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ANTIBACTERIAL EQUIPMENT OF POLYOLEFINS VIA THIOL-ENE CHEMISTRY

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To my family

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Abstract

Many areas of the daily life require control over the microbiological affection. As conventional low molecular weight disinfectants suffer from several disadvantageous properties such as leaching, antibacterial contact polymers, installed in manifold applications as medical devices, water pipe systems or food storage and packaging facilities, gained a lot of interest within the last few years due to several beneficial properties such as long-term stability, no disposal of toxic residues and no volatility.

Herein, the synthesis and characterization of new antibacterial polymers based on post-modified poly(isoprene) and subsequent quaternization of introduced amines is presented. Thereby, the thiol-ene reaction was applied, known for its “click“ characteristics, robustness and wide range of combinable educts. The obtained polymers exhibited good thermal stability and excellent antibacterial properties. Therefore, one polymer, featuring a functionalization degree of 20% and quaternized by alkylation, was chosen for compounding in 2.5 and 5.0 wt% with a commodity polymer. The antibacterial activity could be preserved within this process and a bacteriostatic material was obtained.

In the second part of this work, two main objectives were in the focus of investigations: on the one hand, the development of new thiols, allowing a solvent free thiol-ene modification, on the other hand the formation of an insoluble antibacterial lacquer was accomplished. New thiols were synthesized, starting from cystamine dihydrochloride, either by an Eschweiler-Clark reaction or a reductive amination reaction, subsequent exhaustive methylation and reduction to the free thiol. In this manner, aminothiols with alkylated amines in the range of one to ten carbon atoms were obtained. Thiol-ene reactions with several low molecular weight olefins, however, showed only moderate conversions.

For the development of an antibacterial lacquer, a high vinyl content poly(butadiene) was functionalized with N,N-dimethylcysteamine hydrochloride in a first step. Films were prepared from the latter polymer, natural rubber and a tetra-functional thiol as crosslinking agent. After illumination, networks were obtained and characterized by means of FT-IR, recovery tests, contact angle as well as antibacterial measurements. It could be shown, that predominantly vinyl double bonds underwent crosslinking reactions. Antibacterial activity tests of the pure polymer showed a bacteriostatic to antibacterial activity to diverse bacteria strains, which could not be preserved in the lacquer formulation.

Kurzfassung

In vielen Bereichen des täglichen Lebens ist eine Kontrolle des mikrobiellen Befalls unabdingbar. Da herkömmliche niedermolekulare Desinfektionsmittel Nachteile wie Auswaschen aufweisen, gewannen antibakterielle Kontaktpolymere, eingesetzt für die vielfältigsten Anwendungen wie medizinische Geräte, Wasserrohrsysteme oder Lagerungs- und Verpackungsstationen für Lebensmittel, aufgrund mehrerer vorteilhafter Eigenschaften, wie zum Beispiel Langlebigkeit, keine Freisetzung von toxischen Reststoffen und keine flüchtigen Inhaltsstoffe, an Bedeutung.

In dieser Arbeit wird die Synthese und Charakterisierung neuer antibakterieller Polymere, basierend auf der Post-Modifizierung von Polyisopren und nachfolgender Quaternisierung der eingeführten Amine, präsentiert. Dabei wurde eine Thiol-en Reaktion angewandt, die für ihre „Klick“ Eigenschaften, Robustheit und viele kombinierbare Edukte bekannt ist. Die erhaltenen Polymere wiesen ein gutes thermisches Verhalten sowie exzellente antibakterielle Eigenschaften auf. Deshalb wurde ein Polymer mit einem Funktionalisierungsgrad von 20%, das durch Alkylierung quaternisiert wurde, ausgewählt und in einen handelsüblichen Kunststoff in 2.5 und 5.0 Gew.-% einkompoundiert. Die antibakterielle Aktivität wurde nach diesem Prozess bewahrt und ein bakteriostatisches Material wurde erhalten.

