Responsive hydrogels based on thiolactone chemistry

Thesis submitted to obtain
the degree of Master of Science in Chemistry by

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Academic year 2011-2012

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Copromoter and supervisor: dr. Stefan Reinicke
Acknowledgements

As a thesis means an end of a period, where joys and sorrows were shared, I want to thank a number of people.

At first, I would like to thank professor Du Prez for the opportunity to perform my thesis at his research group. Without his help and remarks, this thesis would not have been possible.

I have to thank Dr. Stefan Reinicke for the guidance of my thesis. His experience and know-how was very useful in the elaboration of this work.

Further on, I would like to thank the support of the friends I have made during my students period.

In particular, I want to thank my girlfriend Joyce for the moral support and the patience during the past 5 years. Without her, I would not be able to reach so far.

As last, a word of gratitude for my parents are in place. Without their financial support, the last period was not possible.
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<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>(P)DEGA</td>
<td>(Poly) di(ethylene glycol)acrylate</td>
</tr>
<tr>
<td>(P)NIPAAm</td>
<td>(Poly) N-isopropylacrylamide</td>
</tr>
<tr>
<td>(P)OEGA</td>
<td>(Poly) oligo(ethylene glycol)acrylate</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom Transfer Radical Polymerization</td>
</tr>
<tr>
<td>CAT</td>
<td>Chloramine T</td>
</tr>
<tr>
<td>CRP</td>
<td>Controlled radical polymerization</td>
</tr>
<tr>
<td>CTA</td>
<td>Chain Transfer Agent</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper catalyzed azide-alkyne cycloaddition</td>
</tr>
<tr>
<td>CVADTB</td>
<td>4-cyano-4-(thiobenzyl) sulfanyl pentanoic acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDMAT</td>
<td>2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid</td>
</tr>
<tr>
<td>DMA</td>
<td>N-N-dimethylacetamide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-Red</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower Critical Solution Temperature</td>
</tr>
<tr>
<td>$M_n$</td>
<td>Number average molecular weight</td>
</tr>
<tr>
<td>NEt$_3$</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>NMP</td>
<td>Nitroxide Mediated Polymerization</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity</td>
</tr>
<tr>
<td>PEG/PEO</td>
<td>Poly(ethylene glycol/oxide)</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible Addition-Fragmentation chain Transfer Polymerization</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Tla</td>
<td>Thiolactone acrylamide</td>
</tr>
<tr>
<td>UCST</td>
<td>Upper Critical Solution Temperature</td>
</tr>
<tr>
<td>wt%</td>
<td>Weight percentage</td>
</tr>
<tr>
<td>TCEP</td>
<td>Tris(2-carboxyethyl)phosphine hydrochloride</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction and objective

In this thesis, the aim was to establish a straightforward synthetic protocol to create multi-responsive hydrogels for sensing purposes. Commonly, such hydrogels require a rather complicated multi-step synthetic protocol. We wanted to overcome this by creating such gels from polymers equipped with thiolactone groups. This functionality provides the opportunity for a simultaneous functionalization and crosslinking of the polymer through a nucleophilic ring opening of the thiolactone ring and a subsequent formation of disulfide bonds from the released thiol groups. The corresponding reaction scheme is represented in scheme 1.1. The thesis includes the synthesis of suitable polymer precursors via RAFT in a first step. With the use of this controlled radical polymerization method, polymers can be synthesized with a low polydispersity. This will later on lead to hydrogels with a more homogeneous structure, and in turn to a quicker and more controllable gel response. Moreover, the RAFT process is undemanding in terms of time and preparation efforts. This gives the opportunity to synthesize a wide variety of polymers in a rather short time, which will help to systematically screen the gel properties in a later stage.

Scheme 1.1: Reaction scheme for the formation of a functionalized gel with S-S bonds as crosslinker.
Afterwards, gels will be prepared through the thiolactone ring opening protocol. This part mainly includes the search for optimum functionalization/gelation conditions. The functional amine targeted will be a compound equipped with a morpholino group, which is supposed to interact with heavy metal ions like Cu$^{2+}$. After optimization of the gelation method and the creation of a method to determine the response with this amine, the more expensive boronic acid derivative could be used in a later stage. This moiety is known to interact with glucose. When the hydrogels are formed, they will be investigated on their molecular characteristics and their responsiveness towards the aimed molecules. Open tasks will include the proof of principle and after that an optimization of the gels with respect to mechanical stability and response behaviour.

This project links the power of the thiolactone chemistry to hydrogel applications. In particular, we believe that our synthetic protocol will help to establish more straightforward methods to create gels for sensing purposes. Our protocol requires in principle no heat, no extra crosslinkers and no environmental unfriendly solvents. Furthermore, no side products are formed and the possibility to vary the ring opening amine opens a huge number of possibilities to the aimed response property. Moreover, thiolactone functionalized monomers are compounds that are easily synthesized from cheap commercial and biological sources. Finally, the hydrogels have the ability to degrade under reductive conditions due to the breaking of the disulfide bonds. This ability can be useful for biomedical applications.
Chapter 2: Theoretical description

2.1 Radical polymerization

In this thesis, polymerization is performed via controlled radical polymerization. In order to obtain a complete background of this method, a theoretical description has been given.

2.1.1 Global introduction

Radical polymerization is a chain growth polymerization in which the active ends of a growing chain bare a free radical. It is accessible for a wide variety of monomers mostly based on vinyl and (meth)acrylate species, i.e. molecules containing a C-C double bond. One can subdivide the radical polymerization mechanism into 3 different steps:

- **Initiation step**
  This step is being introduced by an environment where free radicals are generated within the reaction mixture. This can be done by the use of radical initiators, such as azobisisobutyronitrile (AIBN) (an azo-type initiator) and benzoyl peroxide (a peroxide type initiator). The mentioned initiators, and how they decompose, are shown in scheme 2.1. In the actual initiation step, the formed radical will react with the double bond of one monomer molecule (scheme 2.2) leading to a new radical, which consists of the radical fragment from the initiator and one monomer moiety.

- **Propagation step**
  In this step, the radical chain will continue growing by adding more monomer units until termination or transfer will take place.

- **Termination step**
  This step terminates propagation of a kinetic chain. This can happen by either recombination or disproportionation (scheme 2.2). Recombination is a process where 2 radical chain ends will recombine to form one polymer chain that is no longer a radical and therefore inactive. Disproportionation, on the other hand, is a process where an electron transfer from one radical chain end to the other takes place leading to two inactive chains, one of which has an unsaturated chain end (scheme 2.2).
Besides termination, also inter- or intramolecular electron transfer can take place (scheme 2.2). In that case, new radicals are generated which are still active towards propagation. Those reactions are therefore no termination reactions as the number of active chain ends is not affected. However, the polydispersity broadens and branching of the polymer chains occurs.

![Diagram of radical polymerization](image)

**Scheme 2.1:** The structures and dissociation mechanism of AIBN (above) and benzoyl peroxide (bottom).

2.1.2 **Controlled radical polymerization (CRP)**

2.1.2.1 **Introduction**

The major disadvantage of radical polymerization is the lack of control, which originates from the significant occurrence of termination and transfer. A consequence of this phenomenon is that the received polymer has a high polydispersity and it is difficult to create polymers with a certain functionality, architecture and degree of polymerisation.
A way to solve this problem is to significantly decrease the concentration of the radicals in the solution. Hereby a suppression of the radicals involving side reactions like termination is introduced. Mechanisms including only a low fraction of propagating radicals are called controlled radical polymerizations.

CRP works always on the same principle: an introduction of an equilibrium between an active radical and an inactive, but reactivatable, chain end. In order to have a good control over the polymerization, the inactive side of the equilibrium must be preferred and the conversion between the inactive and the active side has to be very quick. This implies a constant activation and deactivation of the radicals, which means that the active radical concentration is kept low. The most frequently used methods are Atom Transfer Radical Polymerization (ATRP), Nitroxide Mediated Polymerization (NMP) and Reversible Addition-Fragmentation chain Transfer polymerization (RAFT).

In ATRP, Cu(I) or other transition metal ions are used to establish the equilibrium between the activated radical polymer chain and the respective dormant species. The Cu species homolytically cleaves a halogen group from the inactive chain end transforming the latter into the active radical chain. This active chain end will propagate by adding monomer molecules. The disadvantage of this method is the use of the metals, which are toxic above certain concentrations. NMP on the other hand is a method that uses stable radical compounds, in particular nitroxides. These compounds can reversibly bind onto a radical polymer chain end, leaving the latter dormant. This method has certain disadvantages such as the limitation in usable monomers and the high temperature needed. Within this thesis, the focus lied on RAFT. This method therefore will be explained in more detail in the following section.

2.1.2.2 RAFT

The Reversible Addition-Fragmentation Chain transfer (RAFT) method was invented at the CSIRO in Melbourne, by S. Thang, G. Moad and E. Rizzardo. This CRP method uses the same conditions as a conventional radical polymerization with the difference that a so called chain transfer agent (CTA) is added, a compound containing a thiocarbonylthio group. The CTA has the ability to reversibly bind a radical to reduce the number of active radicals in the solution. The resulting intermediate compound is also a radical, but is not active towards propagation.
A general structure of a CTA agent is shown in scheme 2.3, together with the structures of the 2 used CTA’s in this thesis: 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) and 4-cyano-4-(thiobenzoyl sulfanyl) pentanoic acid (CVADTB). Besides the thiocarbonylthio group, a CTA always contains a so called Z- and a R-group. The Z-group is a group that stabilizes the intermediate radical to a certain extent and so helps to balance the chain transfer equilibrium. The R-group on the other hand serves as a leaving group, which can also propagate after cleavage.

![Scheme 2.3: The structure of a general RAFT CTA compound (above) and the structure of DDMAT (left) and CVADTB (right).](image)

The mechanism of a RAFT chain transfer equilibrium is shown in Scheme 2.4. A radical polymer/initiator fragment interacts with the CTA leading to the formation of the intermediate persistent radical compound. This intermediate compound is able to perform a β-scission reaction. This leads to the formation of the original compounds or, via cleavage of the R group, a new propagating radical together with an alternative thiocarbonylthio compound. The formed radical polymer chain can now propagate or again add to the thiocarbonyl group to form a new intermediate radical compound, which again can undergo β-scission, and so on. By the continuous formation of the intermediate compound, the number of propagating polymer chains is being reduced and one gains control of the polymerization.

![Scheme 2.4: The RAFT mechanism.](image)
RAFT is a process that does not require a high preparation time and efforts. This means that the process can be used in a high-throughput workflow. This will aid the project in order to screen and investigate a lot of properties and polymers in a rather short time. RAFT is also tolerant for many functional monomers, thus high molecular weight polymers with a narrow polydispersity can be synthesized. To have optimal conditions, a good choice of the RAFT agent is necessary.

2.2 ‘Click’ chemistry

2.2.1 Introduction

The term click chemistry is used for reactions fulfilling the following criteria defined in 2001 by Nobel prize winner Barry Sharpless: “A click reaction must be modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), are stereospecific, simple to perform and that require benign or easily removed solvent”.

Such reactions are very useful for post-polymerization modification. A lot of new materials with different functionalities and complex architectures could be made in a rather easy and straight-forward way applying a “click” reaction. The impact of the name ‘click’ was so high, that it was used for numerous efficient linking reactions. However, to label a reaction as “click” reaction in polymer chemistry, additional criteria to the ones mentioned above have to be met. For instance, the separation of the polymeric precursors from the final coupling product is often not straightforward. Therefore, a “click” reaction used for polymer-polymer conjugation has to work under strictly equimolar conditions. A modified list of criteria, which have to be met to be able to call a reaction a “click” reaction in polymer chemistry is listed below.

- the mixture owns only stable compounds;
- the reaction owns a high yield;
- the reaction is fast;
- the purification can be done on large scale;
- the reaction has to be equimolar;
- the reaction is modular;
• the reaction is wide in scope;
• the linkage is chemoselective;
• the reaction exists of only 1 reaction.

2.2.2 Huisgen 1,3 dipolar cycloaddition (azide-alkyne ‘click’ chemistry)

One of the most popular reactions being classified as a ‘click’ reaction is the copper catalyzed Huisgen 1,3 dipolar cycloaddition (azide-alkyne click reaction, CuAAc), shown in scheme 2.5.

![Scheme 2.5: Reaction of an azide with a carbon-carbon triple bond.](image)

That particular reaction comes along with no by-products, mild reaction conditions, a high functional group tolerance and water compatibility. The drawback of this reaction is the explosive character of some low molecular weight azides and the use of Cu(I).

2.2.3 Thiol ‘click’ chemistry

Another important ‘click’ reaction is based on the use of thiols. Since 1905, it is known that thiols and carbon-carbon double bonds have a high affinity towards each other. Since then, this highly efficient reaction has been highly investigated. Thiols can react here either as a thiyl radical or as a nucleophilic thiolate anion. In both cases, the reactions fulfil the requirements to call the reaction a ‘click’ reaction, mentioned above, when used for low molecular weight compounds or conjugation of a polymer chain with a low molecular weight compound. Thiols can also react with a number of other
compounds. Scheme 2.6 gives an overview about the common thiol involving conjugation reactions\(^{5b}\).

2.2.3.1 Nucleophilic thiol ‘click’ chemistry

Thiols are nucleophiles and can therefore react with a variety of compounds with electron deficient functional groups. Some examples are the reactions with epoxides (\(S_N 2\) ring opening), isocyanates (carbonyl addition) and electron poor carbon-carbon double bonds (Michael addition). All these reactions require a base that can serve as a catalyst. This base can also be introduced by the use of photolatent bases (scheme 2.7). These are compounds that will form bases when they are exposed to light. This has the advantage that the reaction can be controlled in time and space.

Note that the nucleophicity of the thiolate anion will depend on the total structure where the thiolate is bound onto. This is represented on scheme 2.8\(^{5b}\).
2.2.3.2 Radical thiol ‘click’ chemistry

As stated in scheme 2.8, also thiol compounds exist that have a lower nucleophilicity. This means that they are not suitable for nucleophillic attack and thus only usable for radical thiol chemistry. Typical are the radical involving ‘thiol-ene’ and ‘thiol-yne’ reactions. These are reactions involving conjugation with C-C double and triple bonds. On scheme 2.9\textsuperscript{5b}, one can see a scheme of the reaction mechanism of both thiol-ene and thiol-yne reactions. The mechanism consists of a typical radical reaction, which means that a radical initiator will form a thiyl radical. This radical can react with a double bond to form a new radical. Due to proton transfer, this new radical is stabilized and a new thiyl radical is formed that can start a new reaction cycle. A thiol-yne is analogue to the thiol-ene reaction, with that difference that after the first cycle, the radical formed will own a double bond that also can react with the formed thiyl radicals. Note that the reaction can occur with different types of initiators (photo-, thermal- or redox initiator).

Scheme 2.9: Reaction mechanism of a ‘thiol-ene’ (left) and a ‘thiol-yne’ (right) reaction.
2.2.3.3 Advantages of thiol-ene click chemistry

Because of the fact that this reaction, used to conjugate a polymer chain with a low molecular weight compound, is stated as ‘click’, the reaction owns some advantages linked with ‘click’ chemistry, stated above. If necessary, the thiol-ene chemistry can benefit of the advantages of photo-initiating processes, such as the fact that the process can be initiated at a certain location and time. This means that spatially resolved materials can be formed.

The combination of the high robustness, simplicity and the mechanism makes thiol-ene chemistry perfect to form homogeneous polymer networks through a controllable combination of step-growth and chain growth processes, with simplified polymerisation kinetics. The polymers can also be made with reduced shrinkage and stress and the reaction is insensitive towards oxygen inhibition.

Another important advantage, in comparison with the Huisgen 1,3 dipolar cycloaddition, is that the thiol-ene/yn e reaction makes no use of (toxic) metals and (potentially explosive) azides. As demonstrated, thiol-yn e reactions have the ability to introduce a reaction of 2 thiol compounds with one alkyne compound. This makes the reaction a powerful tool in order to form highly functional and/or hyperbranched materials.

2.3 Thiolactone chemistry

Thiol-ene chemistry is a useful tool in polymer synthesis, however, the use of thiols includes unpleasant smells, a poor availability and a poor shelf life (tendency to form disulfide bridges) of the used compounds. However, it was noticed from the decades old work of Benesch, who wanted to introduce sulhydryl compounds into proteins, that thiolactones can liberate thiols by reacting them with certain nucleophiles such as amines (aaminolysis). Therefore, the thiolactone ring can serve as a kind of protected thiol for thiol-“click” chemistries. After the addition of an amine, the subsequently released thiols can immediately react with a double bond from another compound present in the solution. The reaction protocol is presented in scheme 2.10. The use of thiolactones instead of free thiols avoids the aforementioned disadvantages. Besides, thiolactone compounds can also be received from biological sources.
Since the ring opening amine is added to the thiolactone moiety, it is possible to introduce an extra functionality to the polymer before attending reaction with the released thiol functionality. The full amine-thiol-ene reaction protocol is therefore suitable for the creation of multifunctional polymer materials.

