Facing current challenges in (supra-)macromolecular science: a high-throughput approach

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Facing current challenges in (supra-)macromolecular science – a high-throughput approach –

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Facing current challenges in (supra-)macromolecular science – a high-throughput approach –
by Michael A. R. Meier

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CHAPTER 1

An introduction to combinatorial materials research (CMR)

Abstract

Combinatorial approaches in polymer science are on their way of maturing and recent research examples have shown that combinatorial materials research (CMR) can provide efficient and successful strategies for a large variety of research problems. Generally, polymer chemistry is very well suited for combinatorial approaches since a large parameter space has to be screened in order to evaluate the desired structure-property relationships and to accelerate the design and development of new materials. Therefore, the development and application of high-throughput screening techniques for applications in polymer science is a very suitable approach to accelerate research in an efficient manner. This chapter provides an introduction and overview to combinatorial materials research. Automated and parallel synthetic approaches towards polymer libraries as well as high-throughput screening techniques for these libraries are thoroughly reviewed and discussed. Based on these literature studies the aim and outline of this thesis are formulated.

1.1 Facing current challenges in (supra-)macromolecular science

The control of polymer architecture, molecular weight, end-group and other parameters with the aim to generate defined chemical, physical and biological properties of the studied materials is of major interest in current polymer science. Therefore, controlled and living polymerization techniques teamed up with specific organic reactions and/or supramolecular chemistry and combined with a detailed structure and property characterization will facilitate the design of novel functional materials.

Recent years have shown that it is possible to prepare macromolecules with defined architectures (e.g. linear, star, random, block), composition, molecular weight as well as narrow molecular weight distribution by applying living and controlled polymerization techniques, such as living anionic polymerization or controlled radical polymerization. Per definition, a living polymerization follows a chain growth mechanism with the absence of chain termination and chain transfer processes resulting in most cases in a constant number of growing chains, if the rate of chain initiation \( k_i \) is fast compared to the rate of chain propagation \( k_p \)\(^1\) (generally, a ratio of \( k_p/k_i < 10 \) is required in order to obtain polymers with a narrow molecular weight distribution\(^2\)). Therefore, it is for instance possible to prepare macromolecules with different molecular weights by controlling the monomer conversion and or the ratio between monomer and initiator (M/I ratio) or to prepare block copolymers by sequential addition of different monomers. Moreover, applying selected organic reactions, it is possible to prepare novel monomers, initiators and/or terminating agents that allow the introduction of desired functionalities into polymeric architectures at desired positions, e.g. chain ends or cores of star-shaped polymers, in order to obtain and adjust certain material properties. Last, but not least, supramolecular approaches allow for the introduction of non-covalent binding motifs into the polymeric architecture that might lead to novel functions and the reversible fine tuning of the properties of the studied macromolecules. Therefore, the preparation of well-defined macromolecules with a defined three-dimensional structure possesses several challenges as well as opportunities. The challenge is to design and prepare macromolecules with known and predetermined parameters, such as: composition, architecture and molecular weight as discussed above. The opportunity on the other hand is the development of novel functional materials and their fundamental understanding in areas such diverse as medicine, electronics or nanotechnology to only name a few. However, combinatorial and parallel approaches in combination with efficient screening methods seem to be the only practicable solution to investigate this enormous parameter space of, e.g., different monomers, polymerization techniques, polymer architectures, and introduced functionalities.

This thesis focuses on three diverse main topics including MALDI-TOFMS, PEO-b-PCL block copolymers and terpyridine based supramolecular polymers. The common link in between these topics is the utilization of fast automated and parallel techniques for synthetic
An introduction to combinatorial materials research as well as analytic problems thereby covering the major aspects of combinatorial workflows in polymer science. The following sections of this chapter provide an overview of the efforts made so far by polymer scientists to implement automated polymer synthesis as well as high-throughput screening methods into their work routine, whereas short introductions to the three main topics of this theses will be given in the corresponding chapters.

1.2 Combinatorial approaches in polymer science

Combinatorial materials research (CMR) is still young but has gained large attraction during the last few years.\(^3\)\(^-\)\(^5\) Stimulated by the success of high-throughput screening (HTS) methodologies and combinatorial methods in pharmaceutical research (see e.g. Refs. [6,7]), CMR has advanced to an independent, very promising and highly multidisciplinary field of research. However, the large sample throughput (up to 1 million tests per week) of pharmaceutical ultra high-throughput screening (UHTS) approaches is unlikely to be reached by combinatorial methods in polymer and materials chemistry. Nevertheless, CMR offers very promising approaches in the field of polymer chemistry since it is possible to vary and evaluate a large parameter space in a reduced amount of time. However, the fast preparation and screening of combinatorial material libraries does not necessarily speed-up the time-to-market or understanding of quantitative structure-property relationships (QSPR) of new materials.

Figure 1.1 Schematic representation of a possible workflow in combinatorial materials research covering typical stages of experimentation ranging from the design of the experiments over various steps to data handling and thereby closing the combinatorial cycle.
Therefore, the integration of design-of-experiments (DoE) and data handling solutions is one possibility to complete, optimize and close the combinatorial workflow cycle in CMR as it is depicted in Figure 1.1. The major aspects of this workflow (automated synthesis, high-throughput screening and property investigations) will be discussed and reviewed within the following sections focusing on developments and state–of–the–art in combinatorial material research.

Even if the first examples of combinatorial approaches in material research date back more than one hundred years (Thomas A. Edison and the photochemist Ciamician), CMR was only being utilized intensively during the last decade, since only then the first high-throughput screening techniques for materials became available. Today, combinatorial and high-throughput experimentation is also widely applied in the fields of organic materials, inorganic materials, catalysis as well as polymer research. Especially combinatorial polymer research is a promising approach for the fast preparation and characterization of new materials since a large parameter space can be varied during synthesis, processing, blending, formulation and compounding. Furthermore, numerous important parameters have to be investigated in parallel or fast serial mode in order to be able to establish QSPRs and to reduce the time–to–market for new materials.

1.2.1 Automated and/or parallel polymer synthesis

Automated and parallel synthesis of polymers can provide several advantages for a fast discovery of new materials with certain structure-property relationships. However, some drawbacks regarding the special requirements of the equipment have to be overcome. Polymerizations have to be carried out over a wide range of temperatures and pressures. Furthermore, the automated acquiring of samples in order to monitor polymerization kinetics should be available. Moreover, the strongly varying properties of the polymers (e.g. viscosity or solubility) as well as the handling of solids (e.g. in blends or hybrid materials) are additional challenges. In general, the goal of automated parallel synthesis and formulation in CMR is a faster preparation and property understanding of new polymeric materials and their processing conditions. Literature examples of the automated synthesis of polymers range from simple free radical polymerizations to various controlled and living polymerization techniques. Thereby, both simple manual parallel approaches as well as fully automated and optimized workflows have been applied. In general, the automated and/or parallel synthesis of polymers can be divided into three groups: (i) the parallel synthesis applying standard laboratory techniques; (ii) the semi-automated synthesis using parallel manual approaches; and (iii) the fully automated and parallel synthesis utilizing computer-controlled robotic systems. The applied degree of automation and parallelization will vary strongly and depend on the cost-value ratio for each combinatorial approach to new materials. The following paragraphs will discuss prominent, selected literature examples depending on their degree of automation and/or parallelization, focusing on fully automated approaches.
Manual parallel approaches

The application of commonly available chemical equipment for combinatorial approaches certainly is the easiest and cheapest entry into combinatorial materials research. However, utilizing this standard equipment will lead to a more or less pronounced decrease of some the major advantages of CMR, such as fast, reproducible and automated synthesis of new materials. Nevertheless, these approaches are very well suited for early stages of investigations.

Polycondensation reactions of 14 tyrosine-derived diphenols and eight aliphatic diacids were reported by Kohn and coworkers already in 1997. These monomers were reacted in a manual fashion (up to 32 in parallel) in a water shaker bath on a 200 mg scale to obtain a library of 112 polyarylates with predictable changes in glass transition temperature (T_g), surface wettability as well as cellular response (measured by *in vitro* cell proliferation studies). For instance, it was shown that the T_g as well as the air water contact angle of the polymers increased in a defined fashion as the number of carbon or oxygen atoms in the polymer backbone and pendent chain decreased. Figure 1.2 depicts the gradual variation of the T_g as well as the air-water contact angle values of the whole polymer library as a function of the applied monomers.

![Figure 1.2](image)

*Figure 1.2* Glass transition temperatures (right) and air-water contact angles (left) of a 112-membered polymer library as a function of the polymer pendant chain (x axis) and backbone structure (y axis) (taken from Refs. 25, 26).

The authors mentioned that the identification of these structure-property relationships may be particularly useful in biomaterials research and will facilitate the identification of new biomaterials. Moreover, they mentioned that the time required to prepare and screen all polymers could be significantly decreased by the incorporation of robotic systems. Further investigations of this polymer library showed, e.g., that fibrinogen adsorption to polymer surfaces of this library could be correlated to the polymer structure and moreover that polymer properties were predictable utilizing artificial neural networks.
prepared a 140 membered degradable polymer library consisting of seven diacrylate and 20 amine monomers by performing small scale reactions in parallel.\textsuperscript{29} After disregarding water non-soluble polymers and excluding polymers that did not interact with DNA (electrophoretic DNA binding assay), the remaining materials were further investigated in transfection assays. This led to the discovery of new DNA-complexing materials as well as gene delivery vectors. New polymer supports for liquid-phase organic synthesis (LPOS) were prepared in a manual parallel approach utilizing a combination of conventional and “living” free radical polymerization by Janda et al.\textsuperscript{30} Bifunctional initiators were utilized to prepare a library of copolymers with block or graft structure. Four different free radical initiators that also contained a TEMPO functionality and five vinylic monomers were studied. Generally, first a free radical polymerization at 70 °C was initiated by a α-nitrile diazo (-N=N-) functionality to yield homopolymers, which were subsequently polymerized at higher temperatures (130 °C) utilizing the TEMPO group as free radical mediator. Applying this strategy, polymers with unique solubility profiles that could serve as new supports in LPOS could be prepared. Moreover, Kim and Dordick\textsuperscript{31} reported on the utilization of enzymes as biocatalysts for the combinatorial synthesis as a base of polymeric materials discovery. For these investigations microtiter plates with 96 deep wells (2 mL per well) were utilized to first screen three different enzymes for their ability to catalyze the transesterification of aliphatic and aromatic diols with divinyl adipate in different solvents. Candida antarctica lipase in acetonitrile was identified as most promising enzyme/solvent combination from a first screening and therefore applied for the subsequent library preparation. Twelve diols or polyols were combined with four different diesters in a 12×4 type array. Equimolar amounts of both components as well as 20 mg/mL enzyme in 1.5 mL of acetonitrile were incubated at 45 °C for 8 h in an orbital shaker. The resulting polymers were subsequently analyzed by gel-permeation chromatography showing that this combinatorial approach was capable of producing high molecular weight polymers and therefore might serve as a new tool for materials discovery.

**Semi automated approaches**

Semi automated approaches in CMR have in common the use of not fully automated and sometimes custom built parallel equipment such as special parallel reactors. An advantage of such approaches can be seen in the high degree of adaptation for a certain chemical problem if custom built equipment is utilized, whereas the disadvantage in that case is certainly the lack in flexibility.

General Electric (GE) for instance described a parallel approach for the melt-polymerizations of bisphenol-A (BPA) and diphenylcarbonate (DPC) under inert conditions.\textsuperscript{32,33} Melt polymerizations were carried out at small scale (preferably less than 100 mg) in parallel micro reaction arrays with various temperature programs. After validation of the method,\textsuperscript{32} an automated high-throughput multiparameter optimization of polymerization conditions was performed.\textsuperscript{33} Reaction volumes, ratios of monomers, amount of catalyst, flow rate of inert gas, and dwell time were varied applying DoE strategies. The different polymerizations were
screened noninvasively with fluorescence spectroscopy. Utilizing principal component analysis (PCA) for the data analysis resulted in optimized process parameters. Lavastre et al. reported the synthesis of conjugated polymers from dihalogenated and diethynyl monomers by a Pd-catalyzed carbon-carbon coupling reaction. Therefore, a multichannel pipette system was applied for the charging of 96 vials (1 mL), dispatched in a 12×8 format, with different combinations of monomers and the catalyst. Polymerizations were carried out for 24 h at 60 °C. The resulting polymer library was evaluated for the potential use as red, green and blue emitting materials utilizing a spectrofluorimeter that was able to read 96-well microtiter plates (compare Figure 1.3). Therefore, the relative fluorescence in solution was used applying several combinations of excitation and emission wavelength leading to a fast and unpredictable detection of new green- as well as blue-emitting polymers.

![Figure 1.3 Visual fluorescence of a conjugated polymer library excited with a UV lamp at 254 and 365 nm. The qualitative distinction between fluorescent and non-fluorescent polymers as well as between different emission colors could be obtained from a simple visual test or by the utilization of a 96-well plate reader spectrofluorimeter (taken from Ref. 34).](image)

Moreover, evaluating polymer films with the same strategy led to the identification of polymers showing a blue fluorescence in the solid state (compare Figure 1.3). A multiparallel polymerization system was successfully utilized for suspension polymerizations as reported by Bradley et al.. Within this system, polystyrene resins were prepared in a parallel fashion. After polymerizing divinylbenzene (DVB), styrene and vinylbenzyl chloride in varying ratios (initiated with AIBN), the resulting polymer beads were washed, extracted (Soxhlet) and dried in vacuo. Sieving of these materials resulted in resins of different size distributions in a reproducible manner. Moreover, it was stated that the parallel approach was useful for the rapid design of polymeric bead supports and/or to optimize polymerization conditions.

**Fully automated approaches**

Synthetic strategies for the fully automated preparation of polymeric materials can be found in the literature for all major polymerization techniques (polycondensation, radical polymerization, ring-opening polymerization, polyolefin synthesis as well as for supramolecular polymerization approaches). All of them have in common the utilization of robotic systems for the preparation of the new materials with the advantage of full automation
free radical polymerizations were carried out fully automated in synthetic robots for instance as emulsion polymerizations of styrene and vinyl acetate, as reported by van Herk and Schubert,\textsuperscript{36} or as parallel free radical polymerizations by Symyx (styrene with acrylonitrile present as terminating agent)\textsuperscript{37} and by Long et al. (styrene/methacrylate copolymers).\textsuperscript{38} For the parallel emulsion polymerization, the vortex speed was identified as an important parameter to obtain suitable emulsions and its optimization led to comparable results between polymerizations in the automated synthesizer and ‘classical’ stirred batch reactors regarding the sizes of the obtained polystyrene particles.\textsuperscript{36} Automated synthesis robots have been successfully applied to various controlled radical polymerization techniques like reversible addition fragmentation transfer (RAFT) polymerization,\textsuperscript{39-41} atom-transfer radical polymerization (ATRP)\textsuperscript{42-46} and nitroxide-mediated polymerizations.\textsuperscript{47} RAFT polymerizations of methylmethacrylate (MMA) were controlled and reproducibly performed and monitored in an automated synthesizer.\textsuperscript{40} The polymerization temperature was optimized and chain extension experiments showed the presence of active polymer chains in the reaction mixture. Subsequently, the polymerization of eight different acrylate and methacrylate monomers by RAFT was studied revealing optimized reaction conditions for the preparation of polymers with low polydispersity indices.\textsuperscript{41} Finally, the gained knowledge of these investigations was applied to the synthesis of defined block and random copolymers. Moreover, the RAFT polymerization technique was used to prepare graft copolymers with controlled length and spacing of the grafted chains in a 96-well parallel batch reactor equipped with a liquid dispensing robot.\textsuperscript{39} Therefore, backbones with varying molecular weights were chemically modified in order to attach RAFT control agents with different degrees of modification. These modified polymers were polymerized with different monomers to create a library of graft-copolymers. This procedure was reported to be capable of producing 200-300 materials per day.

Schubert et al. reported on the automated and parallel investigations of reaction conditions and catalysts for the homogeneous ATRP of MMA mediated by CuBr/N-(n-hexyl)-2-pyridylmethanimine,\textsuperscript{45} after they proved the livingness as well as reproducibility of automated ATRP.\textsuperscript{44} In a later stage this method was applied for the screening of reaction conditions of the ATRP of MMA by investigating four different initiators, five different metals salts as well as nine different ligands in a library of 108 different reactions.\textsuperscript{46} This revealed for instance (the expected result) that Cu(I)-mediated systems offered a higher degree of control if compared to Fe(II)-mediated ones under the examined conditions and that the choice of bipyridine ligand is critical for a successful ATRP experiment. Moreover, Symyx demonstrated that it is possible to polymerize styrene and butyl acrylate in parallel and fully automated by ATRP.\textsuperscript{42,43} Robotic systems were used to dispense all reagents and to prepare samples for high-throughput characterization. They mentioned the possibility to create libraries in sizes from 48 to 140 members with volumes of 0.1 to 20 mL per reaction vessel.
Hawker et al.\textsuperscript{47} utilized NMP and combinatorial techniques to control the synthesis of nanoscale materials, namely star-shaped polymers (compare Figure 1.4). By utilizing a macro-initiator for nitroxide-mediated polymerizations in a 4×96 library format, optimized conditions for the formation of star polymers could be obtained. Figure 1.4 depicts a schematic representation of nine different regions identified in this initial library.

\textbf{Figure 1.4} Left: Schematic representation of the synthesis of star-shaped poly(styrene). The first step was performed in a classic way, whereas the star-synthesis was carried out utilizing an automated synthesizer. Right: nine different regions (named A to I) present in a 4×96-membered library of star polymers obtained by nitroxide-mediated polymerization (taken from Ref. 47).

The best results in view of molecular weight and gel-permeation chromatography peak profile could be obtained from the regions G and H. Subsequently, a 168 membered library around the ‘hits’ of the initial library and the investigation of seven different macro-initiators was designed and evaluated resulting in an optimal molecular weight for the utilized macro-initiator. All results led to a fast evaluation of the possibilities and limitations of the described method to prepare tailor-made macromolecules with well-defined 3-dimensional architecture. Moreover, controlled ring-opening polymerizations of lactides\textsuperscript{48} as well as of oxazolines could be preformed in a fully automated fashion.\textsuperscript{49-52} In the case of lactides, an automated synthesizer was utilized for up to 20 parallel reactions for an initial screening of the polymerization of lactides regarding organic catalysts, suitable solvents and a variety of polymerization conditions.\textsuperscript{48} 4-(Dimethylamino)pyridine (DMAP) and 4-pyrrolidinopyridine (PPY) were shown to provide catalytic activity for the polymerization of lactides resulting in polymers with narrow polydispersity indices and molecular weights close to the targeted molecular weight. Schubert and coworkers intensively investigated the living cationic ring-opening polymerization of 2-oxazolines. The polymerizations were performed fully automated in different robotic synthesizer. After demonstrating the living character as well as the reproducibility of the polymerization,\textsuperscript{49} more detailed investigations on the dependence of the polymerization kinetics on four different oxazolines monomers (2-methyl-2-oxazoline, 2-
ethyl-2-oxazoline, 2-nonyl-2-oxazoline and 2-phenyl-2-oxazoline) and four initiators (benzyl bromide, methyl tosylate, methyl triflate and methyl iodide) at two different temperatures (80 and 100 °C) were performed. Furthermore, the utilization of individually heatable reactor blocks resulted in optimal polymerization temperatures as well as activation energies for the investigated polymerization systems.\textsuperscript{51,52} The combination of all results led to a very well understood polymerization system and is useful for the design of new materials such as block copolymers or copolymers with targeted chemical composition and hence targeted material properties.

In order to speed-up the catalyst research in the area of polyolefins, researchers demonstrated that high-throughput screening methodologies for the discovery of new polyolefin catalysts were feasible and benefited of a three stage strategy with a primary (high-throughput, catalyst discovery, 384 experiments), secondary (intermediate-throughput, catalyst optimization, 96 experiments in a focused library) and tertiary (conventional-throughput, laboratory batch reactor, 2 reactions) screening to discover, optimize and evaluate new catalysts.\textsuperscript{53}

More recently, the demonstration of the very demanding anionic polymerization in a parallel and fully automated way to obtain block copolymers\textsuperscript{54} as well as functional polymers\textsuperscript{55} made clear that (nearly) every imaginable polymerization technique can be performed in an accelerated fashion.

\textbf{1.2.2 High-throughput screening}

High-throughput screening (HTS) in combinatorial polymer science cannot be directly compared to HTS in pharmaceutical research since the parameters of interest are rather different. The structural characterization of small molecule libraries can be easily accomplished utilizing gas chromatography/mass spectrometry (GC-MS) or high-pressure liquid chromatography/mass spectrometry (HPLC-MS) techniques. Moreover, if one is screening these libraries for a potential drug candidate, mostly optical screening methods (based on plate reader approaches) are applied in order to obtain qualitative binding information of a certain compound to a certain receptor. On the other hand one of the most important structural parameters of a (synthetic) polymer is its molecular weight and the corresponding molecular weight distribution. Moreover, a large variety of different important properties, such as the glass transition temperature ($T_g$) or melting temperature ($T_m$) need to be determined for polymer libraries. However, the general statement about combinatorial chemistry: “only make in a day what you can screen in a day” is of course also valid for polymer research. Therefore, special high-throughput screening techniques for molecular weight, composition, physical properties and others of synthetic polymers had to be developed. These techniques will be discussed in the following paragraphs.
Screening for molecular weight and polydispersity index

The molecular weight and its distribution are essential characterization parameters for (synthetic) polymers. Size exclusion chromatography (SEC) can be considered as the easiest and most commonly applied way to determine them.\textsuperscript{56,57} The major drawback of this technique considering HTS strategies is the relatively long analysis time of up to 60 minutes for standard systems, which would represent a serious bottleneck for HTS applications (depending on the number of samples). Therefore, much faster SEC systems have been developed in the last years applying one or more of the following approaches: parallelization, shorter columns, flow-injection analysis (FIA) and high-speed SEC columns.\textsuperscript{56-58} Figure 1.5 (left) shows a conventional SEC calibration in comparison with a high speed approach obtained with commercially available columns.\textsuperscript{58,59} This example demonstrates the possible time saving effects in an impressive way. However, by the use of (shorter) high-speed SEC columns or an increase in the flow speed of the chromatographic system, a loss in resolution (plate count) will occur as demonstrated in Figure 1.5.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure1.5.jpg}
\caption{Left: Comparison of a high speed GPC calibration curve with a conventional SEC calibration (taken from Ref. 58). Right: four poly(styrene) standards ($M_p = 2000$ to 12000 Da) investigated with SEC at different flow speeds.}
\end{figure}

It is obvious that the doubling in flow speed utilizing the same SEC column reduced the separation capability of the chromatographic system but also decreased the analysis time by a factor of two. This trend was also observed by even doubling the flow speed again to 2 mL/min, reaching analysis times of less than 7 minutes with a conventional SEC column, but loosing accuracy. In the case of 2 mL/min flow speed the peak maxima of the same poly(styrene) standards as shown in Figure 1.5 (right) were not separated any more. Therefore, it is crucial to choose the column and flow speed of the SEC system according to the needs of the investigation: e.g. if highly accurate kinetic data is required longer columns with lower flow speeds are mandatory. However, if the amount of samples is too high or less accurate data is sufficient, high speed columns at high flow speeds offer a very good alternative for high-throughput determinations of the molecular weight and polydispersity.
index in combinatorial materials research. Moreover, an automated sample preparation technique for high-temperature SEC utilizing robotic systems providing excellent reproducibility has already been described in the literature.\textsuperscript{60} In addition, it was possible to integrate a SEC system into a high-throughput workflow for direct monitoring of polymerization reactions by utilizing the HPLC injection port that was available within an automated synthesizer.\textsuperscript{61} As an application example, it was recently demonstrated that SEC can be a very valuable combinatorial screening method for the identification of active catalysts for olefin polymerization.\textsuperscript{62}

Furthermore, MALDI-TOFMS proved to be perfectly suited for the high-throughput investigation of polymer molecular weights and polydispersity indices. However, these studies will not be summarized in this introduction since these techniques were developed as a part of this thesis’ work and are therefore described in detail in Chapter 2.

Apart from the above described techniques for an automated and/or parallel investigation of the molecular weight of polymers it is also possible to integrate more classical techniques, such as viscosimetry, into the workflow of CMR. Capillary viscosimetry provides an easy and straightforward method for molecular weight determination by measuring the intrinsic viscosity and application of the Mark-Houwink equation. For this purpose completely automated instruments allow the precise measurement of the viscosity using standardized glass capillary viscometers in parallel (e.g. the Processor Viscosity System, see Ref. [63]). Moreover, such systems can be equipped with an autosampler and a completely automated cleaning system, allowing up to 50 samples to be measured per day. Furthermore, automated dilution series can be performed to obtain the intrinsic viscosity. These features make the instrument perfectly suited for the combinatorial workflow and enable a molecular weight determination for polymers that are otherwise difficult to characterize due to e.g. column interactions in SEC or too high molecular weight and/or too broad polydispersity indices for MALDI-TOFMS investigations.

**Screening for polymer composition and polymerization kinetics**

The chemical composition of polymeric materials as well as polymerization kinetics can be conveniently obtained utilizing optical screening methods, such as fourier transform infrared (FTIR) spectroscopy or chromatographic techniques, such as gas chromatography (GC) or high-pressure liquid chromatography (HPLC). In general, optical methods are very well suited for high-throughput screening since the optical signal can be read out continuously for online monitoring purposes. Furthermore, by utilizing parallel approaches (e.g. plate readers or imaging setups), a large number of samples can be evaluated quasi simultaneously. Chromatographic techniques on the other hand are well suited for handling a large number of samples due to the utilization of autosamplers. Moreover, these techniques can be accelerated by taking advantage of commercially available columns with reduced analysis time for HPLC, SEC as well as GC. For the monitoring of polymerization kinetics, both optical and chromatographic techniques can be used in a straightforward manner by measuring $t_0$ samples
An introduction to combinatorial materials research

and subsequently following the process in time relative to the initial sample to obtain monomer conversions or other interesting parameters. It was for instance shown that a flow cell, which is positioned in the working area of the GC autosampler and connected to an injection port inside a robotic synthesizer system, can be used for high-throughput monitoring of cationic ring-opening polymerizations. The reliability and comparability of this online GC setup was investigated by monitoring the cationic ring-opening-polymerization (CROP) of 2-ethyl-2-oxazoline at different concentrations. Ten polymerizations with a monomer to initiator \([\text{M}]/[\text{I}]\) ratio of 60 at different concentration were therefore investigated with both online and offline GC measurements revealing good agreements (less than 5 percent difference). However, the authors stated that for the evaluation of very fast polymerizations this setup might be too slow. Nevertheless, this example clearly demonstrates that online GC monitoring in CMR is feasible. Moreover, the obtained GC characterization results can of course also be correlated to the obtained polymer composition and if the composition in time is analyzed to the polymer structure (e.g. random, block-like, etc.).

Another analytical approach to obtain the polymer composition of polymer libraries as well as monomer conversions of polymerization reactions in a fast and convenient fashion is the application of vibration spectroscopy. Especially infrared spectroscopic techniques are very well suited for these purposes. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy was shown to be a versatile tool for the online monitoring of monomer conversions as well as copolymer compositions for various kinds of polymerization techniques. For instance, emulsion terpolymerizations of butyl acetate, methyl methacrylate and vinyl acetate were monitored by an ATR-FTIR probe and good agreements with traditional gravimetric as well as \(^1\)H NMR investigations were obtained. Moreover, Mülhaupt et al. reported on the high-throughput evaluation of olefin copolymer compositions using ATR-FTIR. Utilizing a multivariate calibration it was possible to evaluate the composition of ethene/propene, ethene/1-hexene and ethene/1-octane copolymers by ATR-FTIR spectroscopy with an error of less than 5% as depicted in Figure 1.6.

![Figure 1.6](image)

**Figure 1.6** Predicted (as determined by IR spectroscopy) vs. actual comonomer content (as determined by \(^{13}\)C NMR spectroscopy) for ethene/propene, ethene/1-hexene, and ethene/1-octene copolymers. The lines indicate the 1σ intervals (taken from Ref. 68).
In addition, it was discussed that it is possible to significantly improve the performance of rapid on-line polymer analysis utilizing near infrared (NIR) spectroscopy in combination with mid infrared (MIR) spectroscopy. Moreover, FTIR was used to monitor living isobutylene and ethylene oxide polymerizations with an acquisition time per FTIR spectrum of as low as 22 seconds. These examples clearly show that the high-throughput screening of monomer conversion as well as polymer composition is feasible by utilizing well known polymer characterization tools, such as chromatography or vibrational spectroscopy that were adopted for the handling of large numbers of samples.

**Polymer property screening**

The property screening of new (polymeric) materials is challenging and special tests have to be developed and/or existing tests have to be adjusted in order to speed up the evaluation of new materials. In particular, the screening of thin films (coatings) for certain properties of interest such as adhesion, crystallization, dewetting or others is of significant importance. Therefore, techniques for the preparation of continuous polymer thin film libraries with gradients in thickness, temperature, composition or others were developed. The advantage of such libraries is their relatively easy preparation and the possibility to obtain material properties over a wide span of different parameters. For the preparation of thin-film thickness libraries, special flow coating devices (based on a velocity-gradient knife-edge coating device) have been designed by Meredith, Karim, and Amis et al.. This equipment is capable of spreading drops of polymer solutions over the substrate at constant acceleration resulting in polymer films with a gradient in thickness (compare Figure 1.7).

**Figure 1.7** Left: thin film combinatorial library preparation (thickness or temperature gradients). Middle: Automated optical microscopy. Right: informatics data reduction (taken from Ref. 72).
A modification of this technique, which utilizes an automated solution premixing setup, was described for the preparation of libraries with gradients in polymer composition. Moreover, the utilization of heating stages allows the application of temperature gradients to the mentioned polymer thin film libraries. Furthermore, different methods for the preparation of surface energy libraries on Si surfaces are available. These are based on buffered oxide etching followed by a gradient Piranha etching (H₂SO₄/H₂O₂/H₂O) or chlorosilane monolayer formation followed by an UV exposure with a gradient in radiation. For both methods the change in surface energy could be validated by contact angle measurements. Finally, the utilization of ink-jet printing or vapor deposition technologies offers advantages for the preparation of polymer thin film libraries with discrete composition variations. These defined libraries are useful for the evaluation of mechanical properties of polymer libraries, contact angle measurements, optical properties and others. In addition, accelerated and/or parallel techniques for a fast evaluation of certain material properties of interest in polymer science including, e.g., the utilization of autosamplers for differential scanning calorimetry (DSC) or thermal gravimetric analysis (TGA) are described in the literature.

Atomic Force Microscopy (AFM) was shown to be a powerful tool for the high-throughput characterization of pattern formation in symmetric poly(styrene)-block-poly(methylmethacrylate) (PS-b-PMMA) diblock copolymer films. Thickness gradient films of copolymers with different molecular weights were prepared and their morphology was screened using AFM and optical microscopy with the result, that new morphology patterns of these materials were observed. AFM was also utilized for the evaluation of two-dimensional thickness-surface energy thin film libraries of poly(styrene)-block-poly(methyl methacrylate). The degree of formation of islands and holes in the film was found to be dependent on differences in surface energy. Moreover, optical microscopy was applied to two-dimensional composition-temperature libraries to detect phase separations and microstructures of poly(styrene)-poly(vinylmethylether) (PS-PVME) blends. An automated setup with a optical microscope (compare Figure 1.7) was also used to evaluate the thin-film dewetting behavior of PS films on silicon. By investigating poly(styrene) (PS) thin-films with orthogonal, continuous variations in thickness and temperature it was possible to obtain the temperature-thickness-time dependence of dewetting structures and kinetics. Moreover, the influence of film thickness as well as temperature on the crystallization behavior of isotactic poly(styrene) (iPS) films was studied. Generally, all results were consistent with conventional experiments. It was observed, for instance, that the crystallization growth rate had a maximum at intermediate temperatures over a wide temperature range. Karim et al. also discussed a combinatorial approach to characterize epoxy curing in films consisting of a constant amount of fluorescent dye, curing agent and epoxy resin. Therefore, FTIR microspectroscopy, confocal microscopy as well as axisymmetric adhesion testing were applied to first study discrete epoxy samples and subsequently study a continuous temperature gradient combinatorial library. Figure 1.8 (a and b) show FTIR maps of the continuous gradient library, revealing a lower degree of curing at lower temperatures.
The same trends in curing degree were found in a corresponding fluorescence map. Furthermore, the work of debonding (WOD, obtained from adhesion testing with polydimethylsiloxane lenses) could be correlated to the curing temperature and therefore the degree of curing. Mechanical properties were also investigated for segmented poly(urethane urea) libraries\textsuperscript{90,91} resulting in structure-mechanical property relationships for libraries with continuous gradients in chain extender composition and/or cure temperature.\textsuperscript{91} The mechanical properties, obtained from a high-throughput mechanical characterization apparatus, could subsequently be correlated to morphology, hydrogen bonding as well as degree of phase separation. As a result, optimum strength and percent elongation were observed at a chain extender composition of 85 mol\%. Furthermore, mechanical properties of a combinatorial library of differently thermally treated isotactic poly(propylene) (iPP) were investigated.\textsuperscript{92} First, the libraries were created utilizing a temperature gradient plate apparatus in a disc-shaped form by cooling from melt to room temperature applying different temperature gradients. Subsequently, the samples were investigated by wide angle X-ray scattering (WAXS) to characterize the changes in crystalline morphology originating from the different thermal treatments. Finally, the mechanical tensile testing was performed on specimens that were punched out from the discs. The results showed that the stress and strain at break could be correlated to the crystal morphology obtained from the WAXS experiments. Moreover, Symyx developed a mechanical thermal analyzer, a fully parallel instrument that is capable of performing 96 simultaneous measurements as a function of varying environmental conditions.\textsuperscript{93} After validation of the new instrument, a case study was performed for tackifier and plasticizer additives to a poly(styrene)-\textit{block}-poly(butadiene)-\textit{block}-poly(styrene) triblock copolymer as possible high performance pressure-sensitive additive. For example it was observed that the addition of the plasticizer dioctyl phthalate to the ABA triblock copolymer led to a decrease in glass transition temperature of the hard styrene domain, whereas it had no

\textbf{Figure 1.8 FTIR maps of the curing degree (CD) (5 mm × 4 mm steps and 400 µm × 800 µm steps, respectively) of a thin film epoxy library depending on the temperature (taken from Ref. 89).}
effect on the soft butadiene domain. The absolute decrease could furthermore be correlated to the amount of plasticizer present in the system. Finally, a recent literature example could show that nanoindentation can provide a general, rapid, precise, and accurate mechanical characterization of a discrete acrylate based polymer library. Symyx also described a generic workflow for the evaluation of synthetic polymers as selective transport agents for targeted delivery of potential drugs to human tissue. After identification of an optimized tissue mimicking substrate as well as a reconstructed biological liquid, the screening of 3000 diverse polymers on two selected mimics (representing two types of biological tissue) was feasible leading to the identification of a polymer with a maximum bioactive uptake (while preserving its high tissue substantivity). The wettability of a poly(urethane) polymer library was evaluated by a new high-throughput screening method consisting of a liquid handing robot, a standard webcam and an automated image processing software. The method includes automated dispensing of a liquid on a spin coated polymer film and subsequent image capture and automated evaluation of the spreading area. Furthermore, the results of this screening method could be correlated to conventional contact angle measurements. High-throughput methods were also established for differential scanning calorimetry (DSC) by means of the development of an automated large sample array DSC (LSA-DSC or LSA). Utilizing this instrument (in combination with a newly developed formulating procedure) 270 manual labor hours could be avoided during the evaluation of the curing kinetics of 275 epoxy formulations. Moreover, a DSC with an autosampler was utilized to study a systematic 2-oxazoline copolymer library prepared with an automated synthesizer. Two copolymer series containing methyl and nonyl (MeOx:NonOx) as well as ethyl and nonyl (EtOx:NonOx) side chains were compared revealing e.g. that the the melting point of pNonOx decreases upon the incorporation of MeOx or EtOx since the crystallinity of the pNonOx is disturbed.

![Surface energies of block co-poly(2-oxazoline)s revealing a considerably lower surface energy for the nonyl containing polymers (taken from Ref. 83).](image-url)
As already briefly mentioned, polymer libraries can be evaluated by means of automated contact angle measurements. A modified commercial contact-angle measuring apparatus that is capable of automatic dispensing, analyzing and aspirating was applied for the evaluation of a 16-membered library of poly(oxazoline) block copolymers. Utilizing diiodomethane and ethylene glycol as test liquids it was possible to convert the measured contact angles into surface energies. The necessary 128 contact angle measurements could be performed within 70 minutes. Figure 1.9 displays the resulting surface energies of this 16-membered poly(oxazoline) library revealing that polymers containing the 2-nonyl-2-oxazoline monomer have a considerably lower surface energy. This is most likely caused by a close packing (and preferential orientation) of the aliphatic nonyl chains on the surface of the samples.