Im zweiten Teil der Arbeit lagen zwei Ziele im Fokus der Untersuchungen: einerseits die Entwicklung neuer Thiole, die eine lösungsmittelfreie Thiol-en Modifizierung erlauben sollten, andererseits die Formulierung eines antibakteriellen Lacks. Neue Thiole wurden ausgehend von Cystamin Dihydrochlorid entweder mit einer Eschweiler-Clark Reaktion oder durch reduktive Aminierung synthetisiert, nachfolgend erschöpfend methyliert und durch Reduktion das Thiol freigesetzt. Auf diese Art wurden Aminothiole mit einem alkylierten Stickstoff mit einem bis zehn Kohlenstoffatome erhalten. Modell Thiol-en Reaktionen mit einigen niedermolekularen Olefinen und den neuartigen Aminothiolen zeigten allerdings nur geringe Umsetzungen.

Für die Entwicklung eines antibakteriellen Lacks wurde in einem ersten Schritt ein Polybutadien mit hohem Vinylanteil mit N-2-(Dimethylamino)ethanthiolhydrochlorid funktionalisiert. Filme aus diesem Polymer, Naturkautschuk und einem tetra-funktionalen Thiol als Vernetzungsreagenz wurden bereitet. Nach Belichtung wurden Netzwerke erhalten, die mittels FT-IR, Wiedergewinnungstests, Kontaktwinkelmessungen und antibakteriellen Untersuchungen charakterisiert wurden. Dabei wurde gezeigt, dass in erster Linie vinylische Doppelbindungen vernetzend reagieren. Antibakterielle Tests des reinen Polymers zeigten eine bakteriostatische bis antibakterielle Aktivität gegen mehrere Bakterienstämme, die in der Lackformulierung nicht bewahrt werden konnte.

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1 BACKGROUND

1.1 THIOL-ENE REACTION

1.1.1 INTRODUCTION

In 1905, Posner described the hydrothiolation of a C=C bond, in other words: the reaction of thiols with reactive carbon-carbon double bonds- enes- to the corresponding thioether.[1] What makes that reaction so widely useable are following attributes: such reactions can proceed under a variety of conditions, such as a radical pathway[2], via catalytic processes mediated by nucleophiles[3], acid/base catalysis[4] or without a catalyst in highly polar solvents such as water or dimethyl formamide[5] or finally via supramolecular catalysis.[6] Secondly, due to that diversity of mechanisms, a high number of enes serve as suitable substrates; depending on the desired product, activated and non-activated species as well as multiple-substituted olefinic double bonds can be employed. Yet, it has to be considered that reactivity strongly depends on the chosen mechanism as well as on the nature of the ene. Further, the range of thiols that can be used for such reactions is almost unlimited; however, efficiency is strongly dependent on the S-H bond strength and the cleavage mechanism.[7] Finally, the rapidness of such reactions (in the range of seconds under optimized reaction

[1] Posner, T. *Ber Dtsch Chem Ges* **1905**, *38*, 646-657.

[2] Hoyle, C. E.; Lee, T. Y.; Roper, T. *J* Polym *Sci, Part A: Polym Chem* **2004**, *42*, 5301-5338.

[3] Sanui, K.; Ogata, N. *Bull Chem Soc Jpn* **1967**, *40*, 1727.

[4] Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *J Polym Sci Part A: Polym Chem* **2008**, *46*, 5093–5100.

[5] [a] Kakwere, H.; Perrier, S. *J Am Chem Soc* **2009**, *131*, 1889-1895. [b] Tolstyka, Z. P.; Kopping, J. T.; Maynard, H. D. *Macromolecules* **2008**, *41*, 599.

[6] Krishnaveni, N. S.; Surendra, K.; Rao, R. *Chem Comm* **2005**, 669-671.

[7] [a] Lowe, A. B. *Polym Chem* **2010**, *1*, 17-36. [b] Lowe, A. B.; Harvison, M. A. *Aust J Chem* **2010**, *63*, 1251-1266.

conditions[8]), the robustness against oxygen and humidity[9] under neat conditions and the proceeding to near quantitative formation of the thioether product make this reaction applicable to a high number of scientific requests.