By converting the released thiol groups into disulfide bridges after their release instead of performing a thiol-ene reaction, it is envisioned that functional, crosslinked structures can be obtained. Here chain functionalization and crosslinking take place in one pot while no additional crosslinker is required. This feature is utilized for this thesis to create functional hydrogels for sensing purposes.

2.4 Stimuli responsive polymers

Stimuli responsive polymers are defined as polymers that respond with a large property change on small changes in their physical and/or chemical environment. In nature, a lot of processes and phenomena are based on molecular responses on external stimuli. Senses like for instance smell, touch, etc. are based on molecular responses on the change of pressure, heat, concentration of volatiles in the air, etc. It is possible to mimic such stimuli responsive behaviour within the field of synthetic polymers by using special polymer structures. Very common are thermosensitive polymers, i.e. polymers changing their hydrodynamic volume by a small temperature shift\textsuperscript{10}. Beside temperature, a lot of other different stimuli exist. For example pH-change, electric fields, light, magnetic fields\textsuperscript{11} and concentration of a chemical reagent are some of the possible stimuli. Besides volume changes, polymers can show other reactions on those stimuli such as degradation, shape change and swelling/collapsing.
2.4.1 Thermoresponsive polymers

One of the most studied and applied stimuli is temperature. Here, a polymer dramatically changes its solubility in the respective solvent upon a slight change in temperature. In other words, a polymer/solvent mixture is transformed from a one phase into a two phase system, i.e. a polymer rich and a polymer poor phase. This is illustrated in figure 2.1. Depending on the particular polymer/solvent combination and a number of other conditions such as the presence of additives, the phase diagram shows a two phase region at high temperatures with a minimum transition temperature (Lower Critical Solution Temperature, LCST) and/or a two phase region at low temperatures with a maximum transition temperature (Upper Critical Solution Temperature, UCST).

By far most important are LCST type polymers. Those are polymers that become insoluble upon temperature increase. The most investigated polymer showing an LCST in water is poly(N-isopropylacrylamide) (PNIPAAm) (scheme 2.11)\textsuperscript{12}.

As shown with the blue lines, PNIPAAm exhibits a hydrophobic backbone and isopropyl side groups. Nevertheless, the amide group of PNIPAAm will ensure the solubility of the polymer in water due to strong hydrogen bonds. With raising temperature, these bonds will break and the polymer will be separated from the solvent leading to the two phase system. This can be explained by the free enthalpy change of the system:

\[
\Delta G = \Delta H - T\Delta S
\]
Simply noted, the gain of entropy at a temperature above the LCST by releasing the water from the polymer is higher than the loss of enthalpy of breaking a hydrogen bond, therefore leading to a positive free enthalpy change.

It is possible to tune an LCST by a number of different methods\textsuperscript{13}. An example is the copolymerization: a comonomer of high polarity will shift the LCST to higher temperatures, while an apolar comonomer will shift it to lower temperatures. PNIPAAm as the most frequently used LCST type polymer has a transition temperature close to body temperature which is potentially useful especially for biomedical applications such as drug release systems. Besides, as mentioned above, the LCST can be easily adjusted giving the opportunity to create tailor made materials\textsuperscript{14}.

The disadvantages of PNIPAAm is that it shows a hysteresis upon a heating/cooling cycle and that it is not biocompatible\textsuperscript{15}. With respect to that, poly(oligo(ethyleneglycol) acrylate) (POEGA) is more useful. This compound is biocompatible, shows no hysteresis and the LCST is tunable by using larger ethylene glycol chains\textsuperscript{16}. Within the research of this thesis, we especially make use of methoxy di(ethyleneglycol)acrylate (DEGA), shown on scheme 2.12. According to literature PDEGA shows an LCST of around 37 °C\textsuperscript{16} making it useful for biomedical applications.

\begin{center}
\textbf{Scheme 2.12: Chemical structure of methoxy di(ethyleneglycol) acrylate.}
\end{center}

\subsection*{2.4.2 Molecule responsive polymers}

Temperature is not the only stimulus that can be applied on certain polymers. With addition of functional side chains, one can design structures that will respond to certain molecules or atoms. These side chains must have specific affinity towards the targeted molecule. In the following is a number chemical groups that show affinity towards certain atoms/molecules listed:
• affinity towards glucose via boronic acid\textsuperscript{17};
• affinity towards alkaline and alkaline earth metals via crown ethers\textsuperscript{18};
• affinity towards CO\textsubscript{2} via amidine groups\textsuperscript{19};
• affinity towards adenosine via uracil groups\textsuperscript{20};
• etc.

Another responsive material can be synthesized by the use of complexing ligands like crown ethers. These functionalities can make a polymer sensitive towards the concentration of alkaline (earth) metal ions in the solution. If this functional group is bound to a thermoresponsive polymer chain, the cloud point of the latter will be strongly influenced by the concentration of the respective heavy metal ion.

Heavy metal responding systems are particularly important for water treatment and other sensing purposes. Also the selectivity towards specific ions play an important role. So is the detection of Cu\textsuperscript{2+} or Ni\textsuperscript{2+} possible via polymers containing morpholine groups\textsuperscript{21}.

Some examples of possible groups that can complex with certain ions are:

• dioxadithioamides with Cd\textsuperscript{2+}-ions\textsuperscript{22};
• 15-crown-5 with Pb\textsuperscript{2+}-ions\textsuperscript{23};
• terphenyl-derived 15-crown-5 with Hg\textsuperscript{2+}-ions\textsuperscript{24};
• morpholine with Cu\textsuperscript{2+} and Ni\textsuperscript{2+}-ions\textsuperscript{25};
• etc.

In this thesis, an amine equipped with a morpholine function will be used for the fabrication of the gels. With the creation of a Cu\textsuperscript{2+} responsive gel, we want to demonstrate the applicability of our synthetic protocol.
2.5 Stimuli responsive hydrogels

2.5.1 Gels

2.5.1.1 Definition of a (hydro)gel

The definition of a gel has been changed a lot in history since the first publication of a definition in 1926 by Dorothy Jordan-Lloyd\textsuperscript{26} and it should be noted that it remains controversial up to date. Jordan-Lloyd noted that a gel is a self-supporting material that is composed of a liquid and a solid compound. In 1993, K. Almdal\textsuperscript{27} came with the following, more exact definition: “A gel is a soft, solid or solid-like material of two or more components, with one that is a liquid, present in a substantial quantity.” The quantity from the definition is not exactly defined, so liquid-like materials (with a high amount of liquid) can be defined as a gel as well. A more useful, technical definition has been recently supplemented by Nishinari\textsuperscript{28}. His definition states that a gel is a system consisting of molecules, particles, chains, etc. which are partially connected to each other in a fluid medium by crosslinks to the macroscopic dimensions. The crosslinks can either be real (covalent) or apparent (micellar solutions).

A gel is called a hydrogel if the liquid phase is water.

2.5.1.2 Different gel classes\textsuperscript{29}

It is possible to separate the gels into different classes according to their internal structures. There are 4 different groups defined by Flory\textsuperscript{30} in 1974:

- covalent polymeric networks;
- physical linked networks;
- highly ordered, non-covalent networks;
- particulate, macrophase separated structures.
**Covalent polymeric networks**

This class includes all the gels whose network consists of a single molecule extended to the macroscopic dimension. Here, the crosslinks are of covalent nature. Such systems are therefore considered as being chemically crosslinked. There are several ways to crosslink the polymeric chains by chemical crosslinking. Next to the use of high energy irradiation, like the use of gamma rays to introduce crosslinking\(^{31}\) and enzymes, for example the crosslinking of lysine end-functionalized PEG\(^{32}\), two other methods of considerable importance exist which will be explained in the following.

A. **Crosslinking by radical polymerisation**

In this approach modified polymers carrying functional side groups, which are accessible to radical involving linking reactions, are used.

In scheme 2.13\(^{29}\) an example involving modified dextran, a polysaccharide with some methacrylate functionalities is shown. When a radical initiator has been added, the double bonds of the methacrylate will react and will form covalent bonds. In that way, every polymeric chain is linked to all other polymeric chains and one macroscopic polymer network is formed. In that particular case, gel formation is reversible since the formed bonds can be cleaved via hydrolysis.

\[\text{Scheme 2.13: Chemical crosslinking by radical polymerization by the use of methacrylate side chains.}\]
B. Crosslinking by a chemical reaction of complementary groups

Similar to the previous method, one uses functional groups on the polymeric chain to crosslink the chains. The difference is the use of a ‘crosslinker’. This is a molecule with complementary functional groups compared to those of the polymeric chain.

A frequently used crosslinker is glutaraldehyde as this molecule reacts readily with alcohols, amines and hydrazine groups (see scheme 2.14).  

![Scheme 2.14](image)

Scheme 2.14: Aldehyde-mediated crosslinking of polymer containing alcohol, amine or hydrazine group (R represents the polymer chain, X is any spacer e.g. (CH$_2$)$_2$ in the case of glutaraldehyde).

There are plenty of useful reactions for crosslinking, not only aldehyde chemistry. Globally, crosslinkers make use of condensation reactions or addition reactions.

C. Crosslinking via formation of disulfide bridges

Disulfide bonds are strong covalent bonds, that are highly common in biochemistry and in nature. The bond is formed by oxidation of two thiol groups, shown on scheme 2.15. In polymer chemistry, this reaction can be used to form good crosslinking between 2 thiol groups from the side chain of a polymer. In that way, a network has been formed and gelation can be introduced.

![Scheme 2.15](image)

Scheme 2.15: Formation of a disulfide bond via oxidation of 2 thiol groups.
The oxidation agents used to form a disulfide bond can be varied. A frequently used oxidation agent for the formation of disulfide bridges is iodine. This compound has the advantage that it tolerates a wide range of reactive functionalities when the reaction is occurring in an inert environment\textsuperscript{37}.

Another oxidation agent is hydrogen peroxide. Because this is a good oxidizing agent, mild conditions have to be applied. If not, higher oxidative states of sulphur can be formed and no disulfide bonds can be formed. Research on the disulfide formation in peptide chemistry\textsuperscript{38} showed that under certain conditions, H\textsubscript{2}O\textsubscript{2} can be applied to form disulfide bonds:

- pH = 8-9;
- less than 2 equivalents of H\textsubscript{2}O\textsubscript{2};
- bubbling of an inert gas.

**Physically crosslinked gels**

In contrast to the chemically crosslinked systems, physical gels do not consist of one single molecule, but of a number of finitely sized polymer chains that are linked in a physical way. The polymers needed for forming a physically crosslinked gel are obliged to be highly functionalised. The different principles of physical crosslinking shall be shortly explained in the following.

A. **Crosslinking by ionic interactions**

Crosslinking by ionic interactions is a method that uses ionic interactions to form a gel. Mostly, one adds a salt into the polymer solution to release ions that will interact electrostatically with complementary ionic groups of the polymer. An example is the crosslinking of alginate (see scheme 2.16), a polysaccharide consisting of 2 acidic components. This component is crosslinked by addition of Ca\textsuperscript{2+} ions\textsuperscript{39}. Note that this is a reversible process, because the gel can be destabilized by the use of a chelating agent.

![Scheme 2.16: Structure of alginate.](image)
B. **Crosslinking by partial crystallization**

Another method is by inducing local crystal formation to link the polymer chains to each other and form a gel. This can be done for instance with poly(vinyl alcohol) (PVA). PVA is a polymer that, once stored in water on room temperature, gradually forms a gel with low mechanical strength. When the solution was being frozen and thawed, a strong and very elastic gel was formed. This is due to the local formation of PVA crystals that act as local physical linkers and thus form a network\(^\text{40}\). This is demonstrated in scheme 2.17\(^\text{41}\).

![Scheme 2.17: Formation of a gel by partial crystallization.](image)

C. **Crosslinking by the use of block and graft copolymers**

Very often physically crosslinked gels consist of block and graft copolymers being mixed with a selective solvent. Here, the insoluble blocks or grafts are collapsed and therefore form the network junctions.

Many different architectures and compositions were tested, one of which are block copolymers consisting of PEG and PLA\(^\text{42}\). These compounds are very popular because of their biodegradability (PLA) and biocompatibility (PEG).
D. Crosslinking by hydrogen bonds

When different polymer chains with the ability to form hydrogen bonds between each other are utilized, it is possible to form a network which can cause gelation. This has been demonstrated for poly(ethylene glycol) with poly(acrylic acid) or poly(methacrylic acid). In this case, the oxygen from the poly(ethylene glycol) can form a hydrogen bond with the carboxylic group of the poly(acrylic acid)/poly(methacrylic acid). Note that this only occurs when the carboxylic groups are protonated, which means that this gel is pH-sensitive. This results in a change of the swelling grade with changing pH and thus protonation of the carboxylic groups.

2.5.2 Applications of stimuli responsive hydrogels

Stimuli responsive hydrogels are used in a wide variation of applications, such as controlled drug release in medicine. This is an application that uses the reversible inclusion of a drug molecule into a polymer aggregate or gel. The release of this drug can be triggered on demand when a stimuli responsive material is used. A temperature responsive gel for instance can be created as such that the drug will be released at physiologic temperature, which is important for the utilization of such a system in the human body.

Stimuli responsive hydrogels can also be used in sensors. A hydrogel based sensor mostly depends on the volume change that occurs with the changing environment. This volume change can be translated into a detectable voltage by using a piezoelectric stripe, which will be deformed when the gel swells. In scheme 2.18, a representation of such a hydrogel based sensor is given.

Scheme 2.18: A hydrogel based sensor with (1): Bending plate, (2) piezoresistive bridge, (3) swellable hydrogel stripe, (4) Si-chip, (5) socket, (6) tube, (7) interconnect and (8) solution.

Other examples of applications are microfluidic devices and actuating systems.
Chapter 3: Results and discussion

3.1 Polymer synthesis

In this thesis, two thiolactone functionalized copolymers with thermoresponsive properties were selected for the formation of multi-responsive hydrogels. These copolymers are poly(methoxy di(ethylene glycol) acrylate – co – N-thiolactone acrylamide) (PDEGA-co-Tla) and poly(N-isopropyl acrylamide – co – thiolactone acrylamide) (PNIPAAm-co-Tla). The reason why these structures are chosen is because of the fact that both polymers own an LCST around body temperature. The DEGA copolymers have the advantage that they own biocompatible properties and the LCST can be tuned by changing the length of the ethylene glycol side chain. Both polymers have been synthesized via RAFT. The RAFT agent was chosen with respect to the type of monomer used. In scheme 3.1, both processes are represented.

![Scheme 3.1: Schematic representation for the formation of PNIPAAm-co-Tla and PDEGA-co-Tla.](image)

The reaction conditions of the synthesis of PNIPAAm-co-Tla were determined in the PCR group in previous experiments. The optimum conditions for the PDEGA-co-Tla however still needed to be established. Also, the monomers for this reaction needed to be synthesized (except for NIPAAm). This first chapter therefore deals with the synthesis of the monomers and the corresponding polymer precursors.
3.1.1 Monomer synthesis

3.1.1.1 Synthesis of methoxy di(ethylene glycol) acrylate (DEGA)

DEGA was synthesized using a recipe established by Hua et al\textsuperscript{16} (scheme 3.2). Here, di(ethylene glycol) monomethylether is reacted with acryloyl chloride in dry dichloromethane in the presence of triethyl amine. Note that the synthesized monomer causes skin irritation during the experiments, even when wearing protecting gloves. The yield of the reaction was 53%, where around 30 g of DEGA monomer was obtained.

![Scheme 3.2: Reaction scheme for the synthesis of DEGA monomer.](image)

3.1.1.2 Synthesis of thiolactone acrylamide (Tla)

Thiolactone acrylamide is synthesized by reacting DL-homocysteine-thiolactone hydrochloride with acryloyl chloride in a 1:1 v/v water/dioxane mixture. The reaction is shown in scheme 3.3. The yield of the reaction was 81%.

