial volume of the emulsion. In other terms, the total volume fraction of droplets in the cream exceeds the value of 74% by volume (reaching 83% in the cream). This is the maximum packing fraction of mono-disperse hard spheres [7]. Emulsions in which the total volume fraction of droplets exceeds the maximum packing fraction are called high internal phase emulsions, HIPE. It is possible for such emulsions to exist only under the condition that the droplet distribution is polydisperse and/or that the droplets are deformable.



Figure 5.2: Photograph of emulsions stabilised by TA-PVP particles. The oil volume fraction (ϕ_{oil}) varies from 0.82, to 0.74, 0.60, 0.50, 0.30 left to right. The vial in the middle is at ϕ_{oil} 0.60 and it is upside down to better show the resistance to flow of this emulsion.

ϕ	H_E mm	H_{Lt_0} mm	H_{Lt_f} mm	$CI(\%)_{t_0}$	$CI(\%)_{t_f}$
0.30	30	5	19	16.7	63.3
0.50	30	3	12	10.0	36.7
0.60	40	1	1	2.5	2.5
0.74	50	0	0	0	0
0.82	45	0	0	0	0

Table 5.1: Creaming index of emulsions measured after 24h from preparation, t_0 , and after 6 months from preparation, t_f . H_E is the total height of the emulsion in the container, H_{Lt_0} and H_{Lt_f} are the heights of the serum layer at t_0 and t_f .

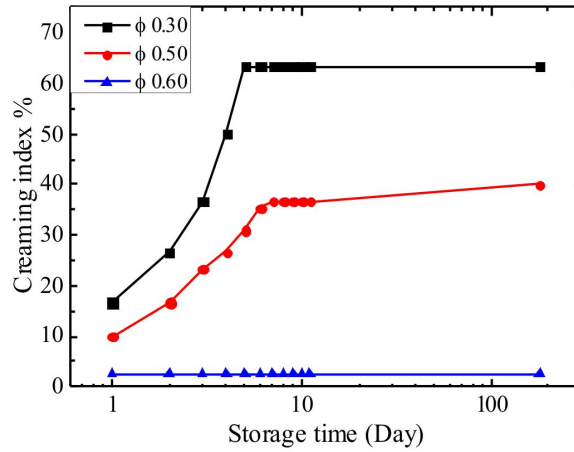


Figure 5.3: Effect of ϕ_{oil} on the creaming index for emulsion at ϕ_{oil} 0.30, 0.50, 0.60 for long storage test.

The emulsions at ϕ_{oil} 0.74 and 0.82 are both high internal phase emulsions (HIPE). The latest did not cream, but showed phase inversion of the emulsion and coalescence. The emulsion at ϕ_{oil} 0.82 separated into a water phase and an oil phase possibly because the interfacial area is too big to be stabilised by the complexes present in the emulsion. Furthermore, PVP-TA particles are dispersed in the lower water phase. Finally, the emulsion at ϕ_{oil} 0.74 presents moderate coalescence at different layers in the vial, but does not completely break up into the two phases.

The emulsion at ϕ_{oil} 0.30 had a creaming index of 16.7 which increased by 63.3 in 5 days. Over the next 6 months the final creaming index was still the same. The creaming index of ϕ_{oil} 0.50 increased from 10 to 36.7 in 7 days. In this case $CI(\%)_{t_f}$ slightly increased during the long storage test from 36.7 to 40. If we look at this data in terms of volume fraction, as we did above

for the emulsion at ϕ_{oil} 0.30, the cream is now occupying 60% of the total initial volume of the emulsion. The volume fraction of oil droplets in the cream has reached 83% as we observed for the emulsion at ϕ_{oil} 0.30. We can conclude that this value is probably the maximum packing fraction for these emulsions.

The emulsion at ϕ_{oil} 0.60, which is the inverted vial figure 5.2, shows a marked resistance to flow. This emulsion is also the one that shows good stability against creaming with a creaming index of 2.5 which is stable through the long-term storage test. For this emulsion we are far from the high internal phase emulsions domain since the ϕ_{oil} in the cream is only.

5.3.3 Flow curves

Flow curves have been obtained as described in Section 5.2.3. We plot the data in overlapping rheological graphs of shear stress [Pa] vs. shear rate (reciprocal second [s^{-1}]) and viscosity [Pa·s] vs. shear rate [s^{-1}]. The flow curves for each sample are shown in figure 5.4.

From these flow curves we can see that emulsions ϕ_{oil} 0.50, 0.60 and 0.74 are shear thinning. Emulsions ϕ_{oil} 0.82, ϕ_{oil} 0.30 and the PVP-TA particle dispersion in water showed almost a Newtonian behaviour and exhibited a small yield stress. Therefore they can be classified as Bingham emulsions [76]. The yield behaviour can be viewed as an unjamming process [116]. Interestingly we found this behaviour at low and zero ϕ_{oil} and again in the emulsion ϕ_{oil} 0.82. The yield behaviour is an indication of elements that are "jamming" in the system. The PVP-TA particles tend to aggregate. The almost Newtonian behaviour of emulsion ϕ_{oil} 0.82 is due to phase inversion and separation of the emulsion.

We found a good fit for all the samples, especially for those at ϕ_{oil} 0.50 and 0.60 with both the Sisko and the Cross models. In figure 5.5 we report the experimental data plotted against both models. The difference between measured and calculated values is very small in the range considered.

The infinite shear viscosities calculated with both models are in agreement with one-another. It is not possible to calculate the zero shear viscosity (η_0) with the Sisko model, so only the one calculated with the Cross model is plotted in figure 5.6. The emulsion at 0.30 ϕ_{oil} has the lowest η_0 , even lower than the PVP-TA particles dispersion. This is an indication

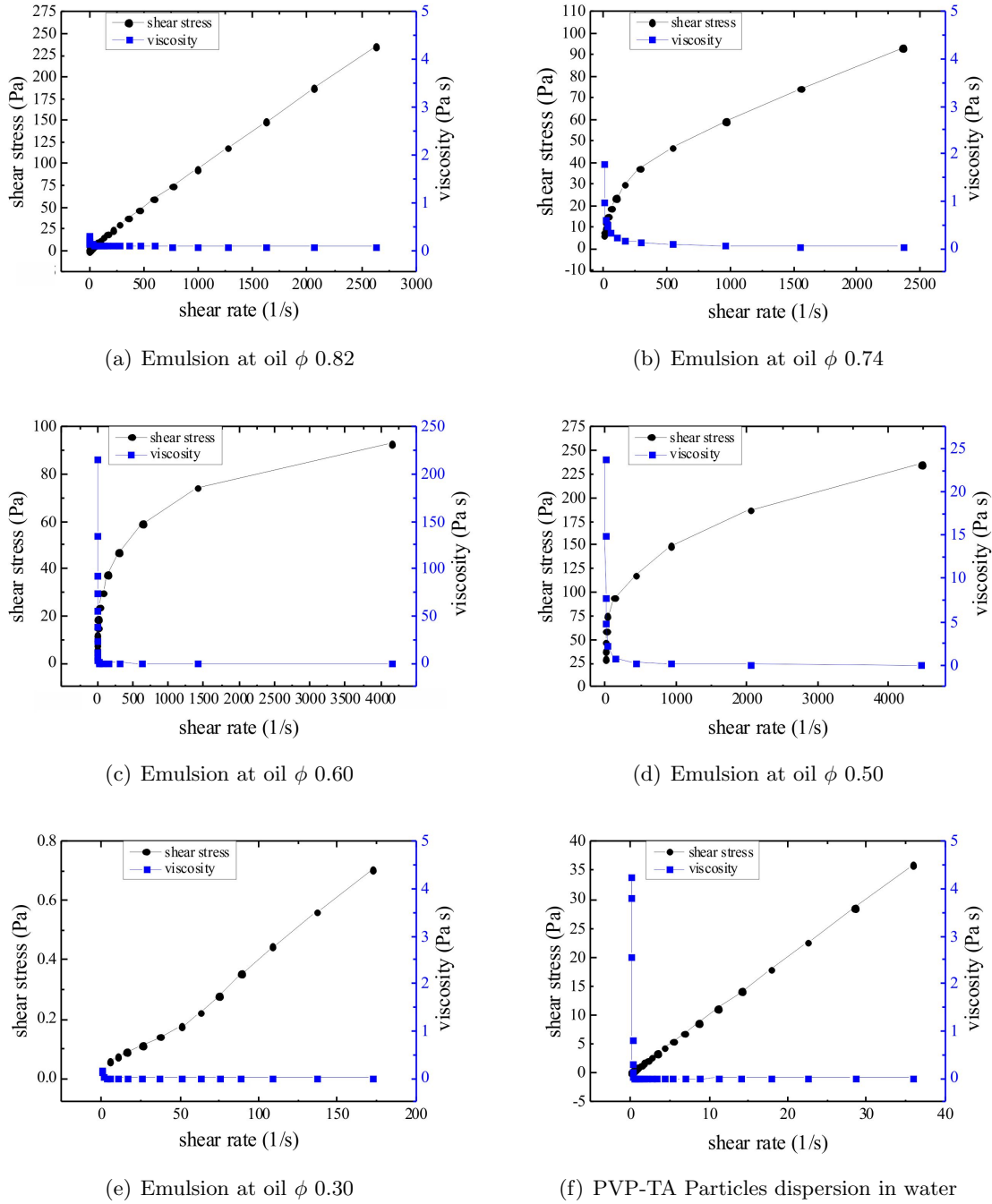
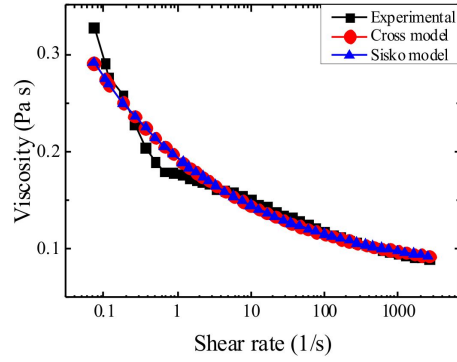
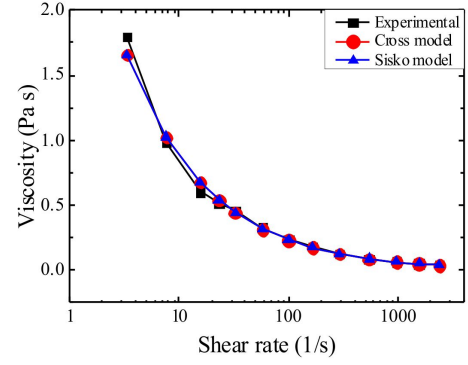
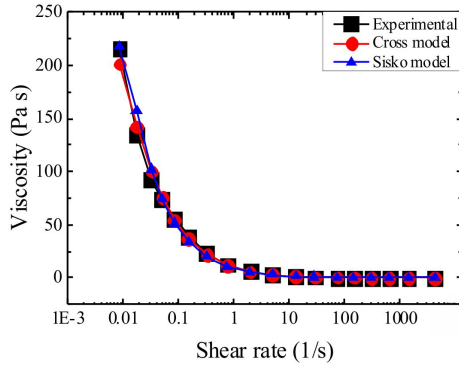
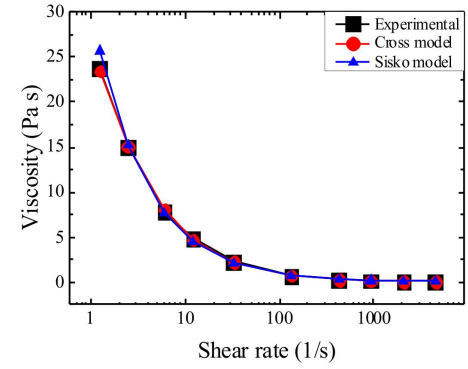
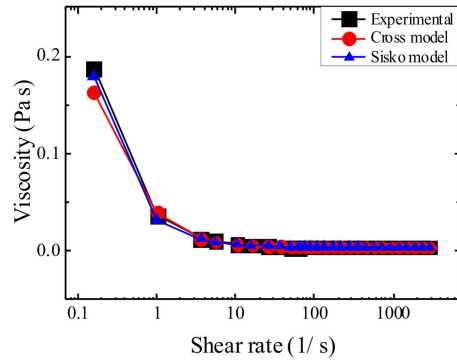
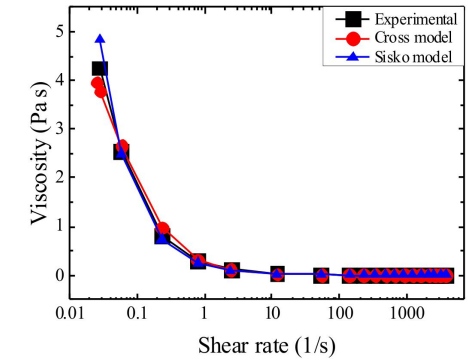