Those mentioned criteria allow considering the thiol-ene reactions as click reactions, by definition by Sharpless *et al*.[10] as i) the obtained yields are high, whereas side products are negligible and easily to be removed, ii) reactions are insensitive towards oxygen and water, iii) regionspecificity and stereospecificity as well as iv) orthogonality is given to other organic synthesis and v) a wide range of educts are available.[11]

1.1.2 Mechanism

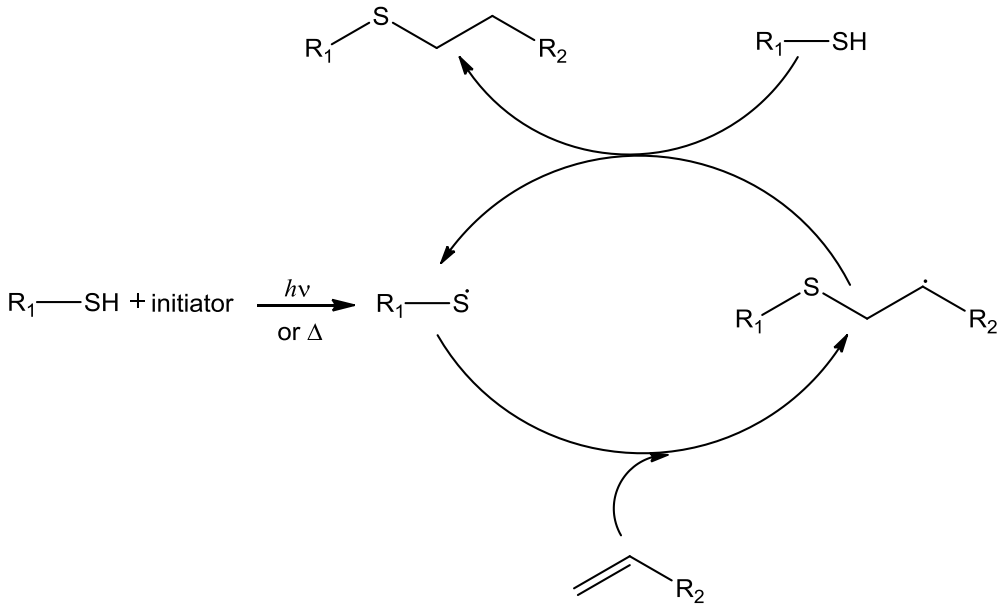
Most likely, the reaction is conducted under radical conditions, either photochemically or thermally induced. The general radical reaction pathway follows the depicted Scheme 1-1.

[8] Cramer, N. B.; Reddy, S. K.; O´Brien, A. K.; Bowman, C. N. *Macromolecules* **2003**, *36*, 7964-7969.

[9] Killops, K. L.; Campos, L. M.; Hawker, C. J. *J Am Chem Soc* **2003**, *130*, 5062-5064.

[10] [a] Kolb, H. C.; Finn, K. B.; Sharpless, K. B. *Angew Chem* **2011**, *113*, 2056-2075. [b] Kolb, H. C.; Finn, K. B.; Sharpless, K. B. *Angew Chem Int Ed* **2001,** *40*, 2004-2021.

[11] Hoyle, C. E.; Bowmann, C. N. *Angew Chem Int Ed* **2010**, *49*, 1540-1573.



Scheme 1-1 Mechanism of radical thiol-ene coupling[12]

By applying radical conditions, the reaction proceeds via a chain process with initiation, propagation and termination steps. By thermal or photochemical initiation, a thiyl radical RS$^{\bullet}$ plus side products are formed. This step can also occur by a thermal lysis of the S-H bond. [13]

