Synthesis and Study of the Aggregation Behavior of Hydrophilically Modified Polybutadienes

Dissertation

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“A good scientist is a person in whom
the childhood quality of perennial curiosity lingers on.
Once he gets an answer, he has other questions.”

Frederick Seitz
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1 INTRODUCTION

Well defined homopolymers and block copolymers containing covalently attached reactive functionalities have attracted great interest for making materials with tailored properties. However, polymers bearing functionality are difficult to prepare, and structural diversity can be introduced only with large synthetic effort. An alternative pathway is to use well-defined polymers, obtained by controlled polymerization techniques, as precursors for further modifications. A convenient tool for a one-step modification of a polymer precursor appears to be “click chemistry” or, for instance, the free-radical addition of $\omega$-functional mercaptanes onto vinyl double bonds. The commercial availability of various functional mercaptanes enables one to produce a toolbox of functional polymers.

The objective of this thesis is to generate a modular platform of hydrophilically modified 1,2-polybutadiene (1,2-PB) homopolymers and block copolymers, containing either poly(ethylene oxide) (PEO) or poly(2-methyl-2-oxazoline) (PMOxa) as a second block segment. The following investigations concern the influence of the introduced hydrophilic groups on the self-organization of the modified products. The ability to synthesize polymers with desired architecture facilities as well as the ability to manipulate their aggregation behavior, provides the key to nanotechnology.

Hydrophilically modified polybutadienes, which are closely related to the polymeric amphiphiles introduced by Ringsdorf et al., should be useable for the formation of polymer colloids.

Having a toolbox of various functional block copolymers in hand allows for a systematic study of the aggregation behavior. Additionally, prepared block copolymers bearing mutually interacting ionic groups provide an interesting opportunity to prepare self-assembled systems by weak electrostatic interactions. Interestingly, polyion complexation of oppositely charged polyelectrolyte block copolymers could provide aggregates with broken or reduced symmetry, that are Janus-type micelles or vesicles with a microphase-separated membrane, if the solvating corona comprises two chemically different chains.

In Section 4.1, an overview of functionalization of 1,2-PB-based polymers by radical addition of $\omega$-functional mercaptanes will be given. Different polymers with acid, primary amine, amino acid, diol functionalities are described and mechanism of the radical addition is discussed.
Section 4.2 describes the formation of homopolymer assemblies. The aggregation behavior of obtained ionic homopolymers in water is described. Then, the self-organization of diol modified 1,2-PB in ethanol/water mixture is presented.

Section 4.3 describes block ionomers self-assembly. Formed aggregates in aqueous solutions of block copolymers with introduced charge functionality are characterized.

In Section 4.4, the ability of the formation of polyion complexes with (i) PEO/PEO and (ii) PEO/PMOxa corona via electrostatic interaction is emphasized. Structural diversity in these systems can be generated in a simple way, i.e. by mixing components caring complementary recognition sites. A challenging task was to obtain asymmetric polymeric aggregates with broken or reduced symmetry.
2 FUNDAMENTALS

2.1 Block copolymers

Prior to the early 1920’s, chemists doubted the existence of molecules having molecular weights greater than a few thousand. Hermann Staudinger, who formulated a polymeric structure for rubber, based on a repeating isoprene unit, challenged this limiting view. The terms polymer was derived from Greek roots poly (many) and meros (part).

Block copolymers are a special type of polymers in which each molecule consists of two or more different segments of varying monomers joined in some architecture by a covalent bond. Hence, block copolymers can be classified by the number of blocks and their arrangement. Block copolymers with two, three, and more blocks are called diblocks, triblocks, and multiblocks. Some topology can be linear, in which the blocks are connected end-to-end, and stars, where blocks are connected via one of their ends at a single junction. More complicated arrangements like brushes, (4-)miktoarm stars, or H-shape are also possible. Various schematics arrangements of block copolymers are presented in Figure 2.1.

![Figure 2.1](image)

---

Hence each block represents different physicochemical behavior it is possible to obtain wide range of interesting properties in single macromolecule.

Recently, many synthesis techniques have been widely extended to prepare block copolymers with well-defined composition, molecular weight, and structure. Beside classical synthetic routes as for example living anionic and cationic polymerization in recent years new polymerization methods have generated intense interest among polymer chemists. Rapidly developing field in polymer chemistry is controlled radical polymerization, including three main types of techniques, as Nitrooxide Mediated Polymerization (NMP), Atom Transfer Radical Polymerization (ATRP), and Reversible Addition-Fragmentation Chain-Transfer
Polymerization (RAFT). The main advantage of these methods is their tolerance to a large variety of functional groups, low sensitivity to impurities and wide range of used reaction solvents including water.\textsuperscript{12,13} Beside controlled polymerization, block copolymers can be also prepared by coupling end-functionalized polymers or for instance chemical modification of a given homopolymer by hydrogenation or hydrolysis.\textsuperscript{14}

### 2.2 Anionic Polymerization

Anionic polymerization has been the first technique for the preparation of well-defined polymers. The processes of anionic polymerizations include three main kinetic steps as: initiation, propagation, and termination. The initiators of anionic polymerization are mainly organometal compounds, alkoxides, amides, and their compositions with various additional agents. The propagation proceeds when the terminal atom of growing chain has a partial or complete negative charge. The main types of active centers in anionic polymerization are contact ion pairs, solvent separated ion pairs, and free carboanions. The coexistence of various forms of active centers is presented via Winstein diagram\textsuperscript{15} (Figure 2.2).

![Winstein diagram](image)

Figure 2.2. Winstein diagram illustrating coexistence of various active sites in anionic polymerization.

Each from these forms of reaction intermediates (ion pairs) can attend in anionic polymerization as reactive propagating species. Equilibrium between different ion pairs affects on polymerization rates as well as stereospecificities and can be controlled by the monomer type, reaction medium (solvent and additional agent, ligands, salts, etc.) and temperature.\textsuperscript{16,17,18}

Anionic polymerization can be also performed in the “living way”, what was described first time in 1956 by Szwarc et al.\textsuperscript{19} In ideal case, there is neither a termination reaction nor chain transfer. After an instantaneous initiation polymers retain their activity after reaction has been
completed, and continue their propagation after the new monomer addition. The kinetics of such an ideal system is represented by following equation:

\[-\frac{d[M]}{dt} = k_p[P^*][M]\] (2.1)

where $[M]$ is the concentration of monomer, $[P^*]$ the concentration of active centers, and $k_p$ the rate constant of propagation.

The degree of polymerization, $DP$, of the synthesized polymer can be calculated from the initial molar molar ratio of monomer, $[M]_0$ and initiator, $[I]_0$, and the monomer conversion, $X_P$:

\[DP = \frac{[M]_0}{[I]_0} X_p\] (2.2)

Living anionic polymerization provides control of molecular weight, leading nearly monodisperse polymers, characterized by a Poissonian distribution, as:

\[
\frac{M_w}{M_n} = 1 + \frac{DP}{(DP + 1)^2}
\] (2.3)

for $DP>>1$

\[
\frac{M_w}{M_n} = 1 + \frac{1}{DP}
\] (2.4)

Living anionic polymerization became possible as a result of perfecting the methods of reagents purification and most importantly, because of the development of the fundamental concepts of the mechanism of single stages in anionic processes.

Among the polymers that have been prepared by anionic polymerization are for instance polystyrene (PS), polybutadiene (PB), poly(methacrylate) (PMA), poly(ethylene oxide) (PEO) and poly(dimethylsiloxane) (PDMS).^{10,20,21}

A wide range of block copolymers is also available by anionic polymerization. They can be obtained by sequential addition of the monomers, either by adding directly the second monomer on the living first block,^{22,23} or by end-capping the first block with suitable reagents.^{24,25} Some of the block copolymers have become commercially available, for instance PS-PB-PS (Kraton) which are used as thermoplastic elastomers, PEO-PPO-PEO (Pluronics).
used as surfactants and steric stabilizers, or PS-PB star (Styrolux) from BASF which is suitable for injection molding, extrusion and thermoforming.

The main limitation in the synthesis of block copolymers by anionic polymerization is its application to a limited number of monomers. Difficulties in polymerization can be encountered in reactions with polar monomers, bearing carboxyl, amino or hydroxyl groups, which may participate in termination or chain transfer, but using suitable protecting groups can solve this limitation. In addition, relative reactivity of the monomers has to be taken into account during polymerization of block copolymers to obtain product with desired sequence.

Anionic polymerization combined with other polymerization methods is an efficient tool for obtaining new polymer materials as well as single elements of complex polymer system.

### 2.3 Cationic polymerization

The discovery of living cationic polymerization in the mid-1980s has provided a valuable new tool in the synthesis of well-defined macromolecules with controlled molecular weight, narrow molecular weight distribution, and high degree of compositional homogeneity. Cationic polymerization is initiated by electrophilic agents such as the protonic acids, Lewis acids and compounds capable of generating carbenium ions.

The propagation proceeds when the terminal atom of growing chain bears a positive charge. The most common active sites are either carbenium ions or oxonium ions. Carbenium ions possess strong electrophilic character which effect in very rapid reaction with monomer, what can cause many ineligible side reactions as chain transfer or even termination. Stabilization of active sites can be obtained using nucleophilic additives (counterions, esters, and ethers), which provide reversible termination/reinitiation in propagating chains. Reversible deactivation of the active centers, in meaning of dynamic equilibrium between active and dormant species, as illustrated in Figure 2.3, decreases the rate of propagation resulting in better control of the polymerization process.
Figure 2.3. Schematic illustrations of active-dormant species equilibrium in living cationic polymerization.

Such a stabilization of the carbocationic active centers yields conditions of cationic polymerization about living.

The monomers suitable for cationic polymerization include all those with electron donating substituents, in which the polarization of the double bond makes them sensitive on electrophilic attack by a carbenium site. Well known monomers used for cationic polymerization are isobutylene\textsuperscript{29,27}, styrene\textsuperscript{30}, vinyl ethers.\textsuperscript{31} Also a wide range of heterocyclic monomers can be polymerized by cationic polymerization. A typical example of cationic polymerization is the ring-opening polymerization of 2-alkyloxazolines.\textsuperscript{32} Kim et al. has mentioned the preparation of biocompatible block copolymers as poly(2-ethyl-2-oxazoline)-b-poly(L-lactide).\textsuperscript{33}

Solvents used in cationic polymerizations should be stable towards acids and unable to react with electrophiles. Preferred are halogenated solvents (methylene chloride, methyl chloride, ethylene chloride, carbon tetrachloride), nitro compounds such as nitromethane or nitrobenzene, but also dioxane.

### 2.4 Polymer analogue reactions

Development of living polymerization technique has provided wide range of material with interesting properties. However, the range of monomers, which are used for the preparation of functionalized block copolymers, is limited. Polymer analogue reaction has captivated both academic and industrial interest towards broadening the diversity of functional polymers. Preferably, the reactions start from well-defined polymers with predetermined molecular weight, narrow molecular weight distribution and desired architecture. Using chemical modification, polymer precursors are further transformed into a material with eligible functionality or solubility, without any degradation.\textsuperscript{1} Admittedly, the molecular architecture and polydispersity ought to stay unchanged.
An example of polymer analogue reaction is the hydrolysis of poly((meth)acrylic ester)s to obtain poly((meth)acrylic acid). Ramireddy et al. reported hydrolysis of the tert-butyl esters with concomitant elimination of iso-butylene. Hydrolysis of a trimethylsilyl methacrylate block with aqueous methanol leads to the formation of a methacrylic acid block. Another important class of polymer analogue reactions is the hydrogenation of unsaturated polymers. Polydienes can be hydrogenated with tosylhydrazide, by homogeneous Wilkinson’s catalyst, or by heterogeneous Pd/CaCO₃ catalyst.

In many polymer analogue reactions the final product is obtained after sequential reactions. First, the precursor block is transformed into reactive intermediates, which can then be reacted with an appropriate functional component. Reactive intermediates obtained, for example, by epoxidation allow a variety of oxirane ring opening reaction to be applied to introduce different functionalities. Another possibility to introduce functional side groups is hydroxylation via hydroboration with 9-borabicyclo[3,3,1]nonane, followed by oxidation with H₂O₂/NaOH. Also esterification of the hydroxyl groups in polymers with functional acid chlorides leads to the transformation of a precursor polymer into a material with different properties. The polymer analogue reactions are not only limited to one block. They can be prepared in one polymer on different blocks with different functionalities, however in a sequential, selective manner. Müller et al. reported preparation of poly(styrenesulfonate)-b-poly(ethylethylene) block copolymer by anionic sequential polymerization of styrene and butadiene (1,2-polymerization) followed by hydrogenation of the remaining double bonds of the butadiene block and subsequent sulfonation of the styrene block.

An efficient tool for a one-step modification of polymer precursors is the free-radical addition of ω-functional mercaptanes or alkyl iodides onto vinyl double bonds. Serniuk et al. used the addition of thyl radicals on the 1,2-units of a polybutadiene (PB) chain to obtain further information on the structure of butadiene polymers and copolymers. Mercaptanes were also used for the modification of natural and synthetic rubbers. Boutevin et al. used 2-mercaptopoethanol to increase the hydroxyl functionality of PB. The same group also described the synthesis of phosphonated PB by grafting PB with a phosphorus-containing mercaptan, an interesting material for the preparation of fire retardant polyurethane networks. However, in neither case did the addition of the mercaptan go to completion. Schapman et al. carried out the modification of PB with (3-mercaptopropyl)triethoxysilane and studied the influence of the mercaptan and initiator concentration on the progress of the
In the case of the modification of butadiene containing copolymers, the free radical addition of mercaptanes has been employed to introduce carboxyl and ester functionalities into styrene/butadiene random copolymers (SBR).\cite{51,52} Schlaad et al. demonstrated the applicability of the free-radical addition of \( \omega \)-functional mercaptanes onto 1,2-PB based homopolymers and block copolymers as a modular synthetic pathway toward a toolbox of diverse functional block copolymers.\cite{53,54} Functional group included, for instance electrolytes (carboxylic acid and amine), L-amino acids, and fluorocarbons.

An important contribution towards the modification of polymers after the successful achievement of a polymerization process represents the azide/alkyne 'click' reaction (also termed the Sharpless 'click' reaction).\cite{55} Of the reactions comprising the click universe, the “perfect” example is the Huisgen 1,3-dipolar cycloaddition between C–C triple, C–N triple bonds, and alkyl-/aryl-/sulfonyle azides.\cite{56,57}

The combination of anionic polymerization with click reactions has been reported by Thomson et al.\cite{58} They used 1-Ethyl-4- hydroxybenzene as a monomer in several block copolymers (e.g., PMMA, PS) and subsequently transformed into the corresponding oxypropynes by classical Williamson or Mitsunobu strategies. The click reactions could then be performed on these substrates. Jordan has reported an example of cationic polymerization of (1,3-oxazolines) using 2-(pent-4-ynyl)-2-oxazoline as the monomer.\cite{59} Matyjaszewski and co-workers demonstrated a combination between ATRP and an azide/alkyne click reaction using the reaction between pendant nitrile-moieties and sodium azide.\cite{60} In the Huisgen 1,3-dipolar cycloaddition reaction, the azide was fixed onto the side chains of polyacrylonitrile or poly(acrylonitrile-styrene) block copolymers. Lutz et al. reported the click reaction with various terminal acetylenes (propargylic alcohol, propiolic acid, 2-methylbut-1-en-3-yne) conducted on azide end-group in polystyrene using the CuBr/4,40-di-(5-nonyl)-2,20-bipyridine catalytic system in tetrahydrofuran (THF) as solvent.\cite{61,62}
2.5 Phase behavior

2.5.1 In bulk

Amphiphilic molecules have received considerable attention for their ability to partition chemically immiscible compounds into complex periodic geometries with long-range order. A block copolymer consisting of chemically different blocks joined together in a single molecule exhibits the tendency for the blocks to be separated, inducing amphiphilic behavior. Various arrangement of microphase-separated block copolymers have been documented. The classical structures are lamellae (LAM), hexagonally packed cylinders (HEX), body-centered cubic (BCC) array of spheres, and a bicontinuous gyroid structure (Figure 2.4).

![Common geometries of microphase-separated block copolymers](image)

Figure 2.4. Common geometries of microphase-separated block copolymers.

For many years, considerable effort has been made to develop a microscopic theory of the molten block copolymers. Helfand has proposed a self-consistent field method to obtain the free energy of molten block copolymers in terms of molecular parameters as molecular mass, concentration, monomer length, and monomer interaction energy. However, the application of the method was limited only to ordered structure. In 1980, Leibler has extended the study of microphase separation to disordered phase. He proposed that the basic parameters, which determine the size and shape of the microdomains for AB-diblock copolymers, are the following:

- degree of polymerization, \( N \), resulting from the sum of the degrees of polymerization of the individual blocks, \( N = N_A + N_B \)

- copolymer composition, \( f \), describing the geometry factor, \( f_A = N_A / N \)

- Flory-Huggins interaction parameters, \( \chi \), representing the strength of repulsive interaction between two blocks.
According to Leibler, the microphase separation of block copolymers can be illustrated in a schematic phase diagram, parameterized in terms of $\chi N$ versus the block length ratio (Figure 2.5). Matsen and Bates derived an exemplary phase diagram illustrating formation of different types of bulk morphologies.\textsuperscript{71}

\textbf{2.5.2 In solution}

Block copolymers in solvents that are selective for one of the block may exhibit self-assembly behaviors,\textsuperscript{72,73,74} wherein spherical and cylindrical micelles as well as more complex, vesicular structures are usually observed (Figure 2.6).