Figure 5.4: Flow rheograms (plots of shear stress vs. shear rate) and viscosity curves.

that PVP-TA particles are flocculating and aggregating in the dispersion more than in the emulsion at low ϕ_{oil} and this phenomenon gives a higher η_0 for the dispersion. We speculate that since more particles are required to stabilise the oil water interface, less are available to form aggregates. The zero shear viscosity increases as a function of ϕ_{oil} up to 0.74, where the oil droplet maximum packing fraction is reached. For emulsion at ϕ_{oil} 0.82 the η_0 drops back to 81

(a) Emulsion at oil ϕ 0.80(b) Emulsion at oil ϕ 0.74(c) Emulsion at oil ϕ 0.60(d) Emulsion at oil ϕ 0.50(e) Emulsion at oil ϕ 0.30

(f) PVP-TA Particles dispersion in water

Figure 5.5: Comparison between the experimental data and the Sisko and Cross model simulation

$Pa \cdot s$ which is similar to the η_0 we have found for emulsion at ϕ_{oil} 0.50.

The infinite shear viscosities (η_∞) slowly increase from 0 ϕ_{oil} to 0.50 ϕ_{oil} . At 0.60 ϕ_{oil} , η_∞ sharply decreases to increase again for emulsions at ϕ_{oil} 0.74 and 0.82. In other words η_∞ drops for ϕ_{oil} 0.60. This emulsion is the more stable in term of creaming index and no coalescence has

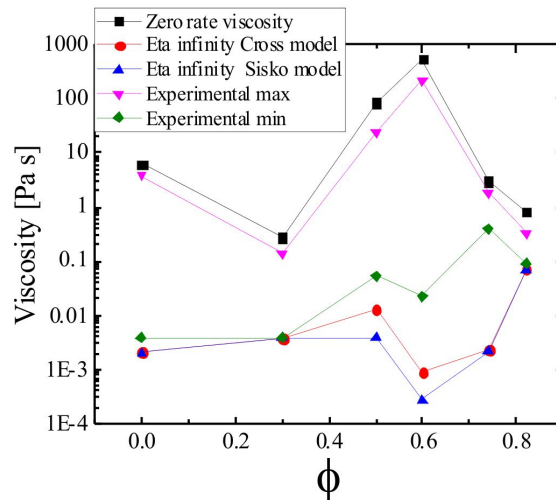


Figure 5.6: Zero shear viscosities calculated with Cross model versus ϕ_{oil} and infinite shear viscosities calculated with both Sisko and Cross model vs ϕ_{oil}

been observed.

5.3.4 Determine the Pseudo-Linear Viscoelastic Region (LVR)

To determine the Pseudo-Linear Viscoelastic Region (LVR) we have performed an Oscillatory Stress Sweep (OSS) imposing the parameters detailed in the Experimental Method (Section 5.2.3). The rheological graphs for each sample are reported in figure 5.7.

From these curves we have visually identified the pseudo-Linear Viscoelastic Region (LVR) for each sample and we used this information to choose suitable values of oscillatory stress for subsequent testing. It is important to identify the LVR because only within the LVR at a particular oscillation frequency is the amplitude of the applied stress directly proportional to the amplitude of the measured strain and this condition is relevant for model fitting purposes since the majority of mathematical models to fit oscillatory frequency experimental data are valid only under these conditions. For the following tests we therefore imposed oscillatory stress, σ , within the LVR and specifically: $\sigma = 0.9$ Pa for emulsion ϕ_{oil} 0.82, $\sigma = 0.5$ Pa for emulsion ϕ_{oil} 0.74, $\sigma = 3$ Pa for emulsions ϕ_{oil} 0.60, 0.50, and 0.30 and $\sigma = 0.1$ for the particle dispersion in water. The experimental data for these tests are plotted in figure 5.7.

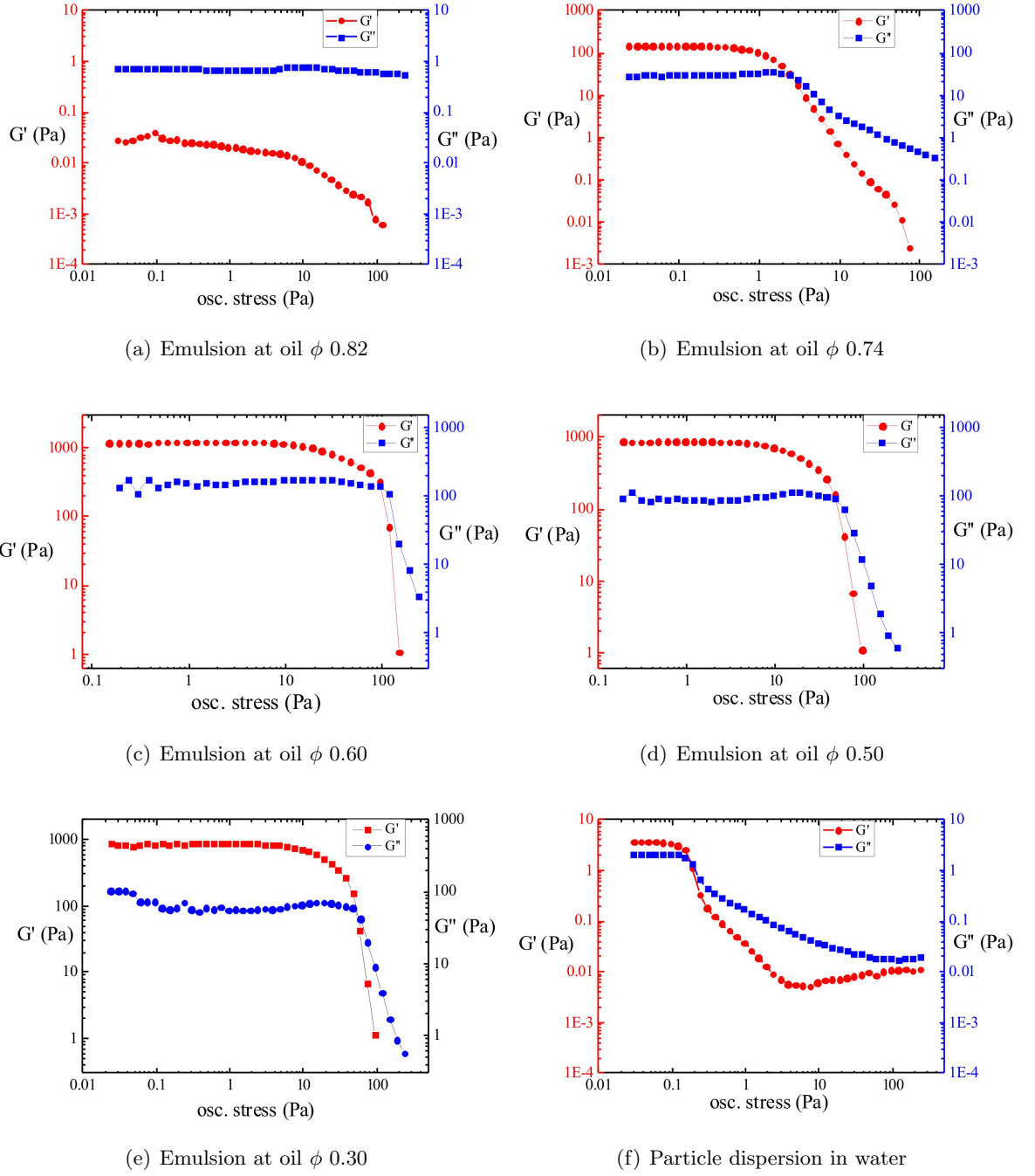


Figure 5.7: Preliminary tests to determine the pseudo-Linear Viscoelastic Region(LVR)

5.3.5 Oscillatory Time Sweep results

Oscillatory Time Sweep (OTS) were performed imposing the parameters detailed in Section 5.2.3. The results of these tests are shown in figure 5.8. On the left we plot the Oscillatory Time Sweep curves of the elastic modulus, G' , measured for each emulsion during the OTS and

on the right we plotted the initial and the final G' measured in the OTS versus the oil volume fraction.

