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Diploma thesis

Hydrogels based on poly(2-oxazoline)s as ultrasound-responsive materials for drug delivery

Carried out at the

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Abstract

Controlled drug delivery systems (DDS) were developed to release a defined amount of drug into the body of the patient with a certain release rate. To release the drug on demand, so-called triggered DDS are used, where a responsive material is triggered by a stimulus. The release in short-term, high-dose bursts takes place whenever the chosen stimulus is applied. External stimuli, such as light, magnetic field or ultrasound (US) can help to release the drug locally as they are applied only at the desired area.

Poly(2-oxazoline)s (POx) are an aspiring polymer class especially in the field of biomedical applications, also as DDS. Their structure is similar to polypeptides, they are biocompatible, some of them are water soluble and additionally it is easy to introduce functional groups. The polymerization of 2-oxazolines by living cationic ring opening mechanism leads to well defined molecular weights, a narrow distribution and the possibility to synthesize block copolymers.

The aim of this work was to develop hydrogels based on POx that can be used as DDS. The release of the respective drug should be triggered *via* US, as this is a cheap and non-invasive way and can be applied in many medical facilities. Therefore, POx backbones with US cleavable crosslinking moieties based on thiols as well as coumarin were considered.

In conclusion, two thiol-containing 2-oxazolines were synthesized using different protecting groups: a methoxybenzyl group and a nitrobenzyl group. Unfortunately, their copolymerization with 2-ethyl-2-oxazoline was not successful.

Coumarin-modified POx were synthesized using two different strategies, (1) modification of polymerized 2-ethyl-2-oxazoline and (2) synthesis and copolymerization of a coumarin-containing 2-oxazoline. Samples resulting from both strategies were crosslinked by irradiation with light with a wavelength above 360 nm, as coumarin moieties are undergoing a [2+2] cycloaddition. Additionally, some samples were crosslinked after addition of poly(ethylene glycol) diacrylate, as this functional group can also cycloadd to coumarin moieties. Despite having a rather low gel content, the obtained hydrogels had a moderate swellability. Furthermore, the degradation study in the ultrasonic bath showed that the coumarin dimers within the hydrogel networks could be cleaved by ultrasonication. Finally, the responsibility to US was confirmed by sonorheology, where a significant loss in the storage modulus was observed during ultrasonication. These results exhibited the potential of coumarin-containing POx as materials as US-triggered DDS.

Kurzfassung

Drug-Delivery-Systeme (DDS) wurden entwickelt, um eine bestimmte Menge eines Arzneimittels mit einer bestimmten Rate an den Körper des Patienten abzugeben. Um den Wirkstoff nur bei Bedarf freizusetzen, werden sogenannte getriggerte Systeme verwendet, bei denen ein spezielles Material auf einen Reiz reagiert. Die Freisetzung in kurzzeitigen, hochdosierten Schüben erfolgt immer dann, wenn der gewählte Stimulus eingesetzt wird. Externe Reize wie Licht, Magnetfeld oder Ultraschall (US) können dazu beitragen, das Medikament lokal freizusetzen, da sie nur an der gewünschten Stelle angewendet werden.

Poly(2-oxazoline) (POx) sind eine aufstrebende Polymerklasse, insbesondere im biomedizinischen Bereich. Ihre Struktur ähnelt Polypeptiden, sind sie biokompatibel, im Falle von kurzen Seitengruppen wasserlöslich und außerdem problemlos mit funktionellen Gruppen modifizierbar. Die Polymerisation von 2-Oxazolinen durch lebende kationische Ringöffnungspolymerisation führt zu gut definierten Molmassen, einer engen Molmassenverteilung und der Möglichkeit zur Synthese von Blockcopolymeren.

Ziel dieser Arbeit war es, Hydrogele auf der Basis von POx zu entwickeln, die als DDS verwendet werden können. Die Freisetzung des jeweiligen Medikaments sollte über US ausgelöst werden, da dies eine kostengünstige und nicht-invasive Methode ist und in vielen medizinischen Einrichtungen angewendet werden kann. Daher wurden POx mit US-spaltbaren Vernetzungseinheiten auf Thiol- und Cumarinbasis in Betracht gezogen.

Zwei thiolhaltige 2-Oxazoline mit verschiedenen Schutzgruppen wurden synthetisiert: einerseits mit einer Methoxybenzylgruppe und andererseits mit einer Nitrobenzylgruppe. Leider war ihre Copolymerisation mit 2-Ethyl-2-oxazolin nicht erfolgreich.

Cumarin-modifizierte POx wurden mittels zwei verschiedener Strategien synthetisiert: (1) Modifizierung von Poly(2-ethyl-2-oxazolin) und (2) Synthese und Copolymerisation eines Cumarin-haltigen 2-Oxazolins. Die aus beiden Strategien resultierenden Proben wurden durch Bestrahlung vernetzt, da die Cumarin-Einheiten eine [2+2]-Cycloaddition durchlaufen. Zusätzlich wurden einige Proben nach Zugabe von Poly(ethylenglykol)diacrylat vernetzt, da diese funktionelle Gruppe ebenfalls an Cumarin cycloaddiert werden kann. Trotz eines eher geringen Gelgehalts wiesen die erhaltenen Hydrogele eine mäßige Quellfähigkeit auf. Darüber hinaus zeigte die Abbaustudie im Ultraschallbad, dass die Cumarin-Dimere in den Hydrogel-Netzwerken durch US gespalten werden konnten. Anschließend wurde die Reaktion auf US durch Sonorheologie bestätigt, wobei ein signifikanter Verlust des Speichermoduls während der Ultraschallbehandlung beobachtet wurde. Diese Ergebnisse bestätigen den potentiellen Einsatz von Cumarin-haltigen POx als Materialien für US-getriggerte DDS.

Table of contents

Introduction

1 Controlled drug delivery systems

Pharmacotherapy refers to the use of drugs to achieve a specific effect on the body to support the treatment of a medical issue.[1] The duration of the treatment and the concentration of the drug are important to reach the desired therapeutic outcome, while keeping the side effects during and after the treatment minimal. In conventional drug delivery, the release rate of a drug follows first order kinetics. That type of drug delivery limits the duration of a treatment because the entire dose of drug is released immediately into the bloodstream. In addition, the concentration of the drug changes over time, which leads to an uneven therapeutic effect and undesired side effects. These two features are the main disadvantages for patients who need multiple dosing throughout the day.[2]

The solution for this problem can be controlled drug delivery. This technique can control the rate of drug release, sustain the duration of therapeutic activity and/or target the delivery of a drug to a tissue.[3]

There are two main types of the controlled drug release: Sustained release means a delayed and/or prolonged release of the drug.[4] The goal is to reach a stable concentration of the drug in the blood, what ensures a constant rate of drug release over a longer period. The drug is released into the bloodstream over a certain amount of time with constant rate. This includes the advantages of a lower dosing frequency because of the longer therapeutic duration and increased patient safety because of the constant amount of released drug.[2] For a sustained release, it is necessary to have some sort of drug depot system, such as hydrogels, liposomes, micelles[1] and semi-solid drug depots.[5]

In some cases, the release of the drug has to be turned off on demand, for instance for diabetes. Li *et al.*[6] investigated a drug delivery system (DDS) sensitive to a change in the pH. An increase of glucose concentration in the blood of the patient leads to decrease of pH and triggers the hydrogel to release the encapsulated insulin. When enough insulin is released, the pH increases, and the drug release is stopped.

These systems are so-called triggered DDS, drugs can be released in short-term, high-dose bursts (visualized in Figure 1 compared with conventional and sustained release).[7] In that case, stimuli-responsive materials act as drug carriers. Besides the flexibility of these triggered DDS, also the specific needs of the patient can be easier considered, and the risk of an overdose is much lower. In some cases, the patients can even regulate the drug release themself, what is especially beneficial *e.g.* for pain killers.[8]

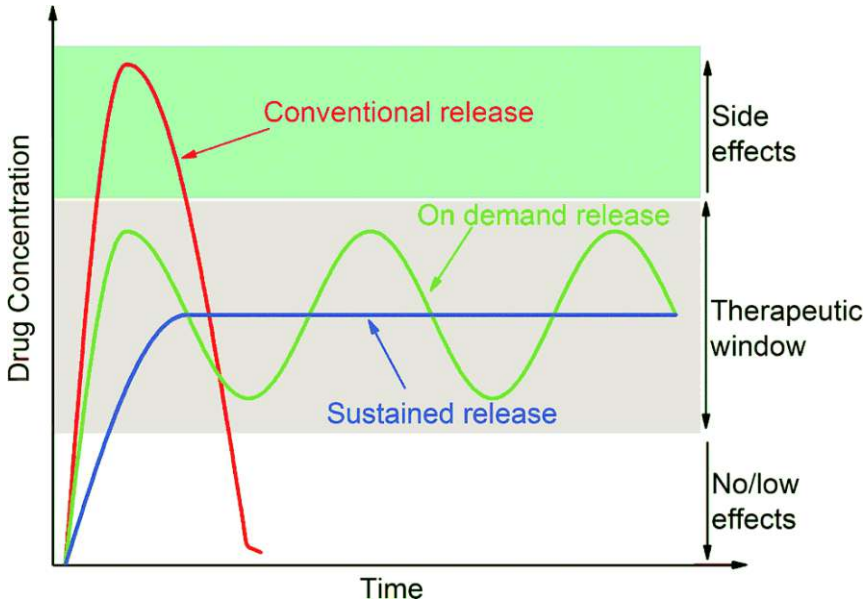


Figure 1: Comparison of the drug concentration after conventional release, sustained release and on demand release[9]

Stimuli such as pH change, reductive or oxidative state, or enzymatic activities are internal stimuli, since they rely on the change of internal environment in the body. DDS that are triggered by internal stimuli to control drug release nevertheless can bear disadvantages. The local environment of the target site where the drug is meant to be released can differ for each patient. In addition, the release kinetics can vary for the same reason and cannot be precisely predicted. External stimuli can overcome these problems.[10]

External stimuli, such as light, magnetic field, heat or ultrasound, also known as physical stimuli, can help to release the drug locally, as displayed in Figure 2.[11] These stimuli are applied externally to the body. Heat can be used as stimulus for thermoresponsive polymers, due to the chemical and/or physical change of their properties. For instance, the temperature in tumour tissue is known to be higher (40 – 42 °C) compared to healthy human tissue (37 °C), what could trigger the release locally.[12] For the release triggered by light, UV light[13] can be used as well as NIR light[14] and can be either reversible or irreversible, depending on the release mechanism. The biggest disadvantage of light triggered DDS is the low penetration depth.

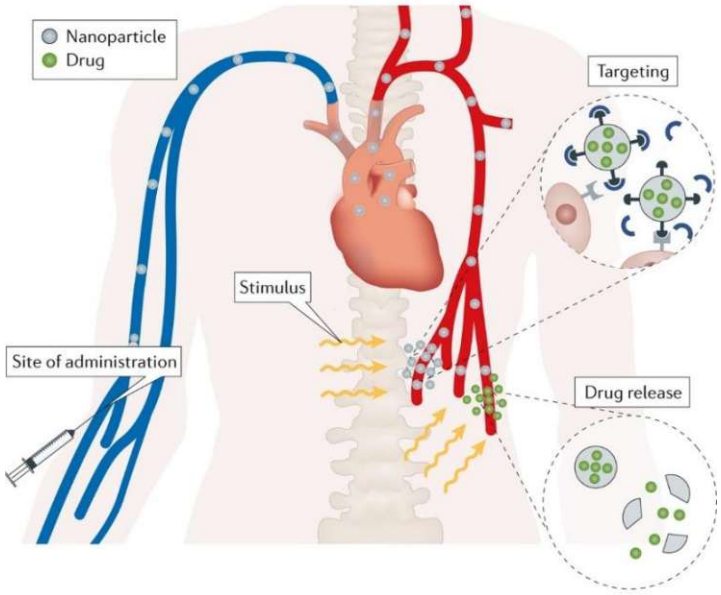


Figure 2: Drug release caused by a stimulus at the side of administration[10]

Ultrasound (US) as external stimulus combines advantages from other stimuli without their disadvantages, such as the deep penetration depth into soft tissues, the availability all over the world, the non-invasiveness as well as the low costs per application.

2 Ultrasound

Ultrasound (US) is a sound wave with a frequency above 20 kHz, that is above the audible limit of humans. In medicine it is mostly known as imaging modality (sonography), for instance to detect kidney stones, and as physiotherapeutic tool for muscles and ligaments.[10] The use of US as stimulus for DDS was first reported in 1989 and is steadily growing.[10, 15] The advantages of triggering the drug release by US are mainly the non-invasiveness and the deep penetration into soft tissues. High-intensity US can also achieve a therapeutic effect without using drug carriers, because it can induce the drug uptake by cells by affecting the membrane transport. It should be noted, however, that for all these applications, the parameters of US have to be carefully controlled, to avoid the side effects. The side effects range from skin irritations to transient pain and even nerve injury. To minimize these side effects, the intensity and the frequency of the treatment should be selected very carefully.[16] The frequency range for US in medical applications is limited up to approximately 15 MHz.[17] The limit for the intensity of diagnostic US is located at 720 mW/cm².[18] As this range is available in medical facilities, it is also desirable to use US in this range as trigger for the drug release from DDS.

US affects the materials by two distinct mechanisms: *via* the thermal and *via* the mechanical effect. US-triggered DDS can respond to only one or also both of these effects.

US stimulates cells, tissues and other medium to oscillate with the same frequency and amplitude as the waves themselves. These pressure oscillations are one part of the **mechanical effects**. Another important process inducing mechanical effects for drug delivery US is the cavitation of bubbles. In this process, gaseous cavities, i.e. bubbles grow, oscillate and eventually burst. When the bubble grows and bursts, it is called inertial cavitation (Figure 3, b and c), if it oscillates and grows in size, it is called stable cavitation (Figure 3, a).[19]

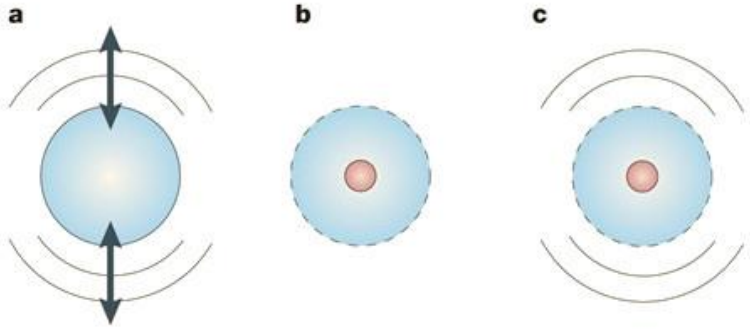


Figure 3: Mechanical effect of ultrasound on gaseous cavities: a) oscillation, b) sudden collapse causing high temperatures in the core, c) sudden collapse due to shock waves, inducing a rising temperature and interference with tissue[19]

The **thermal effects** are caused by the absorption of US waves by the medium. Due to this absorption the temperature rises, known as local hyperthermia, depending on the absorption coefficient of the

material. This increase of temperature can be further used for the drug release. As an example, liposomes can be triggered by the thermal effect of US by increasing their permeability (Figure 4).[20]

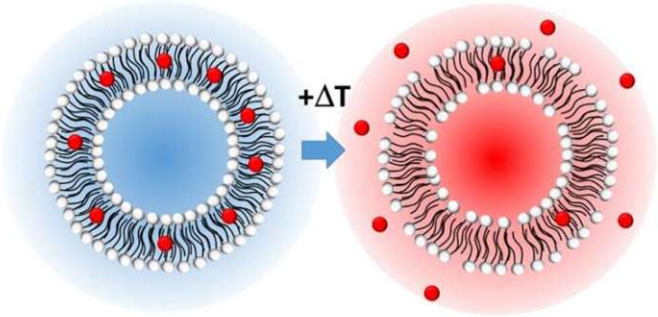


Figure 4: Thermal effect of US on a DDS based on liposomes, resulting in an increase of the temperature inside the liposome and subsequent release of the cargo[21]

These two effects, the mechanical and the thermal, are also able to degrade polymer-based systems, which release encapsulated drugs as a result.[7] First reported in 1989, Kost *et al.*[15] studied the US-enhanced degradation of biodegradable polyanhydrides, polyglycolides, and polylactides. Many researchers were inspired by these first investigations to develop ultrasound-responsive DDS. However, to reach this goal, also the selection of the proper polymer backbone is crucial.

3 Poly(2-oxazoline)s

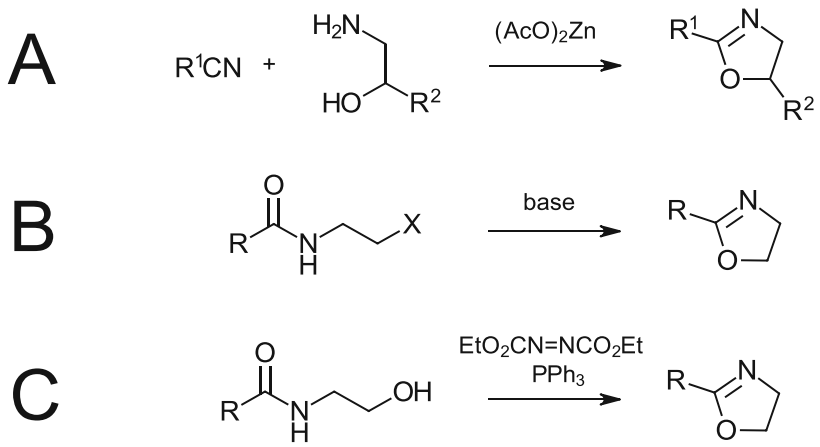
The polymers used for biomedical and pharmaceutical applications must fulfil strict requirements, such as biocompatibility, controllable properties and low batch-to-batch differences. Traditionally, a material of choice for these biomedical applications is poly(ethylene glycol) (PEG), as it is biocompatible, and available as well-defined polymer because of its synthesis *via* living anionic polymerization. In this field, PEG is considered to be the "gold standard",[22] although there are several disadvantages.[23] The PEG backbone can be degraded by oxidative processes what results in toxic by-products. The wide use of PEG, known as PEGylation, for instance in the cosmetic industry, is responsible for the allergic reaction of a non-negligible number of people.[24]

To overcome these drawbacks, numerous polymer classes have been and are still studied for biomedical applications, including poly(2-oxazoline)s (POx). They are structural isomers of polypeptides, and due to many *in vitro* and *in vivo* tests they seem to be biocompatible, what makes them promising for this application field. An advantage compared to PEG is the relatively little commercial use so there are no allergies against POx reported. Numerous research groups are focusing on study POx as drug or gene carriers, protein conjugates, hydrogels for tissue engineering. In addition to this academic interest, POx-drug conjugates have already being tested for use in human medicine. The first in-human study has started in 2015[25] using SER-214, a POx conjugate of rotigotine, as a treatment against the Parkinson's disease. Even if the study is not concluded yet, the proof of concept of Moreadith *et al.*[26] looks promising.

Several strategies to synthesize a 2-oxazoline monomer with a desired functional group were developed. Three of the most common routes are displayed in Scheme 1. Different 2-oxazoline monomers have been synthesized involving an appropriate nitrile and 2-aminoethanol (Scheme 1, A).[27] This synthesis route was first investigated in 1974 by Witte and Seeliger using a Lewis acid catalyst.[28] Nowadays, mostly zinc acetate is used as catalyst.

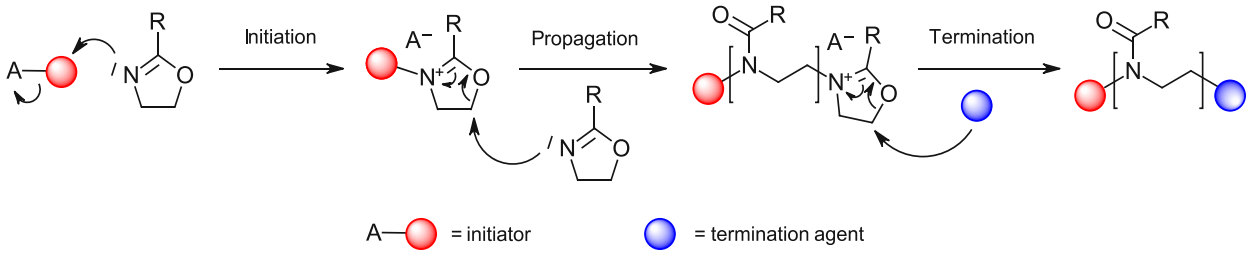
Strategy B (Scheme 1) shows the cyclization of haloamides,[29] where the 2-oxazoline ring is formed starting from N-(2-haloethyl)amides and a base, e.g., KOH or K_2CO_3. The type of selected base, depends on the substituent R. A similar route is the ring formation using a hydroxyamide (Scheme 1, C).[30] Thereby, diethyl azodicarboxylate and triphenylphosphine are used for the dehydration under mild conditions.

For both of these strategies, an amide has to be synthesized previously. Typically, the corresponding acid is used as starting material. One method, already reported in 1952,[31] is to add an acyl chloride to receive mixed anhydride. These can be converted into amides by a reaction with the desired amine. Another, nowadays more popular method is also a two step-strategy, where the acid is converted with NHS and subsequently with the corresponding amine.[32]



Scheme 1: Three common synthesis routes of 2-oxazolines, containing the reaction between 2-aminoethanol and a corresponding nitrile (A), the ring closing of a haloamide (B) and the ring formation via dehydration of a hydroxyamide (C)

POx have been synthesized successfully since 1966[33, 34, 35] *via* cationic ring-opening polymerization (CROP). If performed under appropriate conditions, this polymerization exhibits living character, what is reflected in the well-controlled properties of the polymer. The living polymerization is a type of chain growth polymerization where no termination occurs during the propagation. The advantages of this polymerization are a narrow molar mass dispersity, a well-defined molar mass and the possibility of end group modification. In addition, another advantage is the easy introduction of various functional groups into the POx structure.



Scheme 2: Living cationic ring opening polymerization (CROP) of 2-oxazolines divided into the parts initiation, propagation and termination

Scheme 2 shows the reaction mechanism of the polymerization of 2-oxazolines, divided into three steps. The first step of this polymerization mechanism is the initiation, what is caused by strong electrophiles, such as Lewis acids, strong protic acids and alkyl halides.[36] Typically used initiators are methyl p-toluenesulfonate (methyl tosylate, MeOTs), methyl 4-nitrobenzenesulfonate (methyl nosylate, MeONs) and methyl trifluoromethanesulfonate (methyl triflate, MeOTf), as depicted in Figure 5. All three of them are commercially available and provide a very fast initiation reaction.

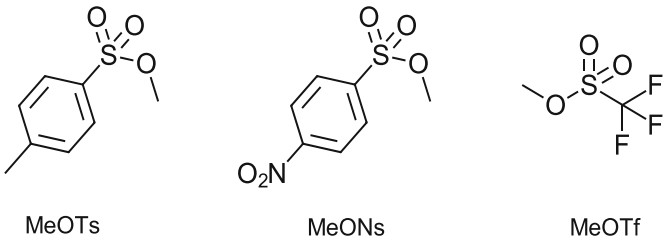


Figure 5: Commonly used initiators for CROP of 2-oxazolines

The counterion influences the propagation rate by with its nucleophilicity. The less nucleophile the counterion is, the faster is the polymerization, in case of the three mentioned initiators the order is MeOTs < MeONs < MeOTf.[37]

The initiation reaction has to be significantly faster than the propagation in order to receive an even growth of each polymer chain. If the polymer chains start to grow at the same time, the propagation can occur for the same duration for all the chains until either no monomer is left or termination takes place. This means that the polymer chains theoretically have the same length as each polymer chain reacted with the same number of monomers. If the initiation is not fast enough, some polymer chains are initiated while others are already growing, what leads to a broad molar mass distribution.

The following second step is the chain growth, known as propagation (Scheme 2, Propagation). The ring of newly formed 2-oxazolinium species is opened by a nucleophilic attack of another monomer molecule. This step is repeated until all the monomer molecules are consumed or a termination reaction is induced. The rate of the propagation depends among others on the side group of the 2-oxazoline. Beside the effect of the side group, the polymerization rate is affected also by the used solvent and the reaction temperature.

The increase of the temperature accelerates the reaction rate of the propagation, what makes the polymerization economically useful.[38] The boiling point of the solvent limits this reaction rate, what can lead to the undesirable effect of long reaction times. To overcome this limitation, microwave reactors[38, 39] enabled to use higher temperatures.

Unlike other polymerization mechanisms, there are no significant chain transfer reactions during CROP. To prevent unintentional termination of the polymer chains, it is necessary to exclude nucleophiles, such as water. Water can be easily introduced during the polymerization reaction from the air as moisture. Therefore, CROP of 2-oxazolines is performed without exception under inert atmosphere.

Under appropriate conditions, the termination reaction does not occur until a nucleophile is added intentionally to the polymerization mixture (Scheme 2, Termination). Depending on the added nucleophile, functional groups can be added onto the end of the polymer chain. The termination agent

opens the ring of the last attached monomer, but pairs with the cation so that there is no charge anymore.

One of the main characteristics of a living polymerization is the first-order polymerization kinetics.[35] Due to the fast initiation and the absence of termination reactions during the propagation, the number of the growing cationic species remains constant. The logarithmic monomer concentration is plotted as a function of time, where a linear trend is obtained (Figure 6). Plotting $\bar{M}_n$ vs. conversion, there is also a linear trend visible for living polymerizations. In contrast, this plot shows that no chain transfer or coupling reactions take place. The living character is also responsible for the narrow dispersity Đ, typically below 1.20 for living polymerizations. This includes the control of the molar mass *via* the ratio of monomer to initiator.

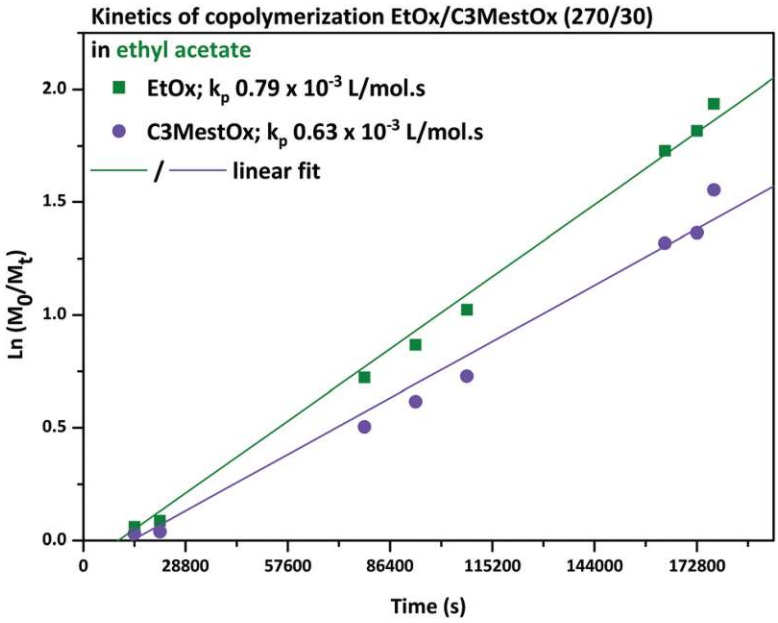


Figure 6: First-order kinetics of the polymerization of 2-oxazolines via CROP with living character on the example of 2-ethyl-2-oxazoline (EtOx, green squares) and 2-methoxycarbonylpropyl-2-oxazoline (C3MestOx, blue spheres) at 60 °C in ethyl acetate (4.8 M)[40]

In the last 50 years, effects of various monomers[41] and solvents[40] on the polymerization kinetics have been investigated. Wiesbrock *et al.*[41] investigated that 2-alkyl-2-oxazolines are polymerizing at a similar rate, whereas 2-aryl-2-oxazolines show a significantly slower rate (Figure 7). Aromatic 2-oxazoline monomers, such as 2-phenyl-2-oxazoline, are polymerizing significantly lower than aliphatic 2-oxazolines, like 2-ethyl-2-oxazoline (EtOx), due to the stabilizing +M effect of the propagating species. Additionally, also spherical hindrance can influence the polymerization rate.

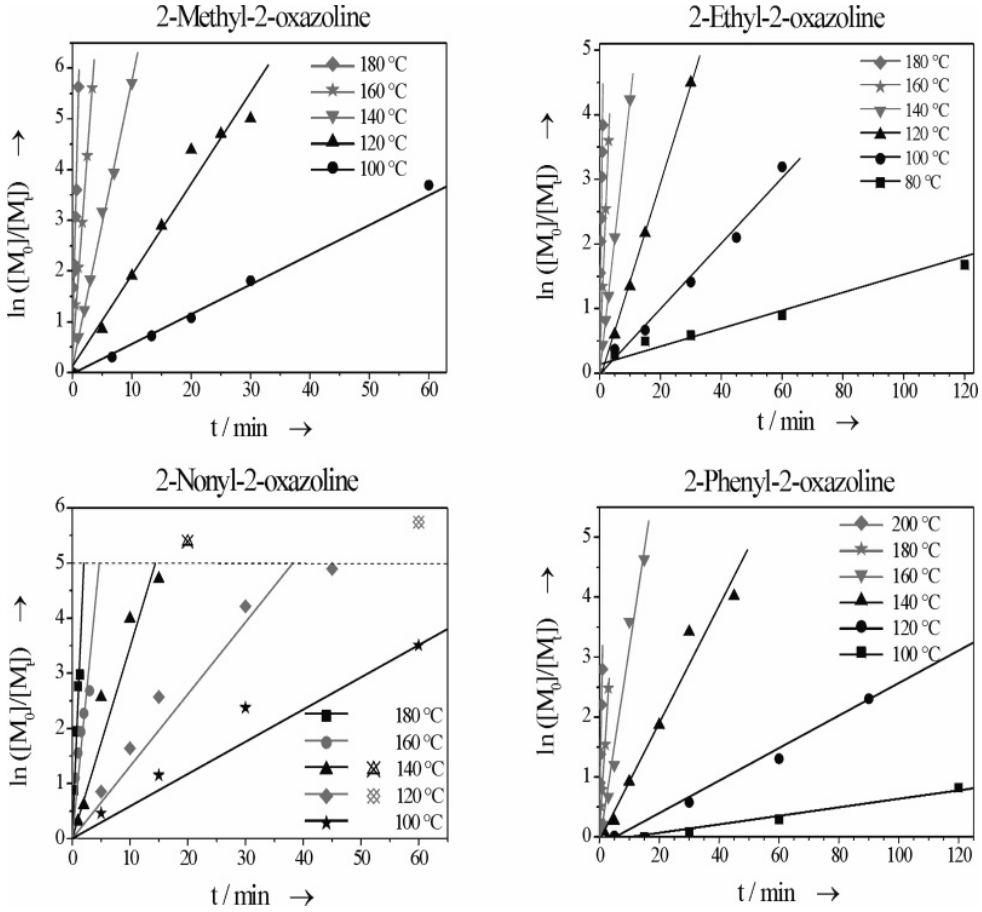


Figure 7: Comparison of the polymerization rates at various temperatures (80 – 200 °C) of 2-methyl-2-oxazoline, 2-ethyl-2-oxazoline, 2-nonyl-2-oxazoline and 2-phenyl-2-oxazoline in acetonitrilewith MeOTs as initiator ([M]:[I] = 60)

Currently acetonitrile, chlorobenzene and benzonitrile are used as solvents for polymerizing 2-oxazolines.[40] They provide good solubility and even more importantly, stabilize the cation of the propagating species.[42] They are influencing the propagation rate with their boiling point as limiting factor. As already mentioned, microwave assisted polymerization is used to avoid this limitation. Recently, also ethyl acetate was proposed as new green solvent that can be used for CROP of 2-oxazolines.[40]

Besides the homopolymerization, also the copolymerization of different 2-oxazoline monomers follows CROP with a living character. Usually, the copolymerization of 2-oxazolines proceeds close to "ideal copolymerization", which means that the resulting polymer chain structure does not depend on the living chain end, only on the reactivity of monomer.[43] Depending on the reactivity of the used monomers, there are two main structures that can be achieved by one-pot copolymerization. If the reactivity of the used monomers is similar, they are incorporated equally into the polymer chain, forming statistical copolymers. Vice versa, when the reactivity of the monomers is significantly different, the product will have the structure of gradient copolymers (Figure 8, A and C).[44]

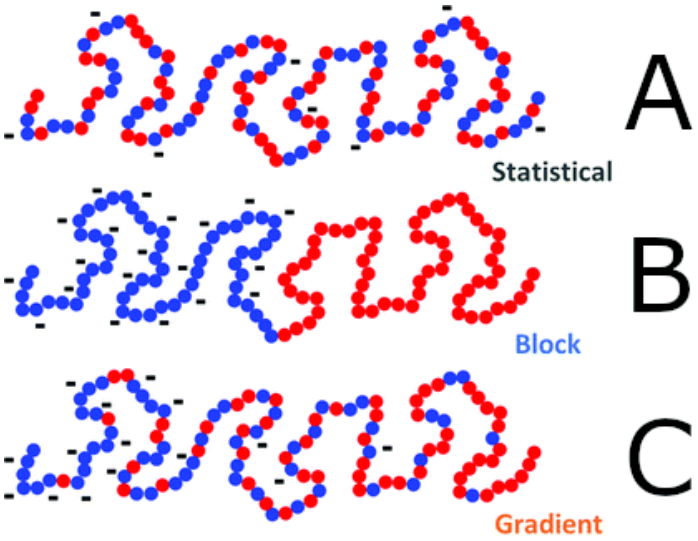


Figure 8: Depiction of the composition of statistical (A), block (B) and gradient (C) copolymers[45]

Another possible structure that is formed *via* copolymerization of different 2-oxazolines is a block copolymer (Figure 8, B). The block copolymer is prepared by polymerizing of one monomer type until full conversion and subsequent addition of the second monomer. However, this is only possible if the living character of the polymerization is maintained. Depending on the desired properties, either di/triblock copolymers or various other structures can be synthesized.

(Co)poly(2-oxazoline)s have different properties depending on the type of monomers, their amount and their arrangement. Especially, the substituent in the position 2 of the ring affects the polarity and hydrophilicity of resulting polymers. Poly(2-Methyl-2-oxazoline) (PMeOx) is well soluble in water, poly(2-ethyl-2-oxazoline) (PEtOx) is also still soluble, but shows first thermoresponsive properties.

Another interesting property of some POx is their thermoresponsibility, exhibited not only by block copolymers. Mostly, the thermoresponsibility is represented by the lower critical solution temperature (LCST), where a POx in aqueous solution becomes turbid using a certain concentration and chain length when the temperature is increased.[46] This is known especially for (co)polymers with medium-sized 2-oxazoline side chains, i.e. with two or three carbon atoms. For example, EtOx and 2-isopropyl-2-oxazoline are soluble at lower temperature but result in a turbid solution at 61 – 69 °C (EtOx)[47] respectively 26 – 34 °C (2-isopropyl-2-oxazoline),[48] known as cloud point. This cloud point depends on the concentration of the polymer in solution, on the chain length and on the architecture of the polymer.[49]

PMeOx does not show a LCST in water as it is too hydrophilic, and poly(2-butyl-2-oxazoline) in contrast is too hydrophobic so it is not soluble even at lower temperatures. These hydrophobic 2-oxazolines are used for instance as hydrophobic part in amphiphilic block or gradient copolymers.[50]

Amphiphilic block and gradient copolymers self-assemble into various structures in the solvents selective to one block, as displayed in Figure 9. As they consist of a hydrophilic and a hydrophobic part, the polymer chains are arranging themselves in solvents. Thereby, the part of the polymer that is soluble in the used solvent, *e.g.* the hydrophilic part in water, will have as much contact with the solvent as possible. The other part of the polymer, *e.g.* the hydrophobic part in water, will be repelled from this hydrophilic part so the contact with the solvent is as small as possible. A very well-known example for a self-assembled structure are micelles.

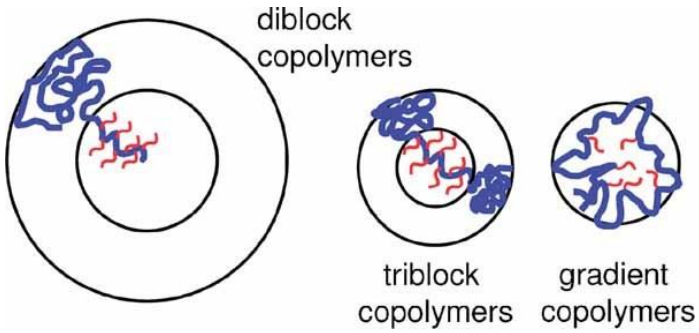


Figure 9: Self-assembly of di-, triblock and gradient copolymers[51]

Thus, amphiphilic POx are also used as surfactants, as investigated already in 1986 by Kobayashi *et al.*[52] After discovery of amphiphilic POx, many research groups studied their properties, such as critical micelle concentration, surface tension and stabilization of emulsions. Gradient copolymers behave similar in solutions,[51] except that the diameter and the shape of the micelles is different compared to the block copolymers of a same composition. This can be explained by the different self-assembly shown in Figure 9. The used solvents affect the diameter and the shape, as well as the critical micellar concentration (CMC) due to the solubility of the polymer.[53]

Typically, POx micelles can be used as drug delivery system (DDS) especially for hydrophobic drugs, as they can be encapsulated in the micelles. Micelles have a spherical shape with a diameter between 10 and 100 nm, what is one of the smallest types of DDS. The amount of encapsulated drug depends on the chemical composition of polymer and drug. Therefore, for each drug various systems have to be investigated to reach the maximum performance of the DDS.[54]

As an alternative to micellar DDS, drug loaded hydrogels can be used whenever a local drug release is needed. Compared to micelles, the hydrogel can be placed directly near the target site and does not distribute in the whole body. For *in vivo* studies, the hydrogels have to be reproducible in structure, crosslinking degree and functionalization to be considered as DDS.

Crosslinked polymer chains are considered to be a gel if a solid, interconnected matrix is swollen in a solvent. For hydrogels, the liquid phase has to be water, thus hydrophilic polymers are used for crosslinking.[55]

In general, two main types of hydrogels are recognized according to crosslinking method. One type is known as physical or non-covalent crosslinking. Non-covalent crosslinking includes hydrogen bonding, hydrophobic interactions, metal complexation or ionic interactions.[56] The second type are chemically crosslinked gels, which are crosslinked *via* covalent bonds.

As an example of physically crosslinked hydrogels, certain block POx show so-called thermogelation.[57] In certain concentrations, chain lengths, compositions and temperatures, the copolymers form gels with significantly higher viscosity than before the transition, i.e. sol-to-gel transition. This transition is usually caused by the decrease of the temperature, but also some systems gelling upon the increase of the temperature were reported. This is known as "inverse gelation".[58] A popular example for thermogelation is the ABA triblock copolymer Pluronic®F127, consisting of poly(ethylene glycol) (PEG) as A blocks and poly(propylene glycol) (PPG) as B block. Lübtow *et al.*[59] reported a similar ABA triblock copolymer based on 2-oxazoline, where poly(2-methyl-2-oxazoline) (PMeOx) were used as hydrophilic A block and poly(2-isobutyl-2-oxazoline) as hydrophobic B block.