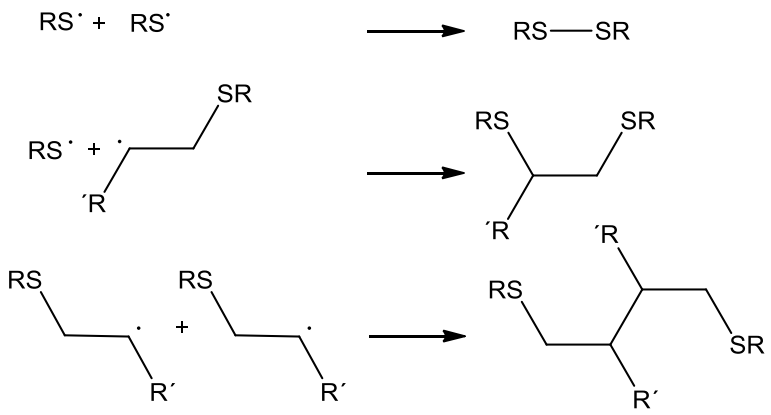
The propagation involves two steps, first the direct addition of the thiyl radical across the C=C double bond and second a chain transfer via a carbon centered radical. In an ideal reaction the propagation of the thiyl radical across the ene functionality and the chain transfer take place alternatingly. The addition reaction of the thiyl radical is exothermic with reaction enthalpies between -10.5 kcal mol^{-1} and -22.6 kcal mol^{-1} depending on the electronic state of the double bonds,[2,11] whereas electron rich double bonds react faster than electron poor ones. So, an intermediate carbon centered radical is yielded. A chain transfer to a newly emerging thiol then yields the next generation thiyl radical and the *anti*-Markovnikov orientated thiol-ene product is obtained.[7] The cyclic nature of these two alternating steps (propagation and chain transfer) and the overall rates, which are as required equal, lead to the following assumption: if one of the reaction steps is inherently slower, this step is rate-determining and also determines the relative concentration of the two thiyl radical species

[12] source: Kade, M. J.; Burke, D. J.; Hawker, C. J. *J Polym Sci Part A: Polym Chem* **2010**, *48*, 743-750.

[13] Cramer, N. B.; Scott, J. P.; Bowman, C. N. *Macromolecules* **2002**, *35*, 5361-5365.

present. Three cases are therefore possible: i) both steps exhibit equal reaction rates, ii) the chain transfer is rate determining and iii) the propagation is rate determining. In all cases the reaction exhibits a first order kinetics in respect to the concentration of the ene as well as the thiol. However, the indeed reactivity is dedicated by the nature and reactivity of both radicals and ene functional groups. Cramer *et al*.[8] showed, that thiols with less abstractable hydrogen atoms, *e.g.* alkyl thiols tend to have reduced chain transfer rates, whereas less reactive enes cause a slow propagation reaction and therefore this reaction is rate determining. More detailed remarks are given in chapter 1.1.3 and 1.1.4.

Termination reactions involve typical radical- radical coupling processes.



Scheme 1-2 Possible termination reactions of thiol-ene reaction

1.1.3 Influence of the Ene Structure

What makes the thiol-ene reaction so outstanding is the ability to convert almost any type of ene. Back in 1977 Morgan *et al*. originally observed a relationship between the chemical structure of the ene, the thiol and the reactivity.[14] An ordering by reactivity against three types of thiols (alkyl 3-mercaptopropionates, alkylthioglycolates and alkylthiol) was done by Hoyle and coworkers.[2,15] Although this ordering is generally supported, it has to be considered, that differences in the practical experiment or the thiol structure might lead to changes.

[14] Morgen, C. R.; Magnotta, F.; Ketley, A. D. *J Polym Sci, Polym Chem Ed* **1977**, *15*, 627-645.

[15] Cramer, N. B.; Bowmann, C. N. *J Polym Sci Part A: Polym Chem* **2001**, *39*, 3311.

Norbornene > Vinyl ether > Propenyl > Alkene ≈ Vinylester > N-Vinyl amides > Allyl ether ~ Allyltriazine > Allylisocyanurate > Acrylate > Unsaturated ester > N-substituted maleimide > Acrylonitrile ~ Methacrylate > Styrene> Conjugated dienes

Generally, the reactivity decreases with decreasing electron density of the carbon-carbon double bond. The first entry, norbornene, demonstrates a peculiarity to some extent, as the combination of the significant relief of ring-strain due to the addition of the thiyl radical across the double bond and the subsequent fast hydrogen- abstraction rate of the thiol hydrogen by the carbon- centered radical lead to extraordinare high conversion rates.

For methacrylate, styrene or conjugated dienes, as placed at the very other side of the ordering, the carbon-centered radicals are very stable and the according hydrogen abstraction rates are lower.