1.2.3 Concluding remarks

Polymer libraries with a known and systematic variation of certain properties are one of the two possibilities for a reliable and accelerated determination of quantitative structure-property relationships in combinatorial materials research since in this case the correlation of the obtained results to the structure of the investigated polymeric materials can often be achieved in a straightforward manner. The second possibility is the application of design-of-experiments and other computational approaches for the reduction of the number of necessary experiments while the whole parameter space is still covered. Both approaches have their pros and cons, e.g. the coverage of the whole parameter space by the computational approaches with the disadvantage that the large number of experiments that have to be performed might still not be sufficient to identify the desired structure-property correlations because, e.g., the descriptors were not chosen correctly. On the other hand, the investigation of small and defined libraries (“targeted libraries”) will certainly reveal structure-property relationships, but only within a limited parameter space and the observed correlations might not be transferable to other materials. Nevertheless, whatever approach is chosen, combinatorial materials research did already show that these efforts can address a large variety of different problems in materials research, including, e.g., the addressing of the extremely large parameter space that is available. Finally, I would like to especially point out the importance of the discussed screening approaches, since (i) it would not be possible to investigate polymer libraries and to reveal the desired structure-property relationships within a reasonable time frame without them and (ii) the efforts taken to optimize, miniaturize and parallelize analytical techniques generally result in a better understanding, improved analytical results and/or increased applicability of these techniques, which is not only very useful for the combinatorial researcher but also provides significant advantages for the whole scientific community.
1.3 Aim and outline of the thesis

As demonstrated with the literature study provided above combinatorial materials research is a very promising and still young field of research. Several approaches for the automated and parallel synthesis of polymer libraries are known, making use of different degrees of automation. Therefore, also an easy access into this field of research is possible by utilizing, e.g., the described manual parallel approaches. Nevertheless, if the productivity of a single researcher is enhanced due to these approaches the need for specially developed and adopted high-throughput screening tools is obvious. However, these screening tools were far less developed four years ago, when this project was initiated. Therefore, the major aim of this thesis was the development, implementation and application of new high-throughput screening tools that allow the combinatorial polymer researcher a fast and accurate determination of important polymer parameters. Moreover, an objective was the execution of automated and/or parallel synthetic approaches in combination with fast (self developed) screening approaches in order to demonstrate the feasibility of combinatorial approaches in polymer science and enhance the productiveness (and therefore reduce the costs) of polymer research in general.

Chapter 2 describes the development and evaluation of a new sample preparation technique for MALDI-TOFMS that allowed the integration of MALDI-TOFMS as a high-throughput screening technique into the workflow of CMR. Therefore, this technique could be automated and integrated into synthetic robots for the screening of polymerization reactions and the characterization of polymer libraries. In a final step of automation and miniaturization this sample preparation technique could be transferred to ink-jet printing technology opening the way for ultra high-throughput applications. In general, this multiple-layer spotting technique provided significantly improved analytical results for a large variety of synthetic polymers, saved valuable sample preparation time and was therefore very well suited for screening approaches in CMR.

Chapter 3 discusses the synthesis and characterization of block copolymers of poly(ethylene glycol) and poly(ε-caprolactone). In particular, linear and star-shaped block copolymers were prepared and their micellar encapsulation properties were investigated utilizing self-developed high-throughput screening assays. The star-shaped block copolymers showed an interesting reversed unimolecular micellar behavior and were able to encapsulate and phase transfer a large variety of different guest molecules. This encapsulation behavior could be correlated to the macromolecular structure and it was for instance observed that the maximum loading of guest molecules within a reversed unimolecular micelle was independent of the chain length of the poly(ε-caprolactone) comprising the corona of these micelles. Moreover, these star-shaped block copolymers could be used as templates for the synthesis of palladium nanoparticles, which were successfully applied as catalysts for C-C coupling reactions utilizing automated synthesizer robots.
Chapter 4 addresses the synthesis and detailed characterization of metal-containing supramolecular polymers based on the terpyridine chelating ligand. The investigations were started due to the need of improved characterization methods for these polymers. A MALDI-TOFMS model study revealed the relative binding strength of the terpyridine ligand for a large variety of different transition metal ions that are frequently used in these supramolecular polymers, whereas a size exclusion chromatography (SEC) study resulted in optimized chromatographic conditions for ruthenium-containing polymers. Especially the SEC study was crucial for the success of a subsequently performed parallel optimization of the reaction conditions of RuCl\textsubscript{3} with a defined low molecular weight bis-terpyridine ligand where it was applied as the major screening technique. These investigations led to the development of a new type of supramolecular ABA triblock copolymer based on a simple polycondensation strategy that revealed amphiphilic behavior and formed micelles in water. Finally, the investigation of a star-shaped supramolecular polymer by means of parallel high-throughput techniques led to the accelerated development of a new sensoric system for transition metal ions. This system is based on the encapsulation of fluorophores within a terpyridine end-group modified polymer and quenching of the fluorescence due to metal complex formation.

1.4 References

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[38] Argonaut Technologies, Application note, No. 28.


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CHAPTER 2

MALDI-TOFMS as high-throughput screening tool

Abstract

The possibilities of the integration of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) as a high-throughput screening tool into the workflow of combinatorial materials research are discussed. A multiple layer sample preparation technique for MALDI is described in detail and its possibilities of automation and miniaturization are evaluated. Automated MALDI sample preparation could be performed within an automated synthesizer robot as well as with an ink-jet printer. The first approach offers the possibility of online reaction monitoring, whereas the second approach gives the opportunity of applications in ultra high-throughput environments. Moreover, examples of high-throughput screening of a polymerization reaction and a polymer library by MALDI-TOFMS are discussed.

2.1 Introduction

In recent years the mass spectroscopic analysis of (biological) macromolecules became feasible due to the invention of soft ionization methods, such as electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI), which was rewarded with the Nobel price in chemistry in 2002 "for the development of methods for identification and structure analyses of biological macromolecules" to J. B. Fenn, K. Tanaka and K. Wüthrich.1 Today, MALDI time-of-flight mass spectrometry (MALDI-TOFMS) is perhaps the most important mass spectroscopic technique currently used for polymer analysis2 and is a very useful tool for the determination of absolute molecular weights, molecular weight distributions, and end-groups of synthetic polymers.2-4 A successful MALDI experiment is a multistep-event consisting of: sample preparation, sample excitation and disintegration of the condensed phase, generation and separation of charges and ionization of the analyte molecules followed by extraction, separation by the mass-to-charge (m/z) ratio and detection of the molecules within the mass spectrometer.5 An important parameter for the excitation part of the MALDI experiment is unsurprisingly a sufficient absorption of the MALDI matrix at the applied laser wavelength5,6 and generally it is tried to match matrix and analyte polarity for good analytic results.3 However, one of the most difficult parts in MALDI analysis seems to be the sample preparation, since this step is crucial for the success of the MALDI experiment.2

![Figure 2.1 Schematic representation of a MALDI experiment.](image)

Figure 2.1 schematically depicts such a MALDI experiment: the sample was prepared as film on a sample target and afterwards the sample is irradiated with a pulsed laser to form a plume in the vacuum. Subsequently, this plume (or “trail of smoke”) physically expands in the vacuum leading to less and less collisions of primary formed ions or excited state matrix molecules with neutral analyte molecules. Simulations of this plume have revealed that a UV MALDI plume can be described as a very fast (and almost explosive) solid to gas phase transition.7,8 Figure 2.2 displays results of such a simulated laser ablation/desorption and plume formation process in time after a 15 ps laser pulse.8 It is clearly visible that not only single molecules are desorbed, but also larger clusters of molecules are ablated. Moreover,
these simulations revealed that at low applied laser energies/cm$^2$ (fluences) the plume was mainly formed due to desorption, whereas at higher fluences more ablation was observed leading to larger clusters in the plume.$^8$

Figure 2.2 Plume expansion (to the top) in time ($a=50$, $b=100$, $c=250$, $d=500$ and $e=1000$ ps, respectively) after a 15 ps laser pulse. The total fluence is approximately twice the threshold fluence for ablation (taken from Ref. 8).

The most likely reactions to form the mentioned primary ions are e.g.: energy pooling of two excited matrix molecules to yield matrix radical cations (see equation 2.1) or excited state proton transfer reactions (see equation 2.2).$^9$

\[
\text{Matrix} + \text{Matrix} + 2h\nu \rightarrow \text{Matrix}^* + \text{Matrix}^* \rightarrow \text{Matrix} + \text{Matrix}^+ + e^- \quad \text{(equation 2.1)}
\]

\[
\text{Matrix} + \text{Matrix} + h\nu \rightarrow \text{Matrix}^* + \text{Matrix} \rightarrow (\text{Matrix-H})^- + (\text{Matrix+H})^+ \quad \text{(equation 2.2)}
\]

Subsequently, mainly secondary reactions form the analyte ions in the expanding plume that are then separated and detected by a time-of-flight (TOF) mass spectrometer. One explanation that the ionization part and therefore the observed ion distributions of a MALDI experiment might be dominated by secondary reactions in the MALDI plume and do not occur during the primary very short (3-5 ns) laser pulse is the fact that reactions between ions and neutral species will continue as long as there are collisions in the expanding plume and the time required to reach collision free densities in the plume is many microseconds.$^{10}$

\[
(\text{Matrix+H})^+ + \text{Analyte} \rightarrow \text{Matrix} + (\text{Analyte+H})^+ \quad \text{(equation 2.3)}
\]
These secondary reactions include e.g. proton transfer (probably the most important secondary reaction, see equation 2.3) of primary protonated matrix and analyte molecules, cation transfer (for synthetic polymers, cationizing agents are often added deliberately to the sample preparation) or electron transfer reactions. They are mainly responsible for the observed ion distribution in a MALDI-TOF mass spectrum.

2.2 Development of a suitable sample preparation technique

As described in the previous chapter, combinatorial materials research (CMR) can be considered as a rapidly growing and highly multidisciplinary field of research. The discussed examples clearly demonstrate that it is feasible to synthesize and investigate a large variety of different polymeric materials in a combinatorial fashion. However, until recently no feasible technique was described for the high-throughput determination of molar masses, molar mass distributions as well as end-groups of synthetic polymers by mass spectrometry. The oldest, simplest and maybe mostly utilized sample preparation technique for MALDI-TOFMS is the dried-droplet (DD) method. For this method solutions of analyte, matrix and ionizing salt (most likely in different solvents) are mixed by volume and an aliquot of the mixture (usually 0.5-1.0 µL) is deposited on the MALDI target and air dried. Within this chapter the development and evaluation of a multiple-layer spotting technique for MALDI-TOFMS of synthetic polymers that is ideally suited for applications in combinatorial materials research will be discussed. This method is able to significantly reduce the time required for sample preparation, to improve the analytical results as well as to be automated and miniaturized.

2.2.1 General requirements

The key factor and therefore the most important requirement for any successful MALDI experiment is an easy applicable and reproducible sample preparation technique. As already briefly mentioned, the most widely applied sample preparation for MALDI-TOFMS is the dried droplet method. One particular problem of this method for the analysis of synthetic polymers is the solvent selection, since all three compounds of the sample should be well soluble in a certain solvent. However, salts are for instance not well soluble in common organic solvents making the DD method difficult for hydrophobic synthetic polymers. This can lead to a small amount of polymer non-solvent in the final mixture, which can affect the signal reproducibility. Therefore, other sample preparation techniques for improved reproducibility and analytic results were developed including vacuum drying, overlayer (two-layer or seed layer), or fast evaporation. All mentioned methods have in common the effort to control the crystallinity of the utilized matrix and therefore the improvement of the analytical results. In this respect, it was for instance proposed that proteins (and other
analytes) bind to the surface of matrix crystals and that an increase of the mainly hydrophobic interactions between crystal and analyte improve the spectral quality in MALDI experiments. In addition, it was observed that a successful UV-MALDI experiment requires an intimate contact between matrix and analyte molecules but does not necessarily require analyte incorporation into the matrix crystals. Moreover, it was observed that non-incorporated analytes provided better MALDI results if the surface-to-volume ratio of the matrix was high. Detailed confocal microscopy studies suggested e.g. that a protein analyte was not homegeniously distributed within microcrystals formed by the fast evaporation sample preparation method but across the entire sample deposition area the protein was more uniformly distributed than with the dried-droplet method. This sample was prepared in two layers and the addition of the analyte in as the second layer redissolved a small amount of matrix molecules in the outer layer of the crystals and these redissolved matrix molecules incorporated the analyte efficiently during the a rapid, nonequilibrium recrystallization process resulting in the nonuniform analyte distribution in individual crystals. However, on a macroscopic scale, the large number of microcrystals made the analyte distribution within the entire sample more homogeneously. Therefore, more densely packed, smaller crystals would be statistically in favor of more uniform analyte distribution across the sample area and this homogeneous distribution is favorable for reproducible MALDI-TOFMS results. Concerning the high-throughput analysis of libraries by MALDI-TOFMS, especially the seed-layer technique could be automated for the analysis of peptides and proteins as well as of a compound library of organic molecules. In general, the requirements for an automated MALDI-TOFMS analysis of combinatorial polymer libraries are similar to the above discussed ones. However, for combinatorial approaches a reproducible and widely applicable sample preparation technique is even more relevant, since a large variety of different compounds or, in the case of polymers, a large variety of different molecular weights has to be analyzed utilizing the same sample preparation technique. Moreover, the applied technique should be easy to automate, to integrate into the combinatorial workflow, and be as fast as possible. In order to meet these challenges a multiple-layer spotting technique was developed, evaluated and later on automated and integrated into the workflow of combinatorial material research.

### 2.2.2 Multiple-layer spotting for MALDI-TOFMS

A new multiple-layer spotting technique for MALDI-TOFMS is described and evaluated within this section. Generally, it consists of a completely decoupled handling of the three main components of a MALDI-TOFMS sample, namely the matrix, the doping salt and the analyte. These three components are spotted on top of one another from different solutions allowing the free choice of solvent for every component. Figure 2.3 depicts a schematic representation of the multiple-layer spotting approach compared to a conventional dried droplet sample preparation technique.
Figure 2.3 Schematic representation of (a) conventional and (b) multiple-layer sample preparation for MALDI-TOFMS.

It should be noted that this representation is oversimplified since re-dissolving of previous layers can occur to a certain extent. Nevertheless, the free choice of solvent for the crystallization of the matrix is a significant advantage of the developed technique and provides clearly improved analytical results, as it will be discussed later. To evaluate the method, resulting MALDI spectra were evaluated in terms of signal intensity and signal-to-noise ratio. The evaluation and comparison of this new sample preparation approach was achieved by analyzing MALDI spectra from five poly(ethylene glycol) standards (Table 2.1) obtained with the same instrument settings while changing the sample preparation. The resulting signal intensities signal-to-noise ratios were then averaged over at least 1000 laser shots and the complete sample surface was investigated in order to circumvent the misinterpretation of sample inhomogeneities, so called hot-spots. One important result of this new multiple-layer spotting technique is the prevention of difficulties in the solubility of analyte, salt and matrix in different solvents.

Table 2.1 Molecular weight data of poly(ethylene glycol) standards: SEC (provided by the manufacturer); MALDI-TOFMS (obtained from optimized sample preparation technique).

<table>
<thead>
<tr>
<th>PEG</th>
<th>M_n (SEC)</th>
<th>M_w (SEC)</th>
<th>M_n (MALDI)</th>
<th>M_w (MALDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2000</td>
<td>2200</td>
<td>1911</td>
<td>1952</td>
</tr>
<tr>
<td>2</td>
<td>4800</td>
<td>5200</td>
<td>5010</td>
<td>5071</td>
</tr>
<tr>
<td>3</td>
<td>9600</td>
<td>9900</td>
<td>10648</td>
<td>10864</td>
</tr>
<tr>
<td>4</td>
<td>18700</td>
<td>20000</td>
<td>17070</td>
<td>17231</td>
</tr>
<tr>
<td>5</td>
<td>29000</td>
<td>30800</td>
<td>34174</td>
<td>34425</td>
</tr>
</tbody>
</table>

The choice of matrix is very important and can be critical for the success of polymer MALDI experiments. Therefore, five commonly used matrices \([\alpha\text{-}cyano\text{-}4\text{-}hydroxycinnamic acid (CHCA), 2\text{-}(4\text{-}hydroxy\text{-}phenylazo)\text{-}benzoic acid (HABA), trans\text{-}3\text{-}indoleacrylic acid (IAA), 2,5\text{-}dihydroxybenzoic acid (DHB), 1,8,9\text{-}anthracenetriol (Dithranol)]\) were chosen and evaluated also investigating different solvents for the matrix crystallization. The multiple layer spotting was performed as follows: first, the matrix (0.5 µL, 50 mM in chloroform-ethanol 1:1) was spotted on the MALDI target. This solvent combination was chosen for the first experiments since all investigated matrices revealed good solubilities in this solvent combination.
Maldi-tofms as high-throughput screening tool

Figure 2.4 MALDI-TOFMS spectrum obtained from PEG 3 (compare Table 2.1) using the multiple-layer spotting technique and dithranol as matrix (crystallized from chloroform).

As the second layer, 0.5 µL of a saturated NaCl solution in acetone was applied, and thereafter the poly(ethylene glycol) (PEG) solution (25 mg/mL in dichloromethane) was added on top as the third and final layer (0.5-0.7 µL). Sufficient time for solvent drying was allowed (~90 seconds) before the next layers were applied. It is known that all the above mentioned matrices can produce useful MALDI spectra of PEG (Mw = 3350 Da). However, it was observed that the optimum choice of matrix is dependent on the molecular weight of the PEG polymers. Most importantly, the higher molecular weight poly(ethylene glycol) standards showed a clear preference for dithranol as the matrix, since only dithranol was able to ionize the PEG standards 3 to 5 with this spotting approach. The resulting spectrum of PEG 3 with dithranol as matrix is illustrated in Figure 2.4. Moreover, in further investigations it was observed that the solvents used for crystallization of dithranol highly affected the quality of the resulting MALDI-TOFMS spectra. Generally, fast solvent evaporation is advantageous for MALDI sample preparation. Investigated solvents were acetone, chloroform, dichloromethane, ethanol, methanol and tetrahydrofuran, with the result that acetone and particularly chloroform were the most suitable solvents to prepare the first layer of dithranol on the MALDI target. It was found that thin dithranol films crystallized from chloroform provided the best results and thin dithranol films crystallized from ethanol or methanol showed the worst results in terms of signal-to-noise ratio as well as signal intensity, most likely because the latter solvents were not able to produce a continuous matrix film. Therefore, all further experiments were performed with dithranol as matrix and chloroform as
the matrix solvent. This observation also correlated to a certain extent with optical microscopy pictures taken from crystallized matrix spots (see Figure 2.7 for pictures), since the film prepared from dithranol revealed a continuous and crystalline structure, whereas the film prepared from methanol was discontinuous. Furthermore, it was observed that the order of spotting the three layers (matrix, sample and doping salt) greatly influenced the quality of the obtained MALDI spectra. For these experiments NaI was used (saturated in acetone) as doping salt solution, and PEGs were applied as solutions (25 mg/mL) in dichloromethane. The three different layers of polymer, dithranol and doping salt were applied to the MALDI target in all possible combinations.

**Table 2.2** Evaluation of the spotting order (layers of polymer, doping salt and matrix with respect to relative signal intensity and signal to noise ratio of the resulting spectra for PEG 2 (see Table 2.1).

<table>
<thead>
<tr>
<th></th>
<th>relative signal-intensity</th>
<th>signal/noise-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-NaI-Dithranol</td>
<td>34.5</td>
<td>2.2</td>
</tr>
<tr>
<td>NaI-PEG-Dithranol</td>
<td>17.4</td>
<td>1.4</td>
</tr>
<tr>
<td>NaI-Dithranol-PEG</td>
<td>78.7</td>
<td>3.4</td>
</tr>
<tr>
<td>PEG-Dithranol-NaI</td>
<td>67.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Dithranol-PEG-NaI</td>
<td>14.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Dithranol-NaI-PEG</td>
<td>100.0</td>
<td>3.7</td>
</tr>
<tr>
<td>conventional: mixing</td>
<td>49.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>

All sample spots were then measured with the same instrument settings, and the resulting PEG spectra evaluated with respect to signal-to-noise ratio and relative signal intensity (compare Table 2.2). The results were also compared with those obtained using the conventional sample preparation method by mixing all components (1:1:1), revealing that the multiple layer approach in some cases offered greater signal intensity as well as better signal-to-noise ratio due to the avoidance of solvent incompatibilities. Moreover, with the multiple-layer spotting approach, solvent incompatibilities can be neglected. According to the results summarized in Table 2.2, the best results were obtained if the analyte was the top layer and dithranol the first layer of the multiple layer sample preparation technique for PEGs (probably due to an undisturbed crystallization and film formation of the dithranol layer). Therefore, this order of spotting was used for all further experiments. Different salts were utilized to screen for the most suitable doping additive for the ionization of poly(ethylene glycol). For these experiments, the order of the spotted layers was matrix – doping salt – polymer, as described above. For a first screening, alkali metal salt solutions (LiCl, NaCl, KCl, CsCl, all saturated in acetone) were utilized. The results revealed that sodium chloride showed the highest signal intensity and best signal-to-noise ratio for higher molecular weight PEGs. Potassium chloride, on the other hand, always gave very low signal intensities; sometimes, no peaks were detected at all. Lithium and cesium chlorides showed comparable signal-to-noise ratios and signal intensities. Figure 2.5 displays the cesiated (top-left) and lithiated (bottom-
left) spectra obtained from PEG 2. The peaks are shifted with respect to another by $126\pm0.5$ Da, which is in agreement with the corresponding atomic masses of Li and Cs. In the literature, the effect of different cationizations of a PEG ($M_n = 1.450$ g/mol) sample with different matrices is discussed. In that case, lithiated peaks showed higher signal intensities than the cesiated ones, if equimolar amounts of Cs$^+$ and Li$^+$ were used for sample preparation with dithranol as matrix. This effect was reversed if different matrices (i.e. DHB) were utilized. This sample preparation approach did not reveal a preference for either Li$^+$ or Cs$^+$. This might be an effect of the different concentrations applied during the sample preparation, since CsCl has a higher solubility in acetone than LiCl. Nevertheless, NaCl showed the greatest potential to ionize PEG if samples are prepared as mentioned above. Furthermore, lithium addition to PEG samples can result in complicated spectra, since PEG samples are often contaminated with sodium and therefore both lithiated and sodiated peaks are observed (see e.g. Ref. [22]). Moreover, a saturated NaCl solution was also compared to a saturated NaI solution (both in acetone) as doping agents. Here, NaI showed better ionization of the PEG samples, most likely due to a higher solubility as well as a higher heat of formation ($\Delta H_f(NaCl) = -411$ kJ/mol; $\Delta H_f(NaI) = -288$ kJ/mol). Therefore, NaI represents the most suitable doping agent out of the tested salts for PEG cationization in MALDI, at least if a multiple layer sample preparation technique is utilized. The data obtained for the five poly(ethylene glycol) standards, utilizing the optimized sample preparation method (dithranol saturated in chloroform, NaI saturated in acetone and PEG in dichloromethane), are shown in Table 2.1. For comparison, the number averaged molecular weight ($M_n$), weight average molecular weight ($M_w$), and the polydispersity index (PDI) obtained by SEC of the investigated standards, are also listed in Table 2.1.

![Figure 2.5](image.png)

**Figure 2.5** Left: MALDI-TOFMS spectra of the cesiated (top) and lithiated (bottom) PEG 2 (compare Table 2.1). The peaks are shifted by $126\pm0.5$ Da, which is in agreement with lithiated and cesiated polymer distributions. Right: MALDI-TOFMS spectra of high molecular weight poly(ethylene glycol) standards. Top: PEG 5; bottom: PEG 4.
The difference between two adjacent mass peaks was found to be 44 Da, which corresponds to the mass of ethylene glycol monomer units (this resolution was obtained up to 20 kDa). Moreover, end-group calculations clearly revealed $\alpha$-CH$_3$ and $\omega$-OH functionalities for PEOs 1-4. Furthermore, even high molecular weight poly(ethylene glycol) standards could be analyzed with monomer resolution up to 20 kDa (see Figure 2.5 bottom-right) and without monomer resolution up to 35 kDa (see Figure 2.5 top-right). These results represent an important and significant improvement compared to known literature procedures, since it is known that the analysis of higher molecular weight PEGs is difficult with MALDI-TOFMS. Finally, it should be mentioned that the above described optimization experiments revealed only little dependence on the laser intensity used for the investigations and that the general trends of signal intensities within one set of experiments had a good reproducibility.

In subsequent investigations the sample preparation of poly(methyl methacrylate) (PMMA) was optimized in the same fashion as discussed for PEG. Screening the same five matrices including (CHCA, HABA, IAA, DHB, Dithranol) revealed a clear preference for dithranol by means of signal intensity and signal to noise ratio. Reasonable results were also obtained with DHB, whereas the other three matrices were not able to ionize a 14 kDa PMMA standard with the chosen MALDI-TOFMS instrumental settings. With respect to salt additives the best result was obtained utilizing sodium iodide, probably for the same reasons as described above. In short, it turned out that the same settings as for PEG, namely dithranol (saturated in chloroform) as first layer, NaI (saturated in acetone) as second layer and PMMA (20 mg/mL in dichloromethane) as third layer, showed the best results. Furthermore, it was observed that a large variety of different polymers (e.g. poly($\varepsilon$-caprolactone), poly(styrene) or poly(oxazolines)) could be successfully analyzed with this optimized multiple-layer spotting approach.

In order to investigate the above mentioned matrix crystallization effect in more detail the crystallization behavior of six different MALDI matrices (CHCA, HABA, IAA, DHB, Dithranol as well as trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB)) from six different solvents was investigated. DCTB was added to the investigated matrices since this matrix is known to provide good results for difficult to ionize analytes. Figure 2.6 clearly illustrates the correlation of matrix crystallinity and MALDI-TOFMS spectrum quality for the CHCA matrix as an example. PEG 2 was analyzed by MALDI-TOFMS applying exactly the same instrument settings for the measurements (e.g. laser intensity, acceleration voltage, etc.) and the multiple-layer spotting technique (with NaI as salt) for the sample preparation. The only difference was the solvent utilized to prepare the first (matrix) layer of the sample: for the sample resulting in the spectrum displayed in Figure 2.6 (left) CHCA was deposited from acetone, whereas in Figure 2.6 (right) it was deposited from methylene chloride. It is obvious that CHCA (and other matrices) is able to ionize PEG providing well resolved MALDI spectra as displayed in Figure 2.6 (left). In this case acetone was applied for the crystallization of CHCA resulting in a continuous and crystalline layer of
CHCA (compare microscopy picture: inset Figure 2.6, left). On the other hand, if methylene chloride was used to prepare the matrix layer of CHCA a discontinuous film showing hardly any crystalline features was obtained (compare microscopy picture: inset Figure 2.6, right). Only baseline signals could be detected from this poorly crystallized matrix film as shown in Figure 2.6 (right).

This correlation between matrix crystallinity and MALDI-TOFMS spectral quality can also be observed for other matrices and is moreover described in the literature (compare also discussion above).\textsuperscript{2,3} A possible explanation for this behavior is the adsorption of the analyte molecules on the matrix crystal surfaces, which might be favorable for successful MALDI-TOFMS experiments.

Figure 2.7 (top) shows optical microscopy pictures of the matrix layer of all six investigated matrices leading to a 36 membered library of MALDI-TOFMS samples that were prepared utilizing the multiple layer approach. Figure 2.7 (bottom) displays the resulting relative signal intensities of this library when NaI in acetone and PEG 2 in dichloromethane were spotted on all 36 positions as second and third layer, respectively. Please note that the relative scale in Figure 2.7 (bottom) is cut off at 25 % for better visualization. For these MALDI-TOFMS experiments, spectra of all 36 samples were taken with the same instrument settings (e.g. laser intensity and delay time) and the corresponding signal intensities were subsequently compared. It was observed that DHB and HABA as matrices showed comparably low signal intensities, which is in good agreement with the above described results. Moreover, the combination of dithranol as matrix with chloroform as solvent, which is the above described optimized result, provided the second highest signal intensity if the previously investigated matrices (dithranol, DHB, IAA, HABA, CHCA) are taken into account. However, the combination of CHCA as matrix and acetone as solvent revealed even higher signal intensities.
Figure 2.7 Top: optical microscopy pictures of layers of different matrices crystallized from different solvents. Bottom: the corresponding MALDI-TOFMS signal intensities for PEG 2 (compare Table 2.1). All MALDI spectra for this experiment were recorded with the same instrument settings for comparison reasons (DCM = dichloromethane; THF = tetrahydrofuran).
This well-suited sample preparation was not identified in the previous evaluation since then a stepwise optimization was performed and not the whole library of samples was investigated as in this experiment. However, the most striking result is that DCTB is the superior matrix and is not very selective on the solvent used for crystallization. All DCTB solvent combinations, except DCTB with methanol, were able to produce an at least 10 fold higher signal intensity if compared to all other sample preparations. It should also be noted that the combination of DCTB and methanol (in contrast to the other DCTB solvent combinations) was not able to produce a crystalline and continuous surface as shown in Figure 2.7 (top), which resulted in the poor MALDI result for reasons already discussed above. Moreover, the trend that DCTB is able to produce considerably higher signal intensities if compared to other matrices is described in the literature for fullerene derivatives\textsuperscript{26} and was also observed for other classes of polymers. Therefore, DCTB in combination with the discussed multiple layer spotting approach for MALDI-TOFMS of synthetic polymers is the method of choice for high-throughput screening of synthetic polymers.

2.3 Integration into the combinatorial workflow

2.3.1 Automated synthesizer

In order to integrate MALDI-TOFMS monitoring possibilities into the CMR workflow, the multiple-layer sample preparation technique was integrated into an automated robotic synthesizer (Chemspeed ASW 2000). This technique makes it possible to obtain and spot samples during polymerization reactions in the synthetic robot and therefore allows monitoring of polymerization reactions with MALDI. For example, samples can be spotted directly from the polymerization vessels onto the MALDI target. In general, the spotting of the sample onto the MALDI target was accomplished utilizing the multi-layer approach (compare section 2.2.2). The respective solutions are aspirated and subsequently spotted on a defined position onto the MALDI target, which is located in the workspace of the robotic system in a custom made MALDI target holder in microtiter plate format. These positions were programmed in the software of the automated synthesizer on a \(xyz\) basis. This software allows for programming the robot in a sequential fashion including for instance the setting of temperature, reflux, vacuum or gas supply in its reactors. Moreover, it controls the liquid handling of the robotic arm in order to perform tasks such as the aspiration and dispensing of reactants from stock solutions into the reactor vessels or to take samples during reactions from the reactors. For this first feasibility study, the spotting order was analyte – salt – matrix, in contrast to the above described order, since the sample preparation was at that time not yet optimized. Nevertheless, the feasibility of this procedure could also be demonstrated utilizing these settings. The time required by the robotic system to finish all sample spots (e.g. from 16...
parallel polymerization reactions) was long enough (about 90 seconds/spot) to ensure complete drying of the spots before the next layer was applied. Moreover, the applied multiple-layer spotting approach has the advantage that no pre-mixing step of the components of the MALDI sample is required, therefore saving time (about 90 seconds/sample) and space with the synthesizer robot. Figure 2.8 (left) shows the needle, which is attached to the robotic arm of the synthetic robot, spotting the matrix solutions onto the MALDI target in the custom made rack.

The time required for spotting additive and matrix solutions (about 45 seconds/spot) is shorter than the time required for sample spotting, because no additional rinsing steps of the robot needle are required between the drop-casting of individual spots. The feasibility of this procedure was first studied with poly(styrene) standards. Therefore, 1 µL aliquots of stock solutions of dithranol (20 mg/mL solution in chloroform), poly(styrene) (5 mg/mL in chloroform) and AgPF₆ (saturated in acetone) were spotted on top of each other by the robotic system and the MALDI spectra were subsequently measured from these automatically prepared samples. AgPF₆ was chosen, since it is known that silver salts are very well suited for the cationization of poly(styrene).²,³ Five poly(styrene) standards with known molar mass (between 1.000 and 10.000 Da) and low polydispersity indices were used for this feasibility study. The samples were measured after the automated spotting resulting in good resolved spectra of the poly(styrene) standards. The difference between two peaks was found to be 104 mass units, which corresponds to the mass of styrene monomer units. Mₙ and Mₘ were calculated with the Data Explorer™ software and were found to be in good correlation with the data supplied by the manufacturer of the standards (not shown). In addition, the multiple layer approach was carried out manually in the same way and the same amount of solutions as for the automated approach. Figure 2.9 displays a poly(styrene) standard (Mₙ = 3.000 Da) from manually (top) and automatically (bottom) spotted samples.
Figure 2.9 Comparison of a manually (top) and automatically (bottom) spotted poly(styrene) standard ($M_n = 3000$ Da). The manually spotted standard reveals a better signal/noise ratio.

These results clearly show that the molecular weight distribution is not changed due to the sample preparation. Nevertheless, the manually spotted samples revealed better signal intensities as well as signal-to-noise ratios than the automatically spotted ones at the same MALDI instrument settings (see Figure 2.9). This could be an effect of the more accurate manual spotting, which resulted in more defined spots due to less solvent spreading (compare Figure 2.7, left). Generally, hand spotted samples are prepared with 0.2-0.5 µL of solvent utilizing Eppendorf pipettes that are able to handle 0.1 µL of solvent accurately, whereas the utilized robotic system can only handle volumes down to 1 µL accurately. This effect could be overcome in the future by using more accurate robotic systems and/or other solvents for the sample preparation. Nevertheless, no problems were observed if the optimized conditions were applied for the monitoring of reactions or the screening of libraries as it will be discussed in section 2.5. In summary, automated MALDI sample preparation technique is feasible and this spotting technique can be integrated into the workflow for combinatorial polymer research, offering fast (about 3 min/sample) and easy sample preparation as well as the possibility of high-throughput screening of polymerization reactions as well as of polymer libraries (see section 2.4).
3.2.3 Ink-jet printing technology

A new multiple-layer spotting technique for MALDI-TOFMS utilizing ink-jet printing technology was developed and evaluated. For this study, the multiple-layer sample preparation technique was transferred to an ink-jet printing device in order to automate and further improve the sample preparation for the mentioned polymers. A photo of the inkjet printer together with the experimental setup of microtiter plate and printed MALDI samples is shown in Figure 2.10. The three respective layers of matrix, salt additive and polymer were deposited sequentially on top of each other by printing a 4x4 array of spots of each layer onto the MALDI-target. Each spot consisted of five individual drops printed on top of each other and the spots were printed at regular intervals of 0.5 mm. The distance between the arrays was 5 mm. After drying the matrix layer was covered with a dopant layer, which was printed in exactly the same way as the matrix. Finally, the analyte was printed on top of the two previous layers.

Figure 2.10 Photo of the ink-jet printer used in this study (left). The glass micro-titer plate for stock solutions as well as MALDI sample target on the stage of the inkjet printer are shown in the top right part. Right-bottom: close-up on the MALDI target.

Generally, this resulted in reproducible sample spots of about 180-200 µm size (see Figure 2.11) and in comparable analytical results for the respective polymeric analytes. The size of the ink-jet printed sample spots is therefore significantly smaller than the size of hand prepared spots (4-5 mm) allowing the deposition of at least 400 times more samples on one target plate (~4000 samples in the case of the MALDI target utilized for the present study) and therefore opening application possibilities for ultra high-throughput screening (UHTS) applications. All obtained results were compared with the optimized settings for both investigated polymer classes, poly(ethylene glycol) and poly(methyl methacrylate), as they
are described in section 2.2.2. As a first step of optimization, different solvents were utilized for the printing of the already discussed five matrices (dithranol, DHB, IAA, HABA, CHCA). The solvents used up to now for matrix crystallization, i.e. chloroform or THF, are not compatible with the ink-jet printer since they evaporate too quickly. Generally, the rate of evaporation of the solvent used should be relatively low to prevent the solution from drying out and thereby clogging the nozzle of the ink-jet printer. A too low rate of evaporation on the other hand might have a negative impact on the matrix performance since solvent residues might be trapped in the sample. Therefore, solutions of five different matrices (CHCA, HABA, IAA, DHB and Dithranol) in different solvents (acetophenone, anisole, methylbenzoate and toluene) that can be used in the ink-jet printer were investigated. After printing these matrix solutions, optical microscopy pictures were taken revealing significant differences in spot shape as well as in continuity and crystallinity of the obtained matrix films. Figure 2.11 (left) clearly shows that certain matrix-solvent combinations can result in very defined spots (~180-200 µm in diameter, see Figure 2.11) with high homogeneity and crystallinity.

![Figure 2.11](left: optical microscopy pictures of ink-jet printed matrix solutions after drying. Right: corresponding relative signal intensities obtained from MALDI-TOFMS spectra with varying ink-jet printed sample preparation for a PEG 3 kDa and a PMMA 4 kDa standard.]