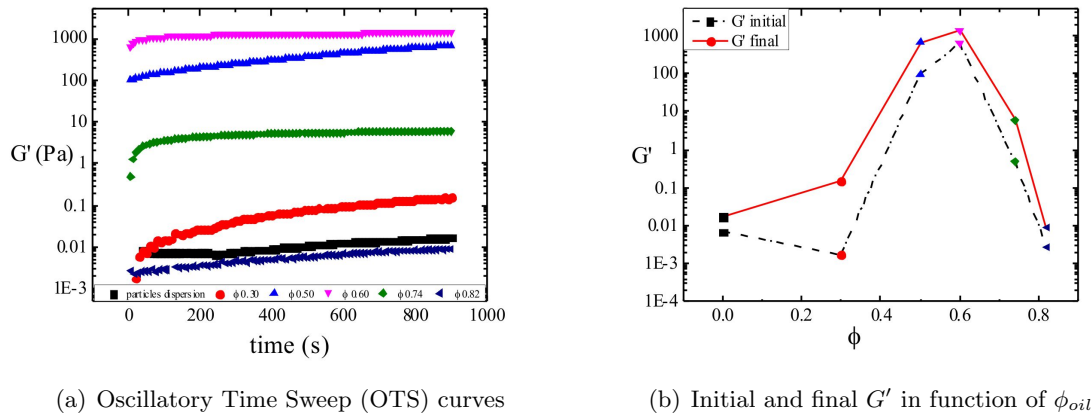


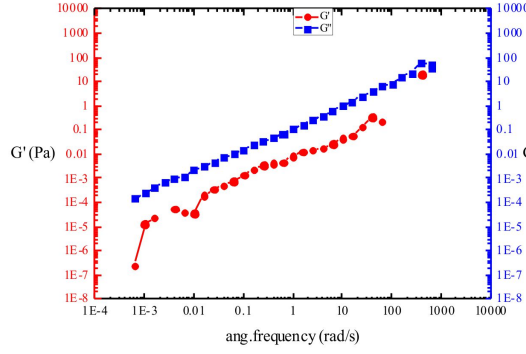
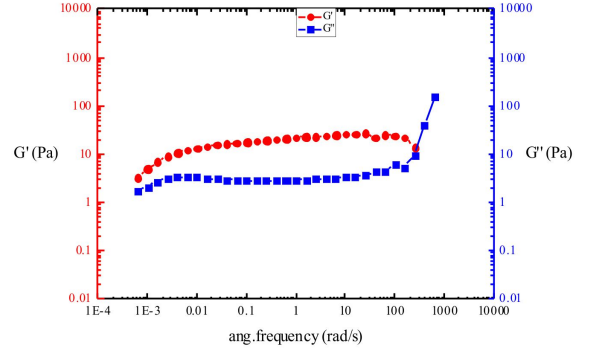
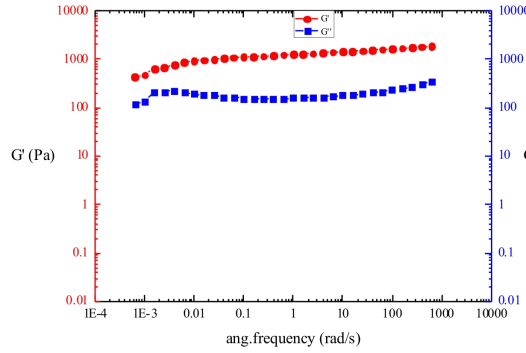
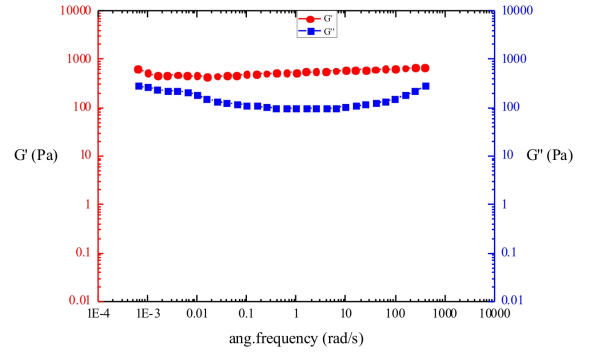
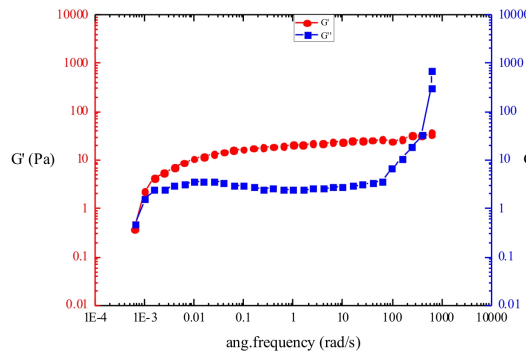
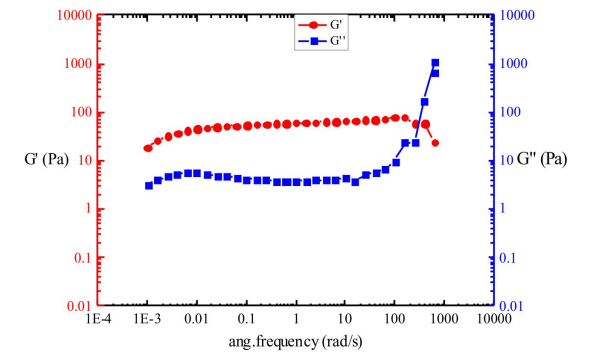
Figure 5.8: (a) Oscillatory Time Sweep (OTS) curve for different oil volume fraction emulsions, ϕ_{oil} 0, 0.30, 0.50, 0.60, 0.74, 0.82. (b) Plot of initial and final elastic modulus G' measured with OTS versus oil volume fraction.

After pre-shear and initiation of the oscillatory stress sweep test, the material begins to form a structure, as shown by an increase in elastic modulus. As already mentioned, OTS tests give information about how the material properties are changing over time, for example if aggregation or sedimentation occurs in the samples. The amount of time to form a stable structure and time dependent rheological properties can be probed. For emulsions ϕ_{oil} 0.74 and 0.60 a stable structure is formed and the OTS curves reach a plateau. For emulsion ϕ_{oil} 0.74 this is a fast and sharp ramp which lead to a constant G' . The highest G' was measured for emulsion ϕ_{oil} 0.60. The curve soon becomes flat so that a stable structure quickly forms and is maintained throughout the experiment. The elastic moduli of emulsions ϕ_{oil} 0.50 and 0.30 increase constantly throughout the experiments showing that for both emulsions the structure is building up throughout the experiment. For the particle dispersion and the emulsion ϕ_{oil} 0.82 the elastic modulus is relatively small and constant which indicates a prominent liquid like behaviour and no building up of any structure. In the next section we will discuss the results of the Oscillatory Frequency Sweep OFS.

5.3.6 Oscillatory Frequency Sweep results

To perform Oscillatory Frequency Sweep (OFS) tests we have used the protocol reported in Section 5.2.3 choosing values of shear stress within the LVR evaluated with the Oscillatory

Stress sweep shown in Section 5.3.4. The experimental results of OFS tests are reported in figure 5.9.

(a) Emulsion at oil ϕ 0.80(b) Emulsion at oil ϕ 0.74(c) Emulsion at oil ϕ 0.60(d) Emulsion at oil ϕ 0.50(e) Emulsion at oil ϕ 0.30

(f) PVP-TA Particles dispersion in water

Figure 5.9: Oscillatory Frequency Sweep tests for different oil volume fraction emulsions.

Theoretically the best fit for emulsions is the Oldroyd (or Jeffreys) model, but from a first visual observation of these rheological graphs it is obvious that we are in the rubbery plateau region and this is best fitted by the power law model. As already mentioned in Chapter

2, a power law gives the best fit for flocculated dispersions and polymeric gels. Under these experimental conditions most of our emulsions gel and exhibit broad power-law responses with the only exception being the emulsion at ϕ_{oil} 0.82, for which the liquid like behaviour is clearly dominant on the range of angular frequencies probed. This is due to the phase inversion of this emulsion. For emulsion ϕ_{oil} 0.50 and 0.60 the elastic modulus is dominant all throughout the OFS. For emulsions at ϕ_{oil} 0.30, 0.74 and the particle dispersion at high frequencies we see a cross over point indicating the start of the glassy region where G'' is once again dominant and the liquid-like behaviour becomes dominant. The result of the fits are reported in table 5.2.

Emulsion	Consistency k (<i>Pas</i>) ⁿ	Frequency index n
0.82	0.12	1.009
0.74	0.55	0.016
0.60	31.80	0.014
0.50	7.61	0.001
0.30	0.52	0.012

Table 5.2: Value of consistency and frequency index calculated by fitting the OFS data with power law.

5.4 Conclusions

In this chapter we have investigated the rheological properties of PVP-TA particle stabilised emulsions as a function of oil volume fraction. We have demonstrated that it is possible to stabilize emulsions with fractal PVP-TA particles across a range of oil volume fraction. The best stability is achieved for ϕ_{oil} 0.60. For higher volume fractions we have observed coalescence and phase inversion. At lower ϕ_{oil} the system is prone to creaming and flocculation. The different rheological tests showed a consistent picture of this behaviour and the oscillatory frequency test highlighted for the stable emulsions a behaviour typical of flocculated dispersions and polymeric gels.