![Scheme 3.3: Reaction scheme for the synthesis of Tla monomer.](image)

3.1.2 Optimization of the synthesis of PDEGA-co-Tla

Since the Tla monomer has not been copolymerized with DEGA before, the first task was to establish a suitable polymerization protocol ensuring a controlled polymerization. For a test polymerization 4-cyano-4-(thiobenzyl) sulfanyl pentanoic acid (CVADTB) was used as RAFT chain transfer agent and AIBN as initiator. This compound has been chosen because this is one of the RAFT agents that is preferred for the polymerization of acrylates. Moreover, the reaction with CVADTB is more controlled compared to a PDEGA-co-Tla copolymerization with 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT), which is the preferred RAFT chain transfer agent for the synthesis of PNIPAAm-co-Tla.
At first, a DEGA/Tla = 50/50 m/m mixture was polymerized. NMR samples for conversion monitoring were taken at 0, 30, 60, 120, 180 and 360 minutes. In order to determine the conversions, the evolution of the peaks representing the Tla and the DEGA double bonds respectively were followed. In order to get reliable data, an internal standard, namely toluene, was used.

In figure 3.1, the parts of interest of the NMR spectra taken of the reaction mixture at the beginning and after 1 hour reaction time of the DEGA/Tla = 50/50 reaction are shown.

![Figure 3.1: 1H NMR spectra of the PDEGA-co-Tla copolymer (DEGA/Tla m/m 50/50 composition) at the beginning (upper) and 1 hour (lower) in CDCl3.](image-url)
From both spectra, it is evident that polymerization of both monomers takes places as all peaks representing the monomeric double bonds decrease with time with respect to the internal standard. Another proof of polymerization is the appearance of the broader polymer peak at 4.0-4.1 ppm, representing the formed polymer.

With the obtained values, it is possible to calculate the conversion (in %) of the sum of the monomers as a function of time with the following formula:

\[
Conversion\ at\ \text{time } t \ (\%) = \left(1 - \frac{RI \text{ at time } t}{RI \text{ at time } 0}\right) \times 100
\]

with \(RI\) being the relative integral of the sum of the \(^1\text{H}\) NMR peaks representing the double bond of the observed monomers.

The results are plotted on figure 3.2.

**Figure 3.2:** Conversion plot as a function of time of both monomers during the formation of copolymer PDEGA-co-Tla with DEGA/Tla ratio = 50/50.

This graph shows that the conversion will level off at around 70%. If the molecular weight as a function of the obtained conversion is a straight line, one of the conditions to obtain a controlled polymerization is validated. The other condition includes that the first-order time-conversion plot should give a straight line:

\[
[M] = [M_0]e^{-kt} \Rightarrow \ln \left(\frac{[M]}{[M_0]}\right) = -kt \Rightarrow \ln \left(\frac{[M_0]}{[M]}\right) = kt
\]
To calculate the logarithm from the conversion at a time $t$ for the DEGA monomer, one uses:

$$\ln \left( \frac{[M_0]}{[M]} \right) = \ln \left( \frac{2.2M \times x(\text{DEGA})}{2.2M \times x(\text{DEGA}) \times \left( 1 - \frac{C(\text{DEGA})}{100} \right)} \right)$$

with $w(\text{DEGA}) = \text{original fraction DEGA in total monomer concentration (here: } x(\text{DEGA}) = 0.5)$

and $C(\text{DEGA}) = \text{conversion of the DEGA monomer at time } t$

The values for the Tla monomer were calculated with a similar formula. Using the obtained data, the molecular weight as a function of the conversion and $\ln([M_0]/[M])$ for both monomers as a function of time can be plotted. The result is shown in figure 3.3.

![Figure 3.3](image-url)
Indeed, the molecular weight as a function of the conversion tracks a straight line. Also \( \ln(M_0/M) \) for both monomers depends linearly on time for the first 3 hours. At higher reaction times, most likely termination is occurring at a higher rate and the curve starts to deviate from the straight line. With this information, one can conclude that the polymerization is controlled at least for conversions up to around 60%.

For the investigation of the copolymerization behaviour, the experiment has been repeated for the following DEGA/Tla ratios: 90/10 – 80/20 – 60/40 – 40/60 – 20/80 and 10/90. However, the 2 latter experiments failed, because of the lack of solubility of the formed polymer in 1,4-dioxane.

In figure 3.4 a plot of the conversion of each monomer as a function of the initial DEGA/Tla ratio is given at 10% overall conversion.

![Figure 3.4: Plot of the conversion of DEGA and Tla as a function of the initial DEGA/Tla monomer ratio at 10% overall conversion.](image)

From this plot, it becomes clear that the incorporation of the Tla monomer is favoured compared to the DEGA monomer, because of the higher conversion of that monomer in the copolymer formed at 10% overall conversion.
Data obtained from the successful polymerizations were used to determine the reactivity ratios of the copolymerized monomers in this particular reaction system. To do so, we employed the Fineman-Ross method. This method is only suitable for rough estimations as it based on the terminal model and hence neglects the influence of penultimate units on the reactivity of the growing polymer chain. However, we consider this method still suitable as a detailed analysis of the copolymerization behaviour is out of scope of this thesis and we only want to get an idea about the final copolymer composition distribution. The Fineman-Ross equation is a linearized version of the Lewis-Mayo equation:

\[
\frac{(f - 1)F}{f} = r_1 \frac{F^2}{f} - r_2 \text{ with } F = \frac{X_{0,\text{DEGA}}}{X_{0,Tla}} \text{ and } f = \frac{x_{\text{DEGA}}}{x_{Tla}}
\]

\(X_0\) and \(x\) represent the molar fraction of a compound in the initial mixture and after a certain overall conversion, respectively. Here, this conversion is fixed at 10%, as the Fineman-Ross method neglects the change of monomer composition with time.

In the Fineman-Ross equation, \(r_1\) and \(r_2\) represent the reactivity ratios of the DEGA and Tla monomer, respectively. Figure 3.5 shows the corresponding plot from which these values were extracted after the linear fit.

We obtained \(r_1 = 0.4124\) and \(r_2 = 0.7273\). These values confirm that the Tla monomer is slightly favoured for addition to the chain compared to the DEGA monomer.

![Figure 3.5: Fineman-Ross plot of the PDEGA-co-Tla copolymerization at 10% monomer conversion.](image)
Both values are smaller than 1, indicating that we deal with an azeotropic copolymerization. Here, a slight tendency for an alternating comonomer addition is observed. In figure 3.6, the copolymerization plot of this kinetic study is shown. Also a theoretical curve obtained through the calculated reactivity ratios has been plotted.

![Copolymerization plot of the DEGA monomer for the PDEGA-co-Tla copolymerization.](image)

Figure 3.6: Copolymerization plot of the DEGA monomer for the PDEGA-co-Tla copolymerization.

The data received from the experiments are following the theoretical line, which means that the assumption of the presence of an azeotropic copolymerization can be confirmed. Unfortunately, we were unable to record data points for the monomer compositions with high Tla content due to the failure of the respective polymerization (as mentioned before). This means that we have no data points confirming the theoretical curve below the azeotropic point.

We can conclude that above the azeotropic composition, the Tla incorporation is favoured whereas at low DEGA ratios, the latter will be preferred. However, it is also clear from the copolymerization plot, that the preference of one of both monomers to be incorporated in the growing chain is not very pronounced at any monomer feed composition. Thus, we can expect polymers, whose composition is not altering too strongly along the chain and so can be called more or less statistical.

### 3.1.3 Synthesis of PNIPAAm-co-Tla and PDEGA-co-Tla

With the earlier obtained results from the PCR group for the PNIPAAm copolymers and the results from the previous chapter, it is possible to synthesize a number of copolymers with different thiolactone contents. The number of thiolactone units will later on determine the crosslinking density of the formed hydrogel as well as the degree of functionalization.
The thiolactone contents in the final polymers were determined in each case via elemental analysis, checking for the sulphur content, or by $^1$H-NMR. In table 3.1, the synthesized polymers and their characteristics are shown. For the synthesis of the PNIPAAm-co-Tla copolymers, N,N-dimethylacrylamide was added in an equimolar amount compared to the added Tla monomer, this to increase the LCST of the synthesized polymer, so the polymer is soluble in water at workable temperatures.

<table>
<thead>
<tr>
<th>Code</th>
<th>Type polymer</th>
<th>% Tla*</th>
<th>$M_n$ (Da)**</th>
<th>$M_n^{\text{theor}}$ (Da)</th>
<th>PDI**</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT2.5</td>
<td>PDEGA-co-Tla</td>
<td>2.5</td>
<td>15000</td>
<td>18000</td>
<td>1.25</td>
</tr>
<tr>
<td>DT9</td>
<td>PDEGA-co-Tla</td>
<td>9</td>
<td>16000</td>
<td>18000</td>
<td>1.30</td>
</tr>
<tr>
<td>DT22</td>
<td>PDEGA-co-Tla</td>
<td>22</td>
<td>17000</td>
<td>18000</td>
<td>1.29</td>
</tr>
<tr>
<td>NT3</td>
<td>PNIPAAm-co-Tla</td>
<td>3</td>
<td>22000</td>
<td>17000</td>
<td>1.10</td>
</tr>
<tr>
<td>NT11</td>
<td>PNIPAAm-co-Tla</td>
<td>11</td>
<td>17000</td>
<td>18000</td>
<td>1.16</td>
</tr>
<tr>
<td>NT22</td>
<td>PNIPAAm-co-Tla</td>
<td>22</td>
<td>20000</td>
<td>18000</td>
<td>1.13</td>
</tr>
</tbody>
</table>

*PDEGA copolymers: analysed via elemental analysis
*PNIPAAm copolymers: analysed via 1H-NMR
**Analysed via DMA GPC analysis – PMMA standards

The mentioned code will imply the type of polymer and the amount of Tla in the polymer. The first character will denote that the copolymer is a DEGA (D) or a NIPAAm (N) copolymer, whereas the last character, together with the digit, will denote the thiolactone content in the copolymer.

### 3.2 Gelation of the polymer

#### 3.2.1 Introduction

The aim of this thesis is to obtain a gel, that is responsive towards certain triggers. Preferably, this gel should be formed in a one-pot reaction. This means that the polymer should be dissolved in water and all the necessary compounds to crosslink the polymer should be added in that solution in one single step. In this thesis, the possibilities of the gelation of PNIPAAM-co-Tla and PDEGA-co-Tla in water are being investigated in order to obtain a recipe to create stable, homogeneous and responsive hydrogels. For this purpose, model reactions were performed including low molecular weight thiolactone compounds.
and, for the kinetics of the aminolysis/disulfide formation reaction cascade, a T1a end functionalized poly(ethylene glycol).

The gelation process can be divided into two parts, the aminolysis and the subsequent disulfide formation. Both steps come along with certain difficulties, being treated in the following paragraphs.

### 3.2.1.1 Aminolysis and hydrolysis

As mentioned before, the thiolactone ring is opened by an amine, in order to release a thiol and a potential functionality linked to the used amine. This, however, is not the only way in which a thiolactone ring can be opened. At a high pH, hydrolysis instead of the aimed aminolysis can occur\(^\text{48}\). This is represented on scheme 3.4.

![Scheme 3.4: Schematic representation of the aminolysis and hydrolysis of the thiolactone ring.](image)

When hydrolysis takes place, the ring is opened with a release of a thiol group, similarly to aminolysis. This means that gelation can also take place when only hydrolysis would take place in the first step. However, in that case the gel will not be functional, as the functionality is introduced via the amine.

### 3.2.1.2 Saponification of PDEGA

In the case of PDEGA, there exists a risk of saponification of the ethylene glycol side chain with the presence of hydroxyl ions. Previous NMR experiments on PDEGA confirmed this (not shown here). This strengthens the necessity to work at moderate pH in the case of DEGA based polymers.
3.2.1.3 Disulfide formation

The disulfide bonds formed from the released thiols by aminolysis of the thiolactones will, as explained in the theoretical chapter of this thesis, serve as crosslinker to obtain a gel. The disulfide formation is an oxidative process, thus an oxidation agent is needed.

In scheme 3.5, different oxidation states of sulphur are represented. Here, one can see that sulphur is not in the highest oxidation state when being part of a disulfide bridge. This means that the selected oxidizing agent has to be strong enough to oxidize the thiol-functionality, but has to be mild enough in order not to overoxidize the sulphur compounds. Oxygen is considered to be such an oxidant.

![Scheme 3.5: A selection of possible oxidation states of sulphur bonds with oxidation state of the sulphur.](image)

To improve the speed and control of oxidation of the thiol groups, additional oxidation agents can also be used. In literature a number of oxidation agents are utilized that promise a disulfide formation with high selectivity. In the following, a description of all the investigated oxidation agents and their outcome are being given:

- Hydrogen peroxide (H\(_2\)O\(_2\)): In biochemistry, mostly hydrogen peroxide is used to perform an oxidation. Because of the high oxidation potential, the amount of hydrogen peroxide should be kept as low as possible. An alternative is a compound that forms in situ hydrogen peroxide, viz. sodium perborate (scheme 3.6).

![Scheme 3.6: Chemical structure of sodium perborate.](image)
3.2.2 Kinetics of Tla ring opening and disulfide formation.

In order to have a full description of the gelation process, both reactions should be investigated in detail. For that purpose, 3 types of model reactions were performed.

3.2.2.1 Aminolysis with low molecular weight compounds

In order to investigate the thiolactone ring opening reaction, an online IR device was utilized. For that purpose, model mixtures of DL-N-acetylhomocysteine and 5 eq. of propyl amine were analysed at three different pH, namely 7, 8 and 9. The stability of the pH during the reaction was checked with pH indicator paper.

Scheme 3.8 shows the reaction. The amine acts as a Brønsted base and is therefore partially present in the protonated form. Depending on the pH, different amounts of the active, deprotonated form will be available. In order to follow the ring opening reaction, the formation of thiol was monitored. The thiol peak is represented by a relatively weak band at 2434 nm. The results are shown in figure 3.7.
Figure 3.7: IR thiol peak height evolution as a function of the reaction time at different pH.

The graph basically shows the evolution of the IR thiol peak as a function of the reaction time. At pH = 7 and 9, a slow increase of the content of free thiols was observed, which is different from the results obtained at pH = 8. At the latter pH, the peak increase with time is much more pronounced. From these observations, we can postulate the following:

At pH = 7, free amine and hydroxyl ions are almost absent, due to the low pH. This results into a very low speed of thiolactone ring opening and in consequence almost no release of free thiol. This is obvious from the fact that the free thiol IR peak stays almost absent during the reaction. Then, at pH = 8, the amount of free amines as well as hydroxide ions is of course higher. For that reason, the speed of ring opening will be higher compared to pH = 7. On the other hand, the formed thiols will be converted into disulfide bridges with time. The fact that the obtained curve shows a significant raise of the thiol peak within the first 2-3 hours, leads to the conclusion that ring opening occurs in a reasonable time, while disulfide formation is not fast enough to consume the liberated thiols immediately. Finally, at pH = 9, the amount of free amines/hydroxide ions is significantly higher compared to pH = 7 and 8. This implies a high speed of aminolysis, which would induce a high free thiol concentration in the beginning of the reaction. However the speed of disulfide formation is also occurring at a much faster rate. This results in the fact, that almost no free thiol is detectable during the entire reaction, as seen from the respective curve.

In summary, the higher the pH, the higher the amount of active amine and hydroxide ions and therefore the higher the aminolysis/hydrolysis speed. However, also the disulfide formation occurs faster. Furthermore, it seems that only at pH = 8, thiol is liberated without being immediately consumed.
Note that these conclusions have a postulative character. In order to verify our assumptions, other experiments were performed.

3.2.2.2 Model reaction to monitor the aminolysis/disulfide formation reaction cascade (gelation process)

In order to get a better idea about the gelation progress at different pH, a model to monitor the evolution of the opening of the thiolactone ring and the formation of disulfide in a polymeric environment as a function of time was needed. For that purpose, PEG (4500 g/mol) was end functionalized with thiolactone according to scheme 3.9. Upon aminolysis and disulfide formation, this polymer should dimerize (scheme 3.10). In that way, the reaction can be monitored nicely via GPC by determining the ratio between uni- and dimer.

The functionalization of the PEG precursor was achieved by reacting the OH end group of the polymer with thiolactone isocyanate. The latter has been previously synthesized by dr. Pieter Espeel (PCR).

![Scheme 3.9: Reaction scheme of the synthesis of the PEO model precursor.](image)

![Scheme 3.10: Reaction scheme of the aminolysis of the thiolactone ring substituted on the PEG-OMe chain end resulting in dimerization by disulfide linkage.](image)
The purpose of this model reaction is to know how fast the crosslinking occurs after addition of the amine in aqueous polymeric environment. For that we need to be able to stop the aminolysis/disulfide formation at certain times. This had been achieved with the help of n-ethyl maleimide. In certain solvents, such as DMA, this compound was found to react readily with thiols and so traps them. Apart from that, remaining amines are also converted. Samples were therefore taken by adding a few droplets of the reaction mixture to DMA containing an excess of n-ethyl maleimide. This mixture could then directly be analysed by GPC.

