1 INTRODUCTION

The understanding of the principles governing the kinetics of chemical reactions in confined geometries is of fundamental interest both in biology and the rapidly emerging field of nanotechnology. In these confined geometries, chemical reactions take place under crowded conditions [1-3] or on a single-molecule level within environments of nano- to micrometer dimensions [4] (for recent reviews, see e.g. [5,6]). The outcome of a chemical reaction is strongly affected by this compartmentalisation, i.e. the spatial constraint on the reaction and the availability of reaction partners on the relevant reaction time scales. Furthermore, the dimensionality of the space in which the reaction occurs influences strongly the reactions in confined media [7]. In recent years, many effects of compartmentalisation on reaction kinetics were theoretically predicted. For instance, *Stange* et al. calculated that the activity of enzymes is synchronised when several enzymes are enclosed in a sufficiently small compartment so that diffusive mixing of regulative molecules occurs on the time-scale of the enzymatic reaction [8]. Furthermore, molecular evolutionary processes like the amplification of DNA or RNA [9] are expected to be affected by their isolation on a single-molecule level for the time-scale of the slowest replication event; thus, slow replicators do not compete with fast replicators anymore but proliferate without competition [10]. On the contrary, the cooperatively coupled amplification of pairs of DNA-templates is predicted to be stabilized when being spatially isolated for a certain time: the functional templates form fast replicating pairs which are protected by the spatial isolation instead of being exploited by mutant amplification [11].

Experimentally, the sustainable proliferation of molecular evolutionary systems was shown to depend on their spatial isolation [12]. Synchronisation within a large ensemble of discrete chemical oscillators with non-linear kinetics was also recently reported [13]. As another example, a similar chemical reaction with non-linear kinetics was performed in a water-in-oil microemulsion and yielded stationary *Turing*-patterns which were shown to be controlled by the composition of the microemulsion, namely the radius and the number density of the microemulsion droplets [14]. Of course, spatial constraints are particularly relevant for biological systems, since every living cell or cell organelle represents a reaction compartment, which is usually occupied by only a few molecules of the same type per reaction site. Accordingly, fluctuations in the number of reaction partners and accordingly reaction events are expected to be important, as discussed for biological processes in general [15,16] and gene expression in particular [17]. All these examples show how spatial constraints interfere with the kinetics of chemical and biochemical reactions subjected to such constraints. Obviously, a gradual change of connectivity between single molecule reaction sites should lead to interesting, measurable effects.

Many different kinds of microscopically compartmentalized media have been utilized as a reaction environment for various purposes, e.g. self-assembled systems like micellar- [18,19] and vesicle solutions [20], emulsions [21-24], microemulsions [25], polymer networks [26] and gels [27], or structures like porous materials [28] and microfluidic channels [29]. In these studies (see e.g. [25] for a review), microemulsions were mainly used with the aim to mediate mixing of polar and non-polar reactants to accelerate their reaction by the formation of a large interfacial area. However, microemulsions and micellar solutions have also been applied as reaction media for RNA-polymerisation [30]. In one publication it was reported that even gene expression has been performed in a water-in-oil microemulsion [31]. Most experimental research with the aim to exclusively study confinement effects on chemical kinetics was performed with surfactant-, lipid- and polymer-vesicles [5]. Even complex biological reactions and processes like DNA/RNA-amplification [32-35], catalysis by enzymes [36,37] and ribozymes [38], gene expression [39-41] and expression of a cascade of genes in a gene network [42] have been conducted in vesicles.

Due to their large diameters of a few micrometers up to a few 100 micrometers vesicles allow for a simple observation, manipulation and addressing of the reaction compartments and accordingly relatively simple control of the experiments [43,44]. Furthermore, networks of vesicles interconnected by thin lipid tubes have been generated [45-47] to study the influence of mass exchange between the different compartments on the reaction pathway. However, in such an ensemble of connected vesicles the number of reaction sites is inherently limited. Furthermore, the diffusive transport across the connecting nanometer-sized lipid tubes requires sufficiently large concentrations of diffusing molecules within the compartments in order to form stationary concentration gradients which are necessary to avoid a stochastic coupling between neighbouring sites. Unconnected vesicles show an only very limited mass-exchange across their amphiphile bilayer which represents a barrier especially for extended molecules as proteins or DNA [48]. Thus, both ensembles of isolated vesicles and networks of interconnected vesicles are not suitable to investigate how a gradually changing connectivity by tuneable mass-exchange between the reaction sites affects chemical reactions isolated to a single-molecule level.