The effective way to modulate morphology of block copolymers in solution includes varying chemical structures, compositions and architectures of copolymers.\textsuperscript{75,76,77,78,79,80,81} Thermodynamically, the final structure of assemblies is determined by a delicate balance among the interfacial energy of the soluble/insoluble interface, intercoronal chain interactions, and
entropy losses due to the insoluble blocks packed into aggregate microdomains. The balance is signified by the so-called critical packing parameter, \( P \):\(^82\)

\[
P = \frac{v}{a \cdot l}
\]

(2.5)

where \( v \) is the hydrophobic volume of the amphiphile, \( a \) the interfacial area, and \( l \) the chain length normal to the interface. It determines the curvature of the hydrophobic-hydrophilic interface as described by its mean curvature, \( H \), and its Gaussian curvature \( K \), which is given by the two radii of curvature \( R_1 \) and \( R_2 \) (Figure 2.7).

\[
H = \frac{1}{2} \left( \frac{1}{R_1} + \frac{1}{R_2} \right)
\]

\[
K = \frac{1}{R_1 R_2}
\]

Figure 2.7. Description of amphiphile shape in terms of packing parameter and its relation to the interfacial mean curvature (H) and Gaussian curvature (K):\(^83\)

The critical packing parameter is related to the curvature by:\(^84\)

\[
P = 1 + Hl + \frac{Kl^2}{3}
\]

(2.6)

The illustrative examples of aggregate morphologies related to packing parameter are presented in Table 2.1.

<table>
<thead>
<tr>
<th>Packing parameter ( P )</th>
<th>Molecule Geometry</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/3</td>
<td>![image]</td>
<td>Spherical micelles</td>
</tr>
<tr>
<td>1/3 – 1/2</td>
<td>![image]</td>
<td>Cylindrical micelles</td>
</tr>
<tr>
<td>1/2 - 1</td>
<td>![image]</td>
<td>Vesicles, flexible bilayers</td>
</tr>
<tr>
<td>1</td>
<td>![image]</td>
<td>Lamellae, planar bilayers</td>
</tr>
<tr>
<td>&gt; 1</td>
<td>![image]</td>
<td>Reverse structure</td>
</tr>
</tbody>
</table>
For micellar aggregation, an important parameter related to the size of the micelles is the aggregation number, $Z$, which is described as:

$$Z = Z_0 N_A^2 N_B^{-0.8}$$  

(2.7)

where $N_A$ is the degree of polymerization of the insoluble block and $N_B$ is the degree of polymerization of the soluble block. The parameter $Z_0$, related to the interaction parameter $\chi$, the monomer volume and the packing parameter is characterized as follow:

$$Z_0 = 36\pi N_B^{6\varepsilon} \left(\frac{V}{a l}\right)^3$$  

(2.8)

Depending on the relative block lengths of the individual segments $N_A$ and $N_B$, three types of micellar structures can be distinguished:

- if $N_A << N_B$, meaning that the core is much smaller then the corona, a “hairy micelles” are formed
- conversely, if $N_A >> N_B$, where the core is much bigger then the corona, a “crew cut micelles” are formed
- “amphiphilic micelles”, when the large interaction parameter $\chi$ is observed

The three different micellar types exhibit characteristic scaling relations with the radius, $R$, the degree of polymerization and the aggregation number, as summarized in the Table 2.2.

<table>
<thead>
<tr>
<th>Micelles</th>
<th>Radius, $R$</th>
<th>Aggregation Number, $Z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy micelles</td>
<td>$R \sim N_A^{3/5}$</td>
<td>$Z \sim N_A^{4/5}$</td>
</tr>
<tr>
<td>Crew cut micelles</td>
<td>$R \sim N_A^{2/3}$</td>
<td>$Z \sim N_A$</td>
</tr>
<tr>
<td>Amphiphilic micelles</td>
<td>$R \sim N_A$</td>
<td>$Z \sim N_A^2$</td>
</tr>
</tbody>
</table>

A vesicular structure can be compared to a pulled bag. The formation of vesicles proceeds in two steps. First a bilayer is form and then the bilayer can bend to form closed vesicles. Figure 2.8 illustrates the schematic formation of bilayers and their closure to vesicular structure.
There are numerous shapes of vesicles, as spherical, tubular, oblate, or stable high-genus vesicle characteristic for polymers. Architecture of polymer vesicles has been studied by Eisenberg.87

Polymer vesicles present numerous possibilities of adjustable physical, chemical and biological properties by variation of block lengths and chemical structure. Many function can be introduce simultaneously either in a single polymer chain, or for instance by mixing another polymer into bilayer. This open a broad field for functionalizing polymer aggregates for wide application.83

### 2.6 Polyion complex aggregates

The controlled association of diblock copolymers has attracted much interest in the fundamental and applied fields of polymer science.75,88 However, the phase behavior can be significantly changed when additional energy from noncovalent interactions comes into play. Electrostatic interactions appearing in the mixture of oppositely charged chains were found to act as a driving force for the formation of polyion complexes (PIC) or interpolyelectrolyte complexes (IPEC) of oppositely charged block ionomers. One of the main advantages of such a complexation is their ability to form a very broad range of micellar and vesicular morphologies and other unusual superstructures. Kataoka et al. have reported the preparation of stable and monodispersive PIC micelles from a mixture of oppositely charged poly(ethylene glycol)-poly(L-lysine) and poly(ethylene glycol)-poly(α,β-aspartic acid) block copolymers in aqueous medium89 and have studied chain length recognition in the micellar core.88 In their presented system micellization was only possible between matched block lengths. Justynska90 has prepared polyion complex using poly(ethylene oxide)-polyanion and poly(ethylene oxide)-polycation block copolymers with unequal lengths of the oppositely charged block. She has observed that formation of PIC in her system has been independent on the length of charged blocks. Interestingly self-assembly behavior through electrostatic interaction has been presented by Voets et al. who prepared disc-shaped micelles by mixing in water two linear, double hydrophilic block copolymers, that has been poly(acrylic acid)-
poly(acryl amide) and poly(2-methylvinylpyridinium iodide)-poly(ethylene oxide). Most interestingly, the obtained micelles had a core consisting of mixing oppositely charged chains, and an unusual asymmetric water-swollen corona, which has been microphase-separated into distinct domains or faces. The overall structure is called “Janus-type” (Figure 2.9).

The group of Gohy has also prepared block copolymer micelles of the second type, which are based on core-forming \textit{IPEC}, mixing a polystyrene-block-poly(2-vinylpyridine)-block-poly(ethylene oxide) triblock copolymer, with a triblock copolymer consisting of a poly(acrylic acid) central block and PEO macromonomer-based outer blocks. Electrostatic interaction between PAA and the P2VP block resulted in the formation of a dense inner micellar core. The outer corona contained both PS and PEO chains has not led to the previously reported “Janus” morphology, but has segregated into interesting comb-like structure (Figure 2.10).

The concept of polyanion complex aggregation with reduced symmetry has been applied by the group of Schlaad, who prepared asymmetric vesicles from pair of oppositely charged ionomers having different solvating blocks. Obtained vesicles were amphiphilic in nature and possessed an inside/outside broken symmetry (Figure 2.11). Solvent changes or specific
binding to the outer polymer could lead to spontaneous vesicle inversion. Luo and Eisenberg have also reported on vesicular structures with preferentially segregated acidic and basic corona chains.93,94

The described examples illustrate that controlled application of secondary interaction provides an effective tool for obtaining interpolyelectrolyte complexes just by mixing linear, flexible water-soluble copolymers. Such a modular concept allows producing a library of complex superstructures with adjustable properties for special applications.
3 CHARACTERIZATION METHODS

3.1 Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC), also called gel permeation chromatography (GPC), is a chromatographic method in which particles are separated based on their hydrodynamic volume into their discrete components. This method is considered one of the most important techniques for the determination of molecular weight ($M_w$) and molecular weight distribution of polymers. The analytes are dissolved in good solvent (mobile phase) and therein transported through a column packed by porous particles (stationary phase). The stationary phase can be either microporous glass beads or a swollen polymer gel. Inside the column, molecules are separated by whether or not they can fit within the pore size of the packing material. Smaller molecules can penetrate the pores and hence its average migration rate is slow. When a molecule experiences steric hindrance in permeation of a pore, then is excluded and continues following the solvent flow. Hence, in SEC, molecules with larger hydrodynamic volume will reach the end of the column before the smaller molecules. In ideal SEC, there is no enthalpic interaction ($\Delta H=0$).

The concentration of the eluted molecules, monitored by a suitable detector, is plotted as a function of time or elution volume ($V_e$). The separation process is illustrated in Figure 3.1.

\[
V_e = V_0 + K_{SEC} V_i
\]  

(3.1)

Where $V_0$ is exclusion volume of the mobile phase, $V_i$ is the volume inside the pores, and $K_{SEC}$ is the distribution coefficient.
SEC is a relative method, therefore molecular weight of analyzed polymers can be determined after calibrating the system in terms of the elution volume. Calibration is performed using narrow molecular-weight polymers standards, which have been previously well characterized regarding their molecular weight, using absolute methods, such as NMR, osmometry, LS. Plot of the molecular weight ($M_w$) of the standards versus their elution volume gives the calibration curve ($logM = f(V_e)$). The calibration curves start with the totally excluded $M_w$ and ends with the totally penetrating $M_w$. With a calibration curve, it is possible to determine the molecular weight for a polymer. The SEC curve of a polymer is divided into vertical segments of equal retention volume. The high or area of each segment and the corresponding average molecular weight, calculated from the calibration curve, are the used for molecular weight calculation. If we assume that the molecules in each slice, $i$, following separation by SEC are monodisperse, the mass moments of each peak selected are calculated by definitions\textsuperscript{95,96} as:

\[
M_n = \frac{\sum n_i M_i}{\sum n_i} = \frac{\sum c_i}{\sum c_i / M_i}
\] \hspace{1cm} (3.2)

for the number-average molar mass, where $n_i$ is the number of molecules of mass $M_i$ in slice $i$ and the summations are over all the slices present in the peak; the concentration $c_i$ of the $i$th species, therefore, is proportional to $M_i n_i$. 

\[
M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} = \frac{\sum c_i M_i}{\sum c_i}
\] \hspace{1cm} (3.3)

for the weight-average molar mass; and 

\[
M_z = \frac{\sum n_i M_i^3}{\sum n_i M_i^2} = \frac{\sum c_i M_i^2}{\sum c_i M_i}
\] \hspace{1cm} (3.4)

for the z-average („centrifuge“) molar mass.

The samples in which all of the molecules have a single molecular weight ($M_w = M_n$) are called monodisperse polymers. The degree of polydispersity, the ratio of the weight to number average ($M_w/M_n$) obtained from equations (3.2) and (3.3), respectively, describes the spread of the molecular-weight-distribution curve.
In case of polymers, for which a suitable calibration curve is not available, it is possible to apply a so-called “universal calibration”. The concept of universal calibration allows one to estimate the molar mass of an eluting fractionation by measuring only the intrinsic (or “limiting”) viscosity, \([\eta]\) and plotting the logarithm of the hydrodynamic volume, \(M[\eta]\) against the elution volume. The intrinsic viscosity can be calculated as follow:

\[
[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}
\]

where \(c\) is the concentration and \(\eta_{sp}\) is the specific viscosity defined by:

\[
\eta_{sp} = \frac{\eta - \eta_0}{\eta_0}
\]

where \(\eta\) is the solution viscosity and \(\eta_0\) is the viscosity of the pure solvent.

For linear polymers, the intrinsic viscosity is related to the polymer molecular weight by the Mark-Houwink-Sakurada equation:

\[
[\eta] = KM^\alpha
\]

where \(K\) and \(\alpha\) are the Mark-Houwink-Sakurada coefficients.

The molecular weights and PDI of the homopolymers described in this thesis were determined using a calibration curve from 1,2-PB standards. Molecular weight values for diblock copolymers were calculated combining SEC with \(^1\)H NMR. Knowing the molecular weight of the first block, the \(DP\) of the second block was calculated from the peak integrals assigned to the different comonomer in the \(^1\)H NMR spectrum.

### 3.2 Light scattering methods

Light scattering occurs when polarizable particles in a sample are bathed in the oscillating electric fields of a beam of light. The varying field induces oscillating dipoles in the particle, and these radiate light in all direction.

In 1869 John Tyndall found that different sized particles scattered light in different ways. Two years later, Lord Rayleigh described the first theory of light scattering based on the theory of electromagnetic waves.
3.2.1 Dynamic light scattering (DLS)

One of the most popular techniques in colloid and polymer analysis is Dynamic Light Scattering (DLS). This method enables the determination of the hydrodynamic radii and polydispersity of a colloidal system. In DLS, monochromatic light with intensity $I_0$, passes through a dilute solution of suspended particles, and the latter scatters light in all directions relative to the incident beam. The intensity of the scattered light $I(\theta)$ is detected at an angle $\theta$ to the incident beam direction and at the distance $r$ between sample and detector, as shown in Figure 3.2.100

![Figure 3.2. Schematic illustration of the scattering process.](image)

The scattered light coming from each particle has a specific amplitude and phase. At the detector, all theses small scattered light amplitudes interfere and add up to a different sum, which depends on the relative phases of the moving particles. The variation in the intensity of scattered light $I(\omega)$ over a frequency range from $-\infty$ to $+\infty$ is given by:

$$I(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} g_1(t) \exp(-i\omega t) dt$$

(3.8)

where $\omega$ is the difference between the angular frequency of the scattered and that of the incident light angle. $g_1(t)$ is the electric field autocorrelation function.

The intensity-time correlation function $g_2(t)$ is expressed as:

$$g_2(t) = \frac{\langle I(t)I(t+\tau) \rangle}{\langle I(t) \rangle^2}$$

(3.9)

The correlation function of the electric field $g_1(t)$ can be derived using the Siegert relation.101

$$g_1(t) = \sqrt{\frac{g_2(t)-b}{b}}$$

(3.10)
where $b$ is the experimentally determined baseline.

For a highly diluted and monodisperse polymer system with $qR_g < 1$, $g_1(t)$ can be expressed as a single exponential function:

$$g_1(t) = B \cdot \exp(-q^2Dt)$$  \hspace{1cm} (3.11)

where $B$ is the signal to noise ratio and $D$ is the translational diffusion coefficient.

For large particles and polydisperse polymer systems ($qR_g > 1$), $g_1(t)$ has a multi-exponential decay. Thus, this function has to be considered as a superposition of several exponential functions with different diffusion coefficients:

$$g_1(t) = \left\{ e^{-\Gamma t} \right\} = \int_0^\infty e^{-\Gamma t}G(-\Gamma t)d\Gamma$$  \hspace{1cm} (3.12)

where:

$$\Gamma = D_{app}(q)q^2$$  \hspace{1cm} (3.13)

In the limit of small $q$ one obtains:

$$\lim_{q \to 0} D_{app}(q) = D_z$$  \hspace{1cm} (3.14)

where $D_z$ is the z-average diffusion coefficient.

Assuming that molecules obey Brownian motion, and considering $R_h$ to be the radius of a sphere with equivalent friction, by using Stokes-Einstein equation the average hydrodynamic radius, $R_h$, of spherical particles can be calculated:

$$R_h = \frac{k_B T}{6\pi \eta_0 D}$$  \hspace{1cm} (3.15)

where $k_B$ is the Boltzmann constant, $T$ absolute temperature, and $\eta_0$ the viscosity of the solvent.

For the evaluation of the data in this thesis, intensity-weighted particle size distributions were calculated from the measured time-correlation functions, using FASTORT.EXE.\textsuperscript{102,103}
3.2.2 Static light scattering (SLS)

Static light scattering (SLS) is a technique used for the characterization of particles in the solution according their mass, size, and shape. This method is based on the polarizability generated within the molecules when an electromagnetic wave interacts with their electrons, inducing a dipole moment. The intensity of elastically scattered light is measured as a function of scattering angle. The ratio of the scattered light $I(\theta)$, to the incident light $I_0$ per scattering center is described with:

$$\frac{I(\theta)}{I_0} = \frac{16\pi^2 \alpha^2}{\lambda_0^2 r^2}$$

(3.16)

where $\alpha$ is the polarizability of the molecules, $\lambda_0$ is the vacuum wavelength of the light, and $r$ is the distance between sample and detector. Debye\textsuperscript{102} showed that for a liquid two-component system the observed light scattering intensity is related to the osmotic pressure of the polymer as:

$$\frac{Kc}{R(\theta)} = 1 + \frac{\partial \Pi}{\partial c}$$

(3.17)

where $R(\theta)$ is called Rayleigh’s ratio, $K$ is an optical constant, $R$ is the gas constant, $T$ is the absolute temperature, $c$ is the concentration, and $\Pi$ is the osmotic pressure.

The Rayleigh’s ratio can be expressed as:

$$R(\theta) = \frac{I(\theta) r^2}{I_0 V_S}$$

(3.18)

where light intensity observed at angle $\theta$ is scattered from a volume $V_S$.

The optical constant $K$, particular for each polymer/solvent system is given as:

$$K = \frac{4\pi^2}{\lambda_0 N_A} n_0^2 \left( \frac{\partial n}{\partial c} \right)^2$$

(3.19)

where $n_0$ is the refractive index of the solvent and $N_A$ is Avogadro’s number.

The refractive index increment is measured off-line by means of a differential refractive index operating at the same wavelength as the one used for the multiangle light-scattering
measurement. It represents the incremental refractive index change $\partial n$ of the solution (solvent plus solute) for an incremental change $\partial c$ of the concentration in the limit of vanishing small concentration.

The term $(\partial \Pi / \partial c)_T$ is called the osmotic module and can be expanded into a power series:

$$
\left( \frac{\partial \Pi}{\partial c} \right)_T = RT \left( \frac{1}{M_w} + 2A_2c + 3A_3c^2 + ... \right)
$$

(3.20)

where $M_w$ is the weight-average molecular weight of the solute and $A_2$, $A_3$, etc. are virial coefficients, which describe interactions between molecules.