This qualitative result correlated with the observed spectrum quality for both a PEG (3 kDa) and a PMMA (4 kDa) polymer standard. Both polymers were printed as final layer on top of the matrix layer crystallized from different solvents and NaI (20 mg/mL water). Subsequently, MALDI-TOFMS spectra were measured with the same instrument settings for all 20 different sample preparations consisting of different matrix/solvent combinations (see Figure 2.11, left) for both polymers. These spectra were evaluated by means of signal intensity and signal-to-noise-ratio resulting in a preferred matrix-solvent combination for each polymer. Some of the results are visualized in Figure 2.11 (right). It is clearly visible that certain
combinations of solvents and matrices show a preference for a certain analyte. For instance toluene is a very bad solvent for crystallization of any matrix (see Figure 2.11, left) and therefore also the obtained results from MALDI-TOFMS measurements with matrices crystallized from toluene are poor (see Figure 2.11, right). On the other hand, acetophenone seems to be a good solvent for matrix crystallization since homogeneous as well as crystalline spots are observed for almost all matrices (see Figure 2.11, left). This behavior can be very well correlated to the relative signal intensities if this solvent is utilized for matrix crystallization (see Figure 2.11, right). In general it seems that the previous conclusion that high matrix crystallinity positively influences the MALDI spectra still holds if an ink-jet printer is used for sample preparation. Furthermore, optimized settings were obtained for MALDI-TOFMS multiple-layer sample preparation with ink-jet printing for the two investigated polymer classes: i) dithranol in acetophenone, NaI in water and PEG in acetophenone; ii) DHB in acetophenone, NaI in water and PMMA in acetophenone (the layers were printed in the given order). The case of poly(ethylene glycol) is very well comparable with results obtained from hand-spotted samples; the solvent had to be changed to acetophenone to enable ink-jet printing, but the preference of poly(ethylene glycol) for dithranol remained. In the case of PMMA, the matrix preference changed from dithranol to DHB for the ink-jet printed samples. This can be explained by the higher crystallinity of DHB if acetophenone is utilized for sample preparation instead of the volatile solvents (chloroform, dichloromethane, acetone, diethyl ether, THF and methanol) utilized for hand spotted samples. If DHB in acetophenone was utilized for hand-spotted sample preparation (2 µL matrix solution was applied to the target) evaporation of the solvent took more than 1.5 h. This resulted in poor spectrum quality as compared to a sample prepared with dithranol (in chloroform) as the matrix. This could be an effect of the uncontrolled crystallization as well as solvent residues in the sample. The ink-jet printed samples on the other hand evaporated relatively fast (~3-5 min) and highly controlled due to the very high surface-to-volume ratio. Therefore, it is understandable that the preferred matrix for hand-spotted samples is not necessarily the same as for ink-jet printed samples. To further investigate the usefulness of these optimized sample preparation techniques higher molecular weight polymers were investigated by MALDI-TOFMS. Figure 2.12 displays MALDI-TOFMS spectra of two PMMA standards with a molecular weight of 14 kDa (top) and 30 kDa (bottom) obtained with optimized setting for the ink-jet printing sample preparation technique, respectively. Moreover, applying the optimized settings for PEG it was possible to detect a 37 kDa PEG standard. Nevertheless, higher molar masses were not accessible with the described sample preparation techniques. Therefore, the multiple-layer ink-jet printing technique is an advanced and automated sample preparation method for MALDI-TOFMS of synthetic polymers, providing good analytic results and leading to the detection of high molecular weight polymers. An important advantage of multiple-layer sample preparation is the possibility to use immiscible solvents for the same reasons as described in previous sections.
MALDI-TOFMS as high-throughput screening tool

Furthermore, ink-jet printing gives access to higher boiling and slower evaporating solvents for sample preparation. This could be of advantage in the future for polymers that are poorly soluble in ‘standard’ solvents. Another advantage is the possibility to fully automate ink-jet printing by utilizing the macro language of the ink-jet printer and therefore its straightforward for integration into the workflow of combinatorial polymer research. Moreover, the small droplet sizes allow applications in UHTS applications due to the possibility to increase the number of samples per MALDI target by a factor of at least 40.

2.4 Application examples

The above described new multiple-layer spotting technique for MALDI-TOFMS of synthetic polymers found several application examples, including the monitoring of the reversible addition-fragmentation chain transfer polymerization of methyl methacrylate\textsuperscript{27} or of the cationic ring opening polymerizations of 2-nonyl-2-oxazoline.\textsuperscript{28} Moreover, the described technique could also be applied in an off-line fashion as standard characterization technique, providing useful insights in polymer molecular weight, architecture and composition.\textsuperscript{29-31} The following sections will address two application examples in order to discuss the advantages as well as limitations of the described techniques.
2.4.1 A library of poly(2-oxazoline)s

This section will focus on the example of a library of poly(2-oxazoline)s that was prepared utilizing a microwave reactor in an automated sequential fashion.

![Figure 2.13 Schematic representation of the investigated poly(2-oxazoline)s.](image)

The resulting library was investigated by MALDI-TOFMS utilizing the automated, robotic spotting technique as described in section 2.3.1 with DCTB as matrix for reasons described in section 2.2.2. The general structure of the investigated polymers based on the 2-nonyl, 2-phenyl, 2-ethyl and 2-methyl-2-oxazoline monomers is depicted in Figure 2.13. Different M/I ratios were investigated in order to prepare series of polyoxazoline polymers with different molecular weights for the four different 2-oxazoline monomers. These four polymer series were subsequently investigated utilizing the automated MALDI-TOFMS sample preparation (compare section 2.3.1) in order to obtain absolute molecular weight information and to avoid SEC calibration and solubility problems. Therefore, MALDI should be the method of choice, since it is an absolute analytical technique in terms of molar mass and molar mass distribution. However, it should be noted here that the investigated polymers need to fulfill certain criteria (most importantly a low polydispersity), since otherwise mass discrimination effects due to instrumental as well as sample preparation parameters will in most cases result in the calculation of too low molecular weights. Moreover, insolubility problems of poly(2-nonyl-2-oxazoline)s in an optimized SEC system with reduced column interactions (DMF with NH₄PF₆) or the tailing of poly(2-methyl-2-oxazoline)s in all available SEC setups (DMF with 5 mM NH₄PF₆ as well as CHCl₃ with 4% NEt₃ and 2% isopropanol) that try to keep column interactions at a minimum, can be avoided by utilizing MALDI-TOFMS. Due to the optimized multiple-layer sample preparation (with DCTB as matrix), it was possible to investigate poly(2-oxazoline)s with relatively high number averaged molecular weights by MALDI-TOF mass spectrometry. Figure 2.14 summarizes all MALDI-TOFMS results of these investigations. Generally, a linear correlation between observed and targeted molecular weights was obtained for all four polymer series indicating the livingness of the cationic ring opening polymerization technique. Moreover, Figure 2.14 (top-right) exemplarily illustrates some of the SEC problems with poly(2-ethyl-oxazoline) as example.
Figure 2.14 Dependence of the number averaged molecular weight ($M_n$) and PDI values on the initial [monomer]:[initiator] ratios for polymers derived from 2-methyl-, 2-ethyl-, 2-nonyl-, and 2-phenyl-2-oxazoline (analysis by MALDI-TOF mass spectrometry).

It is obvious that the utilized standards for the SEC calibrations were not suitable for an investigation of these polymers and that only MALDI-TOFMS was able to provide accurate molecular weight data. Similar effects were found for this and the other investigated polymers in both available SEC systems with all available polymer standards (PEG, PMMA, PS). Moreover, the number average molecular weights obtained by MALDI-TOFMS technique revealed a good agreement between theoretical and experimental data and the MALDI-TOFMS $M_n$ values correlated better than the values obtained by SEC (compare Figure 2.14, top-right and see Ref. [33] for details). This very good fit can mainly be attributed to the above described advantages of MALDI if compared to SEC and is a good indication that the performed polymerizations were living. However, the samples that could be analyzed by MALDI-TOF mass spectrometry were limited by the number averaged molecular weights ($M_n < 30$ kDa) and average molecular weight distributions (PDI < 1.30) as it is common for this technique.
Figure 2.15 MALDI-TOF mass spectra of a series of poly(2-nonyl-2-oxazoline)s composed of 5, 10, 15 and 25 monomers (left) as well as 51, 76, 101 and 152 monomers (right), respectively.

In general, all mass spectra showed the expected signal spacings for the respective monomers of the investigated poly(2-oxazoline)s (85 Da for 2-methyl-, 99 Da for 2-ethyl-, 147 Da for 2-phenyl- and 197 Da for 2-nonyl-2-oxazoline). As an example the MALDI-TOFMS spectra of a series of eight poly(2-nonyl-2-oxazoline)s with molecular weights ranging from 1.000 to 30.000 Dalton are depicted in Figure 2.15. This example clearly demonstrates the added value of fast MALDI-TOFMS investigations in CMR as they can provide accurate molecular weight data for a large variety of different polymers (e.g. different molecular weights and compositions).

2.4.1 Monitoring of RAFT polymerizations

Reversible addition-fragmentation chain transfer (RAFT) polymerizations of methyl methacrylate (MMA) were performed in an Accelerator SLT100 automated synthesizer at various conditions and were monitored by SEC and be the above described MALDI-TOFMS techniques. During these polymerizations, samples were taken for automated SEC as well as MALDI investigations. Figure 2.16 describes the important steps involved in a RAFT polymerization. Classical initiation is followed by an equilibrium step where radical addition to the thioester results in an intermediately radical species B, which can fragment either to the active chain radical A formed in the initiation step or to the radical obtained from the leaving group of the RAFT-agent D. This new radical species can subsequently react with the monomer to form a new propagating radical E. The equilibrium between the dormant polymeric RAFT species F and G and the P_n• and P_m• radicals is illustrated in step IV. The dithioester transfer between the active and dormant chains is important to retain the ‘controlled’ character of the polymerization. Termination via disproportionation or combination (step V) are always operative to some extent but can be largely eliminated by maintaining appropriate conditions that suppress the radical concentration.
**Figure 2.16** Schematic presentation of the reaction mechanism during Reversible Addition-Fragmentation Transfer (RAFT) polymerization of methyl methacrylate (MMA) using azoisobutyronitrile (AIBN) as initiator and 2-cyano-2-butyl dithiobenzoate (CBDB) as transfer agent.

Investigating different raft-agent to initiator ratios *in duplo* (4-fold) revealed an optimum in this ratio 1:0.25 regarding polymerization speed and obtained polydispersity indices (PDI < 1.2). Moreover, both MALDI-TOFMS and SEC revealed the reproducibility of these duplo reactions. Further investigation of these conditions by monitoring four reactions with the optimal conditions in time revealed a curved increase of molecular weight in time for both SEC and MALDI-TOFMS. An example of reaction monitoring in time by MALDI-TOFMS is shown in Figure 2.17. Ionization of higher molecular weight polymers proved to be difficult and not all molecular weights nor the corresponding molecular weight distribution could be calculated accurately. Therefore, peak molecular weights were utilized for the visualization of the polymer chain growth in time (see inset, Figure 2.17). However, it was possible to ionize macromolecules with a molecular weight higher than 10,000 Dalton. These results were highly comparable for all four reactions performed under equal conditions.
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Figure 2.17 MALDI-TOF mass spectra obtained from the reaction monitoring of automated raft polymerizations of MMA at different reaction times. The insert (right top) shows the peak molecular weight ($M_p$) (obtained from MALDI-TOFMS spectra) as function of the reaction time (Matrix: DCTB).

Subsequently, end group analysis of the observed species in MALDI (see Figure 2.18) revealed three distributions. Each distribution showed a spacing corresponding to the MMA monomer unit (100 Dalton). The two main distributions correspond to the double-bond terminated polymer chains, initiated with AIBN (Figure 2.18, structure C) or the leaving group of the RAFT-agent (Figure 2.18, structure A). The latter distribution (initiated with the leaving group of the RAFT-agent) shows the highest peak intensities in the MALDI TOFMS spectra as it was expected since the ratio of RAFT-agent to initiator was 1:0.25. Due to the excess of RAFT-agent compared to the initiator, more chains will be initiated with the leaving group of the RAFT-agent. The double bonded end groups could be ascribed to termination by disproportionation (e.g. species E of Figure 2.16 with itself), but this is in discrepancy with the increase in molecular weight in time (see Figure 2.17). Moreover, if the observed end groups originated from termination by disproportionation, MALDI-TOFMS spectra should also show the related hydrogen-terminated structures. Therefore, the observed double bond terminated polymer chains are most likely formed during the MALDI-TOFMS experiments as it was also reported previously in the literature.\textsuperscript{34,35} Beside these two main distributions a third distribution with lower signal intensity could be observed corresponding to the dormant polymer chains (RAFT terminated polymer chain initiated with the leaving group of the RAFT-agent, Figure 2.18, structure B).
Moreover, MALDI investigations were able to visualize automated chain extension experiments. In general, the results obtained from this MALDI monitoring experiments were highly comparable and provided additional proofs for the controllability and reproducibility of the investigated polymerization system. This example of high-throughput screening with MALDI-TOFMS nicely demonstrates that automated MALDI-TOFMS is a valuable tool for the investigation of polymerization kinetics and that even screening results can provide useful insights into the polymer structure.

2.5 Conclusion

In summary, this chapter describes the development, evaluation and application of a new sample preparation technique for MALDI-TOFMS of synthetic polymers. The investigated multiple-layer spotting technique generally provided good to excellent analytic results for a large variety of different synthetic polymers. Straightforward optimization procedures revealed preferred matrix/solvent combinations for the sample preparation leading to improved analytical results if combinations leading to good matrix crystallinity were chosen. Therefore, the free choice of solvents due to a decoupled handing of matrix, dopant and analyze solutions was identified as key advantage with respect to the quality of the obtained
MALDI spectra. Moreover, especially the analysis of higher molecular weight polymers became feasible utilizing this approach. The described technique could be automated and integrated into the workflow of combinatorial materials research due to its ease of application offering the possibilities of online reaction monitoring, fast and automated library characterization as well as possibilities for UHTS environments. On the one hand it was possible to develop an automated spotting procedure utilizing automated synthesizer robots and on the other hand the technique could be automated and further miniaturized by the application of an ink-jet printer. These techniques were subsequently applied as standard techniques in our laboratories for the investigation and high-throughput screening of a large variety of different polymer libraries and polymerizations.

The last section of this chapter describes the screening of a library of poly(2-oxazoline)s as well as the online monitoring of a controlled RAFT polymerization as examples, revealing the usefulness of the described techniques and that high-throughput screening results are not necessarily of bad quality.

2.6 Experimental

Chemicals and Reagents
Matrices and inorganic salts were purchased from Sigma Aldrich (Oakville, On, Canada). The investigated matrices were: trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB), α-cyano-4-hydroxycinnamic acid (CHCA), 2,-(4-hydroxy-phenylazo) benzoic acid (HABA), trans-3-indoleacrylic acid (IAA), 2,5-dihydroxybenzoic acid (DHB), and 1,8,9-anthracenetriol (Dithranol). Analytical grade solvents were purchased from Biosolve LTD (Valkenswaard, The Netherlands). Narrowly distributed poly(ethylene glycol), poly(methyl methacrylate) and poly(styrene) standards were obtained from Polymer Standards Service GmbH (PSS, Mainz, Germany) and from Polymer Source (Polymer Source Inc., Dorval, Canada).

Instrumentation
All MALDI experiments were carried out on Voyager-DE™ PRO and STR Biospectrometry™ Workstations (Applied Biosystems, Foster City, CA, USA) time-of-flight mass spectrometers. All spectra were obtained in positive ion mode using linear mode for operation. Ionization was performed with a 337 nm pulsed nitrogen laser. All data were processed using the Data Explorer™ software package (Applied Biosystems, Foster City, CA, USA).

MALDI samples were prepared using a multiple-layer spotting approach by depositing the three components of a MALDI sample (matrix, doping salt and analyte) sequentially on top of each other. Care was taken that the previous layers were dried completely, before the next
layer was applied. Moreover, for all techniques (hand spotting or automated spotting) all matrix solutions were prepared freshly prior to the experiments since some matrices are known to degrade in time.

The automated spotting was carried out on a Chemspeed ASW2000 (Chemspeed Technologies, Augst, Switzerland) automated synthesizer. For the spotting a needle with 0.8 mm diameter was utilized in combination with a custom made MALDI target holder in a microtiter plate format (now commercially available from Chemspeed). The sample positions of the MALDI target were programmed on a $xyz$ basis in the Chemspeed software (Gilson 735 Sampler Software V 2.10.). For the spotting, 1 µL aliquots from stock solutions or directly from the reactors were deposited by the liquid handling system of the robot.

Ink-jet printing was carried out using the AutoDrop drop-on-demand printer (microdrop Technologies, Norderstedt, Germany). The setup consists of a MD-P-705-L $xyz$-stage, on which a holder for the print head is mounted. An AD-K-501 micropipette was used as print head (nozzle diameter 70 µm). This micropipette can aspirate a total sample volume of 25 µL by applying a slight underpressure. Ink-jet printing of the MALDI samples was carried out as follows. First all solutions were filtered over a 5 µm dust filter (Schleicher & Schuell, Düren, Germany) and filled into a 96-well glass microtiter plate. Subsequently, the matrix was printed on the MALDI-target. Dots consisting of 5 drops of matrix solution were printed at regular intervals of 0.5 mm. MALDI samples consisted of 16 dots each, grouped into a 4×4 array. The distance between the arrays was 5 mm. After drying, the matrix layer was covered with a doping agent layer, which was printed in exactly the same way as the matrix. Finally, the analyte solution was printed on top of the two previous layers. For cleaning, the micropipette was rinsed one time with the corresponding solvent, and then 2 times with acetone to remove the solvent. Finally air was pumped through the micropipette for 20 seconds to remove the acetone.

The poly(2-oxazoline)s were prepared as described in the literature by Dr. R. Hoogenboom and Dr. F. Wiesbrock. DCTB (10 mg/mL) in dichloromethane, NaI (saturated in acetone) and the polymers in dichloromethane (10 mg/mL) were spotted on top of each other by an ASW 2000 automated synthesizer robot to prepare the MALDI-TOF samples.

The RAFT polymerizations were performed as described in the literature by Ing. Martin Fijten utilizing a Chemspeed Accelerator™ SLT100 automated synthesizer. During the polymerization, samples were taken to 1 mL vials that were prefilled with chloroform. These samples were then used for an automated spotting onto a MALDI-TOFMS target utilizing DCTB (10 mg/mL in THF) as matrix and NaI (saturated in acetone) as cationizing salt.
2.7 References


CHAPTER 3

PEO-b-PCL block copolymers with different architectures

Abstract
This chapter describes the (parallel) synthesis of block copolymers of poly(ethylene oxide) and poly(ε-caprolactone) by applying the controlled ring-opening polymerization of ε-caprolactone with PEO macroinitiators. Block copolymers with different architectures were prepared, characterized in detail and subsequently investigated for their micellar properties. The capability of the micelles obtained from these biocompatible block copolymers to encapsulate guest molecules was then investigated applying high-throughput screening techniques. For the linear block copolymers a structure-property relationship between the guest encapsulation capability of their micelles and the guest hydrophilicity was found. Moreover, the star-shaped block copolymers acted as unimolecular reversed micelles in different solvents. The unimolecular micellar behavior was investigated by dynamic light scattering, parallel guest extraction studies as well as by analytical ultracentrifugation experiments revealing that these unimolecular micelles can encapsulate a large variety of different guest molecules and that they can transport these molecules. In addition, the capability of these unimolecular micelles to template the formation of palladium nanoparticles was investigated showing that stabilized and small nanoparticles could be formed only in the presence of these templates. Moreover, the catalytic behavior of these palladium nanoparticles for Heck cross-coupling reactions was investigated applying parallel and automated techniques. Finally, the synthesis and characterization of four and six armed PCLs and their possible use as building blocks for novel polymeric core-shell architectures is discussed.

3.1 Introduction

Block copolymers are an interesting class of materials since their applications are manifold and reach from the compatibilization of immiscible and/or incompatible polymer blends\textsuperscript{1,2} for commodity products to high tech applications in medicine\textsuperscript{3} or micellar catalysis.\textsuperscript{4} However, all these applications take advantage of the specific chemical and physical properties of block copolymers, e.g. their amphiphilicity and the resulting phase separation behavior. The formation of micelles from amphiphilic block copolymers in a selective solvent due to the good solubility of one and the poor solubility of the other block in the selected solvent\textsuperscript{5} is of particular interest since the resulting nanometer sized micelles are for instance able to solubilize (encapsulate) hydrophobic substances\textsuperscript{5-8} that are otherwise insoluble in an aqueous environment if water is utilized as the selective solvent. This behavior is not only used in every day products, such as non-ionic detergents, but utilizing this approach also novel high-tech applications are being developed. One of these applications that also takes advantage of the possibility to design the size, encapsulation behavior and other parameters is the development of drug carriers that might release their active components due to external stimuli, such as pH\textsuperscript{6} or temperature.\textsuperscript{7} For such micellar drug carriers based on block copolymers the biocompatibility as well as the biodegradability of the polymeric building blocks are of essential importance. Therefore, the widely studied and applied block copolymer system of poly(ethylene oxide) (PEO) and poly(\varepsilon-caprolactone) (PCL) is of special interest since PEO is biocompatible and offers a ‘stealth’ behavior \textit{in vivo} due to its ability to minimize cell and protein interactions,\textsuperscript{9-12} whereas PCL is both biocompatible and biodegradable due to the enzymatic and/or pH dependant breakdown of ester bonds.\textsuperscript{13,14} It was for instance shown that micelles prepared from PEO-\textit{b}-PCL polymers are internalized by cells and mainly distributed to the cytoplasm but not to the nucleic department.\textsuperscript{3} Furthermore, it has been shown that micelles assembled from PEO-\textit{b}-PCL polymers are carriers for a variety of different drugs\textsuperscript{15-17} and, for instance, that it was possible to obtain a slow and steady release of dihydrotestosterone that continued over a one-month period.\textsuperscript{17} On the other hand unimolecular core-shell architectures (unimolecular micelles) have gained large attraction during the last years because of their manifold application possibilities in fields as diverse as drug delivery,\textsuperscript{18,19} stimuli responsive release,\textsuperscript{20} catalysis\textsuperscript{21,22} or phase transfer.\textsuperscript{23-25} Compared to micelles formed from aggregates of amphiphilic molecules, core-shell dendrimers or polymeric unimolecular micelles offer a higher stability in solution since these unimolecular micelles contain covalently fixed branching points. Therefore, no dynamic equilibrium between the individual amphiphile and the self-assembled micellar structure can occur.\textsuperscript{26,27} Dendritic naonocarriers for the above mentioned applications are monodisperse organic compounds and offer precise control over the chemical structure. However, polymeric systems with core-shell architecture can offer similar properties and do not require a complex multistep synthesis and are therefore accessible in larger scale. For drug delivery applications
dendritic macromolecules might have advantages concerning, for instance, the solubilization of non-soluble drugs, which is one of the current problems with the delivery of low molecular weight drugs, or the reduction of toxic effects. This chapter describes the synthesis, characterization as well as encapsulation behavior of micelles and unimolecular reversed micelles based on PEO and PCL. Moreover, the ability to stabilize metal-nanoparticles by star-shaped block copolymers and an application of such nanoparticles in catalysis is described. Finally, an outlook towards new star-shaped block copolymer systems is addressed by discussing the synthesis of 4- and 6-arm PCL star-shaped polymers. All described polymeric materials were obtained by utilizing alcohol functional co-initiators for the stannous octoate (Tin(II) 2-ethylhexanoate, Sn(Oct)$_2$) catalyzed controlled ring-opening polymerization of ε-caprolactone. Although this synthetic method is already applied for the preparation of defined block- or star-polymers since a long time (see e.g. Ref. [32] for an early example) its mechanism was heavily debated for the last three decades and basically every imaginable mechanism was proposed, including e.g. a cationic mechanism. The two major discussed mechanisms were the activated monomer and the coordination-insertion mechanism. Both mechanisms were thought to be alcohol initiated, since the degree of polymerization is clearly dependant on the monomer to alcohol ratio. In the course of the activated monomer mechanism a donor-acceptor complex would be formed between Sn(Oct)$_2$ and the monomer, thereby activating the monomer for a nucleophilic alcohol attack. Subsequently, the OH end groups of the resulting macromolecules would reinitiate the polymerization and chain growth would proceed. In the nowadays accepted coordination-insertion mechanism an alcohol functionality can react in a rapid equilibrium with Sn(Oct)$_2$ to form an alkoxide, covalently bound to tin (compare Figure 3.1), which is most likely the actual initiator.

\[
\text{Sn(Oct)$_2$} + \text{HO-R} \leftrightarrow \text{Oct-Sn-O-R} + \text{Sn(Oct)$_2$-alkoxide}
\]

**Figure 3.1 Rapid equilibrium between Sn(Oct)$_2$ and an alcohol to form tin-alkoxides.**

The formation of these tin-alkoxide complexes could, for instance, be confirmed by MALDI-TOFMS and the liberation of octanoic acid could be confirmed by $^{13}$C NMR supporting this mechanism. Subsequently, the polymerization proceeds via acyl-oxygen cleavage of the lactone with insertion of the monomer into the metal-oxygen bond of the initiator. A schematic representation of this mechanism is depicted in Figure 3.2. Moreover, other experimental findings, such as an increased polymerization rate upon addition of alcohol or the decreased polymerization rate upon addition of carboxylic acid to Sn(Oct)$_2$ are in good agreement with this mechanism. The propagation of the polymerization can then proceed...
with the newly formed tin alkoxide (Figure 3.2, right) by the coordination of a new monomer and subsequent ring-opening until all monomer is consumed. This coordination-insertion mechanism is not only supported by experimental findings as described above, but could also be confirmed by a modeling study.40

![Figure 3.2 A Tin-alkoxide formed from Sn(Oct), and the co-initiating alcohol (compare Figure 3.1) initiates the polymerization of ε-caprolactone.](image)

### 3.2 Linear PEO-\textit{b}-PCL block copolymers

The PEO-\textit{b}-PCL block copolymer system is widely studied for applications in drug delivery due to the biocompatibility of both building blocks. Recent examples have for instance shown that micelles of these block copolymers can penetrate cell walls (and therefore deliver active compounds into the cell) and are subsequently distributed in the cell plasma, but not in the nucleus, thereby demonstrating that such systems are capable of targeting certain domains \textit{in vivo}.3 The following sections describe the synthesis and detailed characterization of this block copolymer system, their micelle formation as well as the investigation of the encapsulation behavior of the obtained micelles with different guest molecules applying a novel high-throughput screening assay.

#### 3.2.1 Synthesis and characterization

Diblock copolymers of poly(ethylene glycol) PEO and poly(\(\varepsilon\)-caprolactone) PCL were synthesized utilizing a \(\alpha\)-methoxy-\(\omega\)-hydroxy-poly(ethylene glycol) polymer as macroinitiator for the controlled ring-opening polymerization of the second \(\varepsilon\)-caprolactone block in the presence of stannous octoate as catalyst. Four different poly(ethylene glycol)-\textit{b}-poly(\(\varepsilon\)-caprolactone) block copolymers (compare Figure 3.4B for a chemical structure) were synthesized with different monomer to initiator (M/I) ratios resulting in four different block copolymers with varying length of the PCL block. The series of PEO-\textit{b}-PCL block
copolymers was analyzed by $^1$H NMR, SEC as well as MALDI-TOFMS. Table 3.1 summarizes the obtained results. For all block copolymers, a unimodal molecular weight distribution was observed in the SEC chromatograms (compare Figure 3.3, left) suggesting complete initiation of the macroinitiator. However, block copolymer analysis by means of SEC without molar mass sensitive detector is difficult since usually no suitable standards are available. Therefore, only the combination of SEC with other techniques will lead to a profound understanding of the investigated polymers. $^1$H NMR was used to calculate number averaged molecular weights ($M_n$) by analyzing the integral ratios of protons belonging to the PEO and PCL repeat units according to peak assignments described in the literature (see e.g. Ref. [41]). The resulting $M_n$ values are reported in Table 3.1 revealing a good agreement with theoretically expected molecular weights.

**Table 3.1** Analytical data obtained from various techniques for a series of four linear PEO-b-PCL block copolymers (molecular weights are reported in g/mol).

<table>
<thead>
<tr>
<th></th>
<th>M/I</th>
<th>$M_n$ (theor.)</th>
<th>$M_n$ (SEC)</th>
<th>PDI (SEC)</th>
<th>$M_n$ ($^1$H NMR)</th>
<th>$M_n$ (MALDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO</td>
<td>0</td>
<td>--</td>
<td>2.670</td>
<td>1.05</td>
<td>--</td>
<td>2.650</td>
</tr>
<tr>
<td>P1</td>
<td>9.4</td>
<td>3.720</td>
<td>3.940</td>
<td>1.10</td>
<td>3.790</td>
<td>4.070</td>
</tr>
<tr>
<td>P2</td>
<td>18.5</td>
<td>4.767</td>
<td>4.780</td>
<td>1.13</td>
<td>4.870</td>
<td>4.950</td>
</tr>
<tr>
<td>P3</td>
<td>27.9</td>
<td>5.837</td>
<td>5.780</td>
<td>1.11</td>
<td>6.020</td>
<td>5.950</td>
</tr>
<tr>
<td>P4</td>
<td>37.5</td>
<td>6.682</td>
<td>6.680</td>
<td>1.24</td>
<td>7.210</td>
<td>6.950</td>
</tr>
</tbody>
</table>

Moreover, Table 3.1 and Figure 3.3 (left) demonstrate that narrowly distributed block copolymer with low polydispersity indices were obtained. Therefore, the combination of $^1$H NMR and SEC results allow the conclusion that the polymerization of $\varepsilon$-caprolactone was indeed controlled.

**Figure 3.3** Left: SEC traces of the prepared PEO-b-PCL block copolymers. Right: dependence of the number averaged molecular weight ($M_n$) on the initial monomer ($M$) to initiator ($I$) ratio for polymers P1-P4. The MALDI result of polymer P4 is presented in brackets since it was only possible to determine the peak maximum molecular mass for the polymer by means of MALDI-TOFMS.
Moreover, Figure 3.3 (right) reveals a linear correlation between targeted and obtained $M_n$ values for SEC, $^1$H NMR as well as MALDI-TOFMS thereby proving the control over the polymerization procedure. Especially MALDI-TOFMS, an absolute analytical technique in terms of molar mass determination, proved to be a very useful tool for the characterization of the obtained block copolymers. Only a few examples are known from literature (see e.g. Refs. [42, 43]) where MALDI could be applied for the analysis of copolymers since the interpretation of copolymer MALDI spectra can be very difficult due to the presence of two distributions, namely a molecular weight and a chemical composition distribution. Nevertheless, it was possible to investigate the synthesized block copolymers by MALDI-TOFMS utilizing a recently developed multiple layer spotting technique (compare chapter 2) in combination with a program written in Visual Basic 6.0\textsuperscript{44} resulting in a full copolymer characterization for polymer $\textbf{P1}$ as well as a good understanding of the other block copolymers. Figure 3.4A displays the MALDI-TOFMS spectra of the PEO macroinitiator as well as polymer $\textbf{P1}$. It is clearly visible that the whole distribution of polymer $\textbf{P1}$ is shifted to a higher molecular weight if compared to the macroinitiator and moreover how complicated the spectrum of polymer $\textbf{P1}$ becomes due to the additional presence of the distribution in its chemical composition. Whereas the macroinitiator only shows masses that are spaced according to PEO repeat units, the spectrum of polymer $\textbf{P1}$ reveals spacings according to both PEO and PCL repeat units. Figure 3.4B shows an enlarged view of the spectrum of polymer $\textbf{P1}$. Figure 3.4B, top does indeed reveal the repeat unit spacings of PEO (44 Da) as well as of PCL (114 Da) as indicated by the arrows. Moreover, Figure 3.4B, bottom displays the calculated isotopic pattern of the depicted species, which can also be found back in the actual spectrum of polymer $\textbf{P1}$, thereby proving its structure. Furthermore, it was possible to calculate a complete copolymer fingerprint of polymer $\textbf{P1}$ (for details see Ref. [44]). Figure 3.4C shows this fingerprint clearly revealing the maximum of both the PEO and PCL repeat units. The PEO block shows its maximum at around 65 repeat units as it was already seen for the PEO macroinitiator (see Figures 3.4A, 3.4C and 3.4D). Therefore, one can conclude that the PEO was unchanged during the polymerization and that the PCL block just added to the PEO block. The maximum in the distribution of the $\varepsilon$-caprolactone units of the PCL blocks can be found at approximately 9 units (see Figures 3.4C and 3.4D) and is in good agreement with both $^1$H NMR and SEC results. Unfortunately, it was not possible to obtain similar fingerprints for polymers $\textbf{P2-P4}$ since the spectral quality decreased with increasing molecular weight. Nevertheless, the spectral quality was good and end-group calculations as shown in Figure 3.4B were possible for polymers $\textbf{P2}$ and $\textbf{P3}$. Only polymer $\textbf{P4}$ revealed a too high molecular weight for end-group calculations to be feasible and only the peak maximum of this polymer could be determined by means of MALDI-TOFMS. However, MALDI-TOFMS was able to provide very useful information concerning the quality of the obtained polymers as well as their absolute molecular weights. Figure 3.3, right also depicts the molecular weight obtained by MALDI providing additional proof that the polymerization of $\varepsilon$-caprolactone utilizing a PEO macroinitiator was controlled.
Figure 3.4 MALDI-TOFMS analysis of polymer P1 including end-group analysis. A: MALDI-TOFMS spectra of the PEO macroinitiator and polymer P1. B: comparison of the theoretical (bottom) with the experimentally obtained (top) isotopic pattern of the depicted polymeric structure with 68 PEO and 9 PCL repeat units. C: MALDI-TOFMS copolymer fingerprint for polymer P1. D: cross-sections of the MALDI-TOFMS copolymer fingerprint for polymer P1. Matrix: dithranol.
In summary, all obtained analytical results are fitting perfectly together and one can conclude that a defined set of polymers with the targeted molecular weights was obtained. Moreover, especially MALDI-TOFMS proved to be a very useful tool for the characterization the described block copolymers providing detailed insights in their structure and composition.

3.2.2 Guest encapsulation screening

The obtained block copolymers were investigated for their micellar behavior, in particular the ability of the micelles to encapsulate guest molecules. It is known that PEO-b-PCL block copolymers form micelles in water and that the size of the obtained micelles is strongly depending on their preparation method. Here, it was chosen to prepare micelles starting from a THF solution of the polymer that was subsequently dropwise diluted with deionized water to finally result in micellar solutions with a 0.5 wt% concentration of polymer. This procedure starts from molecularly dissolved polymer chains in the good solvent THF. The subsequent addition of water (a non-solvent for PCL) results in a continuous decrease in the solubility of the PCL blocks and induces its self-assembly, finally leading to the micellar structure. Generally, amphiphilic block copolymers are also capable of vesicle formation, however such bilayer aggregates are only favorable for block copolymers with a (very) low content of the hydrophilic block. The above described method resulted in defined micelles with reproducible sizes for polymers P2-P4. However, polymer P1 tended to form large aggregates in the order of >350 nm as revealed by DLS. This is an effect of the short PCL block that is not prone to fast precipitation upon water addition and therefore favors the formation of larger aggregates. In contrast, polymers P2-P4 reproducibly formed defined micelles with a size in the range of 20-25 nm. However, no clear structure-property relationship concerning the micellar size and the block copolymer composition was found for these samples. Similar findings with no clear correlation between micelle size and block length were reported for the studied block copolymer system in the literature. Subsequently, the encapsulation behavior of these micelles (compare Figure 3.5) was investigated by performing a high-throughput screening experiment with 12 different potential guest molecules. A similar screening, investigating star-shaped block copolymers, will be discussed in detail in section 3.3.3. This screening was performed as a feasibility study and to carefully evaluate this new kind of high-throughput screening approach revealing, e.g., that no false positives were identified during the screening. Here, a similar approach for the evaluation of the micellar encapsulation capabilities of block copolymer micelles is described. The screening was performed in a microtiter plate format investigating the potential guest molecules in the presence and absence of micelles. An acidic as well as basic aqueous environment was chosen for this experiment to ensure that changes in the microenvironment of the investigated guest molecules could be detected even for pH responsive guest molecules. In general, the encapsulation of a guest molecule within a host can be probed by the evaluation of changes in the guests’ microenvironment with techniques such as UV/Vis- and
fluorescence spectroscopy, NMR spectroscopy, or others. Therefore, it is necessary to provide different microenvironments in order to detect changes of the guest spectroscopic behavior upon encapsulation as it will be discussed in more detail in section 3.3.3.

**Figure 3.5** Schematic representation of the encapsulation of small guest molecules in the core of polymeric micelles.

Figure 3.6 displays the layout of this experiment as well as an image of the resulting microtiter plate. Figure 3.7 (top) shows the investigated guest molecules (consult experimental section for the chemical names of the investigated guest molecules). The detailed preparation procedure for the layout of the screening as depicted in Figure 3.6 (left) is explained in the experimental part of this section. An image of the microtiter plate as it was used for the screening experiment is shown in Figure 3.6 (right).

**Figure 3.6** A schematic representation (left) of the layout that was applied for the high-throughput screening of the guest encapsulation of micelles formed from polymer P3 as well as an image of the resulting microtiter plate (right) used to screen for guest encapsulation within polymeric micelles.

Already a visual inspection can reveal that some of the molecules depicted in Figure 3.7 (top) are encapsulated by the micelles formed from block copolymer P3. For instance, guest 4 in the acidic environment shows a color change from brownish (positions A4 and E4, compare Figure 3.6) to yellow (positions B4 and F4, compare Figure 3.6) in the presence of micelles, which is indicative for an encapsulation event. Figure 3.7 (bottom-right) shows the
encapsulation of guest 3 in the basic environment. A significant change in the spectral behavior accompanied with a precipitation of the guest without the micelles in the water phase let to the conclusion that guest 3 is indeed encapsulated in the core of the polymeric micelles.