In an alternative approach to study the behaviour of a large collection of single molecule reaction sites with continuously variable connectivity, water-in-oil microemulsions with droplet microstructure might be utilized. Microemulsions are thermodynamically stable, macroscopically isotropic, but microscopically structured (length scale 1 – 50 nm) fluids containing at least the three components water, oil and surfactant of which the latter allows for simultaneous solubilisation of the two immiscible components (water and oil) [49-51]. Within these macroscopically homogeneous mixtures nanometer-scale water- and oil-domains coexist, separated by an amphiphilic film which is formed by a monolayer of the surfactant molecules. This surfactant film reduces the interfacial tension of water and oil, which leads to an increase of the interfacial area and thus stabilizes the formation of a variety of different microstructures. Determined by the sample composition as well as the temperature-dependent spontaneous curvature and bending elastic constants of the amphiphilic film, globular-, cylindrical-, network-like- or locally flat structures are found, with the latter being bicontinuous or lamellar [50,51]. In microemulsions with non-ionic surfactant the microstructure can be simply changed by just varying an external parameter like temperature. In this fashion the connectivity of the structure can simply be switched from discrete to connected and vice versa.

Discrete reaction compartments for aqueous reaction media are naturally found in oil-rich, i.e. water-in-oil microemulsions with droplet microstructure. To perform the mentioned studies on biologically relevant processes like enzymatic reactions, sufficiently large water droplets with droplet radii of the order of 50 nm are required to allow for the solubilisation of biological macromolecules. During the last three decades it has turned out that microemulsion systems characterized by large structural length scales can be formulated by using non-ionic surfactants with a large hydrophobic chain length [51-53]. These surfactants are very efficient with respect to solubilisation of both oil and water. However, the large bending rigidity of the amphiphilic film formed by these surfactants also stabilizes liquid crystalline mesophases as e.g. the lamellar phase [51]. Thus, if the hydrophobic chain length of the surfactant molecule exceeds 12 to 14 carbon atoms, extended regions in the phase diagram are occupied by the lamellar phase. This in turn makes the formulation of water-in-oil microemulsions with large structural length scales increasingly difficult. Very recently it has been discovered that amphiphilic block copolymers increase the length scale of the bicontinuous microstructure in microemulsions while lyotropic mesophases are suppressed [54-57]. In these systems, the solubilisation efficiency of the polymer-surfactant mixture is increased, compared to the efficiency of the bare surfactant. Using this efficiency boosting effect of the amphiphilic block copolymers, bicontinuous microemulsions with structure sizes of the order 100 nm have been formulated [58]. Based on measurements of the phase behavior and neutron scattering experiments, the increase in solubilisation efficiency has been interpreted as an increase of the bending elastic constants which, in turn, is caused by the adsorption of the amphiphilic block copolymers to the oil/water interface [59].

Beside the enhanced solubilisation efficiency of surfactant-block copolymer mixtures, another motivation for using these amphiphilic block copolymer additives is the principle of sterical stabilisation of colloidal particles which prevents their aggregation. This effect is well known since in ancient *Egypt*ian recipes the polymeric gum-arabicum was used for stabilization, i.e. for preventing precipitation of carbon black ink (see e.g. chapter 9 in [60] and references therein). Consequently the adsorption of amphiphilic block copolymers to the droplet interface might prevent the microemulsion droplets from coalescence and formation of transient dimers. The rates of inter-droplet mass-exchange might thus be reduced by sterically stabilizing the droplets. Since the typical mass exchange-rates for microemulsions made only from non-ionic surfactants are of the order $k_{ex} \sim 10^6\ s^{-1}$ [61], droplet microemulsions will only provide a compartmentalized environment for reactions which occur faster than the mentioned exchange rates. Thus, for using droplet microemulsions to study the effect of compartmentalisation on slower reactions, the sterical stabilisation of the droplets might be suitable to keep the reaction under spatially discrete conditions for longer reaction time scales of 0.1 - 10 sec. >> 1/ k_{ex}. Careful choice of chain length and chemical properties of the block copolymers and their concentration in the microemulsion will allow to tune the rates of mass-exchange and thus droplet connectivity within the desired range.