Chapter 6

Microfluidics monodisperse microgel stabilised emulsions

6.1 Introduction

Microfluidics is the science of designing and manufacturing devices and processes that deal with volumes of fluids of the order of nanolitres or picolitres. A considerable development of applications in the pharmaceutical, biomedical, biotechnological and chemical engineering domain has arisen in recent years and new techniques such as lab-on-a-chip [115] and cell manipulation devices [123] have seen a surge.

Lab-on-a-chip research aims to reduce in scale single or multiple lab processes down to chip-format. Analytical or preparative methods such as chemical labelling and separation processes. This scaling down involves flow and transport necessary for multiple chemical analyses, mixing, detection and separation.

The ability to create uniform drops using capillary microfluidic devices has been exploited to fabricate monodisperse spherical microparticles such as poly(Nisopropylacrylamide) (PNIPAm) microgels by an in situ polymerization reaction [124]. PNIPAm microgels are thermoresponsive cross-linked polymeric particles that are formed by polymerizing NIPAm monomer and a cross-linker. The polymerization reaction is chemically initiated and is generally performed at elevated temperatures.

Moreover, microfluidics is an interdisciplinary domain in which interesting physics can be done [125] because it becomes possible to isolate and investigate physicochemical effects relevant to soft matter systems and perform proof-of-concept (PoC) experiments that aim to demonstrate the efficacy of new approaches. For instance, many innovative experiments on colloidal interfaces in confinement [126] and liquid crystals have been done [127, 128].

Some commercially available examples of systems and processes that employ microfluidics technology include ink-jet printers, blood-cell-separation equipment, biochemical assays (e.g. enzymatic immunoassay for detecting glucose and protein), chemical synthesis, genetic analysis, drug screening, electrochromatography, surface micro-machining, laser ablation, and mechanical micro-milling [129, 130].

The spatial domain of microfluidics lies between 10^{-6} to 10^{-3} metres. As dimensions shrink, the relative importance of surface to volume forces increases [131]. In addition, there

are unknown factors involved, especially concerning micro-scale heat transfer and mass transfer [132]. Molecular transport also changes dramatically at this scale, convective mixing does not occur and diffusion kinetics is predictable [130].

In the next sections we will discuss some basic theories relevant to microfluidics and we will see why at this scale the flow is laminar and relatively easy to control, the droplets are spherical and the gravity is negligible (section 6.2). After this we will discuss the techniques we have used to design and fabricate microfluidic devices for the production of emulsions and double emulsions (section 6.3). Finally, we report on our successful production of monodisperse emulsions stabilized by PVP-TA microgels particles and double emulsions (section 6.4).

6.2 Microfluidic background

To better understand the microfluidic domain it is useful to introduce some basic concepts of fluid dynamics best illustrated through the Reynolds number and the capillary number.

6.2.1 Reynolds Number

The Reynolds number (Re), named after the physicist Osborne Reynolds, compares inertial forces to viscous forces [133], and it is useful to predict the type of flow different fluids will experience under different flow conditions. It is proportional to the characteristic velocity of the fluid and the length scale of the system (cross section), and it is inversely proportional to the fluid viscosity. It is defined as:

$$Re = \frac{\rho UL}{\eta} = \frac{UL}{\nu}, \quad (6.1)$$

where ρ is the density of the fluid, U is the mean velocity of the fluid, L is the characteristic linear dimension of the channel, η is the dynamic viscosity of the fluid, and ν is the kinematic viscosity. The Reynolds Number can be used to determine if a flow is laminar, transient or turbulent, the flow is:

1. laminar, when $Re < 2300$
2. transient, when $2300 < Re < 4000$

3. turbulent, when $Re > 4000$.

In microfluidics, the Reynolds number is usually small (often on the order of unity or smaller). For example, in a channel with height $100\ \mu\text{m}$, flow with water (shear viscosity $\eta \approx 1\ \text{mPa s}$) at a typical speed of $10^{-2}\ \text{m/s}$, the Reynolds number is of unity. [134]. Since the velocity usually is less than centimetre per second and the widths of the micro-channels are within the tens of micrometres, Reynolds number usually does not exceed 2300. Therefore the controllable laminar flow regime is common.

6.2.2 Capillary Number

The Capillary number is given by the ratio between the viscous stress and the surface tension at the interface between two immiscible liquids or a liquid and a gas and it is defined as:

$$Ca = \frac{\eta U}{\gamma}, \quad (6.2)$$

where η is the dynamic viscosity of the continuous phase ($\text{kgm}^{-1}\text{s}^{-1}$) and U is the mean velocity of the carrier fluid (ms^{-1}) and γ is the interfacial tension [135].

In a microfluidic device, droplet breakup occurs when the shear stress (σ) applied by the continuous phase overcomes the interfacial stress γ/R (where γ is the interfacial tension and R is the radius of curvature at the interface) [136].

Within this framework the capillary number can be rewritten as:

$$Ca = \frac{\sigma}{\gamma/R} = \frac{\eta \dot{\gamma} R}{\gamma}, \quad (6.3)$$

where $\dot{\gamma}$ is the shear rate. For low capillary numbers ($Ca \rightarrow 0$), flow is dominated by capillary forces whereas for high capillary number viscous forces are dominant [137]. A more viscous continuous phase would aid droplet break-off through better shearing, therefore a higher shear rate is typically required if either a less viscous continuous phase, a smaller curvature of the interface or a larger interfacial tension are present.

6.2.3 Droplet generation

The main difference between droplet generation in microfluidic devices and bulk emulsification is that with the former geometrical constraints are introduced while with the latter the process of breakup into droplets is directly linked to the mechanical energy imposed rather than the geometry of the main container. Bulk emulsification processes have the advantage of high-throughput performances at the expense of limited control over the size distribution of the droplets. On the other hand in microfluidic devices the geometrical confinement enables the production of monodisperse droplets. Moreover, the geometrical confinement, which is given by the cross-section of the microfluidic channel, makes the wetting properties and interfacial stresses fundamentally important. Since gravity and inertia are proportional to the mass of the fluid and surface forces are proportional to the surface area, at micro scales the latter are more important. There are several microfluidic geometries suitable for producing droplets e.g. co-flow, T-junctions and flow focusing geometries [135]. A co-flow geometry typically uses glass-capillaries and a 3D design. Many authors have reported very interesting experiments using this geometry, such as fabrication of monodisperse vesicles, liquid crystal shells, particles with different internal structures, synthesis of monodisperse hollow microcapsules, single, double, and higher order emulsions [138] [124]. Since we have chosen to use soft-lithography (see Section 6.3) to fabricate our devices this type of geometry was not considered in this work. T-junctions and flow focusing are both suitable to be designed in 2D and constructed using polydimethylsiloxane (PDMS) channels, and soft lithography using the technique described in section 6.3.1. We have used flow focusing geometries, since they have been shown to be more stable and controllable than T-junction geometries which are passive break-up configurations [139]. In a flow focusing geometry both phases are forced through a narrow channel and the continuous phase shears the to-be-dispersed phase with a symmetrical action. Depending on the flow rates of the two fluids three possible flow regimes can occur [140]: dripping regime, jetting regime and stable co-flow regime as shown in figure 6.1. In the dripping regime monodisperse droplets are formed close to the intersection. The channel width conditions the droplets diameter. In the jetting regime, shearing of the dispersed phase is less efficient and droplet break-off occurs at some point downstream from the intersection. The stable co-flow regime occurs when the instabilities causing droplet break-off are sufficiently suppressed and the laminar flow is dominant. The size of the droplets can be tuned by choosing an appropriate ratio of the flow rate and the total rate of flow in the

device. their diameter strongly depend from the size of the channel at the junction.

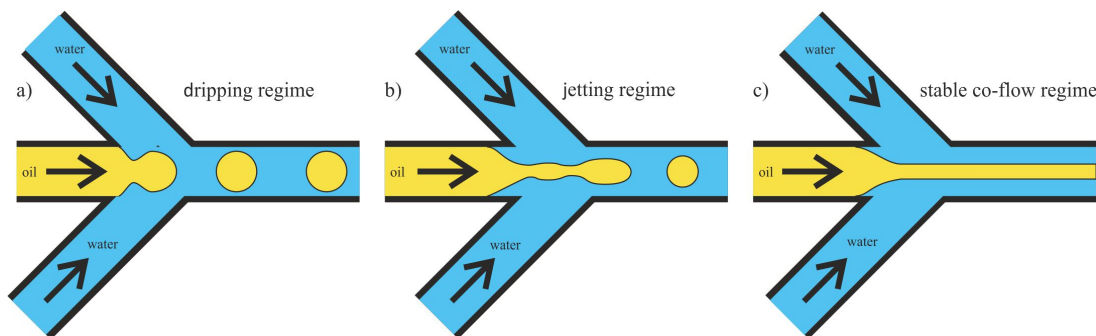


Figure 6.1: Diagram of the different possible flow regimes in a flow focusing geometry.

6.3 Experimental section

6.3.1 Microfluidic devices fabrication: Photo-lithography and soft-lithography

Photo-lithography has been used to fabricate moulds using SU-8 photoresist on silicon wafers. Such moulds were subsequently used for soft-lithography to fabricate poly(dimethylsiloxane) (PDMS) channels. The channels were then sealed with glass cover-slides by means of plasma cleaning. SU-8 is the commercial name of the negative-tone, chemically amplified resist [141] that we have used for the fabrication of the mould for soft-lithography. Negative-tone are photosensitive polymer materials, which are used to perform pattern transfer. The transfer of the pattern from a transparent photo-mask is achieved by selectively irradiating a thin film of resist. The choice of using SU-8 as photoresist is due to its well known qualities such as excellent coating, mechanical and chemical stability [142]. Fully cross-linked SU-8 has a glass-transition temperature around $\sim 200\text{ }^{\circ}\text{C}$, degradation temperature $\sim 380\text{ }^{\circ}\text{C}$ and a Young's modulus $E \sim 45\text{ GPa}$. SU-8 photoresist is supplied by MicroChem Corporation and is available in a wide range of formulations to achieve different film thickness: range from $2\text{ }\mu\text{m}$ to $300\text{ }\mu\text{m}$ in a single coating process or to 3 mm by multicoating processes [143].