Figure 3.7 Top: Chemical structure as well as number code of the investigated potential guest molecules. Bottom-left: encapsulation of guest 3 by polymeric micelles visualized by UV/Vis spectroscopy as well as a macroscopic precipitation (see inset). Bottom-right: encapsulation of guest 6 by polymeric micelles visualized by fluorescence.

Furthermore, encapsulation could be detected due to the increase of the fluorescence intensity of encapsulated guest molecules (e.g. guest 6, see Figure 3.7, bottom-left). The final
assessment of all guest molecules regarding their encapsulation behavior is given in Table 3.2 revealing that 8 of the 12 investigated molecules did encapsulate in the core of the polymeric micelles. The fact that the eight guest molecules that did encapsulate also revealed a macroscopic precipitation in water without the presence of micelles and the other four investigated potential guests did not precipitate, is a good indication that hydrophobicity is the key requirement for a guest molecule to be encapsulated in the core of these polymeric micelles.

Table 3.2: Final assessment of the encapsulation of different guest molecules into PEO-b-PCL block copolymer micelles. (+) guest can be encapsulated (-) guest cannot be encapsulated

<table>
<thead>
<tr>
<th>Guest</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulated (+/-)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Subsequently, in order to show that an encapsulated guest molecule can be transported within the micelles, an analytical ultracentrifugation experiment was performed on micelles formed from P3 and loaded with guest 8. The fact that the sedimenting micelles could be detected by the absorption scans at 262 nm undoubtedly proves that guest 8 has to be encapsulated and transported together with the micelles since the investigated block copolymers are not UV light absorbing. Moreover, the experiment revealed that approximately 100 block copolymer molecules are associated to one micellar aggregate. Such a transportation of guest molecules is of course necessary if such micelles are to be used as drug delivery agents. In conclusion, the described results demonstrate that it is feasible to investigate the encapsulation of guest molecules with block copolymer micelles in an accelerated fashion. Moreover, such screening approaches might be interesting for pharmaceutical applications.

3.3 Star-shaped PEO-b-PCL block copolymers

Star polymers can be defined as polymers with a defined number of arms that are linked together via a low molecular weight core molecule. One of the characteristics of such polymers is their smaller hydrodynamic volume in solution if compared to linear analogues. This peculiar behavior results in interesting physical properties including e.g. lower crystallinity and lower melt viscosity (see e.g. Ref. [49]) when compared to the corresponding linear systems. Furthermore, the higher end-group functionality of star-shaped macromolecules compared to linear polymers of the same molecular weight offers the possibility of covalent attachment of functional groups and/or other polymers to a higher extent.
3.2.1 Synthesis and characterization

Five arm star-shaped block copolymers were prepared in an automated synthesizer to obtain core-shell like reversed unimolecular micelles with PEO inner and PCL outer blocks (see Figure 3.8).

![Figure 3.8 Structure of the investigated core-shell nanocarriers consisting of a poly(ethylene oxide) core and a poly(ε-caprolactone) shell.](image)

Therefore, a five arm star-shaped poly(ethylene) glycol macroinitiator (prepared by anionic polymerization of ethylene oxide utilizing diethylenetriamine as initiator) was utilized for the controlled ring-opening polymerization of ε-caprolactone. However, before actually carrying out the parallel polymerizations the structure of the utilized 5-arm star shaped macroinitiator was confirmed by MALDI-TOFMS. Figure 3.9 shows the respective MALDI-TOFMS spectrum with the assigned structure as well as the expected ethylene oxide repeat units of 44 Da. The absolute number averaged molecular weight (M_n) of this MALDI measurement (M_n = 2150 g/mol) was utilized for the calculation of all monomer/initiator ratios. Now having a strong indication that the structure of the 5-arm initiator is correct, the controlled polymerization of ε-caprolactone was performed on the ASW2000 automated synthesizer in order to investigate the controllability as well as reproducibility of the polymerization technique in the robot system. All polymerizations were performed in 13 mL reactors under a double inert atmosphere at 130 °C in 2.5 mL of ε-caprolactone monomer utilizing the 5-arm star-shaped poly(ethylene glycol) macroinitiator and 1/20th of tin catalyst (according to –OH functional groups). Monomer/Initiator ratios were adjusted and varied to target for different molecular weights. The initiator as well as the catalyst were transferred as stock solutions (in dichloromethane) to the reactors by the liquid handling system of the robot. After the automated evaporation of the transfer solvent, the monomer was added and the bulk polymerization was performed at 130 °C. This method of automated solvent evaporation actually allows the transfer of solids, even if the robotic synthesizer does not support this feature by specially designed hardware, such as the Chemspeed Accelerator™.
First, the reproducibility of the applied automated polymerization technique was investigated by a set of two fourfold experiments with two different targeted molecular weights. The first set with a monomer/initiator ratio of 45 showed only slight variations in the obtained molecular weights and polydispersity indices (PDIs) (both less than 5%) obtained by size exclusion chromatography (SEC). Moreover, the second set of reproducibility experiments with a targeted degree of polymerization (DP) of 60 revealed even lower variations of less than 2.5% for the obtained molecular weights as well as the PDI values. Considering the error of SEC measurements of typically 5% one can conclude that the automatically conducted polymerizations were highly reproducible. Moreover, the control over the parallel polymerization technique can nicely be demonstrated by the fact that it was possible to target for certain degrees of polymerization. Figure 3.10 (left) depicts size exclusion chromatograms of a series of six star-shaped block copolymers with different molecular weights revealing unimodal molecular weight distributions and moderate polydispersity indices (PDIs) of approximately 1.4 as it can be expected if polymeric materials (5-arm PEO-initiator: \( M_n(MALDI) = 2.150 \text{ g/mol; PDI(MALDI) = 1.1} \) are utilized as initiators. The number averaged molecular weight obtained by SEC is increasing with increasing monomer/initiator (M/I) ratio in a linear fashion for \( P5-P10 \) (see Figure 3.10, right) as it can be expected for a controlled polymerization technique. It is known that the hydrodynamic volume of star-shaped polymers is almost independent of their number of arms (if all arms bear the same molecular weight) and that therefore the resolution of the separation of these star-shaped
polymers by SEC is rather limited.\textsuperscript{50} However, if the number of arms is constant for the investigated system, it is possible to utilize SEC measurements for the comparison of molecular weights of a series of star-shaped polymers and even to obtain linear correlations between targeted and observed molecular weights.\textsuperscript{51} Furthermore, one could expect an underestimated molecular weight from SEC measurements due to a smaller hydrodynamic volume of star-shaped macromolecules if compared to their linear analogues.\textsuperscript{52,53} However, here higher molecular weights than expected were obtained. This effect can be attributed to the nature of the PCL blocks in the polymers. For instance, linear PCL homopolymers consistently show higher molecular weights (factor 1.8 to 1.9 relative to the PS calibration) from SEC measurements on the utilized chloroform based SEC system if compared to MALDI-TOFMS results.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_10.png}
\caption{Left: SEC traces of the star-shaped block copolymers P5-P10 with PEO core and PCL shell. Right: dependence of the number averaged molecular weight (M_n) on the initial monomer (M) to initiator (I) ratio for polymers P5-P10.}
\end{figure}

Despite all these difficulties in utilizing SEC in order to access the molecular weight of star-shaped block copolymers, SEC was able to reveal narrow, monomodal molecular weight distributions for all investigated polymers in an automated and therefore convenient way for combinatorial material research (CMR) purposes. Moreover, the advantages of star-shaped block copolymers over similar dendrimer systems can be demonstrated by the fact that upscaling of the described reaction procedure to multi-gram scale was possible resulting in a defined polymer in quantitative yield (P11: M_\text{n}(SEC) = 10.550 \text{ g/mol}; PDI(SEC) = 1.42; yield = 33 \text{ g}).\textsuperscript{54} Before a detailed investigation of polymers P5-P10 was performed by \textsuperscript{1}H NMR as well as MALDI-TOFMS, the materials were analyzed by a FT-IR plate reader setup. This allowed the fast evaluation of copolymer compositions by first normalizing the FT-IR spectra to the C=O stretch vibration at 1735 cm\textsuperscript{-1} (significant for the PCL block; see Figure 3.11, left). Subsequently, three signals could be used to analyze the composition of the block copolymers P5-P10 being the C-O stretch vibrations of the PEO monomer units at 1105 cm\textsuperscript{-1},
the C-H vibrations at 2940 and 2870 cm\(^{-1}\) resulting from both monomer units and the broad signal at 3500 cm\(^{-1}\) resulting from the R-OH end-groups of the polymers.\(^{55}\) The normalized FT-IR spectra of polymers P5-P10 are shown in Figure 3.11 (left) and reveal a decrease of all three described signals with increasing molecular weight of the polymers. Therefore, the block copolymer composition changed with the M/I ratio as expected. Moreover, \(^1\)H NMR revealed co-monomer ratios as it was expected from the initiator and monomer feed ratios for polymers P5-P10. The number-averaged molecular weight as obtained by \(^1\)H NMR analysis of the investigated polymers as well as additional analytical data is summarized in Table 3.3.

**Figure 3.11** Left: Normalized FT-IR spectra of star-shaped block copolymers P5-P10. Right: \(^1\)H NMR spectrum of P7 in CDCl\(_3\) with peak assignment. Peak integral values: a: 18H, a’: 9H, b: 200H, c: 10H, d: 104H, e: 211H, f: 107H, g: 96 H.

**Table 3.3** Monomer/Initiator (M/I) ratio, \(M_n\) (obtained by \(^1\)H NMR) as well as PDI\(_{\text{SEC}}\) (obtained by SEC) of the investigated polymers. The hydrodynamic diameter \(D_h\) was measured by dynamic light scattering (DLS) in CHCl\(_3\) from data extrapolated to zero concentration (molecular weights are reported in g/mol).

<table>
<thead>
<tr>
<th></th>
<th>M/I</th>
<th>(M_n) (theor.)</th>
<th>(M_n) ((^1)H NMR)</th>
<th>PDI (SEC)</th>
<th>(D_h) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5</td>
<td>15</td>
<td>3.860</td>
<td>4.200</td>
<td>1.25</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td>P6</td>
<td>30</td>
<td>5.575</td>
<td>6.350</td>
<td>1.37</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td>P7</td>
<td>45</td>
<td>7.285</td>
<td>8.200</td>
<td>1.35</td>
<td>5.1±1.1</td>
</tr>
<tr>
<td>P8</td>
<td>60</td>
<td>9.000</td>
<td>9.600</td>
<td>1.45</td>
<td>6.0±0.9</td>
</tr>
<tr>
<td>P9</td>
<td>75</td>
<td>10.710</td>
<td>11.200</td>
<td>1.36</td>
<td>6.8±0.7</td>
</tr>
<tr>
<td>P10</td>
<td>90</td>
<td>12.425</td>
<td>13.100</td>
<td>1.44</td>
<td>8.1±1.0</td>
</tr>
</tbody>
</table>

Figure 3.11 (right) shows the \(^1\)H NMR of P7 in CDCl\(_3\) with assigned signals for one of the five polymer arms. All peak integrals revealed values as expected for the polymeric structure provided in Figure 3.8. The integral values of signals c and f were used to calculate \(M_n\) values for polymers P5-P10 as they are reported in Table 3.3. Furthermore, \(^1\)H NMR revealed a linear correlation between targeted and obtained number averaged molecular weight \((M_n)\),
thereby proving the control over the polymerization procedure (Figure 3.10, right). Moreover, the prepared star-shaped block copolymers were investigated by MALDI-TOFMS revealing peak molecular weights in good agreement with theoretically expected ones for \( \text{P5-P7} \) (see Figure 3.12). Higher molecular weight star-shaped block copolymers could not be analyzed by MALDI-TOFMS, most likely due to their relatively broad polydispersity index, which leads to mass discrimination effects during the MALDI experiments. Figure 3.12 (left) depicts the MALDI-TOFMS spectrum of polymer \( \text{P5} \) clearly revealing the difficulties in the analysis of these polymers by MALDI-TOFMS.

![Figure 3.12](image)

**Figure 3.12** Left: MALDI-TOFMS spectrum of \( \text{P5} \). Right: MALDI-TOFMS spectrum of a low molecular weight fraction of \( \text{P5} \).

The presence of two overlapping distributions (molecular weight distribution and composition distribution, compare also section 3.2.1) and the broad polydispersity indices did not allow the calculation of accurate molecular weight distributions or the assignment of end groups. Therefore, only peak maximum molecular weights were taken into account as discussed above. However, it was possible to analyze a MALDI-TOFMS spectrum of a fractionated sample of polymer \( \text{P5} \). Therefore, \( \text{P5} \) was cut into three equal fractions utilizing a SEC system with fraction collector. The MALDI-TOFMS spectrum of the lowest molecular weight fraction (\( M_d(\text{SEC}) = 3.350 \text{ g/mol} \); \( \text{PDI}(\text{SEC}) = 1.2 \); fraction collected from 7.7 to 8.6 minutes retention time; compare also with Figure 3.10, left) could then provide additional proof that the synthesized polymers contained the anticipated structures due to a correct end-group analysis (see assigned signal in Figure 3.13) and the presence of both ethylene oxide and \( \varepsilon \)-caprolactone repeat units in the oligomer distribution of \( \text{P5} \) as depicted in Figure 3.13. The differently sized arrows in Figure 3.13 indicate the difference mass steps of the two respective repeat units present in the molar mass distribution of star-shaped block copolymer \( \text{P5} \).
3.3.2 Unimolecular micellar behavior

Unimolecular micelles are most commonly referred to as core shell architecture that are not in equilibrium with individual amphiphiles, but do still posses micellar properties such as, e.g., the ability to encapsulate guest molecules. Literature examples range from end-group functionalized dendrimers and hyperbranched polymers to unimolecular core-shell objects consisting of a poly(ethylene) core and a PEO corona that were prepared utilizing a chain walking catalyst. All systems have a core-shell architecture in common that can provide significantly different microenvironments in their core if compared to their corona. Moreover, these systems were shown to be able to encapsulate dye molecules in their core.

Dynamic light scattering (DLS) data (see Table 3.3) in CHCl₃, a non-selective solvent for the block copolymers P5-P10, are in agreement with the formation of unimolecular nano-objects in which both the core and corona are solvated. The angular dependence of the DLS signal revealed that these unimolecular micelles should be spherical in shape. Figure 3.14 (left) shows the dependence of the size of the investigated nano-objects in CHCl₃ on their PCL.

**Figure 3.13** Zoom in the MALDI-TOFMS spectrum of fractionated P5 with end-group assignments.

The combination of automated synthesis and screening techniques allowed an accelerated preparation as well as characterization of novel star-shaped block copolymers with PEO core and PCL corona. Moreover, the combination with conventional analytic techniques revealed that the described polymers have the anticipated structure and that the performed polymerizations were controlled and it was possible to target for certain molecular weights.
chain length revealing a linear correlation and that it is possible to prepare unimolecular
micelles of different sizes in the nanometer range. Moreover, Figure 3.14 (right) displays
modeled structures of polymers \textbf{P5} and \textbf{P7} obtained by a simulated annealing procedure
utilizing MS Modeling 3.1. The gas phase structures showed a good agreement with the
anticipated core-shell architecture and the obtained sizes were close to the ones obtained by
DLS (see Table 3.3). The structures displayed in Figure 3.14 (right, top) are color coded by
elements: C: gray, O: red, and N: blue, respectively. The structures displayed in Figure 3.14
(right, bottom) display PEO chains in red and PCL chains in gray clearly revealing the PEO in
the core with PCL chains “wrapped” around. To investigate the host-guest properties of the
star-shaped block copolymers \textbf{P5-P10}, the pH indicator dye methyl orange\textsuperscript{59} (MO, acid
orange 52, 4-[p(dimethylamino)-phenylazo] benzenesulfonic acid, sodium salt) was chosen
for extraction studies in the two-phase system chloroform/water. In this case the mentioned
dye stays in the acidic water phase, whereas upon addition of one of the prepared polymers
the dye is extracted into the chloroform phase. This behavior can be explained by
encapsulation of the dye in the hydrophilic core of the investigated materials and its phase
transfer as depicted in Figure 3.15.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3_14.png}
\caption{Left: linear dependence of the size of the investigated unimolecular micelles depending on
their molecular weight. Right: modeled structures of \textbf{P5} ($D_m = 2.5$ nm) and \textbf{P7} ($D_m = 4.4$ nm).
$D_m$: Diameter obtained by modeling.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3_15.png}
\caption{Encapsulation and phase transfer behavior of the investigated star-shaped block
copolymers \textbf{P5-P10} with methyl orange.}
\end{figure}
The color change can be explained by a change in the dyes microenvironment and is an additional indication that methyl orange is encapsulated by the star-shaped block copolymers. Furthermore, it was possible to evaluate the decreasing concentration of methyl orange in water as a function of added polymer to the two-phase system. Therefore, the overall absorption of the water/chloroform system in transmission mode with an UV/Vis plate reader was measured. This allowed a fast and parallel screening of the extraction properties as well as reproducible quantification of the maximum load (ML) of all investigated polymers. For these experiments 100 µL of a 0.1875 mg/mL solution of methyl orange and different amounts (0-70 µL in 10 µL steps, in rows A to H, respectively) of polymer containing chloroform stock solutions with (2-3 mg/mL, depending on the polymer) were pipetted into the wells of the microtiter plate. For each measured microtiter plate also a calibration was recorded. Figure 3.16 (top) shows a filled microtiter plate as utilized for the polymer extraction property screening and some resulting UV/Vis spectra. Column one of the microtiter plate was utilized for calibration purposes \((R^2 = 0.993)\), whereas all other rows contain different polymers at different concentrations.

**Figure 3.16** Microtiter plate screening of extraction properties of the investigated polymers. Top: filled microtiter plate (column 1: calibration; column 2-12: different polymers; rows A-H: increasing concentration of polymers). Bottom-right: resulting UV/Vis spectra of row 7 of the microtiter plate. Bottom-left: concentration of encapsulated methyl orange as a function of the concentration of polymers P8 and P10.
The concentration of the investigated polymers is increasing from row A to row H. It is clearly visible that, upon addition of a star-shaped block copolymer, the absorption of methyl orange at 504 nm decreases and the absorption maximum of the dye shifts from 504 to 420 nm (see Figure 3.16, bottom-left). This behavior can be explained by an encapsulation and extraction of methyl orange to the organic phase by the polymer. Figure 3.16 (right-bottom) shows an increase of the methyl orange concentration upon addition of polymers P8 and P10. The concentration of methyl orange was obtained from its decrease in absorption at 504 nm utilizing the simultaneously recorded calibration data. The leveling off of both curves is in agreement with a full extraction of all dye molecules from the water into the chloroform phase. Both findings indicate that these star-shaped block copolymers behave as unimolecular micelles. Moreover, DLS was performed on polymer P10 loaded with methyl orange (5 molecules MO per polymer molecule) in chloroform. The D_h (extrapolated to zero concentration) of 10.7 ± 1.3 nm revealed a swelling of these nano-objects upon loading with guest molecules (compare Table 3.3). A few percent of larger aggregates were also detected (2%, D_h ~ 30 nm). This indicates that the described findings of phase transfer of dye molecules by these star-shaped block copolymers is to large extent an unimolecular micellar effect.

To determine the maximum loading (ML) capacity of all investigated polymers accurately UV/Vis extraction studies in the above described microtiter plate format were performed in duplo. The results are summarized in Table 3.4 and represent average values of seven parallel measurements for each polymer.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>INI</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML</td>
<td>0.5</td>
<td>7.2</td>
<td>7.2</td>
<td>6.8</td>
<td>7.1</td>
<td>6.9</td>
<td>6.6</td>
</tr>
</tbody>
</table>

The error of these loading values was found to be 0.25 (molecules methyl orange/molecule polymer). These data reveal that all investigated block copolymers show the same extraction capacity of approximately 6.9 dye molecules per molecule of polymer. This can be explained by the unchanged size of the core of the star-shaped block copolymers and indicates that the size of the shell is of minor importance for the loading properties of unimolecular micelles. The fact that the initiator with the same star-shaped structure has a significantly smaller loading capacity proves that only a core-shell architecture can provide micellar like behavior. Moreover, it was observed that a linear PEO (M_n = 2.150 g/mol; PDI = 1.1) did not show any detectable extraction of methyl orange in the chloroform/water system. This is in perfect agreement with the comparative study of linear and hyperbranched esterified polyglycerols by Frey et al.\textsuperscript{60} and an additional confirmation that a branched polymer structure can freeze in the confirmation that is required to obtain unimolecular micellar behavior. Moreover, the
described findings arise the question if a smaller shell of, for instance, one caprolactone unit or an alkane ether would already be sufficient for an unimolecular micellar behavior. Literature has shown that end-group modifications of dendrimers with alkane chains can provide core shell architectures that can encapsulate dyes or act as catalysts. However, the attachment of PCL chains of different length to the branched PEO core allows the adjustment of solubility and maybe even release parameters of these star-shaped block copolymers. In addition, the utilization of PCL provides biocompatibility and biodegradability of the investigated polymers.

In order to investigate the transport capabilities of the star-shaped block copolymers \textbf{P5-P10}, analytical ultracentrifugation (AUC) experiments were performed on their chloroform solutions. Therefore, polymers \textbf{P5-P10} were loaded with one molecule methyl orange per polymer molecule as described above. Sedimentation equilibrium (SE) measurements were then performed at 50,000 rpm at an absorption wavelength of 415 nm. As a consequence, only polymers with encapsulated MO can be measured by the AUC detector, since polymers \textbf{P5-P10} are non absorbing in this wavelength range and pure MO is insoluble in chloroform (and would therefore sediment immediately due to the applied centrifugal forces).

![Figure 3.17 Sedimentation equilibrium profile of polymer P6 loaded with MO and the corresponding fit to the data using the c(M) model.](image)

Generally, a SE-AUC separation is based on mass and density of the species to analyze and the applied centrifugal forces are sufficient to sediment particles down to the nanometer size regime. Concentration profiles in the sample cell are acquired using absorption optics in time
and the sedimentation equilibrium profile (see Figure 3.17) is then evaluated applying the following equation:

\[ A(r) = \int L(M, r)c(M) dM \quad \text{with} \quad L(M, r) = A(r_0) \exp \left[ M(1 - \bar{v}\rho) \frac{\omega^2}{2RT} \left( r^2 - r_0^2 \right) \right] \]

where \( r \) denotes the distance from center of rotation, \( r_0 \) an arbitrary reference radius, \( \omega \) the angular velocity, \( T \) the absolute temperature, \( R \) the gas constant, and \( A(r_0) \) the absorption at the reference radius.

In order to determine the continuous molar mass distributions, \( c(M) \), as they are depicted in Figure 3.18 (left) for polymers P5-P10, a linear least-square method is applied, as implemented in the used Sedfit software. Figure 3.17 reveals that the investigated unimolecular micelles are floating and not sedimenting in chloroform, which is due to their lower density, if compared to the solvent (CHCl\(_3\) has a rather high density of 1.48 g/cm\(^3\)). The thus obtained \( c(M) \) distributions for the six investigated loaded unimolecular micelles are depicted in Figure 3.18 (left). From these distribution an effective average molar mass, \( M_{\text{eff}} \), can be derived. This effective (uncorrected for buoyancy) molar mass could be converted to an absolute value if \( \bar{v} \) was known for all the loaded micelles according to:

\[ M = \frac{M_{\text{eff}}}{1 - \bar{v}\rho} \quad \text{with} \quad \bar{v} : \text{partial-specific volume and} \quad \rho : \text{density of the solvent} \]

However, since the goal of these studies was to demonstrate that the investigated unimolecular micelles can transport guest molecules, the experimentally tedious determination of \( \bar{v} \) of the investigated polymers was not performed. Nevertheless, all conclusions are still valid, since the obtained effective masses are correct relative to each other (it is fair to assume that \( \bar{v} \) does not change significantly for the different investigated polymers). It is obvious that different effective molar masses were observed for polymers P5-P10 as depicted in Figure 3.18 (left).

**Figure 3.18** Results of AUC investigations of polymers P5-P10. Left: effective molar mass distributions determined from sedimentation equilibrium profiles. Right: average effective molar masses plotted against the degree of polymerization (DP) of the PCL block.
Moreover, a linear correlation was obtained between the degree of polymerization of the PCL block (and therefore the size of these polymers) and the observed effective mass as shown in Figure 3.18 (right). These observations allow the conclusions that i) all investigated star-shaped block copolymers can transport guest molecules and ii) the transport of guest molecules by the star-shaped block copolymers is dependent on the molecular weight and therefore the size of the polymers, e.g. the higher molecular weight polymers sediment faster and therefore their guest molecules are also transported faster.

These results show that the investigated star-shaped block copolymers behave as unimolecular reversed micelles in chloroform that can encapsulate, phase transfer and transport a model guest molecule, methyl orange. Moreover, these findings demonstrate that the guest transport, but not the maximum loading capacity of these polymers, is depending on the degree of polymerization of the PCL block. Both insights might be useful for the design of novel carrier polymers for different applications.

3.3.3 Guest encapsulation screening

The encapsulation of guest molecules within a host can be probed by the evaluation of changes in their microenvironment with techniques such as UV/Vis and fluorescence spectroscopy, NMR spectroscopy, or others. These changes are premised on stabilizing or destabilizing effects of the local microenvironment on electronic states of the guest molecules. The here described primary and fast screening of host guest interactions of 24 organic molecules with the above mentioned star-shaped block copolymers (see sections 3.3.1 and 3.3.2 for preparation, characterization and unimolecular micellar behavior, respectively) is based on an evaluation of the changes in the molecules microenvironments by UV/Vis as well as fluorescence spectroscopy.

Therefore, a microtiter plate screening assay (see Figure 3.19, left for the layout) in combination with a plate reader that allows the fast recording of full UV/Vis and fluorescence spectra in a quasi parallel mode was utilized. This setup allowed recording of all necessary UV/Vis as well as fluorescence spectra for the screening of a 96 well microtiter plate in less than 15 minutes (measuring 96 UV/Vis spectra could be performed in approximately 40 seconds, whereas the recording of 96 fluorescence spectra took roughly two minutes). The investigated unimolecular micelles for this screening approach were star-shaped block copolymers consisting of a poly(ethylene glycol) core and a poly(ε-caprolactone) corona, which was prepared in larger scale (P12: M_n(SEC_DMF) = 3.350 g/mol; PDI(SEC) = 1.21; yield = 55 g). The poly(ε-caprolactone) corona was chosen with only three average repeat units to provide sufficient water solubility of the polymer (the size of the corona was found to have no influence on the host guest properties of the discussed polymers as described above).

To show that the investigated polymer P12 exists as a unimolecularly dissolved species not only in chloroform as described above, but also in an aqueous environment, dynamic light scattering experiments in water were performed. One could expect the formation of larger
aggregates of polymer molecules with the poly(ethylene glycol) part on the outer surfaces and the more lipophilic poly(ε-caprolactone) part in the inside of the aggregate due to the different solubilities of the two block components in water. However, the performed light-scattering experiments did not reveal any aggregates of copolymer \textbf{P12} in a concentration range from 0.001 mg/mL to 100 mg/mL leading to the conclusion that the observed effects can be attributed to a unimolecular behavior even if the absolute conformation of these macromolecules might be considerably different in an aqueous solution. The screening was performed in acidic as well as basic aqueous environment to ensure that changes in the microenvironment of the investigated guest molecules could be detected even for pH active guest molecules (a more detailed discussion of the necessity to utilize different environments for this screening approach is given below). Both the acidic and basic environment could lead to a hydrolysis of the ester bonds of the PCL block of polymer \textbf{P12}. However, no change in the molecular weight distribution of \textbf{P12} could be observed by size exclusion chromatography in DMF after storage in 0.01 M HCl or 0.01 M NaOH for 24 hours. This time is well above the time required to perform all necessary measurements for this screening. Nevertheless, it would be interesting to study the dependence of the degradation behavior of this class of polymers on pH and other factors in the future.

![Image](image.png)

**Figure 3.19** Left: Schematic representation of the applied layout for the screening. Right: optical picture of the microtiter plate used to screen for host-guest interactions.

Figure 3.20 provides the chemical structures of all investigated potential guest molecules together with a digit code for straightforward compound identification (compare experimental section for the chemical names of the investigated guest molecules). The plate was filled with 20 µL equivalents of saturated solutions of all 24 guest molecules in 0.01 M HCl (positions A1 to H6) and 0.01 M NaOH (positions A7 to H12), respectively (compare also with color coding in Figure 3.19, left). Subsequently, the solutions of guest molecules were diluted to 250 µL with 0.01 M HCl (positions A1 to H3), 0.01 M NaOH (positions A7 to H9), 200 mg/mL polymer \textbf{P12} in 0.01 M HCl (positions A4 to H6) and 200 mg/mL polymer \textbf{P12}
PEO-b-PCL block copolymers with different architectures

in 0.01 M NaOH (positions A10 to H12), respectively. This resulted in four sets of the 24
guest molecules, explicitly guest only and guest in the presence of polymer P12 in both acidic
and basic environment. UV/Vis spectra in a range from 250 to 900 nm as well as fluorescence
spectra with different excitation wavelength (390 to 490 nm in 20 nm steps) were recorded
utilizing the described plate reader setup. Moreover, the plate was inspected visually in
daylight and under 254 as well as 365 nm UV irradiation. The screening was completed by
diluting the plate by a factor of 1:10 since some of the measured spectra revealed a too high
UV/Vis absorption. Moreover, the screening was repeated in dichloromethane to avoid any
solubility problems of the guest molecules in the aqueous medium and to investigate the
encapsulation of guest molecules from an organic environment. Subsequently, the recorded
spectra were utilized to evaluate significant changes in the UV/Vis or fluorescence behavior
of the 24 investigated guest molecules. The following discussion will only provide screening
data of selected guest molecules, since the discussion of all 24 molecules in detail would be
too complex and excessively long. The final assessment of all 24 screened molecules as well
as additional screening data is provided in the experimental section. Figure 3.19 (right)
displays an optical picture of the filled microtiter plate as it was utilized for the screening.
Already an optical investigation can clearly reveal that some of the investigated guest
molecules show an interaction with the star-shaped block copolymer.

![Diagram of guest molecules](image)

**Figure 3.20** Chemical structure as well as number code of the investigated potential guest molecules.

For instance the guest molecule 9 (phenolphthalein, a pH indicator) in positions A2 (acidic
environment), A5 (acidic environment in the presence of polymer P12), A8 (basic
environment) and A11 (basic environment in the presence of polymer P12) clearly shows a
color change from red to colorless upon addition of polymer P12 in the basic environment,
but not in the acidic medium. This can be explained by an encapsulation of phenolphthalein
and therefore a change in the dyes microenvironment. However, since phenolphthalein is
colorless in acidic solution this effect is only visible in the basic environment, where this pH indicator shows a red color. The example of phenolphthalein encapsulation also clearly demonstrates the need to screen for host guest interactions in both acidic and basic solutions since otherwise some hits might not be identified in the screening procedure. Generally, it was observed for a number of guests that the capability of being encapsulated by polymer P12 was independent of the environment (e.g. solvent or pH) where the encapsulation took place. However, the environment was important for the detection of the encapsulation as described for the case of phenolphthalein and therefore different environments (solvent and pH) were chosen for this screening approach for a complementary assessment of the host-guest chemistry of the investigated reversed unimolecular micelles. Moreover, the comparison of the “switching” behavior of different pH indicators, such as bromophenol blue (guest 10) and phenolphthalein (guest 9) allows some conclusions about the environment inside the core of the investigated star-shaped block copolymers. Bromophenol blue (pK_a = 7.1) switches from its acidic, yellow form to the blue, basic form in the acidic environment indicating that the pH value in the interior of the star-shaped block copolymer is higher than pH = 7.6 (usually ± half a pH value difference of the indicators pK_a is necessary to observe clear color changes). The behavior of phenolphthalein (pK_a = 9.3) in the basic environment on the other hand allows the conclusion that the pH in the core is lower than 8.8. Therefore, the interior of the reversed unimolecular micelles should be neutral to slightly basic, which is in agreement with the nitrogen based core structure (pentamethyl-diethylenetriamine: pK_a = 8-9). In order to be able to make more accurate determinations about the environment within the core more pH indicator molecules with pK_a values between 7.1 and 9.3 would have to be investigated. A careful investigation of the recorded UV/Vis absorption spectra revealed that seven of the 24 molecules in HCl and twelve of the 24 molecules in NaOH showed a distinct shift (> 25 nm) of the absorption maximum upon addition of the star-shaped block copolymer. This can be interpreted as encapsulation and is recorded as a hit in the sense of the screening.

![Figure 3.21 Encapsulation behavior of guest 4 (positions D7 and D10 in the microtiter plate) in 0.01 M NaOH (left) and guest 10 (positions B8 and B11 in the microtiter plate) in 0.01 M HCl (right) visualised by UV/Vis spectroscopy. (---) Encapsulated; (___) not encapsulated.](image)
Figure 3.21 shows UV/Vis spectra of guest 4 (disperse blue 3, left) as well as guest 10 (bromphenolblue, right) as examples of this behavior. Both spectra reveal a pronounced shift of the absorption maximum due to a change in the microenvironment of these guest molecules upon addition of the host polymer, which acts as a unimolecular micelle and encapsulates the guest molecules. Moreover, the screening for changes in the fluorescence behavior upon encapsulation of guest molecules revealed that five of the 24 guest molecules show a distinct fluorescence increase upon addition of star-shaped block copolymer P12. Figure 3.22 demonstrates this effect for guests 12 (9-(hydroxymethyl)anthracene, right) and 19 (2,5-dihydroxybenzoic acid, left) in the presence and absence of the star-shaped block copolymer P12. This effect can be explained by a reduction of self-quenching due to isolation of these guest molecules upon encapsulation and is an interesting property for sensoric applications as it will be discussed in section 4.4.

Moreover, it was observed that the described changes in UV/Vis and fluorescence behavior depend on both guest and polymer concentration. One example of this behavior is provided in Figure 3.23, where different amounts of polymer P12 are added to a 10.41 mM solution of guest 18 (dithranol) in dichloromethane. Upon addition of the polymer, more and more of the dithranol guest molecules are encapsulated and therefore show a fluorescence increase due to reasons described above. A similar increase was observed if different amounts of dithranol were added to a polymer solution. These concentration dependant effects were not only observed for dithranol but also for other guest molecules and indicate that the investigated polymer class might be a useful material for the development of optical sensors. Some of these concentration dependant examples as well as another example of a phase transfer of a
guest molecule are depicted in Figure 3.24 giving further evidence that the guest molecules are indeed encapsulated and that the encapsulation is based on a unimolecular micellar effect.

**Figure 3.23** Polymer P12 concentration dependent fluorescence increase of guest 18 (dithranol) due to its encapsulation in dichlormethane.

**Figure 3.24** Top: P12 concentration dependant change of guest behavior in the visible as well as under 356 nm UV irradiation. Bottom: encapsulation and phase transfer of guest 10 upon addition of P12 to the two phase system.
Figure 3.24 (top) displays two polymer concentration dependant changes of guest behavior in the visible (guest 10) and under 365 nm UV irradiation (guest 19). Both examples demonstrate that upon addition of polymer more and more of the present guest molecules are encapsulated and show the corresponding change in the absorption or emission behavior, respectively. Moreover, Figure 3.24 (bottom) shows another example of guest encapsulation and subsequent guest transfer from water to an organic chloroform phase.

The complete screening of all 24 molecules by UV/Vis and fluorescence spectroscopy in acidic, basic, and organic environments revealed 17 successful encapsulations (see experimental section for additional screening data). However, to make sure that all hits were correctly identified and no hits were omitted, \(^1\)H NMR investigations in different solvents were performed after the screening was finished.

\[\text{Figure 3.24} \]
\[\text{\(1\)H NMR investigations of guests 16 in CDCl}_3\text{ (left) and 20 in acetone-d6 (right) in the absence and presence of polymer P12. (---) Encapsulated; (___) not encapsulated}\]

Figure 3.25 shows \(^1\)H NMR spectra of guest 16 (5-nitro-1,10-phenanthroline, left) and guest 20 (\(\alpha\)-cyano-4-hydroxycinnamic acid, right) in the encapsulated and the not encapsulated form with the corresponding assigned signals in the aromatic region of the spectrum. A change in the microenvironment and therefore an encapsulation of the guests can once more explain the observed shifts of the aromatic protons of guests 16 and 20. Moreover, it was observed that polymer P12 had a large solubilizing effect on many guests in solvents such as acetone-d6, CDCl\(_3\) or D\(_2\)O, which allows an easy characterization of insoluble molecules in ppm ranges of 4.5 and above if the molecules are encapsulated. In summary, the \(^1\)H NMR investigations could identify five more successful encapsulations, resulting in an overall of 22 hits out of 24 screened molecules (compare experimental section). Moreover, the discussed primary screening identified 77% of all successful encapsulations without any false positives. Especially the facts that i) a large variety of guest molecules can be encapsulated and ii) the observed solubilizing effects in a variety of solvents including water make the investigated unimolecular micelles interesting materials for drug delivery applications.
3.2.4 Stabilized Pd nanoparticles and their catalytic behavior

Nanoparticles are currently investigated for many applications (e.g. increased density storage media, improved wear resistant materials or anti-reflective coatings, to only name few) due to their promising novel properties. These changes in material properties upon size reduction are (for metal nanoparticles) premised on the dominance of surface effects for the small species. More specifically, in a bulk metal, electrons are highly delocalized over large space due to the fact that separation between the valence and conduction bands vanishes, giving the metal its conducting properties. If the size is decreased its electronic motion is restrained and the separation between the valence and the conduction bands increases making the metal a semiconductor and finally an insulator. For noble metals, this size reduction results in an intense absorption in the visible-near-UV region of the spectrum due to the coherent oscillation of the free electrons from one surface of the particle to the other. Therefore, the formation of gold-nanoparticles with the described star-shaped block copolymers P5 to P10 as templates was investigated applying a two step procedure of metal salt incorporation into the core of the unimolecular micelles and subsequent chemical reduction (compare Figure 3.26).