This desired gradually changing connectivity between the reaction sites might not only be achieved by the addition of amphiphilic block copolymers, but also by utilizing structural transitions in water-in-oil microemulsions. Spherical droplets are found for high temperatures in these oil-rich microemulsions (made from non-ionic surfactants), since the spontaneous curvature of the amphiphilic film has large absolute (negative) values. At lower temperatures, however, namely at the phase inversion temperature, the spontaneous curvature becomes zero. In this state, for equal volumes of water and oil the sponge-like, symmetrically bicontinuous structure is found [62]. However, connected, asymmetric water-in-oil structures exist at temperatures slightly above the phase inversion temperature as shown by scattering experiments [63-67], NMR-self-diffusion- [68,69] and conductivity measurements [70-73]. Following theoretical models the formation of these networks is related to the existence of “closed-loop”-two-phase regions in the microemulsion phase diagram [74-79]. Recently, the existence of asymmetrically bicontinuous structures was proved by cryogenic electron transmission microscopy experiments [80]. Since these asymmetrically bicontinuous structures occur for unequal volumes of water and oil, they are made of cylinders interconnected by mainly three-fold junctions. Accordingly, the temperature-induced formation of structures with connected topology might allow a more effective mass transport in the system, compared to the mass exchange between the microemulsion droplets.

It can be concluded that water-in-oil-microemulsions might be a suitable medium to study the effect of variable spatial discreteness on biologically relevant chemical reactions. To establish water-in-oil-microemulsions for this purpose, systematic studies of at least two microemulsion properties are required: first the dependence of droplet properties on water- and block copolymer-content of highly efficient water-in-oil microemulsions, and second the structural properties of networks of branched cylinders.

Motivation and Objectives

It was the goal of this work to structurally characterize water-in-oil microemulsions which in the future might be established as nano-structured reaction media to study the effect of spatial compartmentalisation on biologically relevant, chemical reactions. To obtain suitable reaction compartments for this purpose, water-in-oil microemulsions have to be formulated which contain sufficiently large microemulsion droplets with radii around 50 nm. The connectivity between these reaction compartments might either be gradually reduced by adding amphiphilic block copolymers to sterically stabilise the droplets, or increased by inducing the structural transition from droplets to a network of branched cylinders. Accordingly, the structural characterisation of water-in-oil microemulsions presented here should focus on the questions (i) how large microemulsion droplets can be formed, (ii) how the droplet properties are affected by the addition of amphiphilic block copolymers, and (iii) how the droplets undergo a structural transition to the mentioned network structure of branched cylinders.

To obtain large microemulsion droplets with radii around 50 nm, two different paths can be followed. On the one hand very efficient ternary microemulsion systems, on the other hand the efficiency boosting effect of amphiphilic block copolymers added to ternary microemulsions can be used. Prior to any structural characterization by e.g. scattering methods or electron microscopy, the phase behaviour of the respective microemulsions has to be extensively characterized. To further narrow the temperature range in which discrete globular structures can be found in the phase diagram, temperature dependent measurements of the microemulsions electric conductivity should be carried out.

On this basis, a more detailed structural characterisation by scattering methods, e.g. small angle neutron scattering (SANS), should be conducted. Such experiments should allow to determine the dependence of the droplet radius on the sample composition, namely on the water content in the microemulsion and on the polymer content in the polymer-surfactant mixture. Since small angle neutron scattering experiments yield an intensity distribution in reciprocal space, data analysis requires adequate model expressions to obtain real-space structural information. Although many scattering functions for structures with spherical symmetry already exist, an adaptation of these models to the specific requirements of the sample composition and the structural properties of the microemulsion droplets should be conducted, when necessary. Modifications of existing scattering functions should allow to extract additional information concerning the structural properties of the amphiphilic block copolymer molecules adsorbed to the amphiphilic film.

Small angle neutron scattering experiments can also be applied to characterize the structural properties of the networks of branched cylinders. To follow the transition from droplets to networks, temperature-dependent small-angle neutron scattering measurements should be performed. Again, the extraction of structural information in real-space from the scattering data requires adequate model expressions. Since a form factor formalism to analyse the scattering data from network microemulsions does not exist, it was necessary to derive adequate scattering functions which should then be applied to analyse the experimental data.