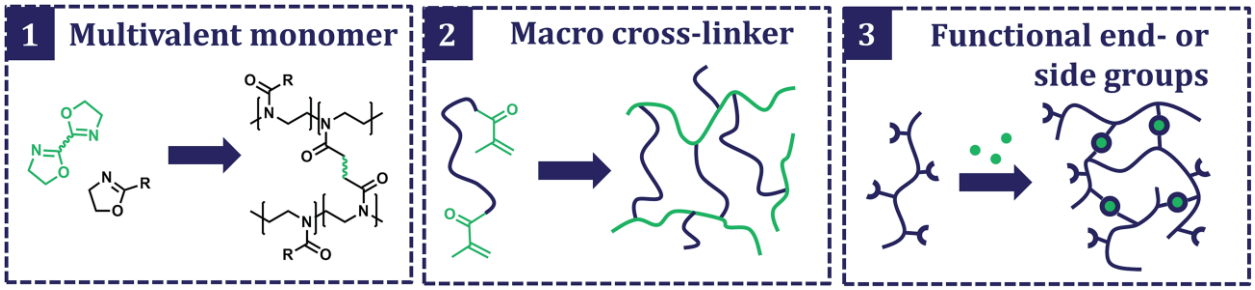


Figure 10: Different strategies to chemically crosslink POx chains containing bis-functional monomers (1), macromolecular monomers (2) and crosslinking via functional groups (3)[60]

Chemically crosslinked gels based on POx were already reported, they can be prepared by one of three different strategies (Figure 10). The most common strategy to receive chemically crosslinked POx is the use of bis-functional monomers, first reported in 1989 by Saegusa *et al.* (Figure 10, 1).[61] The second strategy is to use macro-monomers with functional groups, mostly α- and/or ω-functionalized POx, that are crosslinked in a second polymerization reaction (Figure 10, 2). A common example are POx chains with acrylate end groups that can be crosslinked by a free radical polymerization.[62] The third way to crosslink POx is *via* functional groups, either by end- or side group functionalization (Figure 10, 3). The difference to the second strategy is the particular functional group, as also non-polymerizable functionalities can be used, such as secondary amines crosslinked with bis-functional isocyanides.[63] A nowadays important method of crosslinking *via* functional groups are click reactions, firstly reported for POx by Luxenhofer *et al.*[64] by using azide and alkyne functionalities. Thiol-ene click reaction was also employed for crosslinking of POx with alkene moieties in the side chains and various thiol compounds.[65] Even if POx with thiol moieties have been synthesized in 2007,[66] they have been used in thiol-ene reactions for the first time in 2020 by Jung *et al.*[32]

Objective

The traditional drug delivery systems (DDS) suffer from several drawbacks, namely the immediate release of the drug, the very limited duration of the treatment, the change of the drug concentration during the treatment and the distribution in the whole body.

To address these drawbacks and deliver a controlled amount of drug to a desired location, externally triggered drug delivery systems based on stimuli-responsive hydrogels can be used. One of the external stimuli that can be used is ultrasound (US). This is a non-invasive method with a high penetration depth compared to light for example. US is available in many medical facilities and frequently used due to the low coasts of the treatment.

This thesis aims to extend the library of hydrogel materials suitable for US-triggered drug delivery. To this aim, the proper selection of biocompatible polymer backbone is of huge importance. For DDS and other biomedical applications, poly(ethylene glycol) (PEG) is typically used. However due to the widespread use, this polymer can cause allergies and even immunogenicity due to formation of antibodies, altering thus the function of the DDS. These medical risks can be avoided by using different polymer classes.

A favourable choice to substitute PEG would be poly(2-oxazoline)s (POx). This polymer class in known to be biocompatible due to the polypeptide-like structure. POx with shorter pendant groups, such as poly(2-methyl-oxazoline) (PMeOx) or poly(2-ethyl-2-oxazoline) (PEtOx) are water soluble. Additionally, the synthesized polymer chains show a defined molar mass with a narrow dispersity due to the living character of the cationic ring opening polymerization.

To synthesize US-responsive hydrogels, mechanolabile bonds should be used as crosslinkers of the polymer chains. Considering the application as drug delivery system, also these parts should be biocompatible. Based on literature search of suitable US-responsive moieties, thiols and coumarins were selected for this work.

The first functional group that should be introduced into POx chains are thiols. The material should be synthesized according to Cesana *et al.*[66] by using a thiol-containing monomer. The polymer should be crosslinked *via* redox reactions with disulfide bridges that can be found also in peptides and are therefore biocompatible.

The second group for US-responsive crosslinkers should be coumarin moieties. Coumarin is a biomolecule that naturally occurs in tonka beans, used in food industry and is therefore biocompatible in small doses. The only known example of POx with coumarin moieties in literature was synthesized

by Chujo *et al.*[67] If irradiated, coumarin dimers should be formed that can crosslink the POx chains to receive hydrogels.

A successfully modified polymer should be crosslinked under appropriate conditions and the swelling properties should be studied. The crosslinking process should be optimized to find suitable parameters, such as polymer concentration and solvent for an appropriate formulation. Additionally, crosslinking parameters for polymers with different percentages of functional groups should be compared.

Finally, the ability of prepared materials to respond to US in different set-ups should be studied to assess their potential as drug delivery systems. Therefore, two different methods should be used: for a proof of concept a degradation study should be carried out using an ultrasonic bath. The second method should include a sonorheometer to finally investigate the materials properties under medically justifiable conditions.

State of the art

1 Materials for US-triggered drug delivery systems

Different types of drug carriers that are mostly used as ultrasound (US) triggered DDS are listed in Table 1. Depending on their size and constitution, each of this drug carrier type has its own advantages and disadvantages.

Table 1: Different types of drug carriers used as US-triggered DDS, their size and appearance

Type	Size (diameter)	Appearance
Microbubble[11, 21, 68]	1 – 10 µm	Gas-filled sphere, drug encapsulated in shell
Liposome[11, 21, 68]	100 – 200 nm	Lipoid mono- or bilayer with drug-filled core
Nanoparticle[8, 68]	1 nm – 1 µm	consist of solid, liquid or gaseous compounds, do not contain necessarily gas
Micelle[21]	20 – 50 nm	Spherical, hydrophilic corona and hydrophobic core, made from amphiphilic materials

Microbubbles are gas-filled spheres that can interact with US. The stabilizing shell can encapsulate drugs to apply the system as drug delivery systems (DDS).[21] At certain frequencies the microbubble oscillates and at lower frequencies it even bursts, caused by the mechanical effect of US. When they collapse upon the US trigger, they release the drug. The size of the microbubbles (1-10 µm) limits their applicability in the body. Eisenbrey *et al.*[69] developed a microbubble based DDS made of polylactic acid (PLA), that encapsulated an air bubble inside of 2 µm big bubbles and the drug doxorubicin inside the polymeric shell. After ultrasonication with a resonance frequency of 5 MHz, the microbubbles were destroyed in fragments smaller than 400 µm. The *in vivo* experiments showed a significantly lower doxorubicin concentration in the blood, but also significantly less tumour growth compared to a treatment using doxorubicin directly, without DDS.

With PEGylation of PLA-based microbubbles, it is possible to balance circulation time and acoustic activity. Therefore, ligands are used to modify the microbubble, additionally they are helping to direct it to the target site. Two strategies were investigated by Jablonowski *et al.*[70] for such microbubble modifications, the addition of PEG-PLA copolymers and the incorporation of a PEG lipid. The study of this research group investigates the consequences of modifying of the polymeric shell. The modified microbubbles were treated with US (5 MHz, pulse length 1 µs), showing that there is a border of appliable PEG amount (5 wt% PEG-PLA copolymer, 1 wt% PEG lipid) that can be inserted while maintaining the balance between favorable acoustic properties and immune shielding.

Liposomes are an order of magnitude smaller (approx. 100 – 200 nm) and therefore can penetrate to smaller blood vessels. A lipid mono- or bilayer covers a core, typically a drug-filled core for drug

delivery.[68] The mechanism of the cleavage is mostly the thermal effect of the US, therefore often thermoresponsive materials are used.[10]

Combining liposomes with polymers with a lower critical solution temperature (LCST), the polymer switches from hydrated state with an increase of temperature to aggregated. Grafted onto lipids, the polymer destabilizes the lipid bilayer when insoluble and causes the drug release.[71]

As the tumor tissue is less dense during ultrasonication, some cases showed a higher intratumoral concentration of the applied drug using non-thermoresponsive liposomes with US as triggering stimulus.[72] Some other studies showed no difference by combining the liposomes with US, probably also due to the lower US intensity.[73] So-called sonosensitive liposomes were investigated by Evjen *et al.*[74] consisting of 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine.

Nanoparticles (1 nm to 1 µm) can consist of solid, liquid or gaseous compounds and are small enough to penetrate small capillaries and accumulate into solid tumours. Also combinations of different states of matter are possible, such as in the work of Unger *et al.*,[75] where the authors investigated a DDS composed of a mix of liposome and microbubble, called liposphere. They do not necessarily have a spherical shape, also angular shapes are possible. In comparison to microbubbles, which remain only for minutes in the blood stream, nanoparticles can circulate for several days in the body.

Many nanoparticle types do not contain gas, so they are theoretically transparent to ultrasonication as a stimulus. Practically, most of the prepared DDS contain small amounts of dissolved gas and become therefore acoustically active. If there is additionally to the drug also a gaseous core encapsulated, the nanoparticles are also called nanobubbles. Using perfluoropentane as gas, the nanobubbles can be used to increase the contrast of *e.g.* cancer cells during ultrasonic imaging and simultaneously treat them by releasing the drug. Nowadays, perfluoropentane is mostly replaced by perfluoro-15-crown-5-ether due to its higher boiling point and therefore higher storage stability of the whole system.[76]

Micelles are spherical core-shell structures containing a hydrophilic corona and hydrophobic core. They consist of amphiphilic materials that are dissolved in water, usually amphiphilic block copolymers or ionic surfactants. Their size is in the nano-range (20 – 50 nm), therefore similar to nanoparticles. The size of micelles compared to liposomes and microbubbles is shown in Figure 11. The advantage of using polymeric micelles instead of small surfactant molecules are slower dissolution rates, larger volumes and the possibility of adjusting the hydrophobic and hydrophilic properties. Du *et al.*[77] investigated micelles composed of poly(lactic-co-glycolic acid), poly(ethylene glycol) and poly(L-lysine) (PLGA-PEG-PLL) that encapsulate doxorubicin as well as perfluoropentane as gaseous core, similar to

nanobubbles. The release of the drug was studies *in vitro* at an ultrasonic frequency of 1 MHz and a duty cycle of 50 %.

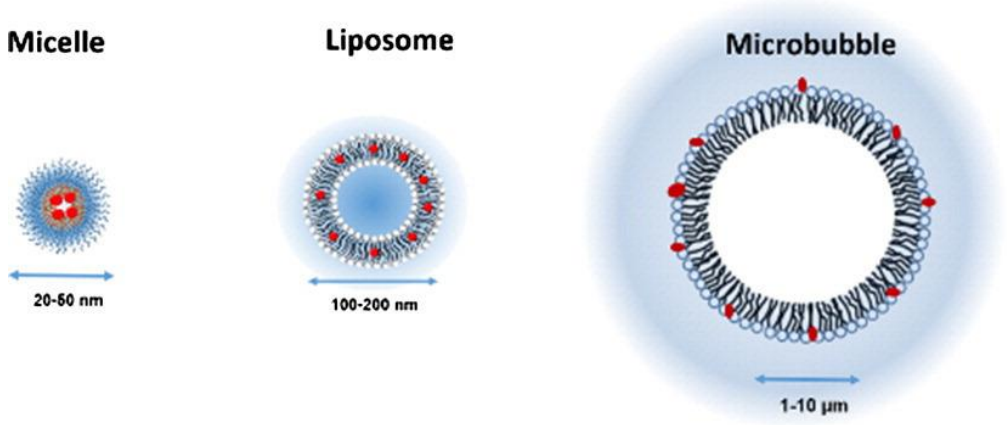


Figure 11: Comparison of the size of microbubbles, liposomes and micelles[21]

Sometimes, microbubbles are combined with nanobubbles, such as in the work of Gao *et al*.[78], using Pluronic® P-105 micelles (PEG-PPG-PEG) or Pluronic® P-105 with PEG-diacylphospholipid in a ratio 1:1. PEG acts as hydrophilic part whereas PPG is the hydrophobic part in the core of the micelle. The hydrophobic drug was released during *in vivo* experiments using 1 or 3 MHz ultrasound locally on the tumour site.

Especially Pluronic® is used very frequently for micellar DDS. As already mentioned in context with nanoparticles, also micelles are fairly transparent to US, only dissolved gas makes the interaction possible and triggers cavitation. Apart from the mechanical effect, also the thermal effect of US can be used by employing thermoresponsive polymers. Chung *et al.*[79] investigated thermoresponsive block copolymers made of poly(N-isopropylacrylamide-b-butylmethacrylate) (PIPAAm-PBMA). The micelles are formed by self-assembly of the hydrophobic PBMA and the hydrophilic PIPAAm that also stabilizes the micelles. If the polymer is heated above its LCST, its solubility decreases immediately and releases the drug through a reversible mechanism.

The main drawback of micelles is their dissociation upon dilution, what is happening right after the injection into the blood stream. A solution to this problem is the covalent crosslinking of micellar core, what prevents the micelles from dissociation.

All the above mentioned DDS are introduced systemically to the human body. On the other hand, hydrogels as DDS have the advantage that they are placed in the body where the treatment should occur. Therefore, the drugs are released only locally. Basically, they consist of hydrophilic polymers that are crosslinked to have a network structure and are therefore insoluble, but swellable in water. An additional advantage is the protection of the trapped drug from the environment in the body.

Hydrogels can be divided into neutral and ionic hydrogels, depending on the type of charges of their pendant group. Some hydrogels containing acidic or basic pendant groups show swelling degrees that are depending on the pH, as the pendant group will be ionized. There are various polymer backbones used for hydrogels as DDS, including PNIPAAm,[80] Pluronic®[81] (both especially for temperature sensitive hydrogels), poly(vinyl alcohol) (PVA)[82] and PEG.[83]

Delaey *et al.*[84] mentioned in 2020 shape-memory polymers which can be also used for drug delivery. The shape-memory effect can anchor the DDS at the target site. The material, e.g. poly(methyl methacrylate-*co*-butyl acrylate), can be triggered by US to stop the release of the drug. The shape of the material is recovered by the thermal effect of the soundwaves.

As these polymers need to be crosslinked to achieve the swelling instead of dissolution, it is necessary to select a suitable crosslinking mode. Keeping the trigger by US in mind, the crosslinking moieties should be responsive to US as the drug should be released by this stimulus.

2 Mechanolabile bonds

To release drugs from polymeric DDS by US it is necessary to ensure ultrasonic degradability, mostly achieved by special functional groups. These groups have so-called mechanolabile bonds, what can be explained as stress-responsive functionalities. A lot of these bonds can be found in the sonochemistry, but not all of them are suitable for DDS, as medically applied US is limited in frequency, amplitude and duration to avoid side effects.

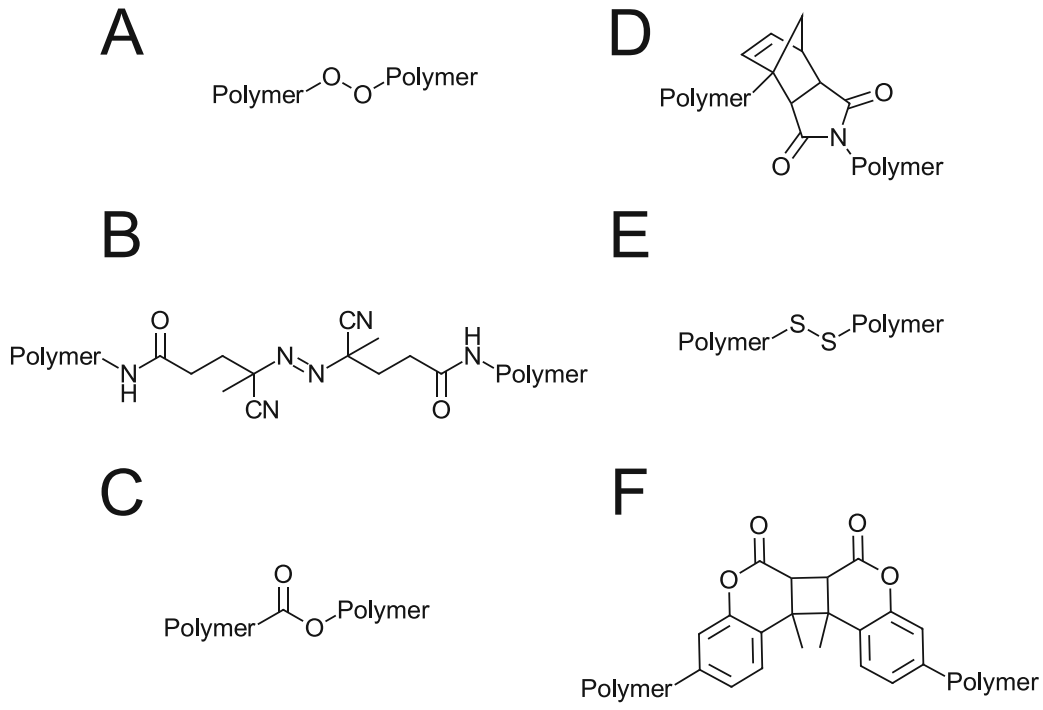


Figure 12: Different ultrasound-responsive bonds: peroxide bonds (A), azo-functionalities (B), ester bonds (C), Diels-Alder adducts (D), disulfide bridges (E) and coumarin dimers (F)

One of the earlies works was the ultrasonic degradation of poly(vinylpyrrolidone) with peroxide linkages in 1980 (Figure 12, A).[85] The peroxide bond is weaker than usual C-C bonds and therefore easier to cleave by the energy of the US.

Berkowski *et al.*[86] established in 2005 US-responsive PEG with azo-functionalities in the polymer chain, displayed in Figure 12, B. During ultrasonication, the polymer is cleaved directly in the middle where the azo-group is located, using 20 kHz and 8.7 W/cm² for 0.5 seconds at 6 – 9 °C in acetonitrile. During this process, gaseous nitrogen is released.

Alginate hydrogels were ionically crosslinked with Ca^{2+} by Hübsch *et al.* to use them as US-triggered DDS.[7] The release of the drug was caused by an ultrasound treatment for 5 minutes with 9.6 mW/cm² once per hour at 25 °C. The material showed self-healing properties, as the polymers re-crosslinked after the US source was switched off. This effect is similar to skin sonophoresis, where US is used to permeabilize the human skin to increase the uptake of macromolecular drugs.

One example of US-responsive functionalities that are already established in DDS are ester bonds (Figure 12, C). Tong *et al.*[87] investigated a Pluronic® type diblock copolymer (PEG-*b*-PPG) with an ester group connecting the blocks. Both thermal effect and cavitation lead to the cleavage of the ester bonds in the prepared micelles at an ultrasonic frequency of 1.1 MHz for 10 minutes at 37 °C.

Diels-Alder adducts, *e.g.* maleimide and furan (Figure 12, D), are mainly known for their thermoreversible Diels-Alder reaction. As this is an equilibrium reaction, the adducts are formed at lower temperatures, whereas the retro-Diels-Alder reaction is favored at elevated temperatures. The thermal effect of US in combination with the pressure generated by US waves can increase the temperature[88] at the administration site and cause there the cleavage of the Diels Alder crosslinkers. The advantage of this cycloaddition is its properties as a click reaction and the absence of any metal catalysts. Duan *et al.*[89] synthesized poly(methacrylate)s with centered Diels-Alder adducts consisting of maleimide and furfuryl alcohol and studied the further cleavage of these adducts with pulsed US (5.52 W at 6 – 9 °C; 1 s on, 2 s off).

The suitability of disulfide bonds (Figure 12, E) was investigated by Li *et al.*[90] in 2010 using PEG-S-S-PLA as biodegradable block copolymer for dual responsive micelles. Disulfide bonds occur also in the human body as peptide crosslinker. This type of bond is also US-responsive due to its low dissociation energy and the enlarged bond length compared to C-C bonds. They can be cleaved by only 10 minutes of ultrasonication at 1.1 MHz and a power of 80 W. Disulfides have already been employed as crosslinking moieties in hydrogels,[91] so it would be very interesting to test them in combination with POx.

Another frequently used US-responsive functionality is coumarin and its derivatives (Figure 12, F). These functional groups can be photodimerized by light with wavelengths above 360 nm, as discovered in 1902 by Ciamician and Silber.[92] Thereby, [2+2] cycloaddition of the double bonds takes place, resulting in different isomers (Figure 13). The first successful crosslinking of polymers with coumarin groups was carried out in the 1960s by Delzenne *et al.*[93] Copolymers of ethyl acetate and 7-acryloxycoumarin as well was poly(hydroxy ethers) and partially hydrolyzed poly(vinyl butyral) with coumarin derivatives were studied and crosslinked *via* irradiation. Additionally to their cleavage by US, coumarin dimers can also be cleaved by irradiation by UV light with wavelengths below 280 nm.[94]

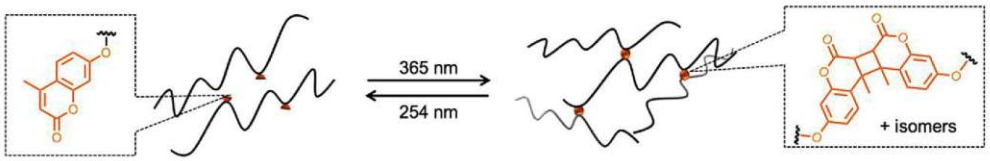


Figure 13: Crosslinking and cleavage of polymers containing coumarin moieties via irradiation with specific wavelengths

The photocrosslinking and subsequent cleavage by US of polymers containing both coumarin and methacrylate moieties was recently investigated.[95] These systems do not only crosslink due to coumarin dimerization, but also due to coumarin-methacrylate [2+2] cycloaddition and radical polymerization of the methacrylates. The cleavage *via* US was investigated using polymer chains equipped with both coumarin and methacrylate groups as well as various mixtures of dicoumarin containing PEG and PEG dimethacrylate. Crosslinked polymers in solution were successfully decrosslinked by breaking the dimerized bonds with 30 kHz sonication. Nevertheless, triggering the materials in gel states with 30 kHz or even 1.1 MHz US, only PEG backbones were fragmented that caused the degradation, but no conversion of the coumarin dimers and adducts occurred.

As coumarin and its derivatives shows antioxidative activity and is additionally used in the pharmaceutical industry, it looks promising as crosslinking moiety for DDS and other biomedical applications. Due to various advantages and literature references dealing with their US-responsiveness, coumarin and thiol moieties were selected to be studied in this diploma thesis.

General Part

Based on literature search, 2 different promising mechanolabile bonds were chosen which will be further used as crosslinking points incorporated into poly(2-oxazoline) (POx) networks. First strategy includes the preparation of POx with thiol moieties in the pendant group. This should be achieved by synthesizing an according 2-oxazoline monomer and copolymerizing it with hydrophilic comonomers. The thiol groups can be crosslinked by redox reactions to result in disulfide bridges.

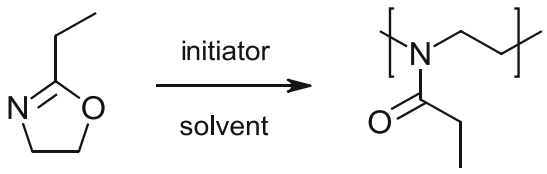
Second strategy was to synthesize POx with coumarin groups for crosslinking the polymer chains. This goal should be reached by homopolymerizing a hydrophilic 2-oxazoline, for instance 2-ethyl-2-oxazoline (EtOx), hydrolyze it partially and attach coumarin derivatives onto the hydrolyzed moieties. These coumarin groups can be crosslinked by irradiation with UV light above 360 nm.

For both strategies, it was necessary to either homo- or copolymerize 2-oxazolines. Therefore, it was necessary to investigate the kinetics of the polymerization of commercially available hydrophilic 2-oxazolines. The living character of the polymerization should be confirmed and suitable polymerization conditions should be selected, such as type of initiator and solvent.

1 Kinetic studies of cationic ring-opening polymerization of poly(2-oxazoline)s

To gain further understanding on POx, the homopolymerization kinetics of EtOx and 2-methyl-2-oxazoline (MeOx) were investigated. The living character of the cationic ring opening polymerization (CROP) was verified to achieve accompanying advantages, like a narrow molar mass distribution and the possibility to adjust the chain length precisely. Additionally, ideal reaction conditions, including the choice of initiator and solvent, were chosen for further homo- and copolymerizations.

1.1 Homopolymerization of 2-ethyl-2-oxazoline



Scheme 3: Homopolymerization of 2-ethyl-2-oxazoline (EtOx)

In this chapter the effect of the solvent and the effect of the initiator on the homopolymerization kinetics of EtOx (Scheme 3) were investigated. The polymerization reactions were carried out under argon atmosphere on the Schlenk line to exclude moisture, what would cause undesired termination reactions. The ratio of monomer to initiator was chosen to be 50, to receive polymer chains with a molar mass around 5000 g/mol. This chain length was also selected for further synthesis of coumarin-modified networks.

The first set of experiments focused on the effect of the solvent. Three different solvents were chosen: acetonitrile (ACN) and benzonitrile are commonly employed in the polymerization of 2-oxazolines. The use of ethyl acetate (EE) was firstly reported in 2020 by Vergaelen *et al.*[40] and showed convincing advantages. It is reported as a green solvent, neither being toxic nor harmful, and the work-up is less time consuming. Vergaelen *et al.* also demonstrated the successful synthesis of well defined high molecular weight poly(2-ethyl-2-oxazoline) (PEtOx) (78 kDa), what is rather difficult with most of the commonly used solvents.

For these experiments, methyl tosylate (MeOTs) was selected as initiator because of its rapid initiation. To achieve living polymerization, it is crucial that the initiation of the polymerization is fast, so it does not affect the reaction rate during the chain growth.

After the components were weighted into a dried flask under argon on the Schlenk line, the mixture was placed in a preheated oil bath. Due to the different boiling points of the solvents, different reaction temperatures were chosen (110 °C for benzonitrile, 75 °C for acetonitrile and 70 °C for ethyl acetate). The highest temperature, 110 °C, was used in case of benzonitrile. Although the boiling point of the solvent is higher, this temperature limit was selected since side reactions may occur during polymerization of 2-oxazolines at higher temperatures.[38]

During the polymerizations, samples were withdrawn in pre-selected time intervals. The withdrawn samples were diluted with $CDCl_3$ to measure ^{1}H-NMR spectra. The spectra showed separate monomer and polymer peaks (see Appendix). Their integrals were compared to calculate the conversion.

The obtained data were plotted into diagrams (Figure 14) and to the semi-logarithmic plots were added linear fits to prove the living nature of the polymerization and to make sure that no side reactions took place that influence the polymerization rate.

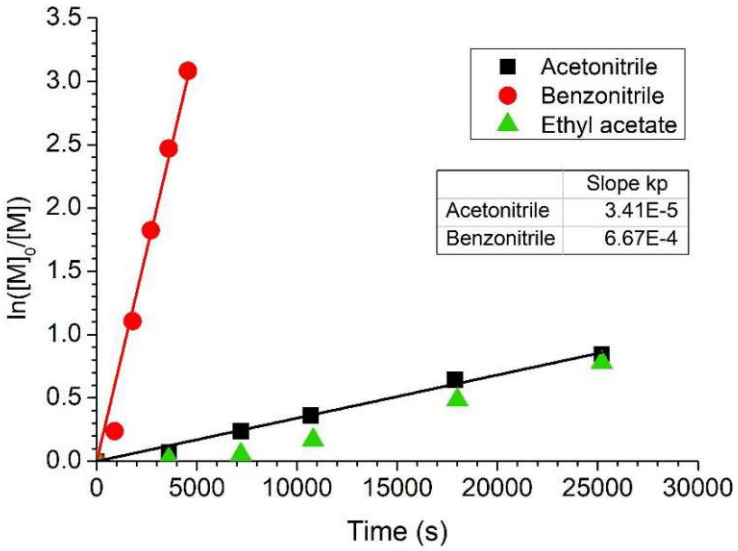


Figure 14: Monomer conversion of EtOx (monomer M), displayed as ln([M]$_0$/[M]) plotted against time in s for three different solvents (3 M) at three different temperatures (75 °C in acetonitrile, 110 °C in benzonitrile, 70 °C in ethyl acetate), polymerized with MeOTs as initiator (I) and a ratio [M]:[I] 50:1.

The polymerization kinetics in ACN and benzonitrile exhibited a linear behaviour in Figure 14, what proves the livingness of the polymerizations. As shown in Figure 14 the polymerization with EE did not follow first-order kinetics, the data deviate from linear fit. The water content of the used ethyl acetate was measured by Karl-Fischer titration (KFT) and resulted in 17.72 ppm water, so termination by moisture can be excluded. Therefore, a possible explanation for these results could be a slow initiation caused by the combination of the solvent with the initiator at this low temperature. Vergaelen *et al.*[40] used also this solvent/initiator system, but the experiments were carried out at different temperatures (60 °C, 100 °C and 140 °C) in a microwave reactor.

Even if the use of EE did not show first order kinetics, there were convincing advantages that justified further experiments with this solvent. The work-up procedure was easier than with benzonitrile and ACN because a precipitation step in diethyl ether was not necessary. Although benzonitrile had a higher reaction rate that shortens the reaction time, the removal of benzonitrile is more difficult than of other solvents because of its high boiling point and immiscibility with water. For this reason, the decision to perform additional experiments with EE as a solvent was made. In order to achieve first-order kinetics, alternative initiators were employed and compared them to MeOTs.

In the second part of this polymerization kinetics study, the initiator was varied whereas the solvent was EE in all cases. The three common initiators selected for these experiments were MeOTs, methyl nosylate (MeONs) and methyl triflate (MeOTf). MeOTs was used in previous experiments with different solvents. The main differences of these chemicals were their states of matter. MeOTf is liquid, MeOTs and MeONs are solid. The difficulty with MeOTs was its melting point at 25 °C, what lead to challenging handling and purification.

The experiments followed the same procedure as the experiments with varying solvents. The plotted data in Figure 15 show the results, where the data with MeOTs were from the same experiment as in the first part of the study.

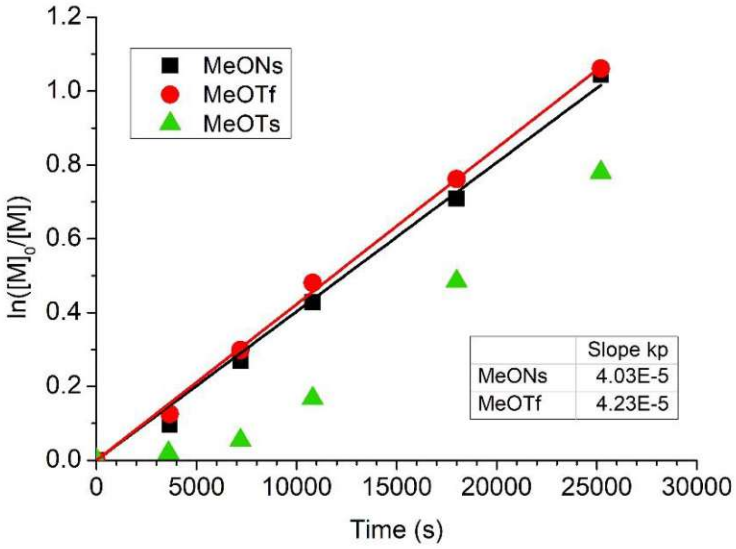


Figure 15: Monomer conversion of EtOx, displayed as ln([M]$_0$/[M]) plotted against time in s for three different initiators, polymerized in EE (3 M) at 70 °C with a ratio [M]:[I] 50:1.

As already discussed before, the experiment with MeOTs does not show a linear behaviour in Figure 15 due to the possible slow initiation based on the initiator/solvent combination at this temperature. MeONs and MeOTf showed both similar slopes (polymerization rate k_p, $4.03 \cdot 10^{-5}$ and $4.23 \cdot 10^{-5}$ L mol^{-1} s^{-1}) and more important a linear behaviour that confirmed the living character of the polymerization.

A similar kinetic study with varying initiators was carried out by Glassner *et al.*[37] This research group used acetonitrile as solvent, EtOx as monomer (4 M solution in ACN) and among others MeOTs, MeOTf and MeONs as initiators ([M]:[I]=50:1). The polymerization took place at 80 °C in a microwave reactor, but the trend of the kinetics was comparable to Figure 15. MeOTf resulted in the highest polymerization rate, whereas MeOTs initiated the polymerization the slowest. Nevertheless, the k_p of MeOTf and MeONs ($4.13 \cdot 10^{-3}$ and $4.35 \cdot 10^{-3}$ L mol^{-1} s^{-1}) are similar to the received k_p values of Figure 15, due to the higher temperature during the experiments of Glassner *et al.* their values are slightly higher.

The same research group[37] investigated the polymerization kinetics with ethyl tosylate (EtOTs) at the same reaction conditions, what lead to a comparable plot as MeOTs in Figure 15, where no linear behaviour was observed. Glassner *et al.* made the hypothesis of a slow initiation and proved it by regression analysis and plotting the $\bar{M}_n$ and Đ against conversion. The $\bar{M}_n$ plot revealed a steeper

increase for low conversions and gel permeation chromatography (GPC) showed peaks with strong tailing towards shorter molar mass with a general peak at higher molar masses.

According to polymerization kinetics experiments, MeOTf was selected as suitable initiator, due to the fastest initiation of all three initiators. Additionally, the purification and handling were pleasing compared to MeOTs for instance. Ethyl acetate was selected as a solvent for following syntheses of PEtOx, since it exhibits linear polymerization kinetics with selected initiator.

After synthesis and purification of the polymers for the kinetic studies, the chain lengths and their distributions were measured. The theoretical and experimental number average molecular weight $\bar{M}_n$ was calculated *via* the used amounts of chemicals, *via* ^{1}H-NMR spectra of the purified polymer and *via* DMSO-GPC analysis. The results and the yield are summarized in Table 2.

An example is shown in Figure 16, where the ^{1}H-NMR spectrum of the polymerization in acetonitrile with MeOTs as initiator at full conversion is displayed.

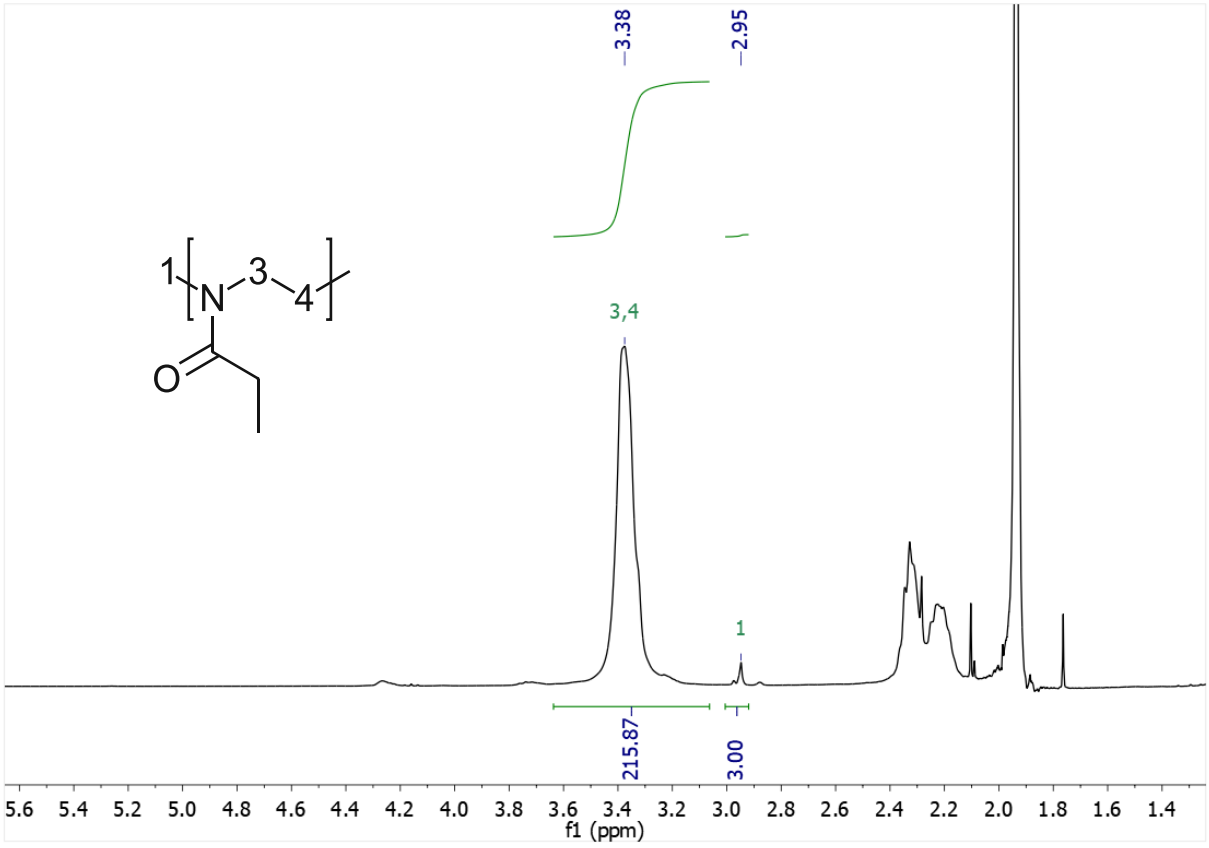


Figure 16: ^{1}H-NMR spectrum of the polymerization mixture of P1 with MeOTs in ACN after purification to show the calculation of the molar mass

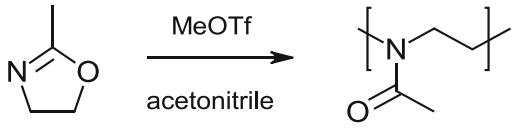
The results of the different methods to examine the molar mass are summarized in Table 2.

Table 2: Comparison of different determined $\bar{M}_n$ with varying initiators and in varying solvents (A=acetonitrile, B=benzonitrile, EE=ethyl acetate), theoretical $\bar{M}_n$ was calculated as $n_M/n_i * M_{Monomer}$; $\bar{M}_n$ from ^{1}H-NMR was calculated from the backbone peak at 3.38 ppm and the methyl group from the initiator at 2.95 ppm.

	Solvent/ Initiator	Theoretical $\bar{M}_n$	$\bar{M}_n^{NMR}$	$\bar{M}_n^{GPC}$	Đ	Yield
		g/mol			-	%
P1	A/MeOTs	4760	5110	5680	1.14	82
P2	B/MeOTs	5790	5430	7020	1.12	88
P3	B/MeOTs	4910	4710	5810	1.12	81
P4	EE/MeOTs	5250	5010	6370	1.17	71
P5	EE/MeONs	4850	5030	6020	1.17	77
P6	EE/MeOTf	5240	4460	7110	1.14	78
P7	EE/MeOTs	4880	4780	6000	1.17	83

The polymerization with benzonitrile as solvent and MeOTs as initiator was carried out twice due to a non-linear behaviour in the first experiment. The reason was that the reaction was cooled to 0 °C every time before a sample was drawn, what slowed down the reaction rate irregularly. In the second experiment, the reaction was not cooled before the samples were drawn. The molar masses values determined *via* ^{1}H-NMR spectroscopy correspond well to the theoretical $\bar{M}_n$, especially for shorter chains as in these experiments. Nevertheless, the calculation of the $\bar{M}_n$ *via* NMR spectroscopy for longer polymer chains should only be a rough comparison as the end group analysis is difficult. Generally, it is obvious that the molar mass measured by GPC in nearly every experiment exceeds those theoretically calculated and measured from ^{1}H-NMR. This difference is not surprising, since the measured $\bar{M}_n$ was relative, based on PMMA standards calibration. The dispersity Đ of all polymers are as narrow as expected, these results also confirm the living character of the polymerization. Almost all the syntheses exhibited similar yields around 70 - 80 %, only P2 reached a slightly higher yield. These yields are in general slightly lower than expected, but product was removed by withdrawing samples for the investigation of the polymerization kinetics. An explanation for the yield of P2 could be the difficult separation of benzonitrile because of its high boiling point. This cause was proven by the slight smell of benzonitrile of the purified polymer.