The chemical structure of the monomer of one of its main component, Bisphenol A Novolak epoxy oligomer (EPONR[®] SU-8 resin, Shell Chemical), is reported in figure 6.2 [141]. Each monomer molecule contains eight reactive epoxy sites (see figure 6.2), and therefore high degree of cross-linking can be obtained after photothermal activation. The process is initiated by hexafluoroantimonic acid which is generated by UV irradiation of triarylsulfonium hexafluoroan-

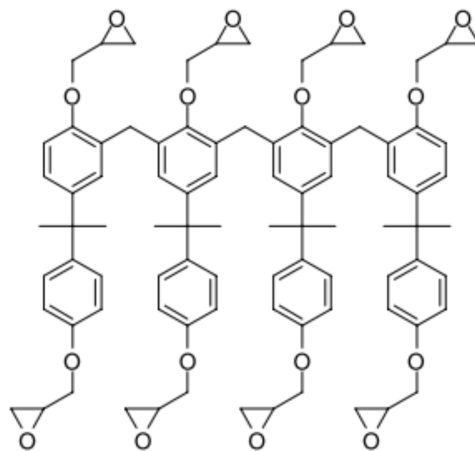


Figure 6.2: Chemical structure of the Bisphenol A Novolak epoxy oligomer (EPONR[®] SU-8 resin, Shell Chemical). After photoactivation the eight reactive epoxy functionalities allow a high degree of cross-linking.

timonate salt (CYRACURER[®] UVI, Union Carbide), which is a photoacid generator present in the formulation of SU-8 typically at 10 wt %. The hexafluoroantimonic acid protonates the epoxides on the oligomer. These protonated oxonium ions are available to react with neutral epoxides in a series of cross-linking reactions after application of heat (post exposure baking). It is important to note that cross-linking occurs only in regions that contain acid catalyst and mainly during post exposure baking and that the interaction between the exposure dose and post exposure bake plays an important role in adhesion between SU-8 resist and the substrate [144].

Details of the process we have used and optimised to fabricate moulds for soft lithography of thickness of 100 μ m on a silicon wafer are given below and schematically reported in figure 6.3. The silicon wafer was first dehydrated in the “pre-baking step” using a hotplate at 95 °C for 10 minutes. Then the wafer was spin coated with 5mL of SU-8. The spin coating process was performed in two steps: pre-shearing at low speed (500 rpm) for 15 second to allow the resistant to spread evenly and then the wafer was accelerated to the final spin speed of 1300 rpm holding it for 38 seconds. The coated wafer was left to “relax” for 10 min at room temperature in order to allow SU-8 to re-flow and settle evenly before soft baking. Soft baking is necessary to remove solvent from the resist layer after spin coating and to optimize adhesion between the photoresist and the silicon wafer [144]. It comprised two steps. The conditions we used were baking on a levelled hotplate for 5 minutes at 65°C and then for 20 minutes at 95°C on a second preheated hotplate. The coated wafer was then irradiated for 28 seconds through a photomask with UV

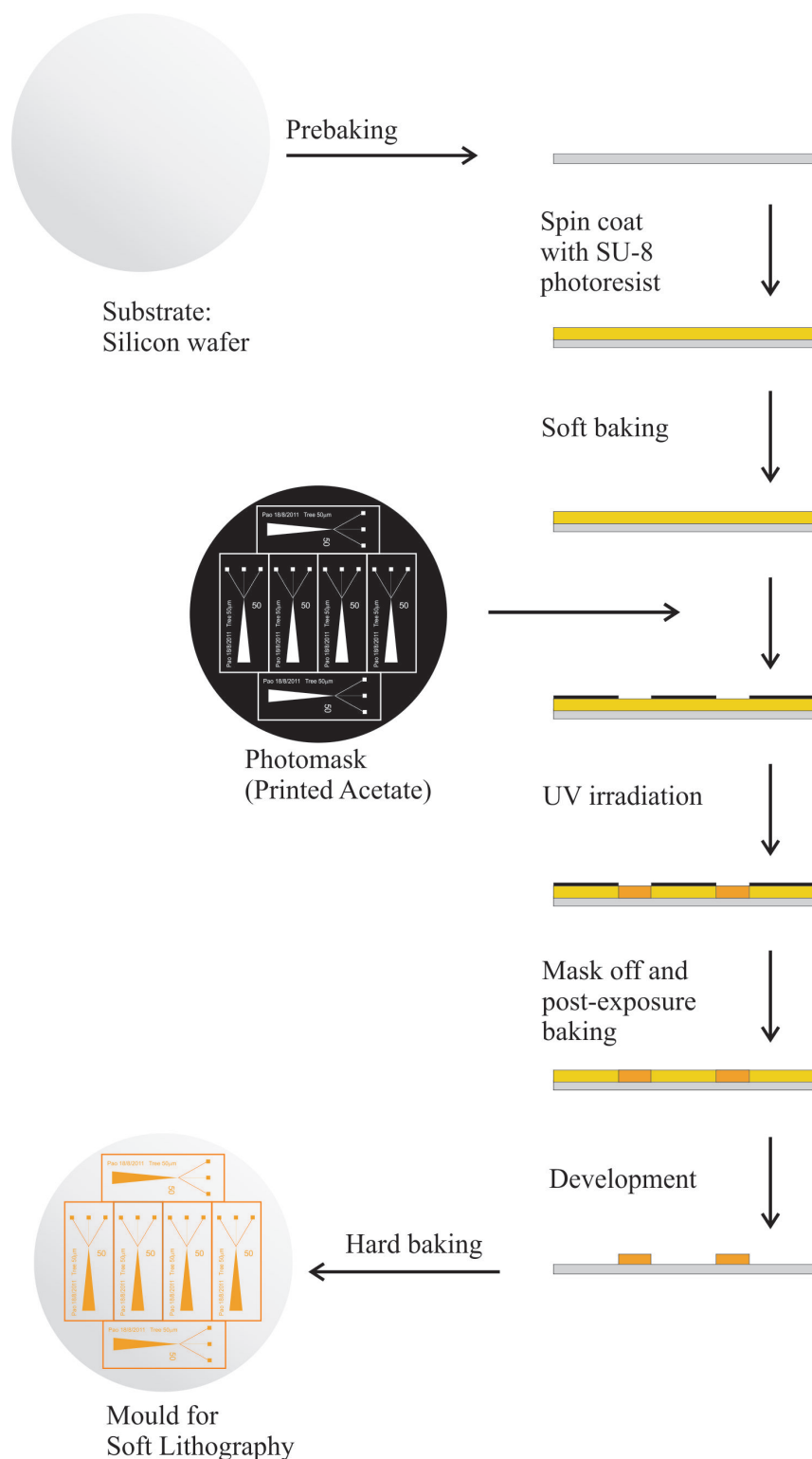


Figure 6.3: Flowchart of photolithography process. In grey the silicon wafer, in yellow SU - 8 photoresist, in orange cross-linked SU-8. Each arrow represents a process that leads to an intermediate step in the fabrication of the mould.

light (130 mJcm^{-2}). The photomasks were designed in CorelDraw and printed onto a sheet of acetate using a high resolution printer by Micro Lithography Services Ltd. Particular attention was taken in placing the photomask with the ink face down. To avoid Fresnel diffraction we used a glass filter held in place by magnets. Post exposure baking, likewise soft baking, comprise two steps: 5 minutes at 65°C then 10 minutes at 95°C . As already mentioned, cross-linking reaction takes place during post exposure baking and it is catalysed by the acid that has been generated during the UV exposure. In the developing step, the unexposed regions of SU-8 were washed away by immersion in propylene glycol methyl ether acetate (PGMEA). The wafer was then washed with PGMEA, rinsed with isopropanol and dried with an air gun. After hard baking at 150°C for 20 minutes, the SU-8 mould was ready to be used multiple times for soft-lithography for the fabrication of PDMS microfluidics devices.

Soft-lithography process details are given below and schematically reported in figure 6.4. The chosen wafer/SU-8 mould was taped up to form a bowl. 30g of PDMS with 1% curing agent was slowly and steadily poured onto the mould after centrifugation at 2000 rpm for 25 minutes to remove any air bubbles generated by the mixing of the PDMS with the curing agent. To avoid formation of air bubbles in the final product, the system was left to settle for two hours before baking overnight at 85°C . When the PDMS was completely polymerised (see figure 6.5), it was peeled off the mould and cut down into individual devices. Inlet and outlet holes were pierced with a flat needle fitted with a piston to eject the PDMS cylindrical cuts. The PDMS devices were then sealed with microscope slides by aid of plasma cleaner. The plasma cleaner works by removing superficial organic contaminants and replacing Si-CH₃ groups on the surface of PDMS with reactive Si-OH groups able to link the glass through Si-O-Si bonds creating an irreversible, water-tight seal [145]. In figure 6.5 we report an example of SU-8 mould and the cured PDMS ready to be peeled off the SU-8 mould.