The following list summarizes the different conditions used for the experiment:

- pH set to 7, 8, 9 or 10 with only oxygen from the air as oxidation agent;
- pH set to 8 with addition of 7 eq. Chloramine T (CAT) upon sample taking;
- pH set to 8, where no amine is used to check the influence of hydrolysis;
- pH set to 9 with addition of 1 eq. of hydrogen peroxide.

In figure 3.8-3.10, the results are shown.

![Figure 3.8: Evolution of the conversion of the PEO model reaction as a function of the reaction time at different pH – Colored lines: guide to the eye - Inset: zoom of the graph at the first 70 minutes.](image-url)
In figure 3.8, the evolution of the conversion as a function of the reaction time is plotted. The pH is the only parameter that has been changed. The first noticeable fact is that the curve at pH = 7 hardly shows a conversion in comparison with the others where more or less full conversion is reached after some time. The main differences between the other reactions occur mainly at an earlier stage of the reaction. This is emphasized in the inset of the figure. Generally, high conversion is reached earlier with increasing pH.
Figure 3.9 shows the evolution of the conversion as a function of the reaction time, where the experiments are performed at pH = 8. It is clear that the conversions are generally higher when chloramine T is added afterwards. However, when CAT is already present in the reaction mixture in equimolar quantities with respect to Tla, no significant difference is observed (results not shown). The other curve is the representation of the experiment where no amine is being used. There, disulfide bonds are formed much slower. However, full conversion is reached as well after two days.

Finally, figure 3.10 shows the evolution of the conversion as a function of the reaction time of two different experiments where the pH in both cases has been stabilized at 9 with one batch containing H$_2$O$_2$ (1 eq. with respect to the number of Tla units). Here, the reaction conversion initially rises significantly faster than in the case where no additional H$_2$O$_2$ is present. However, the conversion levels off at a relatively low value.

From the results, one can conclude:

- the pH of the compound needs to be higher than 7 to achieve gel formation;
- at all pH higher than 7, more or less full conversion is reached;
- CAT in principle can increase the amount of crosslinks when added in excess after the ring opening. During the ring opening, CAT might react with the ring opening amine;
- disulfide formation also takes place at pH = 8 when no amine is present. That means, hydrolysis occurs. However the speed of the reaction is apparently much slower than the speed of the aminolysis;
- hydrogen peroxide will initially improve the speed of disulfide formation, but the amount of formed disulfide bonds will stabilize fast at a rather low level, which is most probably due to overoxidation (scheme 3.5);
- at high pH, the aminolysis/hydrolysis can be seen as the rate determining step.

3.2.2.3 Model reaction to determine the extent of functionalization

A third model reaction was performed to monitor the degree of functionalization in dependence of pH, or in other words, to get an idea about the ratio of aminolysis and hydrolysis at different conditions. Here, the focus lies especially on PDEGA as the ethylene glycol side chains might have an impact on the accessibility of the thiolactone units. DT22
was dissolved in water with a certain amount of propylamine and is stirred for 2 days. The concentration of polymer and amine was relatively high in order to resemble conditions being later established for the gel formation. The formed functionalized polymers were purified and analysed via elemental analysis. The results are shown in table 3.2. This analysis method is normally very accurate, but the results can differ strongly with the presence of impurities in the sample. To take this into account, the following reasoning is made in a semi-quantitative way.

During the reaction, 3 different cases can occur:

- The thiolactone ring stays unreacted;
- the thiolactone ring is aminolyzed;
- the thiolactone ring is hydrolysed.

Each case will change the fraction of S and N atoms in the compound. Therefore, the N/S ratio was calculated for the cases where full aminolysis, full hydrolysis and no reaction was performed. These values are representatively 0.84, 0.435 and 0.44 (scheme 3.11). This means that the higher the ratio for the evaluated polymers, the more functionalization had been occurred.

Scheme 3.11: Overview of the different reactions possible at a thiolactone ring in a DT22 solution with 5 eq. propylamine with the different N/S ratios at full conversion of one of the reactions.
Table 3.2: Overview of the weight percentages of H, N, O and S and the N/S ratio in the aminolysis/hydrolysis extent model reaction, obtained from elemental analysis.

<table>
<thead>
<tr>
<th></th>
<th>wt % Before reaction</th>
<th>wt% at pH = 8</th>
<th>wt% at pH = 9</th>
<th>wt% at pH = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>7.10</td>
<td>7.33</td>
<td>7.54</td>
<td>7.63</td>
</tr>
<tr>
<td>Carbon</td>
<td>50.88</td>
<td>52.1</td>
<td>52.26</td>
<td>52.6</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.16</td>
<td>2.31</td>
<td>2.62</td>
<td>3.1</td>
</tr>
<tr>
<td>Sulphur</td>
<td>4.68</td>
<td>4.76</td>
<td>4.75</td>
<td>4.53</td>
</tr>
<tr>
<td>Oxygen</td>
<td>35.2</td>
<td>33.49</td>
<td>32.83</td>
<td>32.13</td>
</tr>
<tr>
<td>N/S ratio</td>
<td>0.46</td>
<td>0.485</td>
<td>0.55</td>
<td>0.68</td>
</tr>
</tbody>
</table>

In table 3.2, the ratios are calculated from the data obtained at different pH. One can clearly see that the degree of functionalization is increasing as a function of the pH. While the degree of functionalization at pH = 10 seems to be reasonable, almost no amine seems to have attached at pH = 8. From the experiment of section 3.2.2, we know that ring opening also occurs at pH = 8. However, this happens much faster when an amine is present. In other words, also at pH = 8, a significant amount of attachment of the amine should take place. The fact that functionalization at that pH almost totally fails in the case of PDEGA leads to the conclusion that the ethylene glycol side chains effectively shields the thiolactone ring. The hydroxyl ions are expected to be more capable to reach the ring because of their smaller size. They even might not have to diffuse to the thiolactone ring due to a normally fast occurring proton exchange between water molecules and hydroxyl ions.

This model reaction with PNIPAAm-co-Tla polymers is harder to be determined via elemental analysis, because the NIPAAm monomer also owns a nitrogen element in its structure. This leads to a higher nitrogen content whereby the relative change in N/S ratio due to hydrolysis or aminolysis will be lower. This makes the determination of the functionalization indistinguishable. Alternatively, NMR could be performed using an aromatic amine such as benzyl amine. In that case, the distinguishable aromatic signals could help for determining the degree of functionalization. Within the given time, we were however not able to perform these experiments. This will be done in the future.
3.2.2.4 Conclusions from the model reactions

From the results of the previous model reactions, optimal conditions for the construction of the functional gels can be established. Due to the fact that the disulfide bonds will not be formed at pH = 7, the solution needs to exhibit a basic pH. At a pH higher than 7, conversion of released thiols to disulfide bridges proceeds to 100%, however, with a rather low rate at only moderate basic pH. The PEO model reaction shows that at pH = 8, hydrolysis still occurs, but the speed of the aminolysis is significantly faster than the hydrolysis, which means that aminolysis can be in principle performed at pH = 8. Unfortunately, when the thiolactone ring is located in the vicinity of highly hydrated ethylene glycol side chains, as it is the case for PDEGA-co-Tla, the degree of functionalization seems to drop significantly.

A suitable oxidation agent can clearly improve the formation of disulfide bonds, but hydrogen peroxide appeared to be too strong to only form disulfide bonds from the released thiols. Chloramine T can be a good oxidation agent, but it seems to interfere with the amine.

3.2.3 Cu²⁺ response of the morpholino-containing polymers

As stated in the introduction, morpholine moieties own an affinity towards Cu²⁺ and Ni²⁺ ions. Therefore, an additional experiment was performed to test the response of the synthesized morpholino-containing polymers towards the change of the Cu²⁺ concentration in their environment. When the hydrogels are later on tested on their response, this experiment can serve as a comparison. The experiment includes the aminolysis of the thiolactone with propylamine or 3-morpholino propylamine. To prevent disulfide bonding, the released thiols were blocked by the use of n-ethylmaleimide. Because of the reaction of n-ethylmaleimide with amines, the compound is subsequently added to the mixture. This means that disulfide bonding did occur, but in such a low amount that no gel is formed meaning that the obtained polymer is still soluble into different Cu²⁺-containing mixtures. In this way, the turbidimetry measurements can be performed in solution and the mixture becoming turbid will be more pronounced.

Practically, NT3, NT11 and NT22 were modified with 3-morpholino propylamine and purified. The polymer was dissolved in a acetic acid/ammonium hydroxide buffer (pH = 7). This solution was put into the turbidimetry machine and analysed with Cu²⁺ solutions with
different concentrations and a solution without any Cu\textsuperscript{2+}. In figure 3.11, the results for the NT22 polymer are shown.

![Graph showing evolution of turbidimetry experiment response as a function of temperature for a modified NT22 polymer in an aqueous Cu\textsuperscript{2+} solution with a concentration of 0, 10 and 50 ppm.]

*Figure 3.11: Evolution of the turbidimetry experiment response as a function of temperature for a modified NT22 polymer in an aqueous Cu\textsuperscript{2+} solution with a concentration of 0, 10 and 50 ppm.*

The transition temperature was determined by taking the intersection between the tangent of the plateau before the drop and the tangent of the straight line in the drop of the response. The difference of the transition temperature at a mixture with a certain Cu\textsuperscript{2+}-concentration compared to the transition temperature at the mixture of 0 ppm for the different modified polymers has been displayed in figure 3.12.

![Graph showing evolution of the difference in transition temperature in a mixture with a certain Cu\textsuperscript{2+} concentration compared with the transition temperature where no Cu\textsuperscript{2+} is dissolved for different modified polymers.]

*Figure 3.12: Evolution of the difference in transition temperature in a mixture with a certain Cu\textsuperscript{2+} concentration compared with the transition temperature where no Cu\textsuperscript{2+} is dissolved for different modified polymers.*

The results show a trend: the higher the Tla-concentration, the higher the shift of transition temperature at a certain Cu\textsuperscript{2+}-concentration and the higher the range of Cu\textsuperscript{2+}-concentrations where a difference in transition temperature is observed. In this way, for polymers with a sufficient amount of Tla (and thus morpholino groups after aminolysis), the response

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towards the Cu$^{2+}$-concentration has been demonstrated. Nevertheless, these results are only to prove the response possibility of the morpholino-containing polymers. For detailed response curves, more experiments are necessary.

### 3.2.4 Results of the gelation experiments

#### 3.2.4.1 Experimental setup

The experiments performed to investigate the gelation of the synthesized polymers were performed in 5 ml snap cap vials equipped with a needle through the cap in order to ensure sufficient air contact (if no additional oxidation agent was used). The reason of the use of the needle is that the risk of evaporation is higher when the gelation occurs in an open vial, which would vary the concentrations in the mixture. In some cases cooling was needed, since some copolymers with thiolactone contents exhibit cloud points below room temperature. For that purpose, a special setup was used, which is shown in figure 3.13.

![Figure 3.13: Drawing of the setup used to cool the mixture in the gelation attempts.](image)

The setup consists of a thermostated vessel filled with water and connected to a thermostat with which the temperature can be regulated. The sample vials were placed in the middle of the vessel and the whole construction was placed on a stirring plate. In chapter 3.2.4.4, table 3.3 is summarizing all gelation experiments.

#### 3.2.4.2 Influence of solvent

**Gelation in buffered aqueous solutions**

The degrees of functionalization appear to be low in the case of PDEGA when performing the aminolysis at moderate basic pH. However, since PDEGA might be prone to saponification at high pH, we tested gelation first in buffered solution. Unfortunately, it is difficult to establish a moderate pH when working with high concentrations of amines. In order to overcome this problem the protonated version of the respective amine was used. Approaching the slightly
basic regime from the acidic side is much easier, since in that case carbonate or hydrogen carbonate salts can be used for pH adjustment. In some cases, a mixture of the protonated ammonium salt and the deprotonated amine was used.

With this knowledge, gelation was tried with the PDEGA based polymers DT22, DT9 and DT2.5 (see table 3.1). Due to the non-existent risk of saponification in the case of PNIPAAm, only PDEGA was investigated here. Besides, the LCST of PNIPAAm appeared to be too low in buffered solution (influence of ionic strength), so that dissolution of the respective polymer was anyway hard to achieve. This will be solved in future by synthesizing PNIPAAm with a certain content of an additional hydrophilic comonomer.

With each polymer, 3 solutions with 5, 15 and 30 wt% were prepared. Every reaction was performed at pH = 8 and at pH = 9 respectively. The mixtures were stirred and no additional oxidizing agent was used. Even after a waiting time of 4 days, none of the mixtures were in the gel form. This is probably due to acidification of the mixture by uptake of CO₂ when being exposed to air. Due to that acidification, the formation of the disulfide bridges might be hampered and thus gelation does not occur. Of course it should not be forgotten that disulfide formation will be generally slower in the case of PDEGA, once again due to steric problems.

**Gelation in unbuffered aqueous solutions**

In order to test if the gelation with the obtained polymers is possible at all, gelation was performed in unbuffered solution. This means that the polymer is dissolved in Millipore water and the amine is directly added. Here, the pH is much higher (> 10) due to the high amine concentration. As seen from the model studies, disulfide formation occurs quickly at pH = 10. Besides, CO₂ uptake should have much less influence on the pH in that case.

From DT22 and NT22, aqueous solutions of 2, 5 and 10 wt% respectively were prepared. Dissolving higher weight percentages of NT22 in water was not possible, because the LCST of the solution was too low such that cooling via the setup was not able to properly dissolve the polymer. In contrast to that, DT22 could be dissolved at much higher concentrations.

Subsequently to the dissolution of the respective polymer, 5 eq. of propyl amine was added in each case. While the NT22 containing solutions gelled after 2,5 days, no PDEGA-co-Tla
containing solution did so. For this reason, a 30 wt% mixture of the PDEGA-co-Tla was additionally prepared. This mixture, also with 5 eq. of propyl amine finally did gelate. Unfortunately, the synthesized gels appeared unstable in water during the purification step.

We can conclude at this point that the synthesized polymers are able to gelate, but when utilizing PDEGA-co-Tla, a higher polymer concentration in the mixture is needed. This supports the assumption that the ethylene glycol side chains shield the released thiol groups partially (paragraph 3.2.2.3), making it necessary to establish higher concentrations in order to get the thiols groups closer to each other. This shielding might be even pronounced in aqueous solution due to the high degree of hydration of the ethylene glycol side chains, forming some kind of solvent cage. For the same reason, the degrees of functionalization measured appeared to be lower than expected (see section 3.2.2.3).

**Gelation in organic solvents**

So far, the gelation worked only in unbuffered aqueous solution, where gels were obtained, but appeared to be rather unstable. Besides, in the case of PDEGA, rather high concentrations were needed. Therefore, the gelation was tested in organic solvents. Here, no hydrolysis or saponification can take place. Also the amount of disulfide bonds can be increased, because the ethylene glycol side chain of the DEGA monomer are not hydrated anymore and the shielding effect is decreased. The disadvantage of that approach is the necessity of a multi step procedure. After gelation, the solvent has to be removed in vacuum and the gel has to be reswollen in water. Immersing the gel piece directly into water is not an option, due to the of the lack of solubility of both solvents with the latter. Besides, the use of organic solvents makes the approach of course less environmentally friendly.

The solvent used for the gelation of PDEGA-co-Tla is ethyl acetate, for PNIPAAm-co-Tla dichloromethane was used. Both solvents can be easily removed under vacuum. Note that the mixtures in ethyl acetate result in lucent gels.

In this part, every experiment was performed with 20 wt% of polymer. This property was kept stable to have a maximum comparability in the gel properties study. In every attempt gels were formed. However, the gel formed with the DT2.5 or NT3 polymer was unstable in water.
As mentioned already, ethyl acetate and dichloromethane have to be removed in vacuum before swelling the gel in water. However, during the removal of the solvent, the gel gets more concentrated and that might influence the final crosslinking degree. For that reason, THF was tested as alternative solvent, since it is miscible with water. For that test, DT22 and NT22 were used. The gelation worked, but the obtained gels were opaque and showed nearly no swelling in water. This makes them useless for future response studies.

**Summary**

Unfortunately, the control of the gelation in the absence of oxidation agent is in aqueous solutions very hard to achieve. The gelation speed and probably also the degree of crosslinking depends very much on the accessibility of air. Stirring has an influence as well as the geometry of the vial. Bubbling air through the solution should be avoided in order not to get air inclusions. Besides, without additional oxidation agent, the reaction is only completed after several days, which is very slow. Gelation failed completely when buffered at pH = 8 or 9. In unbuffered solution, the resulting gels appeared (especially when the Tla content is low) disintegrated when trying to swell them in water, which points to a insufficient crosslinking degree. This observation is contradictory to the obtained results from the PEO model reaction (chapter 3.2.2.2), where always full conversion was reached. This can be due to the fact that in the model reaction only one thiolactone moiety at the end of the PEO chain needs to be opened compared to the reaction to form a gel, where more thiolactone moieties, that are more difficult to reach because they are on the side chain, have to be opened to form a stable hydrogel. In the case of PDEGA, the strongly hydrated ethylene glycol side chains apparently cause a much more pronounced shielding, which explains the high PDEGA concentrations needed in any case.