1.2 Homopolymerization of 2-methyl-2-oxazoline



Scheme 4: Homopolymerization of 2-methyl-2-oxazoline (MeOx)

As an alternative, the even more hydrophilic monomer MeOx was chosen for the preparation of coumarin-containing networks. Although it is not used as frequently as EtOx, MeOx is important especially for copolymers to adjust the properties of the resulting product.

The kinetics of the homopolymerization of MeOx (Scheme 4) were investigated for comparison with the already performed kinetics of the homopolymerization of EtOx. It was also important to prove the living character of its polymerization due to the already mentioned advantages.

To compare the kinetics of both monomers, it was important to use the same conditions. As already explained in chapter 1.1, the decision for the initiator resulted in the use of MeOTf, but for the solvent acetonitrile was used for both monomers. As the use of ethyl acetate caused problems for higher molar masses, so the commonly used acetonitrile was selected as a solvent for MeOx and further polymerizations.

1 eq. of MeOTf as initiator was weighted into a dried vial under argon atmosphere, acetonitrile as solvent and 50 eq. of MeOx were added to achieve a 3 M solution with a ratio of 1:50 of initiator to monomer. A sample was withdrawn before the polymerization was started by putting the vial into a preheated oil bath at 75 °C. Afterwards, samples were withdrawn every 60 min for 6 hours, resulting in 7 samples in total. After ^{1}H-NMR spectroscopy showed full conversion of the monomer, the reaction was terminated with 1.2 eq. 1 M methanolic KOH and stirred overnight at RT. The product was purified by precipitating it into cold diethyl ether. The polymer was centrifuged and dried at 60 °C overnight, yielding 80 %.

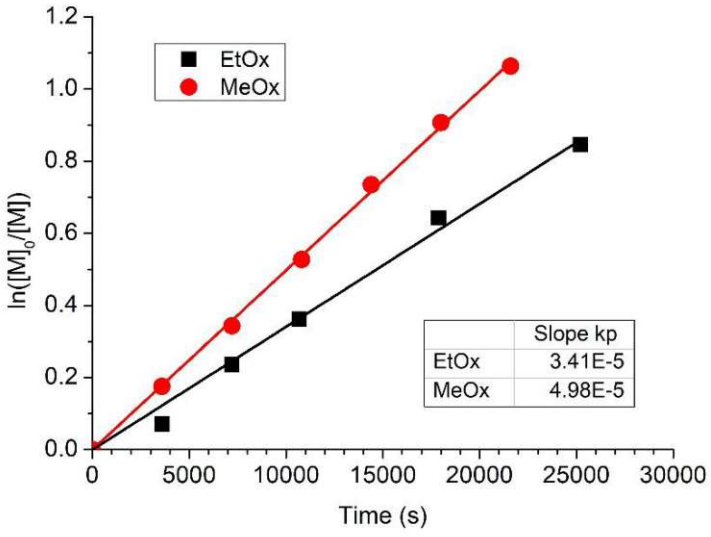


Figure 17: Homopolymerization kinetics of MeOx compared with EtOx with acetonitrile as solvent and MeOTf as initiator at 75 °C with a ratio [M]:[I] 50:1.

Figure 17 shows the polymerization kinetics of MeOx compared to EtOx. Such results were expected, typically MeOx homopolymerization is faster than the homopolymerization of EtOx because of sterical reasons. The linear behaviour proves that the CROP has the desired living character.

After these kinetic investigations, the synthesis of one of the target materials was started, namely the thiol-containing 2-oxazoline. This monomer will be copolymerized with a commercially available hydrophilic 2-oxazoline, for instance EtOx, using the chosen initiator and solvent based on this chapter.

2 Thiol-containing poly(2-oxazoline)s

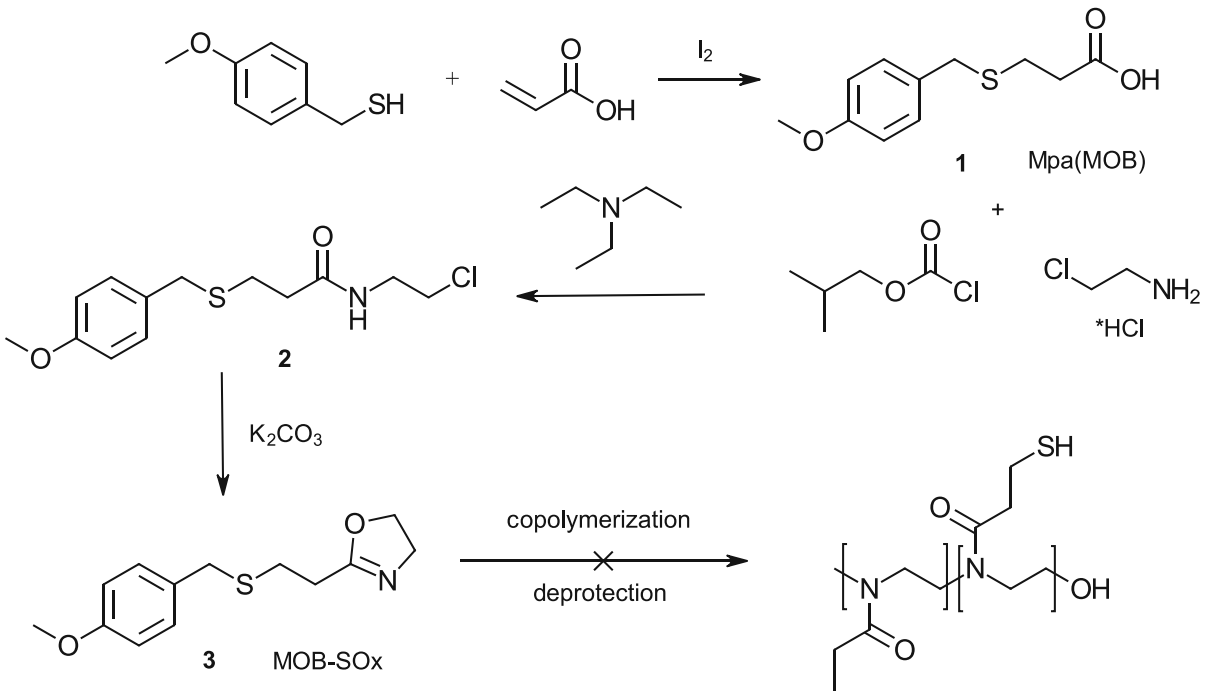
First, the focus was placed on the synthesis of copoly(2-oxazoline)s containing pendant thiol moieties that can be crosslinked to form hydrogels via disulfide bridges. This type of crosslinking for hydrogels is also used with other stimuli, as there is a thiol/disulphide equilibrium controlled by redox potential. This reversibility can be used especially in self-healing materials. Additionally, thiols are mucoadhesive and can form disulfide bonds with cysteine residues of the mucus layer and therefore prolong the delivery process of the drug.[96]

To include thiols during the cationic ring-opening polymerization (CROP) of 2-oxazolines, it is necessary to protect the thiols as they will interfere with the polymerization mechanism and cause unintended termination reactions. To obtain thiol-containing copolymers, first a 2-oxazoline monomer with a protected thiol as a side group will be synthesized. This monomer will be copolymerized with 2-ethyl-2-oxazoline (EtOx) to receive a statistical copolymer with protected thiol side groups. After the thiol is deprotected, the polymer chains can be crosslinked to form disulfide bridges.

2.1 Monomer Synthesis

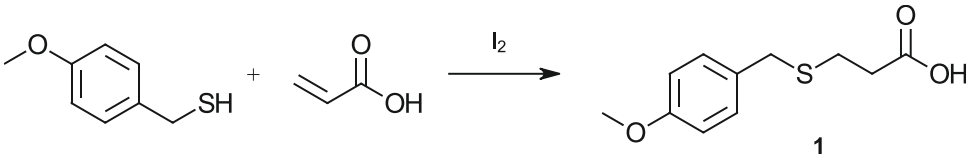
2.1.1 Strategy 1: Thiol-containing 2-oxazoline with methoxybenzyl protecting group

This strategy to synthesize a 2-oxazoline monomer containing pendant protected thiol is inspired by the work of Cesana *et al.*[66] (Scheme 5), where the authors prepared the mentioned monomer protected by a methoxybenzyl group. This monomer was afterwards copolymerized with EtOx yielding in well defined copolymers with the purpose of medical application. The protecting group was cleaved by adding anisole and trifluoroacetic acid under inert atmosphere at elevated temperature. Further, Cesana *et al.* crosslinked the received copolymer with double bonds of N-phenylacrylamide and benzylmaleimide. Compared to that aim, the goal in this thesis was to crosslink the deprotected copolymers *via* redox reactions of the thiol moieties to receive disulfide bridges, which are known to be mechanolabile and therefore cleavable by ultrasound (US).



Scheme 5: Synthesis route for obtaining thiol modified poly(2-ethyl-2-oxazoline) (PEtOx) via copolymerization of EtOx and MOB-SOx

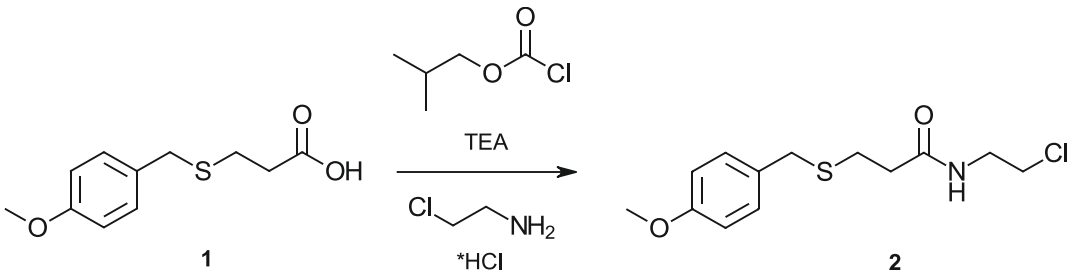
2.1.1.1 Synthesis of 3-[[(4-Methoxyphenyl)methyl]thio]propionic acid (Mpa(MOB)) **1**



Scheme 6: Synthesis of 3-[[(4-Methoxyphenyl)methyl]thio]propionic acid (Mpa(MOB))

The first step to synthesize an oxazoline-based monomer with a protected thiol as a side group was the synthesis of **1** according to Caille *et al.* (Scheme 6).[97] Therefore 4-methoxy-α-toluenethiol and acrylic acid were distilled in vacuo. It was necessary to carry out this reaction under argon atmosphere. To 1 eq. of both compounds 0.25 eq.% of iodine was added under argon counterflow. The yellow reaction mixture was stirred at RT overnight until a white solid precipitated. The precipitation was completed by adding petrol ether. Colourless crystalline needles were collected by filtration and washed with petrol ether. The successful synthesis of **1**, yielding 35 %, was confirmed by NMR spectroscopy and melting point.

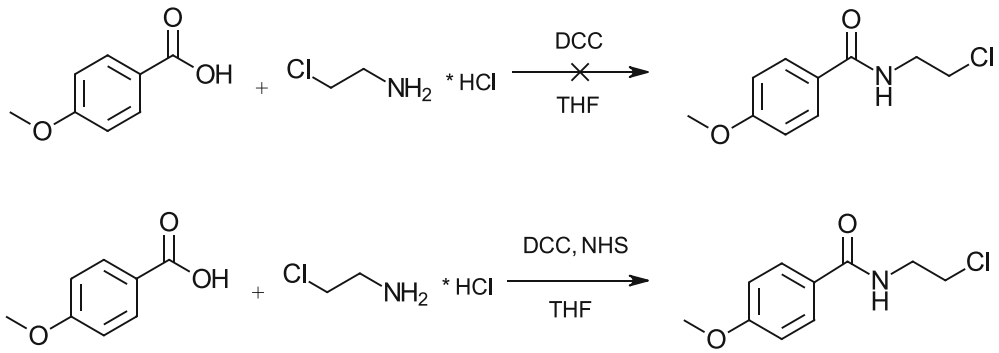
2.1.1.2 Synthesis of N-(2-chloroethyl)-3-(4-methoxybenzylsulfanyl)propionamide **2**



Scheme 7: Synthesis of N-(2-chloroethyl)-3-(4-methoxybenzylsulfanyl)propionamide

The second step (Scheme 7) to synthesize a thiol-based monomer according to Cesana *et al.*[66] started with 1 eq. of **1** that was dried in vacuo. After dissolving it in dry THF and adding 1.1 eq. of dry TEA under argon, the slightly turbid solution was cooled to 0 °C with an ice bath. The first part of the reaction was to synthesize a mixed anhydride, so 1 eq. of isobutyl chloroformate was added, receiving a white turbid mixture. It was stirred for 10 minutes at 0 °C and for further 15 minutes at RT. After cooling the mixture again to 0 °C, 1.1 eq. dry TEA and 1.2 eq. 2-chloroethylamine hydrochloride dissolved in dry DMF were added simultaneously to form an amide. Up to this step it was very important for a successful synthesis to use freshly ordered chemicals without traces of moisture, as this would inhibit the formation of the product.

After stirring the mixture for 1 hour at RT, the solvent was evaporated in vacuo. The yellow-white residue was dissolved in DCM. In contrast to Cesana *et al.*[66] the solid was dissolved completely, due to that it was not filtered. During the work-up procedure, it was also found out that in contrast to the literature, the product did not precipitate in the last step, but the by-product is obtained instead. After removing the white precipitate, the filtrate was dried in vacuo to obtain 115 % of the crude colourless product **2**.

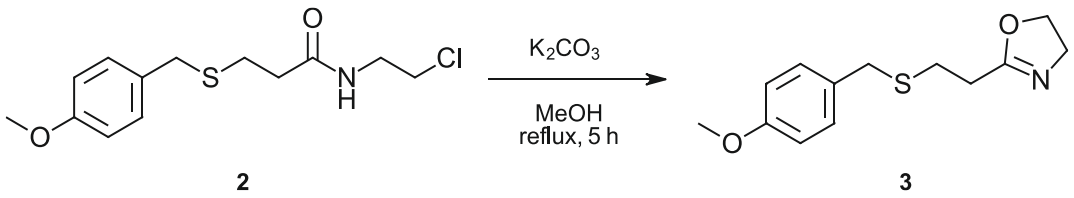


Scheme 8: Amide synthesis with p-anisic acid as model compound using DCC, respectively DCC and NHS for activation

In parallel, the synthesis of amide *via* activation of p-anisic acid as model compound once with DCC and once with DCC and NHS was performed (Scheme 8). The activation with DCC and NHS was

successful, but due to the laborious purification steps, this synthesis route was not used for further work.

2.1.1.3 Synthesis of 2-[2-(4-Methoxybenzylsulfanyl)ethyl]-2-oxazoline (MOB-SOx) **3**



Scheme 9: Synthesis of 2-[2-(4-Methoxybenzylsulfanyl)ethyl]-2-oxazoline (MOB-Sox)

The last step to synthesize a protected thiol-based oxazoline was the ring closing displayed in Scheme 9, what was carried out similarly to Cesana *et al.*[66] Originally, 1 eq. **2** and 2.1 eq. anhydrous K_2CO_3 were put into a dry flask and dry DMF was added. The suspension was put into a preheated oil bath at 70 °C overnight. After cooling to RT, the solvent was evaporated at 60 °C in HV. The residue was redissolved in DCM, filtered and the solvent of the filtrate was evaporated.

As DMF has a high boiling point, it was difficult to remove it completely during the purification. Therefore, another attempt was performed with methanol as solvent and KOH as base. In this case, the ^{1}H-NMR spectrum did not show any conversion, therefore both methods were combined. Using methanol as a solvent and K_2CO_3 as a base showed the best results to receive 45 % of the liquid crude product **3**.

To remove any traces of water that would interfere with living CROP, MOB-SOx was dried overnight with CaH_2 and attempted to be distilled the next day at 5 mbar. After increasing the temperature stepwise to 235 °C, nothing could be distilled because the product turned solid.

Since the product was liquid, a different, probably laborious strategy for purification and drying was required. Additionally, concerns arose concerning the hazardous deprotection after the polymerization containing the addition of anisole and trifluoroacetic acid at elevated temperature. These substances would need to be removed carefully to ensure safe application as drug delivery system. Due to these drawbacks, the synthesis of this monomer was not proceeded, and the search for an alternative 2-oxazoline monomer containing a protected thiol group was started.

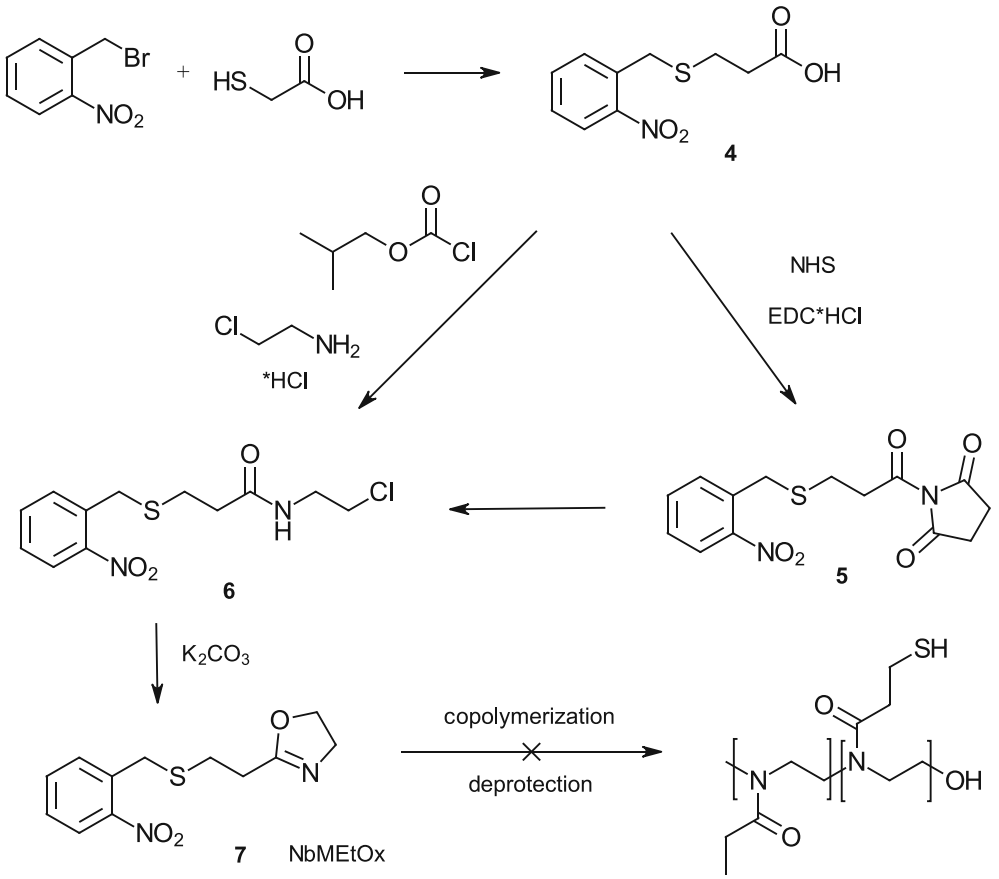
2.1.2 Strategy 2: Thiol-containing 2-oxazoline with nitrobenzyl protecting group

Although the first strategy to synthesize a 2-oxazoline monomer containing a pendant thiol moiety with a methoxybenzyl protecting group (chapter 2.1.1) was not successful, the aim to synthesize poly(2-oxazoline)s (POx) crosslinked with disulfide bridges was still conducted.

Based on recent literature, the synthesis of a 2-oxazoline monomer with an alternative protecting group is possible. Jung *et al.*[32] described the synthesis of POx containing thiol moieties. The difference

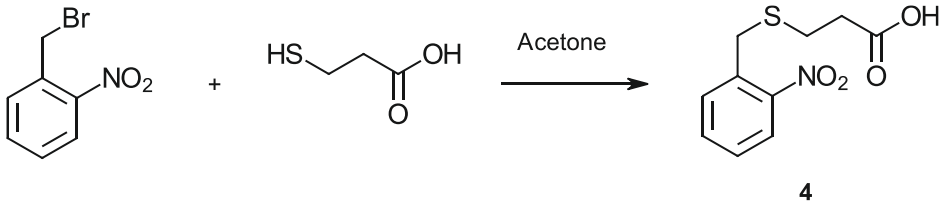
from Cesana *et al.*[66] is a functionality located at the protecting group, a nitro group in ortho position instead of a methoxy group in para position. This difference effects mainly the deprotection conditions: light can be used instead of hazardous chemicals to deprotect this monomer. Also the state of matter is different, as the previously synthesized thiol-based monomer MOB-SOx was liquid, this alternative monomer is known to be solid, therefore the drying process should be easier.

In the first step, 3-mercaptopropionic acid was protected with 2-nitrobenzyl bromide (Scheme 10). The second step of the synthesis was carried out *via* two different methods: the first method was adapted from Cesana *et al.*,[66] where the amide was synthesized from a mixed anhydride. Employing the second method, **6** was also synthesized starting from **4** in a two-step procedure according to Jung *et al.*[32] where an NHS-intermediate could be isolated. Due to the light-sensitivity of the compound, all the following reactions were carried out under exclusion of light in the yellow light lab.



Scheme 10: Alternative synthesis route for obtaining thiol modified poly(2-ethyl-2-oxazoline) (PEtOx) by copolymerization of EtOx with NbMEtOx

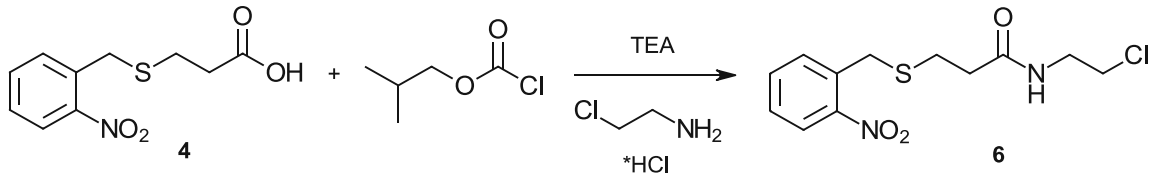
2.1.2.1 Synthesis of 3-[(2-Nitrobenzyl)thio]propionic acid **4**



Scheme 11: Synthesis of 3-[(2-Nitrobenzyl)thio]propionic acid

The first synthesis step depicted in Scheme 11 was performed according to Jung *et al.*[32] A dry Schlenk flask was filled with dry acetone, 2 eq. of freshly distilled 3-mercaptopropionic acid and 1.5 eq. of TEA under argon counterflow. The reaction was cooled to 0 °C with an ice bath for 40 minutes. After removing it from the ice bath, 2-nitrobenzyl bromide was added and the clear solution was protected additionally from light with aluminium foil. After stirring it overnight at RT, the formed white precipitate was filtered off and the solution was dried, resulting in a yellow crude product with both solid and liquid parts. For purification, it was dissolved in DCM and extracted four times with 0.5 M aqueous HCl and one time with saturated aqueous NaCl. After drying the organic phase with $MgSO_4$ and evaporating the solvent, a yellow viscous liquid was received. For further purification, petroleum ether (PE) was added and stirred for 30 minutes after decanting the liquid phase off and drying the remaining solid in vacuo (77 % yield). The product was characterized *via* NMR spectroscopy and melting point.

2.1.2.2 Synthesis of N-(2-chloroethyl)-3-[(2-nitrobenzyl)thio]propenamide **6**

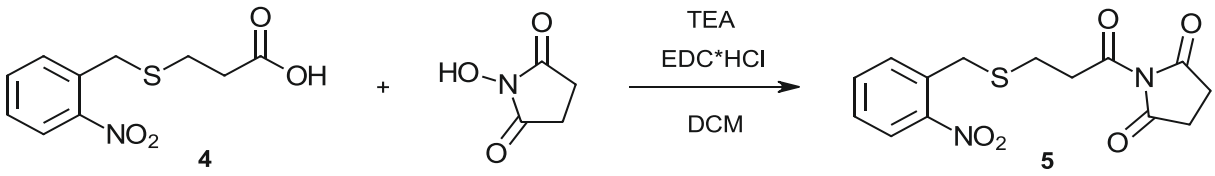


Scheme 12: Synthesis of N-(2-chloroethyl)-3-[(2-nitrobenzyl)thio]propenamide using the mixed anhydride method

The second synthesis step (Scheme 12) of a thiol containing monomer protected by a nitrobenzyl group was similar to the synthesis of **2**.[66] The reaction was carried out in the yellow light lab with dry conditions. 1 eq. **4** was dissolved in DCM and 1.1 eq. TEA was added. The solution was cooled to 0 °C before isobutyl chloroformate was added and stirred for 10 minutes at this temperature. During warming to RT, the solution became turbid, but cleared again after stirring for another 40 minutes at RT. Then, the solution was cooled with an ice bath before 1.2 eq. 2-chloroethyl amine hydrochloride and 1.1 eq. TEA were added. A solid precipitated after this step and the reaction was stirred overnight at RT. The next day, most of this precipitate was dissolved. A few mL of DCM was added to completely dissolve the remaining precipitate. The solution was extracted two times with 1 M HCl, during this step the organic phase became turbid. After extracting it with saturated aqueous NaCl, the organic phase

became clear again, but turned turbid again after extracting it with saturated aqueous $NaHCO_3$. The organic phase was again nearly clear after extracting it again two times with saturated aqueous NaCl, so it was dried and the solvent was evaporated.

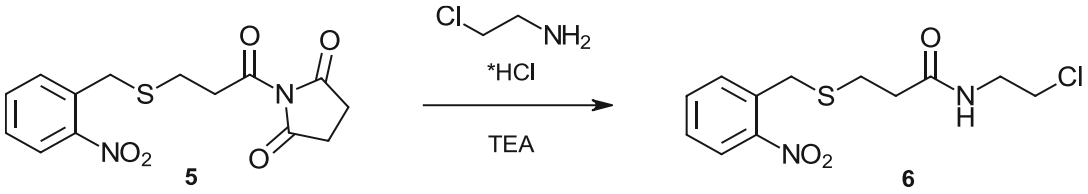
This viscous liquid was not completely pure according to the ^{1}H-NMR spectrum, also it was described as a solid in the previous work.[32] Despite of this, the compound was used for the next step, the ring closing. However, this reaction was not successful as no conversion was observed after 21 hours using K_2CO_3 as base in acetonitrile (ACN) as solvent. Consequently, the two-step strategy to synthesize this intermediate product according to Jung *et al.*[32] was carried out.



Scheme 13: Synthesis of 2,5-dioxopyrrolidin-1-yl 3-[(2-nitrobenzyl)thio]propanoate

The synthesis described in literature[32] contained an intermediate step where **5** can be isolated (Scheme 13). Therefore, 1 eq. of **4** was weighted in and dried carefully in vacuo. 1.6 eq. of each TEA and NHS were added under argon counterflow and dissolved in dry DCM. Afterwards, 1.6 eq. of EDC·HCl was added and stirred at RT overnight. The reaction mixture was extracted with 1 M HCl, water and brine, the combined aqueous phases were extracted with DCM and the combined organic phased dried with $MgSO_4$, filtered and the solvent was evaporated. The result was a yellow viscous liquid that crystallized overnight to a slightly yellow solid. This crude product was used for the next without further purification and characterization yielding 99 %.

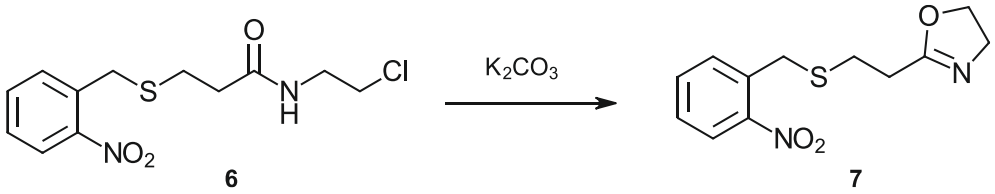
To ensure that the thiol was still attached to the protecting group, the product was tested on free thiols with the Ellman's reagent. Therefore, a few mg of Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)) was dissolved in 2 mL of EtOH and this solution was divided into three parts. One part was used as positive control sample, where 2,2'-(ethylenedioxy)diethane thiol was added, one part was used as a negative control sample, where 4-anisic acid was added, and to the third part **5** was added. The control sample showed a dark yellow colour, the other two samples looked visually equal, featuring transparent appearance. This led us to the assumption that no significant amount of the product **5** was deprotected during previous reactions and work-up procedures.



*Scheme 14: Synthesis of N-(2-chloroethyl)-3-[(2-nitrobenzyl)thio]propenamide using the NHS intermediate **5***

To conclude the synthesis of **6** according to Jung et al.[32] displayed in Scheme 14, 1 eq. of **5** was dissolved in dry DCM and cooled to 0 °C. 2 eq. of TEA and 2 eq. of 2-chloroethylamine hydrochloride were added under argon counterflow. After stirring for 50 min at 0 °C, the clear solution was stirred at RT overnight until TLC showed full conversion (EE:PE 4:1, R_f 0.53). The mixture was diluted with DCM, extracted with 1 M HCl, brine and saturated $NaHCO_3$ before drying the organic phase with $MgSO_4$ and evaporating the solvent. The yellow solid was confirmed *via* NMR spectroscopy, TLC and melting point, yielding 68 %.

2.1.2.3 Synthesis of 2-{2-[(2-Nitrobenzyl)thio]ethyl}-4,5-dihydrooxazole (NbMEtOx) **7**



Scheme 15: Synthesis of 2-{2-[(2-Nitrobenzyl)thio]ethyl} -4,5-dihydrooxazole (NbMEtOx)

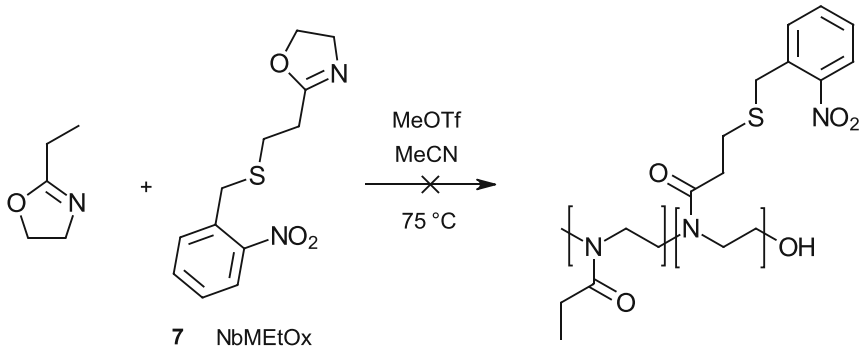
The final step of the synthesis of the thiol-based 2-oxazoline was the ring closing **6** (Scheme 15). Therefore, 1 eq. of **6** was dissolved in acetonitrile and 2.6 eq. of anhydrous K_2CO_3 were added, according to Jung *et al.*[32] The mixture was heated up to 80 °C until TLC showed full conversion (21 hours; EE, R_f 0.29). Afterwards, the suspension was filtered with a syringe filter (PTFE, 0.2 µm) and the solvent was evaporated, resulting in an orange to brownish liquid. The crude product was dried in HV and subsequently dissolved in DCM and again filtered with a syringe filter. After evaporating DCM, the turbid brownish viscous liquid was flashed over silica gel with EE as solvent. The ^{1}H-NMR spectrum showed the presence of impurities in the final product.

As this reaction took significantly longer than the previous ring closing that was carried out according to Cesana *et al.*[66], and there were also impurities that could not be isolated easily, the solvent was changed to dry MeOH. Therefore, also the reaction temperature was decreased to 60 °C. Additionally, the reaction apparatus was equipped with an argon balloon to exclude air and moisture. Confirming the full conversion by TLC, the reaction was stopped after 18 hours. The purification procedure had to be modified as the byproducts could not be separated by flash chromatography, therefore the product was purified by column chromatography (pure EE → EE:MeOH 100:1). The separation was challenging, what lead to numerous mixed fractions. Thus, the amount of completely pure **7** was small (2.5 %), but

necessary to ensure a living polymerization afterwards. In contrast to literature,[32] the product was no solid, but a highly viscous yellow liquid that was confirmed *via* TLC and NMR spectroscopy.

2.2 Polymer synthesis

As already described by Jung *et al.*,[32] the homopolymerization of **7** showed some major issues. As problems have already been reported with methyl triflate (MeOTf) previously[98] due to S-methylation, the reaction carried out by Jung *et al.* was started with an oxazolinium salt made of 2-methyl-2-oxazoline (MeOx) and MeOTf. NbMEtOx was added to the initiation mixture under inert atmosphere, but two out of three times no homopolymer could be isolated. The third attempt yielded 10 % with a broad dispersity of 2.9. Due to the challenging task of this homopolymerization and the initial goal to synthesize copolymers, **7** was directly copolymerized with EtOx according to Scheme 16.



Scheme 16: Copolymerization of the thiol-containing NbMEtOx with EtOx

4 eq. of **7** were placed into a vial and dried on the HV and 96 eq. of EtOx were added. A stock solution of MeOTf in ACN (1 eq. MeOTf, DP 100, 3 M monomer solution) was put into the vial before placing it in a preheated oil bath at 75 °C. The reaction was terminated with 1 M methanolic KOH after 7 days when the ^{1}H-NMR spectrum confirmed full conversion. The polymer was purified by precipitation into ice cooled Et_2O and it was dried overnight at 30 °C under exclusion of light.

To characterize the polymer and determine the exact content of NbMEtOx **7**, ^{1}H-NMR spectrum of the purified polymer was recorded. Interestingly, there was no aromatic peaks of the protecting group visible, although these peaks were visible in the spectra that confirmed the full conversion of the monomers (see Appendix). The hypothesis that the copolymer was deprotected during polymerization or purification could explain these results, despite the fact that the light was excluded during the reactions.

To test this hypothesis, the presence of the free thiols in the copolymer was examined. In order to do this, the test with the Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)) was performed according to chapter 2.1.2.2. The control sample showed a dark yellow colour, the samples with the blind sample and the copolymerized polymer on the other hand remained colourless. This means that there were no free thiols in this copolymer, so most likely there have been difficulties during the copolymerization. As previously reported by Cesana *et al.*[98] analogous S-methylation could have occurred especially with MeOTf as initiator. Thereby the protecting group could have been cleaved after the sulfur was

methylated. Due to the low amount of NbMEtOx in the feed, poly(2-ethyl-2-oxazoline) (PEtOx) could have been synthesized without any thiol content what would explain the behaviour with the Ellman's reagent.

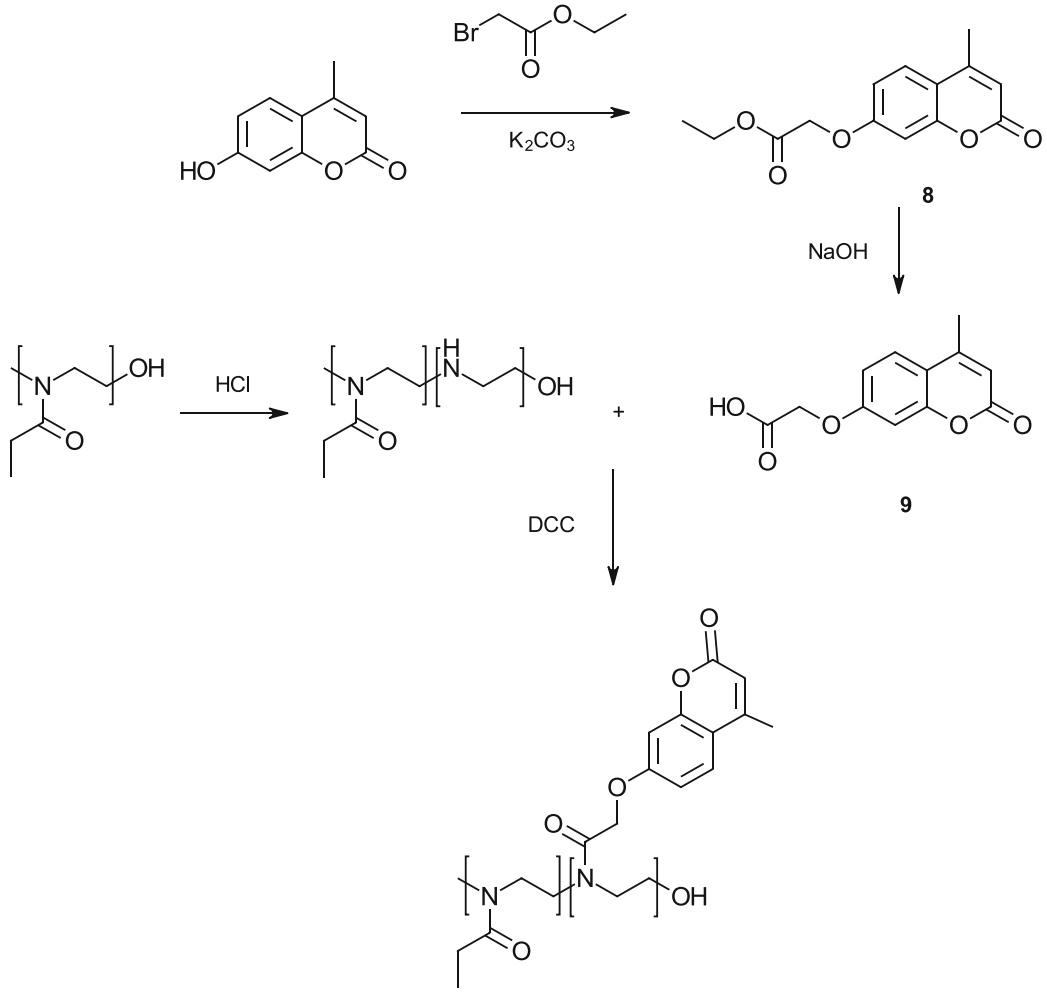
Since the copolymerization of NbMEtOx did not lead to satisfying results, its synthesis and investigation was not proceeded further. In the following chapters, the goal was to synthesize coumarin-based POx in order to prepare US-responsive materials.

3 Coumarin-containing poly(2-oxazoline)s

Poly(2-oxazoline)s (POx) with coumarin moieties can be crosslinked by irradiation with UV light above 360 nm as coumarin groups are dimerizing. The mechanism is a [2+2] cycloaddition of the double bonds that can be additionally cleaved by irradiation with light below 280 nm. Coumarin groups were already used for hydrogels for biomedical applications and tissue engineering[99] and are therefore well investigated. Its decrosslinking by ultrasound (US) was already described in literature,[100] so the general behaviour fulfils the required properties for controlled drug delivery systems.