6.3.2 Materials and methods to produce monodisperse emulsions

To generate specific shear profiles able to produce droplets with our microfluidics devices we used a commercially available syringe pump, the neMESYS Low Pressure Syringe Pump module by Cetoni. This pump is a pulsation-free unit for high-precision dosing of single or multiple fluid streams in the range of mL to pL per min. We worked either with 2 or 3 dispensing modules depending on the geometry of the microfluidics device and the number of inlets. Connection

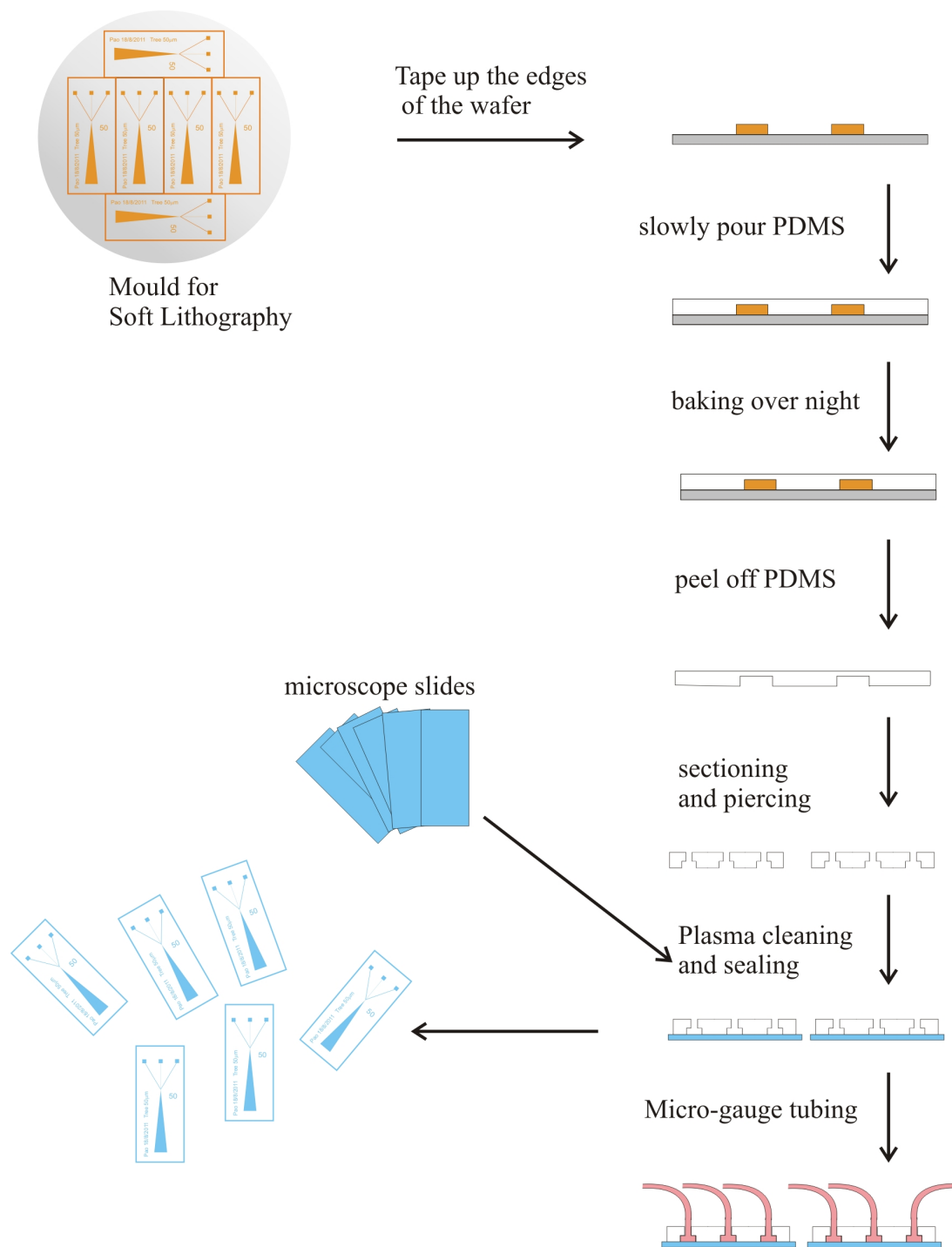


Figure 6.4: Flow chart of soft lithography process. Each arrow represents a process that leads to an intermediate step in the fabrication of the microfluidics devices starting from the SU-8 mould.

between the pump and the PDMS device was made by PTFE tubes with outer diameters of $1/16''$. The water tightness was assured by the elasticity of the PDMS. This type of connection is elegant but fragile, therefore, in some instance, we used a solution of nitrocellulose in butyl

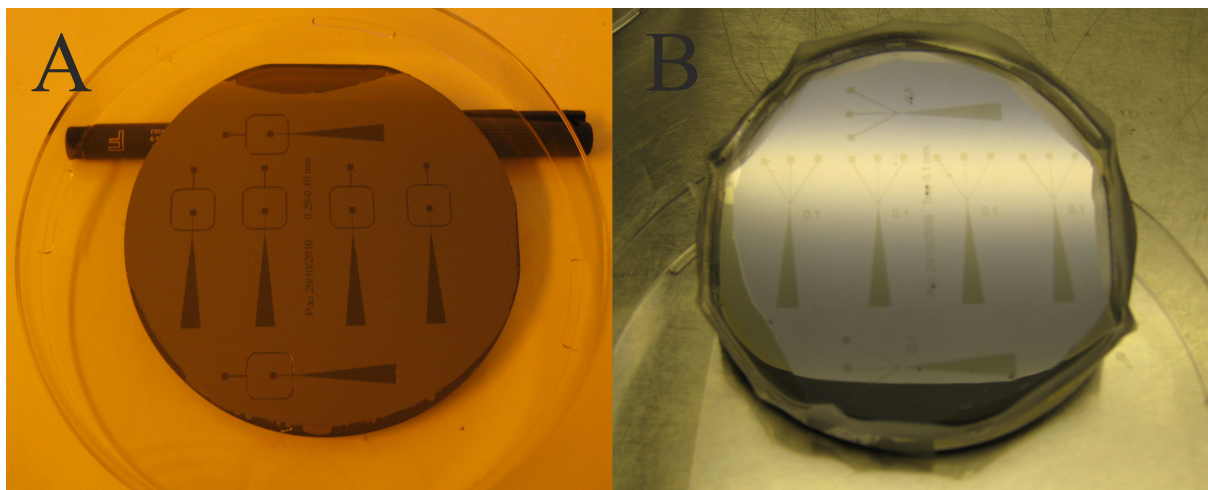


Figure 6.5: (A): final stage of photolithography, SU-8 mould manufactured in cleanroom; (B): Soft lithography: mould with cured PDMS ready to be peeled off

acetate (nail-polish) to increase resiliency and lifespan of the devices.

The flow rates of the different liquids were tuned to give symmetric shearing of the oil phase by the aqueous phases to form oil droplets of approximately $100\ \mu\text{m}$ diameter. The droplets then moved along a channel into the microfluidic device reservoir in which microscopic observation of the forming emulsion could be done. The emulsions were then moved out of the devices through an outlet tube placed at the end of the reservoir and then collected into vials for storage and macroscopic stability tests. The internal diameter of the outlet tubing used was $0.6\ \text{mm}$, which is more than 5 times the diameter of the moving droplets and therefore no relevant shearing was imposed on them at this stage.

6.4 Results and discussions

6.4.1 Characterization and conditioning of the microfluidic devices

The microfluidic devices fabricated with the techniques reported in section 6.3.1 were first checked with optical microscopy for quality control and those units with structural defects were discharged. The channel height was measured with confocal microscopy. By filling the channels with water dyed with rhodamine B, we have performed a Z stack (see chapter 2 for details) and directly measured the height of the micro-channel. The average height of the channels was $101\ \mu\text{m}$ ($\pm 3\ \mu\text{m}$) and the average width was measured with both confocal and light transmission

microscopy and it was $50\text{ }\mu\text{m}$ ($\pm 3\text{ }\mu\text{m}$). An example of confocal 3D image and light transmission microscopy images of one of our microfluidics devices is shown in figure 6.6.

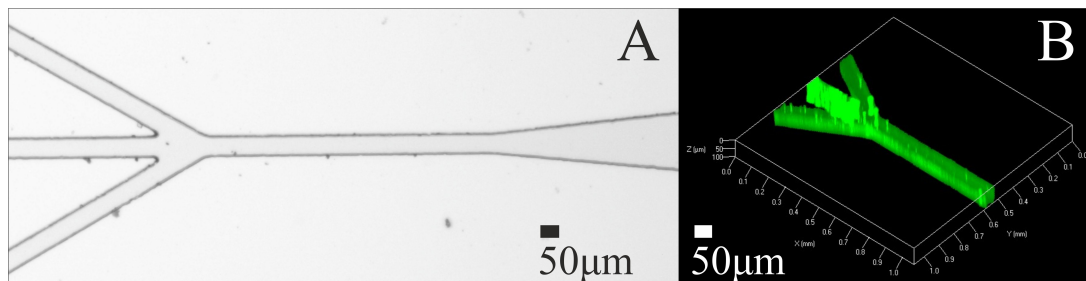


Figure 6.6: Example of flow focusing device: (A) Microscopic image of flow focusing junction; (B) Example of confocal microscopy 3D image of microfluidics channel. This type of image has been used to measure the height of the channels fabricated.

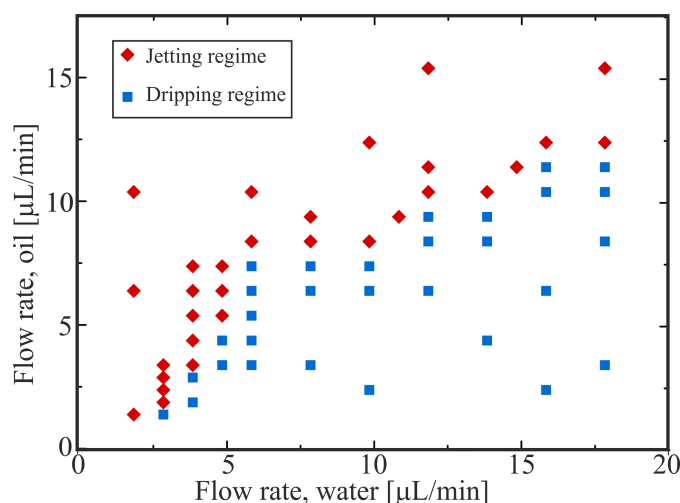


Figure 6.7: Flow diagram of the different possible flow regimes observed in our microfluidic devices.