The gelation in organic solvent had more success, meaning that the formed gels were generally more stable. However, the crosslinking density might be influenced by the additional solvent removal step. Also the goal of this project was to obtain gels in a one pot reaction in water, thus another approach should be searched for. Nevertheless, the obtained gels were stable in water, were not formed via hydrolysis and could be analysed in the gel analysis chapter.
3.2.4.3 Influence of additional oxidation agents

The gelation with air as oxidation agent is slow, both in water and organic solvents. Besides, the disintegration of some of the gels and the general weak appearance of the swollen gels points to generally low crosslinking degrees. Therefore, an additional oxidation agent was used to speed up the gelation and to enhance the crosslinking degree.

*Hydrogen peroxide*

From the model studies, H$_2$O$_2$ appeared not to be the optimum oxidation agent to be used as it lacks selectivity towards disulfide groups. Nevertheless, it was used for a gelation attempt in order to test if the low amount of disulfide groups might already be sufficient to get a stable gel. DT9 was dissolved in water at 5, 15 and 30 wt% at pH = 9 (buffered) containing 1 eq. H$_2$O$_2$ with respect to the Tla content. Here, in contrast from the results in chapter 3.2.3.2, the mixture with the 30 wt% of polymer formed a gel, but it still took about 1 day. Unfortunately, this gel was not very stable and was disintegrating upon immersion in water. Apparently the amount of disulfide crosslinks formed was not high enough.

Also some unbuffered experiments were performed, where 1 eq. H$_2$O$_2$ was added to the reaction mixture described in chapter 3.2.3.2. Here, gelation occurred within the first hour, but the gel was also unstable upon immersion in water. The experiment was repeated once with sodium perborate instead of H$_2$O$_2$. According to literature$^{50}$, Na-perborate is highly selective in producing disulfide groups, however, when testing this compound we obtained more or less the same results as with H$_2$O$_2$.

*Iron(III) + NaI + H$_2$O$_2$*

Based on the article of Kirihara et al.$^{51}$, a reaction was performed with the use of hydrogen peroxide and NaI. The reaction conditions are shown in scheme 3.12.

![Scheme 3.12: Reaction scheme of the disulfide bond formation published by Kirihara.](image-url)
Our approach was slightly different from that scheme, meaning that water was used instead of EtOAc. Unfortunately, this experiment gave the same results: overoxidation was observed. Although we first observed a gel formation within less than an hour, the solution became liquid again a few hours later. Iranpoor et al.\textsuperscript{52}, who used a combination of 10 mole\% FeCl\textsubscript{3} and 20 mole\% NaI, further inspired us to use FeCl\textsubscript{3} as oxidation agent instead of H\textsubscript{2}O\textsubscript{2}. Based on the recipe of Kirihara, another attempt was made, however using 1 eq. of FeCl\textsubscript{3} instead of H\textsubscript{2}O\textsubscript{2}.

The idea was tested with DT22 and benzylamine. After 2 days, a brownish stable gel was formed. With the help of an excess of EDTA, iron can be removed and at first a yellow and later on a colorless stable gel is received. The whole purification procedure is nevertheless very slow. Thus, though this method looks promising from the first results, it is not too useful, due to the lack of an easy purification.

\textit{Chloramine T (CAT)}

Both DT22 and NT22 have been used for the oxidation by CAT, but in both cases, from the moment that CAT is added, precipitation occurs. Probably, this is due to a reaction of CAT with the added amines. This is in accordance with the results from the model studies, were CAT had no effect on the PEG dimerization if not added only after aminolysis and in excess. Unfortunately, a subsequent addition of CAT in such an excess is not helpful for the gel formation neither, as we reach the solubility limit of CAT there.

In an alternative approach, a gel from NT3 (20 wt\%) and benzylamine was formed in a conventional way (in organic solvent (DCM) using air as oxidation agent). Normally this gel would disintegrate after being immersed in water. This time, however, it was immersed into 75 ml water containing an excess of CAT (200 mg). The goal was here to enhance the crosslinking degree on a later stage when the gel is being purified. The gel completely dissolves in water within a few hours when no CAT is present. The gel that was put into the CAT solution seemed a lot more stable on a first glance, however, after 4 days it was finally also disintegrated. Apparently, CAT was able to form additional disulfide bonds, however not in a sufficient quantity.
Global conclusion

The main conclusion is that the use of an additional oxidation agent has a large influence on the success of the gelation trials. The use of the “green” oxidation agent H₂O₂ is not an option, since this oxidation agent is too strong to selectively form disulfide bonds, even when it is added in situ. This also applies for sodium perborate. When using FeCl₃ and NaI a stable gel was obtained, but the purification takes too much time. The last compound tried was chloramine T, which does not show any benefit when added to the reaction mixture in the beginning together with the amine. Treatment of a gel with CAT simultaneous to the purification step, however, can at least enhance the crosslinking degree to some extent.

3.2.4.4 Overview of the gelation attempts.

Table 3.3A: Overview of all the gelation attempts.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>wt%</th>
<th>Buffered?</th>
<th>Oxidation Agent</th>
<th>Amine (always 5x Tla content)</th>
<th>Solvent</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT22, DT9 &amp;</td>
<td>5, 15 &amp; 30%</td>
<td>Yes</td>
<td>O₂ (Air)</td>
<td>Propylamine.HCl</td>
<td>Water</td>
<td>No gelation</td>
</tr>
<tr>
<td>DT22, DT9 &amp;</td>
<td>5, 15 &amp; 30%</td>
<td>Yes</td>
<td>O₂ (Air)</td>
<td>Propylamine.HCl</td>
<td>Water</td>
<td>No gelation</td>
</tr>
<tr>
<td>DT22</td>
<td>2, 5 &amp; 10%</td>
<td>No</td>
<td>O₂ (Air)</td>
<td>Propylamine</td>
<td>Water</td>
<td>Unstable gel</td>
</tr>
<tr>
<td>DT22</td>
<td>20%</td>
<td>No</td>
<td>O₂ (Air)</td>
<td>Propylamine</td>
<td>Water</td>
<td>LCST mixture too low</td>
</tr>
<tr>
<td>DT22</td>
<td>2, 5 &amp; 10%</td>
<td>No</td>
<td>O₂ (Air)</td>
<td>Propylamine</td>
<td>Water</td>
<td>No gelation</td>
</tr>
<tr>
<td>DT22</td>
<td>30%</td>
<td>No</td>
<td>O₂ (Air)</td>
<td>Propylamine</td>
<td>Water</td>
<td>Unstable gel</td>
</tr>
</tbody>
</table>
Table 3.3B: Overview of all the gelation attempts.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>wt%</th>
<th>Buffered?</th>
<th>Oxidation Agent</th>
<th>Amine (always 5x Tla content)</th>
<th>Solvent</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic solvent gelation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT22 &amp; DT9</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>Benzylamine</td>
<td>EtOAc</td>
<td>Stable gel</td>
</tr>
<tr>
<td>DT22 &amp; DT9</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>3-morpholino propylamine</td>
<td>EtOAc</td>
<td>Stable gel</td>
</tr>
<tr>
<td>NT22 &amp; NT3</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>Benzylamine</td>
<td>$CH_2Cl_2$</td>
<td>Stable gel</td>
</tr>
<tr>
<td>NT22</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>3-morpholino propylamine</td>
<td>$CH_2Cl_2$</td>
<td>Stable gel</td>
</tr>
<tr>
<td>NT3</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>Benzylamine</td>
<td>$CH_2Cl_2$</td>
<td>Unstable gel</td>
</tr>
<tr>
<td>NT22</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>Benzylamine</td>
<td>THF</td>
<td>Stable gel</td>
</tr>
<tr>
<td>DT22</td>
<td>20%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>Octylamine</td>
<td>$CH_2Cl_2$</td>
<td>Stable gel</td>
</tr>
<tr>
<td><strong>Additional oxidation agent gelation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT9</td>
<td>30%</td>
<td>Yes</td>
<td>$H_2O_2$</td>
<td>Propylamine.HCl</td>
<td>Water</td>
<td>Unstable gel</td>
</tr>
<tr>
<td>DT9</td>
<td>30%</td>
<td>No</td>
<td>$H_2O_2$</td>
<td>Propylamine</td>
<td>Water</td>
<td>Unstable gel (but very fast gelation)</td>
</tr>
<tr>
<td>DT9</td>
<td>30%</td>
<td>No</td>
<td>$H_2O_2$</td>
<td>3-morpholino propylamine</td>
<td>Water</td>
<td>Unstable gel (Immediate gelation)</td>
</tr>
<tr>
<td>DT22</td>
<td>30%</td>
<td>No</td>
<td>$O_2$ (Air)</td>
<td>3-morpholino propylamine</td>
<td>Water</td>
<td>Unstable gel</td>
</tr>
<tr>
<td>DT22</td>
<td>20%</td>
<td>No</td>
<td>$FeCl_3 + NaI$</td>
<td>Benzylamine</td>
<td>Water</td>
<td>Stable gel (hard to purify)</td>
</tr>
<tr>
<td>DT22</td>
<td>20%</td>
<td>No</td>
<td>CAT</td>
<td>Benzylamine</td>
<td>Water</td>
<td>Precipitation occurring</td>
</tr>
</tbody>
</table>
3.2.5 Swelling degree of the gels

A more quantitative result of the crosslinking density can be obtained from the determination of the swelling degree. To obtain this value, the swollen gels, immersed in Millipore water, are weighted in a cup. This gel piece is then freeze-dried. After all the water has been removed, this gel piece is again weighted and the swelling degree can be determined. The results are shown in table 3.4.

<table>
<thead>
<tr>
<th>Gel piece</th>
<th>Swelling degree (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT22 - Propylamine</td>
<td>300</td>
</tr>
<tr>
<td>DT22 - Benzylamine</td>
<td>200</td>
</tr>
<tr>
<td>DT22 - Octylamine</td>
<td>150</td>
</tr>
<tr>
<td>DT22 - 3-morpholino propylamine</td>
<td>450</td>
</tr>
<tr>
<td>DT9 – Benzylamine</td>
<td>1000</td>
</tr>
<tr>
<td>DT9 – 3-morpholino propylamine</td>
<td>1150</td>
</tr>
<tr>
<td>NT22 – Propylamine</td>
<td>400</td>
</tr>
<tr>
<td>NT22 - Benzylamine</td>
<td>150</td>
</tr>
<tr>
<td>NT22 – 3-morpholino propylamine</td>
<td>500</td>
</tr>
</tbody>
</table>

With the obtained swelling degrees, some trends can be determined:

- For a polymer with the same Tla content, the swelling degree drops with increasing length of the hydrophobic alkyl chain of the used amine;
- amines introducing a group that can be protonated will introduce larger swelling degrees;
- the swelling degree rises with decreasing Tla content.

The appearance of these trends will strengthen the assumption that the crosslinking and the gel formation can be controlled by varying the Tla content in the polymer and the amine. Unfortunately, this is only the case in organic solvents, because this control is very hard to achieve when a gel had to be formed in aqueous solutions, which is represented in chapter 3.2.3.2.
3.3 Study of the synthesized gels

Due to the difficulties occurring when performing gelation in water, gels formed in organic solvent were chosen for the response studies. After purification of the gels, they were first tested on their temperature response abilities. We were particularly interested in the swelling capacities of the formed gels. A high thiolactone content should result in a high degree of functionalization but at the same time, the crosslinking might be too high, meaning the gel is hardly able to swell and in turn useless for future sensing applications. After that, response experiments with the morpholino functionalized polymers towards Cu$^{2+}$ concentration are tested to check if a morpholino functionality is able to show response when it is used as a side chain of a polymer. Also the degradable character of the gels are being tested, because this is one of the main goals of this thesis.

3.3.1 Temperature response of the hydrogels

The functional gels are supposed to be thermosensitive, meaning that they should collapse upon temperature increase. In an initial temperature response test the principal ability of the synthesized gels to do so is demonstrated. For that purpose an optical microscope was utilized, which helps to detect also small changes in the swelling degree. With the use of a heating device being equipped with a petri dish in which a gel piece is immersed in water or a respective analyte solution, the gels can be heated in a semi controlled way. With use of the microscope, one can detect the shrinkage of the gels as a function of time (and through a correlation with the heating rate, thus with temperature). On figure 3.14 a schematic representation is being shown.

![Drawing of the microscope/heating device setup](image)
In figure 3.15 four pictures of a representative experiment are shown. In picture A, one can see a gel based on DT9 and 3-morpholino propylamine at 33 °C. Picture B shows the same gel at 46 °C. One can observe a clear shrinking of the gel piece as response to the temperature change. When these pictures are compared to C and D, also showing a gel but based on a polymer with a higher Tla content (DT22) and propylamine and therefore crosslinking degree, the difference is remarkable. The shrinkage here is not very pronounced, but still detectable (see ruler included in the picture). Apparently, the crosslinking degree of that gel is already too high to show a clear response.

![Figure 3.15: Microscope pictures of the DT9 – 3-morpholino propylamine gel at 33 °C (A) and 46 °C (B) and the DT22 – propylamine gel at 33 °C (C) and 46 °C (D).](image)

### 3.3.2 Cu²⁺ response studies of the morpholino-containing hydrogels

The gels that were formed via aminolysis with 3-morpholino propylamine should obtain a response potential towards the concentration of Cu²⁺ in the mixture where the gel was immersed in. Unfortunately, the determination of this response is very hard. This is normally performed via gravimetry. However, due to a lack of a proper device, this has to be done manually. Since a lot of parameters had to be varied (gel type, temperature, Cu concentration), this procedure would not be very practical. For that reason, alternatives to obtain information about the response were searched. Normally, the response should alter the transition point where the shrinkage of the gel as a function of the temperature starts. Therefore, a trial was made to detect the onset of shrinking as a function of the temperature with the help of an optical microscope and a custom made heating device.
Unfortunately, the transition appears to be rather broad for the gels tested (DT9 – 3-morpholino propylamine and DT22 – propylamine), rendering it almost impossible to detect the exact onset of shrinking with the naked eye.

In the future, turbidimetry can be applied as a potential method. This is based on the fact that when the gel starts to shrink, it becomes more turbid. In that way, the transition point where the shrinkage of the gel starts can be determined with the change of intensity of the detected light, irradiated through the gel piece.

### 3.3.3 Degradability study of the hydrogels

When hydrogels are formed with disulfide bonds as crosslinker, one of the properties of that gel is that it owns a degradable character, when that gel is immersed in a reducing environment. To confirm this property in our synthesized gels, a DT9-3-morpholino propylamine gel is immersed in a 0.8 mM solution of tris(2-carboxyethyl)phosphine (TCEP), which is a water soluble reducing agent (Scheme 3.13). This mixture has been put under the microscope and a picture has taken each 10 minutes. The pictures taken at 0, 60, 120 and 180 minutes are shown on figure 3.16.

![Scheme 3.13: Chemical structure of TCEP.](image)

**Figure 3.16:** Evolution of the degradation of 2 pieces of a DT9 – 3-morpholino propylamine hydrogel in a 0.8 mM aqueous TCEP-solution as a function of time.
The microscope pictures show a slow decrease in size of the gels. In fact, the gel pieces are “peeled off” from the outside, because the TCEP has a low diffusion potential into the hydrogel. After 3 hours, a fair amount of the hydrogel was degraded. When the same mixture was left untouched overnight, the hydrogel was completely degraded and dissolved in the water. This proves the degradable character of the synthesized hydrogel.
Chapter 4: Experimental part

4.1 Materials

Table 4.1A: Overview of the purchased compounds, the source and the purity of the compound.