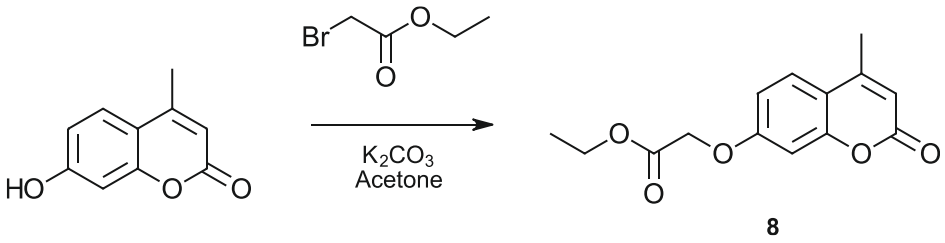
3.1 Postpolymerization modification strategy

After optimizing the polymerization conditions (chapter 1.1) the preparation of coumarin-modified POx was started. The synthesis was carried out according to Chujo *et al.*[67] (Scheme 17). As described, a synthesized coumarin derivative was added onto the PEI units of a partially hydrolyzed PEtOx. The first step to receive the desired material was the homopolymerization of 2-ethyl-2-oxazoline (EtOx) while maintaining a living character according to the kinetic study in chapter 1.1. Different chain lengths were synthesized in order to investigate their influence on the further properties of the finished materials. For the partial hydrolysis of poly(2-ethyl-2-oxazoline (PEtOx), it was necessary to have a closer look at the reaction kinetics before the desired degree of hydrolysis (DH) could be adjusted), where literature of de la Rosa *et al.*[101] was followed.



Scheme 17: Synthesis route for receiving coumarin-modified PEtOx via postpolymerization modification

3.1.1 *Synthesis of (4-Methylcoumarin-7-yloxy)acetate **8***

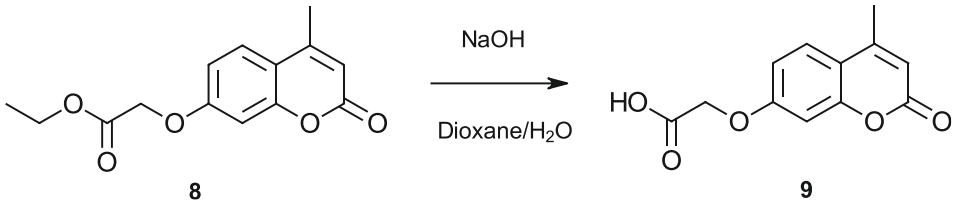


Scheme 18: Synthesis of (4-methylcoumain-7-yloxy)acetate

The first step to obtain coumarin-modified PEtOx according to Chujo *et al.*[67] was the synthesis of **8** from 4-methylumbelliferone and ethyl bromoacetate (Scheme 18). The reaction had to take place under anhydrous conditions, consequently acetone and ethyl bromoacetate were distilled. 1 eq. of 4-methylumbelliferone was dried along with the reaction flask before adding dry acetone, 1.1 eq. of ethyl bromoacetate and 1.05 eq. of anhydrous K_2CO_3. The turbid yellow mixture was heated to reflux for 3 hours before the precipitate was filtered off. The remaining filtrate was still turbid, therefore it was filtrated once again before evaporating the solvent in vacuo. The yellow viscous liquid was cooled with an ice bath to crystallize the crude product. The yellow-white solid was recrystallized from

ethanol, yielding white crystalline needles that were dried at 40 °C in vacuo. The remaining ethanolic solution was concentrated to half and stored at 6 °C overnight. A second fraction of a light-yellow solid was gained, yielding 69 % together. The product was characterized *via* melting point and NMR spectroscopy.

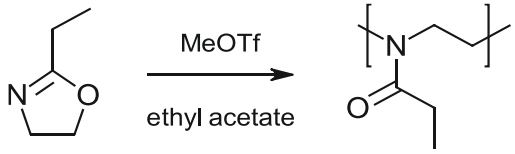
3.1.2 *Synthesis of (4-Methylcoumarin-7-yloxy)acetic acid **9***



Scheme 19: Synthesis of (4-methylcoumarin-7-yloxy)acetic acid

The second step to obtain coumarin-modified polymers was the synthesis of **9** according to Scheme 19, that could be added onto the partially hydrolyzed PEtOx backbone. 1 eq. of the previously synthesized **8** was dissolved with 14.4 eq. of NaOH in 200 mL of a 1:1 mixture of dioxane and water. The yellow solution was stirred overnight at RT. After evaporating the solvent under reduced pressure, the yellow-white solid was dissolved in 20 mL water and acidified with concentrated HCl. The voluminous beige precipitate was filtered and washed several times with cold water. If the product was not washed well enough, NaCl crystals would be visible in the dried solid. The successful synthesis with 69 % yield was confirmed by NMR spectroscopy and melting point measurement.

3.1.3 *Homopolymerization of 2-ethyl-2-oxazoline with varying chain lengths*



Scheme 20: Homopolymerization of EtOx with MeOTf as initiator and ethyl acetate as solvent

The kinetics of homopolymerization of EtOx was examined in chapter 1.1. Ethyl acetate (EE) was selected as a solvent and methyl triflate (MeOTf) was chosen as initiator (Scheme 20). In this chapter, several batches of PEtOx with different chain lengths were synthesized, to investigate later its influence on the properties of the resulting hydrogels. The selected molar masses were 5 kDa, 10 kDa and 20 kDa. According to this specification, the ratio of monomer to initiator was 50, 100 and 200, respectively.

The syntheses followed the same procedure as the polymerizations with EE for the kinetic study in chapter 1.1, except that no samples were collected during the synthesis. The characterization was carried out similar to the analysis of the kinetic study products. The chain length was determined *via* calculation and *via* DMSO-GPC and ^{1}H-NMR spectroscopy (Table 3). In the following, the samples are

termed PEtOx_Xy, where X is approximately the chain length in kDa and y is a letter starting from a counting the polymerization batches with the same chain length if the specific chain length was synthesized more than once.

Table 3: Comparison of the different determined molecular weights polymerized in EE (PEtOx_20b in ACN) with MeOTf as initiator, Đ and the yields of the PEtOx that were further used for coumarin modification

Sample name	Target $\bar{M}_n$	Theoretical $\bar{M}_n$	$\bar{M}_n^{NMR}$	$\bar{M}_n^{GPC}$	Đ	Yield
	g/mol					%
PEtOx_20a	20000	16200	18330	18250	1.83	99
PEtOx_5a	5000	5080	5590	6920	1.13	89
PEtOx_10	10000	10200	7870	12800	1.36	85
PEtOx_5b	5000	5300	5160	6630	1.12	59
PEtOx_20b[1]	20000	19590	15290	25620	1.24	86
PEtOx_20c	20000	23440	41360	22840	1.73	54

[1] ACN as solvent instead of EE

The synthesis of 5 kDa polymer was comparably successful as in case of the kinetic experiments. Theoretical molar masses of PEtOx_5a and PEtOx_5b (5 kDa), correspond relatively well with the molar masses determined *via* NMR spectroscopy and GPC, both polymers also display low Đ of 1.13 and 1.12 respectively. The molar masses of longer polymer chains show higher deviation from the target $\bar{M}_n$. It should be noted that in the case of longer polymer chains, the evaluation *via* ^{1}H-NMR is challenging as the CH_3-peak from the initiator is approaching the resolution limit. However, PEtOx_10 and PEtOx_20a, polymerized in EE, exhibit higher deviation from expected values featured also by the broader Đ. The GPC elugrams of PEtOx_20a and PEtOx_10, those with a molecular weight higher than 5 kDa, are presented in Figure 18. PEtOx_20a exhibited shoulders on both sides of the main peak. PEtOx_10 showed a small peak with an average molecular weight of 103 kDa. To investigate if the used EE influenced the distribution, PEtOx_20b and PEtOx_20c were synthesized. PEtOx_20b was synthesized using ACN as solvent, PEtOx_20c was synthesized in the previously selected EE as a control experiment to evaluate the batch-to-batch reproducibility of the polymerization.

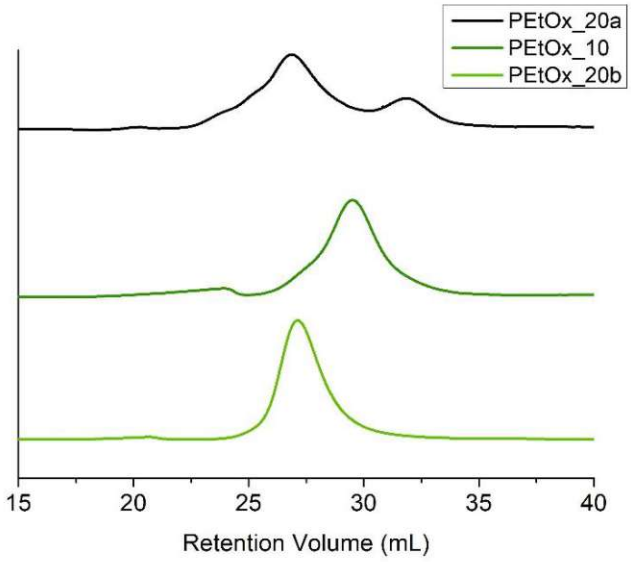


Figure 18: Cut-out from the GPC elugrams of PEtOx_20a(20 kDa), PEtOx_10 (10 kDa, both polymerized in EE) and PEtOx_20b (20 kDa, polymerized in ACN) between 15 and 40 mL retention volume, DMSO as an eluent, PMMA as a standard.

These GPC results in Figure 18 lead to the assumption that the polymerization is different for higher molar masses, side reactions are more likely to occur. Although the GPC elugram of PEtOx_20c did not show such a broad peak, there was nevertheless a small peak at higher molecular weights, similar to the elugram of PEtOx_10. On the opposite, PEtOx_20b did not show abnormalities like shoulders or side peaks, also Đ was narrower. To conclude, although EE acts as suitable solvent for shorter PEtOx chains, for longer chains, the control over the polymer properties is compromised. As a consequence, ACN was used for all further polymerizations.

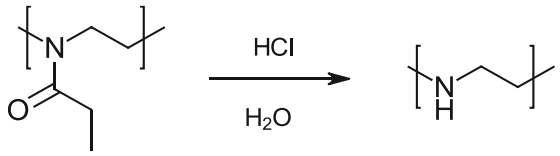
Comparing the yields in Table 3, differences among batches are visible, especially for PEtOx_5b with a molar mass of 5 kDa and PEtOx_20c with 20 kDa. The cause of this very low yield of PEtOx_5b was the dialysis that lasted for 7 days in membranes with a molecular weight cut off (MWCO) of 1 kDa. Even if the difference between the chain length and the MWCO of the dialysis tubes was considered as sufficient, the yield proved the opposite. Compared to the yield of PEtOx_5a, what was dialysed for two days, it is important to dialyze the polymer for shorter times to achieve the expected yield.

The next step was the partially hydrolysis of the obtained homopolymers. To achieve defined degrees of hydrolysis (DH), the kinetics of this reaction mechanism was studied subsequently.

3.1.4 Hydrolysis kinetics of poly(2-ethyl-2-oxazoline)

In order to attach coumarin moieties onto PEtOx chains, it was necessary to hydrolyze PEtOx partially. The secondary amine groups can react with the appropriate molecule including the functionality into polymer chain. The aim was to find the optimal conditions for a controlled partial hydrolysis of PEtOx. This will further allow us to obtain desired percentages of amino groups within polymer chain and to modify them later with functional groups. Hydrolyzing PEtOx fully will result in poly(ethyleneimine)

(PEI), what can be also synthesized *via* ring opening polymerization of aziridine. The difference is the structure of the polymer, where a polymer out of aziridine is branched, but fully hydrolyzed POx results in linear PEI. PEI is in contrast to PEtOx toxic, it is mainly used in detergents and adhesives.



Scheme 21: Acidic hydrolysis of PEtOx

The kinetic study concerning the hydrolysis of PEtOx displayed in Scheme 21 was carried out according to de la Rosa *et al.*[101] and the corresponding master thesis of Eva Bauwens.[102] The principle of the hydrolysis of POx is the acidic or basic catalysed cleavage of the side chain to receive a secondary amine. The two most important factors are the temperature and the concentration of the acid. The chain length of the homopolymer does not affect the hydrolysis.[103]

Similarly to the results of Bauwens master thesis,[102] our first experiment was carried out with PEtOx (DP 182, Đ 1.83) at an amide concentration of 0.48 M and a HCl concentration of 2 M at 100 °C. The oil bath was preheated to 110 °C to ensure that the temperature would not decrease below 100 °C, what would affect the hydrolysis and disturb the kinetics. Samples were withdrawn in pre-selected time intervals, cooled and neutralized with aqueous 5 M NaOH to reach a pH between 8 and 10, frozen overnight at -18 °C and afterwards lyophilized.

For the ^{1}H-NMR spectra measurement, the lyophilized samples were dissolved in methanol-d_6, what was challenging due to the limited solubility of the by-products but successful for lower concentrations. In Figure 19 a typical ^{1}H-NMR spectrum of a sample including PEI parts, unhyrolyzed PEtOx and propionic acid is shown.

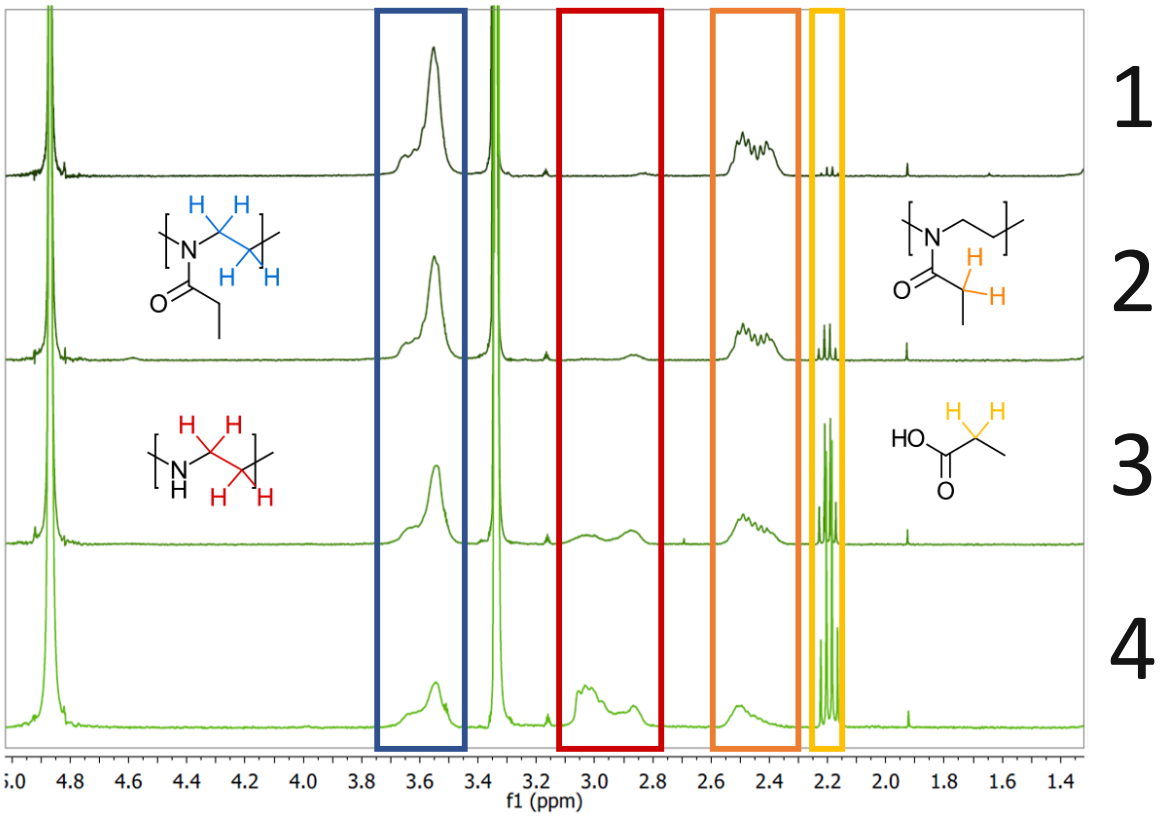


Figure 19: ^{1}H-NMR spectra of the hydrolysis of PEtOx after 15 min (1), 45 min (2), 180 min (3) and 360 min (4) using 2 M HCl to show the calculation of the degree of hydrolysis (DH).

For the sample of the kinetics study three methods to calculate the DH were described in literature.[102] The first method is to divide the integral of the PEI backbone (3.12 – 2.78 ppm, Figure 19 red frame) with the sum of the PEtOx (3.75 – 3.45 ppm, Figure 19 blue frame) and the PEI backbone integrals.

$$DH\ (\%) = \frac{\int(backbone\ PEI)}{\int(backbone\ PEI) + \int(backbone\ PEtOx)} \cdot 100$$

Equation 1: First formula to calculate the DH

Second method is to compare the integral of the PEI backbone (3.12 – 2.78 ppm, Figure 19 red frame) with sum of the CH_2 group integrals of the ethyl moiety (2.60 – 2.30 ppm, Figure 19 orange frame) and the PEI backbone. The CH_2 group was multiplied by 2 to compare its 2 H with the 4 H of the backbone.

$$DH\ (\%) = \frac{\int(backbone\ PEI)}{2 \cdot \int(EtOx\ CH_2) + \int(backbone\ PEI)} \cdot 100$$

Equation 2: Second formula to calculate the DH

The third method is only possible for the samples before purification, because the CH_2 integral of the formed propionic acid (2.25 – 2.15 ppm, Figure 19 yellow frame) and the integral of the CH_2 group of the PEtOx side chain (2.60 – 2.30 ppm, Figure 19 red frame) are used.

$$DH\ (\%) = \frac{\int(propionic\ CH_2)}{\int(propionic\ CH_2) + \int(CH_2\ PEtOx)} \cdot 100$$

Equation 3: Third formula to calculate the DH

The three different methods for calculating the degree of hydrolysis are plotted in Figure 20 separately as semilogarithmic function. The numbers in the legend refer to the order the equations of this chapter are presented (Equation 1 to Equation 3).

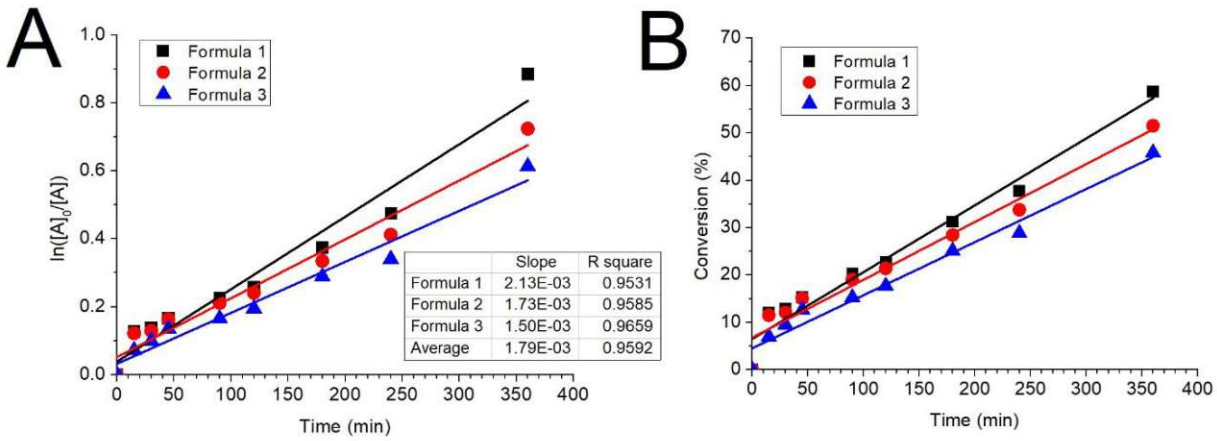


Figure 20: Hydrolysis kinetics of PEtOx (0.48 M amide concentration) in 2 M aqueous HCl at 100 °C, plotted as ln($[A]_0$/[A]) against time (A) and conversion in % against time (B)

The results shown in Figure 20 A followed a linear trend (average R^2 0.9592), as expected. The master thesis of Bauwens[102] showed the same behaviour for lower DH. Above a DH of 35 %, the reaction does not follow this first-order kinetics, however, this region was not important in present work. Nevertheless, this hydrolysis kinetics are not ideal for the desired application, because 10 % hydrolysis was reached in less than an hour (see Figure 20 B). However, for further modifications of the polymers with coumarin units, even lower DH will be needed. In that case, high deviations are expected for such fast reaction rates. Additionally, the hydrolysis rate is significantly different between the three formulas, ranging from $1.50 \cdot 10^{-3}$ to $2.13 \cdot 10^{-3}$ L mol^{-1} s^{-1}. These differences made it even harder to select a suitable time for a certain DH.

The differences in the formulations are the used integrals of the ^{1}H-NMR spectra. The most reliable methods are using Equation 1 or Equation 2 as these are based on polymeric signals. Although these signals are rather broad, the associated product is a solid, whereas propionic acid, whose signals are used for Equation 3, is a liquid. Small parts of the propionic acid can be probably removed by lyophilization, what would explain the less steep slope of the DH calculated with Equation 3.

To achieve slower reaction rate, a second experiment was performed with only 1 M HCl. PEtOx with a DP of 52 was used (sample P6 from chapter 1.1, Đ 1.14).

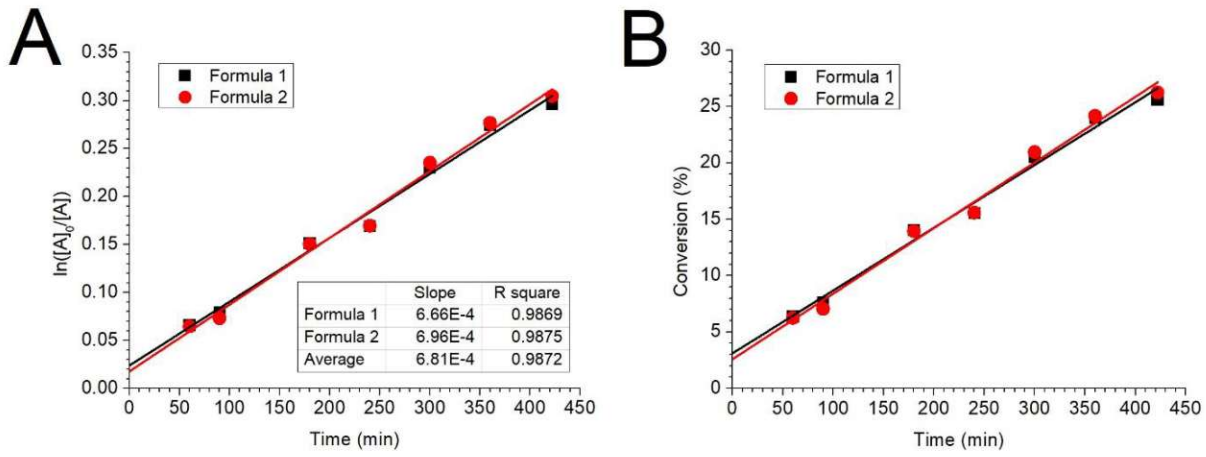
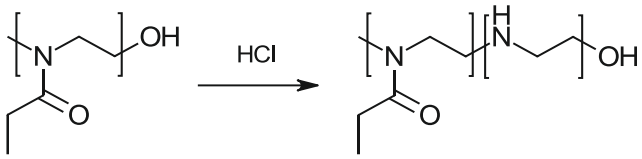


Figure 21: Hydrolysis kinetics of PEtOx (0.48 M amide concentration) in 1 M aqueous HCl at 100 °C plotted as $ln([A]_0/[A])$ against time (A) and conversion in % against time (B)

The most obvious difference of Figure 21 A compared to Figure 20 A is the less steep slope, namely $6.81 \cdot 10^{-4}$ instead of $1.79 \cdot 10^{-3}$ L mol^{-1} s^{-1}, as it was expected. The data was calculated with Equation 1 and Equation 2, as the results calculated with Equation 3 were less reliable. This second experiment provided the appropriate data to partially hydrolyze PEtOx up to 10 % in a controlled and reproducible way. For this reason, it will be used in the following chapter as a calibration to calculate the reaction time for desired DH.

3.1.5 Partial hydrolysis of poly(2-ethyl-2-oxazoline)



Scheme 22: Partial hydrolysis of PEtOx using acidic conditions

As already described previously, PEtOx had to be partially hydrolyzed as displayed in Scheme 22 so that the hydrolyzed parts can react with the synthesized coumarin derivative **9**. The desired DH were selected to be 2.5 %, 5 % and 10 %, what corresponds to the reaction times of 36, 72 and 140 minutes in aqueous 1 M HCl (see chapter 3.1.4). The polymer with a chain length of 5 kDa (PEtOx_5a) was dissolved in water to reach amide concentration of 0.48 M. To receive predicted results and minimize batch-to-batch variations, all three different DH of the polymer were prepared in one flask. After concentrated HCl was added to reach a 1 M solution, the reaction mixture was placed immediately in a preheated oil bath (110 °C). After the calculated time intervals, one third of the solution was collected *via* syringe, cooled and neutralized with an aqueous 4 N NaOH solution to reach pH 8 – 10. The part with the lowest degree of hydrolysis was dialysed for two days, the other two parts for six days (MWCO 1 kDa). The solvent was removed *via* lyophilization.

The experimental DH was calculated using ^{1}H-NMR spectra *via* the first two equations (Equation 1 and Equation 2) in chapter 3.1.4, the third equation (Equation 3) was not used because propionic acid was removed during the dialysis. The samples are named based on the previous polymerization step small changes. In PEtOx_X_Hy, X describes the theoretical chain length in kDa, H marks the partial hydrolysis and y is replaced by the actual DH in %.

Table 4: Yields in % of the partially hydrolyzed PEtOx in the first experiment (PEtOx_5a, 5 kDa PEtOx, 0.48 M amide concentration in 1 M aqueous HCl), dialyzed for 45 hours; theoretical DH calculated from the calibration curve, experimental DH calculated from ^{1}H-NMR spectra, integrals of PEtOx backbone (3.80 – 3.40 ppm) and PEI backbone (3.20 – 2.65 ppm)

Sample name	theoretical DH (%)	experimental DH (%)	Yield (%)
PEtOx_5_H11	2.5	11.1	85
PEtOx_5_H5	5	5.0	40
PEtOx_5_H7	10	6.7	9

Table 4 shows a decreasing yield with an increasing theoretical degree of hydrolysis. As this is not possible due to the workflow, a possible explanation could be that the hydrodynamic radius of the hydrolyzed polymer differs from the one of the homopolymer, so the more hydrolyzed polymer diffused through the membrane of the dialysis bags. This could also explain why the experimental DH are not corresponding to theoretical ones.

The GPC results confirmed this theory of the leakage during dialysis as displayed in Figure 22. The elugrams of the received polymers showed two peaks, one with the original chain length (5 kDa, PEtOx_5a), corresponding to unhydrolyzed part, and one with 15 kDa, what represent a part of the hydrolyzed chains. The dialysis water was evaporated, the residue dissolved in MeOH and filtered to receive the remaining polymer. The elugrams of these samples showed only the peak mentioned above with 15 kDa, what confirms our theory.

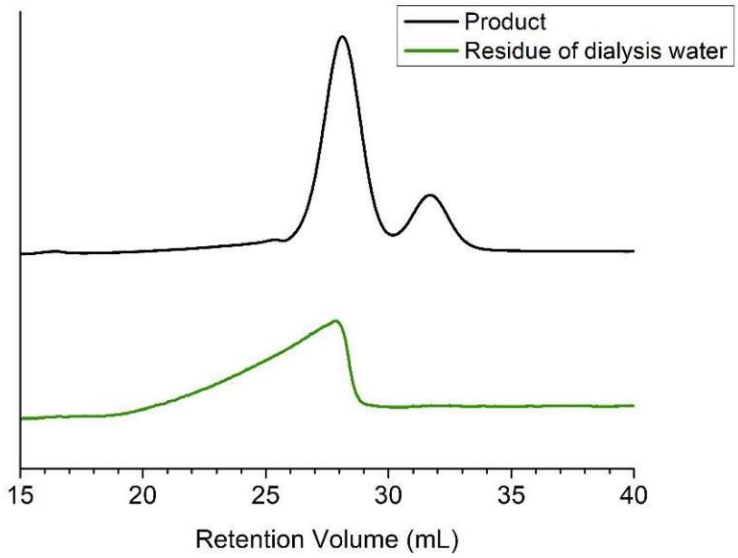


Figure 22: Cut-out from the GPC elugrams of the partially hydrolyzed PEtOx from the first experiment (5 kDa, PEtOx_5a, 5 % hydrolyzed), the obtained product and the residue obtained from the dialysis water, between 15 and 40 mL retention volume, DMSO as an eluent, PMMA as a standard.

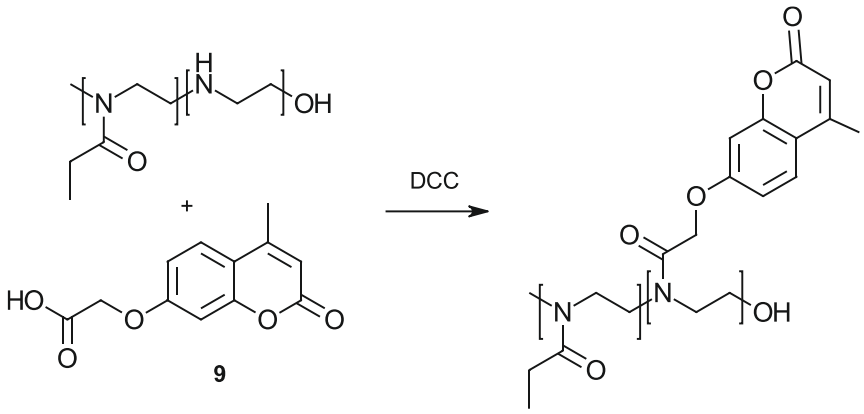
To avoid the problems of the first experiments, the second hydrolysis was carried out in three different flasks, starting with polymer chains of 10 kDa (PEtOx_10). Additionally, the dialysis time was decreased to 24 hours. As a result, the yields were much higher and comparable to each other, as displayed in Table 5.

Table 5: Yields in % of the partially hydrolyzed PEtOx in the second experiment (PEtOx_10, 10 kDa PEtOx, 0.48 M amide concentration in 1 M aqueous HCl), dialyzed for 24 hours; theoretical DH calculated from the calibration curve, experimental DH calculated from ^{1}H-NMR spectra, integrals of PEtOx backbone (3.80 – 3.40 ppm) and PEI backbone (3.20 – 2.65 ppm)

Sample name	theoretical DH (%)	experimental DH (%)	Yield (%)
PEtOx_10_H6	2.5	6.5	97
PEtOx_10_H7	5	7.0	99
PEtOx_10_H19	10	18.6	80

The experimental DHs differ significantly from the theoretical DHs. This difference is probably caused by uncertainty in the calculations *via* the ^{1}H-NMR spectra. This unprecise calculation influences especially low DH, for instance PEtOx_10_H6. These variations occurred also already during the hydrolysis kinetic study, that can lead to variations also at higher DH, such as PEtOx_10_H19.

3.1.6 *Modification of partially hydrolyzed poly(2-ethyl-2-oxazoline)*



Scheme 23: Modification of partially hydrolyzed PEtOx with coumarin moieties

The last step of the synthesis of a coumarin-modified PEtOx according to Chujo *et al.*[67] was the modification of the partially hydrolyzed polymer with the coumarin derivate **9** (Scheme 23). 1 NH-equivalent of a 10 % hydrolyzed polyoxazoline and 2 eq. of **9** were dried along with the reaction flask and set under argon atmosphere. The solids were dissolved in dry DCM and dry DMF and cooled with an ice bath to 0 °C. After adding 3 eq. of DCC in one batch, the solution was stirred overnight at RT. The precipitate was filtered off and the solvent was evaporated in vacuo, resulting in an orange viscous liquid. The crude product was dissolved in methanol and precipitated in diethyl ether. The upper phase was decanted off, leaving an oily orange residue at the bottom of the beaker. The product was dried at 40 °C in vacuo and characterized *via* ^{1}H-NMR and UV/Vis spectroscopy based on calibration to **9** in MeOH at the concentration ranging from 10^{-5} to 10^{-4} mol/L at 318 nm (see Appendix).

This reaction was carried out three times, as listed in Table 6. Polymer batches with different chain lengths and degrees of hydrolysis were used and the finished materials were compared. The following samples are named similar to the partially hydrolyzed samples, PEtOx_X_My, where X is approximately the chain length in kDa, M marks the partial modification with coumarin moieties and y is replaced by the actual coumarin content in % determined *via* NMR spectroscopy.

Table 6: Coumarin content of the samples synthesized via postpolymerization modification determined via ^{1}H-NMR spectroscopy and UV/Vis spectroscopy (both in MeOH)

Name	Hydrolyzed sample	Chain length (kDa)	DH (%)	Coumarin content (%)	
				NMR	UV/Vis
PEtOx_10_M8	PEtOx_10_H7	10	7.0	7.8	4.7
PEtOx_10_M5	PEtOx_10_H6	10	6.5	5.1	1.0
PEtOx_5_M4	PEtOx_5_H11	5	11.1	3.6	3.4

There is a clear difference in the DH of Table 6 and the coumarin content of the same sample. In case of PEtOx_10_M8, the coumarin content determined *via* ^{1}H-NMR spectroscopy exceeds the DH of the

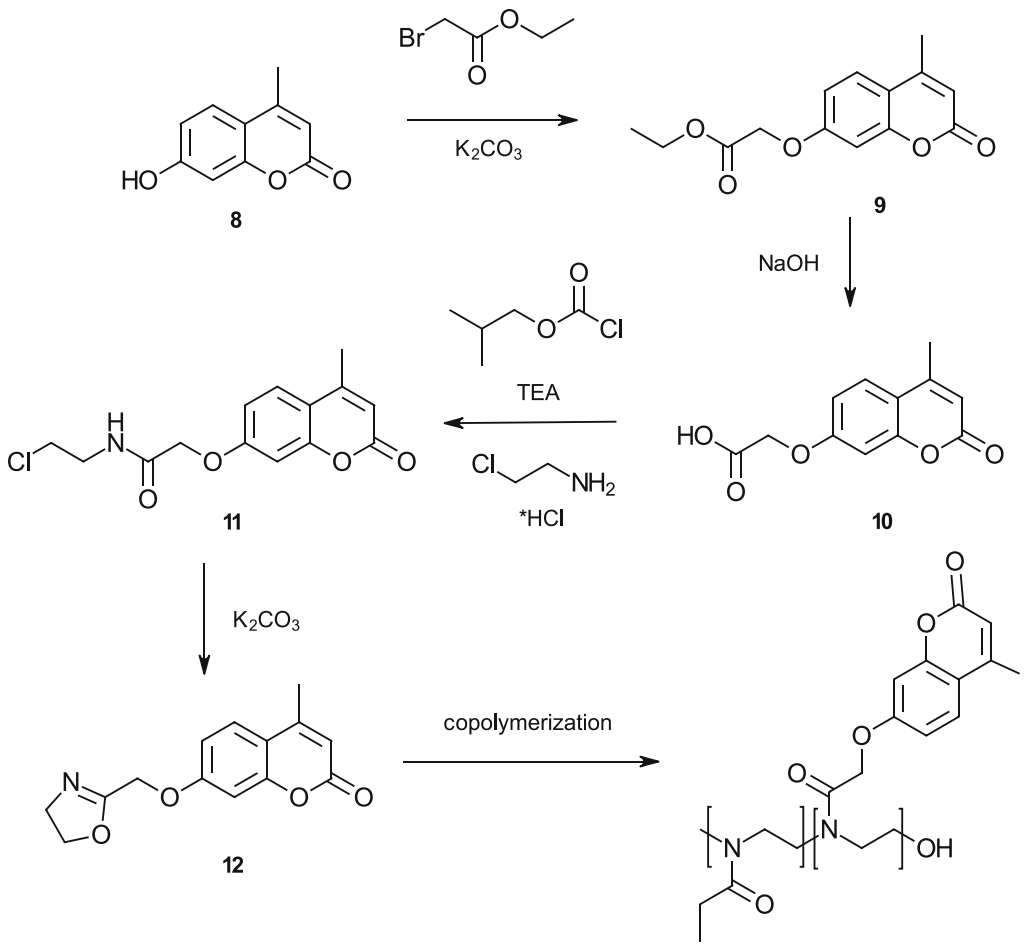
particular copolymer, what is unrealistic. It is also not negligible that the coumarin content determined by NMR spectra is significantly different than the coumarin content determined by UV/Vis spectroscopy for higher coumarin contents. Both the UV/Vis and the NMR spectra were measured in MeOH, consequently the behaviour of the polymer should be similar. Additionally, it is interesting that the sample with the highest DH (PEtOx_5_H11, 11.1 %) resulted in a modified sample with very low coumarin content (PEtOx_5_M4, 3.6 / 3.4 %). Most likely, this postpolymerization modification strategy results for the most cases in only partially modified PEtOx with some PEI units still present.

The difference in the characterization methods can be explained by uncertainties in every method, the DH (*via* NMR), the degree of modification *via* NMR and *via* UV/Vis spectroscopy. Considering a standard deviation for each measurement, the results can be also within the same value range and therefore matching within each sample, although it would be difficult to prove it.

3.2 Copolymerization strategy

During these syntheses *via* the postpolymerization modification strategy, especially during the partial hydrolysis and modification of the polymer backbone, some problems occurred, which were challenging to solve. On one hand, the adjustment and determination of the DH was challenging. On the other hand, the subsequent modification of the hydrolyzed polymer parts was not complete, what resulted in coumarin-modified PEtOx containing PEI units. Therefore, it is not easy to control the coumarin content in the modified samples.

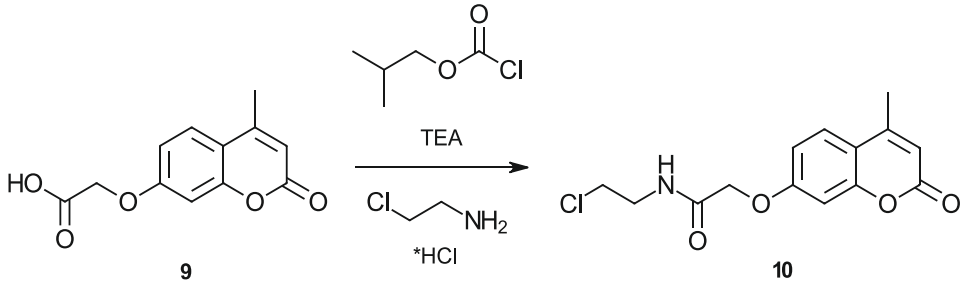
As a solution to these problems, the aim was to synthesize a coumarin-containing 2-oxazoline (Scheme 24). Copolymerization leads to pure coumarin-containing POx without any PEI units. Additionally, the coumarin content can be adjusted easily by using the appropriate ratio of the comonomers in the polymerization mixture, presuming full conversion of the monomes. It should be noted that such 2-oxazoline monomer was not described in the literature yet. The chosen synthetic strategy was inspired by previously prepared thiol-containing 2-oxazoline. The first two steps of this route have been already accomplished in chapter 3.1 Postpolymerization modification strategy (Chujo *et al.*[67]), the amidation with the following ring closing was carried out similar to chapter 2.1.1.2 and 2.1.1.3 according to Cesana *et al.*[66] This monomer can be copolymerized with EtOx and with other oxazoline-based monomers.