After further substitutions and omitting the higher order terms of $c$ one can obtain the basic equation of light scattering applied to polymer solution as follow:104

$$
Kc \frac{R}{R(\theta)} = \frac{1}{M_w} + 2A_2c
$$

(3.21)

The above equation is valid for small particles with diameter smaller than $\lambda/20$. According to the Mie theory one has to introduce a particle scattering or form factor as the scattering becomes sensitive to the shape of the particles.105 The angle dependent form factor $P(q)$ can be expressed by:

$$
P(q) = 1 - \frac{1}{3} \langle R_g^2 \rangle q^2 + ...
$$

(3.22)

where $R_g$ is the radius of gyration and $q$ represents the scattering vector defined as:

$$
q = \frac{4\pi n_0}{\lambda_0} \sin(\theta)
$$

(3.23)

The mean square radius is given by equation (3.24) where the distances $r_i$ are measured from the molecule’s center of mass to the mass element $m_i$.

$$
\langle R_g^2 \rangle = \frac{\sum_i r_i^2 m_i}{\sum_i m_i} = \frac{1}{M_w} \int r^2 \partial m
$$

(3.24)

Introducing $P(q)$ into (3.21) gives:
This equation constitutes the basis for a Zimm plot, \(^{104}\) where \(\frac{Kc}{R(\theta)}\) is plotted versus \(q^2 + kc\) with an arbitrary constant \(k\). The extrapolation \(c \rightarrow 0\) provides initial slope of the curve, which is proportional to \(\langle R_g^2 \rangle\), whereas the slope of the curve by extrapolating \(q^2 \rightarrow 0\) is proportional to \(A_2\). Double extrapolation \(q^2 \rightarrow 0\) and \(c \rightarrow 0\) provides the values of \(M_w\).

The shape of the particles can be distinguished from the shape of the curve in Zimm or Kratky plot (Figure 3.3).\(^{106}\)

All data obtained in this thesis were evaluated by a standard Zimm or Guinier analysis.

![Figure 3.3. a) Zimm plot and b) Kratky plot of a sphere, a coil and a rod having \(R_g = 100\) nm.](image)

### 3.3 Analytical ultracentrifugation (AUC)

Analytical ultracentrifugation is a powerful technique for characterization of particles and polymers. Developed in the 1920s by Svedberg\(^{107}\) and redesigned into a commercial instrument by Edward Pickels,\(^{108}\) it became a central technique for fractionation of the sample into its components either according to their molar masses/particles sizes or to their structure/density. The fractionation enables the measurement of distribution of molar masses, particle sizes and densities providing complimentary physicochemical information.

In AUC a centrifugal force is applied with the simultaneous real-time observation of the redistribution of the macromolecules during sedimentation. The sedimentation process is

\[
\frac{Kc}{R(\theta)} = \frac{1}{M_w} \left(1 + \frac{1}{3} q^2 \langle R_g^2 \rangle \right) + 2A_2 c
\]  

(3.25)
Characterisation Methods

Figure 3.4. Forces acting on the particle during the sedimentation process.

The gravitational force $F_{sed}$ is defined as:

$$F_{sed} = m \omega^2 r$$  \hspace{1cm} (3.26)

with $m$ the particle mass, $\omega$ the rotary angular velocity, and $r$ the distance from the centre of rotation.

The buoyancy force $F_b$ opposes the sedimentation (following Archimedes principle) and is governed by the mass of the displaced solvent, and can be expressed as:

$$F_b = -m \bar{\nu} \rho \omega^2$$  \hspace{1cm} (3.27)

where $\bar{\nu}$ is the effective particle partial-specific volume and $\rho$ is the solvent density.

Finally, the frictional force is governed by the hydrodynamic translational frictional coefficient as well as migration velocity, and is defined as:

$$F_f = s \left( \frac{kT}{D} \right) \omega^2$$  \hspace{1cm} (3.28)

with $s$ the sedimentation coefficient, $k$ the Bolzmann constant, $T$ the absolute temperature and $D$ the diffusion constant.

A key parameter is the sedimentation coefficient, measured in units of Svedberg (1S=10$^{-13}$ seconds) which is a molecular constant defined as:

$$s = \frac{\nu}{\omega^2}$$  \hspace{1cm} (3.29)
with $v$ the absolute migration velocity. From the balance of these three forces, one can derive the Svedberg equation:

$$\frac{s}{D} = \frac{M(1 - v \rho)}{RT}$$  \hfill (3.30)

where $M$ is the molar mass of the sample.

From the shape of the sedimenting boundary, it is possible to calculate the distribution of the sedimental coefficient $g^*(s)$, which gives information about the polydispersity of the particles. Applying the data transformation introduced by Stafford\textsuperscript{109} and further developed by Philo\textsuperscript{110} based on the time derivative $dc/dt$ of the data, the distribution of the sedimental coefficient can be calculated as follow:

$$g^*(s) = \left( \frac{\partial c}{\partial T} \right) c_0 \left( \frac{\omega^2 t^2}{\ln(r/r_m)} \right) \left( \frac{r}{r_m} \right)^2$$  \hfill (3.31)

Where $c_0$ is the initial concentration and $r_m$ is the radial position of the meniscus.

### 3.4 Transmission electron microscopy (TEM)

TEM is a one from widely used technique for imaging a sample with high resolution. In contrast to a light microscope, the electron microscope uses a beam of electrons, which are transmitted through an examined specimen in place of a beam of light. The first practical transmission electron microscope was built by Albert Prebus and James Hillier in 1938 using concepts developed earlier by Louis de Broglie\textsuperscript{111} who proposed that particles like electrons and neutrons could exhibit wave characteristics under certain conditions. According to de Broglie’s theory the wavelength of electrons, $\lambda$, is related to their energy as:

$$\lambda = \frac{\hbar}{p} = \frac{\hbar}{m_e v}$$  \hfill (3.32)

where $\hbar$ is the Planck constant and $p$ is the momentum of the electron equal to the relativistic mass of the electron, $m_e$, multiplied by its velocity, $v$.

The setup of an electron microscope is similar to that of a normal light microscope. A schematic diagram is presented in Figure 3.5. The electrons are generated in a hot cathode and accelerated by application of a high voltage (60 - 200 kV). Electromagnetic lenses focus the
beam on the sample where it is partially deflected. The degree of deflection is higher for a sample with higher electron density or with greater thickness. Therefore, to enhance structural details one can stain the sample selectively with heavy metal-containing substances (e.g. osmium tetraoxide, ruthenium tetraoxide).

For TEM measurements the sample is placed on a carbon-coated copper grid. The thickness of the layer should be smaller than 100 nm, that the lower intensity beam can be used, which prevents the destruction of the specimen due to rising of temperature with electron absorption. After passing the sample the scattered electrons are collected by an objective generating an image, which then appears on a fluorescent screen.

A very powerful preparation technique for the investigation of samples in aqueous solution is the so-called cryogenic TEM. In order to avoid artifacts in sample preparation process, a liquid sample is shock frozen in liquid nitrogen on a TEM grid. The grid is then transferred under liquid nitrogen to the workstation of a cryotransfer system, where it is loaded into the tip of a nitrogen cooled TEM specimen holder. The holder is fitted with a cooled shutter that protects the specimen during cryotransfer to the TEM and his shutter is only opened again when the holder tip is safely within the high vacuum environment of the TEM. The cryotransfer system is designed to maintain the temperature of the specimen below -160 C at all times, so that crystallisation of the vitreous ice does not occur. Similarly there are design features to prevent contamination of the specimen with external ice crystals.

Figure 3.5. Schematic diagram of the setup for TEM.
4 RESULTS AND DISCUSSION

4.1 Free-radical addition of ω-functional mercaptanes

Polymers containing covalently attached reactive functionalities have generated much interest for making materials with tailored properties.\textsuperscript{1,2,114,115}

The synthetic methods allowing the rational design of polymers with respect to molecular weight, molecular weight-distribution, composition, architecture, and functionality include living/controlled ionic,\textsuperscript{116,22} radical\textsuperscript{117} and transition metal-catalyzed polymerization techniques.\textsuperscript{118}

As mentioned in Section 2.2 ionic polymerization can provide block copolymer materials of highest quality, but it also suffers from some experimental drawbacks. It demands high-purity and inert reaction conditions as well as protection of functional groups in monomers.\textsuperscript{16,119} In addition, for most ionic systems, sequential polymerization must follow a strict order in monomer addition so that the relative reactivity of ionic sites is steadily decreasing, thus limiting the number of accessible block copolymers. An alternative pathway is the sequential application of different polymerization methods, that an anionic polymerization can follow cationic a one, or other desirable alternateness.

The applicability of ionic polymerization can be extended by modification reactions as described in Section 2.4. Ideally, a modification of polymers proceeds in quantitative yield and without major side reactions (including preservation of the narrow molecular weight distribution). The most desirable pathway is one general nature, which allows for preparation of a series of different blocks from one precursor copolymer.

Efficient tool for a one-step modification of polymer precursors is the free-radical addition of ω-functional mercaptanes,\textsuperscript{32,44,45,46,47,50,51,52,53,120,121,122} or alkyl iodides\textsuperscript{123} onto vinyl double bonds. The former reaction has also been used for an endfunctionalization of polymers.\textsuperscript{124} The addition of mercaptanes was found to occur on the vinyl double bonds ($1,2$-units), mainly in an anti-Markownikoff fashion, as well as on the in-chain double bonds ($1,4$-units) of PB as presented in Scheme 4.1.
An intermolecular cyclization of two neighboring monomer units, which is evident in the radical addition of alkyl iodides onto 1,2-PB,\textsuperscript{123} or a recombination of polymer chains could not be observed. As demonstrated by Justynska\textsuperscript{125} the conversion of double bonds and thus the degree of functionalization of PB were usually less than quantitative although the mercaptane was used in excess.

In this thesis, the course of the addition of mercaptanes onto 1,2-PB is revisited. In the first part, the mechanism of radical addition of ω-functional mercaptanes onto 1,2-PB is discussed. Amongst previous experiments have been done it was observed that 1,2-PB chains tend to form cyclic adducts, which have a noticeable impact on the degree of functionalization. Importantly, the cyclization reaction does not alter the molecular-weight distribution of the modified polymers. A modular chemical approach of the free radical addition of ω-functional mercaptanes onto 1,2-PB has generated interest to produce a toolbox of “hydrophilically modified polybutadienes”, which are closely related to the polymeric amphiphiles introduced by Ringsdorf et al.\textsuperscript{4} Hence, the first part deals with modifications of 1,2-PB homopolymer precursors to produce polymeric amphiphiles are described.

In the second part, the results obtained for the modifications of 1,2-PB-based block copolymers with ionic groups are described. 1,2-Polybutadiene-\textit{block}-poly(ethylene oxide) (PB-\textit{b}-PEO) and 1,2-polybutadiene-\textit{block}-poly(2-methyl-2-oxazoline) (PB-\textit{b}-PMOxa) copolymers were modified with ω-functional mercaptanes carrying either carboxylate or ammonium groups. Although both presented block copolymers were prepared from the same
1,2-PB precursor, the modification reactions proceeded with different degree of functionalization. The chemical compositions of modified products are discussed.

4.1.1 Modification of polybutadiene

1,3-Butadiene belongs to the group of monomers with conjugated double bonds. The polymerization of this monomer can occur in two different modes, referred to as 1,2- and 1,4 polymerization. Consequently, the obtained products can be of the 1,2-type, where pendant unsaturations occur, or the 1,4-type, where unsaturation is present along the polymer chain. Additionally, 1,4-adducts exhibit cis and trans isomerism (Figure 4.1). The type of product obtained in majority depends on the experimental conditions applied in the polymerization process. Lithium alkyl-based anionic polymerization of 1,3-butadiene in THF produces mostly vinyl unsaturation (1,2-type), while internal bonds in 1,4-type polymer are formed in non-polar solvents such as hexane.

Polybutadiene of the 1,2-type was synthesized by anionic polymerization of 1,3-butadiene in tetrahydrofuran (THF) solution at $-78^\circ$C using sec-butyllithium (sBuLi) as the initiator (section 6.2.1.1). The content of 1,2-adduct in the polymer, calculated on the basis of $^1$H NMR spectroscopy was found to be more then 90%, while the residual contains the trans 1,4-type. The polybutadiene of the 1,4-type was a commercial product from Aldrich, with a 99% content of 1,4-adduct. The molecular characteristic of the precursor homopolymers are summarized in Table 4.1.
Table 4.1. Molecular characteristics of PB precursor homopolymers (n: number-average of butadiene units).

<table>
<thead>
<tr>
<th>Sample</th>
<th>PB 1,2-units (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Average number of repeating units&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Polydispersity index&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB1</td>
<td>97</td>
<td>25</td>
<td>1.10</td>
</tr>
<tr>
<td>PB2</td>
<td>93</td>
<td>40</td>
<td>1.10</td>
</tr>
<tr>
<td>PB3</td>
<td>92</td>
<td>65</td>
<td>1.08</td>
</tr>
</tbody>
</table>

[a] Determined by <sup>1</sup>H NMR according to Ref. 128
[b] Determined by SEC (eluent: THF) using 1,2-PB calibration.
[c] Determined by SEC.

4.1.1.1 Introducing ester functionalities

For the examination of the course of the free radical addition of mercaptanes onto double bonds, both 1,2- and 1,4-types of PB homopolymer samples were modified with methyl-3-mercaptopropionate (HS-MP). Methyl-3-mercaptopropionate was chosen as the exemplary mercaptane because of the easy detection of its ester function applying spectroscopic methods. Additionally both the precursor and the modified product can be analyzed by size exclusion chromatography (SEC) under the same conditions. The free-radical modification reactions were performed in THF at reflux temperature, under inert argon atmosphere in the presence of N,N-azobis(isobutyronitrile) (AIBN) as radical source. The experimental details are described in section 6.2.2.1. Scheme 4.2 depicts the general equation of the performed functionalization reaction.

The mixture containing 1,2-PB samples maintained its low viscosity throughout the whole reaction time, and obtained modified products could afterwards be dissolved in common organic solvents.
Scheme 4.2. The general reaction equation of the radical addition of ester functionality to the PB precursor homopolymer.

Exemplary for PB2-S-MP SEC (Figure 4.2) indicated that modified polymer exhibited a larger hydrodynamic volume but the same molecular weight distribution as the precursor polymer. Any recombination products were not detected. FT-IR spectroscopy showed the presence of the ester carbonyl units ($\tilde{\nu} = 1737 \text{ cm}^{-1}$) and the absence of carbon-carbon double bonds ($\tilde{\nu} = 1640 \text{ cm}^{-1}$) in the modified product.

Elemental analysis revealed a ratio of S/C in the modified product of 0.272, which is 82% the theoretical value assuming quantitative addition of mercaptan onto the double bonds (S/C=0.334). MALDI-TOF mass spectrometry (see the spectrum in Figure 4.4) further revealed that the number-average molecular weight of the sample is $M_n\sim5480$ Da (estimated
from the position of the maximum of the frequency distribution) instead of 6900 Da (number of repeating units, \(x=40\)). Hence, there must be a fraction of monomer units to which no mercaptan was added.

\(^1\)H NMR analysis showed the complete lack of resonances at \(\delta=4.8–5.6\) ppm (see Figure 4.3), indicated the quantitative conversion of both the vinyl as well as the in-chain double bonds. Signals from protons next to the sulfur atom of the newly formed thioether linkage appear at \(\delta=2.6–2.8\) ppm. The signal at \(\delta=2.60\) ppm is split into three lines and can therefore be assigned to the methylene protons \(d'\) of the anti-Markownikoff product (see the chemical structure in Scheme 4.1). Characteristic signals of –SCH\(\sim\) methine protons of the adduct to the in-chain double bonds or of the Markownikoff product (cf. Boileau et al.\(^{122}\)) could not be identified. However a broad peak below 2.2 ppm characteristic for polybutadiene chain can also be related to cyclohexyl or cyclopentyl proton signals.

The most reasonable reaction pathway is given in Scheme 4.3 showing the regular addition of the mercaptane (\(\rightarrow\)unit I) and the formation of a cyclic structure (\(\rightarrow\)unit II, two isomers). Considering the relative thermodynamic stability of secondary vs. primary radical intermediates, the formation of a six-member cycle (anti-Markownikoff) should be preferred over that of a five-member one (Markownikoff).\(^{121}\) However, formation of the five-member
cycles in the functionalized polymer was pointed by Kornfield et al., who used chloroform as a reaction medium. High thiol concentrations should conclude with H-abstraction from RSH prior to the propagation of a chain of cyclization events, thus polycyclic structure should not be produced. It should be noted that if $f>2/3$ the regular addition of mercaptane is higher than the formation of a cyclic structure. A likely random copolymer structure of modified product is supported by MALDI-TOF MS, the spectrum displaying a series of peaks with $\Delta m\sim 54$ Da corresponding to the mass difference between the units I (174.1 Da) and II (228.2 Da). Given the (simplified) chemical structure shown in Figure 4.4 and a degree of functionalization of $f=0.82$, the average composition of the ester modified homopolymer is $I:II=24.8:7.6$ ($I+2II=x$, $I+II=fx$) and thus $M_n\sim 6050$ Da.