By modulating the flow rate and the flow ratio between the water phase and the oil phases we have tested our devices to determine the conditions for establishing the jetting or dripping regimes. In figure 6.7 we show the flow diagram obtained for the geometry shown in figure 6.6. During the experiments that led to the construction of the flow diagram, we observed hysteresis effects. For example, if the first phase that flushed through the device was the oil phase, the wettability of the PDMS channel, which had been made hydrophilic by plasma cleaning, was partially compromised by some big oil droplets stuck on the channel walls. For stable and controllable operation of the microfluidic device, it is of paramount importance that the continuous phase, in this case water, wets the walls of the channel while the phase to be dispersed, oil, does not. To remove the oil from the PDMS a very high water flow must be imposed and this can lead to leaks at the connections between the microfluidic device and the

pump tubing. To prevent this, care was taken to flush the microfluidic devices with water before flowing the oil. The maximum flow rate in the flow diagram was fixed at $20\ \mu\text{L}/\text{min}$, because above this value leaking of the devices was highly probable. The lower limit for the flow was imposed by the pump which had a minimum flow rate of $0.175\ \mu\text{L}/\text{min}$. The oil/water flow ratio, below which the dripping regime was observed, had been evaluated as 1 to 2. This is not surprising since water is less viscous than oil therefore a higher flow rate is required to achieve a shear rate able to generate oil droplets.

6.4.2 Monodisperse emulsions

This set of experiments was done with the aim of producing monodisperse emulsions stabilized with PVP-TA complexes. The flow rate for the water phase was $20\ \mu\text{L}/\text{min}$ and the oil flow rate was $10\ \mu\text{L}/\text{min}$.

To stabilize the emulsions we attempted two approaches:

1. Coating the droplets with PVP-TA complexes formed in situ;
2. Coating the droplets with pre-made PVP-TA complexes.

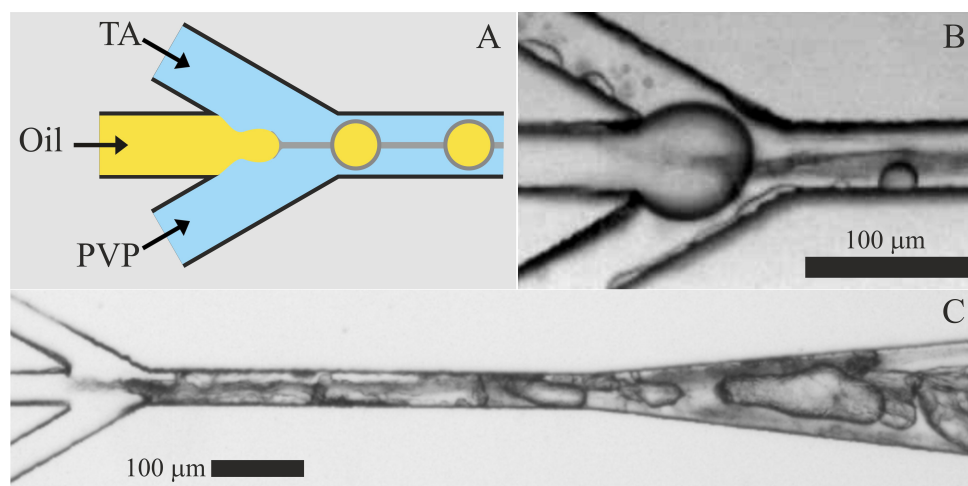


Figure 6.8: Coating the droplets with PVP-TA complexes formed in situ: A extended microgel network blocking the channels of the device

In chapter 3 we saw that the rate of formation of PVP-TA complexes prepared by direct precipitation was almost instantaneous. Furthermore in chapter 4 we saw that the complexes had an affinity for the droplet surface. With this knowledge we set out some experiments to

control the formation of a well defined shell around the emerging droplets to coat them with a film of PVP-TA complexes. We therefore flowed a solution of TA and a solution of PVP and using them to shear the oil tried to produce droplets directly with a shell of PVP-TA complexes. A schematic diagram of this process is shown in figure 6.8 A. Our experiments showed that the PVP and TA solutions exhibited laminar flow and we observed the formation of a continuous film of complexes at the TA/PVP solution interface, see figure 6.8 B. Nevertheless, the emerging droplets were flowing along the film, probably in another plane, but the adsorption at the oil/water interface of the complexes was not fast enough to stabilize the droplets, which coalesced when colliding with the dense microgel structures, generated by the PVP-TA film that was building up in the devices. Unfortunately, in all our experiments the microfluidic system became completely blocked by this extended microgel network and after less than 5 minutes it was no longer possible to flow any of the reagents. This is shown in figure 6.8 C. For this reason the experiments were abandoned and a no characterization of the emulsion was possible.

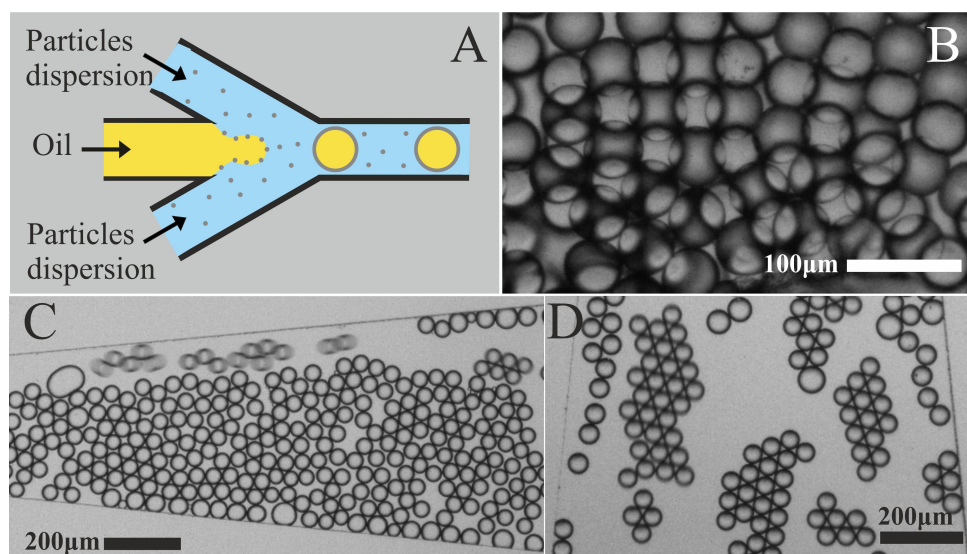


Figure 6.9: Coating the droplets with microgel particles of PVP-TA complexes. A: schematic of the process; B Emulsion observed after collection from the microfluidic devices showed stable coated droplets stacking on top of each other; C PVP-TA microgel stabilized oil droplets in the reservoir of the microfluidics device; D Hexagonally close-packed flocs of droplets moving through the microfluidic device

The second approach was to stabilize the droplets by coating them with microgel particles of PVP-TA complexes. In this case, instead of flowing the TA and PVP solution in the device to shear the oil phase, we use a suspension of PVP-TA microgel particles produced in bulk by direct precipitation as discussed in chapter 3. The dispersions were then centrifuged for 60 minutes at 4000 rpm to separate the bigger complexes not suitable to be pumped in the microfluidic

device. The supernatant liquid was collected with a syringe and stored in a separate vial. This dispersion was then pumped into the microfluidic devices as shown in figure 6.9(A), at a flow rate of 20 $\mu\text{L}/\text{min}$. The oil phase was fed in at 10 $\mu\text{L}/\text{min}$ based on the result of our flow diagram, fig 6.7. With this approach we successfully generated stable coated droplets and we were able to produce 0.67 mL of emulsion in 5 hours. Microscopic images of these droplets are shown in figure 6.9 B, C, D. We observed that these droplets flocculated and packed in hexagonally close-packed arrangements. We speculate that this is due to a short-range interaction between the droplets causing them to stick to one another and flow through the microfluidic reservoir as reported in figure 6.9 C and D. However, this attraction was sufficiently weak to allow the flocs to break up and rearrange themselves in hexagonally close-packed flocs. Interestingly, no coalescence was observed. The origin of this attraction lies in the affinity of the complexes coating the droplets. In chapter 3, we observed that over time PVP-TA particles tend to aggregate into gel agglomerates. We speculate that the affinity of the PVP-TA complexes for the oil/droplet interface and the affinity of the complexes to aggregate are the causes of the formation of flocs of droplets. The long-term storage stability test (refer to section 1.6.1) showed that these emulsions were stable for over 3 weeks. Then partial coalescence was observed, but after 11 weeks the majority of the droplets were still stable.

6.4.3 Double emulsions

Double emulsion are complex colloidal systems which have an important role as delivery systems: they offer possibilities for the controlled release of chemicals dissolved in the internal droplets [146]. They can be water in oil in water emulsions (w/o/w) or oil in water in oil emulsions (o/w/o). We chose to make o/w/o emulsions, starting from emulsions (o/w) prepared with the methods used in chapter 4. Details of the bulk emulsification processes are given in chapter 4. The primary emulsion (o/w) was then flowed in one of our microfluidic devices and, by shearing it with soy-bean oil, we produced monodisperse droplets of oil in water in oil emulsion. For these experiments we used a different geometry from the one used for the monodisperse emulsions. The micro-channel width was 100 μm ($\pm 2\mu\text{m}$) in order to produce bigger, more complex droplets and the geometry was such that we needed only 2 inlets: one for the emulsion and one for the oil phase. Since no surfactants were added to this new system, the new droplets were not stable and therefore had a short "life span". Coalescence occurred in the microfluidic reservoirs as

soon as two droplets collided. An example of one of these unstable double emulsions is reported in figure 6.10.