<table>
<thead>
<tr>
<th>Used chemical</th>
<th>Source</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dioxane</td>
<td>Acros</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>3-morpholino propylamine</td>
<td>Sigma Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Sigma Aldrich</td>
<td>&gt;99.85%</td>
</tr>
<tr>
<td>Acryloyl chloride</td>
<td>Sigma Aldrich</td>
<td>97%</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>Fisher</td>
<td>25 wt% in H\textsubscript{2}O</td>
</tr>
<tr>
<td>Benzylamine</td>
<td>Sigma Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>Chloramine-T hydrate (CAT)</td>
<td>Sigma Aldrich</td>
<td>98%</td>
</tr>
<tr>
<td>Copper(II)bromide</td>
<td>Sigma Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>Di(ethylene glycol) monomethyl ether</td>
<td>Sigma Aldrich</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Dichloromethane (DCM)</td>
<td>Sigma Aldrich</td>
<td>&gt;99.8%</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>Sigma Aldrich</td>
<td>&gt;99.8%</td>
</tr>
<tr>
<td>DL-homocysteine-thiolactone hydrochloride</td>
<td>Acros Organics</td>
<td>&gt;98.5%</td>
</tr>
<tr>
<td>DL-N-Acetylhomocysteine</td>
<td>Sigma Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>Ethyl acetate (EtOAc)</td>
<td>Sigma Aldrich</td>
<td>99.8%</td>
</tr>
<tr>
<td>Hexane</td>
<td>Chemlab</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})</td>
<td>Sigma Aldrich</td>
<td>30 wt% in H\textsubscript{2}O</td>
</tr>
<tr>
<td>Iron(III)chloride</td>
<td>Sigma Aldrich</td>
<td>&gt;97%</td>
</tr>
<tr>
<td>N,N-dimethylacrylamide</td>
<td>Sigma Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>Sigma Aldrich</td>
<td>&gt;98%</td>
</tr>
<tr>
<td>N-isopropylacrylamide (NIPAAm)</td>
<td>Sigma Aldrich</td>
<td>97%</td>
</tr>
</tbody>
</table>
Table 4.1B: Overview of the purchased compounds, the source and the purity of the compound.

<table>
<thead>
<tr>
<th>Used chemical</th>
<th>Source</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(ethylene glycol) monomethyl ether (PEO-OMe)</td>
<td>Fluka</td>
<td>-</td>
</tr>
<tr>
<td>Propylamine</td>
<td>Sigma Aldrich</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Propylamine Hydrochloride</td>
<td>Sigma Aldrich</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>Roth</td>
<td>&gt;99.5%</td>
</tr>
<tr>
<td>Sodium iodine</td>
<td>Sigma Aldrich</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Sodium perborate tetrahydrate</td>
<td>Sigma Aldrich</td>
<td>&gt;97%</td>
</tr>
<tr>
<td>Tetrahydrofuran (THF)</td>
<td>Sigma Aldrich</td>
<td>99.9%</td>
</tr>
<tr>
<td>Toluene</td>
<td>Fisher Scientific</td>
<td>99.9%</td>
</tr>
<tr>
<td>Triethylamine (NEt₃)</td>
<td>Sigma Aldrich</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Triphosgene</td>
<td>Sigma Aldrich</td>
<td>98%</td>
</tr>
<tr>
<td>Tris(2-carboxyethyl)phosphine hydrochloride (TCEP)</td>
<td>Sigma Aldrich</td>
<td>&gt;98%</td>
</tr>
</tbody>
</table>

4.2 Purification of products

All solvents and reagents are used as received, except the following.

N-isopropyl acrylamide is purified by recrystallization from a 50:50 v/v% toluene/n-hexane mixture. After the recrystallization, the monomer is repeatedly washed with pure n-hexane. PEG-OMe or DEG-OMe respectively has been mixed with toluene to remove residual water followed by removal of the toluene with the help of a rotavapor. This procedure was repeated two times. After the third cycle, the purified PEG-OMe/DEG-OMe was dried under high vacuum. DCM was distilled over CaH₂.
4.3 Monomer synthesis

4.3.1 Methoxy di(ethylene glycol) acrylate (DEGA)

DEGA was synthesized through a procedure reported by Hua et al.\textsuperscript{16}

Di(ethylene glycol) monomethyl ether (36.0 g, 0.300 mol), triethylamine (36.4 g, 0.359 mol), and dichloromethane (280 ml) were added to a 500-mL, three-necked flask, which was then placed in an ice/water bath. A solution of acryloyl chloride (32.5 g, 0.360 mol) in dichloromethane (60 ml) was added dropwise to the flask under an N\textsubscript{2} atmosphere. The ice/water bath was then removed, and the reaction mixture was stirred at room temperature overnight.

The precipitate is filtered off and the fluid is mixed with 200g silica. After damping in, the mixture is dried under high vacuum. Then this mixture was transferred to a silica column and the crude product was eluted with EtOAc. After complete removal of EtOAc, hydroquinone was added as inhibitor. As last, the compound was distilled under high vacuum at 60-65 °C. A colorless fluid was obtained.

4.3.2 Thiolactone acrylamide (Tla)

DL-homocysteine thiolactone hydrochloride (7 gram, 45.57 mmol) was dissolved in 100 ml of a 1:1 v/v dioxane/water mixture within a two-necked flask, which was placed in an ice/water bath. After a dropping funnel with acryloyl chloride (7.4 ml, 8.25 g, 91.19 mmol) was placed on the flask sodium bicarbonate (19.11 g, 227 mmol) was added to the reaction mixture, leading to CO\textsubscript{2} liberation. After 30 minutes, the acryloyl chloride was added dropwise. When this was done, the ice/water bath was removed and the mixture was stirred overnight.

After that 200 ml of brine solution were added and a triple extraction with 3x 200 ml EtOAc was performed. Then the organic fractions were collected and dried with MgSO\textsubscript{4}. EtOAc was removed and the remaining solid residue was recrystallized from dichloromethane.
4.4 Polymer synthesis

4.4.1 PNIPAAm-co-Tla

In a typical experiment, NIPAAm, Tla, DDMAT and AIBN were dissolved in 1,4-dioxane establishing a ratio of \([M]/[DDMAT]/[AIBN] = 150/1/0.1\). An equal molar amount of N,N-dimethylacrylamide compared to the Tla molar amount was added to the mixture to increase the LCST. 3-4 freeze-pump-thaw cycles were performed in order to remove the oxygen from the mixture. After that, the mixture was stirred at 65 °C for approximately 4 hours.

The polymerization was stopped by placing the reaction vessel into an ice bath for 5 minutes. Then, the vessel was opened to expose the reaction mixture to air. The pure polymer was obtained by a repeated precipitation in diethyl ether.

4.4.2 PDEGA-co-Tla

The preparation procedure for the synthesis of PDEGA-co-Tla is analogue to the one established for the synthesis of PNIPAAm-co-Tla, except that CVADTB was used as RAFT agent and the lack of additional monomer to alter the LCST. The reaction was performed at 85 °C with a ratio of \([M]/[DDMAT]/[AIBN] = 150/1/0.1\) and run for 2 hours. The reaction was stopped by placing the vessel first in an ice bath and subsequently exposing the mixture to air.

The purification is performed via dialysis of the reaction mixture against Millipore water for 1 week. After the dialysis, the pure polymer is obtained via freeze drying.

4.5 Model and response reactions

4.5.1 Aminolysis with low molecular weight compounds

DL-N-acetylhomocysteine thiolactone (0.25 M) and propylamine hydrochloride (5 eq. with respect to Tla) were separately dissolved in water. Before starting the reaction (addition of propyl amine to the Tla), both solutions were titrated to the desired pH with NaOH\(_{aq}\) (0.2 M). The reaction was followed by online FTIR. A spectrum was recorded every 5 minutes.
4.5.2 Model reaction to follow the aminolysis/disulfide formation reaction cascade (gelation process)

4.5.2.1 Synthesis of 3-isocyanato-dihydro-thiophen-2-one (thiolactone isocyanate) and the thiolactone monofunctionalized poly(ethylene glycol) methyl ether (PEG-OMe-Tla)

DL-homocysteine thiolactone hydrochloride (10 g; 65.09 mmol) was dissolved into 255 ml DCM, where 255 ml saturated NaHCO₃ was added. The mixture was cooled in an ice bath and was constantly stirred. Then, triphosgene (6.38 g; 21.49 mmol) was added (careful: toxic compound). This mixture was stirred for 15 minutes at 0 °C. A separating funnel was used to extract the mixture 4 times with 50 ml DCM where after the organic mixture was dried with MgSO₄. A vacuum distillation was performed to become a yellow oil at 64 °C (0.1 mmHg). Pure thiolactone isocyanate was received.

5 g of purified PEG-OMe was dissolved in 40 ml dry DCM. To that solution, 1.1 eq., compared to the end OH group of each PEG-OMe chain, of the thiolactone isocyanate was added. This mixture was stirred overnight at room temperature. After that the product is precipitated in diethyl ether and dried in a vacuum oven.

4.5.2.2 Elaboration of the aminolysis/disulfide formation reaction cascade model reaction (gelation process)

In a representative reaction, 100 mg of PEO-OMe-Tla was dissolved in 1 ml of a NaHCO₃ or K₂CO₃ solution (0.8 M) of the appropriate pH (adjusted by titration with NaOHₐq and acetic acid) containing already 6.2 mg of propylamine, respectively. Samples were taken by adding three droplets of the reaction mixture to 1ml of DMA containing 0.8 mg of ethyl maleimide.

For the experiment for which chloramine-T was employed, three droplets of the reaction mixture were first mixed with three droplets of water containing a 7-fold excess of chloramine-T with respect to the Tla units. After 5 min, the maleimide containing DMA (1ml) was added.
4.5.3 Model reaction to determine the extent of functionalization

50 mg of DT22 was dissolved in 500 mg of a NaHCO₃ or K₂CO₃ solution (0.8 M) of the appropriate pH (adjusted with 0.5M NaOHₐq and acetic acid) containing already 16 mg of propylamine. This mixture was cooled to 4°C and stirred for 2 days. After the reaction, the polymer was purified via dialysis.

4.5.4 Modification of the copolymers for the analysis of the response towards Cu²⁺ concentration

200 mg of copolymer was dissolved in 20 ml DCM, where 5 eq. of amine is added and was stirred overnight. When the reaction was finished, 7 eq. of n-ethylmaleimide was added. After reaction overnight, the solvent was removed with the rotavapor and the copolymer was dissolved in 10 ml water. This mixture was purified with dialysis and the water was removed by freeze-drying.

4.6 Gelation and gel studies

4.6.1 Gelation in buffered aqueous solution

Both the polymer precursor and the respective amine hydrochloride were dissolved in a NaHCO₃ solution (0.25 M). After adjusting the pH in both cases, the two solutions were mixed in the desired quantities. In a representative sample, the final solution contained 20% of polymer precursor and 5 eq. of amine with respect to the Tla content. If an extra oxidation agent was supposed to be used, this agent was added to the amine containing solution prior to the mixing step. The quantity was 1 eq. with respect to the Tla content. After mixing, air supply was provided with the use of a needle, which was put through the vial cap. While the reaction was proceeding, the mixtures were stirred.

4.6.2 Gelation in aqueous unbuffered and organic solutions

Here, the polymer precursor was dissolved in water or the respective solvent at the desired concentration. When the polymer was completely dissolved, the respective amine is added (5 eq. with respect to the number of Tla units). If an extra oxidizing agent was supposed to be used, this agent was added to the solution (1 eq. with respect to the Tla units) just before
the addition of the amine. For purification, the gels prepared in organic solution were exposed to vacuum in order to evaporate the solvent. When the gels were prepared in water, this step was skipped. After this, the gels were immersed into Millipore water for a week, where each day the water was refreshed.

4.7 Experimental Techniques

4.7.1 ¹H Nuclear Magnetic Resonance (NMR)

The NMR spectra were recorded on a Bruker AVANCE 300 machine (300 MHz) or a Bruker AVANCE 500 (500 MHz). The analysis was performed with the help of the ACD/Spec Manager software of ACD/Labs.

4.7.2 Gel permeation chromatography (GPC)

GPC analysis was performed at 35 °C with three Polymer Standards Services GPC columns, placed in series, (1x GRAM Analytical 30 Å, 10 µm and 2x GRAM Analytical 1000 Å, 10 µm), a Hitachi Column Oven L-7300, a Waters 2414 Refractive Index Detector, a Waters 600 controller and a Waters 610 Fluid Unit. DMA + 0.42 g/l LiBr is used as solvent with a flow rate of 1 ml/min. Calibration was done with PMMA standards (690 g/mol until 1944000 g/mol). The obtained chromatograms were analysed with the Empower software from Waters.

4.7.3 Optical Microscope analysis

The studies with the microscope were performed on a Olympus optical microscope BH2. The heating was performed by a custom made heating device.

4.7.4 Online Fourier Transformation Infra Red (Online FTIR)

Online FTIR measurements were performed on a ReactIR 4000 instrument (Mettler Toledo) with the use of a siliconprobe (SiComp, optical range 4400-650 cm⁻¹).

4.7.5 Elemental Analysis

Elemental analysis was performed with a Thermo Scientific FLASH 2000 Series CHNS/O analyser at the department of Inorganic and Physical chemistry of the UGent.
4.7.6 Turbidimetry measurements

Turbidimetry measurements were performed with polystyrene cuvettes in a Julabo FP50, a programmable thermostat with a thermal stability of 0.01 °C. The temperature was measured with a PT100 temperaturesensor, the light used had a wavelength of 540 nm and the transmittance was detected via an Uvikon 810 UV-VIS spectrophotometer.
Chapter 5: Conclusions and outlook

In the first part of this thesis, a kinetic study of the PDEGA-co-Tla copolymerization was performed. There, different DEGA/Tla compositions were copolymerized via the RAFT polymerization approach. In order to be able to perform these polymerizations, the monomers had to be synthesized beforehand. With the received results, the Fineman-Ross method was applied to obtain the reactivity ratios of this particular copolymerization system. A copolymerization diagram was obtained, showing that we deal with an azeotropic copolymerization. The reactivity ratios were 0.43 for DEGA and 0.67 for Tla. The resulting copolymers can therefore be considered as more or less statistical in composition.

After synthesis of PDEGA-co-Tla and PNIPAAm-co-Tla copolymers with different mol% of Tla, gelation attempts were performed. Preferably, the gelation should take place in water, but that comes along with certain difficulties such as the risk of hydrolysis instead of aminolysis of the thiolactone ring (which results in lower functionalization) and the risk of saponification (only for the PDEGA-co-Tla copolymers). Also, the speed of disulfide formation needed to be controlled and improved with the use of an oxidation agent.

In order to find optimum conditions for a gelation in water, 3 model reactions were done. The first model reaction, involving a low molecular weight thiolactone compound gave information about the speed of the aminolysis of the thiolactone ring. The second model reaction, in which the dimerization of a thiolactone end functionalized PEO upon addition of an amine was monitored by GPC, gave information on the aminolysis/disulfide formation cascade process. In the third part, PDEGA-co-Tla was treated with propyl amine and the resulting polymer composition was measured via elemental analysis. The main conclusion was that the pH of the mixture needs to be above 7 and the use of an additional oxidation agent (e.g. chloramine T) can improve the amount of disulfide bonds and thus crosslink density. In addition, a linear PDEGA-co-Tla copolymer was modified with a morpholine containing moiety, where the thiols were blocked, so no gel was formed. With this polymer, the Cu$^{2+}$ responsive property of the morpholino-containing gels was proven.
With this information, gelation attempts were performed with PDEGA-co-Tla in buffered solutions, but the gelation did not occur, except for high polymer concentrations or upon the addition of an oxidation agent. In any case, nevertheless, the synthesized gel appeared not stable upon immersion in water. PNIPAAm-co-Tla hardly dissolved in buffered solutions. To screen if the gelation can occur at all, the gelation was performed in an unbuffered solution (where the ring opening and the disulfide formation occurs much easier). There, gelation was observed both for PNIPAAm and PDEGA, even without additional oxidation agent, but the formed gels were again not stable, except when FeCl₃ and NaI was used as oxidizing agent. Chloramine T appeared to be another option, but the compound needs to be added after the aminolysis, since the oxidation agent also reacts with the amines. Here, however, the high concentration regime we deal with causes problems, since the solubility of CAT in water is limited.

Due to the difficult gelation in aqueous environment, organic solvents were used to form stable gels, which could be purified and used for analysis. For those gels, the temperature response was tested via an optical microscope and a custom made heating device. The main conclusion there is that a Tla content of 22 mol% might create a too high crosslinking density to obtain a good swell response. This crosslinking density turns out to be controllable in organic solvents by varying the used amine or Tla-content. In the last experiment, the degradability of the synthesized hydrogels has been proven.