![MALDI-TOF mass spectrum and chemical structure of PB2-S-MP obtained by radical addition of HS-MP onto PB2 at 10-fold excess with respect to double bonds.](image)

Figure 4.4. MALDI-TOF mass spectrum and chemical structure of PB2-S-MP obtained by radical addition of HS-MP onto PB2 at 10-fold excess with respect to double bonds.
The composition of the product should be determined by the rates of intermolecular hydrogen transfer and of intramolecular cyclization. The rate of transfer is linearly proportional to $[\text{RSH}]_0$ and, in a first approximation, remains constant during the reaction due to the high excess of mercaptan used. The rate of cyclization does not depend on $[\text{RSH}]_0$ but it will, as a matter of statistics, decrease with increasing conversion of the double bonds. Accordingly, the fraction of structural unit I in the product was found to be larger the higher the concentration of mercaptane (see Table 4.2). However, changes in composition were marginal at $[\text{RSH}]_0/[\text{C}=\text{C}]_0 \geq 10$, and the addition of the mercaptane reached a limit at a conversion of 85% of butadiene units—supposedly due to steric reasons.\textsuperscript{130} Results may differ when the PB chain contains higher amounts of 1,4 units that cannot take part in a cyclization reaction.\textsuperscript{121}

Table 4.2. Chemical composition of products obtained by modification of 1,2-PB, sample PB2 ($x=40$) with methyl-3-mercaptopropionate.

<table>
<thead>
<tr>
<th>$[\text{HS-MP}]_0/[\text{C}=\text{C}]_0$</th>
<th>$\text{C}=\text{C}^a$</th>
<th>$\text{S}/\text{C}^b$</th>
<th>$f^c$</th>
<th>I:II$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>None</td>
<td>0.257</td>
<td>0.77</td>
<td>21.6: 9.2</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>0.272</td>
<td>0.82</td>
<td>25.6: 7.2</td>
</tr>
<tr>
<td>20</td>
<td>None</td>
<td>0.278</td>
<td>0.83</td>
<td>26.4: 6.8</td>
</tr>
<tr>
<td>40</td>
<td>None</td>
<td>0.286</td>
<td>0.86</td>
<td>28.8: 5.6</td>
</tr>
</tbody>
</table>

[a] Determined by $^1$H NMR.
[b] Determined by EA.
[c] Degree of functionalization, determined by EA.
[d] Ratio of structural units I and II (see Scheme 4.3).
In case of 1,4-PB (99% 1,4) strong gelation occurred after 1 h. Seemingly, experimental conditions (especially the high polymer concentration of about 3 wt%) were as such that 1,4-PB chains underwent a radical cross-linking, thus producing an insoluble material.

4.1.1.2 Introducing ionic groups

Three different ω-functional mercaptanes containing ionic groups were utilized for radical modification reaction: 3-mercaptopropionic acid, thiomalic acid, and 2-mercaptoethylamine hydrochloride (see Figure 4.5). All reactions were performed as described in section 6.2.2.1, using THF as the solvent and AIBN as the radical source.

![Chemical structure of the mercaptanes used in the synthesis of ionic homopolymer:](image)

Figure 4.5. Chemical structure of the mercaptanes used in the synthesis of ionic homopolymer: 3-mercaptopropionic acid (HS-MPA), thiomalic acid (HS-TA), 2-mercaptoethylamine hydrochloride (HS-EA·HCl).

The modification reactions were carried out for three precursor homopolymers with average numbers of repeat units of x=25, 40, 65; see Table 4.3. Samples were characterized by means of $^1$H NMR spectroscopy and by elemental analysis (S/C or N/C ratio).

Similar to the addition reaction of methyl-3-mercaptopropionate, modified polymers exhibited the general structure of a random copolymer, as depicted in Figure 4.6, owing to the fact that the intermediate radical species formed via the addition of thyl radical onto a vinyl double bond can either abstract a hydrogen atom from another mercaptane molecule (→ unit I) or add to a double bond in its vicinity (→ six-member cyclic unit II).

The results obtained for the modification of 1,2-PB with different lengths of the 1,2-PB chain do not reveal a dependence of functionalization on the molecular weight. In addition, the chemical nature of the mercaptane does not seem to have a pronounced impact on the pathway of the reaction (see Table 4.3).
Table 4.3. Chemical composition of products obtained by modification of 1,2-PB<sub>x</sub> with mercaptane bearing ionic groups (see chemical structure in Figure 4.6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>R</th>
<th>x&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C=C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S/C&lt;sup&gt;c&lt;/sup&gt;</th>
<th>f&lt;sup&gt;d&lt;/sup&gt;</th>
<th>I:II&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB1-S-PA</td>
<td></td>
<td>25</td>
<td>None</td>
<td>0.26</td>
<td>0.68</td>
<td>9.0: 8.0</td>
</tr>
<tr>
<td>PB2-S-PA</td>
<td></td>
<td>40</td>
<td>None</td>
<td>0.27</td>
<td>0.70</td>
<td>16.0: 12.0</td>
</tr>
<tr>
<td>PB3-S-PA</td>
<td></td>
<td>65</td>
<td>None</td>
<td>0.26</td>
<td>0.69</td>
<td>24.7: 20.5</td>
</tr>
<tr>
<td>PB2-S-TA</td>
<td></td>
<td>40</td>
<td>None</td>
<td></td>
<td></td>
<td>N/C&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>PB1-S-EA·HCl</td>
<td></td>
<td>25</td>
<td>None</td>
<td>0.15</td>
<td>0.77</td>
<td>13.5: 5.7</td>
</tr>
<tr>
<td>PB2-S-EA·HCl</td>
<td></td>
<td>40</td>
<td>None</td>
<td>0.15</td>
<td>0.76</td>
<td>20.8: 9.6</td>
</tr>
<tr>
<td>PB3-S-EA·HCl</td>
<td></td>
<td>65</td>
<td>None</td>
<td>0.14</td>
<td>0.75</td>
<td>32.5: 16.2</td>
</tr>
</tbody>
</table>

[a] Number average of butadiene units determined by SEC (calibrated with 1,2-PB standards).
[b] Residual double bonds determined by ¹H NMR.
[c] Determined by EA.
[d] Degree of functionalization, determined by EA.
[e] Fraction of monomer units I and II, (see Scheme 4.3).

Figure 4.6. General chemical structure of the modified 1,2-polybutadienes (R ionic groups).

In this investigation, it is shown that radical addition of ω-functional mercaptanes represents an alternative method for the production of ionic polymers.

4.1.1.3 Introducing cysteine

In section 4.1.1.2 it has been shown that ammonium and carboxylic groups can be easily applied for producing ionic polymers via radical addition onto double bonds. Cysteine
containing both amine and carboxylic acid functionalities in one molecule was expected to give similar results in a modification reaction. Attempts to attach the free cysteine to the precursor 1,2-PB homopolymer (number-average molecular weight: \( M_n = 2240 \) g/mol; \( x = 40 \), polydispersity index: PDI = 1.1) were unsuccessful. After 24h of reaction time no conversion was observed. However application of cysteine with protected amine functionality – namely \( N\text{-Boc-L cysteine, HS-Cys-Boc} \) (see Figure 4.7) resulted in a high conversion during the modification reaction.

![Chemical structure of the cysteine (HS-Cys) and protected cysteine (HS-Cys-Boc) used for modification reaction.](image)

The successful attachment of the protected cysteine units onto the polymer backbone is supported by \(^1\)H NMR (Figure 4.8) which did not reveal any traces of unsaturated PB 1,2-units, indicating that the addition of HS-Cys-Boc to the PB2 has been achieved in a full conversion of double bonds.

![1H NMR spectrum (400.1 MHz, THF-d8, * signals from THF) of Boc-L-Cys-OH - modified 1,2-PB.](image)
The Boc protecting groups of cysteine were removed by stirring the sample in a mixture of trifluoroacetic acid and dichloromethane (50:50 v/v) for 3 h at room temperature (section 6.2.2.1). FT-IR analysis indicated complete absence of Boc protecting groups (lack of amide I band at $\tilde{\nu} = 1642$ cm$^{-1}$), elemental analysis: S/C = 0.27. The molecular characteristics of the modified polymer are summarized in Table 4.4.

Table 4.4. Chemical composition of products obtained by modification of 1,2-PB$x$ with cysteine (see chemical structure in Figure 4.6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>R</th>
<th>$x$</th>
<th>C=C</th>
<th>S/C</th>
<th>$f$</th>
<th>I:II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2-S-Cys</td>
<td>S</td>
<td>COOH</td>
<td>S</td>
<td>NH$_2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>None</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.27</td>
<td>0.71</td>
<td>16.8:11.6</td>
</tr>
</tbody>
</table>

[a] Number average of butadiene units determined by SEC (calibrated with 1,2-PB standards).
[b] Residual double bonds determined by $^1$H NMR.
[c] Determined by EA.
[d] Degree of functionalization, determined by EA.
[e] Fraction of monomer units I and II (see Scheme 4.3).

Successful addition of cysteine to the 1,2-PB homopolymer represents the initial key for producing polymers with an oligopeptide sequence in the side chains. Polymers with biofunctionalities are of special interest for biomedical application as the so-called “biohybrids” or “molecular chimeras”\textsuperscript{131} where biological and synthetic parts are in single molecule.

4.1.1.4 Introducting diol functionalities

Hydrophilic 3-mercapto-1,2-propanediol (Figure 4.9) was utilized for radical modification reaction onto a 1,2-polybutadiene with an average number of 40 repeat units (number-average molecular weight: $M_n = 2240$ g/mol; polydispersity index: PDI = 1.1).

\[
\text{HS-PD}
\]

Figure 4.9. Chemical structure of 3-mercapto-1,2-propanediol (HS-PD) used for modification reaction.

The reaction was performed as described in section 6.2.2.1, using THF as the solvent and AIBN as the radical source. The resulting product was characterized by $^1$H NMR, FT-IR and EA.
$^1$H NMR (CD$_3$OD) showed the absence of residual double bonds in the modified product. The functional units could be seen by $^1$H NMR (see Figure 4.10) and FT-IR ($\nu$(O–H)~3350 cm$^{-1}$). According to EA, the degree of functionalization is quantitative, S/C=0.38. Obtained product did not contain a random structure, but represented a homopolymer of unit I.

4.1.2 Modification of PB-$b$-PEO copolymers

1,2-Polybutadiene-$block$-poly(ethylene oxide) (see Figure 4.11) (PB-$b$-PEO) copolymers were modified with ω-functional mercaptanes carrying either carboxylate or ammonium groups.

1,2-PB-$b$-PEO were prepared by anionic polymerization of ethylene oxide using 1,2-PB(OH)/phosphazene base $t$-BuP$_4$ as the initiator in THF. The solution was stirred at +50 °C for 2 days and then quenched with acetic acid. The polymer was precipitated into cold acetone, redissolved in water and freeze-dried. Details about the synthesis are described in section 6.2.1.2. The characteristics of all polymers prepared are summarized in Table 4.5. All
modification reactions were done using a 10-fold excess of the mercaptane with respect to PB double bonds (section 6.2.2.2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>PB 1,2-units (%)</th>
<th>Average number of repeating units</th>
<th>Polydispersity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1</td>
<td>97</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>PP2</td>
<td>93</td>
<td>40</td>
<td>132</td>
</tr>
<tr>
<td>PP3</td>
<td>93</td>
<td>40</td>
<td>273</td>
</tr>
<tr>
<td>PP4</td>
<td>92</td>
<td>65</td>
<td>212</td>
</tr>
</tbody>
</table>

[a] Determined by $^1$H NMR.
[b] Determined by SEC (eluent: THF) using 1,2-PB calibration (x) and SEC/$^1$H NMR (y).
[c] Determined by SEC.

Samples modified with 3-mercaptopropionic acid did not contain double bonds according to $^1$H NMR (DMSO-d$_6$). The signals of methylene protons of the thioether displayed at $\delta=2.4–2.7$ ppm. Exemplarily for PP2, the FT-IR spectrum showed the valence vibration band of the carboxylic acid at $\nu=1720$ cm$^{-1}$; after neutralization with NaOH, anti-symmetric and symmetric valence vibrations of carboxylate appeared at a $\nu=1570$ and 1400cm$^{-1}$, respectively. According to EA, S/C ratio, the samples have a degree of functionalization of about 70%, i.e. $f=0.78$ (PP1-S-PA), 0.70 (PP2-S-PA), 0.68 (PP3-S-PA), and 0.72 (PP4-S-PA), similar to that of carboxylic acid-modified 1,2-PB homopolymers ($f=0.7$, cf. Table 4.3). However, a characterization of the samples by SEC could, however, not be achieved due to aggregation phenomena in water. Other solvent was not used as eluent.

In case of 2-mercaptoethylamine hydrochloride, for the modified product $^1$H NMR (D$_2$O) confirmed the absence of double bonds and the presence of functional units, $\delta=3.00$, 2.55 (–CH$_2$SCH$_2$–), and 2.70 ppm (–CH$_2$-NH$_3^+$). Further evidence for the presence of amino-functional groups was established by FT-IR ($\delta$(NH$_3^+$) at $\nu=1580$ cm$^{-1}$ (broad)) and EA (N content). According to EA, the degree of functionalization is close to 80%, i.e. $f=0.82$ (PP1-S-EA), 0.77 (PP2-S-EA), 0.76 (PP3-EA) and 0.75 (PP4-EA). A characterization of the samples by SEC was not possible.

Radical addition of $\omega$-functional mercaptanes bearing electrolytes onto 1,2-PB-$b$-PEO copolymers proceeded with complete conversion of double bonds. However, the degree of functionalization of the block copolymers in the range of 60–80% indicates formation of
cyclic structures in the obtained products. The difference in molecular weight of precursor block copolymers did not reveal a dependence on degree of functionalization. It is assumed that the reaction pathway is the same as for the modification of 1,2-PB homopolymers, thus the functional segments should be composed of the units I and II (see Scheme 4.3). The main characteristics of all described functional block copolymers are summarized in Table 4.6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Functionality</th>
<th>C═C[^a]</th>
<th>S/C[^b]</th>
<th>I:II[^d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP1-S-PA</td>
<td>–COOH</td>
<td>None</td>
<td>0.16</td>
<td>0.78</td>
</tr>
<tr>
<td>PP2-S-PA</td>
<td>None</td>
<td>0.14</td>
<td>0.70</td>
<td>16.0: 12.0</td>
</tr>
<tr>
<td>PP3-S-PA</td>
<td>None</td>
<td>0.09</td>
<td>0.68</td>
<td>14.4: 12.8</td>
</tr>
<tr>
<td>PP4-S-PA</td>
<td>None</td>
<td>0.14</td>
<td>0.72</td>
<td>28.6: 18.2</td>
</tr>
<tr>
<td>PP1-S-EA</td>
<td>–NH₂·HCl</td>
<td>None</td>
<td>0.08</td>
<td>0.82</td>
</tr>
<tr>
<td>PP2-S-EA</td>
<td>None</td>
<td>0.07</td>
<td>0.77</td>
<td>21.6: 9.2</td>
</tr>
<tr>
<td>PP3-S-EA</td>
<td>None</td>
<td>0.04</td>
<td>0.76</td>
<td>20.8: 9.6</td>
</tr>
<tr>
<td>PP4-S-EA</td>
<td>None</td>
<td>0.07</td>
<td>0.75</td>
<td>32.5: 16.2</td>
</tr>
</tbody>
</table>

[^a]: Determined by ¹H NMR.
[^b]: Determined by EA.
[^c]: Degree of functionalization, determined by EA.
[^d]: Ratio of structural units I and II (see Scheme 4.3).

4.1.3 Modification of PB₄₀-b-PMOxa₁₉₀ copolymers

The radical addition of ω-functional mercaptanes was applied for modification of 1,2-polybutadiene-block-poly(2-methyl-2-oxazoline) (PB-b-PMOxa) copolymer.

2-Oxazolines are five-membered cyclic imino ethers (imidates). Cationic ring-opening polymerization of cyclic imino ethers generally involves a thermodynamically favorable isomerization of the imino ether group to the amide.

The PB₄₀-b-PMOxa₁₉₀ copolymer was synthesized in two steps. Firstly, hydroxyl-terminated 1,2-PB was prepared by living anionic polymerization of 1,3-butadiene as described in section 6.2.1.1. The second PMOxa block was synthesized by cationic ring-opening polymerization of MOxa using activated 1,2-PB₄₀ precursor as the initiator in 1,2-dichloroethane, EDC. Details about synthesis are described in section 6.2.1.3. The general structure of the PB-b-PMOxa copolymer precursor is presented in
RESULTS AND DISCUSSION

Figure 4.12. The chemical structure of the copolymer was confirmed by $^1$H NMR analysis ($\delta$/ppm = 1.0–2.0 (CH$_2$ and CH of PB), 2.2, 3.6 (CH$_3$ and CH$_2$ of PMOxa), 5.0, 5.5 (=CH$_2$ and –CH= of PB) (see Figure 4.13).

SEC revealed a monomodal molecular weight distribution with PDI ~ 1.4 (see Figure 4.14).

Figure 4.12. The general structure of the PB-PMOxa block copolymer precursor used for modification with $\omega$-functional mercaptane.