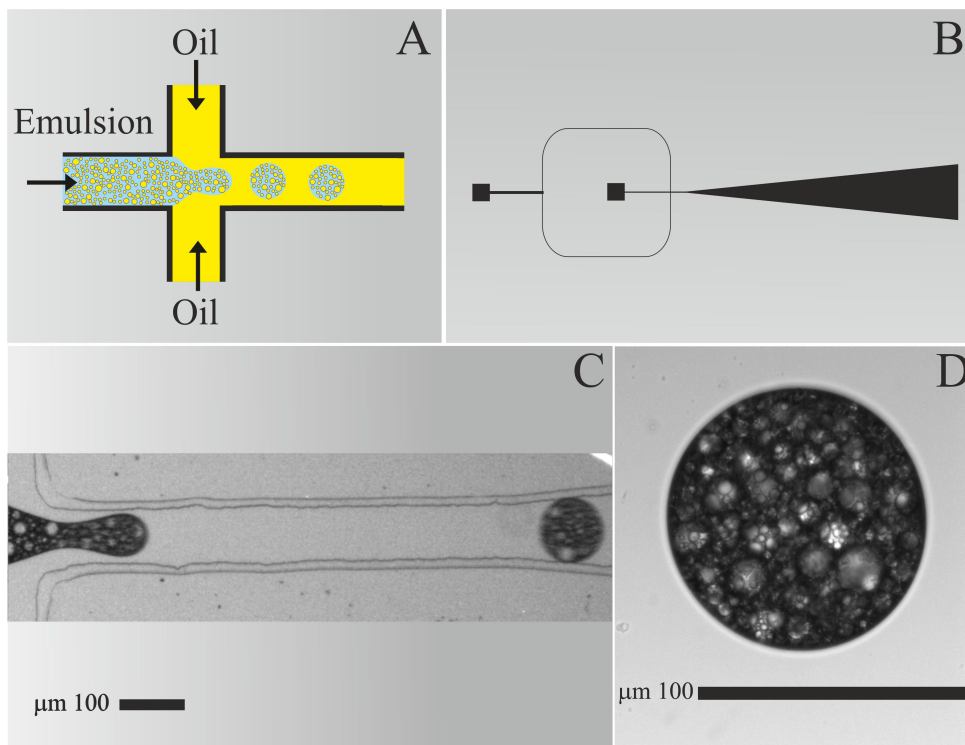


Figure 6.10: Double emulsions: A Schematic of the process for the production of double emulsions; B Example of photo-mask used for the fabrication of mould for microfluidic devices with only 2 inlets; C Micro-channel generating droplets of double emulsion; D droplet of emulsion prepared in bulk, oil in water, in oil phase.

Adding a surfactant to the oil phase would stabilise the system and would make our microfluidic device able to produce double emulsions. However, since the aim of this thesis is to use polyphenol-polymer complexes to replace surfactants, we did not optimize this process using surfactants.

6.5 Conclusion

In this chapter we have introduced a new technique, microfluidics, which is the science of designing and manufacturing devices and processes that deal with volumes of fluids of the order of nanolitres or picolitres. Using photo-lithography and soft-lithography we have designed and fabricated PDMS microfluidic devices for the production of monodisperse emulsions stabilized by PVP-TA complexes and double emulsions. Flow-focusing configuration was used to make

monodisperse emulsions in a controlled fashion. The quality of the devices fabricated, and the width and height of the channels were assessed with light transmission microscopy and confocal microscopy. Two different approaches to stabilize monodisperse emulsions were explored: generating oil droplet and PVP-TA complexes in situ (microfluidic device) and generating oil droplet in situ but flowing a dispersion of PVP-TA particle, produced in bulk, as continuous phase. The aim of the first approach was to make a continuous shell of PVP-TA complexes. In previous work (Chapter 4) this was achieved in bulk emulsification by controlling the TA/PVP molar ratio. Moving the same process to a microfluidic scale, introduced geometrical constraints such that the TA and PVP solutions exhibited laminar flow which led to the formation of a continuous film of PVP-TA complexes along the solution interface. Under these conditions the emerging droplets at the flow-focusing junction were not stabilised by PVP-TA complexes and quickly coalesced. Moreover, the building up of a polymer network inside the microfluidic devices quickly blocked the microfluidic devices.

The latter approaches have been far more successful and monodisperse oil droplets in water stabilized by PVP-TA particles were produced. This was expected since numerous successful attempts to achieve monodisperse droplets are reported by many researchers in the field and the ability of PVP-TA complexes to stabilise emulsion have been extensively proved in this thesis. Therefore the only challenge we had to overcome in this case was the technical difficulty of building and connecting robust microfluidic devices.

Finally, we have shown that more complex structures can be formed using microfluidic devices, such as double emulsions. Since we wanted to stay within the original purpose of this work, i.e. using food grade materials to stabilise the emulsions, we did not explore alternative methods that are needed to stabilise these droplets at the water in oil interface. Nevertheless we proved the principle that it is possible to make oil in water in oil emulsions.

6.6 Acknowledgement

This work was supported by BBSRC (Biotechnology and Biological Sciences Research Council) industrial case studentship and Unilever. I would like to thank Mr Raamanand Chauhan, my Part II student, for his contribution on the flow-diagram and the work on monodisperse emulsions.

Chapter 7

Conclusions

The original aim of the thesis was to explore how to use polyphenol-polymer interactions to synthesise colloidal particles and thin shells with the ability to stabilise emulsions. We have successfully synthesised particle stabilised emulsions with three different techniques: the production of PVP-TA complexes and shells during the emulsification process, the dispersion of different volumes of soy-bean oil in freshly prepared suspensions of PVP-TA particles, and the controlled formation of emulsion using microfluidics.

The polymer-polyphenol interactions used two families of polyphenols: catechins and tannic acid. Two phase diagrams that summarized how the systems behave in relationship to the molar ratio were built. A range of concentrations and molar ratios for both systems were investigated in order to achieve the best colloidal dispersion in term of particle size distribution and stability against sedimentation. The PVP-catechin systems showed a more complex behaviour with the formation of a surface active polymer network at low molar ratio while PVP-TA system gave the best colloidal dispersion in term of stability against sedimentation. For both systems we established that formation of the complexes is due to two types of interaction: hydrophobic interaction between the phenolic and pyrrolidinone rings and hydrogen bonds between the hydroxyl functions of the polyphenols and the CO-N linkages of the PVP. Soluble complexes have been observed for both system at low molar ratio. Increasing the molar ratio leads to the formation of particles. In both cases we found that for more concentrated PVP solutions, a higher amount of polyphenol was necessary to make insoluble complexes suitable for particle formation. This was due to the fact that a critical molar ratio needed to be reached to have a

number of polyphenols molecules sufficient to cover of the PVP molecules and form colloidal size particles. Only in the case of the catechin-PVP systems did we observe the formation of a polymer network before observing turbidity due to the formation of colloidal particles. This more complex behaviour probably stems from the fact that we have used green tea extract which is a mixture of catechin, epicatechin and epigallocatechin gallate, therefore we envisage some sort of competitive reaction among these different polyphenols molecules and the intermolecular complexation. For the PVP-TA system at molar ratio near the transition between soluble complexes and particle formation, over time the particles sediment and compact into a jelly agglomerate at the bottom of the vial. The formation of this polymer network took weeks instead of occurring almost instantaneously as in the case of the catechin system. The study led to an understanding of how to control the particle size by varying the molar ratio and producing soluble complexes, particles and more complex structures.

Chapter 4 studied different emulsification techniques and their optimisation to produce PVP-TA particles and shells able to stabilize surfactant free emulsions. The role of the oil volume fraction as well as the molar ratio has been analysed in relation to emulsion stability, creaming index and microscopic morphology. The complexes have been shown to stabilize emulsions either through the formation of a continuous shell around the droplets or by Pickering stabilization and steric effects which reduce the collision frequency among droplets. The molar ratio between TA and PVP has been identified as an important factor in determining the type of emulsion. At low molar ratio we observed the formation of coated droplets while at higher molar ratio the emulsions were stabilized by microgel particles dispersed in the continuous water phase. After less than a week all emulsions reached a gelling point that arrested any creaming process. All emulsions prepared showed good stability through time and at low pH. Drastic increase of the pH above 10 accelerates destabilization through coalescence. In this range of pH the emulsions were halochromic which is not surprising since TA is halochromic. The advantage in stabilizing an emulsion with these structures is that, having chosen to disperse food grade oil, the emulsion itself is completely biodegradable therefore these emulsions have the benefit of being environmental friendly.

The rheological property of emulsions stabilized by PVP-TA particles was also explored. In this case we focused on the relationship between oil volume fraction and rheological properties. The different rheological tests showed that PVP-TA particle stabilised emulsions behave like

flocculated dispersions and polymeric gels confirming the qualitative observation on emulsion gelation already seen in the previous experiments. Moreover, in Chapter 4 we speculated that these emulsions were stabilized not only by the particle sitting at the inter-phase as in simple Pickering emulsions but also by steric effects of the complexes suspended in the surrounding water phase which promoted kinetic stabilization of the system by reducing the collision frequency of the droplets. This was confirmed by the rheological data that showed flocculated dispersion behaviour.

Finally, in Chapter 6 we introduced a new technique, microfluidics, which is the science of designing and manufacturing devices and processes that deal with volumes of fluids of the order of nanolitres or picolitres. Keeping within the original purpose of this work, i.e. using polyphenol-polymer interactions to stabilise the emulsions, we produced stable monodisperse emulsions stabilised by non-covalently associated microgel particles and double emulsions. Full stabilization of the latter with surfactants would have been possible but beyond the purpose of this thesis.

The primary goal to utilise usually unwanted interactions and the resulting insoluble polyphenol-polymer complexes to design structures and stabilise emulsions has been fulfilled. Nevertheless, based on the experiments and results presented in this thesis, several suggestions for future investigations can be made. In particular further work could be carried out aimed at probing the non-covalent interactions for these interesting systems: new insight into complex structure and folding of individual complex could be obtained by characterizing the soluble complexes with macromolecular mass spectrometry (electrospray ionization mass spectrometry (ESI MS)); relevant techniques have been described in the paper Probing the Nature of Non-covalent Interactions by Mass Spectrometry [147] and other works focusing on Proteins and lipids. Extending the technique to our polymer-polyphenol systems would be of great interest.

On top of the characterisation work mentioned above, an additional interesting extension of the work is represented by a research aimed to the optimization of the particle shape and density. This could be investigated following, for example, the method for the preparation of spherical polyphenol particles described in [148]; the challenge of such approach would be the modification of the method to make it applicable to food grade materials.

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List of publications

To date the following publications based upon work in this thesis have been submitted or are in preparation:

New gel colloidal particles from polymer polyphenols interaction (Chapter 3)

Surfactant free emulsions stabilized by non-covalently associated microgels (Chapter 4)

Bingham plastic surfactant free emulsions (Chapter 5)

Microfluidics for monodisperse microgel stabilised emulsions (Chapter 6)

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