The investigations revealed that the PEO core of P5-P10 can act as templates for the formation of gold nanoparticles in the size range of 3-4 nm with the expected surface plasmon resonances at approximately 530 nm. Moreover, less particle aggregation was observed for the particles templated with higher molecular weight polymers (longer PCL chains) due to steric reasons. The results of these investigations are summarized in Table 3.5 revealing similar sizes of the gold nanoparticles templated with polymers P7-P10 indicating their ability to stabilize these particles due to an increased steric hindrance of the longer PCL chains. Inspired by these results the formation of palladium nanoparticles and their application as catalysts for C-C coupling reactions was subsequently investigated.
**Table 3.5** DP: degree of polymerization of PCL per arm; $D_h$ was measured by DLS in DMF of unloaded micelles from data extrapolated to zero concentration. Diameter ($D$ in nm) of the gold nanoparticles was measured by TEM after reduction with NaBH₄.

<table>
<thead>
<tr>
<th>Template</th>
<th>DP</th>
<th>$D$ (TEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 P5</td>
<td>3</td>
<td>5.9±0.5</td>
</tr>
<tr>
<td>2 P6</td>
<td>6</td>
<td>6.8±0.6</td>
</tr>
<tr>
<td>3 P7</td>
<td>9</td>
<td>3.4±0.2</td>
</tr>
<tr>
<td>4 P8</td>
<td>12</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>5 P9</td>
<td>15</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>6 P10</td>
<td>18</td>
<td>3.6±0.2</td>
</tr>
</tbody>
</table>

The “Heck reaction”, 66,67 a Pd-catalyzed C-C coupling between aryl- or vinyl halides and triflates with alkenes, has evolved to a robust and efficient method for carbon-carbon bond formation and remains a flourishing area of research.68 Several strategies have been described to obtain high-turnover catalysts (HTC) for these reactions since i) the applied Pd catalysts are rather expensive and ii) contamination of the product with organometallics especially for pharmaceutical applications should be as low as possible.69 In particular, a HTC can be defined as a catalyst that can lead to quantitative conversion of starting materials at a load of 0.1 mol%.69 Some recent examples include the design of efficient ligands for palladium complexes70,71 or the stabilization of palladium nanoparticles.72,73 Especially dendrimers and other branched polymers seem to be perfectly suited for the stabilization of defined and small Pd nanoparticles and some of these particles showed high activity for Heck coupling reactions.74-76 In this respect Crooks et al. highlighted the use of dendrimers as hosts for the stabilization of a large variety of metal nanoparticles and their use as homogenous catalysts for, e.g., the catalytic hydrogenation of alkenes in aqueous as well as in organic.77 More specifically, up to 70% conversion within 24 h at 90 °C was observed for Heck coupling reactions of aryl halides with $n$-butyl acrylate utilizing 3-5 mol% dendrimer stabilized Pd nanoparticles with a size of 2-3 nm.75 Moreover, dendrimer stabilized Pd nanoparticles have been reported to be efficient catalysts for hydrogenation reactions as well as Heck couplings in supercritical CO₂78 and hyperbranched polymers were reported to stabilize palladium nanoparticles (size 2 and 5 nm) that were successfully utilized for the hydrogenation of cyclohexene in a continuously operated membrane reactor.79 On the other hand micelles were reported to be able to stabilize palladium particles that were subsequently efficiently applied as catalysts for Heck and other Pd catalyzed reactions.80 Here, the possibility to stabilize palladium nanoparticles with polymers P5-P10 will be discussed.

**Preparation and characterization of Pd nanoparticles**

The formation and stabilization of palladium nanoparticles with star-shaped block copolymers templates P5-P10 was studied. The characterization data of the investigated 5-arm star-shaped block copolymers was summarized in Table 3.3. The palladium nanoparticles were prepared
by adding a known amount of Pd(OAc)$_2$ to solutions of polymers P5-P10 in DMF at a concentration of 1 g/L and a subsequent reduction of Pd$^{2+}$ to Pd$^0$ by NaBH$_4$ (see Figure 3.26). The palladium salt is expected to interact with the oxygen and nitrogen atoms located in the PEO central core of the star-shaped copolymers. After addition of the Pd(OAc)$_2$ to the star-shaped block copolymer the resulting solutions were stirred for 24 h to ensure an effective incorporation of the palladium salt into the micellar core. Subsequently, non-incorporated salt was eliminated by dialysis and the incorporated palladium salt was reduced by the addition of NaBH$_4$. The preparation of the studied palladium nanoparticles was carried out in DMF due to the good solubility of all reactants (polymers, Pd(OAc)$_2$ and NaBH$_4$) in this solvent. Because both PEO and PCL blocks are soluble in DMF, the star-shaped block copolymers were observed as unimolecular objects in DMF by DLS (see Table 3.6) and the hydrodynamic diameter ($D_h$) of the star-shaped block copolymers was increasing with the length of the PCL outer chains. The star-shaped block copolymers loaded with the palladium salt were then investigated by TEM (see Figure 3.27). Electron-irradiation during TEM observation resulted in the reduction of the palladium salt into palladium nanoparticles. Small individual Pd nanoparticles with an average size of approximately 4 nm were observed (see Table 3.6). A Pd(OAc)$_2$/EO loading molar ratio of $\frac{1}{4}$ has been utilized for all following experiments since this loading ratio was optimal to saturate the PEO core of the star-shaped block copolymers, while minimizing the excess palladium salt to be eliminated. Under these conditions, the incorporation of the palladium salt was almost complete (no palladium salt was detected by UV-Vis spectroscopy in the exterior compartment after dialysis). The polydispersity of the palladium nanoparticles was larger whenever star-shaped block copolymers with short PCL blocks were used (NP1 and NP2, Figure 3.27).

Figure 3.27 TEM micrographs of star-block copolymers P5-P10 (resulting in nanoparticles NP1 to NP6) loaded with Pd(OAc)$_2$ at a Pd(OAc)$_2$/EO loading molar ratio of $\frac{1}{4}$. The scale bar in each image is 100 nm.
This effect is thought to result from the merging of a few palladium salt-loaded star-shaped block copolymers in solution and is only observed whenever the stabilizing PCL blocks are too short to prevent aggregation of the composite nanoparticles, i.e. for NP1 and NP2. The chemical reduction of the loaded micelles was performed by adding a known amount of a NaBH4 solution in DMF in order to reduce the Pd\(^{2+}\) to Pd\(^0\). The reduction of the Pd\(^{2+}\) salt proceeded immediately during dropwise addition of NaBH4, as indicated by a color change of the solution from yellow to brown. The amount of added NaBH4 had to be optimized precisely, as reported in the literature for gold nanoparticles.\(^8\) Indeed, the addition of an excess of NaBH4 resulted in an increased clustering of the Pd nanoparticles and the long-term stability of the solutions accordingly decreased from months to days. This was experimentally evidenced by TEM observations in which the size and size polydispersity of the Pd nanoparticles were found to increase. Actually, an excess of NaBH4 certainly can act as a reductant for the PCL chains and therefore destroys the steric barrier against nanoparticle aggregation. Figure 3.28 shows TEM micrographs of palladium nanoparticles templated by polymers P5-P10 with a Pd(OAc)\(_2\)/EO loading molar ratio of \(\frac{1}{4}\) and a Pd(OAc)\(_2\)/NaBH4 molar ratio of \(\frac{1}{2}\) (optimized results). Small Pd nanoparticles with a spherical shape and an average size of approximately 4 nm were formed in the core of all the investigated star-block copolymers. The dimensions of at least 50 palladium nanoparticles recorded on different locations of the TEM grid have been averaged and the corresponding number-averaged diameters are provided in Table 3.6.

**Table 3.6** DP: degree of polymerization of PCL per arm; \(D_h\) was measured by DLS in DMF of unloaded micelles from data extrapolated to zero concentration. Diameter (D in nm) of the Pd nanoparticles was measured by TEM before (1) and after reduction with NaBH4 (2).

<table>
<thead>
<tr>
<th>Template</th>
<th>DP</th>
<th>(D_h) (DLS)</th>
<th>(D^1) (TEM)</th>
<th>(D^2) (TEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP1</td>
<td>P5</td>
<td>3</td>
<td>3.2±0.5</td>
<td>5.8±0.5</td>
</tr>
<tr>
<td>NP2</td>
<td>P6</td>
<td>6</td>
<td>3.4±0.8</td>
<td>4.1±0.4</td>
</tr>
<tr>
<td>NP3</td>
<td>P7</td>
<td>9</td>
<td>4.5±1.0</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>NP4</td>
<td>P8</td>
<td>12</td>
<td>5.1±0.7</td>
<td>4.3±0.2</td>
</tr>
<tr>
<td>NP5</td>
<td>P9</td>
<td>15</td>
<td>6.4±0.9</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>NP6</td>
<td>P10</td>
<td>18</td>
<td>7.4±0.8</td>
<td>3.7±0.2</td>
</tr>
</tbody>
</table>

The Pd nanoparticles observed before and after chemical reduction with NaBH4 look similar (compare Figures 3.27 and 3.28). This observation is a strong indication that the incorporation of the palladium salt in the star-shaped block copolymer is effective. Since the average size of the accordingly prepared nanoparticles directly compares with the size of the templating star-shaped block copolymers, one can conclude that Pd nanoparticles are essentially formed in the star-shaped block copolymers. A closer look at the TEM pictures (Figure 3.27) revealed again a broader polydispersity for the Pd nanoparticles templated by polymers P5 and P6. Aggregated Pd nanoparticles were observed even for freshly prepared solutions if the outer
PCL blocks were very short. This observation highlights the crucial role of the PCL outer block as stabilizing entities for the accordingly formed nanoparticles. A critical PCL block length (DP > 6) has to be reached to prevent contact between two adjacent unimolecular micelles and further coalescence of the embedded nanoparticles.

**Figure 3.28** TEM micrographs of star-block copolymers P5-P10 (resulting in nanoparticles NP1 to NP6) loaded with Pd(OAc)$_2$ at a Pd(OAc)$_2$/EO loading molar ratio of ¼ and after reduction with NaBH$_4$ (Pd(OAc)$_2$/NaBH$_4$ molar ratio of ½). The scale bar in each image is 100 nm.

The long-term stability of the palladium nanoparticles prepared in the star-shaped block copolymers P5-P10 has subsequently been investigated. It was already mentioned in the literature that macromolecule stabilized Pd nanoparticles offer high colloidal stabilities, however these reports are lacking experimental details, such as the storage time at a certain temperature in combination with TEM observations before and after storage of such nanoparticles. The palladium nanoparticles prepared in samples P5-P10 with a Pd(OAc)$_2$/EO loading molar ratio of ¼ and a Pd(OAc)$_2$/NaBH$_4$ molar ratio of ½ are unaffected after three month while clustering is observed for the other samples. Clustering has been followed by TEM but is also visualized by the formation of macroscopic flocks at the bottom of the vessels containing solutions of the Pd nanoparticles. Moreover, the thermal stability of the palladium nanoparticles was also tested at 100 °C for three days (see Figure 3.29). Clustering was clearly accelerated for nanoparticles NP1 and NP2 while nanoparticles NP3-NP6 only showed a minor fraction of aggregated nanoparticles. This effect is exemplarily depicted in Figure 3.29 where Pd nanoparticles templated with polymer P5 (NP1) and polymer P10 (NP6) are shown after they were heated in DMF at 100 °C for three days. It is obvious that polymer P5 revealed a much smaller capability to stabilize the nanoparticles than polymer P10 as evidenced by the fact that more and larger aggregates were present after the heating of particles stabilized by polymer P5 in comparison with particles stabilized by polymer P10.
Moreover, palladium nanoparticles have been prepared by using the same methodology as described above in (i) the presence of the PEO core without stabilizing blocks and (ii) without any polymer. These nanoparticles were characterized by a poor colloidal stability and a strong aggregation was observed just after their preparation, as will be discussed later.

**Figure 3.29** TEM micrographs of palladium nanoparticles prepared with star-block copolymers P5 (A) and P10 (B) after heating to 100 °C for three days in DMF. The scale bar in each picture is 50 nm.

**Catalytic studies with palladium nanoparticles**

The prepared nanoparticles were subsequently evaluated for their capability to catalyze C-C coupling reactions. Consequently, the Heck reaction of 4-bromoacetophenone with styrene was investigated in a parallel and fully automated fashion utilizing a synthesizer robot with varying catalyst amounts and types (see Table 3.7). Therefore, stock solutions of 4-bromoacetophenone and the nanoparticles in DMF as well as bulk styrene and triethylamine were transferred to the reactors of the automated synthesizer by its liquid handling system. Subsequently, all reaction mixtures were brought to a total volume of 4 mL resulting in 1.6 mmol of 4-bromoacetophenone, 1.8 mmol of styrene and 1.92 mmol of NEt₃ in each reactor, respectively with varying amounts and types of catalysts present. These reaction mixtures were degassed by 10 consecutive cycles of vacuum/argon before the reactions were performed under argon at 100 °C in DMF for 48 h. All reactions were monitored by GC-MS utilizing the liquid handling system of the synthesizer to take samples at predefined times. Figure 3.30 (left) shows the layout of the ASW2000 synthesizer as it was used for the catalytic Heck reactions with the reactor block for the 16 parallel reactions, the sample vials for GC-MS analysis and regions for stock solutions and catalysts. A typical GC-MS trace obtained for a catalytic Heck reaction with a conversion of approximately 80% and positive identification of the educt and product is displayed in Figure 3.30 (right) together with a conversion in time plot of the Heck coupling performed with nanoparticles NP3 (compare Table 3.7, entry 3) in the inset of Figure 3.30 (right). The Pd nanoparticles obtained with polymers P5-P10 as templates, Pd(OAc)₂ (reduced and unreduced) as well as Pd(PPh₃)₄ were
investigated at different catalyst loadings. Table 3.7 summarizes representative results of these investigations.

Table 3.7 Results of palladium catalyzed Heck coupling of 4-bromoacetophenone with styrene performed in a parallel fashion utilizing an automated synthesizer. All reactions were performed at 100 °C in DMF at a total volume of 4 mL. 1.6 mmol of 4-bromoacetophenone, 1.8 mmol of styrene and 1.92 mmol of NEt₃ were used for all reactions, respectively. *, **, *** These results were irreproducible and showed large fluctuations between: 0-60%, 0-40% and 0-90% conversion, respectively (see explanation in the text for details).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Mol % Pd</th>
<th>Conversion after 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanoparticle NP1</td>
<td>0.1</td>
<td>*</td>
</tr>
<tr>
<td>Nanoparticle NP2</td>
<td>0.1</td>
<td>*</td>
</tr>
<tr>
<td>Nanoparticle NP3</td>
<td>0.1</td>
<td>94%</td>
</tr>
<tr>
<td>Nanoparticle NP4</td>
<td>0.1</td>
<td>98%</td>
</tr>
<tr>
<td>Nanoparticle NP5</td>
<td>0.1</td>
<td>99%</td>
</tr>
<tr>
<td>Nanoparticle NP5</td>
<td>0.05</td>
<td>67%</td>
</tr>
<tr>
<td>Nanoparticle NP6</td>
<td>0.1</td>
<td>98%</td>
</tr>
<tr>
<td>Core templated</td>
<td>0.1</td>
<td>**</td>
</tr>
<tr>
<td>Pd(OAc)₂</td>
<td>0.1</td>
<td>59%</td>
</tr>
<tr>
<td>Pd(OAc)₂ (red.)</td>
<td>0.1</td>
<td>***</td>
</tr>
<tr>
<td>Pd(PPh₃)₄</td>
<td>0.1</td>
<td>26%</td>
</tr>
<tr>
<td>Pd(PPh₃)₄</td>
<td>1.0</td>
<td>42%</td>
</tr>
</tbody>
</table>
It is obvious that especially the nanoparticles NP3-NP6 are highly active catalysts that meet the requirements to be named HTC\(^{69}\) (compare also with the introduction). The comparison with palladium nanoparticles described in the literature is difficult since a large variety of different reactions were investigated. However, the here described Pd nanoparticles showed a considerably higher activity (full conversion compared to 78% conversion) for the same coupling reaction at even lower temperatures (100 °C compared to 140 °C) in shorter reaction times (24 h compared to 3 days) with lower catalyst loading (0.1 mol% compared to 0.5 mol%) if compared to similarly small Pd nanoparticles stabilized in the core of block copolymer micelles.\(^{80}\) However, some unexpected but explainable effects were also observed. For instance the nanoparticles formed with polymers P5 and P6 also showed relatively high conversions for the investigated Heck coupling reaction. However, these results were irreproducible, especially if different batches of catalysts were compared. This might be due to the fact that only catalysts prepared from polymers P7-P10 have a sufficient capability to stabilize these nanoparticles and to prevent their aggregation. In general, aggregation of particles will of course lead to a reduced surface area of the particles and therefore to a reduced catalytic activity. These results are in good agreement with findings from TEM since nanoparticles NP1 and NP2 showed some aggregates (see also Figures 3.27 and 3.28) and more importantly revealed a smaller capability to stabilize the particles under the applied reaction conditions (see Figure 3.29). Similar findings were observed for Pd(OAc)\(_2\) that was reduced under the same conditions as the templated nanoparticles as well as with the particles obtained from the pure 5-arm PEO core without PCL chains (see Table 3.7). TEM pictures of the resulting nanoparticles are shown in Figure 3.31. It is clearly observable that very small particles can be obtained if no polymer is added for templating during the particle preparation. This explains the high conversions observed with these nanoparticles (see Table 3.7 ***).

![Figure 3.31](image-url) **Figure 3.31** TEM micrographs of palladium nanoparticles prepared without any added copolymer (A) and in the presence of the PEO macroinitiator (Pd(OAc)\(_2\)/EO loading molar ratio of \(\frac{1}{4}\) and Pd(OAc)\(_2\)/NaBH\(_4\) molar ratio of \(\frac{1}{2}\)) (B). The scale bar in each picture is 50 nm.
However, Figure 3.31 (left) also clearly shows that these particles tend to aggregate. Moreover, in some cases the formation of a macroscopic precipitation of Pd upon addition of the reducing agent was observed. These observations explain the irreproducibility of the catalytic results for the reduced Pd(OAc)$_2$. Moreover, large aggregates were found in the case when the PEO core was used for templating (see Figure 3.31, right) explaining the low activity as well as the irreproducibility of the catalytic reactions. Therefore, it can be concluded that PCL chains with a DP > 6 can introduce a sufficient steric barrier for the aggregation of these nanoparticles. Moreover, the stabilized particles that do not show aggregation were highly active catalysts. The higher activity of these particles, if compared to the other investigated particles, is most likely due to a higher surface to volume ratio of the non-aggregating particles. Finally, the high activity of nanoparticles templated by polymers P7-P10 can be confirmed by the fact that a standard catalyst for these reactions, Pd(PPh$_3$)$_4$, showed significantly lower conversions, even at higher loadings of the catalyst (compare Table 3.7). This is especially interesting since the studied polymers are easy to synthesize on large scale and cheap, offering significant advantages to dendrimer stabilized or ligand based catalytic systems (compare e.g. a two step procedure for the preparation of block copolymers with a multi-step procedure for dendrimer synthesis) while still maintaining comparably high activity. Moreover, the general advantage of working with nanoparticles, e.g. the avoidance of toxic and expensive phosphine ligands$^{76}$ to obtain high conversions at low catalyst loadings, is of course also true for the here described system. Therefore, the investigated star-shaped block copolymer system is a very easy to synthesize, less toxic and cheap way prepare and stabilize highly active palladium catalysts.

### 3.4 Four and six arm star-shaped poly(ε-caprolactone)s

In order to obtain a series of 4-arm star-shaped polymers with different molecular weights, polymerizations of ε-caprolactone with pentaerythritol as co-initiator were performed in bulk at 130 °C for 8 h with stannous octoate as catalyst. Figure 3.32 schematically depicts the synthesis of polymers P13-P16 as well as their structure. Monomer to initiator (M/I) ratios of 20, 40, 60 and 80 were stirred at 130 °C for 15 minutes to obtain a homogeneous reaction mixture before stannous octoate was added (n(cat) = 1/20$^{th}$ of n(OH-functional groups) and the polymerizations were performed for 8 hours. Pentaerythritol was used as co-initiator for these polymerizations. All polymerizations were monitored in time by SEC revealing an increase of the molecular weight in time and narrow molecular weight distributions, as it can be expected for controlled polymerization techniques. Figure 3.33 (left) displays the monitoring of the reaction with a M/I ratio of 60 (polymer P15) in time as an example. Figure 3.33 (right) displays SEC results of polymers P13-P16 after workup. Narrow and symmetrical molecular weight distributions were obtained and an increasing molecular weight with
increasing M/I ratio was observed indicating the control over the polymerization system. The analytic data of all synthesized polymers is summarized in Table 3.8.

**Figure 3.32** Synthesis of 4-arm star-shaped poly(ε-caprolactone) polymers.

**Figure 3.33** Left: monitoring of the synthesis of polymer P15 by SEC in time. Right: SEC chromatograms of the final polymers P13-P16.

It is obvious that especially the molecular weight values obtained by SEC are lower than the theoretically expected molecular weights (see Figure 3.34, right). This effect was already explained in detail in section 3.3.1. Figure 3.34 (right) shows the number averaged molecular weight ($M_n$) values obtained by different measurement techniques are plotted against the M/I ratio for polymers P13 to P16. The SEC $M_n$ values show a too low molecular weight due to the calibration of the system with linear standards and a linear correlation between theoretical and experimental molecular weight values was found as expected for the above described reasons. In order to circumvent the mentioned SEC calibration problems, $^1$H NMR as well as MALDI-TOFMS were applied to investigate the synthesized polymers.
Table 3.8 Analytical data of the synthesized star-shaped poly(ε-caprolactone)s: $M_n$ (SEC): obtained using a linear poly(ethylene glycol) calibration; $^1$H NMR values calculated from signals e and f (see Figure 3.34), (molecular weights are reported in g/mol).

<table>
<thead>
<tr>
<th></th>
<th>$M_n$ (SEC)</th>
<th>PDI (SEC)</th>
<th>$M_n$ (MALDI)</th>
<th>$M_n$ ($^1$H NMR)</th>
<th>$M_n$ (theory)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P13</td>
<td>2150</td>
<td>1.09</td>
<td>2.520</td>
<td>3.200</td>
<td>2.420</td>
</tr>
<tr>
<td>P14</td>
<td>3600</td>
<td>1.14</td>
<td>4.570</td>
<td>5.250</td>
<td>4.700</td>
</tr>
<tr>
<td>P15</td>
<td>4650</td>
<td>1.19</td>
<td>6.400</td>
<td>7.550</td>
<td>6.990</td>
</tr>
<tr>
<td>P16</td>
<td>5650</td>
<td>1.19</td>
<td>8.300</td>
<td>10.400</td>
<td>9.230</td>
</tr>
<tr>
<td>P17</td>
<td>2850</td>
<td>1.17</td>
<td>4.510</td>
<td>4.650</td>
<td>4.250</td>
</tr>
<tr>
<td>P18</td>
<td>4330</td>
<td>1.19</td>
<td>7.680</td>
<td>8.100</td>
<td>7.670</td>
</tr>
</tbody>
</table>

Figure 3.34 (left) displays the $^1$H NMR spectrum of polymer P16 with all peaks assigned. The integral of the end-group signal $f$ decreased (compared to the polymer related integrals a-e) with increasing molecular weight of the polymers and was utilized to calculate the molecular weight of polymers P13-P16. Figure 3.34 (right) reveals a linear correlation of calculated number averaged molecular weights obtained from $^1$H NMR (the ratio of signal intensities of signals $f$ and e were utilized for the calculations) and targeted molecular weights (M/I ratio). This linear correlation is a further proof that the polymerizations were well controlled.

Moreover, polymers P13-P16 were investigated by MALDI-TOF mass spectrometry. The $M_n$ values obtained from these measurements are given in Table 3.8 and are plotted against the initial monomer over initiator ratio in Figure 3.33 (right). Also here a linear correlation was observed. However, the higher molecular weight polymers showed a slightly lower molecular weight than expected from theory. This might be explained by a mass discrimination effect during the MALDI experiments of the higher molecular weight polymers since it was especially difficult to analyze polymer P16 by MALDI-TOFMS. Figure 3.35 shows the
PEO-b-PCL block copolymers with different architectures

MALDI-TOFMS spectrum of polymer P13 with corresponding end-group assignment clearly confirming the structure of the obtained polymers. In this case an average repeat unit number of 5 was found per polymer arm which is in perfect agreement with the theoretically expected value. Similar assignments were also possible for polymers P14-P16.

Summarizing the analytical results, it can be concluded that a defined set of 4 arm star-shaped polymers was synthesized and fully characterized. The different measurement techniques independently proved that the polymerization of ε-caprolactone was controlled due to the fact that linear correlations were observed for targeted and obtained molecular weight values (see Figure 3.34, right). All applied techniques have their pros and cons: $^1$H NMR cannot provide polydispersity indices but gives absolute molecular weight values whereas SEC provides only relative molecular weight values but also PDIs. MALDI, as an absolute analytical technique, can provide both PDI and $M_n$ values but difficulties were observed for the higher molecular weight polymers. Therefore, only the combination of all techniques allowed a complete and comprehensive analysis of these polymers.

![Figure 3.35 MALDI-TOFMS spectrum of P13 with end-group assignment (matrix: dithranol).](image)

Having shown that defined star-shaped polymers can be obtained by the ring-opening polymerization of ε-caprolactone with pentaerythritol as initiator, the synthesis of 6 arm star-shaped poly(ε-caprolactone) polymers by applying dipentaerythritol as the initiator was also investigated. Two polymers with different molecular weights were synthesized (P17 and P18). Their analytical data is shown in Table 3.8 and their structure is schematically depicted in Figure 3.36 (left).
For these polymers similar conclusions can be drawn as for the 4 arm polymers. The molecular weight obtained by SEC is underestimated by a factor of two if compared to $^1$H NMR and MALDI-TOFMS for the same reasons as described above. SEC revealed narrow molecular weight distributions indicating controlled polymerizations (see Figure 3.36, right). Moreover, MALDI-TOFMS end-group analysis clearly showed the expected structure of polymers P17 and P18. Figure 3.37 displays the MALDI-TOFMS end-group analysis of polymer P17 with an average repeat unit number of 5 $\varepsilon$-caprolactone units for each polymer arm.
Therefore, it can be concluded that it is possible to prepare and fully characterize star-shaped poly($\varepsilon$-caprolactone)s with different architectures and molecular weights as it was shown in the above discussed examples. The investigated polymers are interesting materials for future research, since star-shaped block copolymers on the basis of these PCL stars might show interesting micellar behaviors. It would for instance be interesting to end-group modify the PCL stars with poly(ethylene oxide)s or with initiators for living polymerization techniques in order to obtain core-shell architectures with a hydrophilic corona and a hydrophobic core – the reversed architecture as discussed in section 3.3. Investigations in that direction are still ongoing.

3.5 Conclusions

Series of PEO-$b$-PCL block copolymers with linear and star-shaped architecture as well as star-shaped PCL homo polymers were synthesized and fully characterized. The polymerization of a $\varepsilon$-caprolactone was shown to be controlled in both cases since the obtained and targeted molecular weights were in good agreement. In particular, MALDI-TOFMS characterization of the block copolymers provided additional insights into the block copolymer architecture. The obtained linear block copolymers were investigated by DLS for their ability to form micelles revealing defined nanometer sized micelles. These micelles were tested for their encapsulation behavior with different guest molecules utilizing a high-throughput screening approach leading to the conclusion that guest encapsulation behavior seems to depend on the hydrophobicity of the guest molecule. For the star-shaped block copolymers the screening of their host guest properties was accomplished by utilizing a new UV/Vis plate reader extraction assay. The investigated polymers showed unimolecular micellar behavior. This unimolecular micellar behavior could be confirmed by dynamic light scattering and analytical ultracentrifugation. Their loading capacity could be evaluated utilizing the developed extraction assay leading to a better understanding of the requirements for unimolecular micellar behavior. Subsequently, a high-throughput screening experiment revealed that the star-shaped block copolymers are able to encapsulate a large variety of different guest molecules. More specifically, the fact that only two of the 24 investigated guests did not show any encapsulation behavior with the star-shaped block copolymers makes the investigated polymer class a highly promising candidate for drug delivery applications. Moreover, the large solubilizing effects, in combination with analytical ultracentrifugation experiments that demonstrated the guest transport capabilities of both block copolymers systems, offer interesting features with regards to potential drug delivery applications, since nonsolubility of drugs can be a major problem when developing a new therapy. Generally, the applied high-throughput screening approaches provided a fast and easy way to evaluate the encapsulation behavior of the investigated guest molecules. Therefore, these approaches
might be useful for pharmaceutical research since they can provide a fast and accurate way to evaluate if a certain drug can be encapsulated within a certain micellar carrier. Moreover, the synthesis and characterization of defined palladium nanoparticles utilizing the star-shaped block copolymers as templates for their formation was demonstrated. TEM revealed nanoparticles with an average size of about 4 nm. These nanoparticles were subsequently tested as catalysts for Heck cross-coupling reactions. The reactions were performed in an automated and parallel manner revealing a high activity of the studied nanoparticles for the studied C-C cross coupling reactions. These results clearly demonstrated that the investigated star-shaped block copolymers are cheap and effective templates for the formation of highly active palladium nanoparticle catalysts. Finally, 4- and 6-armed star-shaped PCL polymers were prepared in a controlled fashion and fully characterized. The further utilization of these materials as starting blocks for novel star-shaped block copolymer architectures is discussed.

In summary, the applied parallel and automated approaches, as they are commonly applied in combinatorial materials research, provided significant advantages for a fast and accurate determination of interesting material properties and led to an accelerated discovery of interesting properties of the investigated polymers.

3.5 Experimental

Chemicals and Reagents

All reagents were used without further purification unless stated otherwise. Solvents were purchased from Biosolve Ltd. (Valkenswaard, The Netherlands). Dithranol, stannous octoate, \(\varepsilon\)-caprolactone, pentaerythritol, dipentaerythritol, 4-bromoacetophenone, styrene, Pd(OAc)_2 as well as Pd(PPh_3)_2 were obtained from Aldrich (Oakville, On, Canada) and used as received. The \(\alpha\)-methoxy-\(\omega\)-hydroxy-poly(ethylene glycol) macroinitiator was obtained from Shearwater polymers (now: Nektar Therapeutics, Bradford, West Yorkshire, England) and dried by co-evaporation with toluene (3x) prior to use. The 5-arm star poly(ethylene glycol) macroinitiator was donated by the BASF AG (Ludwigshafen, Germany) and purified by column chromatography prior to use (\(\text{Al}_2\text{O}_3\), \(\text{CH}_2\text{Cl}_2\)).

Instrumentation

Size exclusion chromatograms for polymers P1-P4 as well as P13-P18 were measured on a Waters SEC system consisting of an isocratic pump, solvent degasser, column oven, 2996 photo diode array (PDA) detector, 2414 refractive index detector, 717plus autosampler and a Styragel HT 4 SEC column with precolumn installed. The eluent was \(N,N\)-dimethyl formamide (DMF) with 5 mM NH_4PF_6 at a flow of 0.5 mL/min. The column temperature was set to 50 °C. PEO standards were utilized for calibration.
Size exclusion chromatograms for polymers P5-P12 was measured on a Shimadzu system equipped with a SCL-10A system controller, a LC-10AD pump, a RID-10A refractive index detector, a SIL-10AD autosampler and a Polymer Laboratories Mixed-D column utilizing a chloroform:triethylamine:isopropanol (93:5:2) mixture as eluent at a flow rate of 1 mL/min and a column temperature at 50 °C. Calibration was performed utilizing linear poly(styrene) standards.

FT-IR spectra were recorded on a Bruker Tensor 37 with HTS-XT extension. Samples were prepared on Silicone microtiter plates by solution drop casting and subsequent solvent evaporation in vacuo.

UV/Vis as well as fluorescence spectra were recorded on a FlashScan 530 (AnalytikJena, Germany) in 96-well microtiter plates (polypropylene, flat bottom) from Greiner (Greiner Bio-One, Germany) in a range from 250 to 800 nm. All spectra were referenced to an empty microtiter plate and measurements were performed with four flashes. The actual time for the measurement of one microtiter plate with 96 full UV/Vis spectra was approximately 40 seconds.

NMR spectra were measured on a Varian Mercury 400 NMR spectrometer in deuterated chloroform unless otherwise stated. The chemical shifts were calibrated to TMS.

MALDI-TOFMS measurements were carried out on a Voyager-DE™ STR Biospectrometry™ Workstation time-of-flight mass spectrometer using reflector mode for operation. All spectra were obtained in the positive ion mode. Ionisation was performed with a 337 nm pulsed nitrogen laser. Samples were prepared in a multiple layer spotting approach with dithranol as matrix as described previously. Because of the complexity of copolymer mass spectra a homemade program written in Visual Basic 6.0 was used for their analysis. This program needs the molecular formulas of the repeat units, end groups, and cation as an input for a full mass spectral analysis.

Dynamic light scattering (DLS) experiments for polymers P1-P4 were performed at 25 °C at an angle of 90° on a Malvern 4700 DLS Particle Size Analyzer apparatus equipped with a 488 nm laser.

DLS measurements for polymers P6-P10 were performed on a Brookhaven Instruments Corp. BI-200 apparatus equipped with a BI-2030 digital correlator and a Spectra Physics He-Ne laser with a wavelength of 633 nm. The scattering angle used for the measurements was 90°. The Stokes-Einstein approximation was used to convert diffusion coefficient into hydrodynamic diameter (Dh).

Analytical Ultracentrifugation was performed using an Optima XL-A instrument from Beckman-Coulter. The sedimentation cell contained a two sector-shaped centerpiece. The sample volume was 300 µL. For polymers P1-P4 sedimentation velocity measurements were performed at 30,000 rpm, at a temperature of 20 °C and at a wavelength of 262 nm. A total of 100 concentration profiles, measured with absorption optics during the sedimentation process, were acquired. For polymers 5-10 sedimentation equilibrium measurements were performed in chloroform at 50,000 rpm, at a temperature of 20 °C and at a wavelength of 415 nm.
Chapter 3

Transmission electron microscopy (TEM) was performed on a Leo 922 microscope, operating at 200 KV accelerating voltage in bright field mode. Samples for TEM experiments were prepared by drop-casting the palladium-loaded micelles before and after reduction with NaBH4 on a carbon-coated TEM grid.

GC-MS analysis was performed on a Shimadzu GC-MS-QP5000, the mass values are reported as mass/charge ratio (m/z). All spectra were measured with a column temperature program from 80-300 °C (25 °C/min) and 3 minutes hold time at 300 °C. The injection temperature was 300 °C and the detector temperature 250 °C.

Automated and parallel reactions were carried out on a Chemspeed ASW2000 automated synthesizer using one reactor block with 16 reactor vessels of 13 mL (up to 16 reactions in parallel). All reaction vessels were equipped with a heating jacket and cold-finger reflux condensers. The ASW2000 was connected with a Huber Unistat 390W cryostat.

Synthesis of polymers P1-P4

The dried α-methoxy-ω-hydroxy-poly(ethylene glycol) (800 mg, 0.27 mmol) was weighed into a dry flask and ε-caprolactone (different amount according to different M/I ratios) was subsequently added. The reaction mixture was stirred for 5 minutes at 130 °C in a preheated oilbath before the catalyst (stannous octoate, 1 drop) was added and the polymerization was performed for 3 hours. The resulting viscous solution was rapidly cooled upon which it solidified. The crude polymer was dissolved in dichloromethane and precipitated in heptane. The isolated yields were in the order of 90-95% for the different polymers. 1H NMR (CDCl3): δ = 1.38 [tt, J = 8.1, 7.3 Hz, COOCH2CH2CH2CH2CH2COO], 1.65 [m, COOCH2CH2CH2CH2CH2COO], 2.31 [t, J = 7.3 Hz, CH2COO], 3.64 [s, PEO-backbone], 4.06 [t, J = 6.6 Hz, COOCH2].

Micelle preparation of polymers P1-P4

The PEO-b-PCL block copolymers were dissolved in the unselective solvent THF and subsequently an at least 50 fold excess of deionized water was added dropwise while stirring. The remaining THF in the resulting micellar solution was removed under a flow of nitrogen. Finally, the concentration of the micellar solution was adjusted to 0.5 wt%.