Figure 4.13. $^1$H NMR spectrum (400.1 MHz, CDCl$_3$) of PB$_{40}$-b-PMOxa$_{190}$. 
The radical addition of 2-mercaptoethylamine hydrochloride to PB<sub>40</sub>-b-PMOxa<sub>190</sub> was carried out similarly as for 1,2-PB homopolymers, using THF and AIBN as radical initiator with a 10-fold excess of the mercaptane with respect to PB double bonds. The experimental details are described in section 6.2.2.3. The obtained product (PM-S-EA·HCl) was analyzed using <sup>1</sup>H NMR spectroscopy and EA, S/C ratio. The <sup>1</sup>H NMR spectrum is presented in Figure 4.15. The signals originating from the unsaturated PB units, δ = 4.80-5.60 ppm, disappear completely upon modification indicating the complete absence of double bonds in the modified product. According to EA the degree of functionalization was quantitative, S/C=0.11. Obtained product was free from the cyclic units, thus did not contain random structure but represented a homopolymer of unit I (see Scheme 4.3). It is assumed that the reaction pathway of radical addition of ω-functional mercaptanes into 1,2-PB unit is influenced by chemical composition of the block copolymers. In case of PB-b-PMOxa copolymer a 10-fold excess of mercaptane with ammonium functionality is sufficient to attach a primary amine function in quantitative yield. The chemical composition of the obtained product is summarized in Table 4.7.
4.2 Self-organization of hydrophilically modified 1,2-PB homopolymers

Bioinspired polymers are raising more and more attention as advanced materials for key applications in materials science or biomedicine. Especially interesting are the so-called “biohybrids” or “molecular chimeras”,\textsuperscript{131} that are polymers containing biological (peptide, sugar, or nucleic acid) and synthetic parts. Polymers with main-chain biological segments can nowadays be rationally designed in high quality\textsuperscript{132,133,134} – but often in low quantity. Polymers with pendent biofunctionalities, on the other hand, are easily available through chemical modifications, basically without limitations in quantity. The most promising strategies involve “click chemistry”, i.e. Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of azides onto
alkyne side chain polymers, or free-radical addition of mercaptanes onto an alkene side chain polymers.

In section 4.1.1 the mechanism of modification of 1,2-PB homopolymer precursors was described. The applied approach enables one to generate a platform of “hydrophilically modified polybutadienes” from readily available starting materials. Such hydrophilically modified polybutadienes are random copolymers that are closely related to the polymeric amphiphiles introduced by Ringsdorf et al. (also compare to amphiphilic homopolymers, polysoaps and hydrophobically modified polymers), as can be recognized by the chemical structure in Figure 4.16. As such, they should be useable for the production of functional polymer colloids by design, especially bioinspired polymer vesicles or membranes—a field so far being reserved to block copolymers. The following studies refer to the self-organization of the hydrophilically modified 1,2-PB. Firstly, the aggregation behavior of the obtained ionic homopolymers in water is described. Then, the self-organization of diol modified 1,2-PB in ethanol/water mixture is presented.

![General chemical structure of the hydrophilically modified 1,2-polybutadienes prepared (R – functional groups) and idealized sketch of a polymeric amphiphile.](image)

**4.2.1 Aggregation behavior in water**

Three polymeric amphiphiles (see Table 4.8) produced by free-radical additions of 3-mercaptopropionic acid (PB2-Ac), 2-mercaptoethylamine hydrochloride (PB2-Am), and N-Boc-L-cysteine (PB2-Cys), onto a 1,2-polybutadiene with an average number of 40 repeat units (number-average molecular weight: \( M_n = 2240 \ \text{g/mol} \); polydispersity index: PDI = 1.1) were investigated according to their aggregation behavior. All reactions were performed as described in section 6.2.2.1.
Table 4.8. List of hydrophilically modified polybutadienes (see chemical structure in Figure 4.16).

<table>
<thead>
<tr>
<th>Sample</th>
<th>mercaptane</th>
<th>C=Ca S/Cb</th>
<th>f</th>
<th>I:II</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB2-S-PA</td>
<td>S(\text{COOH}) none 0.35 0.91 32.8: 3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB2-S-EA</td>
<td>S(\text{NH}_2\cdot\text{HCl}) none 0.35 0.79 23.2: 8.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB2-S-Cys</td>
<td>S(\text{COOH}) none 0.27 0.71 16.8: 11.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[a] Determined by \(^1\)H NMR.
[b] Determined by EA.
[c] Degree of functionalization, determined by EA.
[d] Ratio of structural units I and II (see Scheme 4.3).

Three weak polyelectrolytes were dispersed in water (polymer concentration \(\sim 0.1\) wt %) at room temperature in a certain range of pH, as monitored by eye (clear solution) and by measurement of the light scattering intensity at an angle of 90° (Figure 4.17 a). Flocculation or precipitation of polymer was recognized by a steep increase of turbidity and scattering intensity. The polyanion PB2-S-PA readily dispersed in neutral to basic media when acid residues were in carboxylate form. The polymer started to precipitate when the pH was decreased (using 0.1 N HCl) to a value of 6.0 or lower. The polymer re-dispersed upon addition of 0.1 N NaOH. The scattering intensity was the same as at the beginning of the titration cycle, indicating that the process was fully reversible. According observations were made for the polycation PB2-S-EA, which was dispersable in neutral to acidic media when amine residues were in ammonium form and started to precipitate sharply at pH 8.2. The poly(amino acid) PB2-S-Cys dispersed in very acidic and in basic media; precipitation occurred around the isoelectric point (which for methionine is at pH 5.74) between pH 2.3 and \(\sim 9\).

Evidently, these samples responded to changes in pH as would be expected for weak polyelectrolytes. The peculiarity is that the copolymer chains form aggregates rather than dissolve on a molecular level. Dynamic light scattering analyses (DLS, scattering angle: 90°) of 0.3 wt % solutions of PB2-S-PA and PB2-S-Cys in 0.1 N NaOH and of PB2-S-EA and PB2-S-Cys in 0.1 N HCl showed the presence of aggregates with apparent hydrodynamic radii of \(R_{hh}^{\text{app}} \sim 170\) nm (PB2-S-PA and PB2-S-EA) and \(R_{hh}^{\text{app}} \sim 130/120\) nm (PB2-S-Cys, low/high pH) (Figure 4.17).
Figure 4.17. a) Results of light scattering analysis of dilute aqueous solution of 1) PB2-S-PA, 2) PB2-S-EA, 3) PB2-S-Cys (top to bottom) in dependence of pH; lines are to guide the eye. b) Size distributions of aggregates (DLS) in 0.3 wt % aqueous solutions of 1) PB2-S-PA, 2) PB2-S-EA, 3) PB2-S-Cys at given pH.
Small-angle X-ray scattering (SAXS) indicated the presence of vesicles in 5 wt % solutions of three polymeric amphiphiles (Figure 4.18) (samples were directly dissolved in water and then the pH of the solution was adjusted to either pH 7 (PB2-S-PA and PB2-S-EA) or pH 11 (PB2-S-Cys)). The asymptotes of scattering curves at low scattering vectors \( s = \frac{2}{\lambda} \sin \Theta \) obey the characteristic \( I(s) \propto s^{-2} \) (\( I \): scattering intensity). An additional local maximum arises at \( s \sim 0.13 \text{ nm}^{-1} \), suggesting a multilamellar structure of the membrane (Figure 4.19) with a lamellar spacing of about 7 nm. Evidently, the layers formed by the three polymeric amphiphiles are about as thick as a bilayered membrane of a liposome (~5 nm).
4.2.2 Aggregation behavior in alcohol/water mixtures

For investigations on the self-organization of polymeric amphiphile in alcohol/water mixture diol modified 1,2-polybutadiene with an average number of 40 repeat units PB2-S-PD (number-average molecular weight: $M_n = 2240$ g/mol; polydispersity index: PDI = 1.1) was used. The characteristic of the product is described in section 4.1.1.4. PB2-S-PD sample could be dissolved in EtOH (polymer concentration ~0.1 wt %) at room temperature but not in water.

The onset of self-assembly was followed by measurement of the change of scattering intensity of the solution as a function of water content. To construct the scattering intensity diagram deionized water was added dropwise to the polymer solution. After the addition of the desired amount of water, the solution was left to equilibrate for 10 minutes. The scattering intensity and the size of aggregates were then measured using DLS. The cycle of water addition was continued until turbidity of solution did not disappear and a high jump of scattering intensity was detected.

Figure 4.20 shows the scattering intensity and diagrams of apparent hydrodynamic radii obtained for a solution depending on the water content. The diagrams show an increase of both scattering intensity and size of aggregates with increasing water content. A sharp jump in scattering intensity occurred at a water content of about 70%, and as monitored by eye, the solution became turbid. DLS measurement revealed formation of aggregates with the apparent hydrodynamic radius in range from about 140 nm to 500 nm. Evidently, this sample responded to changes in water content. This growing process can be explained by coagulation, which occurs because larger particles, with their greater volume to surface area ratio, exhibit a lower surface free energy than smaller particles. To minimize the energy, small particle tend to diffuse through solution and binds together forming larger particles. Therefore the growing process of the aggregates is observed.
Light microscopy measurements of PB2-S-PD sample in a mixture of ethanol/water 50:50 (w/w) shows the presence of micron-sized spherical aggregates (see Figure 4.21). Size and shape of the obtained aggregates suggest that these are vesicles.

4.3 Self organization of block ionomers

The creation of new interesting morphologies is connected with the synthesis of well-defined functional block copolymers. A variety of block copolymer material with high quality and well-defined structure can be obtained by a combination of ionic polymerization and radical addition techniques. Amphiphilic 1,2-polybutadiene-based block copolymers with either a poly(ethylene oxide) or a poly(2-methyl-2-oxazoline) hydrophilic segment, made by ionic polymerization, were modified with a series of mercaptanes, as described in section 4.1.2 and section 4.1.3. For the self-assembly investigations copolymers with carboxylic acid and primary amine functions have been selected. 1,2-Polybutadiene-block-poly(ethylene oxide)
(PB-b-PEO) copolymer was modified with ω-functional mercaptanes carrying either a carboxyl HS-PA (polyanion_{27-b-PEO_{273}}) or an ammonium group HS-EA (polycation_{30-b-PEO_{273}}). 1,2-Polybutadiene-block-poly(2-methyl-2-oxazoline) (PB-b-PMOxa) copolymer was modified with ω-functional mercaptan HS-EA (polycation_{40-b-PMOxa_{190}}). As was described in section 4.1.1.1, the functional segment in the modified products represents a random copolymer structure composed of the units I and II, thus the number of introduced functional ionic groups, $x'$ onto 1,2-PB segments of the precursor copolymers corresponds the sum of the units I and II.

The chemical compositions of products utilized in these studies are summarized in Table 4.9.

Table 4.9. Chemical composition of products obtained by modification of 1,2-PB_{40}-based copolymers with mercaptanes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C=C</th>
<th>S/C</th>
<th>f</th>
<th>I:II</th>
<th>$x'$</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB_{40-b-PEO_{273}}/carboxylate</td>
<td>none</td>
<td>0.05</td>
<td>0.68</td>
<td>14.4:12.8</td>
<td>27</td>
<td>Polyanion_{27-b-PEO_{273}}</td>
</tr>
<tr>
<td>1,2-PB_{40-b-PEO_{273}}/ammonium</td>
<td>none</td>
<td>0.08</td>
<td>0.76</td>
<td>20.8:9.6</td>
<td>30</td>
<td>Polycation_{30-b-PEO_{273}}</td>
</tr>
<tr>
<td>1,2-PB_{40-b-PMOxa_{190}}/ammonium</td>
<td>none</td>
<td>0.11</td>
<td>1</td>
<td>40:0</td>
<td>40</td>
<td>Polycation_{40-b-PMOxa_{190}}</td>
</tr>
</tbody>
</table>

[a] Determined by $^1$H NMR.
[b] Determined by EA.
[c] Degree of functionalization.
[d] Ratio of structural units I and II (see Scheme 4.3).

The block copolymers prepared consist of a weak polyelectrolyte block and a neutral hydrophilic block. In aqueous solution at neutral pH, the polyelectrolyte block should be fully ionized, and the samples should thus be double-hydrophilic in nature. However, the charged groups are connected to a hydrocarbon backbone via a relatively long hydrophobic thioether spacer, these polymers are rather amphiphilic- hydrophilic polymers (see modified 1,2-PB described in section).

4.3.1 Polycation_{40-b-PMOxa_{190}} copolymer

Shape and size of block copolymer aggregates depend not only on polymer-related properties, such as molecular weight or relative block length, but also on solution conditions. The addition of salts results in a shielding of charges along the ionized chains and consequently in a reduction of electrostatic repulsion, thus can be used to control the morphology of
To investigate the formation of aggregates based on the manipulation of solution conditions, the effect of salts on the aggregation behavior of polycation$_{40-b}$-PMOxa$_{190}$ was investigated. Salts were selected based on the Hofmeister series, which ranks the salts according to their effectiveness to precipitate for colloidal system\textsuperscript{148} (See Scheme 4.4). Ions used for salt formation are marked with a circle in Scheme 4.4.

![Scheme 4.4. Hofmeister series. (This is a partial listing; many more salts have been studied).](image)

Three different salts were selected, which were arranged in the series starting from cosmotropic (ammonium dihydrogenphosphate, NH$_4$H$_2$PO$_4$) through sodium chloride (NaCl), which is located in the middle of series, to finish with chaotropic one (Guanidine thiocyanate, NH$_2$C(=NH)NH$_2$*HSCN). To give a distinguishable Hofmeister effect of ion type on the aggregation behavior of polycation$_{40-b}$-PMOxa$_{190}$, salts were used in a high concentration of 1 N.

1 N salt solutions were prepared by directly dissolving the appropriate salt in bi-distilled water, pH~6.5. Then, PM-S-EA was dissolved in the aqueous salt solutions at a concentration of 1 g/L. After 24 h of stirring, the solutions were examined by dynamic (DLS) and static (SLS) light scattering to obtain information on the size and shape of aggregates. DLS showed the presence of large aggregates with apparent hydrodynamic radii $R_{\text{h,app}}$ ~205 nm, 180 nm and 99 nm going from cosmotropic to chaotropic salt solution (Figure 4.22).

SLS provided further information on the examined samples, such as the radius of gyration, $R_g$, and the apparent molecular weight of the aggregates $M_w^{\text{app}}$, results are summarized in Table 4.10. However, the large size and high polydispersity of obtained aggregates led to difficulties in data evaluation. Guinier plots obtained from SLS are presented in Figure 4.23.
RESULTS AND DISCUSSION

AUC sedimentation velocity runs were performed to obtain apparent sedimentation coefficient distributions, $g^*(s)$, of the assemblies formed by polycation$_{40}$-b-PMOxa$_{190}$ depending on the type of salt.

For PM-S-EA dissolved in cosmotropic salt solution, AUC revealed a multimodal $g^*(s)$, suggesting the presence of different kind of species. In the sodium chloride and the chaotropic salt solution only one kind of species could be observed (Figure 4.24); mean values of sedimentation coefficient, $s$ [Sved] (1S=1 Svedberg=10$^{-13}$ second), were 0.5 and 1, respectively. Aggregates in sodium chloride solution sedimented at a slower rate than that in chaotropic salt solution, and additionally represented a narrower sedimentation distribution. Experimental data obtained for polycation$_{40}$-b-PMOxa$_{190}$ sample depending on the nature of the aqueous salt solution are summarized in Table 4.10.

Figure 4.22. Apparent particle size distributions, measured by DLS, for 0.1 wt % PM-S-EA sample, in a) NH$_4$H$_2$PO$_4$, b) NaCl, c) NH$_2$C(=NH)NH$_2$HSCN salt solutions.
Figure 4.23. SLS data (Guinier plots) obtained for 0.1 wt % PM-S-EA sample, in a) NH₄H₂PO₄, b) NaCl, c) NH₂C(=NH)NH₂·HSCN salt solutions.

Figure 4.24. Apparent sedimentation coefficient distributions g*(s) of polycation-b-PMOxa₁₉₀ aggregates (concentration: 0.1 wt %) in three different salt solution.
Table 4.10. Experimental data obtained from LS and AUC measurements for PM-S-EA sample depending on kind of aqueous salt solution.

<table>
<thead>
<tr>
<th>Salt type</th>
<th>( R_h^{\text{app}} ) [nm]</th>
<th>( R_g ) [nm]</th>
<th>( M_w^{\text{app}} ) [g/mol]</th>
<th>( s ) [Sved]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium dihydrogenphosphate</td>
<td>205</td>
<td>300</td>
<td>( 6 \times 10^{10} )</td>
<td>0.4, 0.9, 1.1</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>180</td>
<td>305</td>
<td>( 1 \times 10^{10} )</td>
<td>0.5</td>
</tr>
<tr>
<td>Guanidine thiocyanate</td>
<td>99</td>
<td>203</td>
<td>( 1 \times 10^{10} )</td>
<td>1</td>
</tr>
</tbody>
</table>

A low value for sedimentation coefficient indicates that the molar mass of particles is very low or that the aggregates contain large amounts of solvent. As indicated by SLS, molar masses of the aggregates are very high, thus compact particles should sediment faster than observed. LS and AUC results together suggest that the aggregates formed are vesicles. Calculated \( R_g/R_h \) ratios are significantly higher than 1, what may suggest elongated shape of vesicles.

Exemplary for the aggregates formed by polycation_{40-b}-PMOx_{190} in sodium chloride solution, the concentration of the salt was decreased to 1 mN. SLS provided values of \( R_g=328 \) nm and \( M_w^{\text{app}}=5.1 \times 10^9 \) g/mol. DLS revealed formation of aggregates with apparent hydrodynamic radius, \( R_h^{\text{app}}=183 \) nm. Sedimentation coefficient, as measured by AUC, were found to be as low as \( s \approx 0.5 \) Sved (see Figure 4.25).

According to these results, there is no concentration salt dependence in 1 mN-1 N region for polycation_{40-b}-PMOx_{190} aggregates.
For a visualization of the aggregates formed by polycation$_{40}$-$b$-PMOxa$_{190}$ copolymer in 1mN NaCl solution confocal microscope was applied. For this purpose, amine groups in the sample were labeled with a fluorescence dye by reaction with fluorescein 5(6)-isothiocyanate (FITC). Details about the labeling are described in section 6.2.3. Confocal microscopy measurement confirmed presence of aggregates (see Figure 4.26), but an accurate measurement of their size could not be achieved.