In the future, more tests should be made with the chloramine T as oxidation agent. The disadvantage is that this is a reaction in two steps, while a one-step reaction is preferred. However, the use of such a compound hopefully leads to a better control of gelation. Gelation should be further more explored at a pH in the range of 10, since here, faster gelation can be expected. PDEGA might not be suitable at all in the end, because of the strong shielding effect of the thiolactone/thiol groups. We will therefore focus now on PNIPAAm based gels. In any case, these action points will also help to obtain gels of better stability and higher mechanical strength. Also detailed response studies with the morpholino functionalized gels have to be carried out in order to test their suitability for heavy metal ion sensing. When the proof of principle has been achieved, the concept can be extended to gels bearing boronic acid functions for glucose sensing.
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Responsieve Hydrogelen gebaseerd op de Thiolactonchemie

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Abstract: Gebruik makende van de thiolactonchemie werden er responsieve hydrogelen met een gecontroleerd degradeerbaar karakter gesynthetiseerd via een efficiënte synthesestrategie. Allereerst werd er een kinetische studie uitgevoerd om een controle te verkrijgen over de copolymerisatie van hydrofiele monomeren met een thiolacton-bevattend monomeer. Vervolgens werd de invoering van de functionaliteiten in de zijketen van het gevormde copolymer onderzocht. Tenslotte werden de gevormde hydrogelen getest naar hun responsief en degradeerbaar karakter.

Trefwoorden: RAFT, hydrogelen, responsieve polymeren, disulfide bruggen, thiolacton.

Introductie

Stimuli-responsieve hydrogelen zijn vernette polymeerstructuren met een grote variëteit aan applicaties, zoals sensoren (1) en geneesmiddel afgiftesystemen (2). Deze hydrogelen worden echter ontwikkeld door ingewikkelde meerstapsreacties met toevoeging van externe factoren zoals warmte, externe vernetters of milieuonvriendelijke solventen. Dit kan omzeild worden door middel van de thiolactonchemie. Deze chemie stelt ons in staat om functionaliteiten aan een polymeerketen toe te voegen, zodat deze keten een responsief karakter verkrijgt. Dit gebeurt via aminolyse van de thiolactonfunctie met een amine die een functionaliteit naar een bepaalde stimuli bevat. Hiervoor kan bijvoorbeeld 3-morpholino propylamine gebruikt worden, wat een morpholinfunctie bevat die affiniteit vertoont tegenover Cu$^{2+}$ en Ni$^{2+}$-ionen (3). Bovendien komt door de aminolyse een thiogroep vrij die de mogelijkheid geeft om disulfidebruggen te vormen. Deze dienen dan als vernetter van de polyemeerketens, waardoor er een stimuli-responsieve hydrogel gevormd wordt. Als de gebruikte polymeerketen op zich reeds een functionaliteit bevat die aanleiding kan geven tot een responsief karakter, zou er zelfs via deze methode een multi-responsieve hydrogel ontwikkeld kunnen worden. Twee bekende thermo-responsieve polymeren (respons tegenover verandering in temperatuur) zijn poly(N-isopropylacrylaamde) (PNIPAAm) (4) en poly(di(ethyleenglycol)acrylaat) (PDEGA) (5) (figuur 1).

Figuur 1. Chemische structuren van poly(N-isopropylacrylaamde) (PNIPAAm) (links) en poly(di(ethyleenglycol)acrylaat) (PDEGA) (rechts).
Door copolymerisatie NIPAAm of DEGA met een thiolacton gesubsitueerde acrylaatfunctie (Tla) verkrijgt men een thermoresponsief polymeer met mogelijkheid om een extra functionaliteit toe te voegen, afhankelijk van het gekozen amine. De gebruikte copolymerisatiemethode is reversiebare additie-fragmentatie ketentransfer polymerisatie (RAFT) omdat deze aanleiding geeft tot een lage polydispersiteit. Dit zorgt voor een hogere homogeniteit, wat leidt tot een snellere respons van de gelen. Op figuur 2 is een volledig overzicht van de gelsynthese weergegeven.

Figuur 2. Schematisch overzicht van de vorming van een gefunctionaliseerde gel die vernet is via disulfidebruggen.

Om het percentage aan thiolactonzijgroepen in de keten van het gesynthetiseerde copolymeer volledig te kunnen controleren, dienen er kinetische studies uitgevoerd te worden. Dit zal het eerste deel van dit artikel in beslag nemen. Aan de hand van de resultaten van deze studie werden er copolymeren gesynthetiseerd met verschillende percentages aan Tla zijgroepen.

In het tweede deel werden drie modelreacties opgesteld om de aminolyse, de disulfidevorming en het aantal functionele zijgroepen na ringopening te controleren. Aan de hand van deze resultaten werden gelen gevormd uit de gesynthetiseerde copolymeren.

Tenslotte werden de gesynthetiseerde gelen getest op hun respons en degradeerbaarheid.

**Experimenteel**

**Materialen:**
Propylamine (Sigma Aldrich, >99%), Benzylamine (Sigma aldrich, 99%), 3-morpholino propylamine (Sigma Aldrich, 99%), propylamine waterstofchloride (Sigma Aldrich, >99%), n-ethylmaleïmide (Sigma Aldrich, >98%), chloramine T hydraat (Sigma aldrich, 98%), 1,4-dioxaan (Acros, >99%), N,N-dimethylacrylamide (Sigma Aldrich, 99%), DL-N-Acetylhomocysteine (Sigma Aldrich, 99%), DL-N-Homocysteine thiolacton waterstofchloride (Acros Organics, >98,5%), Trifosgeen (Sigma Aldrich, 98%), chloramine T (Sigma Aldrich, 98%) en waterstofperoxide (Sigma Aldrich, 30 wt% in water) werden gebruikt zoals verkregen. Solventen werden aangekocht van Sigma Aldrich en werden gebruikt zoals verkregen, tenzij anders vermeld.
N-isopropylacrylamide werd voor gebruik opgezuiverd door herkristallizatie met een 50/50 v/v% toluene/n-hexaan mengsel. Poly(ethyleenglycol) methylether werd opgezuiverd door het drie keer op te lossen in toluene en te drogen onder vacuüm. Dichloormethaan werd voor gebruik gedestilleerd over CaH2 en di(ethyleen glycol)acrylaat werd gesynthetiseerd volgens de literatuur (5).

Synthese van poly(N-isopropylacrylamide-co-thiolacton acrylaat) (PNIPAAm-co-Tla):

In een typisch experiment werden N-isopropylacrylamide (NIPAAm), thiolactone acrylaat (Tla), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT) en AIBN in een verhouding van [M0]/[DDMAT]/[AIBN] van 150/1/0.1 opgelost in 1,4 dioxaan. Een equimolaire hoeveelheid aan N,N-dimethylacrylamide, vergeleken met de Tla-hoeveelheid, werd toegevoegd om de LCST te verhogen. Na het mengsel 3-4 keer te ontgassen via de vries-pomp-dooi cyclus, ging de polymerisatie door voor 4 uur bij 65 °C. Daarna werd het mengsel voor 5 minuten afgekoeld in een ijsbad en werd het reactievat geopend. Het pure copolymeer werd bekomen door precipitatie in diëthylether.

Synthese van poly(di(ethyleen glycol)acrylaat-co-thiolacton acrylaat) (PDEGA-co-Tla):

De synthese is analoog aan deze beschreven voor PNIPAAm-co-Tla, uitgezonderd het gebruik van 4-cyano-4-(thiobenzyl) sulfanyl pentanoic acid (CVADTB) als RAFT keten transferreagens. Er werd ook geen extra monomeer gebruikt om de LCST te verhogen. De reactie werd uitgevoerd bij 85 °C gedurende 2 uur. Het polymeer werd gezuiverd door dialyse met Millipore water gedurende 1 week. Door middel van vriesdrogen werd het zuivere copolymeer bekomen.

Model reacties om de condities van de gelering te bepalen:

Aminolyse met stoffen die een laag moleculair gewicht bevatten. DL-N-Acetylhomocysteine (0,25M) en propylamine waterstofchloride (5 eq.) werden afzonderlijk opgelost in water. Alvorens de reactie te starten werden de oplossingen getitmeerd met 0,2 M NaOHaq. De reactie werd gevolgd via FTIR, waarbij er elke 5 minuten een spectrum opgenomen werd.

Modelreactie om de aminolyse/disulfidebrugvorming cascade te volgen. DL-homocysteine thiolacton waterstofchloride (10 g; 65,09 mmol) werd opgelost in 255 ml dichloormethaan, waaraan 255 ml verzadigde NaHCO3 werd toegevoegd. Het mengsel werd onder constant roeren gekoeld in een ijsbad. Daarna werd er trifosgeen (6,38 g; 21,49 mmol) toegevoegd (opgelet: giftige stof) en werd er geroerd voor 15 minuten. Een scheidtrechter werd gebruikt om het mengsel 4 keer te extraheren met 50 ml dichloormethaan, waarvan de organische fase gedroogd werd met MgSO4. Via vacuüm destillatie werd er een gelige olie (zuiver thiolacton isocyanaat) bekomen bij 64 °C (0,1 mmHg).

5 g van opgezuiverd poly(ethyleen glycol) methyl ether (PEG-OMe) werd opgelost in 40 ml droge dichloormethaan. In deze oplossing werd 1,1 eq. (in vergelijking met de hydroxygroep op het einde van de PEG-OMe keten) thiolacton isocyanaat toegevoegd. Na een nacht reageren bij kamertemperatuur werd het gemonificeerd polymeer (PEG-OMe-Tla) geprecipiteerd in diëthylether en gedroogd.
100 mg PEG-OMe-Tla werd opgelost in een 1 ml 0.8 M NaHCO₃/K₂CO₃ oplossing (ratio afhankelijk van de beoogde pH, titratie met verdunnd NaOH of azijnzuur). Hieraan werd 6.2 mg propylamine toegevoegd. De monsteropnames werden uitgevoerd door drie druppels toe te voegen in een mengsel met 1 ml DMA, waarin 0.8 mg n-ethylmaleïnide is opgelost. Voor de experimenten met chloramine T werden de drie druppels bij monsteropname eerst in een zevenvoudige overmaat aan chloramine T, in vergelijking met de Tla-functionaliteiten, gebracht. Na 5 minuten werd dit mengsel in een oplossing van 0.8 mg n-ethylmaleïnide in 1 ml DMA gebracht.

**Modelreactie om de functionaliteitsgraad te bepalen:** 50 mg van het polymeer werd opgelost in 500 mg van een NaHCO₃/K₂CO₃ oplossing (ratio afhankelijk van de beoogde pH, titratie met verdunnd NaOH of azijnzuur), waaraan 16 mg propylamine werd toegevoegd. Dit mengsel werd gekoeld tot 4 °C onder constant roeren gedurende 2 dagen. Na de reactie werd het polymeer opgezuiverd via dialyse.

**Geleringsstudies in gebufferde oplossingen:**

Het polymeer en het amine-waterstofchloride zout werden afzonderlijk opgelost in een NaHCO₃-oplossing (0.25M). Nadat de mengsels getitreerd zijn tot de juist pH-waarde, werden ze samengevoegd in de vereiste verhoudingen. Als een extra oxidans gebruikt werd, werd 1 eq., in vergelijking met het aantal Tla-functionaliteiten, toegevoegd in de amine-waterstofchloride zout oplossing. Na het samenvoegen werd het reactievat gesloten en werd de luchtaanvoer verzekerd door een naald, waarna de reactie doorging onder constant roeren.

**Geleringsstudies in niet-gebufferde oplossingen en organische solventen:**

Nadat het polymeer is opgelost in de vereiste concentratie werd er 5 eq., in vergelijking met het aantal Tla-functionaliteiten, van het amine toegevoegd. Hierna werd er eventueel 1 eq. oxidans aan toegevoegd. Als de gelen gevormd werden in organisch solvent, werd dit solvent bij opzuivering verwijderd in vacuüm. Bij oplossingen in water werd deze stap overgeslagen. De zuivere gel werd bekomen door dialyse uit te voeren in Millipore water gedurende 1 week, waarbij het water elke dag ververst werd.

**Modificatie van de copolymeren voor de analyse van de respons tegenover de Cu²⁺-concentratie:**

Er werd 200 mg polymeer opgelost in 20 ml dichloormethaan, waaraan, in vergelijking met het aantal Tla-functionaliteiten, 5 eq. amine toegevoegd werd. Dit mengsel werd voor 1 nacht geroerd, waarna er 7 eq. n-ethylmaleïnide toegevoegd werd. De reactie ging opnieuw 1 nacht door. Daarna werd het solvent aan de rotavapor verwijderd en werd het verkregen copolymeer opgelost in 10 ml water. Het zuivere copolymeer werd verkregen door dialyse gedurende 1 week, waarbij het water verwijderd werd via vriesdrogen.

**Instrumentatie:**

¹H-NMR spectra werden verkregen met een Bruker AVANCE 300 machine (300 MHz) of een Bruker AVANCE 500 (500 MHz). GPC werd uitgevoerd bij 35°C op drie Polymer Standard Services GPC kolommen (1x GRAM analytical 30 Å, 10 µm and 2x GRAM Analytical 1000 Å, 10 µm) die in serie geplaatst zijn. DMA + 0.42 g/l LiBr werd gebruikt als solvent met een vloeisnelheid van 1 ml/min en er werd gekalibreerd met PMMA standaarden. De gebruikte
microscoop was een Olympus BH2 optische microscoop. Online IR metingen werden verkregen via een ReactIR 4000 instrument van Mettler Toledo. Elementaire analyse werd uitgevoerd met een Thermo Scientific FLASH 2000 series CHNS/O analyser in de vakgroep Fysische en Anorganische Chemie aan de UGent. Turbidimetrie experimenten werden met polystyreen cuvetten uitgevoerd in een programmeerbare thermostaat, die een PT100 temperatuursensor bevat. De lichtstraal heeft een golflengte van 540 nm en werd gedetecteerd door een Uvikon 810 UV-VIS spectrofotometer.

Resultaten en discussie

Kinetiekstudie van de DEGA/Tla copolymerisatie:

Om controle van het aantal Tla groepen in een copolymerketen te verkrijgen, werd een kinetiekstudie uitgevoerd. Deze studie voor de copolymerisatie van NIPAAm en Tla werd reeds voltooid in niet-gepubliceerde experimenten in de PCR-groep. Hierdoor werd enkel de copolymerisatie van DEGA en Tla behandeld. De conversie werd via $^1$H-NMR bepaald. Hierbij werd de verandering van de oppervlaktes van de pieken die de dubbele bindingen van DEGA en Tla vertegenwoordigen in functie van de tijd bekeken.

Vervolgens wordt de Fineman-Ross methode gebruikt om de reactiviteitsverhoudingen van de monomeren te bepalen. Uit deze methode blijkt dat de reactiviteitsverhouding van DEGA gelijk is aan 0.4124 en deze voor Tla aan 0.7273. Dit betekent dat men in een copolymerisatieplot, weergegeven in figuur 3, een azeotropisch punt moet bekomen, aangezien beide waarden lager zijn dan 1. Practisch bewijzen de bekomen data de aanwezigheid van het azeotropisch punt niet, maar doordat ze de theoretische trend volgen, kan dit aangenomen worden.

Figuur 3. Copolymerisatieplot van het DEGA monomeer in de DEGA/Tla copolymerisatie

In bovenstaande figuur is af te leiden dat bij elke molaire fractie de afwijking, waar er geen voorkeur voor een bepaald monomeer bestaat, dusdanig laag is dat men mag aannemen dat de copolymerisatie nagenoeg statistisch verloopt.

Modelreacties om de condities van de gelering te bepalen:

Aminolyse met stoffen die een laag molecular gewicht bevatten. Om de snelheid waarmee de thiolacton ring wordt geopend via aminolyse te bepalen bij verschillende pH- waarden werd DL-N-acetylhomocysteïne opgelost samen met 5 eq. aan propylamine, waarbij de vorming van vrije thiolen gevolgd werd via online IR spectroscopie. De resultaten staan uitgedrukt in figuur 4.
Figuur 4. Evolutie van de online IR thiolpiekhoogte in functie van de reactietijd.

Men kan concluderen dat bij pH 7 en 9 de aanwezigheid van thiolen vrij laag blijft, terwijl bij pH 8 er duidelijk meer thiolen aanwezig zijn. Bij pH 7 zal dit waarschijnlijk zijn door de lage concentratie aan vrij amine, terwijl bij pH 8 deze concentratie duidelijk groter zal zijn. Bij pH 9 krijgt men een nog grotere snelheid aan ringopening, maar de lage hoeveelheid thiolen kunnen duiden op het feit van snelle disulfidebrugvorming.

Modelreactie om de aminolyse/disulfidebrugvorming cascade te volgen. Om bovenstaande resultaten te testen in een polymeeromgeving werd poly(ethyleen glycol) methylether (PEG-OMe) gefunctionaliseerd met een thiolactonfunctionaliteit (PEG-Tla). Dit gebeurde via de vorming van een urethaanbinding uit de vrije hydroxylgroep op het einde van de PEG-OMe polymeerketen. Door de aanwezigheid van de thiolactonfunctionaliteit kunnen deze structuren na aminolyse en disulfidebrugvorming dimeren vormen die zichtbaar zijn via GPC. Om een correct beeld te krijgen van het aantal disulfidebruggen bij het nemen van een monster op een bepaald tijdstip, werden de vrije thiolen geblokkeerd via reactie met n-ethylmaleimide. Deze reactie werd uitgevoerd bij verschillende omstandigheden (verschillende pH, gebruik van oxiderende stoffen) waarvan de resultaten staan uitgebeeld in figuur 5.