The thesis is organized as follows: at the beginning, the fundamentals of microemulsions and scattering theory are briefly reviewed. Both the detailed characterisation of the phase behaviour and the temperature-dependent measurements of the electric conductivity of the investigated water-in-oil microemulsions precede a detailed description of the development of the tools to analyse the scattering experiments. The scattering data are then presented and analysed using the different novel scattering functions. Finally, the results of this analysis allow for the systematic structural characterisation of microemulsions with both droplet- and network-microstructure.

2 MICROEMULSIONS

Microemulsions are thermodynamically stable, macroscopically homogenous mixtures of at least three components (see e.g. [49-51] an overview). Of these three components, two are mutually immiscible, like a polar component (e.g. water) and a non-polar component (e.g. an alkane). The third component is an amphiphilic component, i.e. a surfactant, which is miscible with the two other ones and thereby mediates their mixing. These ternary systems show an amazingly rich phase behaviour of one-phase microemulsion states (and lyotropic mesophases), and two- and three-phase states. Thus the understanding of microemulsions requires to study the phase behaviour. Due to its amphiphilic nature, the surfactant preferentially adsorbs to the interface between the two immiscible components. Accordingly, an amphiphilic film is formed that reduces the interfacial tension between these two components and allows the interfacial area to increase. As a consequence, microstructures with a variety of morphologies are formed. In recent years it turned out that the phase behaviour of microemulsions and the temperature-dependent evolution of the microstructure are closely related. It is the aim of this chapter to briefly review this relation.

2.1 Phase behaviour

The main properties of microemulsions can be observed for simple ternary systems that contain water (A), an alkane (B) and a non-ionic surfactant (C) [81]. The phase behaviour of all systems of this type is fairly universal. Under isobaric conditions, the phase behaviour of a ternary system can be represented in a phase prism with the *Gibbs* phase triangle A-B-C as the base and the temperature axis as the ordinate [49,82]. The phase behaviour of the ternary system can be understood by regarding the phase behaviour of the three binary systems water-oil, water-surfactant and oil-surfactant that represent the three sides of the phase prism, as shown in Figure 1 [49,83].

The binary system water-oil (A-B) shows a miscibility gap over the complete experimental temperature range. The phase diagram of the binary oil-surfactant (B-C) mixture shows a lower miscibility gap with an upper critical point cp_α, with a critical temperatures T_α which is mostly below zero degrees *Celsius* (at ambient pressure). The binary system water-surfactant (A-C) shows a lower miscibility gap with an upper critical point. At ambient pressure, the critical temperature is mostly below the melting point of the mixture. For higher temperatures, a closed miscibility gap with lower critical point cp_β at the critical temperature T_β is found. At ambient temperature (and

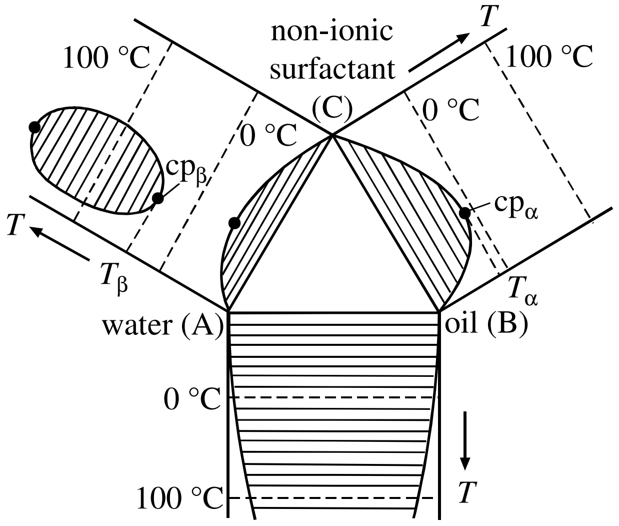

Figure 1: schematic drawing of the unfolded phase prism of the ternary mixture A-B-C, showing the phase diagrams of the three corresponding binary mixtures (taken from [49]).

low surfactant mass fractions, see [84]), however, homogenous mixtures of water and surfactant are found.