![Figure 4.26](image)

Figure 4.26. The confocal microscopy picture obtained for polycation$_{40}$-$b$-PMOxa$_{190}$ sample labeled with FITC.

Studies on the self-organization of hydrophilically modified 1,2-PB homopolymers (section 4.2) revealed the formation of vesicular structures with very thin multilamellar membrane, formed by hydrophobic backbone, which was covered by hydrophilic ionic function (Figure 4.19). In the case of the block copolymers, the hydrophobic backbone can assemble into a mono- or a bilayer, and is covered by water-soluble ionic groups and PMOxa blocks (see Scheme 4.5).

![Scheme 4.5](image)

Scheme 4.5. Schematic model of the structure of vesicles formed by polycation$_{40}$-$b$-PMOxa$_{190}$ in 1 mN NaCl aqueous solution.

However if the two hydrophilic parts undergo phase separation, the membrane can be asymmetric: one side predominantly covered by ionic groups and the other by PMOxa.
The membrane should then have a monolayered structure. Additionally, the question arises, which hydrophilic segment is oriented toward the outside and which to the inside (see Scheme 4.6).

Scheme 4.6. Schematic model of the possible vesicle morphologies of polycation_{40-b-PMOxa_{190}}.

The polycation_{40-b-PMOxa_{190}} sample revealed a positive electrokinetic zeta-potential with a value of about +23 mV, suggesting that the ammonium groups are on the outside of the vesicles. However, the membrane of vesicles should be very thin, which could result in a positive zeta-potential value, even though ammonium groups are on the inside of the vesicle wall. Positive values can also be affected by a dynamic flip-flop of polycation_{40-b-PMOxa_{190}} chains, or in other words: there is no phase separation. Taking into account the above mentioned consideration the orientation of the block copolymers within the vesicular walls cannot be judged.

4.3.2 Polyanion_{27-b-PEO_{273}} copolymer

The polyanion_{27-b-PEO_{273}} copolymer was dissolved in 1 mN NaCl salt solution (pH~6.5) at a concentration of 0.1 wt %. After 24 h of stirring, the solution was examined by LS and AUC. SLS revealed presence of aggregates with $R_g = 254$ nm, and $M_{w,app} = 1.9 \times 10^9$ g/mol. However, the high polydispersity of aggregates again led to difficulties in data evaluation. DLS showed
the presence of large aggregates with an apparent hydrodynamic radius of about 122 nm. The AUC measurement revealed low sedimentation coefficient of 0.5 Sved, typical for vesicles (see Figure 4.27).

Figure 4.27. a) Apparent particle size distributions (DLS) and b) sedimentation coefficient distribution of the polyacid-b-PEO₂₇₃ (concentration: 0.1 wt %).

Cryo-TEM analysis of polyanion₂₇-b-PEO₂₇₃ sample revealed the presence of spherical aggregates with sizes in the range of 70-200 nm. However, due to the low contrast characteristic rim for vesicles was not detected (Figure 4.28 a). Vesicular aggregates could be observed on representative TEM picture presented in Figure 4.28 b. Due to no contrasting applied the interior of the aggregates stayed bright.

Figure 4.28. a) Cryo-TEM image of a vitrified 0.1 wt % PP3-S-PA suspension, b) TEM micrograph of the vesicular aggregates of the PP3-S-PA sample.

Like for the polycation₄₀-b-PMOxa₂₉₀ system (section 4.3.1), the polymer vesicles are formed with a hydrophobic backbone membrane, which is covered by hydrophilic PEO chain and by ionic groups. However, if the hydrophilic parts undergo phase separation, the vesicles could have an asymmetric membrane. In order to get information about the orientation of the
diblock copolymer within the vesicular walls, the morphology of the vesicles was studied by fluorescence spectroscopy. The end chain of the PEO block was labeled with a fluorescent dye: anthracene. Details about the synthesis are described in section 6.2.4. A fluorescence quenching experiment was used to determine whether the labeled PEO block was oriented toward inside or toward the outside of the vesicles*. N-dodecylpyridinium chloride was added to the external solution of the labeled polyanion 27-b-PEO273 sample. N-dodecylpyridinium chloride is known to quench the fluorescence of anthracene.150 However, only the anthracene located at the outer surface of the vesicles should be quenched.

Figure 4.29 shows the fluorescence decays and emission spectra of anthracene in labeled PP3-S-PA sample.

![Fluorescence decays and emission spectra of anthracene in PP3-S-PA sample](image)

Figure 4.29. a) Fluorescence decays and b) emission spectra of anthracene in PP3-S-PA sample labeled with anthracene, (the mean lifetime \( \tau \) was evaluated from the emission at 417 nm, excitation at 370 nm).

The Stern-Volmer plot of the time-resolved fluorescence and the modified Stern-Volmer plot are presented in Figure 4.30.

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* Experiment was done in Department of Physical and Macromolecular Chemistry, Charles University in Prague.
The evaluation yields that about 80% of the labeled PEO chains were quenched. Due to the hydrophobic character of anthracene, the remaining 20% could be buried in the hydrophobic membrane, forming some kind of loop from PEO chains. In that case, vesicles with a corona of segregated polymer chains could be obtained, the PEO blocks being oriented toward the outside, and the ionic blocks toward the inside of the vesicles. This could agree with geometrical consideration: due to the curvature of the vesicle walls, it is favorable that the blocks with lower volume segregate toward the interior.\textsuperscript{151} However, the solution of polyanion\textsubscript{b}-PEO\textsubscript{273} sample exhibited a negative electrokinetic zeta potential of about -20 mV. Results suggest that the morphology of the obtained vesicles could be partially mixed, with 20% of PEO chains being oriented toward the interior. Nevertheless, 80% of PEO blocks oriented to the outside of the vesicles means that the vesicle membrane has a reduced symmetry.

Nevertheless, one has to remember the dynamics of the system and the very little thickness of the membrane, which can be penetrated by quencher. In that case, the morphology of the obtained vesicles cannot be judged.

\subsection*{4.3.3 Polycation\textsubscript{30}-b-PEO\textsubscript{273} copolymer}

The polycation\textsubscript{30}-b-PEO\textsubscript{273} copolymer was dissolved in 1 mN NaCl salt solution (pH≈6.5) at a concentration of 0.1 wt %. After 24 h of stirring, the solution was examined by electrokinetic zeta-potential, LS and AUC. The electrokinetic zeta-potential was found to be + 15 mV. Guinier analysis was applied to evaluate SLS yield $M_w^{\text{app}}$ $3.1\times10^9$ g/mol and $R_g$ 257 nm.
RESULTS AND DISCUSSION

However, high polydispersity led to difficulties in data evaluation. DLS showed the formation of unimodal aggregates with an apparent hydrodynamic radii about 200 nm. The sedimentation coefficient, as measured by AUC, were found to be as low as $s \sim 0.6$ Sved, indicating that the molar mass of particles is very low or that aggregates contain large amounts of water. DLS and AUC results together suggest that aggregates are vesicles (Figure 4.31).

![Figure 4.31. a) Apparent particle size distributions (DLS), b) apparent sedimentation coefficient distribution of the polybase-$b$-PEO$_{273}$ (concentration: 0.1 wt %).](image)

Cryo-TEM analysis of the polycation$_{30}$-$b$-PEO$_{273}$ revealed the presence of spherical aggregates with sizes in the range of 150-300 nm. A representative cryo-TEM image is shown in Figure 4.32. As for the polyanion$_{27}$-$b$-PEO$_{273}$ copolymer (section 4.3.2), neither the outer surface nor the characteristic rim of the membrane could be detected.

![Figure 4.32. Cryo-TEM image of a vitrified 0.1 wt % PM1-S-EA suspension.](image)

Taking into account the similarity of this system to the polyanion$_{27}$-$b$-PEO$_{273}$ system (described in section 4.3.2) one would expect a partially mixed morphology of vesicles, with
the majority of PEO segments oriented toward outside and the cationic segment toward inside. However, the dynamics of the system and the thin membrane cannot be neglected.

### 4.4 Polyion complexes of block copolymers ionomers

The ability of amphiphilic block copolymers to self-assemble into colloidal objects in selective solvents can be used for a variety of applications. Applying the concepts of molecular recognition to the self-assembly of macromolecules can lead to greater structural complexity and functionality. For example, electrostatic interactions occurring in the mixture of oppositely charged chains were found to act as a driving force for the formation of interpolyelectrolyte complexes with a very broad range of micellar and vesicular morphologies and other hierarchal superstructures. 6,75,152,153,154

In the following study, 1,2-polybutadiene-\textit{block}-poly(ethylene oxide) (PB-\textit{b}-PEO) and 1,2-polybutadiene-\textit{block}-poly(2-methyl-2-oxazoline) (PB-\textit{b}-PMOxa) copolymers modified with \(\omega\)-functional mercaptanes carrying either carboxyl or ammonium groups are utilized for the formation of complexes based on electrostatic interaction. The formation of polyion complexes with (\textit{i}) PEO/PEO and (\textit{ii}) PEO/PMOxa corona by mixing oppositely charged ionic block copolymers is described.

#### 4.4.1 Polyion complex with PEO/PEO corona

For the formation of polyion complexes with a PEO/PEO corona, polyanion-\textit{b}-PEO and polycation-\textit{b}-PEO copolymers were used. Oppositely charged segments of the block copolymer pair are preferred to have matched chain length, compensating for the counter charge in a stoichiometric manner. To satisfy this condition of matched chain length, both the anionic and the cationic block copolymer were prepared from the same precursor copolymer PB-\textit{b}-PEO.

Anionic and cationic components were prepared from PB-\textit{b}-PEO precursor by radical addition into 1,2-PB block of \(\omega\)-functional mercaptanes bearing carboxylate and ammonium group, respectively (section 4.1.2). The polyanion\textit{27-b-PEO}_{273} and polycation\textit{30-b-PEO}_{273} copolymers were separately dissolved in 1 mN NaCl aqueous solution at a concentration of 0.1 wt %. PICs were prepared by mixing the polymer solutions at pH~7, in a double-jet...
syringe pump; the molar ratio \([+]/[-]\) was unity. The final concentration of the complex was 0.06 wt %.

Electrostatically neutral conditions were calculated based on the number of functional groups in modified block copolymers used for complex formation, and were confirmed by Zeta-potential titration (Figure 4.33).

![Zeta-potential titration](image)

Figure 4.33. Zeta-potential titration of polyanion\(_{27}\)-b-PEO\(_{273}\) with polycation\(_{30}\)-b-PEO\(_{273}\) (■), and back-titration of polycation\(_{30}\)-b-PEO\(_{273}\) with polyanion\(_{27}\)-b-PEO\(_{273}\) (▲).

The Guinier analysis of SLS data obtained for the mixture of polyanion\(_{27}\)-b-PEO\(_{273}\) and polycation\(_{30}\)-b-PEO\(_{273}\) revealed \(R_g=233\) nm and \(M_w^{\text{app}}=2.1\cdot10^9\) g/mol. DLS showed the presence of aggregates with \(R_h^{\text{app}}\sim144\) nm (Figure 4.34). The results of the AUC measurements for polyanion\(_{27}\)-b-PEO\(_{273}\) and polycation\(_{30}\)-b-PEO\(_{273}\) have been already discussed in sections 4.3.2 and 4.3.3. In case the polymers did not form a PIC, the sedimentation coefficient distribution in the mixture should correspond to that of the separated copolymers. Otherwise, a different sedimentation coefficient distribution should be observed. Figure 4.35 depicts the apparent sedimentation coefficient distributions of the samples polyanion\(_{27}\)-b-PEO\(_{273}\), polycation\(_{30}\)-b-PEO\(_{273}\) and the mixture, PIC-PEO/PEO. The AUC measurement for the mixed sample PIC-PEO/PEO revealed the presence of a single species with a different apparent sedimentation coefficient distribution than in the solutions of block ionomers. The presence of new species, sedimenting with a mean \(s\) value of 1 S confirmed that polyion complexation took place. The low value of the sedimentation coefficient and the large size (DLS) suggested that PIC aggregates were vesicles.
Figure 4.34. Apparent particle size distributions, measured by DLS, for 0.1 wt % PIC-PEO/PEO in 1 mN NaCl aqueous solution.

Figure 4.35. Apparent sedimentation coefficient distributions of PIC-PEO/PEO, polyanion_{27-b}-PEO_{273} and polycation_{30-b}-PEO_{273} in 1 mN NaCl solution.

Cryo-TEM indicated presence of spherical aggregates, which are about 100-200 nm in diameter (Figure 4.36). However, the contrast is too low to render the rim and the corona of vesicles.
4.4.2 Polyion complex with PEO/PMOxa corona

As was described in section 4.4.1, the polyion complex prepared from two block ionomers with PEO as the second block led to the formation of vesicular aggregates with PEO corona. In case there are two different solvating blocks, one might obtain polyion complexes with an asymmetric structure, like “Janus micelles”\textsuperscript{155} or vesicles having a microphase-separated membrane, as illustrated in Scheme 4.7.

![Scheme 4.7. Schematic formation of PIC with segregated corona.](image)

The anionic and cationic components of the polyion complex with PEO/PMOxa corona were prepared from PB-\textit{b}-PEO and PB-\textit{b}-PMOxa copolymer precursors by radical addition into 1,2-PB block of ω-functional mercaptanes bearing carboxylate and ammonium groups
respectively (sections 6.2.2.2 and 6.2.2.3). The polyion complex with PEO/PMOxa corona,
PIC-PEO/PMOxa, was prepared by mixing polyanion$_{27}$-$b$-PEO$_{273}$ and polycation$_{40}$-$b$-
PMOxa$_{190}$.

The polyanion$_{27}$-$b$-PEO$_{273}$ and polycation$_{40}$-$b$-PMOxa$_{190}$ copolymers were separately
dissolved in 1 mM NaCl aqueous solution. The PIC with PEO/PMOxa corona was prepared
by mixing the polymer solutions in a double-jet syringe pump in an equal unit ratio of anionic
and cationic groups, such that the final concentration of the complex was 0.06 wt %.
Electrostatically neutral conditions were calculated based on the number of functional groups
in the modified block copolymers used for complex formation, and were confirmed by Zeta-
potential titration (Figure 4.37). Additionally, the slight difference in back-titration may be
due to the bulky PMOxa segment that can obstruct the access to the cationic functions.

![Figure 4.37. Zeta-potential titration of Polyanion$_{27}$-$b$-PEO$_{273}$ with Polycation$_{40}$-$b$-PMOxa$_{190}$ (■),
and Polycation$_{40}$-$b$-PMOxa$_{190}$ with Polyanion$_{27}$-$b$-PEO$_{273}$ (▲).](image)

The aggregates of PIC-PEO/PMOxa were analyzed by LS and AUC. According to SLS, the
value of $R_g$ was 285 nm (Guinier analysis). The molecular weight of aggregates was $3.9 \times 10^9$
g/mol. DLS showed the presence of aggregates with $R_{h}^{app}=134$ nm (see Figure 4.38). AUC
revealed the presence of a PIC-PEO/PMOxa species sedimenting with a mean $s$ value of 1 S.
The apparent sedimentation coefficient distribution is different from that of separated
copolymers, confirming that polyion complexation took place (see Figure 4.39).
RESULTS AND DISCUSSION

Figure 4.38. Apparent particle size distributions (DLS) of PIC-PEO/PMOxa in 1 mN NaCl solution.

DLS/AUC results suggested that PIC-PEO/PMOxa aggregates were vesicles. Cryo-TEM indicated spherical aggregates (Figure 4.40).

Figure 4.39. Apparent sedimentation coefficient distribution of PIC-PEO/PMOxa, polyanion\textsubscript{27-b-PEO\textsubscript{273}} and polycation\textsubscript{40-b-PMOxa\textsubscript{190}} in 1 mN NaCl solution.

Figure 4.40. Cryo-TEM image of a vitrified 0.06 wt % PIC-PEO/PMOxa suspension.
The incompatible blocks of PEO and PMOxa can undergo phase separation in aqueous media, as demonstrated by Meier,\textsuperscript{151} thus PIC-PEO/PMOxa vesicles can have an asymmetrical membrane, where one side is predominantly covered by PEO and the other by PMOxa.

To check whether PIC-PEO/PMOxa forms a microphase-separated membrane, \textsuperscript{1}H NMR NOESY experiment was performed. In case there is no phase segregation a NOE between the protons of PEO (\(\delta = 3.7\) ppm) and PMOxa (\(\delta = 1.8\) ppm, (CH\(_3\)), 3.5 ppm (CH\(_2\)) should be detected. Otherwise, there should be no cross peak in 2D spectrum.\textsuperscript{5} As can be seen in Figure 4.41, there is cross peak between corona blocks PEO and PMOxa. Hence, there is no phase separation of PEO and PMOxa chains.

**Figure 4.41.** 2D \textsuperscript{1}H, \textsuperscript{1}H NMR NOESY contour plot of a 1 wt % aqueous solution of polycation\textsubscript{40}\textsuperscript{-}b-PMO\textsubscript{190} and polyanion\textsubscript{27}\textsuperscript{-}b-PEO\textsubscript{273}. Circles indicate cross peaks between PEO and PMOxa blocks. * THF traces (from the synthesis).
5 SUMMARY AND OUTLOOK

In this work, the modular synthesis of functional polymers by radical addition of \( \omega \)-functional mercaptanes onto 1,2-PB-based homopolymers and block copolymers is demonstrated. Due to the commercial availability of a large number of different mercaptanes, this method can be used for the generation of a toolbox of polymers with diverse functionalities, including electrolytes as well as chiral amino acids and so on. Polymer samples become available which cannot or can hardly be made by any other synthetic method.