Scheme 24: Synthesis route for receiving coumarin-modified poly(2-ethyl-2-oxazoline) (PEtOx) via copolymerization

3.2.1 Monomer synthesis

3.2.1.1 Synthesis of N-2-Chloroethyl(4-methyl-7-coumarinyloxy)acetamide **10**

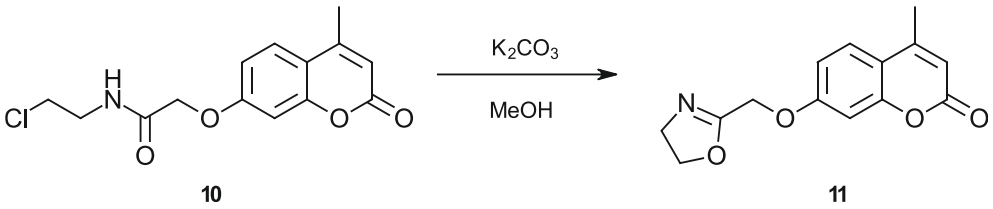


Scheme 25: Synthesis of N-2-chloroethyl(4-methyl-7-coumarinyloxy)acetamide

To obtain the coumarin-containing monomer, **10** is synthesized in a similar reaction (Scheme 25) as Cesana *et al.*[66] used for synthesizing a protected thiol-containing monomer, also applied in chapter 2.1.1.2. To 1 eq. **9** in a dry flask, THF and 1.1 eq. TEA were added to receive a slightly turbid solution that was cooled to 0 °C. 1 eq. isobutyl chloroformate was added and the turbid suspension was stirred for 10 minutes at 0 °C and for further 20 minutes at RT. After cooling it again with an ice bath, 1.2 eq. chloroethylamine hydrochloride in dry DMF and 1.1 eq. TEA were added. The mixture was stirred for 2 hours at RT before the solvent was evaporated to receive a slightly yellow solid crude

product. This was dissolved in DCM and filtered. By-products were precipitated by adding diethyl ether, the product was afterwards precipitated by adding PE, resulting in 74 % of a white product, characterized *via* NMR spectrometry, HR-MS and melting point.

3.2.1.2 Synthesis of 2-[(4-Methyl-7-coumarinyloxy)methyl]oxazoline (CoumarinOx) **11**

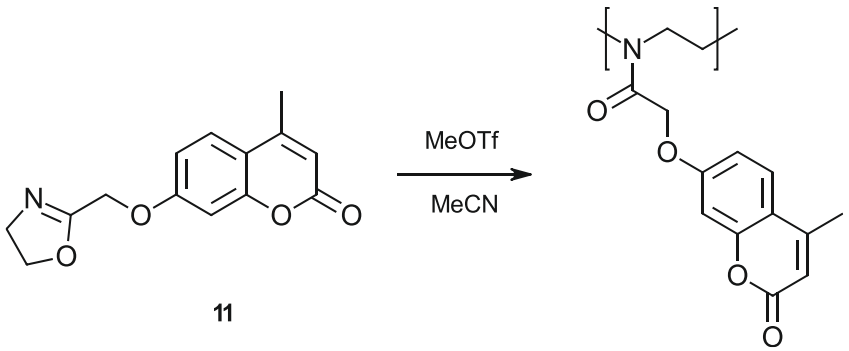


Scheme 26: Synthesis of 2-[(4-methyl-7-coumarinyloxy)methyl]oxazoline (CoumarinOx)

The last step of the synthesis of a coumarin-containing oxazoline was the ring closing depicted in Scheme 26 following Cesana *et al.*[66] similar to chapter 2.1.1.3. 1 eq. of **10** and 2.1 eq. anhydrous K_2CO_3 were weighted in a flask, set under argon and dry methanol was added. The mixture was refluxed for 5 hours, the solvent was evaporated after cooling it to RT. The off-white residue was dissolved in DCM and centrifuged to remove the yellow solid. The solution was dried to yield in 45 % off-white solid that was characterized *via* NMR spectroscopy, HR-MS and melting point.

3.2.2 Polymer synthesis

3.2.2.1 Homopolymerization of coumarin-containing 2-oxazoline (CoumarinOx) **11**



*Scheme 27: Homopolymerization of CoumarinOx **11***

As **11** is not known in literature, the rate of its homopolymerization as shown in Scheme 27 was investigated before proceeding to copolymerize it with any other 2-oxazoline. 50 eq. of **11** were dried on the HV before adding a stock solution of methyl triflate (MeOTf) in dry acetonitrile (ACN, 1 eq. initiator). Due to low solubility of the monomer, more solvent was added until it finally dissolved in the preheated oil bath at 75 °C (overall 0.2 M solution). From this moment on, samples were withdrawn according to Table 7.

*Table 7: Timetable for the drawn samples during the homopolymerization of **11***

No.	1	2	3	4	5	6
Time (min)	0	60	120	180	240	420

During the polymerization it was observed that the withdrawn sample precipitated during cooling down to RT, but it could be redissolved in $CDCl_3$ to measure ^{1}H-NMR spectra. After full conversion in 28 hours, the polymerization mixture was cooled to 0 °C, where again a white solid precipitated, and terminated with 1 M methanolic KOH. The solvent was evaporated, but the polymer was not further purified because it was not possible to dissolve it in any solvent.

The conversion of the samples was determined using NMR spectroscopy and plotted in Figure 23, compared to the homopolymerization of EtOx and 2-methyl-2-oxazoline (MeOx) under similar polymerization conditions, only the concentration of the monomer was lower (0.2 M instead of 3 M) due to solubility issues.

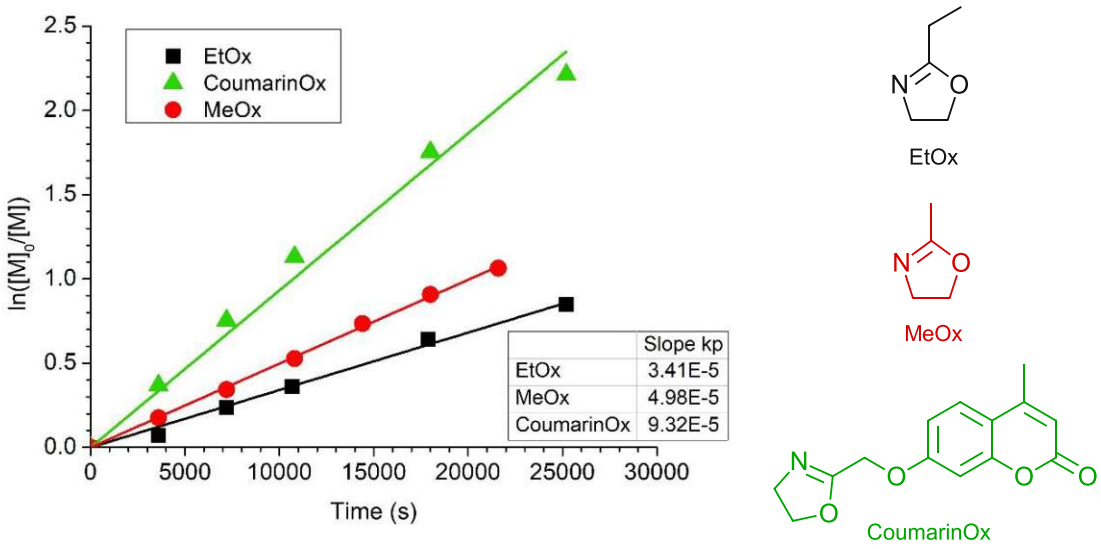
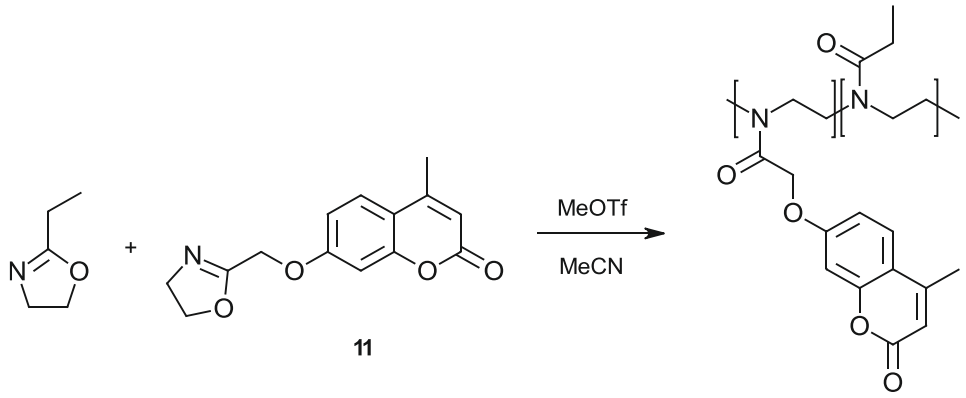


Figure 23: Homopolymerization kinetics of the new CoumarinOx compared with EtOx and MeOx with acetonitrile as solvent and MeOTf as initiator at 75 °C

Surprisingly, CoumarinOx showed a faster polymerization rate than EtOx and MeOx but showed the requested linear behaviour to proof its living character. Bouten *et al.*[104] experienced a similar kinetic behaviour by polymerizing methyl ester containing 2-oxazolines that polymerized also faster than EtOx. This is explained by the interaction of the methyl ester group with the living chain end that is increasing its electrophilicity. Although there is no methyl ester group in the new synthesized coumarin-containing monomer, the ester group could anyway interact with the living chain end and influence thereby the polymerization kinetics.

3.2.2.2 Copolymerization of the coumarin-containing 2-oxazoline (CoumarinOx) **11**



Scheme 28: Copolymerization of CoumarinOx and EtOx using MeOTf as initiator and ACN as solvent

The main goal was to synthesize comparable materials as the ones obtained via postpolymerization modification[67] and compare their properties afterwards. To be sure that the copolymer can be crosslinked after its synthesis, the highest measured coumarin content (from PEtOx_10_M8, 7.8 % determined via ^{1}H-NMR spectroscopy, 5.3 % via UV/Vis spectroscopy) was selected and synthesized according Scheme 28. 8 eq. **11** and 1 eq. MeONs were dried in a vial, dissolved in dry acetonitrile and 92 eq. EtOx were added to achieve a product with a DP of 100 and 8 % coumarin content. The mixture was heated to 75 °C for 26 hours, cooled to 0 °C and terminated with 1 M methanolic KOH. This polymerization time was based on the homopolymerization kinetics study, since the used monomers were supposed to reach full conversion. The slightly yellow, turbid solution was precipitated two times in ice cooled diethyl ether, the yellow residue was dried on the rotary evaporator at 40°C and afterwards in the oven at 45 °C overnight, yielding 67 %.

^{1}H-NMR spectroscopy was employed to determine the coumarin content in synthesized copolymers. 5 different solvents were used, since the formation of amphiphilic gradient copolymer could be expected, as the polymerization rates of the monomers differ significantly. As a result, self-assembly could be expected in solvent selective for one block, which could lead to decreased NMR signal of the inner block, altering the measured copolymer composition. The coumarin content calculated from these spectra is compared in Table 8. The polymer was well soluble in all of the solvents according to visual evaluation.

Table 8: Coumarin content of the copolymerized sample consisting of CoumarinOx and EtOx based on ^{1}H-NMR spectra in different solvents

Solvent	Coumarin content
D_2O	2.6 %
THF	2.0 %
MeCN	2.3 %
DMSO	2.8 %
MeOD	2.6 %

According to Table 8, the coumarin content in the different spectra were all similar, so the formation of micelles could be excluded.

However, the average coumarin content is 2.5 % instead of the theoretical 8 %. As the CROP of 2-oxazolines has a living character, the polymerization reaction is supposed to reach 100 % conversion. Obviously, this was not the case in the previous experiment, so a new copolymerization was set up to determine the reaction kinetics. Additionally, the reaction was set up also with MeOx as copolymer.

20 eq. of **11** were weighted into a vial and dried in HV before adding 80 eq. EtOx or MeOx, respectively, 2 eq. MeOTf as initiator (DP 50) and acetonitrile as solvent (1.5 M solution). The vial was put into a preheated oil bath at 75 °C to start the polymerization and samples were drawn according to Table 9.

Table 9: Withdrawn samples to determine the kinetics of the copolymerization of EtOx and CoumarinOx

No.	1	2	3	4	5	6	7	8
Time (h)	0	16	24	40	48	64	72	88

The withdrawn samples were diluted with $CDCl_3$ and ^{1}H-NMR spectra were measured. The determined conversion was plotted in Figure 24. Additionally, the homopolymerization plots from CoumarinOx, EtOx and MeOx were added for comparison.

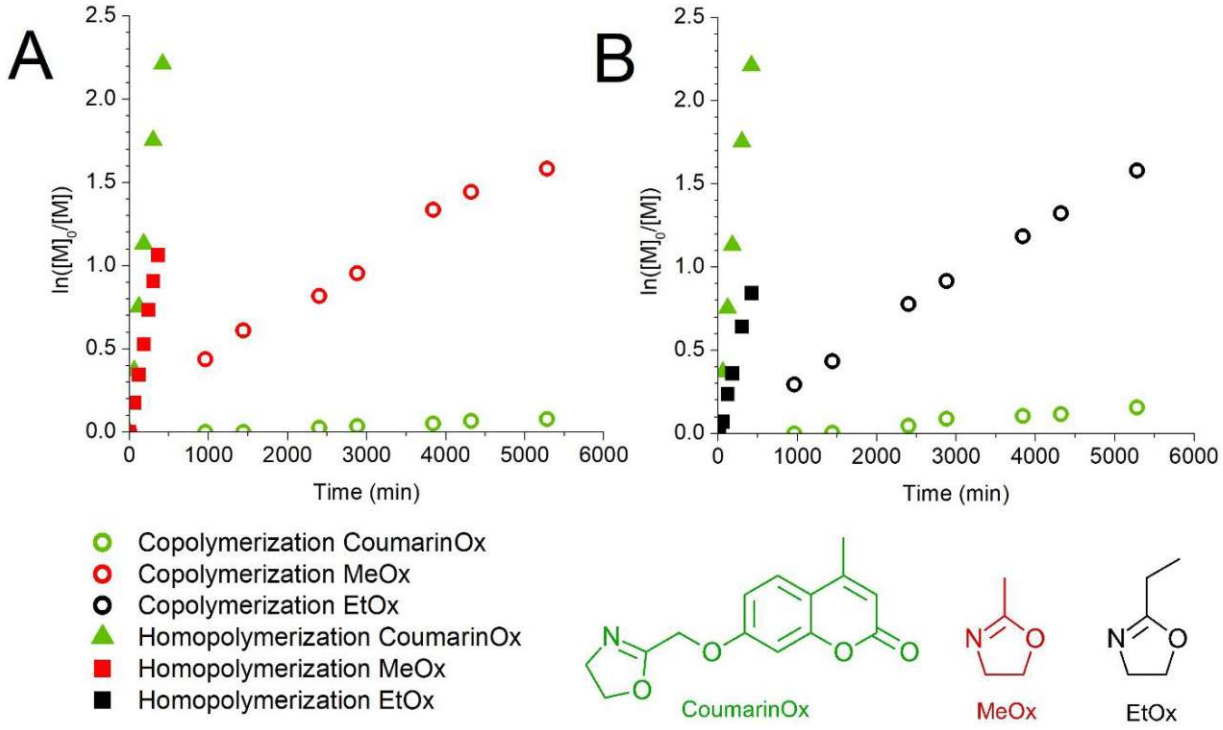


Figure 24: Comparison of the homopolymerization kinetics of the CoumarinOx, MeOx and EtOx with the copolymerization kinetics of CoumarinOx with MeOx (A) and with EtOx (B) in acetonitrile at 75 °C with MeOTf as an initiator ([M]:[I]=50:1).

The most obvious change in Figure 24 is the inversion of the polymerization rates of CoumarinOx, EtOx and MeOx. During the homopolymerization, CoumarinOx is polymerizing faster than the comonomer, but during the copolymerization the rates are reversed. Additionally, the polymerization rates of both MeOx and EtOx during the copolymerization are slower than during their homopolymerization.

A similar inversion of the polymerization rates during a copolymerization was observed by Sedlacek *et al.*[43] concerning the copolymerization of a 2-alkyl-5,6-dihydro-4H-1,3-oxazine with a 2-alky-2-oxazoline, but here the plot did not show linear behaviour. There, the copolymerization resulted in a gradient copolymer due to the different polymerization rates of the monomers.

Based on these kinetic studies, three copolymers were synthesized following the procedure described in this chapter with coumarin contents chosen according to Table 10. The polymerization time was prolonged to reach 100 % conversion. The samples are named according to PEtOx/PMeOx_X_Cy, where PEtOx or PMeOx describe the copolymer of CoumarinOx during the polymerization, X is replaced by the approximate chain length in kDa, C stands for copolymerized sample and y is the coumarin content in % determined by NMR spectroscopy.

Table 10: Coumarin content of the copolymerized samples containing CoumarinOx and either EtOx or MeOx, calculated from the initial weight (theoretical), from ^{1}H-NMR spectra (NMR) and from UV/Vis measurements (UV/Vis)

Name	Comonomer	DP	Coumarin content (%)		
			Theoretical	NMR	UV/Vis
PEtOx_10_C3	EtOx	100	8.0	2.5	2.9
PEtOx_10_C8	EtOx	100	8.0	8.2	5.3
PMeOx_10_C5	MeOx	100	8.0	5.3	4.0

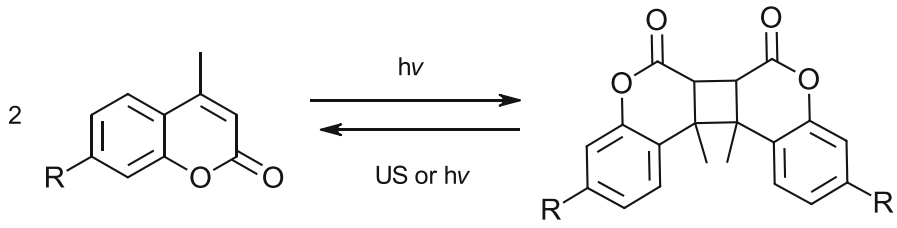
The target coumarin content for copolymerized samples was 8 % to be comparable with selected sample synthesized *via* the postpolymerization modification strategy (PEtOx_10_M8, 4.7 % *via* UV/Vis spectroscopy, 7.8 % *via* NMR spectroscopy). As described earlier, PEtOx_10_C3 was terminated prematurely, so the measured final coumarin content was lower than the feed. The following experiments with EtOx (PEtOx_10_C8) and MeOx (PMeOx_10_C5) were polymerized for a longer time, consequently the measured coumarin content was higher. As the copolymerization kinetics of EtOx and MeOx with CoumarinOx are different, also the coumarin content of the copolymers is different. Comparing the results of the coumarin content determined *via* two different methods, similar inconsistencies as discussed in chapter 3.1.6 were observed. For samples with lower coumarin content, such as PEtOx_10_C3, the results of NMR and UV/Vis spectroscopy are very similar. In contrast, the two other samples with higher coumarin content show significant differences between the two methods. Also in this case, the NMR spectroscopy displayed higher coumarin contents compared to the UV/Vis spectroscopy. Here it has to be mentioned that for the NMR spectra, DMSO was used as a

solvent, and for UV/Vis spectroscopy hexafluoro isopropanol was used due to the solubility problems in other solvents. The polymer is due to the gradient structure likely to behave differently in different solvents, so this could be an explanation of the observed differences in the coumarin content.

3.3 Crosslinking

The coumarin side chains of the modified polymer further served as crosslinking points to create a polymer network. The crosslinking proceeds through dimerization *via* [2+2] cycloaddition which is triggered by irradiation with UV light with a wavelength above 360 nm,[105] shown in Scheme 29. Employing this method, hydrogels can be obtained. Additional to the cleavage by US, coumarin dimers can undergo photo-scission by irradiation with light below 280 nm.

This chapter is divided into two parts. The first part is focused on crosslinking the polymers synthesized *via* the postpolymerization modification strategy. In the second part, the crosslinking of the copolymers prepared *via* the copolymerization strategy is described.



Scheme 29: Reversible dimerization of coumarin groups via [2+2] cycloaddition

3.3.1 Crosslinking of the modified polymers

For the first crosslinking trials, a coumarin-modified poly(2-oxazoline) with 4.7 % (UV/Vis) / 7.8 % (NMR) coumarin content and a molar mass of 10 kDa was selected (PEtOx_10_M8). The used formulations for the first gelation attempts are shown in Table 11. Therefore, EtOH and distilled water (DW) were used as solvent with either 10 or 20 wt% copolymer as formulation.

The solvents for the formulation were chosen according to the solubility of the main polymer chain PEtOx. Additionally, to keep in mind the future application as drug delivery system, DW and ethanol are two of the least toxic solvents. The solvent is also affecting the dimerization rate, therefore different mixtures of DW and EtOH were investigated. Benzophenone was added as a photosensitizer to increase the coumarin dimerization rate. Benzophenone is only soluble in DW and not in EtOH, therefore it was not used with every solvent combination.

Table 11: Formulations for the first gelation study with a coumarin-modified polymer containing 4.7 % (UV/Vis) / 7.8 % (NMR) coumarin (PEtOx_10_M8) using the UV oven for 30 minutes at 94 mW/cm² (320 – 500 nm).

No.	Copolymer (wt%)	Solvent 1	Solvent 2	Ratio of the solvents (vol)	Benzophenone (wt%)	Gel
1	10	EtOH	-	-	-	No
2	10	EtOH	-	-	1	Yes
3	20	EtOH	-	-	-	No
4	20	EtOH	-	-	1	Yes
5	10	EtOH	DW	3:1	-	No
6	10	EtOH	DW	3:1	1	No
7	20	EtOH	DW	3:1	-	No
8	20	EtOH	DW	3:1	1	No
9	10	EtOH	DW	1:1	-	No
10	20	EtOH	DW	1:1	-	No
11	10	H_2O	-	-	-	No
12	20	H_2O	-	-	-	No

The polymer in the formulations number 11 and 12 did not dissolve well using DW as a solvent, resulting in a turbid suspension. For this reason, the solvent was changed to 1:1 mixture of ethanol and water, what led to well-dissolved copolymers. 210 µL of each formulation were put into a silicon mold, closed with a small round glass coverslip and sealed with grease. The silicon mold was put into the UV oven for 30 minutes at 94 mW/cm² (320 – 500 nm). In the molds of the aqueous formulations number 11 and 12, the solid part precipitated during the irradiation. From all the other formulations with ethanol, the solvent evaporated because of the higher temperatures in the UV oven, some of them even formed bubbles on the surface. After another 15 minutes in the UV oven, the gel-like residues were put into excess of water. Only formulation number 2 and 4 resulted in hydrogels, all the other samples dissolved. These crosslinked samples both contained ethanol as a solvent and benzophenone as sensitizer. The copolymer concentration did not play crucial role in the crosslinking efficiency.

Due to the benzophenone content in the successfully crosslinked hydrogels, it is probable that additionally to the coumarin dimers formation, also hydrogen extraction from copolymer backbone and radical crosslinking mechanism took place. Therefore, the following experiments were carried out without benzophenone. The polymer content was increased, supposing to increase the crosslinking rate. Also, the light source was changed from the UV oven to 365 nm LED, on one hand to avoid the evaporation of the solvent caused by high temperatures in the UV oven, and on the other hand to exclude all the wavelengths below 280 nm that would cleave the coumarin dimers.

Another hypothesis for the low crosslinking efficacy of the formulation is that some coumarin derivative is still present in the sample, due to the insufficient cleaning step after postpolymerization modifications of copolymers. As a result, the presence of free coumarin could prevent gel formation. To test this hypothesis, dialysis of the formulation (50 wt% polymer in EtOH) against ethanol was carried out for three hours with the same sample used in previous crosslinking experiment (PEtOx_10_M8, 4.7 % coumarin content, 10 kDa), displayed in Table 12. In these experiments, only 100 µL per sample were used for the hydrogel formation and they were irradiated by LED lamp (365 nm) with 85 % intensity (270 mW/cm²) and a distance of 18 mm with irradiation times between 30 minutes and one hour.

Table 12: Formulation for the second gelation study with a modified polymer (PEtOx_10_M8, 4.7 % (UV/Vis) / 7.8 % (NMR) coumarin content, 10 kDa) without benzophenone, irradiated with the LED lamp (365 nm) with 270 mW/cm² and 18 mm distance

No.	Polymer (wt%)	Solvent	Dialysis	Irradiation (min)	Gel
I	50	EtOH	Yes	60	Yes
II	50	EtOH	No	30	Yes
III	40	EtOH	No	60	Yes
IV	35	Dioxane	No	30	Yes
V	25	Dioxane	No	30	Yes
VI	25	EtOH	No	90	No

The first two samples showed that there was no difference between the dialyzed and the untreated sample, both were crosslinked. The only difference was the irradiation time, as sample No. I was diluted due to the dialysis. However, the irradiation with the LED caused partial evaporation of ethanol. Dioxane was thus further proposed as an alternative solvent because of higher boiling point. Dioxane was indeed evaporating less than other used solvents, and additionally the crosslinking was faster than in any previously used solvent, as observed by comparison of sample V and VI. Formulations with dioxane were used for further experiments, because in this case also lower concentrations of copolymer were successfully crosslinked. Additionally, solvent evaporation was avoided during crosslinking.

For the third crosslinking experiment, formulation of 40 wt% polymer in dioxane was selected. 100 µL of the formulation was irradiated with 365 nm LED lamp for one hour with an intensity of 270 mW/cm². Compared to the hydrogel formation attempts before, samples with different coumarin content and chain length were used (Table 13).

Table 13: Polymers with different coumarin content as a formulation with 40 wt% polymer in dioxane used for the third gelation study irradiated with the LED lamp (365 nm) with 270 mW/cm² and 18 mm distance for one hour

No.	Name	Coumarin UV/Vis (%)	Coumarin NMR (%)	Chain length (kDa)	Gel
A	PEtOx_10_M8	4.7	7.8	10	Yes
B	PEtOx_10_M5	1.0	5.1	10	No
C	PEtOx_5_M4	3.4	3.6	5	No

Only sample A resulted in a hydrogel, so this polymer (PEtOx_10_M8) was selected for further experiments where the gel content and the swelling degree were investigated. The coumarin content of sample No. B was probably too low to result in hydrogels with these conditions of irradiation. According to UV/Vis spectroscopy, sample No. C (PEtOx_5_M4) has a similar, only slightly lower coumarin content as the gelling sample No. A (PEtOx_10_M8). Another difference is the shorter chain length of sample No. C, what can also cause a different behaviour during the gelation by irradiation.

As the second gelation study in Table 12 confirmed, a formulation according to No. V (25 wt% PEtOx_10_M8 in dioxane) was used. The gelation with the LED was carried out for one hour in order to achieve quadruplicates. The gels were put for three hours into EtOH to wash out not crosslinked copolymer chains, and afterwards they were swollen for 24 hours in distilled water, frozen and lyophilized to calculate the gel content and the swelling degree (Table 14).

Table 14: Gel content and swelling degree of gels made with the sample with the highest coumarin content from the third gelation study (PEtOx_10_M8, 4.7 % (UV/Vis) / 7.8 % (NMR) coumarin) in dioxane (25 wt% polymer, Mod) irradiated for one hour with the LED lamp (365 nm) with 270 mW/cm² and 18 mm distance

Sample	Swollen (mg)	Dry (mg)	Gel content (%)	Swelling degree
Mod 1	95.2	3.5	14.0	26.2
Mod 2	110.1	3.7	14.8	28.8
Mod 3	102.5	5.1	20.4	19.1
Mod 4	145.7	5.8	23.2	24.1
Average	-	-	**18.1 ± 4.4**	**24.5 ± 4.1**

Both the gel content and the swelling degree are rather low, the gel content could be increased either by irradiating it with higher intensity or for a longer time, or additionally by using samples with higher coumarin content. As the samples were prepared by postpolymerization modification, it is also possible that some polymer chains have more coumarin moieties than others. In this case, the polymer chains with less coumarin have a lower chance to crosslink than chains with more coumarin. It is also possible that this synthesis route results in some polymer chains without any coumarin moieties.

In contrast, the swelling degree would increase if there would be less crosslinked coumarin moieties. Zahoranová *et al.*[106] investigated the swelling degree of POx based hydrogels crosslinked by

introducing three different bis(2-oxazoline)s. The highest swelling degree in DW was reached by using only 2 % of each bis(2-oxazoline), resulting in 21.3 ± 0.9 for the most swollen gel. For gels with 5 % bis(2-oxazoline), the gel content decreased to 4.7 ± 0.3 for the same sample type. The modified samples in this work showed with a swelling degree of 24.5 ± 4.1 correspond well with previous results, despite it is not exactly known how many coumarin moieties are dimerized.

3.3.2 Crosslinking of the copolymers

3.3.2.1 Coumarin – coumarin crosslinking

Further, the crosslinking of the other sample type was proceeded to study, coumarin-containing copolymers prepared *via* copolymerization of EtOx and the newly synthesized CoumarinOx (see chapter 3.2.1). For the copolymerized samples the goal was the same as for the modified samples: to achieve the crosslinking of the coumarin moieties and receive stable hydrogels.

The first gelation experiments were performed with sample PEtOx_10_C3 (coumarin content 2.9 % (UV/Vis) / 2.5 % (NMR), see Table 10). Similar to the copolymerization of the modified polymers, 25 wt% of this polymer were dissolved in dioxane and 100 µL were irradiated in a silicon mold with a 356 nm LED lamp with 270 mW/cm² for 45 minutes, but no gel was formed. The polymer content was increased to 50 wt% and neither in ethanol nor in methanol a hydrogel could be obtained after more than one hour irradiation with the same conditions. The reason was the low coumarin content, as the same problem occurred in chapter 3.3.1 where no sample below 4 % coumarin was crosslinked successfully.

To achieve crosslinking, the newly synthesized PEtOx_10_C8, with 5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content was used. Simultaneously, also a PMeOx-co-poly(CoumarinOx) (PMeOx_10_C5) with 4.0 % (UV/Vis) / 5.3 % (NMR) coumarin was attempted to be crosslinked. Unfortunately, the solubility of the copolymerized samples with each EtOx and MeOx was lower in majority of the solvents (Table 15).

Table 15: Solubility study of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin) and PMeOx_10_C5 (4.0 % (UV/Vis) / 5.3 % (NMR) coumarin) samples in various solvents and their polarity as electric dipole moment (✓fully soluble, + slightly turbid, ++ little turbid, +++ turbid, ++++ very turbid, - insoluble)

Solvent	Polarity (*10^{-30} Cm)	EtOx copolymer	MeOx copolymer
HFIP	-	✓	✓
DMSO	13.00	+	
H_2O	6.07	++++	+++
$CHCl_3$	3.84	+++	
DCM	5.17	+++	++++
DMF	12.88	++	
DMA	12.41	++	
EtOH	5.77	++++	
MeOH	5.67	++++	
EE	6.27	-	
Acetone	9.54	+++	-
THF	5.84	+++	-
Dioxane	1.50	+++	-

As these solubility experiments confirmed, the polarity and solubility of the copolymerized samples are completely different that PEtOx and PMeOx, but most surprisingly also than the obtained polymers obtained by the postpolymerizaiton modification strategy. As already assumed due to the investigation of the copolymerization kinetics in chapter 3.2.2.2, CoumarinOx is not statistically distributed in the whole polymer chain, but rather concentrated at the end of the polymer chain. This explains also the relative turbidity in various solvents (represented as crosses), as the insoluble part of the polymer formed aggregates. The relative turbidity follows the trend that both copolymers are better soluble in solvents with higher polarity.

Based on these results, DMSO was selected as solvent for formulations because of relatively low turbidity and high boiling point. HFIP was excluded due to the low boiling point and toxicity.

The crosslinking experiments started with PEtOx_10_C8 (chapter 3.1) as it was the most comparable to the modified copolymer that was used previously for the synthesis of hydrogels. The change in the solvent and the polymeric structure resulted in a different crosslinking behaviour, so the conditions used in chapter 3.3.1 were not suitable for this system (365 nm LED with 270 mW/cm² and 18 mm distance) and needed to be further optimize. After various optimization trials, the following conditions were changed to improve the crosslinking rate: The polymer content was increased to 40 wt% instead of the previous 25 wt% and only 50 µL formulation were used instead of 100 µL. Nevertheless, the irradiation intensity was not high enough to crosslink the polymer chains within one hour. Thus, the 365 nm LED was replaced by a broadband lamp (OmniCure 3, 320 – 500 nm) with higher intensity of 5.50 W/cm², the distance kept at 18 mm. To avoid damaging the sample due to high intensity, the

intensity was stepwise decreased to 2.51 W/cm² where the polymer was crosslinked within 30 minutes. Employing these conditions, 6 samples were crosslinked in total. Afterwards, the gels were swollen in DW for at least 24 hours, frozen and lyophilized. The properties of hydrogels containing EtOx are listed in Table 16. Compared with the gels made of modified polymer in chapter 3.3.1 (gel content 18.1 ± 4.4 %, swelling degree 24.5 ± 4.1), the gel content is only slightly lower, but the swelling degree is noticeable lower, although the standard deviation of the copolymerized samples is lower in both cases. As the swelling degree will increase with lower crosslinking density, it is probable that these copolymerized samples contain more dimerized coumarin moieties than the samples made by postpolymerization modification, although the coumarin content is similar. This can be explained by the different structure and distribution of functional groups. The functional groups of a gradient copolymer are closer in proximity than a statistical copolymer, what can reduce the crosslinking time and crosslinking density.

Table 16: Gel content and swelling degree (in water) of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content) in the pure polymer gel (40 wt% in DMSO, CoEt) irradiated for 30 minutes with the OmniCure 3 (320 – 500 nm), 2.51 W/cm² with 18 mm distance

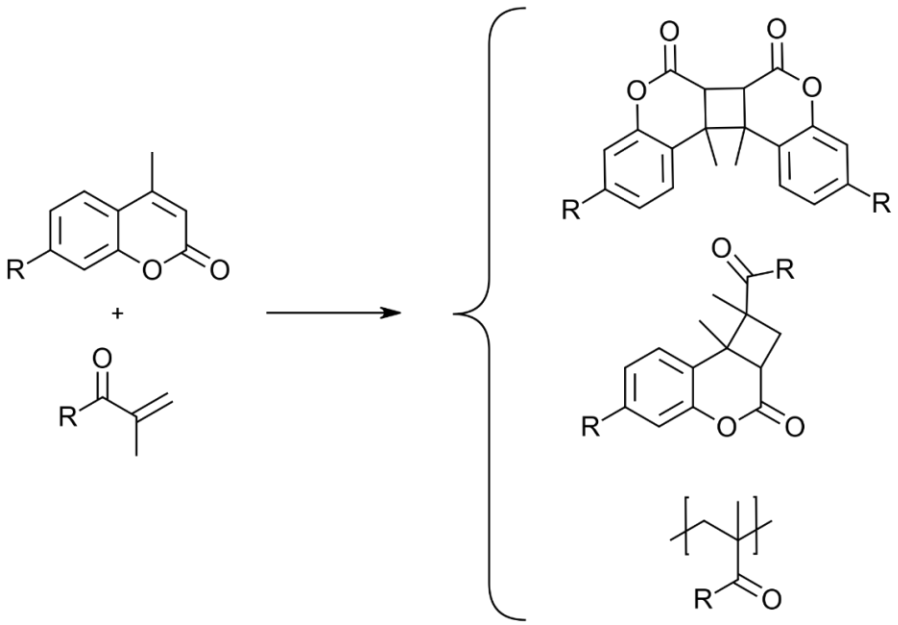
Sample	Swollen weight (mg)	Dry weight (mg)	Gel content (%)	Swelling degree
CoEt 1	61.5	2.8	12.8	21.0
CoEt 2	39.9	3.5	15.9	10.4
CoEt 3	50.3	2.8	12.8	17.0
CoEt 4	53.0	2.9	13.2	17.3
CoEt 5	42.0	2.8	12.8	14.0
CoEt 6	48.7	2.8	12.8	16.4
Average	-	-	**13.4 ±1.3**	**16.0 ± 3.5**

On the other hand, it was not possible to crosslink PMeOx_10_C5 employing these conditions. The possible reason was the lower coumarin content. Another cause is possibly also the different behaviour in DMSO and its affinity to form micelles. PMeOx is even more hydrophilic than PEtOx, therefore also the difference in hydrophilicity between the poly(CoumarinOx) parts and the PMeOx parts in the copolymers is bigger, what increases the probability of micelle formation.

3.3.2.2 Coumarin – acrylate crosslinking

In the previous chapter, the crosslinking of POx copolymers with coumarin moieties *via* UV light was studied. However, the described reaction is rather slow and can also be improved quantitatively as the gel content is very low. Approximately at the same time in 2021, a paper from Eng *et al.*[95] was published where the authors described a system containing both coumarin and methacrylate moieties that can be crosslinked by irradiation and decrosslinked by US. The used polymer consisted of a PEG backbone with both coumarin and methacrylate end groups. During gelation, three reactions took place simultaneously according to the authors: the [2+2] cycloaddition between coumarin and coumarin,

between coumarin and methacrylate and the initiator-free radical polymerization of the methacrylates (displayed in Scheme 30).



Scheme 30: Crosslinking mechanisms of coumarin and methacrylate moieties, resulting in coumarin dimers, coumarin/methacrylate adducts and poly(methacrylate)[32]

To improve the stability of the hydrogels made of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin), PEG diacrylate ($\bar{M}_n$ 700 g/mol) was added to the formulation and the overall polymer content was decreased from 40 to 25 wt% in DMSO. To reach similar conditions as Eng *et al.*[95] the molar ratio of PEG diacrylate to coumarin was set to 1:1, resulting after irradiation of 15 min with 320 – 500 nm, 2.51 W/cm² at a distance of 18 mm in a slightly yellow, stable hydrogel. As a control experiment, a 25 wt% formulation of PEG diacrylate in DMSO was also irradiated and resulted also in a hydrogel, although exhibiting significant shrinkage.

As our work is mainly focused on the reversible crosslinking of coumarin moieties, the PEG diacrylate content was decreased to 12.3 wt% and mixed with 12.0 wt% coumarin copolymer that was also used last in chapter 3.3.2.1. For each of the triplicates, 50 µL of the formulation were put into a round silicon mold with 10 mm diameter and closed with a glass coverslip. The irradiation conditions remained the same as for the coumarin – coumarin crosslinking (30 minutes at 320 – 500 nm, 2.51 W/cm² with 18 mm distance) so the results can be compared. The hydrogels were swollen for three days in distilled water and afterwards lyophilized. The calculated gel content and swelling degree from the swollen and dried weights are summarized in Table 17.