High-Throughput screening of guest encapsulation of polymers P1-P4

The guest encapsulation of polymer micelles of PEO-b-PCL block copolymers was investigated by a high-throughput screening approach. Stock solutions of 12 different guest molecules in THF (10 mg/mL) and of polymer P3 (compare Table 3.1) in THF (100 mg/mL) were prepared. Subsequently, 20 µL of the different guest solutions were mixed with 10 µL of polymer stock solution and transferred to the respective positions in the microtiter plate (see Figure 3.6, guests 1-12 in positions B1 to B12 and D1 to D12, respectively). Afterwards, the solutions of guest molecules were diluted with 200 µL of 0.01 M HCl (positions B1 to B12) or 0.01 M NaOH (positions D1 to D12) to induce the micellization process. Positions A1 to
A12 as well as C1 to C12 were filled in the same fashion without the addition of polymer. Therefore, they contained the pure guests without micelles. Finally, the rows A to D were diluted 1:5 with 0.01 M HCl (rows E and F) and 0.01 M NaOH (rows G and H) into rows E to H. This resulted in eight sets of the 12 guest molecules, explicitly guest only and guest in the presence of micelles formed from polymer P3 in both acidic and basic environment at two concentration levels. UV/Vis spectra in a range from 250 to 900 nm as well as fluorescence spectra with different excitation wavelength (390 to 590 nm in 20 nm steps) were recorded.

The names of the investigated guest molecules are provided in Table 3.9.

Table 3.9 Investigated guest molecules.

<table>
<thead>
<tr>
<th>Guest</th>
<th>Guest Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl orange</td>
</tr>
<tr>
<td>2</td>
<td>Disperse Red 1</td>
</tr>
<tr>
<td>3</td>
<td>Disperse Blue 3</td>
</tr>
<tr>
<td>4</td>
<td>Bromophenolblue</td>
</tr>
<tr>
<td>5</td>
<td>9-(Chloromethyl)anthracene</td>
</tr>
<tr>
<td>6</td>
<td>9-(Hydroxymethyl)anthracene</td>
</tr>
<tr>
<td>7</td>
<td>trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile</td>
</tr>
<tr>
<td>8</td>
<td>Dithranol</td>
</tr>
<tr>
<td>9</td>
<td>2,5-Dihydroxybenzoic acid</td>
</tr>
<tr>
<td>10</td>
<td>α-cyano-4-hydroxycinnamic acid</td>
</tr>
<tr>
<td>11</td>
<td>trans-3-Indoleacrylic acid</td>
</tr>
<tr>
<td>12</td>
<td>2-(4-Hydroxy-phenylazo)-benzoic acid</td>
</tr>
</tbody>
</table>

Synthesis of polymers P5–P10

All polymerizations were performed in an ASW2000 automated synthesizer. Reaction vessels were heated to 120 °C, evaporated in vacuo for 30 minutes and filled with argon. This procedure was repeated three times and the reactions were performed under an argon atmosphere. First, stock solutions of catalyst (n(cat)=1/20th of n(OH-functional groups) and initiator (amount according to targeted molecular weight) (both in CH₂Cl₂) were added and subsequently the solvent was removed in vacuo. Subsequently, the ε-caprolactone (2.5 mL, 2.575 g, 22.6 mmol) was added to the reaction vessels. The reaction mixture was well stirred before the polymerization was carried out for 3 h at 130 °C in bulk. The polymers were purified by column chromatograph (Al₂O₃, CH₂Cl₂). The isolated yields were in the order of 90-95% for all polymers. ¹H NMR (CDCl₃): δ = 1.38 [tt, COOCH₂CH₂CH₂CH₂CH₂COO], 1.65 [m, COOCH₂CH₂CH₂CH₂CH₂COO], 2.31 [t, CH₂COO], 2.55-2.80 [m, 18 H, CH₂OCH₂CH₂NCH₂CH₂N], 3.50 [t, 10 H, CH₂OCH₂CH₂NCH₂CH₂N], 3.55-3.80 [m, PEO-backbone], 4.06 [t, COOCH₂], 4.23 [t, 10 H, COOCH₂CH₂OCH₂].
High-Throughput screening of guest encapsulation of polymers P5-P10

The guest encapsulation behavior of unimolecular micelles of PEO-\(b\)-PCL star-shaped block copolymers was investigated by a high-throughput screening approach. The plate was filled with 20 \(\mu\)L equivalents of saturated solutions of all 24 guest molecules in 0.01 M HCl (positions A1 to H6) and 0.01 M NaOH (positions A7 to H12), respectively. Subsequently, the solutions of guest molecules were diluted to 250 \(\mu\)L with 0.01 M HCl (positions A1 to H3), 0.01 M NaOH (positions A7 to H9), 200 mg/mL polymer P12 in 0.01 M HCl (positions A4 to H6) and 200 mg/mL polymer P12 in 0.01 M NaOH (positions A10 to H12), respectively. This resulted in four sets of the 24 guest molecules, explicitly guest only and guest in the presence of polymer P12 in both acidic and basic environment.

Similarly, a plate with dichloromethane (DCM) was prepared with saturated solutions of the 24 guest molecules in DCM and P12 in DCM resulting in 48 filled wells in the microtiter plate. The remaining 48 wells were used to dilute the first 48 wells with a factor of 1:5.

The investigated guest molecules and the final outcome of the screening are provided in the following Table 3.10. Moreover, Figures 3.38 and 3.39 provide additional data of the performed screening experiment.

<table>
<thead>
<tr>
<th>Guest</th>
<th>Guest Name</th>
<th>Hit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl orange</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Disperse Red 1</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Disperse Orange 3</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Disperse Blue 3</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Disperse Yellow 7</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Solvent Green 3</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>Fat Brown RR</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>Fast Violet B</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>Phenolphthalein</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>Bromophenolblue</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>9-(Chloromethyl)anthracene</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>9-(Hydroxymethyl)anthracene</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>2,2':6',2''-Terpyridine</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>4,4'-Bipyridine</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>1,10-Phenanthroline hydrochloride monohydrate</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>5-Nitro-1,10-phenanthroline</td>
<td>Yes</td>
</tr>
<tr>
<td>17</td>
<td>trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile</td>
<td>Yes</td>
</tr>
<tr>
<td>18</td>
<td>Dithranol</td>
<td>Yes</td>
</tr>
<tr>
<td>19</td>
<td>2,5-Dihydroxybenzoic acid</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>(\alpha)-cyano-4-hydroxycinnamic acid</td>
<td>Yes</td>
</tr>
<tr>
<td>21</td>
<td>trans-3-Indoleacrylic acid</td>
<td>Yes</td>
</tr>
<tr>
<td>22</td>
<td>2,4-(4-Hydroxy-phenylazo)-benzoic acid</td>
<td>Yes</td>
</tr>
<tr>
<td>23</td>
<td>Fluorene</td>
<td>No</td>
</tr>
<tr>
<td>24</td>
<td>Copper(II) phthalocyanine-tetrasulfonic acid tetrasodium salt</td>
<td>No</td>
</tr>
</tbody>
</table>
Figure 3.38 Screening results of two guest molecules in 0.01 M HCl (left) and 0.01 M NaOH (right) visualised by UV/Vis spectroscopy. Only the spectrum of guest 2 reveals a shift of the absorption maximum, whereas guest 24 does not show a shift in its absorption characteristics.

Figure 3.39 Fluorescence intensity increase due to encapsulation of two guest molecules. Left: fluorescence of guest 18 under 450 nm excitation in dichloromethane (DCM). Right: fluorescence of guest 11 under 390 nm excitation in 0.01 M NaOH.

Preparation of palladium nanoparticles
The copolymers P5-P10 were dissolved in DMF at a concentration of 1 g/L. A known amount of a Pd(OAc)₂ solution in DMF (10 g/L) was subsequently added to the copolymer solution and the mixture was stirred for 24 h. The palladium to ethylene oxide (Pd/EO) molar ratio was varied. The excess of Pd(OAc)₂ not interacting with the PEO core was removed by dialysis for 30 min against pure DMF. A known amount of a NaBH₄ solution in DMF was finally added to the copolymer loaded with Pd(OAc)₂. Palladium nanoparticles were also prepared without any added copolymer. For this sample, the amounts of Pd(OAc)₂ and NaBH₄ were identical to the ones used for the metallization of sample 1 with Pd(OAc)₂/EO molar ratio of ¼ and Pd(OAc)₂/NaBH₄ molar ratio of ½. Palladium nanoparticles were finally prepared in the presence of the PEO macroinitiator at 1 g/L without any PCL outer blocks with Pd(OAc)₂/EO molar ratio of ¼ and Pd(OAc)₂/NaBH₄ molar ratio of ½.
Chapter 3

General procedure for catalytic reactions in the ASW2000 automated synthesizer

Reaction vessels were heated to 120 °C, evaporated in vacuo for 30 minutes and filled with argon. This procedure was repeated three times and the reactions were performed under an argon atmosphere. First, stock solutions of 4-bromoacetopheneone (c = 1 mol/L, 1.6 mL, 1.6 mmol) and the palladium nanoparticles (varying concentration) were transferred with the liquid handling system of the synthetic robot to the respective reactors. Subsequently, styrene (0.206 mL, 187 mg, 1.8 mmol) and triethylamine (0.278 mL, 194 mg, 1.92 mmol) were added to the reactors. Finally, the reactors were filled up to a volume of 4 mL with DMF resulting in a 4-bromoacetophenone concentration of 0.4 mmol/mL. The reaction mixtures were then deoxygenized by 10 consecutive vacuum/argon cycles to ensure the inert atmosphere required for these reactions. The reactions were finally performed under an argon atmosphere for 24-48 h at 100 °C and monitored by GC-MS. Therefore, samples (50 µL) were taken out of the reactors and filled into GC vials automatically in predefined intervals. These GC vials were capped and pre-filled with chloroform. After the reaction was finished these vials were transferred to the autosampler of the GC-MS and measured automatically. For all catalytic reactions the nanoparticles were freshly prepared prior to use (not older than 24 h) since ageing of the particles resulted in slightly reduced activities (e.g. 25% less conversion for Pd-nanoparticle 6 after 6 weeks of ageing).

Synthesis of polymers P13-P18

Polymerizations leading to P13-P16 were performed with 20.00 g (20.60 mL, 0.18 mol) of monomer for 8 hours, whereas polymerizations leading to P17-P18 were performed at 1.00 g (1.03 mL, 8.75 mmol) for 3 hours. The initiator amount was calculated according to the respective M/I ratios. The monomer, ε-caprolactone, and the initiator, pentaerythritol for P13-P16 and dipentaerythritol for P17-P18, were added to a flask and stirred at 130 °C for 15 minutes in order to obtain a homogeneous solution. Subsequently, the polymerization was started by adding the catalyst, stannous octoate (n(cat) = 1/20th of n(OH-functional groups)). After the reaction time was elapsed the very viscous reaction mixtures were cooled to room temperature in an ice bath to stop the polymerization. The polymers were purified from residual monomer and catalyst by precipitation from a concentrated dichloromethane solution into ice cold heptane resulting in powdery products for P14-P18 and a waxy solid for P13. All yields were in the order of >95%. The corresponding analytical data is thoroughly described in the article text.
3.6 References

[46] The synthesis of P11 and P12 was performed as described in section 3.3.1 on lab scale with 25 mL (25.8 g, 225.6 mmol) ε-caprolactone. P11: 8.1 g (3.8 mmol) P12: 32.3 g (15.0 mmol) 5-arm poly(ethylene glycol) macroinitiator, respectively and 1/20th of stannous octoate catalyst (according to OH groups) at 130 °C for 6 h.
PEO-b-PCL block copolymers with different architectures

CHAPTER 4

Terpyridine-containing supramolecular polymers

Abstract
The synthesis and detailed characterization of metal-containing supramolecular polymers based on the terpyridine chelating ligand is described. The investigations were started due to the need of improved characterization methods for these polymers. A MALDI-TOFMS model study revealed the relative binding strength of the terpyridine ligand for a variety of different transition metal ions that are frequently used in these supramolecular polymers, whereas a size exclusion chromatography (SEC) study resulted in optimized chromatographic conditions for ruthenium-containing polymers. Especially the SEC study was crucial for the success of a subsequently performed parallel optimization of the reaction conditions of RuCl₃ with a defined low molecular weight bis-terpyridine ligand where it was applied as the major screening technique. These investigations led to the development of a new type of supramolecular ABA triblock copolymer based on a simple polycondensation strategy that revealed amphiphilic behavior and formed micelles in water. Moreover, a grafting to approach utilizing terpyridine metal complexation was investigated. Finally, the investigation of a star-shaped supramolecular polymer by means of parallel high-throughput techniques led to the accelerated development of a new sensoric system for transition metal ions. This system is based on the encapsulation of fluorophores within a terpyridine end-group modified star-shaped polymer and quenching of the fluorescence due to metal complex formation.

4.1 Introduction

The combination of macromolecular and supramolecular chemistry opens ways to obtain new functional materials that can be designed by the application of state-of-the-art controlled and living polymerization techniques teamed up with organic synthesis for the introduction of functional moieties into the polymer architecture. Recent years have shown that polymers containing supramolecular binding units are easily synthetically accessible. These ‘supramolecular polymers’ can generally be defined as polymer chains of small molecules held together via reversible, non-covalent bonds. The most prominent examples of supramolecular polymers can be found in hydrogen bonded systems, systems that utilize metal ligand interactions and systems that apply ionic interactions. All of the mentioned non-covalent interactions have certain advantages and disadvantages. Concerning metal ligand interactions the adjustability of their binding strength (approximately between 25 and 95% of covalent C-C bonds with a bond energy of 350 kJ/mol) as well as their directionality can be considered an advantage, whereas the toxicity of some transition metal ions is a definite disadvantage. Hydrogen bonding motifs on the other hand can generally be considered as less toxic, but span a considerably smaller range of binding strengths (approximately 1-20% of covalent C-C bonds).

Figure 4.1 Schematic representation of the formation supramolecular polymers with self-complementary (top) and non self-complementary binding units (bottom).

Figure 4.1 shows the formation of linear chain extended polymers via self-complementary supramolecular interactions as well as the formation of linear strictly alternating copolymers via non self-complementary interactions as examples. Such systems were for instance realized by the utilization of hydrogen bonded systems with a quadruple hydrogen bonding motif (UPy, self-complementary, see Figure 4.2) or non self-complementary hydrogen bonding motifs (Upy and Napy, see Figure 4.2). This concept is illustrated in more detail in Figure 4.2. The key discovery that allowed the preparation of the alternating copolymers with UPy and Napy is the fact that the Upy/Napy dimer with an ADDA/DAAD binding motif has an improved stability if compared to the homo dimers of Upy (see also Figure 4.2). Moreover, it is possible to utilize metal ligand interactions with terpyridine-ligands and, e.g., iron(II) cations and other transition metal ions (e.g. Ni, Co, Cu) in oxidation state +2 (self-
complementary) or terpyridine-Ru(III) monocomplexes in combination with free terpyridine ligands\textsuperscript{16} (non self-complementary) (see Figure 4.3) for the formation of a variety of supramolecular polymer architectures.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.2.png}
\caption{Illustration of the working principle of self-complementary and non self-complementary quadruple hydrogen bonding units. Left: Upy: 2-ureido-4-pyrimidone. Right: Top: NAPY: 2,7-diamido-1,8-naphthyridine; bottom: Upy tautomer with ADDA binding motif. A: H-Bond Acceptor; D: H-Bond Donor.}
\end{figure}

It should be noted here that these systems do, of course, not only form linear polymers but can also form cyclic structures as it will be discussed later. In addition to the supramolecular binding motifs a variety of different interactions, such as host-guest chemistry\textsuperscript{17} or halogen bonding\textsuperscript{18} were shown to be useful for the construction of supramolecular polymers. Moreover, several examples have demonstrated that these new materials can provide interesting material properties including for instance thermo-, chemo-, and mechano-responsive supramolecular polyelectrolyte gel-like materials that show thixotropic behavior\textsuperscript{19} or solution processable fluorescent polymers with tunable emission wavelength ranging from blue (427 nm) to yellow (565) due to variations of the ligand set of zinc quinolate complexes in the polymer.\textsuperscript{20} For the class of metal-coordinating polymers, terpyridine metal-complexes are particularly widely applied as building blocks in supramolecular and macromolecular chemistry (see Figure 4.3). Generally, 2,2':6',2''-terpyridines are tridentate ligands that can form complexes with a large variety of transition metal ions and their functionalization in the 4'-position (starting from commercially available 4'-chloro-2,2':6',2''-terpyridine) allows a straightforward access to supramolecular polymers and monomers. Some recent literature examples of metal-containing supramolecular polymers include a large variety of different polymer architectures, such as dendrimers,\textsuperscript{21,22} rods,\textsuperscript{23} linear chain extended polymers,\textsuperscript{24,25} copolymers,\textsuperscript{26,27} block copolymers,\textsuperscript{28,29} or graft copolymers.\textsuperscript{30} A highly interesting aspect of this class of supramolecular polymers is the reversibility of the corresponding metal
complexes. Furthermore, by the choice of the appropriate transition-metal ion for the complexation of the terpyridine binding unit, the binding strengths as well as optical and redox properties within the functional material can be varied and adjusted. As already shortly mentioned, it is in principle possible to realize all architectures that are known for classical polymers by applying the terpyridine-metal complexation chemistry. For instance, it has been reported that the preparation of well-defined block copolymers is feasible via selective mono-complex formation of Ru(III)-ions with terpyridine ligands and subsequent reaction with a second terpyridine ligand (under reducing conditions) leading to block copolymer systems with a large variety of different polymeric building blocks (see also Figure 4.3).

The micellar properties of such systems are of particular interest since it is for instance possible to cleave the corona of the micelles with competitive ligands in order to obtain defined nano-objects composed of the micellar core material. This is an interesting example of how the presence of supramolecular binding units can be utilized to prepare new materials that would otherwise be difficult to access.

![Figure 4.3](image)

**Figure 4.3** Illustration of the formation of octahedral bis-terpyridine metal complexes (see three dimensional model: top, left). Top: Self complementary complex formation. Bottom: Non self-complementary formation of heteroleptic bis-terpyridine Ru(II) complexes. Counter ions are omitted for clarity reasons.

The following sections describe the synthesis and detailed characterization of metal-containing supramolecular polymers based on the terpyridine chelating ligand using parallel methods wherever applicable. However, before the synthesis of supramolecular ABA triblock copolymers (section 4.3.1) or the evaluation of a new sensoric system (section 4.3.3) based on terpyridine ligands will be discussed, MALDI-TOFMS as well as SEC model studies will be
described, since improved characterization techniques for the studied metal-containing materials were a prerequisite for the success of these studies.

### 4.2 Development of suitable analytics

In contrast to the significant progress regarding the synthesis of terpyridine and bipyridine containing compounds, the characterization of the corresponding polymer complexes is far less developed. Nevertheless, in order to design new functional materials, a detailed analysis of the obtained compounds is a prerequisite. In particular the molecular weight and molecular weight distribution have to be determined since (as for classical polymers) the material properties of such systems are highly depending on their molecular mass. However, the determination of the molecular weight of such polymers represents a special challenge: size exclusion chromatography (SEC), one of the most frequently applied methods for molecular weight determinations, turned out to be difficult or even impossible in the case of reversible systems such as hydrogen-bonds or weak metal-ligand interactions (e.g. zinc(II) or cobalt(II) bis-terpyridine complexes) since i) column interactions could lead to a breaking of the mentioned non-covalent interactions and/or ii) high molecular weight species of these dynamic systems might re-equilibrate in the course of the measurement due to dilution. Moreover, in equilibrium no fixed molecular weight exists due to a continuous opening and reestablishing of the supramolecular interactions. Therefore, standard SEC analysis of bi- and terpyridine metal complex containing polymers is rather difficult even if several additives are utilized to prevent the mentioned column interactions and the more stable iron or ruthenium complexes are investigated. Many authors tend not to mention the problem or to obtain molecular weights utilizing other techniques than SEC. Nevertheless, some examples of successful analysis are reported in the literature. However, often these successful cases are not reproducible on new SEC columns but only on frequently used columns. This might be an effect of successful blocking of active sites on the columns over prolonged periods of time due to the analysis of thousands of samples. However, this does not mean that the results obtained on these columns cannot be trusted, since separation still takes place on the basis of size exclusion.

Another important method for the analysis of telechelics and polymers is MALDI-TOFMS (see also Chapter 2). Mass spectrometry in general has become a superb technique for the characterization of non-covalent species since soft ionization techniques such as ESI or MALDI have become available. Electrospray ionization mass spectrometry (ESI-MS) is often used to investigate metal-ligand interactions, to determine metal-ligand binding energies or relative affinities of ions towards complexing ligands. However, this technique has some major problems such as the formation of solvent clusters under mild source conditions or the lack of sensitivity under harsh conditions. During the last years, matrix
assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) is used more and more for the investigation of non-covalent interactions, such as transition metal ion-peptide complexes\textsuperscript{49} or metal-ligand complexes.\textsuperscript{50,51} As a soft ionisation method,\textsuperscript{52} the MALDI process provides the mild conditions required for the analysis of these non-covalent assemblies.

4.2.1 A MALDI-TOFMS model study

The goal of this study was a detailed understanding of the above described supramolecular assemblies by means of their fragmentation behavior under MALDI conditions. A new approach to relative binding strength of metal ligand complexes using variable laser energies in MALDI-TOFMS is presented for 4’-(1,4,7-trioxa-octyl)-2,2’:6’,2’’-terpyridine transition metal complexes of the type $[\text{M}^{II}\text{L}_2]^{2+}$ (see Figure 4.4). The relative binding strength is depending on the central ion of the complex and was obtained by taking MALDI mass spectra of the different compounds at varying laser intensities. The signal intensity ratio of ligand to complex ([LH]$^+$/[ML$_2]^+$) is depending on the laser intensity used for the spectrum acquisition. By considering a [LH]$^+$/[ML$_2]^+$ ratio $> 10$ as the point of complete complex breaking it is possible to establish a row of complex stabilities depending on the metal ion used because this point is reached at different relative laser intensities for the different complexes.

![Figure 4.4](image-url) Structure of the studied bis-complexes of 4’-(1,4,7-trioxa-octyl)-2,2’:6’,2’’-terpyridine with different transition metal ions ($M = \text{Mn, Fe, Co, Ni, Cu, Ru}$).

Interpretation of MALDI-TOFMS spectra of the investigated metal-ligand complexes.

The bis(4’-(1,4,7-trioxa-octyl)-2,2’:6’,2’’-terpyridine) metal (Mn(II), Fe(II), Co(II), Ni(II), Cu(II), and Ru(II)) complexes (see Figure 4.4) were investigated measuring MALDI spectra at different laser intensities and calculating [LH]$^+$/[ML$_2]^+$ ratios as described above. The complexes were fully characterized by $^1$H NMR, $^{13}$C NMR, UV/Vis spectroscopy as well as elemental analysis in order to confirm their structure and purity. Spectra of all compounds with assigned peaks are shown in Figure 4.5.
Figure 4.5 MALDI-TOFMS spectra of all investigated bis-4’-(1,4,7-trioxa-octyl)-2,2':6',2''-terpyridine transition metal complexes. Peaks corresponding to the matrix are not assigned.

The signal corresponding to the uncomplexed ligand with one proton attached at a m/z value of 352.2 Da was found in every spectrum, whereas the signal from the mono-ligand-complex [LM]⁺ was only present in the cases of copper and nickel. Peaks corresponding to dithranol adducts of the mono-metal-complex [LM + Dithranol - H]⁺ were detected in all spectra, except the ones of ruthenium. Complexes without counter ions [L₂M]⁺ were detected for all metal ions, whereas signals with one [PF₆]⁻ counter ion [L₂MPF₆]⁺ were observed for all compounds, except manganese. All observed signals were singly positive charged as
confirmed by their isotopic patterns. This special effect, observed even if two counterions are lost, is discussed in detail by Karas et al.\textsuperscript{53} Signals corresponding to the complex without loss of counter ions were not detected in any case.

\textit{Estimation of the relative binding strength}

As described above, all complexes were investigated applying different laser energies during MALDI measurements. A general trend for all compounds was observed: the ratio of the signal intensities belonging to the ligand [LH]\textsuperscript{+} and the complex [ML\textsubscript{2}]\textsuperscript{+} ([LH]\textsuperscript{+}/[ML\textsubscript{2}]\textsuperscript{+}) is depending on the laser intensity used for the spectrum acquisition. Starting from a certain point, this ratio is increasing continuously with increasing laser intensity during the laser desorption process. This behavior may be explained as a breaking or destruction of the complex with increasing applied thermal energy and photon-intensity due to increased laser intensity. It is also known from literature that supramolecular architectures decompose in the MALDI process depending on the metal ion used for complexation.\textsuperscript{54,55} However, this study is the first attempt to quantify and better understand this behavior. Figure 4.6 shows the iron complex at different laser intensities in order to illustrate the described behavior of the complexes. All peaks, except the [L\textsubscript{2}Fe]\textsuperscript{+} (758.25) and [L + H]\textsuperscript{+} (352.17) peaks, were deleted in this figure for a more pronounced demonstration. The signal intensities were normalized to the intensity corresponding to the free ligand. A general trend is clearly observable: the ratio [LH]\textsuperscript{+}/[ML\textsubscript{2}]\textsuperscript{+} increases with increasing laser intensity. All investigated complexes behave in this way, but the [LH]\textsuperscript{+}/[ML\textsubscript{2}]\textsuperscript{+} ratios > 10 are reached at different laser intensities. Matrix reactions with the analyte were considered to not influence the discussed ratios. Typical curves that represent this behavior are shown in Figure 4.7. The discussed ratio of all investigated complexes is plotted versus the relative applied laser intensity. It is obvious that, depending on the metal ion, the different complexes show a different degradation behavior under the applied MALDI conditions. If a [LH]\textsuperscript{+}/[ML\textsubscript{2}]\textsuperscript{+} ratio > 10 is considered as the point of complete complex breaking it is possible to estimate a order of relative complex stabilities depending on the central metal ion: Co > Ru > Fe > Ni > Cu > Mn. This ratio was chosen to assure a high grade of complex dissociation, however, as depicted in Figure 4.7, the order of relative binding strength could also be established at lower values for the [LH]\textsuperscript{+}/[ML\textsubscript{2}]\textsuperscript{+} ratio. The errors made in measuring the ratios can be quite high (up to 15\%). Nevertheless, the row of relative binding strength does not change, even if the “worst” case is considered. Furthermore the order of the discussed relative binding strength was found to be fully reproducible. Moreover, Figure 4.8 shows the ionic species that could be formed during the ionization process and their possible fragmentation patterns. The gray species were not observed in any spectrum and all fragmentation reactions 1 to 6 will be discussed in detail later.
**Figure 4.6** Four MALDI-TOFMS spectra of the bis-4’-(1,4,7-trioxa-octyl)-2,2’:6’,2’’-terpyridine-iron(II) complex investigated at four different laser intensities. All peaks, except the ones belonging to the ligand (LH) and the intact complex (L₂Fe) are omitted for clarity reasons.

**Figure 4.7** Degradation behavior of the investigated complexes under MALDI-TOFMS conditions.
In the cases of ruthenium and cobalt, the 10:1 ratio could not be reached because measuring of the spectra became difficult due to detector overload at the high applied laser intensities. However, extrapolation to this value makes it possible to establish the order of relative binding strength. To assure that this behavior of complex dissociation is not an effect of dithranol, the nickel and iron complexes were also investigated with DHB and CHCA as matrix substance at varying laser intensities. Both complexes showed the same fragmentation of the complex and an increase of the \([\text{LH}]^+\)/[\text{ML}_2]^+ ratio with increasing laser intensity independent of the matrix used. The results also indicated that CHCA has a smaller stabilizing effect on the complexes than dithranol and therefore a higher degree of degradation at lower laser intensities can be observed compared to dithranol. DHB on the other hand showed lower potential to ionize these complexes and as an effect the complexes can only be detected at higher laser intensities, which led to high complex fragmentation. Due to these reasons dithranol was chosen as matrix for the estimation of the relative binding strength of the investigated complexes.

**Discussion of fragmentation patterns**

Figure 4.8 shows possible fragmentation pathways of the generated ions. Reactions 1 to 7 would affect the ligand/complex ratio and therefore change the order of relative binding strength. To ensure that these reactions can be neglected, more calculations of different ratios were performed for the iron and nickel complexes as examples. The considered ratios and their behavior depending on the applied laser energy are shown in Figure 4.9.

**Figure 4.8** Possible fragmentation pathways of generated ions (black). Reactions 1 to 6 would affect the ligand/complex ratio and therefore change the order of relative binding strength. Not observed species are grayed out.
In Figure 4.9 (right) all the mentioned ratios are plotted against the relative laser intensities for the nickel complex. Pathways 1 and 6 to 8 revealed no significant alteration of the corresponding ratio with changing laser intensities. Therefore it can be concluded that no complex or ligand is formed due to fragmentations of \([LM+\text{Dithranol-H}]^+\), \([L_2\text{MPF}_6]^+\) or \([LM]^+\) and that these pathways do not affect the \([\text{LH}]^+/[L_2\text{M}]^+\) ratio. Pathways 2 to 5 resulted in an increase in the respectively ratio. It should be noted here that pathways 3 and 4 as well as 2 and 5 are strongly interconnected since, e.g., the fragmentation \([L_2\text{M}]^+\) does most certainly produce of \([\text{LH}]^+\) and \([LM]^+\) species. However, pathway 5 is the most important one for this study. If the complex \([L_2\text{M}]^+\) breaks with increasing laser intensity, then Ligand \([\text{LH}]^+\) and \([LM]^+\) species are the fragmentation products. Therefore, it is understandable that also the ratios of pathways 2 to 4 increase with increasing laser intensity, because \([LM]^+\) and \([\text{LH}]^+\) species are produced during the fragmentation of the complex. Moreover, pathways 2 to 4 describe the same phenomenon as pathway 5, namely the fragmentation of intact complexes. The iron complex revealed a similar fragmentation behavior as the nickel one, except that the \([L_2\text{MPF}_6]^+\) and the \([LM]^+\) peaks are not observed in the spectra of the iron complex. Therefore, the fragmentation pathways 1-4, 6 and 8 can be neglected and one can conclude that the increasing \([\text{LH}]^+/[\text{ML}_2]^+\) ratio with increasing laser intensity is only affected by the destruction of the complex and not by other fragmentations.

<table>
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<th>Pathway</th>
<th>Corresponding-ratio</th>
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<tr>
<td>1</td>
<td>([L_2\text{M}]^+/[L_2\text{MPF}_6]^+)</td>
</tr>
<tr>
<td>2</td>
<td>([LM]^+/[L_2\text{M}]^+)</td>
</tr>
<tr>
<td>3</td>
<td>([LM]^+/[L_2\text{MPF}_6]^+)</td>
</tr>
<tr>
<td>4</td>
<td>([\text{LH}]^+/[L_2\text{MPF}_6]^+)</td>
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<tr>
<td>5</td>
<td>([\text{LH}]^+/[L_2\text{M}]^+)</td>
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<tr>
<td>6</td>
<td>([\text{LH}]^+/[LM]^+)</td>
</tr>
<tr>
<td>7</td>
<td>([\text{LH}]^+/[LM+\text{Dithranol-H}]^+)</td>
</tr>
<tr>
<td>8</td>
<td>([LM]^+/[LM+\text{Dithranol-H}]^+)</td>
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**Figure 4.9** Left: Occurring species in MALDI mass spectra of the investigated complexes with their corresponding fragmentation pathway. Right: plot of calculated ratios of occurring species in MALDI mass spectra of the nickel complex versus relative laser intensity.

**Comparison of the relative binding strength with literature data.**

Unfortunately, only little is known in literature about the binding strength of these or similar bis-terpyridine complexes, especially if the investigation of complete complex series is taken into account. Only recently, a series of metal salts (Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), and Zn\(^{2+}\)) in combination with unsubstituted 2,2’:6’,2”-terpyridine was investigated by isothermal titration calorimetry revealing binding constants in the order of \(10^8\) M\(^{-1}\) for the mono complex
formation and $10^{10} \text{ M}^{-1}$ for the bis complex formation, respectively.\textsuperscript{56} However, no clear trend for the different metal ions could be established. The here reported relative binding strength is for instance in good agreement with the results from Satterfield and Brodbelt, who investigated the relative binding strength of 2,2':6',2''-terpyridine complexed with Cobalt, Nickel and Copper by using energy-variable collisionally activated dissociation in a quadrupole ion trap mass spectrometer. They found the following relative binding strength for the mentioned complexes: Co $>$ Ni $>$ Cu.\textsuperscript{46} The relative binding strength also correlates with data from thermogravimetric analysis (TGA) of 5,5'-dimethyl-2,2':6',2''-terpyridine metal complexes, which reveals that the cobalt complex has a higher thermal stability than the manganese one.\textsuperscript{57} The data obtained from kinetic studies (Co $>$ Ni $>$ Fe)\textsuperscript{58} only agrees to a certain extent, but this is understandable since it is difficult to compare solution with solid phase properties (in solutions also the kinetic stability of the metal complexes play an important role). The data described above, however, represent complex stabilities under MALDI conditions, which should be more related to thermodynamic stabilities. The knowledge of the obtained order of relative binding strength for the model system is of significant importance. Transferring this knowledge to supramolecular polymers, which contain the investigated terpyridine groups, leads to an improved understanding of these systems and simplifies their characterization as described below.

**Investigation of supramolecular polymers**

Bis-metal-complexes of terpyridine end-functionalized poly(ethylene glycol) (PEG) ($n = 70$) (see Figure 4.10) were investigated with MALDI-TOF. Only the cobalt and ruthenium polymer bis-complexes were easily detectable. This result is consistent with the above mentioned relative binding strength of the terpyridine complexes under MALDI conditions. Cobalt and ruthenium form the two most stable complexes and are therefore the ones that are easier to analyze by MALDI-TOFMS.

![Figure 4.10](image) **Figure 4.10** Structure of the investigated supramolecular ruthenium(II) block copolymer. The two PF$_6$- counterions are omitted.

It was found, that the relative laser intensity that is required to ionize a single PEG ligand is about 90\% (compare also with Figure 4.7) and hence the laser intensity that is required to
ionize the supramolecular polymer is comparably high. Therefore, all complexes, except the cobalt and ruthenium ones, cannot be detected due to pronounced fragmentation. As a result, only the signals corresponding to uncomplexed PEG ligands are observed for iron, nickel, copper, manganese and cadmium complexes of terpyridine end-functionalized poly(ethylene glycol)s.

Figure 4.11 shows the MALDI spectra of the supramolecular ruthenium-polymer at different laser intensities in linear mode. As expected, the ratio of ligand to polymer increases with increasing laser intensity.

The results presented thus far allow an improved understanding of the behavior of the investigated metal-ligand complexes. Especially the knowledge of the relative binding strength will not only facilitate the design of novel supramolecular architectures but is essential for a profound understanding of the investigated class of materials. Moreover, the interpretation of MALDI analytic results will be easier in the future due to the presented model study. However, only the combination with the following SEC study will provide a detailed picture and lead to improved characterization techniques that will also be applicable as screening techniques in parallel approaches.

Figure 4.11 MALDI-TOFMS spectra of a supramolecular bis-terpyridine ruthenium(II) polymer at different relative laser intensities in linear mode; a bimodal distribution is observed due to fragmentation of the terpyridine complexes during MALDI analysis.
4.2.2 *A size exclusion chromatography study*

Terpyridine end-group modified model polymers (poly(styrene) \( M_n = 2.000 \text{ g/mol} \) and poly(ethylene oxide) \( M_n = 3.000 \text{ g/mol} \)) were utilized for this study. These polymers were subsequently used to prepare terpyridine *mono-* as well as *bis-*complexes as already shown in Figure 4.3. The synthetic details of the compounds that were utilized for this study can be found in the literature.\(^{28,59}\) Figure 4.12 shows the polymers that were utilized for this model study consisting of the polymers with free ligands, the *mono-*complexes thereof and several combinations of *bis-*complexes having the metal complex in the center of the different polymer blocks. Apart from the characterization described here, all polymers have been investigated by \(^1\)H NMR, IR- and UV/Vis-spectroscopy revealing the expected chemical compositions and UV characteristics. One distinct feature of the *bis-*terpyridine ruthenium containing polymers is their absorption at 490 nm; the *mono-*terpyridine ruthenium(III) complexes show an absorption at 400 nm. This behavior was very useful for the described SEC experiments, since a photo diode array (PDA) detector in addition to a refractive index (RI) detector can then provide crucial information about the type of complex present in the investigated polymer.

![Structure of the investigated terpyridine functionalized supramolecular polymers and their building blocks.](image)

The SEC analysis of *bis-*terpyridine metal complex containing polymers (see Figure 4.12) is very difficult due to strong interactions of the charged compounds with the SEC column material (mostly poly(styrene)/divinylbenzene cross-linked beads). The successful examples mentioned in literature may have been lucky shots for reasons already mentioned in the introduction: the active sites of the column material have been deactivated, either through extensive use over prolonged periods of time or by using special solvents or additives. Here a systematic approach to reduce these interactions by adjusting parameters such as solvent, temperature and flow rate as well as the use of additives is presented.
A new PS/DVB based column was used for this study in order to circumvent column ageing effects as already mentioned above. Standard SEC conditions, such as THF or CHCl₃ as eluents with a flow rate of 1 mL/min at room temperature, often result in uninterpretable results, when polymers as depicted in Figure 4.12 are investigated. Nevertheless, these conditions were the starting point of the presented investigation, since most synthetic polymers are readily soluble in the mentioned solvents. Under these conditions the fragmentation of the metal-ligand bond was observed, as proven by UV/Vis data from the photo diode array detector (PDA). The next logical step in preventing column interaction was the utilization of additives for the eluent, such as triethylamine and isopropanol or to increase the column temperature. Unfortunately, none of these methods could rule out all interactions of the investigated polymers with the column material completely. However, it was observed that the described methods eliminated the observed fragmentation of the compounds to a certain extent and that uncomplexed terpyridine containing polymers could be investigated without tailing effects. Subsequently, more unusual solvents for SEC, such as the coordinating solvents DMSO and DMF, were investigated. These solvents are also well-suitable solvents for the polymers under investigation.