As evidenced by the results obtained for the model reaction of methyl-3-mercaptopropionate with PB, radical addition of mercaptanes onto the vinyl double bonds occurs mainly in anti-Markownikoff fashion. Modified polymer samples were free of double bonds, thus giving good thermal stability. However, the degree of functionalization was usually less than quantitative, i.e. 70–86%. The deviation between the number of converted double bonds and that of functional groups is due to a side reaction of radicals involving the formation of cyclic units. Importantly, the cyclization reaction does not alter the molecular-weight distribution of the polymer backbone.

The free-radical addition of mercaptanes was applied to produce a platform of hydrophilically modified polybutadiene homopolymers. Firstly, the aggregation behavior of weak polyelectrolytes was described. The investigated samples revealed amphiphilic character and self-assembled into vesicles through direct dissolution in water. The aggregates revealed pH-responsive character. Further investigation on the self-assembly of polymeric amphiphiles involved the aggregation behavior of the diol-modified polybutadiene homopolymer in water/alcohol mixtures. The sample assembled into vesicles, and the size of which responded on changes in water content.

The following investigations concerned the influence of the introduced groups on the self-organization of series of modified block copolymers. 1,2-PB-based block copolymers with either a poly(2-methyl-2-oxazoline) or a poly(ethylene oxide) hydrophilic segment, modified with a series of mercaptanes containing \( (i) \) cationic and \( (ii) \) anionic functionalities, were investigated according to their aggregation behavior.

The block copolymers prepared consisted of a polyelectrolyte block and a neutral hydrophilic block. In aqueous solution at neutral pH, the modified copolymers should be double-hydrophilic in nature. However, the charged groups connected to a hydrocarbon backbone via
a relatively long hydrophobic thioether spacer, influenced on rather amphiphilic-hydrophilic character of these polymers.

The studies on the self-organization of the block ionomers revealed the formation of vesicular structures with a membrane formed by the hydrophobic backbone, and a corona of water-soluble segments. The questions arose if the two hydrophilic parts (electrolyte and PEO or PMOxa) underwent phase separation, thus resulting in the formation of an asymmetric membrane. However, due to very thin membrane and a possible dynamic flip-flop of the block copolymers chains, zeta-potential and fluorescence quenching could not provide information about the orientation of the block copolymers within the vesicular walls. More information about the structure of the vesicular membrane could be provided by small-angle X-ray scattering.

The formation of polyion complexes with (i) PEO/PEO and (ii) PEO/PMOxa corona by mixing oppositely charged block ionomers were described. AUC confirmed that polyion complexation took place, and DLS/AUC results suggested that PIC aggregates are vesicles.

In case of PIC-PEO/PMOxa, which contain the two different solvating blocks, a challenging task was to obtain asymmetric polymeric aggregates with broken or reduced symmetry, that are Janus micelles or vesicles with microphase separated membrane. However, in the system described here, phase separation of PEO and PMOxa blocks could not be observed by 2D-NMR NOESY experiments.

These results provide some insight on the mechanism involved in the radical addition of ω-functional mercaptanes onto PB segments. The versatility of the applied method was confirmed by a number of examples of successfully introduced functionalities onto PB homopolymers and PB-based block copolymers. Due to the modular character of synthesis, this concept can be extended on additions of different functional mercaptanes onto different precursor polymers. Modifications of 1,2-PB polymers with a series of mercaptanes bearing the functionalities, which can be charged under appropriate conditions represents an alternative method for the production of polyelectrolytes. The addition reaction using protected amino acid (cysteine) may represent the first step in the direction of synthetic polymers with introduced oligopeptide sequences. Further development of this approach may lead to the production of so-called “molecular chimeras”, polymers containing synthetic and peptide blocks. In the future, the library of functional polymers may be extended with samples containing, for example phosphonates, oligopeptides, nucleo bases and sugars.
Synthesis of various functional polymers based on the same backbone allows further systematic studies on the aggregation behavior. The method provides also an interesting opportunity to prepare polymers containing suitable complementary functionalities, which may be used for the preparation of polymer complexes via noncovalent interactions. A next step would have been to synthesize and study PIC with a thermo-responsive poly(2-isopropyl-2-oxazoline) (PiPOxa) instead of PMOxa chain. PIC-PEO/PiPOxa could provide an asymmetric structure due to a stronger segregation. Due to biocompatibility and a lower critical solution temperature (LCST) near human body temperature of PiPOxa, this system could be interesting for application in biomedical fields.
6 EXPERIMENTAL PART

6.1 Solvents and Reagents

All solvents used in NMR measurement were commercially obtained and used as received: CDCl₃, D₂O, DMF-d₇, DMSO-d₆, CD₃OD-d₄, THF-d₈ (Deutero GmbH).

Where dry solvents or reagents were used, these were distilled and dried over suitable drying agents: THF Na/K alloy, LiAlH₄, KOH, 2-methyl-2-oxazoline (MOxa), toluene, DCE and n-hexane (CaH₂). Methanol, ethanol, benzene, DMSO, acetone, diethyl ether, NMP, etc. were used as received.

The following reagents were obtained commercially in the highest purity grade available (≥98%) from Sigma-Aldrich, Fluka, or Acros Organics (Germany) and were used as received. Unless otherwise stated: ethylene oxide, 1,3-butadiene, phosphazene base (t-BuP₄), sec-butyl lithium (sec-BuLi), n-butyl lithium (n-BuLi), acetic acid, N,N-azobis(isobutyrilonitrile) (AIBN), 3-mercaptopropionic acid, 2-aminoethanethiol hydrochloride, 2-(dimethylamino)ethanethiol hydrochloride, 2-(diethylamino)ethanethiol hydrochloride, 3-mercaptop-1,2-propanediol, boc-L-cysteine. All inorganic materials used (NaOH, KOH, Na, K, CaH₂, LiAlH₄, HCl) were of standard quality.

6.2 Synthetic procedures

6.2.1 Preparation of precursors by anionic polymerization

All polymerization reactions were performed on the Schlenk line (combined vacuum/argon line) equipped with Rotaflo® Teflon valves. Argon was first dried by flowing through a column with an active drying and adsorption agent (silica gel blue, Fluka) and then “washed” through a system containing Na/K alloy in toluene. Utilized glass reactors were heated under vacuum to about 600 °C to exclude residues of moisture and, if necessary, flushed with dry argon.

6.2.1.1 Synthesis of 1,2-PB precursors

1,3-butadiene was dried over CaH₂ and then purified by cryo-distillation from n-BuLi. THF was purified by distillation from sodium/potassium alloy. The reactor was kept at -78 °C
using ethanol/dry ice bath and then THF and dry 1,3-butadiene were condensed into the reactor. To the solution calculated amount of sec-BuLi initiator was added. After completion of the polymerization (24 h) ethylene oxide was added to cap the living PB chain ends. After stirring for 1h at -78 °C the reaction solution was left to warm to room temperature and was stirred at this temperature for next 3 days till colorless solution developed. The resulting polymer was precipitated in methanol and yellow honey-like product was dried under vacuum to constant weight. According to $^1$H NMR analysis, end capping of the chains was quantitative and content of butadiene 1,2-adduct was found to be $\geq 93\%$.\textsuperscript{156}

6.2.1.2 Synthesis of 1,2-PB-b-PEO precursors

The PB-b-PEO copolymers were synthesized in 2 steps. Firstly, hydroxyl-terminated 1,2-PB was prepared by living anionic polymerization of 1,3-butadiene as described in section 6.2.1.1. Then the second PEO block was synthesized by anionic ring-opening polymerization of EO using activated 1,2-PB precursor. Ethylene oxide was purified successively by distillation from CaH$_2$, sodium mirror, and $n$-BuLi.

The 1,2-PB homopolymer was dissolved in the small amount of dry THF, placed in the reactor and dried overnight under dynamic vacuum. The polymerization solvent, THF, was dried above Na/K alloy and then condensed in the polymerization reactor.

After complete dissolution of the 1,2-PB homopolymer, phosphazene base ($t$-BuP$_4$) was added to affect deprotonation of the terminal –OH function allowing initiation of the anionic ring opening polymerization of ethylene oxide. The reactor was cooled to -78 °C and then the ethylene oxide was condensed in the reactor. The reaction mixture was stirred for 30 min at that temperature, then slowly heated to +40 °C and kept at this temperature for 3 days. The color of the solution turned from yellow to deep violet. The polymerization was quenched with acetic acid. The product was precipitated in cold acetone, re-dissolved in water, washed with the acetic cation exchanger DOWEX 50WX4-100 to extract the protonated $t$-BuP$_4$, ultrafiltered with bi-distilled water and freeze-dried.

6.2.1.3 Synthesis of 1,2-PB-b-PMOxa precursors

1,2-PB precursor was obtained according the procedure described in section 6.2.1.1, then second PMOxa block was synthesized by cationic-ring opening polymerization of MOxa using activated 1,2-PB precursor. MOxa was dried over CaH$_2$. PB(OH) was reacted with
triflic anhydride (Tf$_2$O) in hexane in the presence of triethylamine. After 1h of stirring at 0 °C, the suspension was filtered and access of solvent evaporated in vacuum. MOxa in EDC was added, and the solution was first stirred for 2 h in room temperature and then for 72 h at +45 °C under an argon atmosphere. Polymerization was quenched using 0.5 N KOH/EtOH solution. Sample was precipitated in cold hexane, re-dissolved in water, dialyzed against water and freeze-dried.

6.2.2 Radical addition of $\omega$-functional mercaptanes

Mercaptane (HS-R) and radical initiator N,N-azobis(izobutyronitrile) (AIBN) were added to a 3 wt % solution of polymer in dry THF so that [RSH]$_0$/[C=C]$_0$/[AIBN]$_0$=10:1:0.33. The reaction mixture was degassed and heated to reflux temperature for 24 h under an argon atmosphere.

6.2.2.1 Modification of PB homopolymers

Radical addition of methyl-3-mercaptopropionate (HS-MP) into 1,2-polybutadiene (1,2-PB) 1,2-PB$_{40}$ (PB2) and AIBN were dissolved in THF in the proportions given in Table 6.1. Then HS-MP was added by means of a syringe and the solution was heated to reflux. After 24 h of reaction, the solution was left to cool to room temperature and the solvent was partially evaporated under reduced pressure. The polymer was precipitated twice from methanol, washed with water and dried under vacuum to constant weight. The yellow, honey-like product was isolated in 70 % yield.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB$_{40}$</td>
<td>0.100</td>
<td>2160</td>
<td>4.62·10$^{-5}$</td>
</tr>
<tr>
<td>aHS(CH$_2$)$_2$COOCH$_3$</td>
<td>2.221</td>
<td>120.17</td>
<td>1.85·10$^{-2}$</td>
</tr>
<tr>
<td>bAIBN</td>
<td>0.101</td>
<td>164.21</td>
<td>6.16·10$^{-4}$</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB.
[b] 1/3 equivalents of double bonds of PB.
Radical addition of methyl-3-mercaptopropionate (\(HS\text{-}MP\)) to 1,4-polybutadiene (1,4-\(PB\))

1,4-PB and AIBN were dissolved in THF in the proportions given in Table 6.2. Then \(HS\text{-}MP\) was added by means of a syringe and the solution was heated to reflux. Within 1 h of reaction, a strong gelation of the solution occurred. Thus the mixture was left to cool to room temperature. The gelatinous product was washed thoroughly with THF and dried under vacuum. The obtained product was insoluble in common organic solvents; hence sufficient purification as well as a detailed analysis of the resulting material was not possible.

Table 6.2. Amounts of reagents utilized in the exemplary modification of 1,4-polybutadiene, 1,4-\(PB\), with methyl-3-mercaptopropionate, \(HS\text{-}MP\).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-PB</td>
<td>0.100</td>
<td>3000</td>
<td>3.33\times10^{-5}</td>
</tr>
<tr>
<td>(^a)HS(CH\text{2})\text{3}COOCH\text{3}</td>
<td>2.203</td>
<td>120.17</td>
<td>1.83\times10^{-2}</td>
</tr>
<tr>
<td>(^b)AIBN</td>
<td>0.100</td>
<td>164.21</td>
<td>6.11\times10^{-4}</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB; [b] 1/3 equivalents of double bonds of PB.

Radical addition of 3-mercaptopropionic acid (\(HS\text{-}PA\))

1,2-PB and AIBN were dissolved in THF in the proportions given in Table 6.3. \(HS\text{-}PA\) was added by means of a syringe and the solution was heated to reflux. After 24 h of reaction the solution was left to cool to room temperature and the solvent was partially evaporated under
reduced pressure. The polymer was precipitated twice from bi-distilled water, washed with bi-distilled water and dried under vacuum to constant weight. The yellow, honey-like product was isolated in 65 % yield.

Table 6.3. Amounts of reagents utilized in the exemplary modification of PB homopolymer with 3-mercaptopropionic acid, \( HS-PA \).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>( m ) [g]</th>
<th>( M ) [g/mol]</th>
<th>( n ) [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB_{40}(PB2)</td>
<td>0.200</td>
<td>2164</td>
<td>9.24·10^{-5}</td>
</tr>
<tr>
<td>( ^a )HS(CH_{2})_{2}COOH</td>
<td>3.920</td>
<td>106.14</td>
<td>3.70·10^{-2}</td>
</tr>
<tr>
<td>( ^b )AIBN</td>
<td>0.200</td>
<td>164.21</td>
<td>1.23·10^{-3}</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB.  
[b] 1/3 equivalents of double bonds of PB.

\[ ^1H \text{NMR} (400 \text{ MHz, d}_8-\text{THF}) \ \delta \text{ [ppm]}: \sim2.8 \text{ (m, –CH}_2, \ e), \ 2.6-2.35 \text{ (m, –CH}_2, \ d, \ f), \ 2.20-0.90 \text{ (br, –CH}_2, \ a, \ –CH, \ b, \ –CH}_2, \ c). \]

Radical addition of thiomalic acid (\( HS-TA \))

1,2-PB_{40}(PB2) and AIBN were dissolved in THF in the proportions given in Table 6.4. \( HS-TA \) was added and the solution was heated to reflux. After 24 h of reaction the solution was left to cool to room temperature and the solvent was partially evaporated under reduced pressure. The polymer was converted into the sodium salt by re-dissolving in NaOH aqueous solution (10 fold excess of NaOH with respect to the carboxylic acid groups), and then the polymer was dialyzed (cut off: 1 kDa) against water and freeze-dried. The modified polymer was isolated in 62 % yield.
Table 6.4. Amounts of reagents utilized in the exemplary modification of PB homopolymer with thiomalic acid, H5S-TA.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB₄₀ (PB2)</td>
<td>0.100</td>
<td>2160</td>
<td>4.62·10⁻⁵</td>
</tr>
<tr>
<td>aHSCH(COOH)CH₂COOH</td>
<td>2.776</td>
<td>150.16</td>
<td>1.84·10⁻²</td>
</tr>
<tr>
<td>bAIBN</td>
<td>0.101</td>
<td>164.21</td>
<td>6.16·10⁻⁴</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB.
[b] 1/3 equivalents of double bonds of PB.

1H NMR (400 MHz, D₂O) δ [ppm]: ~3.50 (m, –CH, e), 2.8 (m, –CH₂, f), 2.60-2.35 (m, –CH₂, d), 2.20-0.90 (br, –CH₂, a, –CH, b, –CH₂, c).

Radical addition of 2-mercaptoethylamine hydrochloride (H5S-EA)

1,2-PB₄₀ (PB2), AIBN and H5S-AE (proportions given in Table 6.5) were stirred in THF at room temperature until both polymer and AIBN were dissolved. H5S-EA is insoluble in THF. The mixture was heated to reflux and the reaction was carried out for 24 h. The mixture was then left to cool to room temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in water, dialyzed against water (cut off: 1 kDa) and then freeze-dried. The modified polymer was isolated in 36 % yield.

Table 6.5. Amounts of reagents utilized in the exemplary modification of PB homopolymer with 2-aminoethanethiol hydrochloride, H5S-AE·HCl.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB₄₀ (PB2)</td>
<td>0.100</td>
<td>2160</td>
<td>4.62·10⁻⁵</td>
</tr>
<tr>
<td>aHS(CH₂)₂NH₂·HCl</td>
<td>2.100</td>
<td>113.61</td>
<td>1.84·10⁻²</td>
</tr>
<tr>
<td>bAIBN</td>
<td>0.101</td>
<td>164.21</td>
<td>6.16·10⁻⁴</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB.
[b] 1/3 equivalents of double bonds of PB.
Radical addition of cysteine (HS-Cys)

1,2-PB$_{40}$ ($PB_2$), AIBN and HS-Cys with protected ammonium group by boc were dissolved in THF in the proportions given in Table 6.6. The mixture was heated to reflux and the reaction was carried out for 24 h. The solution was then left to cool to room temperature and the solvent partially was evaporated under reduced pressure. The ammonium groups were deprotected in situ by stirring the polymer in trifluoroacetic acid/dichloromethane (50:50) mixture for 3 h at room temperature. The polymer was precipitated twice from diethyl ether, dissolved in water, dialyzed against water (cut off: 1 kDa) and then freeze-dried. The modified polymer was isolated in 30 % yield.

Table 6.6. Amounts of reagents utilized in the exemplary modification of PB homopolymer with Boc-L-cysteine, HS-Cys.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB$_{40}$ ($PB_2$)</td>
<td>0.1</td>
<td>2160</td>
<td>4.62·10$^{-5}$</td>
</tr>
<tr>
<td>$^a$HS(CH$_2$)NH$_2$CHCOOH</td>
<td>2.044</td>
<td>221.27</td>
<td>9.27·10$^{-3}$</td>
</tr>
<tr>
<td>$^b$AIBN</td>
<td>0.101</td>
<td>164.21</td>
<td>6.16·10$^{-4}$</td>
</tr>
</tbody>
</table>

[a] 5 equivalents of double bonds of PB.
[b] 1/3 equivalents of double bonds of PB.