Table 17: Gel content and swelling degree (in distilled water) of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content) mixed with PEG diacrylate (12.0 wt% coumarin polymer and 12.3 wt% PEG diacrylate in DMSO, CoA) irradiated for 30 minutes with the OmniCure 3 (320 – 500 nm), 2.51 W/cm² with 18 mm distance

Sample	Swollen weight (mg)	Dry weight (mg)	Gel content (%)	Swelling degree
CoA 1	101.7	17.7	66.2	4.7
CoA 2	102.4	19.0	71.1	4.4
CoA 3	109.4	19.2	71.8	4.7
Average	-	-	**69.7 ± 3.0**	**4.6 ± 0.2**

Compared with the hydrogels prepared from pure coumarin-containing polymer (13.4 ± 1.3 %) the average gel content of the coumarin – acrylate hydrogels is much higher. The difference was also visible, as the coumarin – coumarin crosslinked gels were thin and fragile, the coumarin – acrylate gels were thicker and more stable. It is possible that more polymer chains are crosslinked due to the three different mechanisms between (meth)acrylate and coumarin groups during irradiation compared to only one mechanism if the formulation contains only coumarin groups. Additionally, it is possible that the two additional mechanisms are faster than the coumarin dimerization and the gel content is therefore higher.

There was also some shrinkage after crosslinking the acrylate containing gels, what made it easy to remove them from the mold. The swelling degree is compared with the gels in chapter 3.3.2.1 lower, but the relative standard deviation was smaller, for the coumarin – coumarin crosslinked gels the swelling degree was 16.0 ± 3.5.

Both swelling degree and gel content were expected in this range due to the similar results of Eng *et al.*[95] with a gel content of 76 % and a swelling degree of 5.67 (10 wt% PEG with coumarin end groups (3400 g/mol) mixed with 10 wt% PEG dimethacrylate (750 g/mol)). The ratio of functional groups was not the same because of the different coumarin content and different chain length of the coumarin polymer, what means that the coumarin content was much lower in the experiments of Eng *et al.*

3.4 US degradation

As the goal of the thesis was to prepare US-responsive systems based on POx, in the final chapter the most promising materials were selected, using PEtOx-co-poly(CoumarinOx) (8.2 % (NMR) / 5.3 % (UV/Vis) coumarin content) pure and in combination with PEG di(meth)acrylates, and tested their US responsiveness in two different experimental set-ups. First a proof of concept in the ultrasonic bath and further an investigation of the degradation of selected gels with the sonorheometer. The difference between the systems was the duration, frequency and intensity of the US, as the ultrasonic bath applies much harsher conditions onto the materials compared to the sonorheometer, what shows more similar conditions to US used in medical applications.

3.4.1 Degradation in the ultrasonic bath

To proof the assumption that both selected sample types (see chapter 3.3.2.1 and 3.3.2.2, copolymerized PEtOx_10_C8 with and without PEG diacrylates) are decrosslinking during ultrasonication, a degradation study in the ultrasonic bath was carried out. It is possible to investigate and prove the concept of the US-responsiveness with this experimental set-up. The advantage is the common availability and the cheap and easy use of ultrasonic baths, although the US conditions are harsh compared to US for medical applications. Nevertheless, with these experiments the US-responsiveness could be estimated and subsequently the suitability as a DDS.

The samples synthesized in chapter 3.3.2.1 (coumarin – coumarin crosslinking) and 3.3.2.2 (coumarin – acrylate crosslinking) were used for the degradation study in the ultrasonic bath. The samples were pre-swollen in DMSO overnight. The workflow of the degradation study in the ultrasonic bath is shown in Figure 25. Briefly, two pieces of pre-swollen hydrogels were placed In an Eppendorf tube filled with DMSO. Then, the 3 cycles with alternating ultrasonication (15 min on/30 min off) were performed, while DMSO was exchanged after each cycle. The experiment was performed in triplicates (three separate Eppendorf tubes for each sample type).

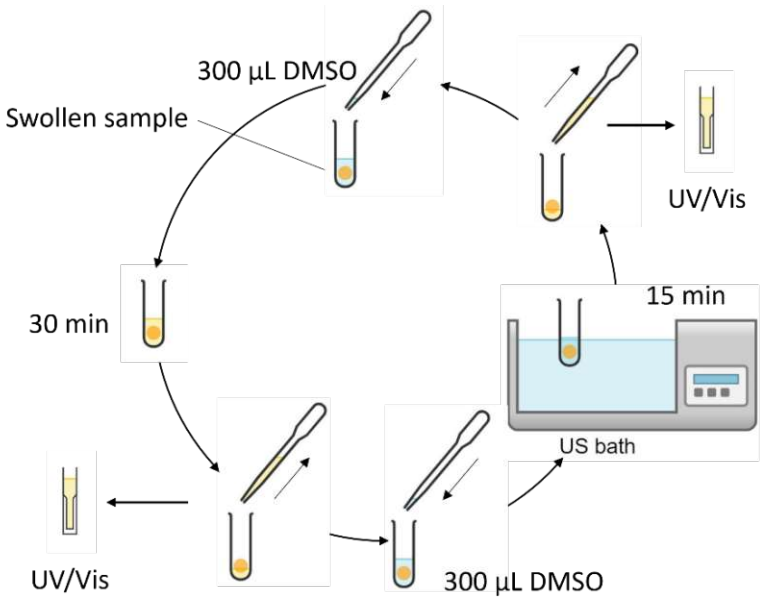


Figure 25: Workflow for investigating the degradation of the coumarin-based gels in the ultrasonic bath in three cycles of ultrasonication

The coumarin content was measured on a UV/Vis spectrometer at 318 nm. The relative cumulative release after each step is visualized in Figure 26.

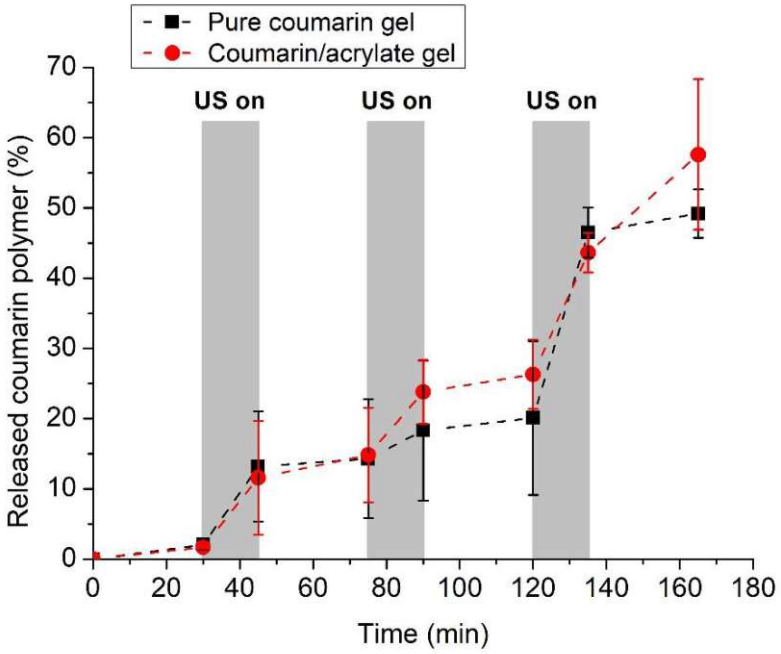


Figure 26: Degradation of gels (copolymerized sample PEtOx_10_C8 with 5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content, DP 100; pure crosslinked polymer and crosslinked polymer with PEG diacrylate content) swollen in DMSO, three cycles of 15 minutes in the ultrasonic bath are indicated in grey. Values are displayed as mean +/- standard deviation from triplicates.

Figure 26 shows the cumulative release of copolymer chains based on the total release over time. In the graph the difference between the release during ultrasonication cycle (grey marked areas) and the release without US is clearly visible. The release during the ultrasonication (grey areas) is in all cases higher than the release without US, as it can be seen on the different slopes of the dashed lines.

The ideal behaviour of a triggered drug delivery system is a controllable and fast release during the triggering and no release without triggering.[107] Both investigated systems showed a significant difference between triggered and not triggered period, but even more important almost no release during the not triggered period. This behavior makes them a promising platform for the further incorporation of the drug. To apply this material as DDS, either the drug will be bound to the polymer network through mechanolabile linkers, or it will be physically loaded into the hydrogel. Physically loaded means that there will be a steady release of this drug due to diffusion, even when no US is applied.

3.4.2 Sonorheology

To further investigate another aspect of US-responsiveness of prepared materials, a different experimental set-up was used. A new custom-made experimental system was proposed, combining rheometer with an ultrasonic transducer (see Figure 27 A), enabling direct simulation of the sample during rheological measurements. It was used in the course of this thesis for the first time. A similar set-up was described only once in literature so far by Gibaud *et al.*[108], although it was used for different types of materials.

Compared to the degradation study with the ultrasonic bath the parameters of the US are more controlled and similar to the US used in medical facilities. This enables an evaluation how the mechanism of the cleavage would work with the US properties for future applications. Additionally, the change in the storage modulus can be tracked in real time during the ultrasonication.

The parameters of the US, which can be varied are especially the amplitude and the duty cycle, in our case the frequency was kept to 1 MHz.

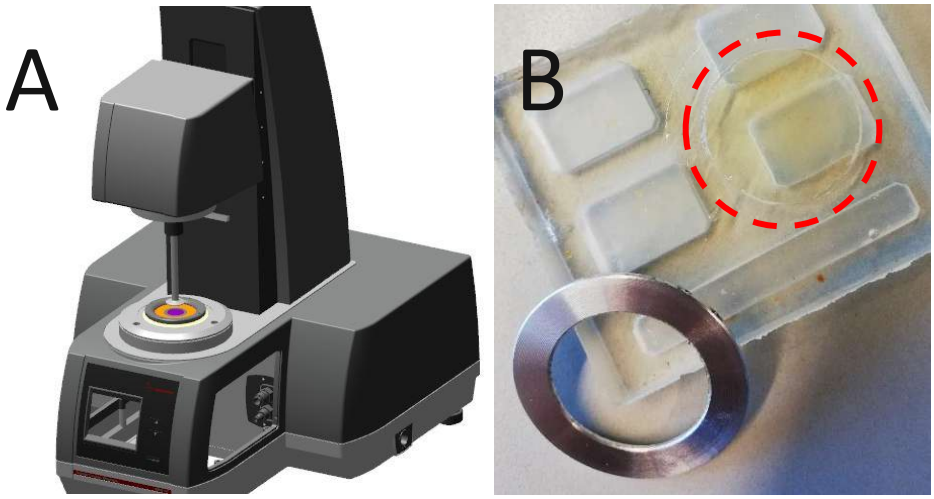


Figure 27: (A) Experimental set-up of the sonorheometer combining a rheometer with an US transducer (picture by Anton Paar), (B) Hydrogel containing a coumarin copolymer and PEG diacrylate with detached aluminium ring

For measuring sonorheology of a system, it was necessary to synthesize gels with 20 mm in diameter to fit the dimensions of ultrasonic transducer. The height of the gels was selected to 1 mm. These special gels were prepared by using an aluminium ring with an inside diameter of 20 mm and a height of 1 mm that was pressed on the plane side of a silicon mold, as shown in Figure 27 B. 314 µL of the formulations were filled into this ring and crosslinked with a broadband lamp (320 – 500 nm, 4.40 W/cm²) at a distance of 8.0 cm.

However, gels made of pure PEtOx-co-poly(CoumarinOx) were too brittle and fragile and could not be prepared in such manner. Therefore, only combined samples with either PEG diacrylate or to the other half PEG dimethacrylate were measured. The used formulations containing either additional PEG diacrylate (MW 700 g/mol) or PEG dimethacrylate (MW 750 g/mol) with DMSO as solvent and their irradiation times are shown in Table 18.

Table 18: Used formulations for sonorheology measurements containing PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin, either PEG diacrylate or PEG dimethacrylate and DMSO as solvent and their irradiation time (broadband lamp with 320 - 500 nm, 2.51 W/cm², 3.2 cm distance between light source and formulation)

Sample	Coumarin polymer (wt%)	PEG diacrylate (wt%)	PEG dimethacrylate (wt%)	Irradiation time (min)
A0	-	25.1	-	45
A1	12.1	12.5	-	30
A2	16.2	8.6	-	45
A3	17.8	6.4	-	60
A4	20.8	4.2	-	90
M0	-	-	25.0	60
M1	12.5	-	12.1	60
M2	15.7	-	8.9	60
M3	17.6	-	6.7	60
M4	20.6	-	4.1	75

All samples listed in Table 18 were swollen in water overnight prior the measurement on the sonorheometer. Additionally, two more samples were prepared directly before the measurement without swelling, they had the same composition as M0 and M4.

As reported in chapter 3.3, the stability of the gels increased by adding polymers with acrylate end groups. However, the crosslinking mechanism is mixed in that case, what hinders the study of cleavage of the coumarin dimers. Therefore, the amount of di(meth)acrylates was decreased stepwise as much as possible, to obtain stable hydrogels.

The used parameters of the rheometer, the ultrasonication and the samples are summarized in Table 19. The intensity of the US is chosen to reach a balance between high enough so some effect can be seen and nevertheless not so high that the system will be damaged. The amplitude and frequency are

also values that are thoroughly used for medical applications. The duty cycle describes the percentage of time how long the US is switched on relied on the whole cycle, called burst period (in our case 2 ms US relied on a burst period of 10 ms, equals 20 % duty cycle).

Table 19: Parameters of the sonorheology experiments

Rheology	Shear strain γ	0.02 %
	Frequency f	1 Hz
Ultrasound	Amplitude	100 mV_{RMS}
	Frequency λ	1 MHz
	Duty cycle	20 %
	Duration	1 min
Samples	Diameter	2 cm
	Thickness	1 mm

The measurement itself consisted of three parts: the first part was a 1 minute measurement without US, the second part was a measurement for 1 minute with US and the third part was a measurement of 3 minutes without US to see the impact and the consequence of the ultrasonication on the systems. For better comparison of the results, the average value of the storage modulus in the first minute was normalized (Figure 28 and Figure 29).

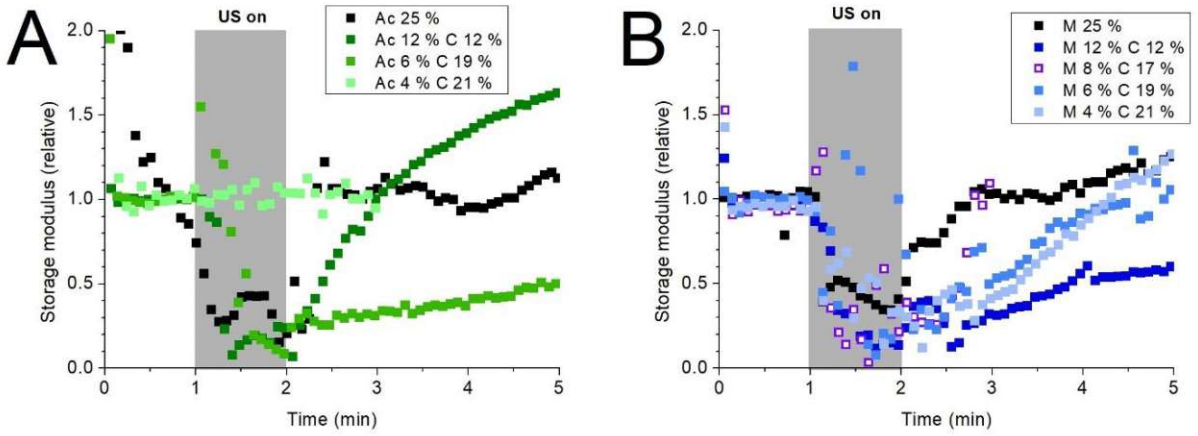


Figure 28: Sonorheology of gels swollen in water based on POx with coumarin side groups with various amounts of PEG diacrylate (A) and PEG dimethacrylate (B), grey area marking the ultrasonication period

Regarding the acrylate samples (Figure 28 A), sample A2 (8.6 wt% PEG diacrylate, 16.2 wt% coumarin copolymer) is not shown because it broke during the application onto the sonorheometer, so the results were not comparable to the results of the other samples. The measurement of A4 (4.2 wt% PEG diacrylate, 20.8 wt% coumarin copolymer) was stopped after 3 minutes as there was no change in the storage modulus during or after the ultrasonication. The sample A3 showed a slow recovery of the relative storage modulus compared to the fast recovery of A0 and A1. Especially sample A1 (12.5 wt%

PEG diacrylate, 12.1 wt% coumarin copolymer) exhibited higher relative storage modulus after the ultrasonication, what means that the sample is seemingly more crosslinked afterwards.

The pure acrylate and the pure methacrylate sample behaved very similarly, the minimal storage modulus was reached at the end of the ultrasonication and they recovered fast afterwards. In Figure 28 B all methacrylate samples showed a change in storage modulus during and after the ultrasonication. The relative storage modulus of M3 recovered after the ultrasonication the fastest, while M1 with the lowest coumarin content recovered the slowest. Also some of the methacrylate samples show a higher relative storage modulus after the ultrasonication than before, similar to the acrylate samples.

Unfortunately, nearly all of the samples were destroyed while removing them from the sonorheometer plate. Because of this, it was not possible to remeasure the same sample.

In general, the decrease of the relative storage modulus was expected due to the ultrasonication that is displayed in nearly every measurement. In contrast, its subsequent fast increase after the ultrasonication is stopped, was not expected as the hydrogels should have a lower storage modulus if at least some of the dimers are cleaved.

A possible explanation was inspired by the work of Eng *et al.*[95] This research group also studied the degradation of coumarin – methacrylate gels *via* US and looked more closely into the degradation. The ^{1}H-NMR analysis did not show the breaking of the cyclobutene ring of the coumarin – coumarin and coumarin – methacrylate dimers. Instead, they discovered fragments of the PEG backbones, what means that these PEG chains were acoustically more responsive than the dimers. Further, Eng *et al.* explained the breakdown of the PEG chains by radicals generated by the ultrasonication. In our case, the presence of radicals can be also expected. These radicals could cause remaining (meth)acrylates to polymerize and subsequently the storage modulus would rise after the ultrasonication.

As there is not really a trend regarding the different ratios of the compounds, also the different swelling properties of the samples could have an influence. As explained in chapter 3.3.2, hydrogels with different acrylate/methacrylate and coumarin content show different swelling degrees. In Figure 29 swollen and non-swollen methacrylate containing samples are compared.

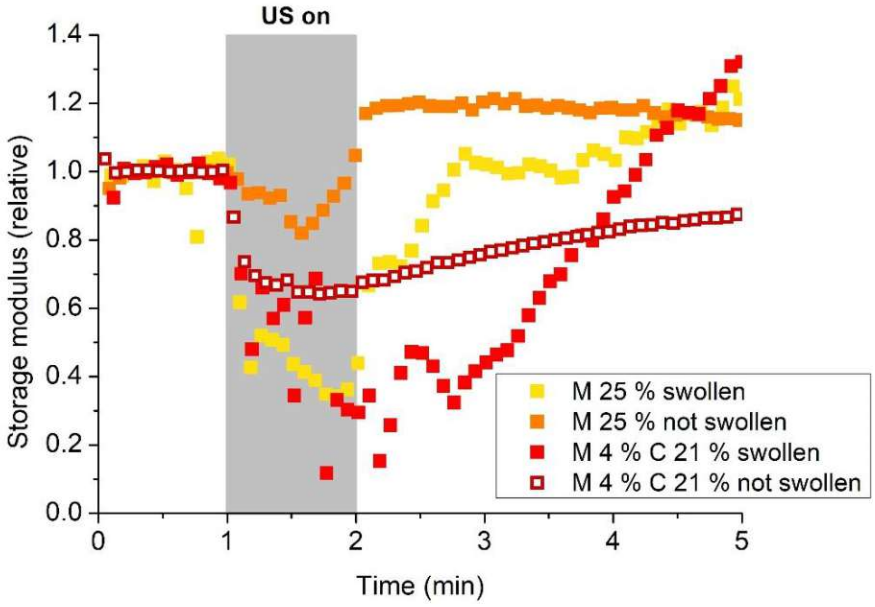


Figure 29: Sonorheology of gels both swollen and directly used after crosslinking (not swollen) based on POx with coumarin side groups with various amounts of PEG dimethacrylate, grey area marking the ultrasonication period

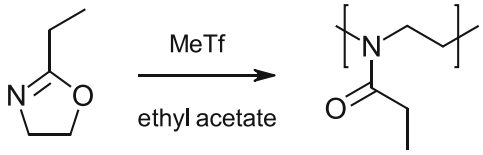
Figure 29 shows that the relative storage modulus is decreasing less during the ultrasonication if the sample is not swollen. The recovery of the relative storage modulus is on the other hand independent from the swelling, as one of the not swollen samples is recovering faster and the other one slower than the swollen sample.

Summarizing these results, the US has definitely some influence on the storage modulus of the synthesized gels. As confirmed by the degradation study in the US bath, the coumarin dimers within the hydrogel network are cleaved nearly exclusively during ultrasonication, what mimics the desired behaviour. Anyway, these are experimental set-ups and the methods need to be established, *e.g.* by using different sample types. The next step would be to load the hydrogels physically with a model drug and study its release under influence of US. A further investigation would be the comparison of different US parameters and to select the most suitable, keeping in mind the application as DDS. Additionally, of course also cytotoxicity of the materials has to be investigated before it can be proposed for real application as a DDS.

Experimental Part

1 Kinetic studies of cationic ring-opening polymerization of poly(2-oxazoline)s

1.1 Homopolymerization of 2-ethyl-2-oxazoline



The polymerizations were carried out in dry Schlenk reactors under argon atmosphere on a Schlenk line. After the calculated amounts of chemicals were injected (Table 20), the flasks were closed and placed in a preheated oil bath (acetonitrile 75 °C, benzonitrile 110 °C, ethyl acetate 70 °C) until the ^{1}H-NMR spectra showed full conversion of the monomer (usually after 24 h).

The general conditions of the polymerization were a 3 M solution of monomer in the respective solvent, the molar ratio of monomer to initiator was calculated to be 50.

Table 20: Amount of used chemicals for the homopolymerization of 2-ethyl-2-oxazoline (EtOx), as weighted before injection (A=acetonitrile, B=benzonitrile, EE=ethyl acetate, MeOTs=methyl tosylate, MeONs=methyl nosylate, MeOTf=methyl triflate)

	Solvent/ Initiator	Initiator (I)		EtOx (M)		Solvent	Ratio M:I
		g	mmol	g	mmol	mL	-
P1	A/MeOTs	0.169	0.907	4.32	43.58	11.5	48
P2	B/MeOTs	0.127	0.682	3.95	39.85	11.5	58
P3	B/MeOTs	0.113	0.607	2.98	30.06	8.7	50
P4	EE/MeOTs	0.071	0.381	2.00	20.18	6.5	53
P5	EE/MeONs	0.090	0.414	2.01	20.28	6.5	49
P6	EE/MeOTf	0.061	0.369	1.93	19.47	6.5	53

Samples in the size of 0.05 mL were taken according to Table 21 to Table 25. In case of acetonitrile and ethyl acetate, the reaction was cooled with an ice bath before. ^{1}H-NMR spectra were measured without further purification and integrated from 4.15 – 4.00 ppm and from 3.75 – 3.60 ppm (summarized integral monomer) and from 3.45 – 3.15 ppm (integral polymer).

Table 21: Change of the conversion during the polymerization of EtOx with MeOTs as initiator and ACN as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	$\ln([M]_0/[M])$
0	0	3.86	0.00	0.00	0.000
60	3600	3.53	0.26	6.86	0.071
120	7200	2.82	0.75	21.01	0.236
178	10680	2.73	1.19	30.36	0.362
298	17880	2.06	1.86	47.45	0.643
420	25200	1.67	2.22	57.07	0.846

Table 22: Change of the conversion during the polymerization of EtOx with MeOTs as initiator and benzonitrile as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	3.00	0.00	0.00	0.000
15	900	3.03	0.81	21.09	0.237
30	1800	1.29	2.61	66.92	1.106
45	2700	0.63	3.27	83.85	1.823
60	3600	0.33	3.57	91.54	2.470
76	4560	0.18	3.75	95.42	3.083

Table 23: Change of the conversion during the polymerization of EtOx with MeOTs as initiator and ethyl acetate as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	3.00	0.00	0.00	0.000
60	3600	1.96	0.04	2.00	0.020
120	7200	1.97	0.11	5.29	0.054
180	10800	1.92	0.35	15.42	0.167
300	18000	1.94	1.21	38.41	0.485
420	25200	1.92	2.27	54.18	0.780

Table 24: Change of the conversion during the polymerization of EtOx with MeONs as initiator and ethyl acetate as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	3.00	0.00	0.00	0.000
61	3660	3.23	0.33	9.27	0.097
120	7200	3.32	1.03	23.68	0.270
180	10800	3.59	1.92	34.85	0.428
300	18000	3.37	3.48	50.80	0.709
420	25200	3.86	7.12	64.85	1.045

Table 25: Change of the conversion during the polymerization of EtOx with MeOTf as initiator and ethyl acetate as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	3.00	0.00	0.00	0.000
61	3660	2.91	0.39	11.82	0.126
120	7200	3.19	1.11	25.81	0.299
180	10800	1.44	0.89	38.20	0.481
300	18000	2.88	3.29	53.32	0.762
420	25200	2.82	5.33	65.40	1.061

When full conversion of the monomer was reached, the reaction was cooled to RT and terminated methanolic 1 M KOH (1.2 eq. of KOH to initiator) for 3 to 4 hours at RT.

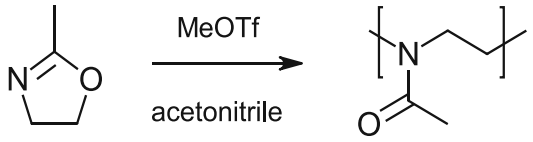
The reaction mixtures in acetonitrile and benzonitrile were precipitated into cooled diethyl ether and dried at 35 °C in a vacuum oven. The polymerization mixtures containing EE were evaporated to dryness on rotary evaporator.

To further purify these poly(2-ethyl-2-oxazoline)s (PEtOx), they were dissolved in 20 mL of water each and filled into a semipermeable cellulose membrane (MWCO 1 kDa, Spectrum Laboratories, Inc.) to perform dialysis against 5 L of distilled water for 22 hours. The water was changed once after one hour. The polymers were filled into falcon tubes and frozen with liquid nitrogen, the water was removed by lyophilization.

Characterization: white powder

^{1}H-NMR (400 MHz, $CDCl_3$, before purification): δ 4.15 – 4.00 (q, 2H, EtOx -C*H*$_2$-CH$_2$-), 3.75 – 3.60 (q, 2H, EtOx -CH$_2$-C*H*$_2$-), 3.45 – 3.15 (m, 4H, PEtOx backbone), 2.40 – 2.08 (m, 2H, side chain -C*H*$_2$-CH$_3$), 1.10 – 0.85 (t, 3H, side chain -CH$_2$-C*H*$_3$)

1.2 Homopolymerization of 2-methyl-2-oxazoline



In a dried vial under argon atmosphere, MeOTf (39.9 mg, 0.235 mmol, 1 eq.) as initiator, acetonitrile (3.9 mL, 3 M solution of the monomer) as solvent and 2-methyl-2-oxazoline (MeOx) (0.9893 g, 11.75 mmol, 50 eq.) were weighted in, sealed, and placed into a preheated oil bath at 75 °C. Samples in the size of 0.05 mL were taken according to Table 26, ^{1}H-NMR spectra were measured without further purification and integrated from 4.15 – 4.00 ppm (integral monomer) and from 3.40 – 3.20 ppm (integral polymer).

Table 26: Change of the conversion during the polymerization of MeOx with MeOTf as initiator and acetonitrile as solvent

Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	1.00	0.00	0.00	0.000
60	3600	1.98	0.38	16.10	0.176
120	7200	2.00	0.82	29.08	0.344
180	10800	2.00	1.39	41.00	0.528
240	14400	2.00	2.17	52.04	0.735
300	18000	1.99	2.94	59.63	0.907
360	21600	2.01	3.81	65.46	1.063

When full conversion of the monomer was reached, the reaction was cooled with an ice bath and terminated methanolic 1 M KOH (1.2 eq. to the initiator) for 4 hours at RT. The reaction mixture was precipitated into cooled diethyl ether, centrifuged and dried at 60 °C in a vacuum oven.

Characterization: white solid

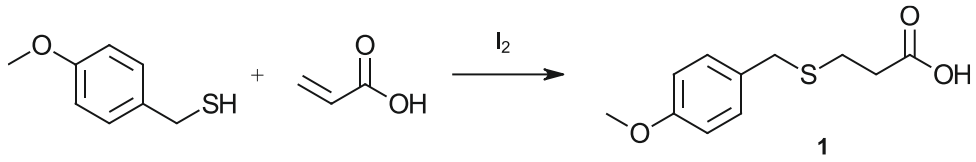
^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.50 – 3.30 (m, 4H, POx backbone -N-CH_2-CH_2-), 1.84 – 1.77 (m, 3H, -CO-CH_3).

2 Thiol-containing poly(2-oxazoline)s

2.1 Monomer Synthesis

2.1.1 Strategy 1: Thiol-containing 2-oxazoline with methoxybenzyl protecting group

2.1.1.1 Synthesis of 3-[[(4-Methoxyphenyl)methyl]thio]propionic acid (Mpa(MOB)) **1**



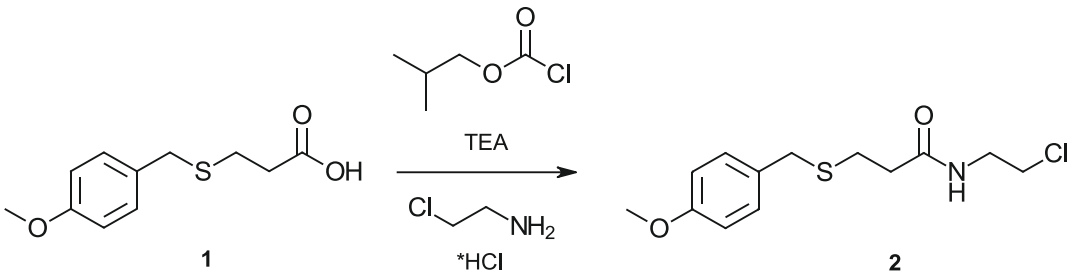
Freshly distilled acrylic acid (2.09 g, 29.0 mmol, 1 eq.) and iodine (0.02 g, 0.08 mmol, 0.25 %) were added to freshly distilled 4-methoxy-α-toluenethiol (4.44 g, 28.8 mmol, 1 eq.) under argon atmosphere according to Caille *et al*.[97] The yellow mixture was stirred for 20 hours until a white solid precipitated. The product was precipitated, washed with petrol ether (PE) and afterwards characterized *via* NMR spectroscopy and melting point.

Yield:	2.22 g (35 %)
Characterization:	colourless crystals
Melting point:	70.4 – 74.0 °C

^{1}H-NMR (200 MHz, $CDCl_3$): δ 12.00 – 9.00 (s, 1H, COOH), 7.31 – 7.15 (m, 2H, Ar), 6.95 – 6.75 (m, 2H, Ar), 3.81 (s, 3H, -OCH_3), 3.71 (s, 2H, -Ar-CH_2-S), 2.80 – 2.48 (m, 4H, S-CH_2-CH_2-)

^{13}C-NMR (101 MHz, $CDCl_3$): δ 178.09 (quart. C), 158.85 (quart. C), 130.04 (CH), 129.97 (quart. C), 114.13 (CH), 55.42 (CH_2), 35.42 (CH_3), 35.84 (CH_2), 34.44 (CH_2), 25.84 (CH_2)

2.1.1.2 Synthesis of N-(2-chloroethyl)-3-(4-methoxybenzylsulfanyl)propionamide **2**



1 (1.00 g, 4.4 mmol, 1 eq.) was weighted into a Schlenk flask and dried in vacuo. The solid residue was dissolved in dry THF (25 mL) and dry TEA (0.7 mL, 4.8 mmol, 1.1 eq.) was added under argon atmosphere.[66] The solution was cooled to 0 °C before adding isobutyl chloroformate (0.58 mL, 4.4 mmol, 1 eq.) and stirred for 10 minutes at this temperature. After stirring further 15 minutes at RT, the mixture was cooled to 0 °C again. 2-chloroethylamine hydrochloride (0.67 g, 5.3 mmol, 1.2 eq.) was dissolved in dry DMF (5 mL) under inert conditions and added simultaneously with dry TEA (0.7 mL, 4.8 mmol, 1.1 eq.) to the reaction. The mixture was stirred at RT for one hour before the solvent was

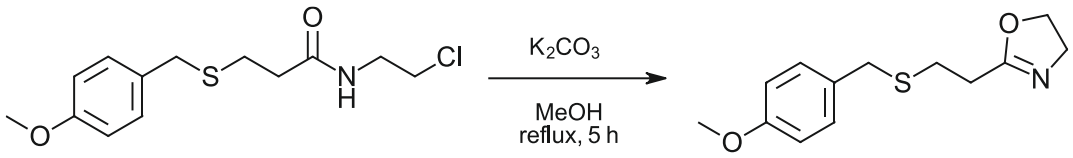
evaporated in vacuo. The residue was dissolved in DCM, the by-products were precipitated by adding each 100 mL of PE and diethyl ether. After filtration, the solvent was evaporated and the product was characterized *via* ^{1}H NMR spectroscopy.

Yield: 1.46 g (115 %, crude)

Characterization: colourless crystals

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.25 – 7.20 (m, 2H, Ar), 6.89 – 6.82 (m, 2H, Ar), 5.96 (s, 1H, -NH), 3.80 (s, 3H, -OCH_3), 3.70 (s, 2H, Ar-C*H*$_2$-S-), 3.64 – 3.57 (m, 4H, N-CH_2-CH_2-Cl), 2.73 (t, J = 7.1 Hz, 2H, S-CH_2-), 2.39 (t, J = 7.1 Hz, 2H, -CH_2-CO-).

2.1.1.3 Synthesis of 2-[2-(4-Methoxybenzylsulfanyl)ethyl]-2-oxazoline (MOB-SOx) **3**



2 **3**

2 (6.45 g, 21.8 mmol, 1 eq.) was dissolved in 87 mL MeOH and anhydrous K_2CO_3 (6.47 g, 45.8 mmol, 2.1 eq.) was added before heating the mixture up to reflux.[66] After 5 hours, the reaction mixture was cooled to RT and the solvent was evaporated. The yellow to orange residue was dissolved in DCM and centrifuged. The liquid phase was dried in vacuo.

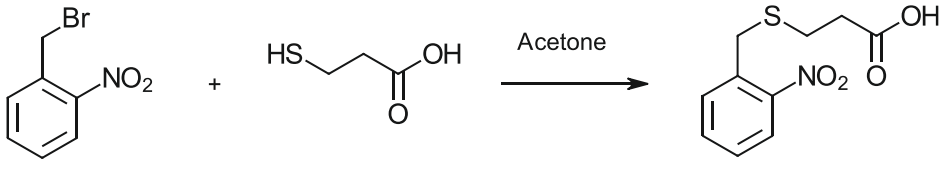
Yield: 2.53 g (45 %)

Characterization: yellow liquid

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.25 – 7.20 (m, 2H, Ar), 6.87 – 6.81 (m, 2H, Ar), 4.27 – 4.17 (m, 2H, -O-CH_2-CH_2-N), 3.86 – 3.79 (m, 2H, -O-CH_2-CH_2-N), 3.79 (s, 3H, -CH_3), 3.70 (s, 2H, Ar-CH_2-S-), 2.72 – 2.66 (m, 2H, -S-CH_2-), 2.52 (tq, J = 7.6, 1.2 Hz, 2H, -S-CH_2-C*H*$_2$-).

2.1.2 Strategy 2: Thiol-containing 2-oxazoline with nitrobenzyl protecting group

2.1.2.1 Synthesis of 3-[(2-Nitrobenzyl)thio]propionic acid **4**



4

A dry three-necked flask was filled with 30 mL dry acetone and 3-mercaptopropionic acid (0.8 mL, 9.26 mmol, 2.0 eq.) under argon counterflow in a lab with yellow light according to Jung *et al.*[32] before it was cooled to 0 °C. After adding TEA (1.0 mL, 6.94 mmol, 1.5 eq.) the mixture was stirred for 40 minutes at this temperature. After reaching RT, 2-nitrobenzyl bromide (0.98 g, 4.63 mmol, 1.0 eq.)

was added slowly, the clear solution was stirred overnight and protected from light with aluminum foil. The white precipitate was filtered, washed with acetone and the solvent was evaporated. The mixture of solid and liquid components was dissolved in 30 mL DCM and washed with 0.5 M HCl (4 times á 20 mL) and brine (20 mL), dried with $MgSO_4$, filtered and dried. To the yellow viscous crude product was added 50 mL PE and stirred for 30 minutes. The resulting solid was separated from the liquid phase and dried in vacuo.

Yield: 0.92 g (77 %)

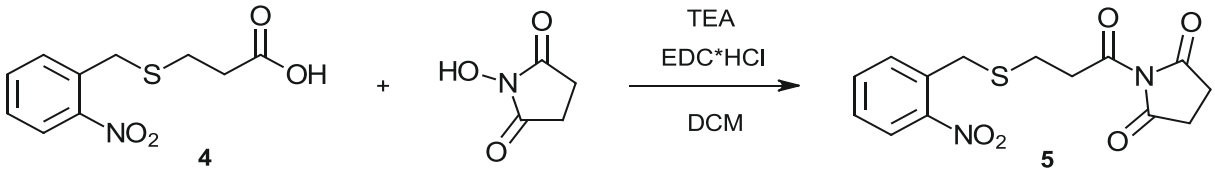
Characterization: light yellow solid

Melting point: 70.0 – 78.0 °C

^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.43 (s, 1H, -COOH), 7.98 (dd, J = 8.2, 1.4 Hz, 1H, Ar), 7.57 (td, J = 7.5, 1.4 Hz, 1H, Ar), 7.51 – 7.39 (m, 2H, Ar), 4.10 (s, 2H, -S-CH_2-), 2.76 – 2.69 (m, 2H, -S-CH_2-CH_2-), 2.65 – 2.58 (m, 2H, -S-CH_2-CH_2-).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 178.08 (quart. C), 148.79 (quart. C), 133.91 (quart. C), 133.22 (CH), 131.99 (CH), 128.46 (CH), 125.54 (CH), 34.46 (CH_2), 33.73 (CH_2), 26.64 (CH_2)

2.1.2.2 Synthesis of 2,5-Dioxopyrrolidin-1-yl 3-[(2-nitrobenzyl)thio]propanoate **5**

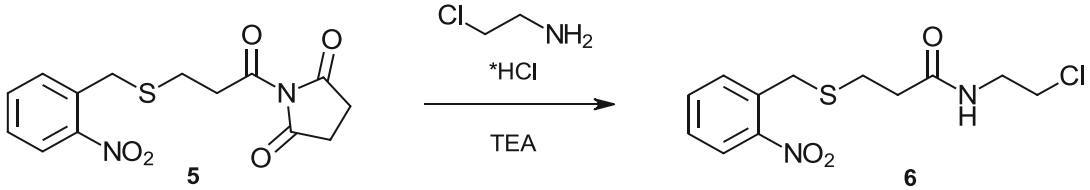


4 (1.01 g, 1.15 mmol, 1 eq.) was dried at HV before TEA (0.92 mL, 6.63 mmol, 1.6 eq.) and NHS (0.78 g, 6.63 mmol, 1.6 eq.) were added and dissolved in dry DCM (29 mL).[32] To the clear solution, EDC*HCl (1.08 g, 6.63 mmol, 1.6 eq.) was added. After stirring the mixture for 23 h under argon atmosphere, it was extracted with 1 M HCl (three times), H_2O (three times) and brine (three times). The combined aqueous phases were extracted once with DCM, the combined organic phases were dried with $MgSO_4$, filtered and the solvent was evaporated. The crude product was used for the next step without further purification.