**Figure 4.13** Size exclusion chromatograms of polymer P6 under different conditions.
In the first case of DMSO, a fragmentation of the Ruthenium containing polymers was not observed. This could also be confirmed with PDA analysis, since the absorption of the Ru(II)-bis-terpyridine complex at 490 nm was clearly visible over the complete polymer distribution. However, also an inexplicably effect was found. All investigated compounds (see Figure 4.12) retained at approximately the same elution volume. This effect could also not be overcome by mixing additives to the eluent. Finally, DMF showed promising results for the SEC of supramolecular polymers. Figure 4.13 displays the SEC curves of P6 under different conditions with DMF as the eluent. Figure 4.13 (top, left) shows the chromatogram of P6 in DMF at 50 °C without additive. Fragmentation of the polymer to a large extent is visible and could also be confirmed by analysis of the PDA data. Elevation of the column temperature to 80 °C decreased the fragmentation and therefore improved the chromatogram (Figure 4.13, top, right). However, only the addition of salts led to the desired chromatogram without fragmentation of the Ru(II)-bis-complex, as also confirmed by PDA analysis. Both NH₄PF₆ (Figure 4.13, bottom, left) and NBu₄PF₆ (Figure 4.13, bottom, right) were able to screen out all column interactions and therefore allowed the successful analysis of the fragile compounds. However, NH₄PF₆ already revealed the desired effect at lower concentrations. As a result the intensity of the solvent/salt peaks in the RI signal could be reduced. Further investigations revealed that 5 mM NH₄PF₆ in DMF at a flow speed of 0.5 mL/min and 50 °C column temperature represents the most optimal setting for the characterization of supramolecular polymers as described in Figure 4.12. Figure 4.14 (left) shows the chromatograms of the investigated supramolecular polymers demonstrating that the analysis of mono- and bis-terpyridine metal complex based supramolecular polymers is feasible with the described SEC method.

**Figure 4.14** Left: Size exclusion chromatograms of the investigated supramolecular polymers under optimized conditions (DMF, 5 mol% NH₄PF₆, 50 °C, flow: 0.5 mL/min). Right: SEC-coupled in-line diode array spectra of P6. The MLCT band at 490 nm is clearly visible.
The results obtained from the SEC-coupled in-line diode array detector of P6 clearly demonstrated (i) the integrity of the supramolecular assembly over the complete polymer distribution as indicated by the MLCT band at 490 nm and (ii) the purity of the compound (see 4.14, right). The integrity of the supramolecular polymers (polymers P4-P6) was confirmed by the presence of the MLCT band of the Ru(II)-bis-terpyridine complex at 490 nm with the PDA detector. With this optimized system it was also possible to analyze the terpyridine-mono-Ru(III) building blocks (P3). This could again be proven by the presence of the MLCT band at 400 nm in the whole polymer distribution.

![Figure 4.15 Schematic representation of the formation of the metallo-supramolecular polymer P8 (counter ions are omitted for clarity).](image)

Subsequently, the developed chromatographic technique was applied to a chain extended metal-containing polymer in order to further evaluate its usefulness for real life applications. Therefore, a bis-terpyridine-functionalized poly(ethylene oxide) (P7) based coordination polymer (P8) that is linked together via bis-terpyridine ruthenium(II) complexes (see Figure 4.15) was investigated applying the developed chromatographic system. These SEC investigations revealed high molecular mass polymers as depicted in Figure 4.16 (left).

![Figure 4.16 Left: Refractive index size exclusion chromatography traces of polymers P7 and P8. Right: photo diode array gel permeation chromatogram results of polymer P8. The inset shows an extracted UV/Vis spectrum at a retention time of 11.5 minutes.](image)
It is obvious that the molecular weight of \( \textbf{P8} \) shifted to a shorter retention time and therefore a higher molecular weight if compared to \( \textbf{P7} \). Moreover, a broadening of the molecular weight distribution was observed. This behavior is explainable since a polydispersity index of 2 is expected for a classical polycondensation and 100% functional group conversion if the theory developed by Flory is taken into account.\(^{60,61}\) Furthermore, the presence of the small shoulder on the high molecular weight side of the molecular weight distribution of \( \textbf{P8} \) might be explained by the presence of different macromolecular species, namely linear chain extended polymers as well as macromolecular rings. Once more, this behavior is typical for polycondensation like polymerization reactions. Using the RI-detector and a linear PEO calibration revealed a number averaged molecular weight \( (M_n) \) of 138 kDa and a polydispersity index \( (\text{PDI}) \) of 1.55 for polymer \( \textbf{P8} \). These results represent the first successful evaluation of a high molecular weight chain extended supramolecular polymer based on a Ru(II)-\textit{bis}-terpyridine connectivity by the optimized SEC method. The calculated molecular weight of \( \textbf{P8} \) corresponds to 15 repeat units of the \( \alpha,\omega\text{-}\textit{bis}\text{-}\text{terpyridine-poly(ethylene glycol)} \) polymer \( \textbf{P7} \) clearly demonstrating that the chain extension of a linear terpyridine functionalized polymer is feasible. In addition to the RI-detector, a photodiode array (PDA) detector was used to study \( \textbf{P8} \) with SEC techniques. Figure 4.16 (right) shows the corresponding three dimensional elution profile of \( \textbf{P8} \). An extracted UV/Vis spectrum at an elution time of 11.5 minutes is displayed in the inset of Figure 4.16 (right) revealing the typical metal-to-ligand charge-transfer (MLCT) band of the \( \textit{bis}\text{-}\text{terpyridine-Ru(II)} \) complex at \( \sim 490 \) nm. Furthermore, the three dimensional plot clearly shows that this characteristic metal-to-ligand charge-transfer (MLCT) band can be found over the whole polymer distribution indicating that the \( \textit{bis}\text{-}\text{terpyridine-Ru(II)} \) type connectivity is present in all observed macromolecular species of \( \textbf{P8} \). Moreover, analytical ultracentrifugation was performed in order to determine the average molecular mass of the metallopolymer \( \textbf{P8} \). Sedimentation equilibrium measurements were carried out and the obtained equilibrium profile was fitted with the exponential describing the ideal sedimentation behavior.\(^{62}\) In this way, an average molar mass of the metallopolymer (which is, in general, different from the known average values \( M_n \) and \( M_w \)) was obtained. The experiments were carried out using \( \text{NH}_4\text{PF}_6 \) as buffer salt to screen out electrostatic interactions. As a result, a molecular weight of 143 kDa was determined. This corresponds to 16 repeat units of the \( \alpha,\omega\text{-}\textit{bis}\text{-}\text{terpyridine-poly(ethylene glycol)} \) \( \textbf{P7} \) in the supramolecular polymer \( \textbf{P8} \) and is in good agreement with the above described results obtained by SEC measurements. In addition, the molecular weight of \( \textbf{P8} \) was evaluated by viscosity measurements.\(^9\) The intrinsic viscosity of \( \textbf{P8} \) was shown to be 120 mL/g and this value was used to calculate a viscosity average molecular weight of 123 kDa (based on Mark-Houwink constants for completely covalent poly(ethylene glycol)).\(^9\) This value corresponds to approximately 14 repeat units of \( \textbf{P7} \) in the supramolecular polymer \( \textbf{P8} \). In general, all obtained results accordingly show the formation of chain extended supramolecular polymers with approximately 15 repeat units of \( \textbf{P7} \), which demonstrates the usefulness of the developed chromatographic technique. Moreover, this technique was found
Terpyridine-containing supramolecular polymers to be suitable for the analysis of Ru(II)-tris-bipyridine complex containing polymers and other metal-complex containing polymers. The analysis of polymers bearing more labile metal complexes, such as bis-terpyridine-iron(II) or bis-terpyridine-cobalt(II) complexes was up to now not possible. Nevertheless, this technique allows for the first time the straightforward molecular weight and polydispersity characterization of bis-terpyridine-Ru(II) metal complex containing polymers and will provide crucial information for the design of novel polymer architectures based on the mentioned supramolecular connectivity in the future.

Moreover, in order to better understand the metal-containing investigated polymers under MALDI conditions, the fragmentation behavior of P6 was investigated in detail by MALDI-TOFMS. These experiments were crucial for a comprehensive analysis of the investigated polymers, since only now it was possible to determine the purity of them accurately by SEC. As discussed above, P6 is a pure compound as can be easily concluded from the SEC chromatogram depicted in Figure 4.14. Nevertheless, under MALDI conditions not only signals corresponding to the bis-complexed polymer but also peaks corresponding to both building blocks are detected. This behavior is shown in Figure 4.17, where the unfragmented polymer distribution is visible in the m/z range from 4.500 to 6.500 Da.

![Figure 4.17 MALDI-TOFMS spectrum of P6. Both building blocks and the supramolecular polymer are detected. Inset: fragmentation behavior of the investigated compound in dependence of the applied laser intensity during MALDI analysis.](image-url)
Both the poly(styrene) and the poly(ethylene glycol) building blocks are visible in the m/z range from 1.500 to 3.000 and 2.800 to 4.200 Da, respectively. The poly(styrene) distribution shows equally spaced peaks with a mass difference of 104 Da, which corresponds to styrene monomer units. The poly(ethylene glycol) distribution also shows equally spaced peaks with a difference of 44 Da, which corresponds to ethylene glycol monomer units. As described in section 4.2.1, bis-terpyridine-metal complexes tend to fragment during MALDI analysis. The supramolecular polymer P6 behaves very similar. This was proven by acquisition of MALDI-TOFMS spectra at varying laser intensities. With increasing laser intensity, the metal complex fragments more and more. Therefore, the signal intensity ratio of the complete block copolymer to the building blocks of poly(styrene) and poly(ethylene glycol) changed. This behavior can be visualized by calculating a ratio of poly(styrene)/block copolymer signal intensities and plotting this ratio versus the relative laser intensity utilized for spectrum acquisition (see inset Figure 4.14). The ratio increases with increasing laser intensity indicating a more pronounced complex dissociation. This finding is in good agreement with the mentioned model studies and therefore demonstrates that the knowledge gained during the model investigations can be transferred to supramolecular polymer system. In conclusion, polymer P6 shows both building blocks in its MALDI-TOFMS spectrum, even if P6 was shown to be a pure compound by SEC. Therefore, only the combination of both novel analytic techniques can provide profound information about the architecture and molecular weight of the investigated polymers, especially if hyphenated chromatographic techniques, such as the PDA detector, are applied.

4.3 Novel terpyridine-containing polymer architectures

As already mentioned in the introduction to this chapter, polymers that contain supramolecular binding units are an interesting field of research and their study has led to several new functional materials. In particular terpyridine (tpy) metal-complexes are widely used as building blocks in supramolecular and macromolecular chemistry resulting in a large variety of different supramolecular polymer architectures. These architectures include linear chain extended polymers, block copolymers, graft copolymers, dendrimers and many others and are schematically depicted in Figure 4.18. The architectures B, C, and E can only be obtained by applying ruthenium and osmium complex chemistries by the preparation of mono-complexes, as already discussed in the introduction. The architectures based on a self-complementary recognition (A and D) can be prepared with a large variety of different transition metal ions.
The most widely studied architectures are the chain extended polymers, block copolymers, as well as the homodimers. Especially the block copolymer systems showed interesting material properties, such as the formation of micelles and the subsequent cleavage of the metal coordination bond in order to obtain polymeric nanoparticles as already addressed earlier. Therefore, the following sections will focus on novel, less studied architectures, namely: graft-copolymers, ABA triblock copolymers as well as a star-shaped architecture.

### 4.3.1 Supramolecular ABA triblock copolymers based on a polycondensation approach

Here, the one step polymerization of 1,16-bis(2,2':6',2''-terpyridin-4'-yloxy)hexadecane 1 with RuCl₃ under reducing conditions was investigated in a parallel fashion in order to optimize the reaction conditions for this polymerization process. This was necessary since known literature procedures for the polymerization of bis-terpyridine ligands require (very) long reaction times and/or an additional step for the activation of the ruthenium species. Subsequently, the gained knowledge was applied for the preparation of a supramolecular ABA triblock copolymer with potentially interesting material properties, such as the formation of micelles due to its amphiphilic character. However, as a first step of investigations the reaction of 1 (and other terpyridine compounds) with RuCl₃ had to be investigated in more detail in order to avoid time consuming procedures and to obtain better control of the polymerization procedure. Therefore, the polymerization conditions of 1,16-bis(2,2':6',2''-terpyridin-4'-yloxy)hexadecane with RuCl₃ were optimized utilizing a carousel reactor. This parallel approach allowed the fast investigation of different additives and concentrations for the polymerization procedure. All polymerizations were monitored by
SEC as well as UV/Vis spectroscopy aiming for an as high as possible molecular weight and monomer conversion whilst maintaining the typical UV/Vis characteristics of the bis-terpyridine-Ru(II) complex (e.g. the pronounced MLCT band at ∼490 nm). It should be noted here that ruthenium was chosen because it forms very stable complexes with terpyridine ligands thereby offering the possibility to investigate the obtained species by an optimized SEC method (see section 4.2.2). Other transition metal ions, such as Co²⁺, Cu²⁺, Fe²⁺ and others, do also form stable complexes with terpyridine ligands. However, to our experience they are not stable enough for an investigation by means of SEC. Nevertheless, even the choice of the very stable ruthenium-complexes allows the opening of complexes after their synthesis utilizing e.g. redox chemistry.66

The first results of these screening reactions revealed that dimethylacetamide (DMA) was a suitable, high boiling solvent for this kind of polymerizations that did not show undesired side reactions and dissolves the monomer, RuCl₃ as well as the resulting polymer very well at elevated temperatures. Table 4.1 summarizes representative results of the more than 20 performed parallel optimization reactions. Generally, the polymerizations in DMA proceeded without any additives, however only very slowly reaching approximately 5-10% conversion after 24 hours of reaction time (see Table 4.1 entry 1).

| Table 4.1 Screening data for six polymerization runs of monomer 1 with RuCl₃ (1:1 molar ratio; 2:1 ratio of terpyridine:Ru) under various conditions at 130 °C. All additives are in 10 fold excess according to 1. *conversions are estimated based on SEC screening results. |
|---|---|---|---|
| additive | c(1) / (mmol/mL) | conversion of 1 after 24 h* / % | λₘₐₓ UV/Vis / nm |
| 1 | - | 0.02 | <10 | 490 |
| 2 | ethanol | 0.02 | 15 | 400, 440, 490, 530 |
| 3 | NH₄PF₆ / butanol | 0.02 | <10 | none |
| 4 | N-ethyl-morpholine / butanol | 0.01 | 27 | 490 |
| 5 | N-ethyl-morpholine / butanol | 0.03 | 39 | 490 |
| 6 | N-ethyl-morpholine / butanol | 0.2 | 80-85 (after 5 h) | 490 |

The addition of alcohols such as ethanol or butanol slightly increased the observed conversions and molecular weights, but led to undefined species as observed by UV/Vis spectroscopy (see Table 1 entry 2). The addition of N-ethyl-morpholine had no effect on the polymerization speed. Furthermore, the addition of salts that could maybe promote an exchange of the counter ions of the obtained bis-terpyridine complexes such as NH₄PF₆ or AgBF₄, did not affect the observed conversions. The addition of salt and alcohol to a reaction
mixture led to the formation of undefined complexes without characteristic features in the UV/Vis spectrum (see Table 4.1 entry 3). The possibility of the formation of defect structures in terpyridine complexes with ruthenium(II) ions was already discussed in the literature\textsuperscript{65} and might be due to the fact that the counter-ions that are present during the complex formation coordinate to the ruthenium cation. Nevertheless, the addition of alcohol and \textit{N}-ethyl-morpholine led to the formation of high molecular weight species with the desired UV/Vis characteristics (MLCT band of the \textit{bis}-Ru(II)-terpyridine complex at 490 nm) providing high monomer conversions. The addition of triethylamine and butanol did not lead to high conversions and high molecular weight species suggesting that not the capability of \textit{N}-ethyl-morpholine to act as a base but to act as a (co)reductant is the driving factor for the observed high conversions. Furthermore, Table 4.1 entries 4 and 5 reveal that an increase in the concentration of \textbf{1} resulted in increased conversions. Further investigating that effect showed that a polymerization performed with a monomer concentration of 0.2 mmol/mL at 130 °C for 5 hours provided a monomer conversion of 80 to 85\% as estimated by both \textsuperscript{1}H NMR and SEC analysis (Table 4.1, entry 6). These results, in combination with a good reproducibility of the performed reactions, led to the conclusion that DMA in combination with butanol and \textit{N}-ethyl-morpholine as reducing additives are a very capable reaction medium for the polymerization of \textbf{1} with RuCl\textsubscript{3} under reducing conditions. Subsequently, utilizing these conditions it was observed that lower monomer concentrations led to lower molecular weights of the obtained polymers. This behavior is in consistence with polymerization theory and can be explained by a favored intermolecular reaction of the reactive polymer chain ends at higher concentration. Figure 4.19 displays SEC results for two polymerizations performed at 130 °C in DMA for 24 hours. It is obvious that the increase in monomer concentration from 0.01 to 0.17 mmol/mL led to the formation of higher molecular weight species and moreover to an increase in the observed monomer conversion. However, it is also clearly visible that a mixture of different species was obtained, which are most likely different sizes of macromolecular rings and linear polymers (see Figure 4.20). Such macromolecular rings were isolated by different authors\textsuperscript{67,68} but not further investigated here. In the course of further investigations it was observed that a monomer concentration of 0.2 mmol/mL with a 10 fold excess of butanol and \textit{N}-ethyl-morpholine as additives in capped conical vials led to the highest molecular weights and largest conversions after a reasonable reaction time of 5 hours. Higher concentrations of monomer could not be applied due to solubility problems. The capped vials were utilized in order to avoid any loss of the lower boiling butanol during the reaction. Moreover, the optimized conditions were also tested on different (also rigid) \textit{bis}-terpyridine ligands and the conditions generally offer very good results. Further investigations in that direction are still ongoing. In conclusion, the optimized reaction conditions represent a crucial improvement over literature procedures since the polymerization time could be reduced from 5 days to 5 hours and since no precipitation was observed during the reaction.\textsuperscript{65}
Figure 4.19 UV/Vis chromatograms of two supramolecular polymerization reactions of 1,16-bis(2,2':6',2''-terpyridin-4'-yloxy)hexadecane 1 illustrating the concentration effect on the polycondensation like polymerization procedure.

Figure 4.20 Overview of the performed polymerization and optimization reactions finally leading to the synthesis of the supramolecular ABA triblock copolymer P9 (counter ions are omitted for simplicity reasons).
After the evaluation of the best reaction conditions for the polymerization of 1,16-bis(2,2′:6′,2″-terpyridin-4′-yloxy)hexadecane 1 with RuCl₃, the supramolecular ABA triblock copolymers could be synthesized via a polycondensation approach utilizing a α-terpyridine-ω-methyl-poly(ethylene glycol) polymer P9 as chain stopper during the polymerization reaction (see Figure 4.20). As a consequence, both ends of the synthesized polymer will be capped with poly(ethylene glycol) chains resulting in the desired A-b-B-b-A structure. Moreover, the utilization of a chainstopper in the course of the polymerization will avoid the formation of macrocyclic rings to a large extent. The most promising results were obtained in the presence of 10 mol% of P9 with respect to 1 and the corresponding amount of RuCl₃ within 5 hours reaction time. The crude polymer was precipitated in acetone and subsequently the counter ions were exchanged from Cl⁻ to PF₆⁻ by refluxing the polymer with an excess of NH₄PF₆ in methanol. This led to the precipitation of the supramolecular triblock copolymer P11 (see Figure 4.20) upon cooling.

*Figure 4.21* Photo diode array size exclusion chromatogram results after workup of polymer P11.

It should be mentioned here, that only changing the counter ions had a significant effect on the solubility of the synthesized polymer: with chloride counter ions it was methanol soluble and acetone insoluble, whereas with hexafluorophosphate counter ions the reverse behavior was observed. Recently it was also reported that an exchange of the counter ions of a bis(terpyridine)ruthenium(II) complex that connects a poly(styrene) block with a PEO block in a diblock copolymer led to a change in the melt morphology from a spherical to a lamellar mesophase.⁶⁹ These are first examples of how the properties of a metal-containing polymer
can be changed by a simple exchange of its counter ions outlining the additional freedom these types of polymers offer for the tailoring of polymer properties. In the case of polymer P11 the exchange to PF₆⁻ counter ions changed its solubility completely making it water insoluble (also upon heating or ultrasonication) thereby introducing the amphiphilicity needed for the preparation of micelles as it will be discussed later. Figure 4.21 displays the chromatogram of polymer P11 obtained with a photo diode array detector revealing the characteristic (terpyridine)₂-Ru MLCT band at 490 nm over the whole polymer distribution. The insets show a UV chromatogram at an absorption wavelength of 310 nm and an UV/Vis spectrum at an elution time of 17.5 minutes, respectively. Moreover, it was observed (see Figure 4.22, left) that polymer P11 eluted faster than polymer P10 indicating its higher molecular weight. However, no suitable calibration was found until now to evaluate the molecular weight of the supramolecular polymer P11 by means of standard SEC techniques. As a point of discussion molecular weight values of polymers P10 and P11 utilizing different calibrations are provided in Table 4.2.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Mₙ</th>
<th>Mₘ</th>
<th>PDI</th>
<th>Repeat units of 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>P10 / PEG</td>
<td>8450</td>
<td>10200</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>P11 / PEG</td>
<td>18400</td>
<td>34200</td>
<td>1.86</td>
<td>9</td>
</tr>
<tr>
<td>P10 / PS</td>
<td>36300</td>
<td>40000</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>P11 / PS</td>
<td>68700</td>
<td>94600</td>
<td>1.38</td>
<td>29</td>
</tr>
<tr>
<td>P10 / PMMA</td>
<td>20700</td>
<td>23300</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>P11 / PMMA</td>
<td>40200</td>
<td>59600</td>
<td>1.48</td>
<td>18</td>
</tr>
</tbody>
</table>

It was observed that the PEO calibration shows comparable molecular weight values to ¹H NMR calculations for polymer P10 but underestimates the molar mass of the central B block in polymer P11, since approximately 30 repeat units of 1 are expected in polymer P11 (compare ¹H NMR results below and Table 4.2). Moreover, both PS and PMMA calibrations are absolutely not suitable for an evaluation of the molecular weight of polymer P10 since they largely overestimate its Mₙ. The PS calibration on the other hand seems to be well suited for molar mass estimations of the central block of P11 since the calculated repeat unit number of 29 is very close to values obtained by ¹H NMR spectroscopy (see below). However, even if none of the applied SEC calibrations are suitable for P11 with DMF as eluent, all results clearly demonstrate the increase in both the molecular weight and the polydispersity index of P11 if compared to P10.
Figure 4.22 Left: size exclusion chromatography traces of polymers P10 and P11. Right: $^1$H NMR analysis of monomer 1 and polymer P11 in acetone-d6 revealing the typical shifts of terpyridine protons due to the complexation with ruthenium ions.

Figure 4.22 (left) also clearly depicts that polymer P11 shows two small shoulders at the low molecular weight side of its molecular weight distribution, which are most likely due to the presence of a minor fraction of macromolecular rings and/or polymer P11 that was only functionalized with PEO on one side resulting in an AB block copolymer like structure. In addition, a broadening of the molecular weight distribution of P11 (PDI ~ 1.6, average of PEO and PS calibration), if compared to P10 (PDI ~ 1.2, PEO calibration), can be observed. Both observations were expected since it is known for many different kinds of polycondensations that cyclic species are formed in different amounts (see e.g. Refs. 70-72 for recent examples) and a polydispersity index of 2 is expected for 100% functional group conversion if the theory developed by Flory is taken into account. $^{60,61}$ A detailed $^1$H NMR analysis of P11 (compare Figure 4.22, right) revealed that the terpyridine signals of monomer 1 as well as of the terpyridine modified PEO shifted upon complexation with ruthenium ions and that the obtained polymer only shows complexed terpyridine signals. This is a good indication that the shoulders observed in the SEC trace of P11 (compare Figure 4.22, left and discussion above) are due to macrocyclic rings and not an A-b-B type supramolecular polymer since the latter would show signals of free terpyridine moieties in the $^1$H NMR spectrum. End-group calculations utilizing the PEO polymer as end-group and assuming that both ends of polymer P11 were functionalized with PEO reveal that in average 33 of the 1,16-bis(2,2':6',2''-terpyridin-4'-yloxy)hexadecane units were built into the polymer backbone. Therefore, the number average molecular weight of polymer P11 can be calculated to be 43 kDa (including counter ions). These results demonstrate that it is feasible to construct supramolecular ABA triblock copolymers utilizing a one pot polycondensation approach applying the optimized reaction conditions.

In order to study the amphiphilic character of the obtained supramolecular triblock copolymer P11 its ability to form micelles was investigated (Figure 4.23, left).
Since the supramolecular polymer was not readily soluble in water, pure water was added dropwise to a solution of P11 in DMSO or acetone and the obtained solution was dialyzed against pure water, leading to frozen micelles. Details about this method are reported in the literature. In short, the supramolecular polymer P11 is molecularly dissolved in the unselective solvent (as confirmed by $^1$H NMR). Upon the addition of water the solvent quality for the central supramolecular block decreases continuously and aggregation is induced. At higher water contents the mobility of the central B segment of polymer P11, forming the core of the micelles, will be very low and the process of polymer self assembly and the equilibrium between micelles and individual polymer molecules will be very slow. Finally, after dialysis of the residual unselective solvent, the micellar structure can be considered as kinetically frozen. Therefore, micelles with a “supramolecular” metal-ion complex containing core and a PEO corona were obtained from triblock copolymer P11.

Figure 4.23 Left: schematic representation of the micelle formation of P11. Right: TEM image of micelles prepared with P11 observed without contrasting.

Figure 4.24 Left: schematic resonant mode topography image of micelles of P11 imaged in solution. Image z-range is 50 nm. Right: cross-section taken at the position indicated by the dotted line. The height of individual micelles is approximately 26 nm.
Dynamic light scattering of the obtained micelles showed the presence of objects with a diameter of 70 nm as well as a minor population of aggregates of these micelles with a size of approximately 500 nm. Moreover, micelles of P11 have been imaged by resonant mode scanning probe microscopy imaging in water. The measurements were performed by immersing the cantilever directly in the micellar solution of P11 in water. Images of these measurements are presented in Figure 4.24 showing individual micelles as well as clusters of micelles. The micelles in their native environment revealed a diameter of 26 nm in average (standard deviation: 6.5 nm). This result might be explained by the fact that the atomic force microscopy (AFM) tip penetrated the swollen PEO corona. Furthermore, transmission electron microscopy (TEM) experiments of unstained micelles revealed spherical objects with a mean diameter of 25 nm (see Figure 4.23, right: standard deviation: 5 nm). This result clearly shows that the investigated micelles have the proposed structure with the supramolecular repeat unit in the core of the micelles since i) a non metal containing repeat unit would show no contrast in TEM and ii) rings would be observed in the case where the metal containing repeat units would form the corona of the micelles. The investigation of micelles of P11 with various techniques provided a good picture of their morphological characteristic features. The diameter of the core of the micelles (~25 nm) was obtained by TEM and AFM. Finally, the total size (core+corona) of the hydrated micelles can be estimated to be around 70 nm as measured by DLS.

The presented results demonstrate that it is feasible to prepare supramolecular triblock copolymers via a one pot approach. This is a significant improvement for the straightforward, fast and reproducible synthesis of a novel class of supramolecular materials. Moreover, it was possible to utilize the amphilicity of the studied polymers to prepare micelles with a metal complex containing core. Therefore, in the future the application of more labile metal ions, such as iron or copper, might lead to pH and redox responsive micelles that disassemble due to the mentioned stimuli opening application possibilities in the field of stimuli responsive release. First results in this direction clearly revealed that it is possible to prepare iron containing supramolecular ABA triblock copolymers with similar properties as the ruthenium ones applying the optimized reaction conditions. Moreover, preliminary results demonstrate that these iron containing ABA triblock copolymers can form micelles with similar morphology and size as the micelles discussed in this chapter.

4.3.1 Supramolecular PVC

Poly(vinyl chloride) (PVC) is a polymer of great commercial importance with a demand of more than 25 million tons per annum (2002, ca. 16.5% of the plastics used in the world). It can be used to produce products with varying properties, e.g. from very rigid to very flexible, and has a variety of applications ranging from containers for blood or urine over cable insulations to packaging materials. Grafting as well as cross-linking of PVC is intensively discussed in the literature for several applications or material property improvements.
instance, it has been shown that the degradation rate of PVC correlates to the degree of modification of PVC with xanthogenate groups as well as the degree of grafting with MMA. Both modifications can have a stabilizing effect on the final polymer.\textsuperscript{78} Covalent modification of PVC for the introduction of functional groups to the polymer main chain can be achieved by nucleophilic substitution reactions of chlorine atoms. Mercapto compounds were shown to be appropriate modification reagents for this strategy.\textsuperscript{82,83} This can lead to a control of certain properties of the resulting materials, such as the gas transport properties, as it was shown for mercapto pyridine modified PVC.\textsuperscript{84}

This section describes an approach for the covalent modification of PVC with terpyridine ligands (see Figure 4.25) in order to obtain multi-functional materials that can be utilized for supramolecular grafting reactions. First, PVC was modified by a nucleophilic substitution reaction with (2-mercapto-phenyl)-methanol (P12) in order to insert OH groups to the polymer backbone, which were utilized for further functionalization with an isocyanate-modified terpyridine (4). Compound 4 was prepared in a two step reaction starting from 4'-chloro-2,2':6',2"-terpyridine by nucleophilic substitution with 6-amino-hexan-1-ol resulting in the NH\textsubscript{2} functionalized terpyridine 2. Subsequently, 2 was reacted with 3 to form the isocyanate-modified terpyridine 4.

Figure 4.25 Synthetic strategy for the preparation of terpyridine modified PVC.

Figure 4.26 shows the proton NMR spectra of the utilized commercial PVC, the modified PVC P13, as well as compound 2.
Characteristic signals that are found for compound 2 are also present in the $^1$H NMR spectrum of polymer P13. Further signals are observed in the aromatic region between 7.4 and 7.8 ppm corresponding to (2-mercapto-phenyl)-methanol. These signals are also present in polymer P12 revealing a degree of functionalization of PVC with (2-mercapto-phenyl)-methanol of 11 mole %. Therefore it can be concluded that the two step modification of PVC with terpyridine units was successful. The signal at 4.5 ppm corresponding to the CH-Cl protons became weaker in intensity for polymer P13 if compared to PVC, while a new signal at 4.9 ppm appeared as a result of the modifications made. This new signal belongs to the CH-S protons (see also Refs. [82-84]). Moreover, in the aromatic region also several additional peaks appeared corresponding to the terpyridine unit and the 2-Mercaptobenzyl alcohol unit. Finally, the urethane proton could be found at 5.3 ppm. $^1$H NMR calculations revealed that the average degree of modification of polymer P13 with terpyridine groups was 4.2 mole %. This percentage corresponds to approximately 30 terpyridine units per 740 vinyl chloride units if the $M_w$ of 49,000 g/mol supplied by the manufacturer is taken into account. SEC investigations with PDA detector (see Figure 4.27) of unmodified PVC, polymer P12 and P13 revealed that the unmodified PVC showed no UV absorption as expected, whereas polymers P12 and P13 showed UV maxima at 267 nm and 277 nm. These maxima are equally distributed over the whole polymer distribution, indicating a successful modification of PVC with (2-mercapto-phenyl)-methanol and terpyridine units, respectively. The obtained
molecular weights and poly dispersity indices (PDI) from the analysis can be found in the experimental section.

**Figure 4.27** SEC curves for unfunctionalized PVC, polymer P12 and P13 as well as graft-copolymer P14 (top). UV/Vis spectra of P12, P13 and P14 extracted from the PDA detector (bottom).

**Figure 4.28** Grafting of RuCl₃-terpyridine mono-complexes to polymer P13.
In order to prove the concept of supramolecular grafting onto the terpyridine binding units, a small organic molecule was grafted onto the PVC backbone (see Figure 4.28). The success of the grafting is clearly visible by SEC analysis of the resulting polymer P14 (see Figure 4.27). Furthermore, photo diode array analysis of P14 revealed the characteristic MLCT band of the Ru(II)-bis-terpyridine complex at 490 nm over the whole molecular weight distribution. This demonstrates, that supramolecular grafting reactions can successfully be applied to high molecular weight polymers. This short feasibility study shows that grafting to terpyridine units is feasible and that the resulting polymers can successfully be characterized with the optimized size exclusion chromatography system. In the future, it might, be interesting to study such materials for their capability to form micelles or their phase behavior in thin films.

4.3.3 Fluorescent sensing of transition metal ions based on the encapsulation of dithranol in a polymeric core shell architecture

The encapsulation of functionalities into polymeric or dendritic core shell architectures is a highly promising field of research due to the manifold application possibilities in drug delivery,85,86 stimuli responsive release,87 catalysis,88,89 or phase transfer.90,91,92 Moreover, Patroni et al. recently described that a lipophilized ion receptor, a surfactant, and a fluorescent hydrocarbon (pyrene), are capable of forming micelles in water with the fluorophore included in them, that respond to Cu²⁺ and Ni²⁺ ions as selective fluorescent sensors.93 Furthermore, it was described in sections 3.3.2 and 3.3.3 that star-shaped block copolymers based on a poly(ethylene oxide) core and a poly(ε-caprolactone) corona are capable of encapsulation and phase transfer of guest molecules. Combining these concepts this section describes the encapsulation behavior as well as the fluorescent sensing application of a terpyridine end-group modified 5-arm star-shaped poly(ethylene oxide) polymer. The synthetic strategy as well as the structure of the resulting polymer are depicted in Figure 4.29.
The hydroxyl end-groups of the PEO starting polymer could be fully functionalized as shown by $^1$H NMR. Moreover, MALDI-TOFMS revealed the anticipated structure of P15 due to a correct end-group analysis with fitting isotopic pattern as well as the expected 44 Da peak spacing of the ethylene glycol repeat units as shown in Figure 4.30. The labelled peak at 3265 Da corresponds to P15 (see Figure 4.30) with 7 repeat units of ethylene oxide per polymer arm. The small distribution that is visible in the low molecular weight region corresponds to singly charged and protonated but not sodiated species (MH$^+$). Terpyridine ligands are capable of forming terpyridine-M$^{2+}$-terpyridine complexes with a variety of transition metal ions. To investigate this behavior with polymer P15, a parallel titration experiment was performed with Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$ as well as Zn$^{2+}$ salts in a microtiter plate. The layout of the microtiter plate, as it was used for this experiment, is shown in Figure 4.31 (left). The experiment clearly revealed that P15, but not the 5-arm PEO, can form transition metal complexes with all investigated transition metal ions. Moreover, Figure 4.31 (right) shows the resulting UV/Vis spectra of this experiment for the case of Co$^{2+}$ ions. The increase of the $\pi-\pi^*$ band at 310 nm upon addition of Co$^{2+}$ ions indicates the complex formation. This change in the absorption behavior is due to the change of the terpyridine ligands to an all-cis configuration and due to the metal-coordination of the ligands. A similar behavior was found for the other investigated metal ions with $\pi-\pi^*$ bands in the region between 300 and 330 nm. Moreover, the titration experiment revealed the typical metal-to-ligand-charge-transfer (MCLT) band at 556 nm for the terpyridine-Fe$^{2+}$-terpyridine complex. For all metal ions, clear isosbestic points were found (see e.g. Figure 4.31, right), indicating that only a single

**Figure 4.30** MALDI-TOFMS spectrum of polymer P15 with corresponding peak assignment.
equilibrium between two species, namely free and complexed terpyridine, occurs during the titration. The formation of these transition metal complexes with P15 leads to the formation of a supramolecularly cross-linked network. However, no undesired effects such as precipitation were observed in the investigated concentration ranges in DMSO.

Figure 4.31 Left: microtiter plate layout applied to the parallel property investigation of polymer P15. Right: complexation behavior of P15 with Co²⁺ ions investigated in a parallel fashion in DMSO.

Subsequently, P15 was also investigated regarding its encapsulation behavior. In general, the encapsulation of a guest molecule within a host can be probed by the evaluation of changes in the guests microenvironment, which can be detected by spectroscopic techniques.⁹⁴ These changes are premised on stabilizing or destabilizing effects of the local microenvironment on electronic states of the guest molecules.⁹⁴ Studies of P15 in that regard revealed that it can encapsulate methyl orange, a pH indicator dye, in the same fashion as described in section 3.3.2 for star-shaped block copolymers based on a poly(ethylene glycol) core and a poly(ε-caprolactone) corona (see this section for details).