2.1.1 The Phase Prism

In Figure 2, a schematic drawing of the phase prism for the ternary system containing water (A), an alkane (B) and a non-ionic surfactant (C) is shown. The overall phase behaviour of these ternary mixtures can be related to the inversion of the surfactant solubility in water and oil when the temperature is increased: with increasing temperature, the solubility of the non-ionic surfactant in oil increases while the solubility in water decreases. The origin of this effect is the partial dehydration of the hydrophilic surfactant head group when the temperature is increased [85,86]. [A second explanation for the temperature-dependent phase behaviour will be given below when the phase behaviour is related to the temperature dependent mean curvature of the amphiphilic film, see subsection 2.2.3.]

Accordingly, the binary water-surfactant mixture phase separates at the critical point cp$_\beta$ when temperature reaches the critical temperature T_β. At this critical point cp$_\beta$, the critical line cl$_\beta$ starts to proceed into the phase prism until it ends in the critical end-point cep$_\beta$ at the temperature T_l. Thus, for low temperatures $T < T_l$, the surfactant-rich oil-in-water microemulsion phase (a) coexists with an oil-excess phase (b) (see Figure 2, test tube at the bottom, and tie lines in the two-phase region).

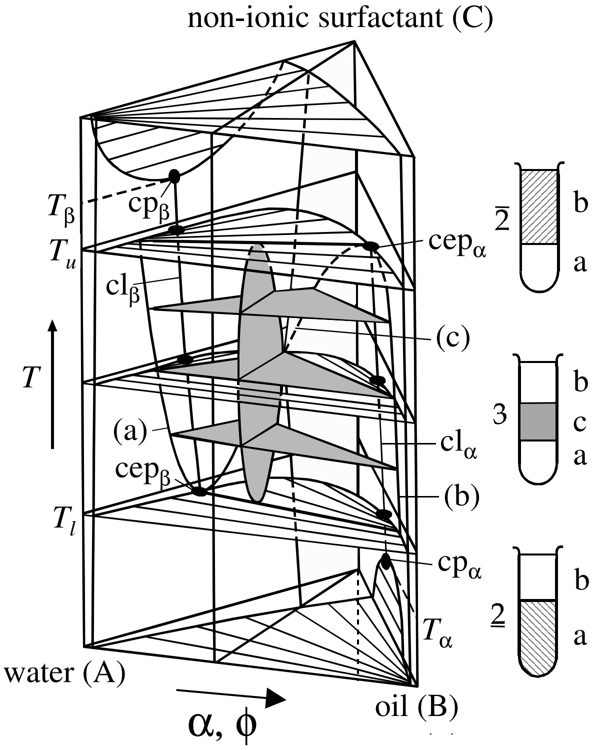

Figure 2: Schematic phase prism illustrating the temperature-dependent phase behaviour of ternary systems containing water (A), oil (B) and surfactant (C). The three test tubes at the right show two- and three-phase states observed experimentally at different temperatures (taken from [50]).

This two-phase state is called $\underline{2}$, since the surfactant-rich microemulsions phase is the high density phase (for an oil-component that has a lower density than water). At the temperature $T = T_\mathrm{l}$, the oil-in-water microemulsion separates into an water-excess phase (a) (see Figure 2, test tube in the middle) and a surfactant-rich microemulsion middle-phase (c), yielding a three-phase state denoted by 3 in Figure 2. Due to the inversion of surfactant solubility with increasing temperature, the major component of the surfactant-rich middle phase (c) changes from water at low temperature $T \approx T_\mathrm{l}$ to oil at high temperature $T < T_\mathrm{u}$: the composition of the microemulsion phase changes from water-rich to oil-rich when the temperature is increased.

Furthermore, with increasing temperature, the composition of the oil-excess phase (b) approaches the composition of the middle-phase (c), until both compositions are equal at the critical end-point cep_α at temperature T_u. At this temperature, the critical line cl_α meets the central miscibility gap. For higher temperatures $T > T_\mathrm{u}$, the surfactant is mainly solved within the oil-rich phase (b), namely the water-in-oil microemulsion which coexists with a water-excess phase (a) (see top test tube in Figure 2 and tie lines in the two-phase region for $T > T_\mathrm{u}$). This two-phase state is denoted $\overline{2}$, since the surfactant-rich phase is the low density phase. The temperature-dependent progression