Yield: 1.37 g (99 %)

Characterization: light yellow solid

2.1.2.3 Synthesis of N-(2-chloroethyl)-3-[(2-nitrobenzyl)thio]propenamide **6**



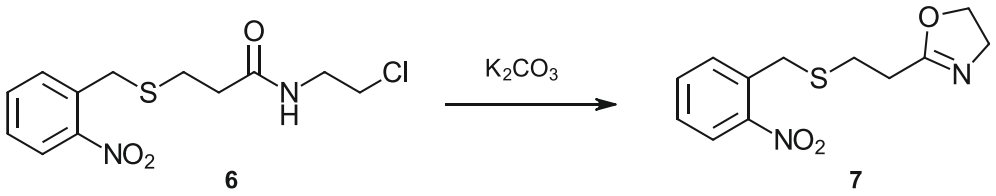
5 (1.37 g, 4.25 mmol, 1 eq.) was dissolved in DCM (15 mL) and cooled to 0 °C before 2-chloroethylamine hydrochloride (1.06 g, 8.5 mmol, 2 eq.) and TEA (1.15 mL, 8.5 mmol, 2 eq.) were added.[32] The reaction was cooled for 50 minutes and afterwards stirred for another 2 h until TCL showed full conversion (PE:EE 1:4). The mixture was diluted with DCM and extracted with aqueous 1 M HCl (three times), brine (one time) and saturated aqueous $NaHCO_3$ (three times). The organic phase was dried with $MgSO_4$, filtered and the solvent was evaporated.

Yield:	0.88 g (68 %)
Characterization:	yellow solid
R_f:	0.53 (PE:EE 1:4)
Melting point:	68.6 – 75.8 °C

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.96 (dd, J = 8.1, 1.4 Hz, 1H, aromatic), 7.56 (td, J = 7.5, 1.3 Hz, 1H, aromatic), 7.50 (dd, J = 7.7, 1.6 Hz, 1H, aromatic), 7.42 (ddd, J = 8.2, 7.3, 1.6 Hz, 1H, aromatic), 6.07 (s, 1H, -CO-NH-), 4.09 (s, 2H, Ar-CH_2-S-), 3.68 – 3.55 (m, 4H, Cl-CH_2-CH_2-), 2.76 (t, J = 7.1 Hz, 2H, -S-CH_2-), 2.44 (t, J = 7.1 Hz, 2H, -CO-CH_2-).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 172.08 (quart. C), 171.38 (quart. C), 133.96 (quart. C), 133.27 (CH), 132.13 (CH), 128.43 (CH), 125.46 (CH), 43.82 (CH_2), 41.38 (CH_2), 36.42 (CH_2), 33.74 (CH_2), 27.73 (CH_2)

2.1.2.4 Synthesis of 2-{2-[(2-Nitrobenzyl)thio]ethyl}-4,5-dihydrooxazole (NbMEtOx) **7**



6 **7**

6 (1.63 g, 5.38 mmol, 1 eq.) was dissolved in dry MeOH (22 mL) and anhydrous K_2CO_3 (1.93 g, 14.0 mmol, 2.6 eq.) was added.[32] The mixture was stirred at 60 °C under Argon atmosphere. After full conversion was proofed *via* TLC (ethyl acetate (EE), R_f 0.29), the solvent was evaporated. DCM was added to the brownish solid to receive a yellow solution with an orange solid. After removing the solid, the solvent was removed yielding a brown turbid liquid. The crude product was purified *via* column chromatography (pure EE → EE:MeOH 100:1).

Yield:	36.3 mg (2.5 %)
Characterization:	yellow viscous liquid
R_f:	0.29 (EE)

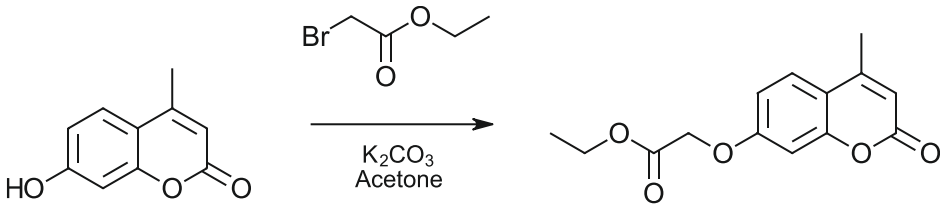
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.97 (dd, J = 8.2, 1.4 Hz, 1H, aromatic), 7.59 – 7.46 (m, 2H, aromatic), 7.42 (ddd, J = 8.1, 7.2, 1.7 Hz, 1H, aromatic), 4.27 – 4.18 (m, 2H, Ar-CH_2-S-), 4.10 (s, 2H, -O-CH_2-), 3.87 – 3.77 (m, 2H, =N-CH_2-), 2.79 – 2.70 (m, 2H, -S-CH_2-), 2.53 (ddt, J = 7.6, 6.3, 1.1 Hz, 2H, oxazoline-CH_2).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 166.66 (quart. C), 158.47 (quart. C), 134.17 (quart. C), 133.19 (CH), 132.01 (CH), 128.36 (CH), 125.56 (CH), 67.55 (CH_2), 54.51 (CH_2), 33.76 (CH_2), 28.55 (CH_2), 28.24 (CH_2)

3 Coumarin-containing poly(2-oxazoline)s

3.1 Postpolymerization modification strategy

*3.1.1 Synthesis of (4-Methylcoumarin-7-yloxy)acetate **8***



8

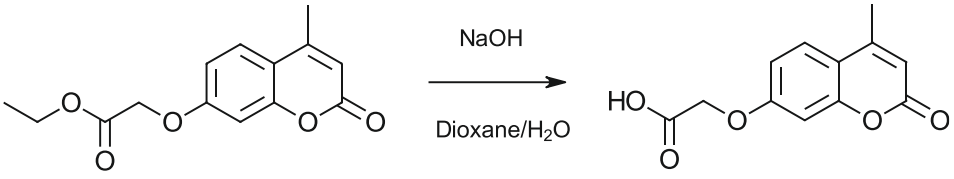
4-Methylumbelliferone (12.78 g, 72.5 mmol, 1 eq.) was dried in a three-necked flask and flushed with argon. It was dissolved in dry acetone (300 mL), then freshly distilled ethyl bromoacetate (8.9 mL, 80.5 mmol, 1.1 eq.) and anhydrous K_2CO_3 (10.55 g, 76.3 mmol, 1.05 eq.) were added under stirring according to Chujo *et al.*[67] The mixture was refluxed for three hours, resulting in a turbid yellow suspension. The precipitate was filtered and the solvent of the filtrate was evaporated in vacuo. The viscous yellow liquid was cooled to 0 °C to crystallize the crude product, afterwards it was recrystallized from ethanol.

Yield:	13.10 g (69 %)
Characterization:	white crystalline needles
Melting point:	103.1 – 104.5 °C

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.52 (d, J = 8.8 Hz, 1H, Ar), 6.90 (t, J = 2.7 Hz, 1H, Ar), 6.78 (d, J = 2.6 Hz, 1H, Ar), 6.16 (q, J = 1.2 Hz, 1H, Ar), 4.68 (s, 2H, CH_2), 4.26 (dq, J = 10.2, 7.1 Hz, 2H, CH_2), 2.40 (d, J = 1.3 Hz, 3H, CH_3), 1.30 (td, J = 7.1, 2.6 Hz, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 168.03 (quart. C), 161.07 (quart. C), 160.70 (quart. C), 155.09 (quart. C), 152.48 (quart. C), 125.86 (CH), 114.44 (quart. C), 112.56 (CH), 101.77 (CH), 65.41 (CH_2), 61.76 (CH_2), 18.72 (CH_3), 14.21 (CH_3)

*3.1.2 Synthesis of (4-Methylcoumarin-7-yloxy)acetic acid **9***



8 **9**

Sodium hydroxide (5.07 g, 137.1 mmol, 14.4 eq.) and **8** (2.50 g, 9.5 mmol, 1 eq.) were dissolved in each 100 mL water and dioxane.[67] The solution was stirred overnight and the solvent was evaporated in vacuo, leaving a white-yellow solid. The residue was dissolved in distilled water (20 mL) and acidified

with concentrated HCl. The product precipitated as a beige voluminous solid that was filtrated, washed with cold water (200 mL), and dried at 60 °C overnight.

Yield: 4.29 g (96 %)

Characterization: beige solid

Melting point: 195.0 – 210.0 °C (lit. 209 – 213 °C)

^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.97 (s, 1H, COO*H*), 7.69 (d, J = 8.5 Hz, 1H, Ar), 7.04 – 6.87 (m, 2H, Ar), 6.22 (d, J = 1.6 Hz, 1H, Ar), 4.83 (s, 2H, CH_2), 2.40 (d, J = 1.4 Hz, 3H, CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 169.67 (quart. C), 160.79 (quart. C), 160.08 (quart. C), 154.54 (quart. C), 153.33 (quart. C), 126.47 (CH), 113.52 (quart. C), 112.27 (CH), 111.38 (CH), 101.50 (CH), 64.85 (CH_2), 18.12 (CH_3)

3.1.3 Homopolymerization of 2-ethyl-2-oxazoline with varying chain lengths

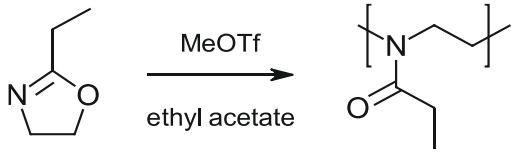


Table 27: Amounts of used chemicals to synthesize poly(oxazoline)s with different chain lengths

Desired molar mass	Ratio M:I	MeOTs		EtOx		Ethyl acetate
g/mol		g	mmol	g	mmol	mL
5000	50	0.295	1.80	9.14	92.2	25
10000	100	0.158	0.96	9.82	99.1	25
20000	200	0.102	0.62	10.07	101.6	25

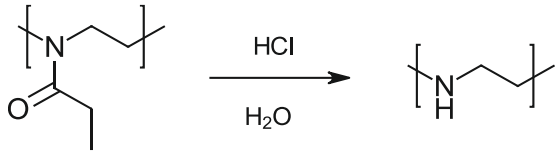
A Schlenk flask was dried on HV on a Schlenk line with a heat gun before weighting in MeOTs, dry ethyl acetate and dry EtOx under argon atmosphere (Table 27). The flask was put into a preheated oil bath (70 °C) for 12 to 48 hours (depending on the desired molar mass). The polymerization was terminated with 1 M methanolic KOH solution (1.2 eq. regarding the initiator) and stirred for at least 3 hours at RT. The polymer was purified by evaporating the solvent, dissolving the solid residue in water and dialysis for 24 hours against distilled water. The PEtOx solution was frozen with liquid nitrogen and lyophilized for at least 48 hours.

Yield: 89 %

Characterization: white powder

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.36 (m, 4H, N-CH_2-CH_2-), 2.27 (m, 2H, -CO-CH_2-), 1.13 – 0.91 (m, 3H, CH_3).

3.1.4 *Hydrolysis kinetics of poly(2-ethyl-2-oxazoline)*



PEtOx (0.9552 g, 9.64 mmol) was dissolved in water (20 mL, 0.48 M amide) in a three-necked flask. Concentrated hydrochloric acid (1.8 mL) was added to receive a 1 M acidic solution[101] and the first sample was withdrawn. The flask was placed in a preheated oil bath (110 °C) immediately to start the hydrolysis. Starting from this point, samples were withdrawn after selected time intervals according to Table 28. The used integration intervals and used equations (Formula 1 F1 and Formula 2 F2) can be found in General part chapter 3.1.4.

Table 28: Change of the PEI content during the hydrolysis using a 1 M aqueous HCl solution

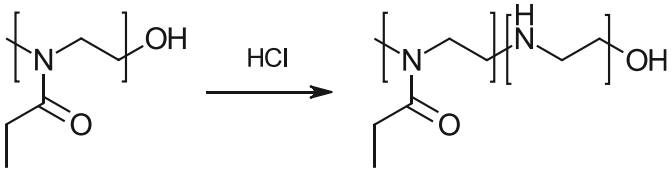
Time	Integrals				DH (%)		ln([A]$_0$/[A])	
min	PEtOx backbone	PEI backbone	EtOx CH_2	Propionic CH_2	F1	F2	F1	F2
60	3.96	0.27	2.02	0.19	0.064	0.063	0.066	0.065
90	3.75	0.31	2.04	0.22	0.076	0.071	0.079	0.073
180	3.79	0.62	1.91	0.25	0.141	0.140	0.152	0.150
240	3.69	0.68	1.84	0.29	0.156	0.156	0.169	0.170
300	3.67	0.95	1.79	0.38	0.206	0.210	0.230	0.235
360	3.29	1.04	1.63	0.5	0.240	0.242	0.275	0.277
422	3.45	1.19	1.67	0.49	0.257	0.263	0.296	0.305

The samples were put into vials that were prefilled with a 1 M aqueous NaOH solution to reach a pH between 8 and 10. The solutions were frozen with liquid nitrogen and lyophilized before characterizing them *via* ^{1}H-NMR spectroscopy.

Characterization: white powder

^{1}H-NMR (400 MHz, CD_3OD): δ 3.80 – 3.40 (m, 4H, PEtOx backbone), 3.20 – 2.65 (m, 4H, PEI backbone), 2.55 – 2.30 (m, 2H, CO-C*H*$_2$-CH_3 side chain), 1.20 – 1.00 (m, 3H, -CH_3).

3.1.5 *Partial hydrolysis of poly(2-ethyl-2-oxazoline)*



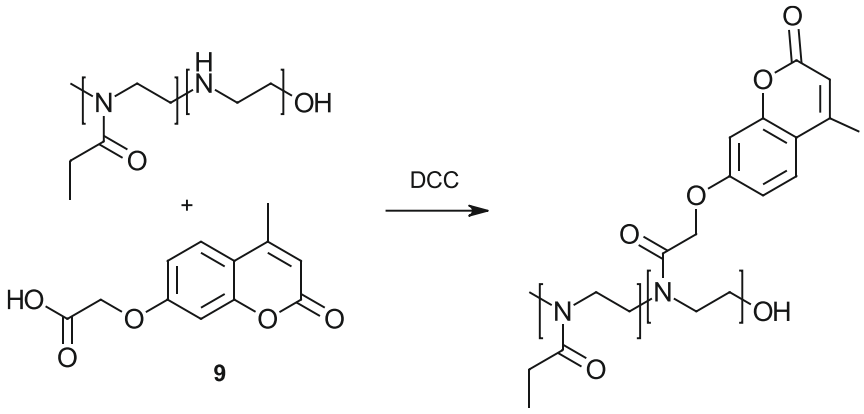
PEtOx (2.70 g, 27.2 mmol) was dissolved in water (57 mL, 0.48 M amide concentration) and concentrated hydrochloric acid (5.2 mL) was added to receive a 1 M acidic solution.[101] The flask was

placed in a preheated oil bath (110 °C) immediately to start the hydrolysis. After the corresponding time intervals (see general part chapter 3.1.4) to reach a selected degree of hydrolysis (DH), the solution was cooled rapidly to RT with an ice bath. 1 M aqueous sodium hydroxide was added to the polymer solution until a pH of 8-10 was reached. The product was purified by dialysis for 24 hours, frozen with liquid nitrogen and lyophilized.

Yield:	2.66 g (99 %, PEtOx_10_H7, see General Part chapter 3.1.5)
Degree of hydrolysis:	6.5 % (36 minutes)
	7.0 % (72 minutes)
	18.6 % (140 minutes)

^{1}H-NMR (400 MHz, CD_3OD): δ 3.8 – 3.4 (m, 4H, PEtOx backbone), 3.2 – 2.65 (m, 4H, PEI backbone), 2.55 – 2.3 (m, 2H, CO-C*H*$_2$-CH_3 side chain), 1.2 – 1.0 (m, 3H, -CH_3)

3.1.6 Modification of partially hydrolyzed poly(2-ethyl-2-oxazoline)



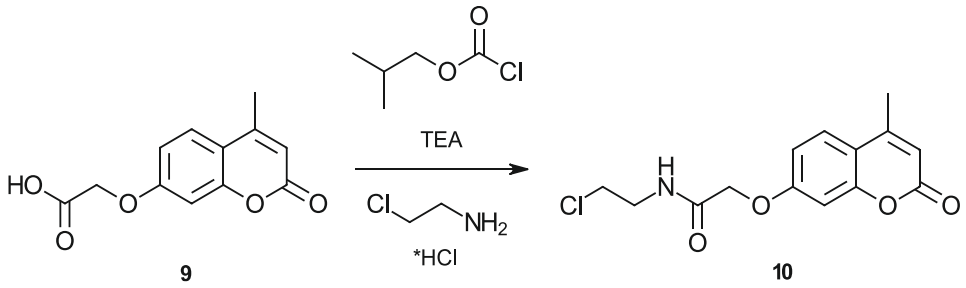
The partially hydrolyzed polymer (1.97 g, DH 10 %, 1 eq. amine) and **9** (1.00 g, 4.3 mmol, 2 eq.) was weighted into a three-necked flask and dried over HV. The solids were dissolved in dry DCM (64 mL) and dry DMF (19 mL) and cooled to 0 °C. After DCC (1.34 g, 6.5 mmol, 3 eq.) was added rapidly, the solution was stirred overnight at room temperature. The off-white precipitate was filtered, washed with DCM and the solvent of the yellow filtrate was evaporated in vacuo, resulting in a pale orange residue. After dissolving the solid in 100 mL methanol, it was precipitated into 300 mL diethyl ether.

Yield:	1.12 g (38 %)
Characterization:	orange solid

3.2 Copolymerization strategy

3.2.1 Monomer synthesis

3.2.1.1 Synthesis of N-2-Chloroethyl(4-methyl-7-coumarinyloxy)acetamide **10**



9 10

3 (1 g, 4.27 mmol, 1 eq.) was dissolved in 45 mL dry dioxane and TEA (0.56 mL, 0.48 mmol, 1.1 eq.) was added under argon atmosphere before the solution was cooled to 0 °C.[66] Isobutyl chloroformate (0.65 mL, 4.27 mmol, 1 eq.) was added, receiving a turbid solution that was stirred 15 minutes at 0 °C and further 15 minutes at RT. After cooling it again to 0 °C, 2-chloroethylamine hydrochloride (0.61 g, 5.12 mmol, 1.2 eq.) dissolved in 4 mL dry DMF and TEA (0.56 mL, 0.48 mmol, 1.1 eq.) were added slowly. The suspension was stirred for 2 hours at RT, afterwards the solvent was evaporated. The slightly yellow solid was dissolved in DCM and filtered. 100 mL diethyl ether were added and the white precipitate was filtered off. The product was precipitated by adding 100 mL of petrol ether, the white solid was dried at 60 °C overnight.

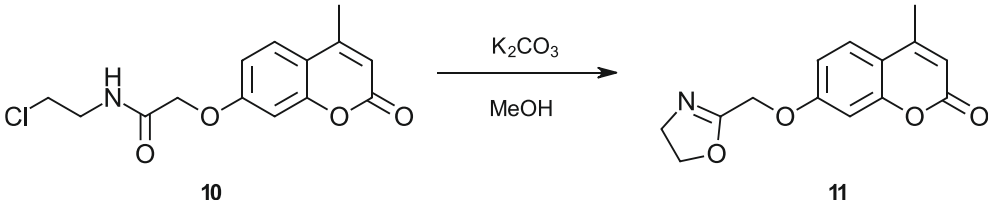
Yield:	6.45 g (74 %)
Characterization:	white solid
Melting point:	158.4 – 208.8 °C (decomposition)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 (d, J = 8.8 Hz, 1H, Ar), 6.91 (dd, J = 8.7, 2.6 Hz, 2H, Ar), 6.87 (d, J = 2.5 Hz, 1H, -NH), 6.19 (q, J = 1.3 Hz, 1H, -CO-CH=), 4.58 (s, 2H, -O-CH_2-CO-), 3.77 – 3.64 (m, 4H, -CH_2-CH_2-Cl), 2.41 (d, J = 1.2 Hz, 3H, -CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 167.51 (quart. C), 160.95 (quart. C), 159.85 (quart. C), 155.26 (quart. C), 152.30 (quart. C), 126.19 (CH), 115.07 (quart. C), 113.11 (CH), 111.95 (CH), 102.69 (CH), 67.57 (CH_2), 43.69 (CH_2), 40.94 (CH_2), 18.83 (CH_3)

HR-MS: (ACN, ESI+, m/z): calc.: 296.7239 $[M+H]^+$; found: 296.0686 $[M+H]^+$ (diff 0.38 ppm)

3.2.1.2 Synthesis of 2-[(4-Methyl-7-coumarinyloxy)methyl]oxazoline (CoumarinOx) **11**



10 11

6 (6.45 g, 21.8 mmol, 1 eq.) and anhydrous K_2CO_3 (6.47 g, 45.8 mmol, 2.1 eq.) were flushed with argon before 87 mL dry MeOH was added.[66] The mixture was refluxed for 5 hours and after cooling down to RT the solvent was evaporated. The residue was dissolved in DCM, centrifuged and the supernatant liquid was dried in vacuo.

Yield:	2.53 g (45 %)
Characterization:	off-white solid
Melting point:	170.0 – 220.0 °C (decomposition)

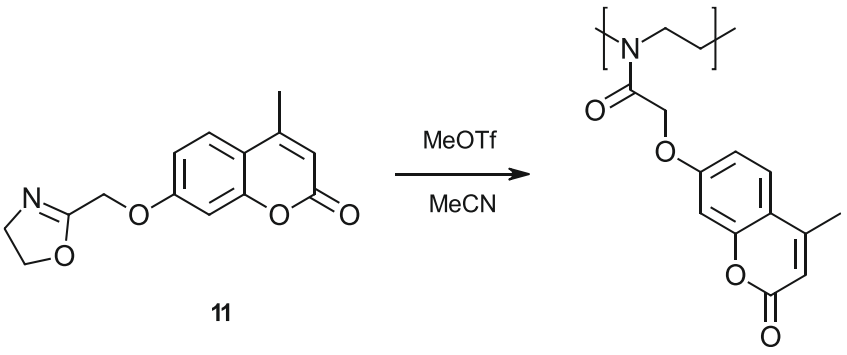
^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.52 (d, J = 8.8 Hz, 1H, Ar), 6.96 (dd, J = 8.8, 2.6 Hz, 1H, Ar), 6.89 (d, J = 2.5 Hz, 1H, Ar), 6.16 (q, J = 1.2 Hz, 1H, -CO-CH=), 4.78 (t, J = 1.4 Hz, 2H, -O-CH_2-), 4.43 – 4.30 (m, 2H, -O-CH_2-CH_2-N), 4.02 – 3.89 (m, 2H, -O-CH_2-CH_2-N), 2.40 (d, J = 1.3 Hz, 3H, -CH_3).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 163.08 (quart. C), 161.21 (quart. C), 160.94 (quart. C), 155.19 (quart. C), 152.52 (quart. C), 125.85 (CH), 114.48 (quart. C), 112.70 (CH), 112.58 (CH), 102.01 (CH), 68.29 (CH_2), 62.88 (CH_2), 54.64 (CH_2), 18.79 (CH_3)

HR-MS: (ACN, ESI+, m/z): calc.: 282.2458 $[M+Na]^+$, found: 282.2798 $[M+Na]^+$ (diff 2.35 ppm)

3.2.2 Polymer synthesis

3.2.2.1 Homopolymerization of coumarin-containing 2-oxazoline (CoumarinOx) **11**



11

11 (0.340 g, 1.31 mmol, 50 eq.) was weighted into a vial, flushed with argon and dried carefully in HV for 30 minutes. 0.43 mL of initiator stock solution (10 mg/mL of MeOTf in dry acetonitrile; 0.026 mmol, 1 eq.) was added. The suspension was put into a preheated oil bath at 75 °C, where the monomer dissolved. Samples were taken after 1, 2, 3, 5 and 7 hours according to Table 29 in the size of approximately 0.05 mL and analyzed *via* ^{1}H NMR spectroscopy (integral monomer 4.27 – 4.20 ppm and 3.86 – 3.76 ppm, integral polymer 3.65 – 3.40 ppm). The general conditions of the polymerization were a 3 M solution of monomer in the respective solvent, the molar ratio of monomer to initiator was calculated to be 50.

Table 29: Change of the conversion during the polymerization of CoumarinOx with MeOTf as initiator and ACN as solvent

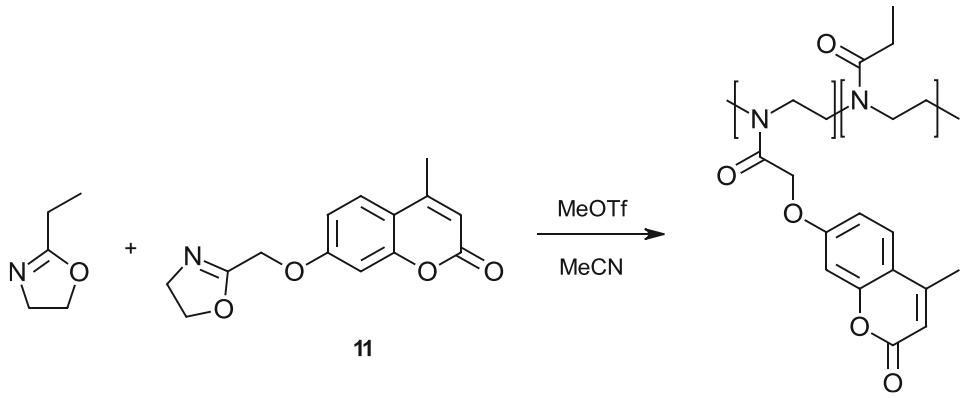
Minutes	Seconds	Integral monomer	Integral polymer	Conversion (%)	ln([M]$_0$/[M])
0	0	3.86	0.00	0.00	0.000
60	3600	4.41	1.97	30.88	0.369
120	7200	4.15	4.67	52.95	0.754
180	10800	4.59	9.65	67.77	1.132
300	18000	3.58	17.10	82.69	1.754
420	25200	1.70	13.84	89.06	2.213

After 28 hours the polymerization was cooled to RT and terminated with 1.2 eq. 1 M KOH in MeOH overnight. The solvent was evaporated, further purification and analysis were not possible due to the insolubility of the product.

Characterization: off-white solid

^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.28 (m, Ar), 6.92 – 6.47 (m, Ar), 6.01 – 5.75 (m, -CO-CH=), 5.01 – 4.54 (m, polymer backbone), 3.71 – 3.40 (m, -O-CH_2-), 2.35 – 2.10 (m, -CH_3).

3.2.2.2 Copolymerization of the coumarin-containing 2-oxazoline (CoumarinOx) **11**



11

CoumarinOx was copolymerized simultaneously once with EtOx and once with MeOx. For the copolymerization with EtOx, MeOTf (31 mg, 0.19 mmol, 1 eq.) and **11** (387 mg, 1.49 mmol, 8 eq.) were weighed into a vial and dried in HV. Acetonitrile (6.2 mL) and EtOx (1.718 g, 17.15 mmol, 92 eq.) were added and the reaction was started by heating the mixture up to 75 °C.

The copolymerization with MeOx was started by weighting MeOTf (52 mg, 0.22 mmol, 1 eq.) and **11** (450 mg, 1.74 mmol, 8 eq.) into a vial, adding acetonitrile (7.2 mL) and MeOx (1.707 g, 19.98 mmol, 92 eq.). This reaction mixture was started at the same time as the copolymerization with EtOx under the same conditions.

For the investigation of the copolymerization kinetics (CoumarinOx with both EtOx and MeOx), samples were drawn according to chapter 1.1 in time intervals, integrals and calculated conversion of each monomer listed in Table 38 and Table 39 (Appendix).

After 26 hours, the reactions were cooled to 0 °C, terminated with 1 M methanolic KOH (0.5 mL) and stirred for another 3 hours. The mixtures were precipitated two times into ice cooled diethyl ether and dried in vacuo at 50 °C.

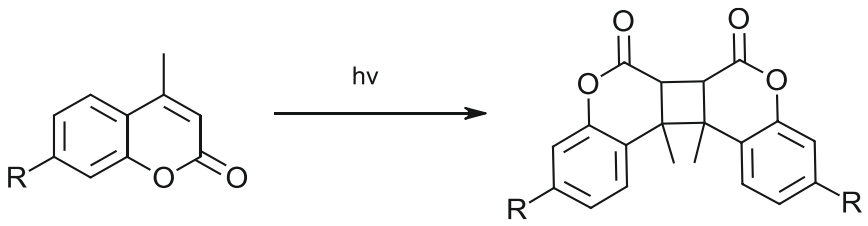
	EtOx	**MeOx**
Yield:	2.08 g (98 %)	2.12 g (98 %)
Characterization:	slightly yellow solid	yellow solid
Coumarin content:	NMR 8.2 %	NMR 5.3 %
	UV/Vis 5.3 %	UV/Vis 4.0 %

EtOx: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.28 (m, Ar), 6.92 – 6.47 (m, Ar), 6.01 – 5.75 (m, -CO-CH=), 5.01 – 4.54 (m, coumarin backbone), 3.71 – 3.45 (m, -O-CH_2-), 3.45 – 3.15 (m, ethyl backbone), 2.35 – 2.10 (m, ethyl-CH_2 and coumarin-CH_3), 1.10 – 0.85 (m, ethyl-CH_3).

MeOx: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.28 (m, Ar), 6.92 – 6.47 (m, Ar), 6.01 – 5.75 (m, -CO-CH=), 5.01 – 4.54 (m, coumarin backbone), 3.71 – 3.45 (m, -O-CH_2-), 3.45 – 3.03 (m, ethyl backbone), 2.35 – 2.10 (m, coumarin-CH_3), 1.14 – 1.03 (m, ethyl-CH_3).

3.3 Crosslinking

3.3.1 Crosslinking of the modified polymers



A formulation containing of 25 wt% PEtOx_10_M8 (4.7 % (UV/Vis) / 7.8 % (NMR) coumarin content, 10 kDa) and dioxane as solvent was mixed and 100 µL were put into a silicon mold (round, 10 mm diameter). After placing a microscope cover glass on top to prevent the evaporation of the solvent, the formulation was irradiated with 365 nm for 60 minutes with an intensity of 270 mW/cm². The resulting gels were put for 3 hours into ethanol to wash out the parts of the polymer that were not crosslinked and afterwards it was swelled in distilled water for 24 hours. After weighting the slightly orange hydrogels, they were frozen and lyophilize to determine the dry weight.

Using the determined values, gel content and swelling degree were determined and displayed in Table 30 using following equations:

$$Gel\ content\ (\%) = \frac{m_{dry} \cdot 100}{m_{formulation} \cdot polymer\ content\ (\%)}$$

Equation 4: Calculation of the gel content in % using the dry weight m_{dry} of the samples, the weight of the used formulation $m_{formulation}$ and the polymer content in the formulation

$$Swelling\ degree = \frac{m_{swollen} - m_{dry}}{m_{dry}}$$

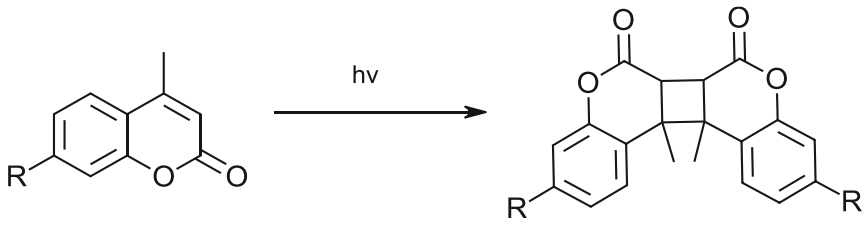
Equation 5: Calculation of the swelling degree using the swollen weight $m_{swollen}$ and the dry weight m_{dry} of the gels

Table 30: Gel content and swelling degree (in water) of the sample with the highest coumarin content from the third gelation study (PEtOx_10_M8, 4.7 % (UV/Vis) / 7.8 % (NMR) coumarin) irradiated for one hour with the LED lamp (365 nm) with 270 mW/cm² and 18 mm distance

Sample	$m_{swollen}$ (mg)	m_{dry} (mg)	Gel content (%)	Swelling degree
Mod 1	95.2	3.5	14.0	26.2
Mod 2	110.1	3.7	14.8	28.8
Mod 3	102.5	5.1	20.4	19.1
Mod 4	145.7	5.8	23.2	24.1
Average	-	-	**18.1 ± 4.4**	**24.5 ± 4.1**

3.3.2 Crosslinking of the copolymers

3.3.2.1 Coumarin – coumarin crosslinking

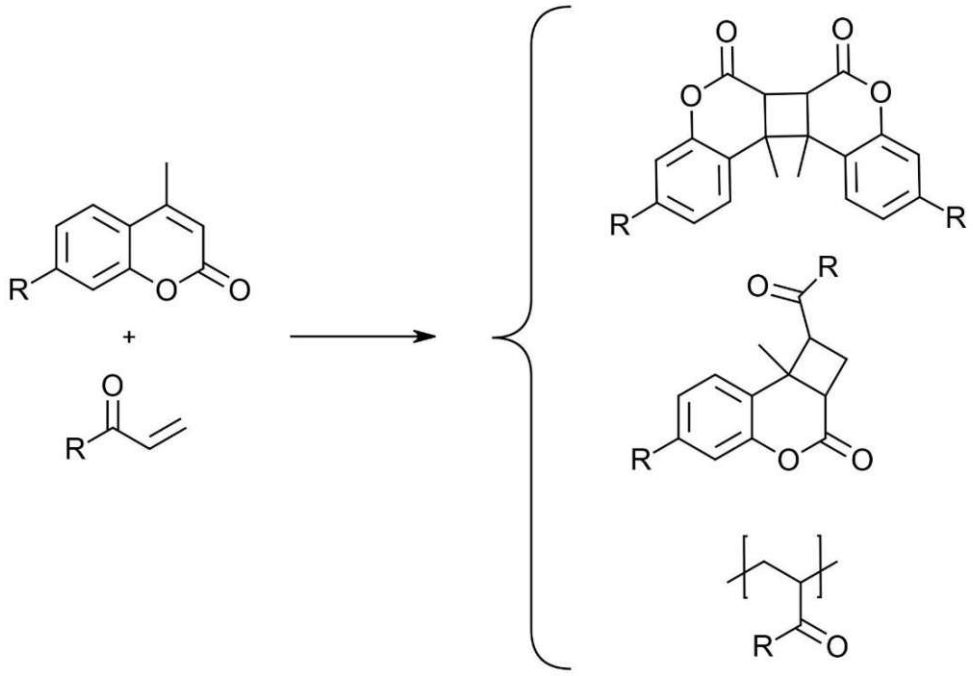


A copolymerized sample, PEtOx_10_C8, with 5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content (DP 100) was used in a 40 wt% formulation with DMSO as solvent. 50 µL were put into a silicon mold (round, 10 mm diameter) and irradiated at a distance of 1.8 cm with a broadband lamp (320 – 500 nm) with 2.51 W/cm² for 30 minutes. The brown gels were swollen in water for three days, afterwards they were frozen and lyophilized to determine the gel content and the swelling degree (Table 31) according to chapter 3.3.1.

Table 31: Gel content and swelling degree (in water) of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content) in the pure polymer gel (40 wt% in DMSO, CoEt) irradiated for 30 minutes with the OmniCure 3 (320 – 500 nm), 2.51 W/cm² with 18 mm distance

Sample	$m_{swollen}$ (mg)	m_{dry} (mg)	Gel content (%)	Swelling degree
CoEt 1	61.5	2.8	12.8	21.0
CoEt 2	39.9	3.5	15.9	10.4
CoEt 3	50.3	2.8	12.8	17.0
CoEt 4	53.0	2.9	13.2	17.3
CoEt 5	42.0	2.8	12.8	14.0
CoEt 6	48.7	2.8	12.8	16.4
Average	-	-	**13.4 ±1.3**	**16.0 ± 3.5**

3.3.2.2 Coumarin – acrylate crosslinking



12.0 wt% of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin, DP 100) and 12.3 wt% PEG diacrylate (MW 700 g/mol) were dissolved in DMSO and 50 µL were filled into a silicon mold (round, 10 mm diameter). The formulation was protected with a microscope cover glass and irradiated at a distance of 1.8 cm with a broadband lamp (320 – 500 nm) with 2.51 W/cm² for 10 minutes. The stable gels were swollen in water, frozen and lyophilized to investigate the gel content and the swelling degree according to chapter 3.3.1 displayed in Table 32.

Table 32: Gel content and swelling degree (in distilled water) of PEtOx_10_C8 (5.3 % (UV/Vis) / 8.2 % (NMR) coumarin content) mixed with PEG diacrylate (12.0 wt% coumarin polymer and 12.3 wt% PEG diacrylate in DMSO, CoA) irradiated for 30 minutes with the OmniCure 3 (320 – 500 nm), 2.51 W/cm² with 18 mm distance

Sample	$m_{swollen}$ (mg)	m_{dry} (mg)	Gel content (%)	Swelling degree
CoA 1	101.7	17.7	66.2	4.7
CoA 2	102.4	19.0	71.1	4.4
CoA 3	109.4	19.2	71.8	4.7
Average	-	-	**69.7 ± 3.0**	**4.6 ± 0.2**

3.4 US degradation

3.4.1 Degradation in the ultrasonic bath

The dried samples from the chapters 3.3.2.1 and 3.3.2.2, both containing the copolymer PEtOx_10_C8 with 5.3 % (UV/Vis) / 8.2 % (NMR) of coumarin units, were swollen overnight in DMSO and put into 1.5 mL Eppendorf tubes filled with fresh DMSO.

For each crosslinking method (coumarin – coumarin and coumarin – acrylate) triplicates were performed, where one sample of coumarin – coumarin crosslinking consisted of two gels due to the low weight. 300 µL fresh DMSO per sample were added and taken out in before and after each step displayed in Table 33.

The first part of the cycle was a 30 minutes period without ultrasound (US) (step No. 1, 3 and 5). Afterwards, the DMSO solution was taken out of the Eppendorf tube and 300 µL fresh DMSO was added before the second part took place: a 15 minutes period in the ultrasonic bath at 25 °C (step No. 2, 4 and 6). Again, after this period the DMSO solution was separated and fresh DMSO was added, until three cycles had taken place (three times without and three times with US). At the end of the study, there was again a 30 minutes period without US (step No. 7), but after removing the DMSO the gels were smashed with a spatula. 300 µL DMSO were added and the tubes were put to the ultrasonic bath for 90 minutes to simulate a total release (step No. 8).

Table 33: Steps of the degradation study in the ultrasonic bath at 25 °C, including periods with and without US and a simulated total release in step No. 8

Step No.	Duration (min)	Ultrasound
1	30	Off
2	15	On
3	30	Off
4	15	On
5	30	Off
6	15	On
7	30	Off
8	90	On

The 8 DMSO solutions from each sample were measured on the UV/Vis spectrometer at 318 nm in a 1 mm thick cuvette with pure DMSO as reference. The summarized coumarin content from all 8 solutions (inclusive total release, step No. 8) were normalized as 100 %. The measured absorption (see Appendix) represents the amount of the decrosslinked polymer chains (free polymer chains present in the solution). The relative cumulative release after each step is displayed in Table 34.