Uit de resultaten blijkt dat de pH-waarde van het mengsel hoger dan 7 moet zijn. Hoe hoger de pH-waarde, hoe sneller de disulfidebrugvorming, maar uiteindelijk wordt eenzelfde conversie bereikt. Er kunnen ook disulfidebruggen optreden zonder aanwezigheid van amines via hydrolyse, maar dit is echter aan een lagere snelheid. Tenslotte kan het aantal disulfidebruggen ook opgevoerd worden door na aminolyse chloramine T toe te voegen aan het mengsel. Waterstofperoxide zal initieel het aantal vermogen, maar bereikt uiteindelijk lage conversies.

Modelreactie om de functionaliteitsgraad te bepalen. Een thiolactonring kan geopend worden door hydrolyse (6) of aminolyse (Figuur 6). Beide reacties zullen aanleiding geven tot een vrije thiolfunctie die kan reageren tot een disulfidebrug. In het geval van hydrolyse zal er echter geen functionaliteit aan de polymeerketen toegevoegd worden. Op figuur 6 is het zichtbaar dat de N/S ratio in het polymeer varieert naargelang er geen reactie, aminolyse of hydrolyse doorgaat. Aangezien de N/S ratio van de hydrolyse en het geval zonder reactie nagenoeg gelijk zijn, kan de functionaliteitsgraad dus op semi-kwalitatieve manier bepaald worden. De verhoudingen bij verschillende pH-waarden van een gefunctionaliseerd PDEGA-co-Tla polymer met 22% Tla werd bepaald via elementaire analyse, weergegeven in tabel I.

Figuur 6. De verschillende mogelijke reacties met een thiolacton in een water/aminemengsel, met hun respectievelijke N/S verhouding waarbij de thiolacton voor 22% als zijketen voorkomt in een PDEGA-co-Tla copolymeer.
TABEL I. N/S verhoudingen van de gemodificeerde PDEGA-co-Tla polymeren (22% Tla) met 5 eq. propylamine bij verschillende pH-waarden.

<table>
<thead>
<tr>
<th>N/S ratio</th>
<th>Origineel polymeer</th>
<th>Reactie bij pH = 8</th>
<th>Reactie bij pH = 9</th>
<th>Reactie bij pH = 10</th>
</tr>
</thead>
</table>

Uit de resultaten blijkt dat de functionalisatie stijgt bij hogere pH-waarden, aangezien de N/S verhouding dichter bij 0.84 komt te liggen, wat de N/S verhouding is voor een reactie met volledige aminolyse. Nochtans werd aan de hand van de vorige reactie verwacht dat de functionalisatie hogere waarden zou aannemen. Deze toont namelijk aan dat in het begin van de reactie de aminolyse veel vlugger verloopt dan de hydrolyse. Deze uitkomst kan verklaard worden doordat de sterk gehydrateerde ethyleenglycol zijketen van het DEGA monomeer de thiolacton functionaliteiten zal beschermen. Hierdoor kan het amine de ring moeilijker bereiken. De hydroxyl ionen daarentegen moeten omwille van de snelle protonuitwisseling tussen water- en hydroxyxilonen niet diffunderen naar de thiolactonring. Daardoor zal de hydrolyse geen hinder ondervinden door de aanwezige ethyleenglycol zijketen.

Samengevat kan men stellen dat de aminolyse en disulfidebrugvorming moet gebeuren bij een pH-waarde die gelijk is aan 8 of hoger. Helaas blijkt de ethyleenglycol zijketen van het DEGA-monomeer de functionalisatie sterk te verminderen omdat deze de diffusie van het amine naar de thiolacton functionaliteit verlaagt. Daarnaast zal de stabilititeit van het gevormde hydrogel vergroten door chloramine T toe te voegen na de aminolyse, omdat dan extra disulfidebruggen gevormd worden.

Cu$^{2+}$ respons van de morpholinebevattende polymeren:

Polymeren die een morpholine groep bevatten, kunnen in oplossing de eigenschap hebben responsief te zijn tegenover de concentratie Cu$^{2+}$ in die oplossing (3). Om dit te testen werd een PNIPAAm-co-Tla polymeer met 3, 11 en 22% Tla opgelost in dichloormethaan, waaraan er 5 eq. 3-morpholino propylamine is toegevoegd. Na een dag reageren werd hieraan een overmaat n-ethylmaleimide toegevoegd om de vrijgekomen thiolfunctionaliteiten te blokkeren, zodat geen gelvorming via disulfidebruggen kon optreden. Het gezuiverde polymeer werd dan in een CuBr$_2$-oplossing met een bepaalde concentratie opgelost, bij een constante pH-waarde van 7. Via turbidimetrie kon de temperatuur van het transitiepunt, waar de troebelheid van de oplossing begint toe te nemen, bepaald worden. Zo is het mogelijk om het verschil in transitiemisstemperatuur tussen een oplossing met een bepaalde Cu$^{2+}$-concentratie en degene zonder Cu$^{2+}$, te bepalen. Hiervan zijn de resultaten weergegeven in figuur 7.

Figuur 7. Evolutie van het verschil in transitiemisstemperatuur voor drie morpholinegesubstitueerde PNIPAAm-co-Tla copolymeren (3, 11 & 22 mol% Tla) in een Cu$^{2+}$-oplossing met een bepaalde concentratie, vergeleken met deze copolymeren in een oplossing zonder Cu$^{2+}$ aanwezig.
Bovenstaande resultaten bewijzen dat morpholine-gesubstitueerde copolymeren een respons kunnen vertonen tegenover de Cu$^{2+}$-concentratie in de oplossing waarin ze opgelost zijn, op voorwaarde dat het percentage aan Tla-groepen (en dus morpholinglegroepen, ingevoerd via aminalyse) hoog genoeg is. Dit experiment werd uitgevoerd om de mogelijkheid tot respons te testen, echter om een gedetailleerd responsverloop te verkrijgen moeten er meer experimenten uitgevoerd worden.

Resultaten van de geleringsstudie:

invloed van het solvent op de gelering. PDEGA-co-Tla copolymeren werden getest op gelering bij gebufferde waterige oplossingen, omwille van de lage functionalisatie bij pH < 7 en de kans op verzeep in sterk basische oplossingen. Aangezien de kans op verzeep bij PNIPAAm-co-Tla copolymeren onbestaande is, is dit experiment bij dit copolymeer nutteloos. Het resultaat was dat geen enkele oplossing van 5, 15 of 30 gewichtsprocent van een copolymer (2.5, 9 of 22 % Tla) bij een pH-waarde van 8 of 9 met 5 eq. propylamine waterstofchloride een gel vormde na 4 dagen.

Om te controleren of gelering toch mogelijk was, werd een experiment uitgevoerd bij niet-gebufferde oplossingen. Hierbij was de pH-waarde hoger dan 10, wat volgens de modelstudies aanleiding zou moeten geven tot een hoge snelheid aan disulfidevorming. PNIPAAm-co-Tla (22 % Tla) gaf in een oplossing met 2 gewichtsprocent aan copolymer met 5 eq. propylamine reeds aanleiding tot een gel, terwijl bij PDEGA-co-Tla (22 % Tla) de gel pas gevormd werd in een 30 gewichtsprocent oplossing door de aanwezigheid van de ethyleenglycol zijketens. De gevormde gelen, ook bij hogere concentraties, bleken helaas niet stabiel in water bij de opzuivering van de gelen. De maximale gewichtsconcentratie van de PNIPAAm-co-Tla gelen was 10%, omdat de LCST bij hogere concentraties daalde tot een niet-werkbare temperatuur.

Omdat hydrolyse en verzeep niet kunnen voorkomen bij organische solventen, werd er een poging gedaan om gelen te vormen in ethylacetaat (voor PDEGA-co-Tla) en dichloormethaan (PNIPAAm-co-Tla). Het nadeel van deze methode is dat het solvent na gelering nog moet verwijderd worden om daarna opgezuiverd te worden in water. Hier werd elke gelering uitgevoerd bij 20 gewichtsprocent aan copolymer om later een goede vergelijking te kunnen uitvoeren. Deze methode gaf echter wel aanleiding tot gelen, waarvan enkel de gelen met een lage Tla-inhoud (2.5-3%) onstabiel leken in water bij zuivering.

invloed van additionele oxidantia. Om de stabiliteit van de gelen te verhogen kan men oxiderende componenten toevoegen om het aantal disulfidebruggen te verhogen. Waterstofperoxide is in de biochemie een vaak gebruik oxidans (7), maar elke poging om een gel te vormen met 1 eq. oxidans in de oplossing mislukte doordat er telkens een onstabiele gel gevormd werd. Hoogstwaarschijnlijk is waterstofperoxide als oxidans te sterk, waardoor er overoxidatie optreedt bij de zwavelbevattende functionele groepen. Als men het mildere Fe$^{3+}$ gebruikte als oxidans werd er wel een stabiele gel gevormd, maar de zuivering van het gel was zeer moeilijk. Analoog aan de kinetiekstudie werd dan chloramine T gebruikt. Aangezien deze stof reageert met amines kan deze stof enkel gebruikt worden na de aminolyse. Hieruit blijkt dat een onstabiele hydrogel gevormd uit een PNIPAAm-co-Tla copolymeer met 3% Tla 3 dagen langer stabiel bleef bij de zuivering in water. Dit is mogelijk door deze gel in een chloramine T oplossing (200 mg in 75 ml) te brengen. Chloramine T is dus in staat om het aantal disulfidebruggen voor een gering aantal te verhogen.
Onderzoek naar de controle van de vernettingsdichtheid. Een semi-kwantitatieve methode om het aantal knooppunten te bepalen is via de zwelgraad. Uit experimenten, zichtbaar in tabel II, blijkt dat, zoals verwacht, hoe langer de hydrofobe alkylketen van het gebruikte amine is, hoe lager de zwelgraad is, tenzij men een amine gebruikt waarop een protoneerbare functionaliteit zit (bv 3-morpholino propylamine). Daarnaast zal het gebruik van een copolymer met een lagere Tla-inhoud aanleiding geven tot een hogere zwelgraad. Hieruit blijkt dat het mogelijk is om de zwelgraad (en dus de vernettingsdichtheid) te controleren door variatie van het gebruikte amine en de Tla-inhoud in het copolymer.

### Tabel II. Zwelgraden van de onderzochte gelen.

<table>
<thead>
<tr>
<th>Gel</th>
<th>Zwelgraad (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDEGA-co-Tla (22% Tla) - Propylamine</td>
<td>300</td>
</tr>
<tr>
<td>PDEGA-co-Tla (22% Tla) - Benzylamine</td>
<td>200</td>
</tr>
<tr>
<td>PDEGA-co-Tla (22% Tla) - Octylamine</td>
<td>150</td>
</tr>
<tr>
<td>PDEGA-co-Tla (22% Tla) - 3-morpholino propylamine</td>
<td>450</td>
</tr>
<tr>
<td>PDEGA-co-Tla (9% Tla) - Benzylamine</td>
<td>1000</td>
</tr>
<tr>
<td>PDEGA-co-Tla (9% Tla) - 3-morpholino propylamine</td>
<td>1150</td>
</tr>
<tr>
<td>PNIPAam-co-Tla (22% Tla) - Propylamine</td>
<td>400</td>
</tr>
<tr>
<td>PNIPAam-co-Tla (22% Tla) - Benzylamine</td>
<td>150</td>
</tr>
<tr>
<td>PNIPAam-co-Tla (22% Tla) - 3-morpholino propylamine</td>
<td>500</td>
</tr>
</tbody>
</table>

Resultaten van de studie op de gesynthetiseerde gelen:

**Temperatuurrepons van de gelen.** Aangezien de copolymeren een hoog aantal aan zijketens bevatten die aanleiding geven tot thermoresponsieve materialen, werden deze materialen gecontroleerd op hun thermoresponsief karakter. Hiervoor werd een stuk gel, ondergedompeld in water, onder de microscoop geplaatst. Met behulp van een zelf gemaakt opwarmingselement kon de gel opgewarmd worden op een gecontroleerde manier. Bij een thermoresponsieve gel zou er krimp moeten optreden bij hogere temperaturen. Figuur 8 toont vier foto’s van twee PDEGA-co-Tla gelen (boven: 9% Tla met 3-morpholino propylamine, onder: 22% Tla met propylamine) bij 33 °C (links) en 46 °C (rechts). Bij beide gelen is er een krimp waarneembaar, wat hun thermoresponsief karakter bewijst. Bij de gelen C en D is het moeilijker zichtbaar, maar aan de hand van de meetbalk is het verschil detecteerbaar.

![Figuur 8](image)

**Degradeerbaarheid van de gelen.** Een van de eigenschappen van een gel, gevormd via disulfidebruggen, is de mogelijkheid om de gel gecontroleerd te degraderen door middel van reducerende stoffen. Om deze eigenschap te testen werd er een gel gevormd uit 20 gewichtsprocent PDEGA-co-Tla met 9% Tla en 5 eq. 3-morpholino propylamine. Deze werd in een 0.8 mM oplossing van tris(2-carboxyethyl)phosphine (TCEP) in water gebracht. Onder de microscoop werd de evolutie van de gel in deze oplossing bekeken. In figuur 9 staan de foto’s van 0, 1, 2 en 3 uur.

![Figuur 9](image)
Figuur 9. Overzicht van de degradeerbaarheid van een PNIPAAm-co-Tla hydrogel met 3-morpholino propylamine met 9% Tla in een 0.8 mM oplossing van TCEP in water.

Op de figuur is een trage verkleining van de gel waar te nemen. Dit betekent dat de gel een degradeerbaar karakter vertoont in een reducerend midden, maar dat de snelheid van degradatie traag is omwille van de trage diffusie van het TCEP in de gel. De gel was na een nacht volledig gedegradeerd en het copolymeer was opgelost in het water.

Samenvatting

Het doel van dit werk is om een responsieve hydrogel te synthetiseren door gebruik te maken van thiolactonchemie. Via deze methode werd de synthese van hydrogelen in een waterig milieu getest. Het responsief karakter werd ingevoerd door gebruik te maken van een molecule die zowel een functionele groep, die aanleiding geeft tot responsiviteit, als een aminefunctie bevat. Deze laatste was dan in staat om aminolyse toe te passen op de thiolactonketen die zich op de zijketen van een copolymer bevond. Dit copolymeer bestond ook uit een monomeer (N-isopropylacrylamide (NIPAAm) of di(ethyleenglycol) acrylaat (DEGA)) dat zijketens heeft die thermoresponsieve eigenschappen bevat. Hierdoor werd de mogelijkheid tot een multi-responsief materiaal geopend.

Aan de hand van kinetiekstudies, deels uitgevoerd in dit artikel, deels in eerdere experimenten in de PCR-groep, konden copolymeren gesynthetiseerd worden met een bepaald percentage aan Tla. Deze copolymeren konden dan omgevormd worden tot gelen. Via 3 modelreacties, die de snelheid van aminolyse, de snelheid van disulfidebrugvorming en de functionalisatiegraad bepaalden, werden optimale condities ontwikkeld voor deze gelvorming: de pH-waarde van de oplossing moest 8 of hoger zijn en het gebruik van chloramine T als oxidans na de aminolyse kon de stabiliteit van de gelen verhogen.

Via deze voorwaarden werden experimenten uitgevoerd om gelen te vormen. Aangezien de gelen onstabiel bleken te zijn in waterig midden, werden ze vervolgens gevormd in organisch solvent. Ook verschillende oxidantia werden getest, waaruit opnieuw blijkt dat chloramine T kan gebruikt worden om na aminolyse het aantal disulfidebindingen nog op te voeren. Vervolgens werd de controle van de zwelgraad door variatie van het gebruikte amine of het percentage Tla in het copolymeer aangetoond. Ten slotte kon men aantonen dat lineaire copolymeren die een morpholinegroep bevatten een respons vertonen tegenover de Cu$^{2+}$ concentratie in het water waarin het copolymer is opgelost.

In het laatste deel werden er met de gevormde gelen testen gedaan. Deze toonden aan dat de vernettingsdichtheid kan gecontroleerd worden door variatie van het gebruikte amine en de hoeveelheid Tla-monomeren. Uit de testen bleek ook dat de copolymeren een thermoresponsief karakter vertonen. Als laatste werd ook het degradeerbaar karakter van de gevormde hydrogelen bewezen.
Bronnen