$^1$H NMR (400 MHz, D$_2$O) $\delta$ [ppm]: 3.0 (m, –CH$_2$, e), 2.80 (m, -CH$_2$, d), 2.54 (m, –CH$_2$, f), 2.20-0.90 (br, –CH$_2$, a, –CH, b, –CH$_2$, c).
Radical addition of 3-mercapto-1,2-propanediol (HS-PD)

1,2-PB₄₀ (PB₂) and AIBN were dissolved in THF in the proportions given in Table 6.7. HS-PD (90 wt % aqueous solution) was added by means of a syringe and the solution was heated to reflux. After 24 h of reaction, the solution was left to cool to room temperature, and the solvent was partially evaporated under reduced pressure. The polymer was precipitated twice from bidistilled water, washed with bidistilled water and dried under vacuum to constant weight. The modified polymer was isolated in 78 % yield.

Table 6.7. Amounts of reagents utilized in the exemplary modification of PB homopolymer with 3-mercapto-1,2-propanediol, HS-PD.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-PB₄₀ (PB₂)</td>
<td>0.200</td>
<td>2160</td>
<td>9.24×10⁻⁵</td>
</tr>
<tr>
<td>¹HSC₃H(OH)CH₂OH</td>
<td>3.992</td>
<td>108.16</td>
<td>3.70×10⁻²</td>
</tr>
<tr>
<td>¹H₂AIBN</td>
<td>0.200</td>
<td>164.21</td>
<td>1.23×10⁻³</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB.
[b] 1/3 equivalents of double bonds of PB.
**Modification of PB-b-PEO copolymers**

Radical addition of 3-mercaptopropionic acid (HS-PA) was carried out using PB40-b-PEO273 (PP3) and AIBN. The reagents were dissolved in THF in the proportions given in Table 6.8. HS-PA was added by means of a syringe and the solution was heated to reflux. After 24 h of reaction, the solution was left to cool to room temperature and the solvent was partially evaporated under reduced pressure. The polymer was precipitated twice from cold n-hexane, dissolved in water, dialyzed (cut off: 1 kDa), and freeze-dried from the water solution. The modified polymer was isolated in 85% yield.

Table 6.8. Amounts of reagents utilized in the exemplary modification of PB-b-PEO copolymer with 3-mercaptopropionic acid, HS-PA.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB40-b-PEO273 (PP3)</td>
<td>0.2</td>
<td>14 370</td>
<td>1.39·10^{-5}</td>
</tr>
<tr>
<td>HS(CH2)2COOH</td>
<td>0.590</td>
<td>106.14</td>
<td>5.56·10^{-3}</td>
</tr>
<tr>
<td>^bAIBN</td>
<td>0.030</td>
<td>164.21</td>
<td>1.85·10^{-4}</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB;
[b] 1/3 equivalents of double bonds of PB.

**1H NMR** (400 MHz, CD3OD) δ [ppm]: 3.70 (m, CH, f), 3.70-3.50 (br, –CH2, g), 2.75 (m, –CH2, e) 2.60 (m, –CH2, d), 2.00-0.90 (br, –CH2, a, –CH, b, –CH2, c).
**Radical addition of 2-mercaptoethylamine hydrochloride (HS-EA)**

PB_{40-b-PEO_{273}} (PP3), AIBN and HS-AE (proportions given in Table 6.9) were stirred in THF at room temperature until both polymer and AIBN were dissolved; HS-EA is insoluble in THF. The mixture was heated to reflux and the reaction was carried out for 24 h. The mixture was then left to cool to room temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in water, dialyzed against water (cut off: 1 kDa) and then freeze-dried. The modified polymer was isolated in 93 % yield.

Table 6.9. Amounts of reagents utilized in the exemplary modification of PB-b-PEO copolymer with 2-aminoethanethiol hydrochloride, HS-AE-HCl.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB_{40-b-PEO_{273}} (PP3)</td>
<td>0.100</td>
<td>14 370</td>
<td>6.9·10^{-6}</td>
</tr>
<tr>
<td>(^a) HS(CH_2)_2NH_2·HCl</td>
<td>0.316</td>
<td>113.61</td>
<td>2.78·10^{-3}</td>
</tr>
<tr>
<td>(^b) AIBN</td>
<td>0.015</td>
<td>164.21</td>
<td>9.28·10^{-5}</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB;
[b] 1/3 equivalents of double bonds of PB.
**6.2.2.3 Modification of PB-b-PMOxa copolymers**

Radical addition of 2-mercaptoethylamine hydrochloride (HS-EA) 

PB_{40-b-PMOxa}_{180} (PM), AIBN and HS-AE (proportions given in Table 6.10) were stirred in THF at room temperature until both polymer and AIBN were dissolved; HS-EA is insoluble in THF. The mixture was heated to reflux and the reaction was carried out for 24 h. The mixture was then left to cool to room temperature and the solvent was evaporated under reduced pressure. The residue was dissolved in water, dialyzed against water (cut off: 1 kDa) and then freeze-dried. The modified polymer was isolated in 78% yield.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB_{40-b-PMOxa}_{190} (PM)</td>
<td>0.300</td>
<td>16 660</td>
<td>1.80\times 10^{-5}</td>
</tr>
<tr>
<td>$^{a}$HS(CH$_2$)$_2$NH$_2$HCl</td>
<td>0.818</td>
<td>113.61</td>
<td>7.20\times 10^{-3}</td>
</tr>
<tr>
<td>$^{b}$AIBN</td>
<td>0.039</td>
<td>164.21</td>
<td>2.40\times 10^{-4}</td>
</tr>
</tbody>
</table>

[a] 10 equivalents of double bonds of PB; $^{b}$1/3 equivalents of double bonds of PB.
\textbf{EXPERIMENTAL PART}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{ExperimentalDiagram.png}
\caption{Chemical structure of the polycation 40-b-PMOxa190 copolymer.}
\end{figure}

\textbf{\textsuperscript{1}H NMR} (400 MHz, D$_2$O) $\delta$ [ppm]: 3.40-3.00 (br, $-\text{CH}_2$, d), 2.75 (m, $-\text{CH}_2$, g), 2.50 (m, $-\text{CH}_2$, f), 2.30 (m, $-\text{CH}_2$, h), 1.60-1.90 (br, $-\text{CH}_3$, e) 1.90-0.40 (br, $-\text{CH}_2$, a, $-\text{CH}$, b, $-\text{CH}_2$, e).

\subsection*{6.2.3 Labeling of ammonium groups in the polycation 40-b-PMOxa190 copolymer with the fluorescence dye}

The polycation 40-b-PMOxa180 (PM-S-EA), was dissolved in bidistilled water (concentration 0.9 wt %). Fluorescein 5(6)-isothiocyanate was dissolved in DMF. While stirring the polymer solution, 50 $\mu$L of the reactive dye solution was slowly added (proportions given in Table 6.11). The reaction was carried out for 1 h at room temperature with continuous stirring. To terminate the reaction and to remove weakly bound probes, 0.1 ml of freshly prepared 1 N hydroxylamine, pH 8.5, was added. The solution was stirred for 1 h, and then dialyzed against water (cut off: 1 kDa). After dialysis, the solution was pinkish, as monitored by eye.

\begin{table}
\centering
\begin{tabular}{lll}
\hline
Reagents & m [g] & M [g/mol] & n [mol] \\
\hline
Polycation 40-b-PMOxa190 (PM-S-EA) & 0.01 & 21190 & 4.7\times10^{-7} \\
\textsuperscript{a}Fluorescein 5(6)-isothiocyanate & 0.00005 & 389.38 & 1.3\times10^{-7} \\
\hline
\end{tabular}
\caption{Amounts of reagents utilized in the labeling of ammonium groups in polycation 40-b-PMOxa190 copolymer with fluorescence dye.}
\end{table}

\begin{table}
\centering
\begin{tabular}{lll}
\hline
Reagents & m [g] & M [g/mol] & n [mol] \\
\hline
Polycation 40-b-PMOxa190 (PM-S-EA) & 0.01 & 21190 & 4.7\times10^{-7} \\
\textsuperscript{a}Fluorescein 5(6)-isothiocyanate & 0.00005 & 389.38 & 1.3\times10^{-7} \\
\hline
\end{tabular}
\caption{Amounts of reagents utilized in the labeling of ammonium groups in polycation 40-b-PMOxa190 copolymer with fluorescence dye.}
\end{table}

\[\text{[a]}\ 0.25\ \text{equivalents of functional groups of PM-S-EA.}\]

\subsection*{6.2.4 Labeling of the end chains of PEO blocks in the polyanion 27-b-PEO273 copolymer with the fluorescence dye}

PB$_{40}$-b-PEO$_{273}$ (PP3) with OH function in the end of PEO chains and t-BuP$_4$ (proportions given in Table 6.12) were stirred in THF at room temperature until the polymer was dissolved. To the solution 9-anthracene carboxaldehyde was added. The mixture was heated to the reflux and the reaction was carried out for 24 h. The mixture was then left to cool to
room temperature, the product was precipitated in a cold acetone, re-dissolved in water, washed with the acetic cation exchanger DOWEX 50WX4-100 to extract the protonated t-BuP₄, ultrafiltered with bi-distilled water and freeze-dried.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>m [g]</th>
<th>M [g/mol]</th>
<th>n [mol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyanion₂₇₋₇₋PEO₂₇₃</td>
<td>0.300</td>
<td>14 170</td>
<td>2.2·10⁻⁵</td>
</tr>
<tr>
<td>9-anthracene carboxaldehyde</td>
<td>0.004</td>
<td>206.24</td>
<td>2.2·10⁻⁵</td>
</tr>
<tr>
<td>Phosphazene baze</td>
<td>0.012</td>
<td>633.73</td>
<td>2.2·10⁻⁵</td>
</tr>
</tbody>
</table>

### 6.3 Experimental techniques

**Nuclear Magnetic Resonance (¹H NMR)** spectra were recorded at room temperature on a Bruker DPX-400 spectrometer operating at 400.1 MHz. For classifying the signalson the NMR spectra, abbreviation such as s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad) were used. The solvents for NMR used were CDCl₃ (δ = 7.26 ppm), DMSO-d₆ (δ = 2.62 ppm), DMF-d₇ (δ = 8 ppm), CD₃OD-d₄ (δ = 4.78 and 3.30 ppm), D₂O (δ = 4.67 ppm), THF-d₈ (δ = 1.73 and 3.58 ppm).

**Fourier Transform Intra Red (FT-IR)** spectra were recorded on BioRad 6000 FT-IR. All samples were measured in the solid state using a Single Reflection Diamond ATR. For classifying the molecular vibrations of the signals on IR spectra, abbreviations such as ν (stretching), δ (bending) and γ (“out of plane” bending), s (symmetrical), as (asymmetrical) were used.

**Size Exclusion Chromatography (SEC)** was performed on Thermo Separation Products setups being equipped with UV (TSP UV1000) and differential refractive index (Shodex RI-71) detectors. THF and 0.1N aqueous NaNO₃ were used as the eluent, at a flow rate of 1.0 mL/min at 25 °C.

The column set for analysis in THF consisted of three 300 x 8 mm columns, MZ-SD plus (spherical polystyrene particles with an average diameter of 5 μm) with pore size of 10³, 10⁵, 10⁶ Å.

All samples were filtered prior use and 100 μL of approximately 0.2% w/v of polymer solution was injected in the SEC column. For calibration 1,2-PB, PEO, PS standards (PSS
GmbH, Mainz, Germany) were used. Molecular weights and molecular weight distributions were calculated using Program-Packed NTeqGPC V5.1.5 (hs GmbH, Oberhilbersheim, Germany).

**Dynamic Light Scattering (DLS)** measurements were carried out on a home-built goniometer with fixed scattering angles, 30°, 50°, 60°, 90° and 120° at 25 °C. The instrument was connected via a single mode fiber to a single photon detector (ALV/So-SIPD), the output of which was fed to a multiple-tau digital correlator (ALV 5000). The light source (Polytec, 34 mW) was a HeNe laser operating at a wavelength of $\lambda = 633$ nm. From the measured time-correlation functions, intensity-weighted particle size distributions were calculated using FASTORT.EXE.103

**Static Light Scattering (SLS)** commercial spectrometer from ALV consisting of an ALV goniometer with temperature controller (+/- 0,05 K) and an ALV 5000 multi-tau corrector was used. The refractive index increment $dn/dc$ was measured using a NFT-Scanref differential refractometer. SLS experiments were performed at scattering angles from 15° to 150° at 5° intervals. Data were evaluated by a standard Zimm or Guinier analysis.

All solvents were filtered through 0.45 μm millipore filters prior measurement. The cylindrical quartz cuvettes (1 cm diameter) were extensively cleaned first with THF and ethanol followed by cleaning with ultrasound using a Tensid solution (Hellmanex, Hellma). They were subsequently washed several times with distilled water to completely remove any Tensid remained. Finally, they were washed with acetone in a dust-free fountain for 20 minutes and placed in desiccators.

**Analytical Ultracentrifugation (AUC) sedimentation velocity** measurements were performed on a Beckman Optima XL-I (Beckman Coulter, Palo Alto, CA) at 25 °C, using self-made 12mm titanium centerpieces. Detection of the sedimentation boundary was carried out using UV-Vis absorption and interference optics. Data were evaluated using the SEDFIT software package.10

**Light Microscopy (LM)** was performed on Olympus Microscope (Model BX50) directly connected with a digital camera.

**Transmission Electron Microscopy (TEM)** micrograms were obtained with a Zeiss EM 912 OMEGA instrument operating at an acceleration voltage of 120 kV. The diluted colloidal
solutions were placed on a 400-mesh carbon-coated copper grid and left to dry; no further contrasting was applied.

**Cryogenic Transmission Electron Microscopy (Cryo-TEM)** specimens were prepared by vitrification of thin liquid films supported on a TEM copper grid (600 mesh) in liquid ethane at its freezing point. The specimen was inserted into a cryo-transfer holder (CT3500) and transferred to a Zeiss EM922 EFTEM. Examination were carried out at temperatures around 90 K. The TEM was operated at an acceleration voltage of 200 kV. All images were recorded digitally by a bottom-mounted CCD camera system (UltraScan 1000).

**Small-angle X-ray scattering (SAXS)** was performed on a polymer solution in a capillary of Boron-silicates. SAXS data were acquired using a rotating anode (Cu K\(_\alpha\), \(\lambda = 0.154\) nm) instrument with pinhole collimation at room temperature. A 2 MAR CCD detector was used, and the 2D SAXS data were radially averaged. Fitting of data was done based on an approach described by Förster and Burger.\(^{157}\)

**Time-Resolved Fluorescence Decay** was recorded on a ED 299T time-resolved fluorometer, Edinburgh Instruments, Inc., equipped with a nanosecond coaxial discharge lamp filled with hydrogen at 0.5 atm (half-width of pulse ca. 1.2 ns).\(^{158}\)
7 APPENDIX

$^1$H NMR SPECTRA (REFER SECTION 4.1)
8 ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_2$</td>
<td>Second virial coefficient</td>
</tr>
<tr>
<td>AIBN</td>
<td>$N,N$-azobis(izobutyronitrile)</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerization</td>
</tr>
<tr>
<td>AUC</td>
<td>Analytical ultracentrifugation</td>
</tr>
<tr>
<td>BuLi</td>
<td>Butyl lithium</td>
</tr>
<tr>
<td>$cmc$</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion coefficient</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>$DP$</td>
<td>Degree of polymerization</td>
</tr>
<tr>
<td>EDC</td>
<td>1,2-Dichloroethane</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier-transform infrared spectroscopy</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>HTPB</td>
<td>Hydroxyl-terminated polybutadiene</td>
</tr>
<tr>
<td>IPEC</td>
<td>Interpolyelectrolyte complex</td>
</tr>
<tr>
<td>LS</td>
<td>Light scattering</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>$M_a$</td>
<td>Number averaged molecular weight</td>
</tr>
<tr>
<td>$M_w$</td>
<td>Weight averaged molecular weight</td>
</tr>
<tr>
<td>$M_w^{app}$</td>
<td>Apparent averaged molecular weight</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PB</td>
<td>Polybutadiene</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>PIC</td>
<td>Polyion complex</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>PTFE</td>
<td>Poly(tetrafluoroethylene)</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible addition-fragmentation chain-transfer polymerization</td>
</tr>
<tr>
<td>$R_g$</td>
<td>Radius of gyration</td>
</tr>
<tr>
<td>$R_h$</td>
<td>Hydrodynamic radius</td>
</tr>
<tr>
<td>$R_h^{app}$</td>
<td>Apparent hydrodynamic radius</td>
</tr>
<tr>
<td>RI</td>
<td>Refractive index</td>
</tr>
<tr>
<td>$R(\theta)$</td>
<td>Rayleigh ratio</td>
</tr>
<tr>
<td>$s$</td>
<td>Sedimentation coefficient</td>
</tr>
<tr>
<td>SEC</td>
<td>Size exclusion chromatography</td>
</tr>
<tr>
<td>SLS</td>
<td>Static light scattering</td>
</tr>
<tr>
<td>$t$-BuP$_4$</td>
<td>Phosphazene base P$_4$</td>
</tr>
<tr>
<td>T</td>
<td>Absolute temperature</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-visible</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Flory-Huggins interaction parameter</td>
</tr>
<tr>
<td>$Z$</td>
<td>Aggregation number</td>
</tr>
<tr>
<td>$Z^{app}$</td>
<td>Apparent aggregation number</td>
</tr>
</tbody>
</table>
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