Table 34: Relative cumulative release of decrosslinked coumarin copolymers during the degradation study in the ultrasonic bath for both the coumarin - coumarin crosslinked samples (CoEt) and the coumarin - acrylate crosslinked samples (CoA) based on the total release after all steps

Time (min)	CoEt		CoA	
	Average release (%)	Standard dev. (%)	Average release (%)	Standard dev. (%)
30	2.0	0.8	1.7	0.6
45	13.2	7.9	11.6	8.1
75	14.3	8.5	14.8	6.8
90	18.3	10.0	23.8	4.5
120	20.1	10.9	26.3	4.9
135	46.5	3.6	43.6	2.8
165	49.2	3.4	57.6	10.7
255	100	-	100	-

3.4.2 Sonorheology

To investigate the rheological properties of the synthesized hydrogels, they had to be synthesized in a special shape. To fit between the ultrasonic transducer and the stamp of the rheometer the gels had to be round with a diameter of 20 mm and 1 mm thick. The formulations (see Table 35) were cured in this shape with a broadband lamp (320 – 500 nm) at 4.40 W/cm² in 8.0 cm distance. Afterwards the gels were swollen in water overnight. Two gels with the same composition as M0 and M3 were crosslinked directly before the measurement and were not swollen.

Table 35: Used composition of the hydrogels measured on the sonorheometer containing a copolymerized coumarin-polymer (PEtOx_10_C8, 5.3 % (UV/Vis) / 8.2 % (NMR) coumarin, DP 100) and either PEG dimethacrylate (M) or PEG diacrylate (A) in dioxane

Name	PEtOx_10_C8 (wt%)	Polymer 2 (wt%)
A0	-	25.1 A
A1	12.1	12.5 A
A2	16.2	8.6 A
A3	17.8	6.4 A
A4	20.8	4.2 A
M0	-	25.0 M
M1	12.5	12.1 M
M2	15.7	8.9 M
M3	17.6	6.7 M
M4	20.6	4.1 M

The gels were placed on the sonorheometer and a measurement of 5 minutes was started. The rheological parameters were a shear strain of 0.02 % and a frequency of 1 Hz. The first minute was measured without ultrasonication, the second minute was with US (sonication duration), and the last three minutes were again without US. During the ultrasonication, pulsed US in form of a sin wave with a frequency of 1 MHz was used. This pulsed waves were necessary not to overheat the system, as the temperature is rising during the measurement depending on the parameters of the US. As shown in Figure 30, one pulse (burst period) was set to 10 ms, and with a duty cycle of 20 % the effective ultrasonication took 2 ms. The amplitude of the driving voltage of waveform generator was set to 200 mV_{RMS}. The precise output of the intensity could not be measured due to reflection and absorption of the current set-up.

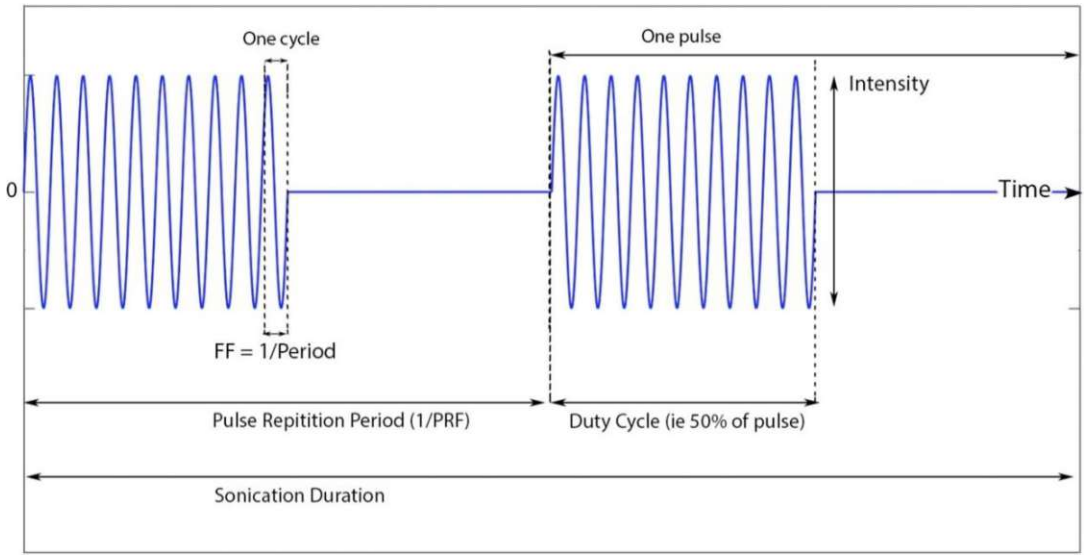


Figure 30: Depiction of pulsed US used in the sonorheology experiments[109]

Materials and Methods

2-Ethyl-2-oxazoline, 2-methyl-2-oxazoline (both Sigma-Aldrich) and acetonitrile (Arcos Organics) were dried with CaH_2 overnight, distilled under argon and stored over molecular sieves. Benzonitrile (Arcos Organics) was dried with phosphorus pentoxide overnight, distilled in vacuo and stored over molecular sieve (3 Å, Merck). Methyl tosylate and methyl triflate (purchased from Sigma-Aldrich) were distilled *via* Kugelrohr distillation and stored under argon. Methyl nosylate, purchased from Sigma-Aldrich, was dried on HV prior to use. Dry ethyl acetate was purchased from Acros Organics and stored under inert gas atmosphere.

4-Methoxy-α-toluenethiol and acrylic acid were purchased from Sigma-Aldrich, distilled in vacuo prior to use and stored under argon. Iodine was purchased from Merck and used as received. Triethylamine (TEA) was purchased from Roth, distilled under argon and stored over molecular sieves excluded from light. Isobutyl chloroformate and chloroethylamine hydrochloride were purchased from Sigma-Aldrich and stored under inert atmosphere. Dry DMF was purchased from Acros Organics and stored over molecular sieves under argon. K_2CO_3 and p-anisic acid were purchased from Merck and used as received.

2-Mercaptopropionic acid was purchased from Fluka, distilled prior to use and stored under argon, 2-nitrobenzyl bromide was purchased from Sigma-Aldrich and used as received. N-hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich and EDC*HCl was purchased from abcr, both used as received and stored at -18 °C. Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid)) was purchased from Sigma-Aldrich and used as solution in ethanol for qualitative analysis of free thiols.

4-Methylumbelliferone was purchased from Aldrich and used as received. Ethyl bromoacetate was purchased from Fluka and distilled in vacuo prior to use. Acetone was dried with anhydrous K_2CO_3 overnight, distilled under argon and stored over molecular sieve. Dioxane was purchased from Acros Organics and used as received. DCC was purchased from Sigma-Aldrich and stored over silica gel.

Commercial grade dichloromethane (DCM, Donau Chemie), Methanol (MeOH, Donau Chemie) and tetrahydrofuran (THF, Donau Chemie) were dried using a PureSolv system (Inert, Amesbury, MA).

Chloroform-d, DMSO-d_6, Methanol-d_4, D_2O, THF-d_8 and acetonitrile-d_3 were purchased from Eurisotop and used as received.

Yellow light laboratory was used as location for all synthesis and preparation of the formulations. All windows of this laboratory were covered with adhesive foils (company: IFOHA), and the fluorescent lamps were type Osram lumilux with chip control light colour 62 (filtration of wavelengths below 480 nm)

Karl Fischer titrations (KFT) were done using an Envirotech CA-21 moisture meter with the anode solution "Aquamicron AX Karl Fischer Reagent for Coulometric Moisture Meter" containing methanol, propylene carbonate, 2,2'-iminodiethanol, sulfodioxide and iodine. As a cathode solution, the reagent "Aquamicron CXU Karl Fischer Reagent for Coulometric Moisture Meter" was used, which contains methanol, ethane-1,2-diol and choline chloride. In order to measure the water content, an appropriate amount of reagent (0.1 – 0.3 mL) was weighed in a syringe with mg accuracy and injected into the device. Liquid reagents could be measured without further preparation steps. The titration device displayed the amount of water in mg and simultaneously in ppm referring to the sample weight that was entered.

Thin layer chromatography (TLC) was done on silica-coated aluminium foils (silica 60 F_{254}) produced by the company Merck.

Column chromatography was carried out using a preparative column using Silica gel 60 (Merck, 40 – 64 µm). The used eluents are mentioned at the corresponding chapter in the experimental part of the purified product.

Dialysis was carried out by dissolving the concerning polymer in deionized water and filling it into dialysis membranes with a MWCO of 1 kDA (Spectrum Laboratories, Inc.). The closed membranes were put into a bucket with 5 L water that was changed after one hour. After another 23 hours the membrane was removed from the bucket and opened into a beaker to receive the purified polymer.

For **lyophilization** the polymer solutions were stored in round bottom flasks and the hydrogel samples were stapled in a freeze-drying flask from Christ MartinTM (volume of 600 mL). The samples were either frozen with liquid N_2 or in a freezer at -20°C. For the sublimation a Christ freeze-drying system Gamma 2-20 at a pressure of ~0.5 mbar and a collector temperature of -45 °C was used.

HR-MS analysis was performed from the monomer samples, dissolved in HPLC-grated acetonitrile (concentration: 10 µM), by using an HTC PAL system autosampler (CTC Analytics AG, Zwingen, Switzerland), an Agilent 1100/1200 HPLC with binary pumps, degasser, and column thermostat (Agilent Technologies, Waldbronn, Germany), and an Agilent 6230 AJS ESI-TOF mass spectrometer (Agilent Technologies, Palo Alto, CA).

NMR spectra were recorded on a Bruker DPX-200 FT-NMR spectrometer at 200 MHz for ^{1}H and 50 MHz for ^{13}C, as well as on a Bruker Avance DRX-400 FT-NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The signals are recorded according to their chemical shifts, which were reported in ppm (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, sep = septet, m = multiplet, bs = broad singlet) in comparison to tetramethyl silane (d = 0 ppm). The spectra were then referenced on the used NMR-solvent [^{1}H: $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm), MeOD (3.3 ppm), ^{13}C: $CDCl_3$ (77.16 ppm)].

Analysis of the spectra was performed with the program MestRe Nova v12.0.4 from Mestrelab Research S.L.

Melting Points (or decomposition onsets) were conducted with an OptiMelt Automated Melting Point System (Standford Research System) at a heating rate of 1 °C min^{-1}.

Gel permeation chromatography (GPC) measurements were carried out in DMSO (purchased from PanReac AppliChem, 99.5 % for synthesis) on a Viscotek GPCmax device using three columns (2 AppliChrom ABOA DMSO-Phil-P-250, 100 – 70000 Da, 300 x 8 mm and 50 x 8 mm and AppliChrom DMSO-Phil-P-350, 5 – 1500 Da, 300 x 8 mm) equipped with a RI detector (Waters 2410), 1 mL/min at 22 °C. Samples were prepared as 2 – 10 mg/mL DMSO solutions that were syringe-filtered. A calibration with polymethylmethacrylate (PMMA) standards was used to determine the molecular weight of the polymers.

For irradiating the samples, an OmniCure LX400 **UV-LED-spot curing system** was used with a 365 nm LED. In order to measure the light intensity an Ocean Optics 2000+ USB device was used with the SpectraSuit.

Additionally, an OmniCure 2000, equipped with a 320-500 nm filter and a single-tube liquid filled light guide with a diameter of 8 mm, was used. The **UV source** was calibrated at least once a day with an Omnicure R2000 radiometer.

For the UV-Vis measurements samples were dissolved in pure methanol or hexafluoro isopropanol and measured in either 10 mm or 1 mm quartz cells on a UV-1800 **UV-Vis spectrometer** by Shimadzu (scanning mode) from 250 to 450 nm at a slit width of 2 nm. The software Spectrum from PerkinElmer v10.03.07 was used to process the data.

The degradation studies were performed in a **digital ultrasonic cleaner (ultrasonic bath)** "MH-009S" by Vevor with a rated voltage of 200 – 240 V and 50 Hz and ultrasonic power of 60 W at a temperature of 25 °C.

Measurements on the **sonorheometer** were performed on an Anton Paar MCR 302 device in a plate-plate geometry, equipped with a US transducer (frequency 1 MHz, active diameter 23 mm, Precision Acoustics Ltd, UK) serving as a bottom plate. The US transducer was connected in series with a 75 Watt RF power amplifier (model 75A400, Amplifier Research Inc. Souderton, PA, USA) and a waveform generator (33220A, Agilent Technologies, Santa Clara, CA, USA). For the rheology a PMMA stamp with 20 mm diameter was used, oscillatory measurements were done with a frequency of 1 Hz and a shear strain of 0.02 %. During the measurements, the US transducer was driven by a pulsed sin wave with a voltage adjusted to 200 mV_{RMS}, and a duty cycle of 20 % (2 ms on, 8 ms off). During the first minute,

only the rheological properties of the gels were measured without ultrasound, then one minute was measured with ultrasonication (sonication duration) and further three minutes without ultrasound.

Abbreviations

ACN	Acetonitrile
CMC	Critical micellar concentration
CoumarinOx	2-[(4-Methyl-7-coumarinyloxy)methyl]oxazoline
CROP	Cationic ring-opening polymerization
Đ	Dispersity
DCC	N,N‘-dicyclohexylcarbodiimide
DCM	Dichloromethane
DDS	Drug delivery system
DH	Degree of hydrolysis
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DW	Deionized water
EDC*HCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EE	Ethyl acetate
eq.	Equivalent
EtOH	Ethanol
EtOTs	Ethyl p-toluenesolfonate (ethyl tosylate)
EtOx	2-Ethyl-2-oxazoline
GPC	Gel permeation chromatography
HV	High vacuum
KFT	Karl-Fischer titration
k_p	Propagation rate constant (in L mol^{-1} s^{-1})
LCST	Lower critical solution temperature
LED	Light emitting diode
MeCN	Acetonitrile
MeOH	Methanol
MeONs	Methyl 4-nitrobenzenesulfonate (methyl nosylate)
MeOTf	Methyl trifluoromethanesulfonate (methyl triflate)
MeOTs	Methyl p-toluenesulfonate (methyl tosylate)
MeOx	2-Methyl-2-oxazoline
m_{dry}	Weight of dried gel
$m_{formulation}$	Weight of used formulation
$\bar{M}_n$	Number average molecular weight
$m_{swollen}$	Weight of swollen gel
MOB-Sox	2-[2-(4-Methoxybenzylsulfanyl)ethyl]-2-oxzoline
%	Molar percent
Mpa(Mob)	3[[(4-Methoxyphenyl)methyl]thio]propionic acid
MWCO	Molecular weight cut off
NbMEtOx	2-{2-[(2-Nitrobenzyl)thio]ethyl}-4,5-dihydrooxazole
NHS	N-hydroxysuccinimide
NMR	Nuclear magnetic resonance
PE	Petrol ether, hexanes
PEG	Poly(ethylene glycol)
PEI	Poly(ethyleneimine)
PEtOx	Poly(2-ethyl-2-oxazoline)
PIPAAm-PBMA	Poly(N-isopropylacrylamide-b-butylmethacrylate)

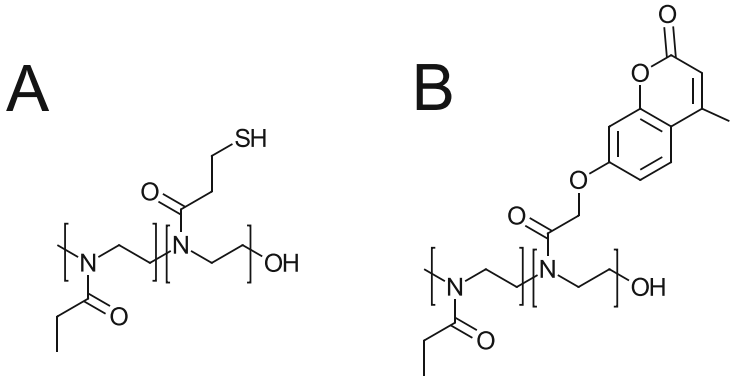
PLA	Polylactic acid
PMeOx	Poly(2-methyl-2-oxazoline)
PMMA	Poly(methylmethacrylate)
POx	Poly(2-oxazoline)
PPG	Poly(propylene glycol)
ppm	Parts per million
PTFE	Poly(tetrafluoroethylene)
PVA	Poly(vinyl alcohol)
R_f	Retarding front value
RT	Room temperature
TEA	Triethylamine
THF	Tetrahydrofuran
US	Ultrasound
UV	Ultraviolet
wt%	Weight percent

Summary

The aim of this work was the investigation of new materials for ultrasound-triggered drug delivery systems (DDS) based on poly(2-oxazoline)s (POx). Their peptide-like structure makes them highly favourable for biomedical applications, but due to their slow polymerization rates they nearly fell into oblivion. As the polymerization via microwave reactors was introduced, POx are now an option as substitute *e.g.* for poly(ethylene glycol) (PEG).

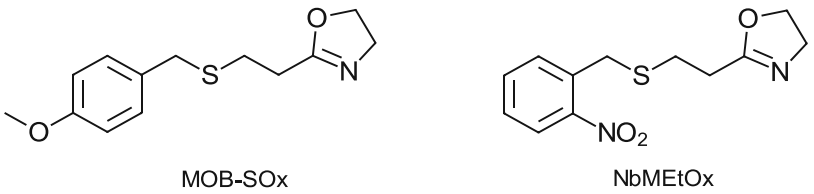
2-Oxazolines are polymerized *via* cationic ring-opening polymerization (CROP) with a living character. That results in well defined polymers with a narrow dispersity, but also special reaction conditions, as nucleophiles like moisture would terminate the reactions and interfere with the living character.

Two strategies were envisaged to synthesize POx hydrogels responsive to ultrasound (US): firstly, to introduce thiol moieties and secondly to use coumarin moieties (Scheme 31).



Scheme 31: Poly(2-oxazoline) modified with thiol moieties (A) and with coumarin moieties (B)

A known strategy to receive thiol-containing POx is to synthesize a 2-oxazoline monomer with a protected thiol. The first attempt according to Cesana *et al.*[66] was the synthesis of 2-[2-(4-methoxybenzylulfanyl)ethyl]2-oxazoline (MOB-SOx; Scheme 32).



Scheme 32: Synthesized 2-oxazoline monomers with protected thiols: 2-[2-(4-methoxybenzylulfanyl)ethyl]2-oxazoline according to Cesana et al.[66] (MOB-SOx) and 2-{2-[(2-nitrobenzyl)thio]ethyl}-4,5-dihydrooxazole (NbMEtOx) according to Jung et al.[32]

MOB-SOx was synthesized successfully, but unfortunately the purification was challenging as the liquid product had to be dried with CaH_2 and distilled afterwards. In addition, the remove of the protecting group is carried out with toxic substances that had to be removed completely before the material could be used in medical applications.

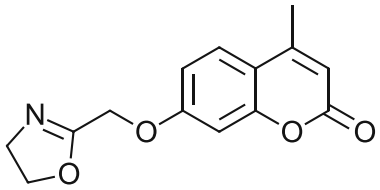
The second thiol-based monomer was 2-{2-[(2-nitrobenzyl)thio]ethyl}-4,5-dihydrooxazole (NbMEtOx; Scheme 32) according to Jung et al.[32] The largest benefit of this 2-oxazoline was its possible deprotection with irradiation, so no toxic chemicals were needed. Also, this synthesis was successful after three steps, although a low yield was achieved (3.6 %). Somehow, the product was obtained as yellow viscous liquid, different than the slightly orange solid described in literature. Nevertheless, 4 eq. NbMEtOx were copolymerized with 96 eq. 2-ethyl-2-oxazoline (EtOx) using 1 eq. initiator (DP 100). The received polymer did not contain any thiol moiety according to a test with the Ellman's reagent and NMR spectroscopy. Form this point on, the focus lied on the synthesis of coumarin-containing POx.

The second attempt to synthesize ultrasound-responsive hydrogels was to synthesize coumarin-containing POx. The coumarin containing POx were already prepared by Chujo *et al.*,[67] however, to the best of our knowledge, the POx-based hydrogel materials responsible to US have not been not reported yet.

The strategy here was to homopolymerize EtOx, hydrolyze it partially and attach coumarin derivatives onto the poly(ethylamine) (PEI) units.[67] To define the ideal conditions of both homopolymerization and hydrolysis, kinetic studies were performed, using three different solvents and three different initiators for the EtOx homopolymerization. With the hydrolysis kinetic study, the respective time was investigated to receive the desired degrees of hydrolysis (DH).

The desired polymers were synthesized with different chain lengths and coumarin content. The next step was to crosslink the polymers, but only one batch of polymer (4.7 % coumarin determined *via* UV/Vis spectroscopy, 7.8 % coumarin determined *via* ^{1}H-NMR spectroscopy, DP 100) resulted in a hydrogel (25 wt% polymer in dioxane, 365 nm with 270 mW/cm² and 18 mm distance). The gels were swollen in water and afterwards lyophilized to determine the swelling degree and the gel content. Nevertheless, this strategy was challenging regarding the partial hydrolysis and the determination of the DH and the coumarin content.

Therefore, similar polymers were synthesized by copolymerizing a coumarin-based 2-oxazoline, 2-[(4-methyl-7-coumarinyloxy)methyl]oxazoline (CoumarinOx, Scheme 33), with each EtOx and 2-methyl-2-oxazoline (MeOx). Additionally, also the kinetics of the homopolymerization of CoumarinOx and both of the copolymerizations were investigated.



CoumarinOx

Scheme 33: Synthesized coumarin-containing 2-oxazoline for copolymerization (CoumarinOx)

The received copolymers showed a low solubility in over 10 different solvents, they were only completely soluble in hexafluoro isopropanol and nearly soluble in DMSO. Thus, the formulation solvent was switched to DMSO and the polymer content was increase to 40 wt% to receive homogenous gels. Due to the change of the solvent, also the irradiation parameters had to be changed, therefore a broadband lamp with 320 – 500 nm was used with 2.51 W/cm². The CoumarinOx/EtOx copolymer (5.3 % coumarin content via UV/Vis, 8.2 % coumarin via NMR spectroscopy, DP 100) resulted in hydrogels after 30 minutes of irradiation, in contrast to the crosslinking of the CoumarinOx/MeOx copolymer (4.0 % coumarin content via UV/Vis, 5.3 % coumarin content via NMR spectroscopy, DP 100) what was not successful.

According to Eng *et al.*[95] it is also possible to crosslink coumarin moieties with methacrylates by irradiation. Three main reactions take place during the crosslinking: the dimerization of two coumarin moieties, the [2+2] cycloaddition of coumarin and methacrylate as well as the radical polymerization of the methacrylates. Subsequently, a formulation with CoumarinOx/EtOx copolymer and PEG diacrylate ($\bar{M}_n$ 700 g/mol) was mixed and irradiated with the same conditions as the formulation without acrylates. The result was a successfully crosslinked gel that was more stable than the gels made of pure coumarin-containing polymer.

Similar to the gels made of the modified polymers, the crosslinked gels were swollen in water and lyophilized to compare the gel content and the swelling degree (Table 36).

Table 36: Comparison of the swelling degree in water and the gel content of the hydrogels made of two different synthesized coumarin-containing polymers (P) and three different formulations, using PEG diacrylate (A) as second polymer, irradiated with 365 nm and 270 mW/cm² (modified polymer) or with 320 – 500 nm and 2.51 W/cm² (copolymerized polymer)

Polymer	**Coumarin content**	**Formulation**	**Gel content (%)**	**Swelling degree**
Modified	4.7 %	25 wt% P in dioxane	18.1 ± 4.4	24.5 ± 4.1
Copolymerized	5.3 %	40 wt% P in DMSO	13.4 ± 1.3	16.0 ± 3.5
		12.0 wt% P, 12.3 wt% A in DMSO	69.7 ± 3.0	4.6 ± 0.2

Comparing the gel content of the three types of gel in Table 36, the gel with acrylate content has the highest gel content. The cause could be the three different crosslinking mechanisms that are more

effective than only the coumarin dimerization. The swelling degree on the other hand is the lowest for the acrylate containing gels, here is also a significant difference between the modified and the copolymerized samples.

The further aim was the investigation of the degradation by US. Therefore, the crosslinked gels made of the copolymerized sample, both with and without acrylates, were swollen in DMSO. The swollen gels were put into 300 µL DMSO and placed alternating 30 minutes outside and 15 minutes inside the ultrasonic bath, replacing the DMSO after each step. The coumarin content of the decrosslinked polymer chains in the solution was measured *via* UV/Vis spectroscopy and displayed in Figure 31.

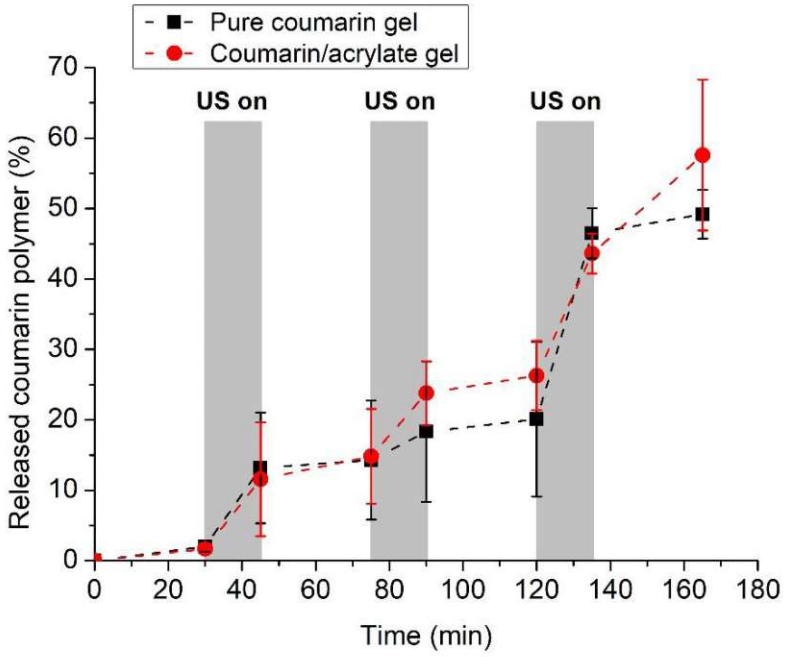


Figure 31: Degradation of gels (copolymerized samples with 5.3 % (UV/Vis)/8.2 % (NMR) coumarin content, DP 100; pure crosslinked polymer (black) and crosslinked polymer with PEG diacrylate (red)) swollen in DMSO during three periods in the ultrasonic bath (US on, grey bars)

In Figure 31, the relative amount of coumarin-containing polymer chains is shown that were cleaved over time. The diagram shows a nearly ideal behaviour what was desired in a similar way. The release during the periods with US (grey marked areas) is significantly higher than the release without US, what shows a very low rate. As the frequency and intensity of the ultrasonic bath is different than the US in medical facilities, this degradation study act as a proof of concept that the synthesized gels are degradable with US.

The next step was the investigation of the rheological properties of the gels during and after ultrasonication. As the gels made of pure coumarin-containing POx were very fragile and could not be transferred without destruction, only gels containing different amounts of PEG diacrylate or PEG dimethacrylate were measured on the sonorheometer. Special gels with 20 mm diameter and 1 mm

height were synthesized and swollen in DMSO to fit the area on the sonorheometer. The measurements consisted of 1 minute rheology without US (γ = 0.02 %, f = 1 Hz), 1 minute measurement with US (100 mV_{RMS}, f = 1 MHz, 20 % duty cycle) and further 3 minutes without US to investigate the changing properties.

Both samples with PEG diacrylates and PEG dimethacrylates behaved similar, the storage modulus decreased after one minute, as the US was switched on. After the US was switched off again, the storage modulus recovered to values similar to the rheology before the ultrasonication. The storage modulus after the ultrasonication even increased compared to the storage modulus before. As Eng *et al.*[95] proofed, nearly no coumarin dimer or coumarin – methacrylate addition was cleaved by US than rather the PEG backbones. Simultaneously, also swollen and non-swollen gels were compared with the same composition, resulting in less decrease of the storage modulus during the ultrasonication. This decrease could be explained by the increase of temperature from 25 to 52 °C during the treatment with US.

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Appendix

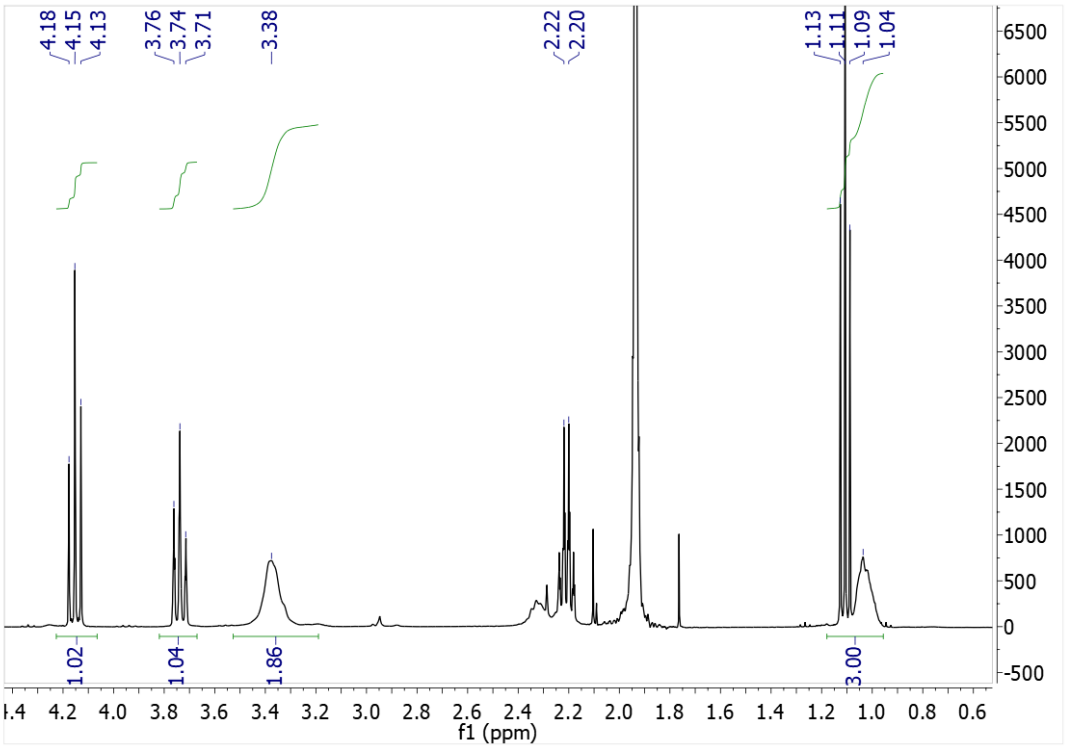

Figure 32: Determination of the homopolymerization kinetics of EtOx (3 M) using acetonitrile as solvent and MeOTs as initiator ([M]:[I] = 50) after 5 hours at 75 °C

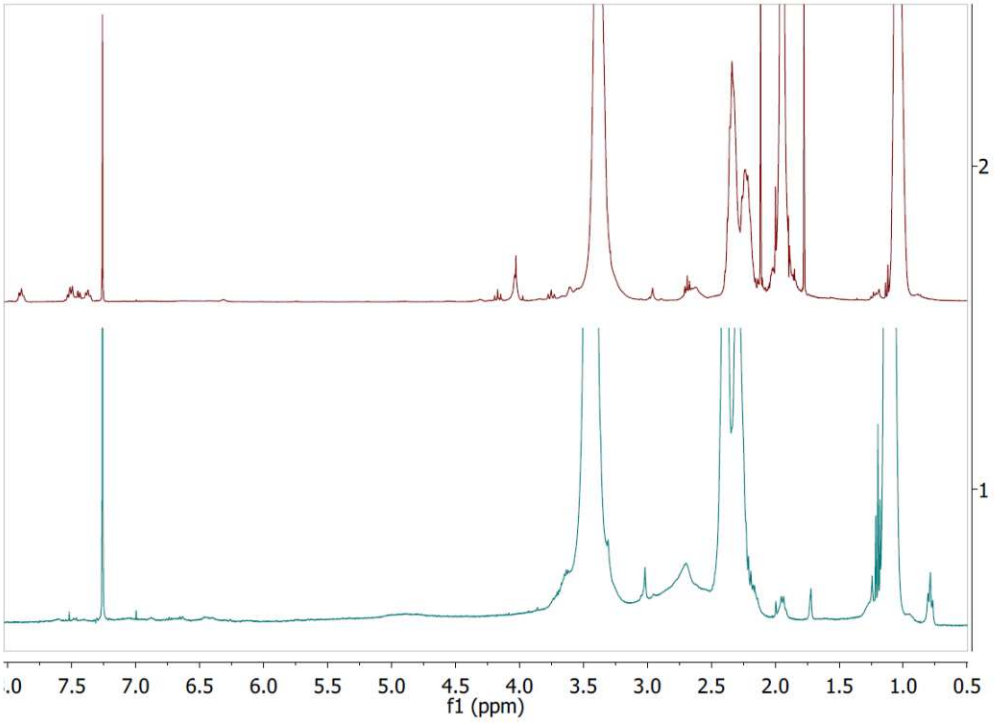

Figure 33: ^{1}H-NMR spectra of PEtOx-co-poly(NbMEtOx) before purification (2) with still visible aromatic peaks (between 8.00 and 7.30 ppm) and after purification (1) without visible aromatic peaks

Table 37: UV/Vis calibration values at 318 nm using (4-methylcoumarin7-yloxy)acetic acid in MeOH in three different dilution series with concentrations ranging from 10^{-5} to 10^{-4} mol/L

A		B		C	
Concentration (mol/L)	**max. absorbance**	**Concentration (mol/L)**	**max. absorbance**	**Concentration (mol/L)**	**max. absorbance**
$1.00 \cdot 10^{-4}$	1.531	$9.99 \cdot 10^{-5}$	1.629	$9.99 \cdot 10^{-5}$	1.522
$7.50 \cdot 10^{-5}$	1.240	$7.49 \cdot 10^{-5}$	1.186	$7.49 \cdot 10^{-5}$	1.172
$5.00 \cdot 10^{-5}$	0.768	$4.99 \cdot 10^{-5}$	0.826	$4.99 \cdot 10^{-5}$	0.772
$2.50 \cdot 10^{-5}$	0.432	$2.49 \cdot 10^{-5}$	0.456	$2.49 \cdot 10^{-5}$	0.418
$1.00 \cdot 10^{-5}$	0.182	$9.99 \cdot 10^{-6}$	0.185	$9.99 \cdot 10^{-6}$	0.180

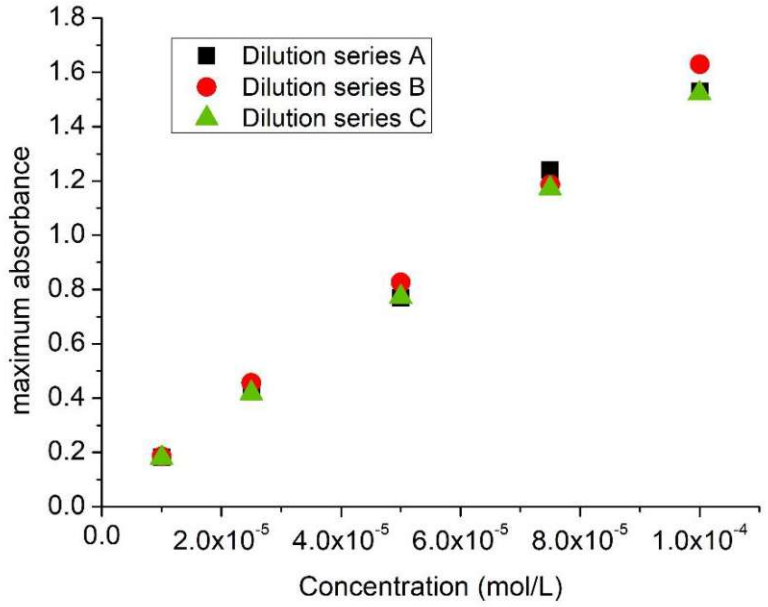

Figure 34: UV/Vis calibration (318 nm) using (4-methylcoumarin7-yloxy)acetic acid in MeOH in three different dilution series with concentrations ranging from 10^{-5} to 10^{-4} mol/L

Table 38: Investigation of the copolymerization kinetics of EtOx and CoumarinOx using MeOTf as initiator and ACN as solvent, 20 eq. CoumarinOx and 80 eq. EtOx, DP 50

Time	Integrals				Overall conv.	EtOx		CoumarinOx	
h	CoumarinOx	EtOx	EtOx CH_3	Polymer	(%)	Conv.	ln	Conv.	ln
0	2.06	10.69	16.27	0.00	0.000	1.44	0.01	0.00	0.00
16	2.02	8.84	17.81	5.81	17.427	25.55	0.30	0.00	0.00
24	1.99	7.37	17.07	7.65	22.487	35.24	0.43	0.50	0.01
40	1.91	5.42	17.70	12.05	31.089	54.07	0.78	4.50	0.05
48	1.83	4.44	16.66	12.94	33.680	60.02	0.92	8.50	0.09
64	1.80	3.48	17.09	16.2	37.709	69.46	1.19	10.00	0.11
72	1.78	3.17	17.88	17.61	39.029	73.41	1.32	11.00	0.12
88	1.71	2.58	18.82	20.03	41.180	79.44	1.58	14.50	0.16

Table 39: Investigation of the copolymerization kinetics of MeOx and CoumarinOx using MeOTf as initiator and ACN as solvent, 20 eq. CoumarinOx and 80 eq. MeOx, DP 50

Time	Integrals			Overall conv.	MeOx		CoumarinOx	
h	CoumarinOx	MeOx	Polymer	(%)	Conv.	ln	Conv.	ln
0	2.10	8.18	0.00	0.000	0.00	0.00	0.00	0.00
16	2.01	5.28	6.98	24.457	35.45	0.44	0.00	0.00
24	2.01	4.44	9.07	29.220	45.72	0.61	0.00	0.00
40	1.95	3.61	13.66	35.536	55.87	0.82	2.50	0.03
48	1.93	3.15	14.81	37.230	61.49	0.95	3.50	0.04
64	1.90	2.15	13.68	38.579	73.72	1.34	5.00	0.05
72	1.87	1.93	14.89	39.834	76.41	1.44	6.50	0.07
88	1.85	1.68	16.25	41.077	79.46	1.58	7.50	0.08

Table 40: Intensity of the solutions obtained from the release of decrosslinked coumarin copolymers during the degradation study in the ultrasonic bath measured on the UV/Vis spectrometer for both the coumarin - coumarin crosslinked samples (CoEt) and the coumarin - acrylate crosslinked samples (CoA)

	CoEt			CoA		
Time (min)	I	II	III	A1	A2	A3
30	0.020	0.041	0.067	0.009	0.041	0.004
45	0.205	0.357	0.057	0.016	0.357	0.031
75	0.020	0.032	0.012	0.019	0.032	0.016
90	0.089	0.090	0.048	0.074	0.090	0.025
120	0.045	0.040	0.011	0.016	0.040	0.008
135	0.415	0.252	1.000	0.058	0.252	0.107
165	0.048	0.055	0.057	0.100	0.055	0.071
255	0.800	1.050	1.199	0.169	1.050	0.146