Figure 4.32 Encapsulation behavior of polymer P15. Left: encapsulation and phase transfer of methyl orange. Right: encapsulation of dithranol measured by fluorescence spectroscopy.
Figure 3.32 (left) shows this encapsulation behavior of methyl orange in polymer P15 in a two phase (water/chloroform) system in a macroscopic fashion. The hydrophilic pH indicator dye is dissolved in the water phase, whereas upon addition of P15 to the system the dye is encapsulated in the polymer and phase transferred since the polymer is more soluble in the chloroform phase than in the water phase. This encapsulation is accompanied by a color change of methyl orange from red ($\lambda_{\text{max}} = 504 \text{ nm}$) to yellow ($\lambda_{\text{max}} = 420 \text{ nm}$) indicating a change in the dyes microenvironment. It was already discussed in section 3.3.2 that a hydrophobic barrier is necessary to obtain this encapsulation behavior for the same starting 5-arm PEG polymer, but that the size of the hydrophobic barrier did not show any influence on the encapsulation behavior of star-shaped block copolymers. Therefore, the observed behavior of P15 suggests that the terpyridine ligands represent a sufficient hydrophobic barrier to obtain the described encapsulation and phase transfer behavior. Moreover, it was observed that P15 can encapsulate anthracene derivatives (such as dithranol or 9-hydroxymethylnanthracene) from DMSO or chloroform solutions as it was also described in section 3.3.3 for the corresponding star-shaped block copolymers. Figure 3.32 (right) shows the encapsulation of dithranol within P15 and the resulting increase of the fluorescence of the guest molecule depending on the concentration of added polymer. It was observed that dithranol:P15 ratios higher than 1:1 led to a decrease in the guests fluorescence, probably due to self quenching effects. Therefore, a dithranol:P15 ratio of 1:1 was chosen for all further experiments, since this ratio provided the highest fluorescence intensities. The combination of the two described concepts of guest encapsulation and transition metal binding possibilities of polymer P15 were subsequently investigated in a microtiter plate format utilizing a combined UV/Vis and Fluorescence reader. These studies revealed that the fluorescence intensity of encapsulated dithranol is decreasing upon addition of transition metal salts to the host guest complex of dithranol and P15. This fluorescence quenching effect was observed for the binding of all investigated transition metal ions ($\text{Mn}^{2+}$, $\text{Fe}^{2+}$, $\text{Co}^{2+}$, $\text{Ni}^{2+}$, $\text{Cu}^{2+}$, $\text{Zn}^{2+}$) and is depicted in Figure 4.33. DMSO was used for these studies due to the good solubility of all components in this solvent. Dithranol was selected for these experiments, since its excitation wavelength of 410 nm does not overlap with any absorption bands of the investigated terpyridine metal complexes. Moreover, $^1$H NMR studies showed that dithranol is not released from its host-guest complex upon binding of transition metal ions providing further evidence that the observed decrease in the fluorescence intensity is indeed due to a quenching effect. As described in the literature and already mentioned above similar effects have been utilized for the sensoric detection of transition metal ions utilizing self assembled micelles. Fluorescent sensors are widely applied in different fields of research, such as the detection and quantification of proteins, pH measurements as well as for anion and cation quantification. Most often, these cation and anion sensors are based on a covalent linkage of a fluorophore and a receptor site. The here described approach utilizes the self organization and encapsulation of a fluorophore within a polymer and requires only one synthetic step to functionalize the polymer with a receptor molecule. This approach has similar advantages as
described for self-assembled micelles from lipophilized receptors, a surfactant and a fluorophore,\textsuperscript{93} e.g. the choice of the fluorophore without synthetic effort as well as the variation of the receptor in only one synthetic step. However, this system cannot be used in water due to the low solubility of P15 in water. Nevertheless, a significant advantage is the avoidance of the dynamic equilibrium between the individual small amphiphiles and the self-assembled micellar structure, which might lead to undesired effects depending on temperature or ionic strength due to shifts in the individual molecule micelle equilibrium.

\textbf{Figure 4.33} Fluorescence quenching behavior of the dithranol-P12 host-guest complexes depending on the binding of transition metal ions to the terpyridine ligands.

Figure 3.43 displays the response of the described sensor system to FeCl\textsubscript{2} in DMSO. Figure 3.43 (left) depicts the UV/Vis spectral changes upon addition of Fe\textsuperscript{2+} ions to the sensoric system.

\textbf{Figure 4.34} Left: UV/Vis response of the investigated sensoric system to Fe\textsuperscript{+2} ions. Right: fluorescence response of the investigated sensoric system to Fe\textsuperscript{+2} ions.
As expected the MLCT band at 556 nm is increasing until full bis-complexation of all terpyridine ligands is reached. Figure 3.35 (left) clearly reveals an equivalence point at 100% complexation. Figure 2.35 (right) shows the corresponding fluorescence signal of dithranol. The intensity is quenched upon addition of the transition metal salt until no detectable signal is observed (corresponding to full complexation of the ligands). This decrease in the fluorescence intensity of the encapsulated dithranol was not only observed for Fe$^{2+}$ ions but for all investigated transition metal ions (Mn$^{2+}$, Fe$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$).

**Figure 4.35** Response characteristic of the sensoric system of dithranol encapsulated in P15. Left: UV/Vis response to Fe$^{2+}$ ions. Right: fluorescence response to Fe$^{2+}$ ions.

Figure 3.35 (right) shows the decrease of the fluorescence intensity at its maximum at 535 nm for the case of Fe$^{2+}$ ions. A linear correlation between this intensity and the concentration of transition metal ion was observed. Moreover, the dynamic range of this sensoric system was found to be in the micro-molar range of analyte. The described sensor is not selective to a specific species but might be useful for the quantitation of the overall content of the mentioned transition metal ions. However, the linear response in the micro-molar range makes this system quite sensitive and detection of small quantities of the mentioned species is feasible.

### 4.5 Conclusions

This chapter describes results on the characterization as well as synthesis of terpyridine metal complex based supramolecular polymers.

In a MALDI-TOFMS model study the relative binding strength of non-covalent metal-ligand interactions was investigated. Bis-complexes of 4’-(1,4,7-trioxa-octyl)-2,2’:6’,2”-terpyridine of different transition metal ions have been evaluated at different laser intensities during their analysis with MALDI-TOFMS leading to the estimation of a row of relative binding strength,
depending on the central metal ion of the complex. This knowledge allows a better understanding of the degradation behavior of such supramolecular assemblies under MALDI conditions. Therefore, it is for the first time possible to interpret MALDI spectra of the corresponding supramolecular polymers which contain terpyridine binding units, in particular if weaker binding metal complexes are investigated. Moreover, advances made for the characterization of terpyridine supramolecular polymers via SEC are reported. In this particular case the choice of solvent and additive was crucial for a successful characterization of the supramolecular systems. A more exotic chromatographic system with 5 mM NH₄PF₆ in DMF at 50 °C was shown to yield the optimal results by screening out all non-specific interactions with the column material. Therefore, the separation of the investigated polymers only depending on size (excluding chromatographic effects) is now possible. Furthermore, it could be shown that the novel chromatographic system can provide accurate analytic results by comparison of SEC with AUC and viscosity data. Therefore, the developed system became a routine analytic tool for the evaluation of the investigated metal-containing polymers allowing for the first time their straightforward characterization by the most common technique for molecular weight and polydispersity characterization: SEC.

Moreover, it was shown that a parallel approach utilizing the developed SEC technique as well as UV/Vis spectroscopy as major screening methods for the optimization of the polymerization conditions of 1 was successful and led to the designed synthesis of the supramolecular ABA triblock copolymer P11. The optimized reaction conditions led to higher molecular weight polymers within a reduced polymerization time revealing the bis-terpyridineRu(II) type connectivity in its backbone. The triblock copolymer P11 was composed of approximately 33 repeat units of 1 outlining the usefulness of the applied optimized conditions for its synthesis. Furthermore, P11 proved to be amphiphilic and it was possible to prepare micelles from P11 in a straightforward approach. The resulting micelles were investigated utilizing DLS as well as AFM and TEM as imaging techniques. The obtained results demonstrate that it is possible to prepare ABA type block copolymer architectures utilizing the supramolecular terpyridine metal type of connectivity and open ways for the synthesis of novel kinds of block copolymer structures by i) applying different kinds of terpyridine functionalized polymers as chain stopper and/or ii) applying different kinds of bis-terpyridine functionalized supramolecular monomers.

In the last section of this chapter, a new material based on a 5-arm star-shaped poly(ethylene glycol), which was functionalized with terpyridine binding units, was synthesized and evaluated. This material is capable of forming metal complexes with a variety of transition metal ions. Moreover, it was observed that the mentioned polymer allowed encapsulation of functional molecules, such as fluorescent anthracene derivatives. The combination of the two concepts led to a new fluorescent sensor system for transition metal ions with a linear response range in the micro-molar range. Finally, the utilization of the parallel micro titerplate
approach allowed an accelerated evaluation of these material properties and demonstrates once more the advantages of combinatorial approaches in materials science.

4.6 Experimental

**Chemicals and Reagents**
1,8,9-Anthracenetriol (dithranol), 2,5-dihydroxybenzoic (DHB) acid, α-cyano-4-hydroxycinnamic acid (CHCA) and 4'-chloro-2,2':6',2"-terpyridine were purchased from Sigma Aldrich (Oakville, On, Canada). Analytical and HPLC grade solvents were purchased from Biosolve LTD (Valkenswaard, The Netherlands).

**Instrumentation**
All MALDI experiments were carried out on a Voyager-DE™ PRO Biospectrometry™ Workstation (Applied Biosystems, Foster City, CA, USA) time-of-flight mass spectrometer using reflector mode for operation unless otherwise stated. All spectra were obtained in the positive ion mode. Ionization was performed with a 337 nm pulsed nitrogen laser. All data were processed using the Data Explorer™ software package (Applied Biosystems, Foster City, CA, USA). Polymer samples were prepared in a multiple layer-spotting approach as recently reported.98 The order of spotting was dithranol (saturated in chloroform), NaI (saturated in acetone) and analyte (10 mg/mL in dichloromethane). For the small organic molecules described in section 4.2.1 samples were prepared as follows: matrices were used as 20 mg/mL solutions (dithranol in chloroform, 2,5-dihydroxybenzoic in acetone and α-cyano-4-hydroxycinnamic acid in methanol). Fresh matrix solutions were prepared prior to every measurement because dithranol is known to decompose rapidly. Solutions of the complexes were prepared at a concentration of 2 mg/mL in a 1:9 acetonitrile/chloroform mixture. 10 µL of the matrix solution and 10 µL of the sample solution were combined in an Eppendorf tube and vortexed for at least 20 seconds. A 2.5 µL aliquot of this mixture was dropcasted on a stainless steel MALDI sample plate and dried under a gentle stream of air.

Size exclusion chromatograms were measured on a Waters SEC system consisting of an isocratic pump, solvent degasser, column oven, 2996 photo diode array (PDA) detector, 2414 refractive index detector, 717plus autosampler and a new Styragel HT 4 GPC column with precolumn installed. Samples were dissolved in the respective eluent at concentrations varying from 1-3 mg/mL and measured with instrument settings as described below. For comparison reasons, all investigated samples were of the same batch.

NMR spectra were measured on a Varian Mercury 400 NMR spectrometer in various deuterated solvents. The chemical shifts were calibrated to TMS.

UV/Vis and fluorescence spectra were recorded on a FlashScan 530 (AnalytikJena, Germany) in 96-well microtiter plates (polypropylene, flat bottom) from Greiner (Greiner Bio-One,
Terpyridine-containing supramolecular polymers

Germany) in a range from 250 to 800 nm. The UV/Vis spectra were referenced to an empty microtiter plate and measurements were performed with four flashes. The actual time for the measurement of one microtiter plate with 96 full UV/Vis spectra was approximately 40 seconds.

Dynamic light scattering (DLS) experiments were performed on a Malvern CGS-3 equipped with a He-Ne laser (633 nm). The measurements have been performed at an angle of 90 degrees and at a temperature of 25 °C.

Analytical ultracentrifugation was carried out with a Beckmann Optima XL-A ultracentrifuge. Sedimentation equilibrium measurements were performed at 20 °C and a rotor speed of 5000 rpm. The absorption was measured at 488 nm (metal-to-ligand charge transfer band of the terpyridine ruthenium(II) complex). Ammonium hexafluorophosphate or tetrabutyl ammonium hexafluorophosphate (20 mmol) were added to screen out the electrostatic interactions in the solution.

AFM imaging in liquid was performed using an Ntegra SPM (NT-MDT, Moscow, Russia) using AC40TS-type (SiN, 0.1 N/m, Olympus, Tokyo) cantilevers. To avoid tip convolution effects, the diameter of the micelles was estimated from the observed height.

Transmission electron microscopy (TEM) was performed on a LEO 922 microscope, operating at a 200 kV accelerating voltage in bright field mode. The images were formed by unscattered electrons only. Samples for TEM experiments were prepared by spin-coating a drop of diluted solution of micelles on a carbon-coated TEM grid. The samples for TEM measurements were not stained.

Data Acquisition for experiments described in section 4.2.1

All data used for the estimation of the relative binding strength was obtained in the following way: three mass spectra with 50 laser shots per spectrum were recorded for each laser intensity. The relative signal intensities for the ligand (L) and complex (ML₂) peaks were taken from the calibrated mass spectra to calculate the [LH]⁺/[ML₂]⁺ ratios. Afterwards these ratios were averaged over the three spectra corresponding to one laser intensity. The acceleration potential was left constant at 25 kV throughout these measurements.

Preparation of model complexes and polymers studied in section 4.2

The model complexes with the 4’-(1,4,7-trioxa-octyl)-2,2’:6’,2’’-terpyridine ligand as well as P1-P6 that were studied in section 4.2 were prepared and fully characterized as described in the literature by Dr. B. G. G. Lohmeijer. Polymers P7 and P8 that were used for the verification of the SEC system as described in section 4.2.2 were prepared and fully characterized by Dr. H. Hofmeier.

SEC and UV/Vis screenings described in section 4.3

Samples (20 µL for each measurement) were taken directly from the reaction mixtures at various times and diluted with SEC eluent to a volume of 1 mL for SEC measurements or
diluted with DMF to 250 µL in polypropylene microtiter plates for UV/Vis measurements. Subsequently, UV/Vis spectra were recorded in parallel whereas SEC samples were transferred to the autosampler of the GPC system to be measured and processed.

**Synthesis of 1,16-bis(2,2’:6’,2”-terpyridin-4’-yloxy)hexadecane (I)**

I was prepared according to a modified literature procedure. To a stirred suspension of powdered KOH (449 mg, 8 mmol) in dry DMSO (25 mL) at 60 °C 1,16-hexadecanol (517 mg, 2 mmol) was added. After 15 min 4’-chloro-2,2’:6’,2”-terpyridine was added (1.17 g, 4.4 mmol) and the mixture was stirred for 24 h at 60 °C and subsequently poured into cold water (600 mL). The product precipitated and was collected by filtration and afterwards washed with deionized water and methanol. After drying in vacuo I was obtained as a white solid (1.26 g, 85%). ^1H NMR (CDCl₃): δ = 1.25-1.42 [m, 20 H, OCH₂CH₂CH₂(CH₂)₁₀CH₂CH₂CH₂O], 1.50 [tt, 4 H, J = 8.1, 7.3 Hz, OCH₂CH₂CH₂O], 1.85 [tt, 4 H, J = 7.3, 6.6 Hz, OCH₂CH₂(CH₂)₁₀CH₂CH₂CH₂O], 4.22 [t, 4 H, J = 6.6 Hz, OCH₂CH₂(CH₂)₁₀CH₂CH₂CH₂O], 7.32 [m, 4 H, H₅,₅”], 7.84 [m, 4 H, H₄,₄”], 8.01 [s, 4 H, H₃’,₃”], 8.61 [d, 4 H, J = 8.1 Hz, H₃,₃”], 8.69 [d, 4 H, J = 3.7 Hz, H₆,₆”]. MALDI-TOFMS: (matrix: dithranol): m/z (%) = 721.9 (MH⁺, 35%), 743.9 (MNa⁺, 100%).

**Screening polymerizations of I**

I was polymerized with RuCl₃ in various stoichiometries and under diverse conditions in a 12 place carousel reaction station from Radleys. Different solvents, temperatures, reactant ratios as well as various additives, such as different salts and different alcohols were investigated.

**Synthesis of α-terpyridine-ω-methyl-poly(ethylene glycol) (P9)**

P7 was prepared and fully characterized as described in the literature by Dipl.-Chem. C. Ott.

**Synthesis of bis-(α-terpyridine-ω-methyl-poly(ethylene glycol))Ru(II) (P10)**

RuCl₃ (0.12 mmol, 24.9 mg) and P7 (0.12 mmol, 360 mg) were added to a solvent mixture of dimethylacetamide (DMA) (414 µL), n-butanol (110 µL, 1.2 mmol, 88.9 mg) and N-ethyl-morpholine (76 µL, 1.2 mmol, 69.1 mg) utilizing a conical glass vial that can be capped with a septum. The reaction was performed for 5 hours at 130 °C. The product was purified by preparative size exclusion chromatography (BioBeads SX-1, dichloromethane). Analytical results were according to the literature values.

**Synthesis of supramolecular ABA triblock copolymer (P11)**

RuCl₃ (0.132 mmol, 27.4 mg), I (0.12 mmol, 86.5 mg) and P7 (0.012 mmol, 36 mg) were added to a solvent mixture of dimethylacetamide (DMA) (396 µL), n-butanol (121 µL, 1.32 mmol, 97.8 mg) and N-ethyl-morpholine (83 µL, 1.32 mmol, 76.0 mg) utilizing a conical glass vial that can be capped with a septum. All components became readily dissolved after stirring the mixture for 3 minutes at 70 °C. The polymerization was performed for 5 hours at
130 °C. The resulting product was first precipitated in acetone and then redissolved in methanol. NH4PF6 was added in 10 fold excess and the mixture was refluxed for 1 hour. After cooling to room temperature P9 precipitated and was collected by filtration. Excess NH4PF6 was removed by preparative size exclusion chromatography (BioBeads SX-1, Acetone) yielding P9 as a red powder (105 mg, 59%). 1H NMR (acetone-d6): δ = 1.25-1.55 [m, 792 H, OCH2CH2CH2(CH2)10CH2CH2CH2O and OCH2CH2CH2], 1.67 [m, 132 H, OCH2CH2], 3.57 [s, 480 H, PEG-backbone], 4.62 [t, 136 H, J = 6.6 Hz, OCH2], 7.31 [m, 136 H, H5,5'"], 7.74 [d, 136 H, J = 5.1 Hz, H6,6"], 8.03 [t, 136 H, J = 8.1 Hz, H4,4"], 8.68 [s, 136 H, H3',5'"], 8.82 [d, 136 H, J = 8.1 Hz, H3,3'"].

Synthesis of 6-Aminohexyl 4'-2,2':6',2''-terpyridinyl) ether (2)

To a stirred suspension of powdered KOH (22 4.4 mg, 4.0 mmol) in dry DMSO (30 mL) at 60°C, 6-amino-hexan-1-ol (131.2 mg, 1.0 mmol) was added. After 30 min, 4'-chloro-2,2':6',2''-terpyridine was added (267.7 mg, 1.0 mmol) and the mixture was stirred for 4 h at 60°C and then precipitated drop wise into 0.5 L of ice-cooled, distilled water. After 2 h stirring, the precipitate was collected and washed with distilled water. The pure product was obtained in 93% yield after column chromatography (CHCl3, Al2O3). 1H NMR (CDCl3): δ = 1.34-1.48 [m, 6 H, OCH2CH2CH2CH2CH2NH2], 1.87 [tt, 2 H, J = 6.6, 7.3 Hz, OCH2CH2], 2.72 [t, 2 H, J = 6.6 Hz, CH2NH2], 4.23 [t, 2 H, J = 6.63 Hz, OCH2], 5.33 [s, 2H, NH2], 7.33 [m, 2 H, H5,5'"], 7.82 [ddd, 2 H, J = 1.5, 6.6, 8.13 Hz, H4,4"], 8.01 [s, 2 H, H3',5'"], 8.61 [d, 2 H, J = 8.13 Hz, H3,3"], 8.70 [d, 2 H, J = 3.93 Hz, H6,6"]. 13C NMR (CDCl3): δ = 26.10 [OCH2CH2CH2C], 26.82 [OCH2CH2C], 29.25 [CCH2NH2], 33.94 [OCH2C], 42.37 [CH2NH2], 68.36 [OCH2], 107.66 [C3',5'"], 121.63 [C5,5'"], 124.05 [C3,3'"], 137.08 [C4,4"], 149.27 [C6,6"], 156.44 [C2,2"], 157.29 [C1',5'"], 167.55 [C4']. MALDI-TOFMS (m/z): 349 (MH+).

Modification of PVC with (2-mercapto-phenyl)-methanol (P12)
PVC (2 g, 32 mmol) and (2-mercapto-phenyl)-methanol (560 mg, 4 mmol) were dissolved in 50 mL of dried cyclohexanone. 1.6 g of K2CO3 was added and the reaction was stirred under Argon atmosphere at 60°C. The reaction was stopped after 24 h by precipitating the mixture in cold methanol/water (2:1 v/v). The modified polymer 4 was purified using THF/methanol as solvent-precipitant system. The characterization results are described in the section 4.3.2.

Synthesis of terpyridine functionalized PVC (P13)

In the first step of the synthesis, the isocyanate 4 was prepared by reaction of 2 (175 mg, 0.5 mmol) with an excess of di-tert-butyltricarbonate 3 (158 mg, 0.6 mmol) in dichloromethane (20 mL) at room temperature. The 1H NMR showed a shift of the CH2NH2 triplet from 2.72 to 3.32 ppm after 15 min reaction time, indicating the successful formation of the isocyanate 4. Subsequently, 250 mg of P12 in dichloromethane (100 mL) were added to 4, without isolation of the isocyanate (the byproducts of the isocyanate formation are
harmless to the next reaction step). Dibutyltindilaurate (0.2 mL) was added as a catalyst and the reaction mixture was stirred overnight at room temperature. Polymer \textbf{P13} was precipitated in methanol after the reaction mixture was concentrated by solvent evaporation \textit{in vacuo}. Purification of \textbf{P13} was accomplished by solvation in THF and subsequent precipitation in MeOH (two times). The characterization results are described in the section 4.3.2.

\textbf{Synthesis of grafted PVC (P14)}

20 mg of \textbf{P13} were dissolved in CHCl$_3$. After addition of 5 (8.5 mg, 0.015 mmol) (prepared according to literature procedures by Dr. B. G. G. Lohmeijer) in CHCl$_3$/EtOH (1:1) and 0.5 mL of \textit{N}-ethyl-morpholine the reaction mixture was refluxed for 4 hours. All solvents were evaporated and the polymer was purified by preparative size exclusion chromatography (Biobeads SX-1, CH$_2$Cl$_2$). The characterization results are described in the section 4.3.2.

\textbf{Synthesis of star-shaped polymer (P15)}

To a stirred suspension of KOH (224.4 mg, 4.0 mmol) in dry DMSO (30 mL) at 65 °C, the starting 5-arm poly(ethylene glycol) polymer (131.2 mg, 1.0 mmol) was added. After 30 min, 4’-chloro-2,2′:6′,2′′-terpyridine was added (267.7 mg, 1.0 mmol) and the mixture was stirred for 16 h at 65 °C, added to dichloromethane and subsequently washed with water (3x). The organic layer was dried over MgSO$_4$. The pure product was obtained after column chromatography (CH$_2$Cl$_2$, Al$_2$O$_3$). $^1$H NMR (CDCl$_3$): $\delta$ = 2.55-2.80 [m, 18 H, CH$_2$OCH$_2$CH$_2$NCH$_2$CH$_2$N], 3.50 [t, 10 H, $J$ = 5.86 Hz, CH$_2$OCH$_2$CH$_2$NCH$_2$CH$_2$N], 3.55-3.80 [m, 180 H, CH$_2$OCH$_2$CH$_2$OCH$_2$], 4.39 [t, 10 H, $J$ = 4.39 Hz, tpy-OCH$_2$CH$_2$O], 3.93 [t, 10 H, $J$ = 4.39 Hz, tpy-OCH$_2$CH$_2$O], 7.32 [m, 10 H, H5,5′], 7.84 [m, 10 H, H4,4′], 8.04 [s, 10 H, H3’,5’], 8.61 [d, 10 H, $J$ = 8.06 Hz, H3,3′], 8.67 [d, 10 H, $J$ = 4.93 Hz, H6,6′]. $M_n$ (SEC) = 3100 Da, PDI (SEC) = 1.15; $M_n$ (MALDI) = 3350 Da, PDI (MALDI) = 1.03.

### 4.7 References


Terpyridine-containing supramolecular polymers


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List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AFM</td>
<td>atomic force microscopy</td>
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<tr>
<td>AUC</td>
<td>analytic ultracentrifugation</td>
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<tr>
<td>CMR</td>
<td>combinatorial materials research</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
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<td>DoE</td>
<td>design-of-experiments</td>
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<td>FT-IR</td>
<td>Fourier-transform infrared</td>
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<td>GC</td>
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<td>HTS</td>
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<td>M/I ratio</td>
<td>monomer/initiator ratio</td>
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<td>MALDI</td>
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<td>PCL</td>
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<td>PDA</td>
<td>photo diode array detector</td>
</tr>
<tr>
<td>PDI</td>
<td>polydispersity index (Mₘ / Mₙ)</td>
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<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>QSPR</td>
<td>quantitative structure property relationship</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible Addition-Fragmentation Chain Transfer</td>
</tr>
<tr>
<td>ROP</td>
<td>ring opening polymerization</td>
</tr>
<tr>
<td>SEC</td>
<td>size exclusion chromatography</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TOF</td>
<td>time-of-flight</td>
</tr>
<tr>
<td>tpy</td>
<td>terpyridine</td>
</tr>
<tr>
<td>UHTS</td>
<td>ultra high-throughput screening</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>ultra violet/visible</td>
</tr>
</tbody>
</table>
Summary

Current challenges in polymer science focus on the control of polymer architecture, molecular weight, end-group and other parameters. Scientists have realized that the synthesis of such complex and well-defined macromolecules possess major challenges as well as opportunities since novel chemical, physical and biological properties of the studied materials might be obtained in a controllable fashion. The advances made in the preparation of macromolecules due to the invention of controlled and living polymerization techniques in combination with specific organic reactions for end-group modifications or the preparation of functional monomers offer the synthetic chemist the right tools to address these challenges. However, this enormous parameter space is difficult to tackle and requires the combination of the synthetic techniques with a detailed structure and property characterization. Therefore, combinatorial and parallel approaches in combination with efficient screening methods seem to be the perfect solution to investigate this parameter space. This thesis describes several aspects of combinatorial, parallel and automated approaches in polymer science covering the necessary development of new detailed characterization techniques as well as the synthesis of novel macromolecules with defined architectures and their property evaluation. In particular, automated and parallel approaches in three different fields of research were addressed, namely: high-throughput screening by MALDI-TOFMS, micellar systems of biocompatible block copolymers and supramolecular metal-containing polymers.

The first part describes the development and evaluation of a new sample preparation technique for MALDI-TOFMS that allowed the integration of MALDI-TOFMS as a high-throughput screening technique into the workflow of combinatorial materials research (CMR). Therefore, a novel multiple-layer sample preparation technique for synthetic polymers was invented and evaluated revealing improved analytic results. Subsequently, this technique could be automated and integrated into synthetic robots in order to provide a new screening technique for polymer libraries. In a final step of automation and miniaturization this sample preparation technique could be transferred to ink-jet printing technology opening the way for ultra high-throughput approaches. Moreover, two application examples of the developed techniques are discussed, namely the investigation of a library of poly(2-oxazoline)s and the online monitoring of reversible addition fragmentation transfer (RAFT) polymerizations of methyl methacrylate (MMA). In general, this multiple-layer spotting technique provided significantly improved analytical results for a large variety of synthetic polymers, could be automated in straightforward fashions, saved valuable sample preparation time and was therefore very well suited for screening approaches in CMR.

The second part describes the synthesis and characterization of block copolymers of poly(ethylene glycol) and poly(ε-caprolactone). In particular, linear and star-shaped block copolymers were prepared and characterized in detail. Their micellar properties were investigated utilizing self-developed high-throughput screening assays revealing structure-
property relations concerning the guest encapsulation behavior for the linear block copolymers. Moreover, the star-shaped block copolymers showed an interesting reversed unimolecular micellar behavior and were able to encapsulate and phase transfer a large variety of different guest molecules. This encapsulation behavior could be correlated to the macromolecular structure and it was for instance observed that the maximum loading of guest molecules within a reversed unimolecular micelle was independent of the chain length of the poly(ε-caprolactone) comprising the corona of these micelles. Moreover, the guest transport of both micellar systems could be confirmed by analytical ultracentrifugation experiments. Finally, the star-shaped block copolymers could be used as templates for the synthesis of defined palladium nanoparticles, which could successfully be applied as catalysts for C-C coupling reactions.

The last part of this thesis describes the synthesis and detailed characterization of metal-containing supramolecular polymers based on terpyridine ligands. The investigations were started due to the need of improved characterization methods for these polymers. A MALDI-TOFMS model study revealed the relative binding strength of the terpyridine ligand for a large variety of different transition metal ions that are frequently used in these supramolecular polymers, whereas a size exclusion chromatography (SEC) study resulted in optimized chromatographic conditions for ruthenium-containing polymers. Especially the SEC study was crucial for the success of a subsequently performed parallel optimization of the reaction conditions of RuCl₃ with a defined low molecular weight bis-terpyridine ligand where it was applied as the major screening technique. These investigations led to the development of a new type of supramolecular ABA triblock copolymer based on a simple polycondensation strategy that revealed amphiphilic behavior and formed micelles in water. Finally, the investigation of a star-shaped supramolecular polymer by means of parallel high-throughput techniques led to the accelerated development of a new sensoric system for transition metal ions. This system is based on the encapsulation of fluorophores within a terpyridine end-group modified star-shaped polymer and quenching of the fluorescence due to metal complex formation.

The described results clearly demonstrate that automated and parallel approaches in polymer science provide one possible solution to efficiently address current challenges in materials science. Generally, the study of the three different topics by these techniques led to a better understanding of the investigated subjects within a reduced time frame and revealed the effectiveness of the chosen approaches. In conclusion, the results obtained during the study of these subjects significantly extended the scientific knowledge in all three sub disciplines. Moreover, the addressed new application possibilities of the prepared macromolecular architectures as well as the developed screening techniques are not only useful for the combinatorial researcher but also provide significant advantages for the whole scientific community.
Samenvatting


Het eerste gedeelte beschrijft de ontwikkeling en evaluatie van een nieuwe monsterbereiding voor MALDI-TOFMS welke het integreren van MALDI-TOFMS als hoge-doorvoer screening methode in het gebied van combinatorieel onderzoek mogelijk maakte. Daarvoor was een nieuwe multipel-laag monsterbereiding ontworpen en getest dat betere analytische resultaten mogelijk maakte. Daarna werd deze techniek met behulp van synthese robots geautomatiseerd en geïntegreerd, om het screenen van polymeer bibliotheken mogelijk te maken. In de laatste stap van automatisering en miniaturisatie werd de monsterbereidingsmethode toegepast op ink-jet printen met als resultaat dat MALDI-TOFMS gebruikt kon worden voor ultra hoge-doorvoer applicaties. Bovendien worden twee applicatie voorbeelden behandeld: het onderzoek van een bibliotheek van poly(2-oxazoline)s en de gekoppelde karakterisatie van RAFT polymerisaties van MMA. Algemene voordelen van deze multipel-laag monsterbereiding waren betere analytische resultaten voor een groot aantal verschillende synthetische polymeren, het ongecompliceerd automatiseren en het besparen van monsterbereidingstijd. Deze techniek is daarom heel goed toepasbaar voor screening applicaties in het combinatoriële polymeer onderzoek.

Het tweede gedeelde behandelt de synthese en karakterisatie van block-co-polymeren van poly(ethylene glycol) en poly(ε-caprolacton). Hier zijn lineaire polymeren zoals ster-vormige
Samenvatting

block-co-polymeren gemaakt en in detail gekarakteriseerd. De micelaire eigenschappen zijn daarna met behulp van zelf-ontwikkelde hoge-doorzet screening methodes onderzocht met als resultaat dat de lineaire block-co-polymeren alleen water onoplosbare gastmoleculen konden insluiten. De ster-vormige block-co-polymeren gedroegen zich als unimoleculaire micellen en waren in staat een grote hoeveelheid verschillende gastmoleculen in te sluiten, en deze van een waterfase naar een chloroformfase te transporteren. Het was mogelijk dit insluitgedrag in verband te brengen met de structuur van de onderzochte macromoleculen. Bovendien was het mogelijk het transportgedrag van de block-co-polymeer systemen met behulp van analytische ultra-centrifugatie te onderzoeken. Ten slotte was het mogelijk de ster polymeren als sjabloon te gebruiken voor het maken van goed gedefinieerde en gestabiliseerde palladium nano-deeltjes. Deze nano-deeltjes gedroegen zich als effectieve katalysatoren voor C-C koppeling reacties.

Het laatste gedeelte van dit proefschrift behandelt de synthese en gedetailleerde karakterisering van metaal-houdende supramoleculaire polymeren gebaseerd op terpyridine liganden. Het onderzoek was gestart omdat de karakterisatie-mogelijkheden van dit soort polymeren verbeterd moest worden. De relatieve bindingssterkte van de terpyridine liganden met een aantal verschillende overgangsmetaal-ionen was de uitkomst van een MALDI-TOFMS onderzoek. Verder zijn optimale condities voor grote-uitsluit-chromatografie voor de karakterisatie van ruthenium-houdende polymeren gevonden. Vooral de chromatografische techniek was zeer belangrijk voor het vervolgonderzoek naar de parallelle optimalisatie van de reactiecondities van RuCl₃ met een bis-terpyridine ligand omdat deze techniek de hoofd-screening methode was. Het resultaat van deze optimalisatie was de ontwikkeling van en nieuw soort ABA triblock co-polymer gebaseerd op een simpele polycondensatie techniek waardoor micellen in water gevormd werden. Ten slotte was de uitkomst van het parallelle hoge-doorvoer onderzoek aan een ster-vormig supramolecular polymer de uitvinding van een nieuw sensorisch systeem voor de detectie van overgangsmetaal-ionen. Dit systeem is gebaseerd op het insluiten van een fluorescerend molecuul in een terpyridine eind-groep gemanipuleerd ster-vormig polymer en het onderdrukken van de fluorescentie in verband met metaalcomplex vorming.

De beschreven resultaten laten duidelijk zien dat geautomatiseerde en parallelle toepassingen voor polymeeronderzoek een van de mogelijkheden zijn om efficiënt met de recente uitdagingen in het gebied van materiaal onderzoek om te gaan. Met behulp van deze technieken zorgt het onderzoek voor een beter inzicht in alle drie de verschillende onderwerpen in een korte tijd en laten de effectiviteit van de gekozen toepassingen zien. Als conclusie heeft het onderzoek naar deze onderwerpen tot een verbetering van de vakkundige kennis in alle drie de onderzoeksgebieden bijgedragen. Ten slotte zijn de nieuwe macromoleculen met de behandelde toepassingen en de ontwikkelde screening-technieken niet alleen nuttig voor de combinatoriële onderzoeker maar ook gunstig voor de hele vakkundige gemeenschap.
Curriculum vitae

Michael A. R. Meier was born in Ingolstadt (Germany) in 1975. After finishing his general qualification for university entrance (Abitur) in 1995, he graduated in chemistry from the University of Regensburg (Germany) in 2002. His diploma thesis dealt with the fluorosensing of ammonium ions via molecular recognition in polymeric emulsion membranes and was carried out at the Institute of Analytical Chemistry, Chemo- & Biosensors at the University of Regensburg with Prof. O. Wolfbeis. In May 2002 he started his Ph.D. project with Prof. U. S. Schubert at the Eindhoven University of Technology, The Netherlands. The most important results of this work are described in this thesis.
List of Publications

Refereed Journal publications (in chronologic order):


### Selected oral presentations (in chronologic order):


M. A. R. Meier, U. S. Schubert, Automated Synthesis, Parallel Screening and Application Possibilities of Star-Shaped Block Copolymers, *Young European Scientists meeting (YES 2005)*, Cracow, Poland, September 13-17, **2005**.

M. A. R. Meier, U. S. Schubert, Application possibilities of reversed unimolecular micelles based on star-shaped block copolymers, *Dutch Polymer Days 6*, Lunteren, The Netherlands, February 6-7, **2006**.
Acknowledgement

Thousands of MALDI and UV/Vis spectra, hundreds of NMRs and SECs, dozens of experiments, presentations and publications, 8 GB of data, 4 years and 1 thesis later I am ready to obtain the highest (not awarded) academic degree ... am I? Whatever the answer to this question is, I would not have come so far without the help, support and encouragement of a lot of people and I would like to take this opportunity to express my gratitude to everybody who contributed to this work in whatever fashion.

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¹ Para: Latin for "against" or "counter"; nimf: Dutch for "charming girl", "mythology derived figure" or "insect"