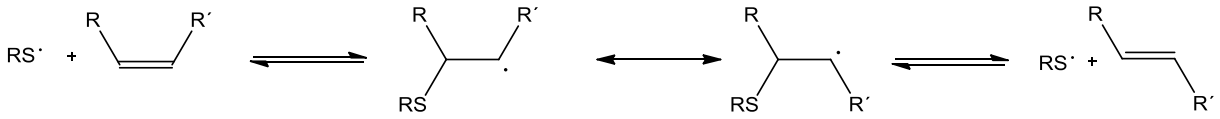
In order to learn about the reaction behavior of aliphatic enes, hexenes, in particular 1-hexene, *trans*-2-hexene and *trans*-3-hexen, were chosen as model components and copolymerized with a monofunctional thiol. It could be shown, that reactivity decreases remarkably from the 1-hexene to the *trans*-2-hexene and the *trans*-3-hexene. There, also the increasing steric hindrance must be considered as important factor.

Application of the reaction to *cis* double bonds leads to the isomerization to the respective *trans* structure due to a reversible addition of the thiyl radical (*cf*. Scheme 1-3).[16] The rate constant for the decomposition of the intermediate carbon-centered radical is 20x larger than the rate of chain transfer, however, the rate constant of the decomposition yielding the less reactive *trans* ene is even 80 times higher. This obtained *trans* structure reacts efficiently in the addition step with thiyl radicals, however this step is reversible and not as fast as with terminal enes. The rate limiting step in that context therefore is the abstraction of the thiol hydrogen by the carbon-centered radical. Johansson brought several examples using unsaturated fatty acids as substrates.[17] Metzger *et al*. showed recently the isomerization of *cis*-2-methyl oleate without initiator in the presence of dodecanthiol by the formation of an electron donor/acceptor complex formed by a thiol and an alkene, followed by a hydrolysis

[16] Walling, C.; Helmreich, W. *J Am Chem Soc* **1959**, *81*, 1144-1148.

[17] [a] Claudino, M.; Johansson, M.; Jonsson, M. *European Polymer Journal* **2010**, *46*, 2321-2332. [b] Samuelsson, J.; Jonsson, M.; Brinck, T.; Johansson, M. *J Polym Sci Part A: Polym Chem* **2004**, *42*, 6346-6352. [c] Bexell, U.; Olsson, M.; Johansson, M.; Samuelsson, J.; Sundell, P. E. *Surf Coat Technol* **2003**, *166*, 141-152.

reaction of this complex, yielding an alkyl and a sulfuranyl radical (RS$^{\bullet}$-S(H)R), which in turn is in an equilibrium with the thiyl radical, catalyzing the isomerization reaction.[18]

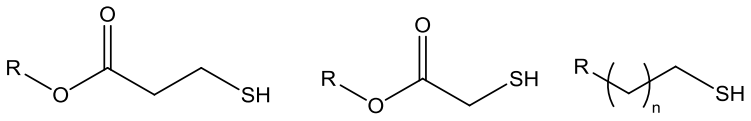


Scheme 1-3 Thiol-ene reaction with *cis* olefins

However, although this reaction is investigated for more than 50 years, there are still significant voids in the understanding of kinetics and rate processes involved.

1.1.4 Influence of the Thiol Structure

Generally, any kind of thiol imaginable can be utilized for thiol-ene reactions, although, comparable to the ene-side of the reaction, reactivity depends a lot on the electronic and steric surrounding of the thiol. The most common types of thiols used in photo-polymerizations are alkyl 3-mercaptopropionates, alkyl thioglycolates and alkylthiols.



Scheme 1-4 General structure of the three most often used thiols for thiol-ene photo polymerization (left: alkyl 3-mercaptopropionate, middle: alkyl thioglycolate, right: alkylthiol)

One very prominent representative of the first group is the tetra-functional pentaerythritol tetrakis(β-thiopropionate) **PETMP** (*cf.* Scheme 2-4), belonging to the most widely used and commercially available thiols. There, the assumption that an intramolecular H-bonding leads to a weakening of the thiol S-H bond and thereby to an increase of the reactivity[14] cannot be

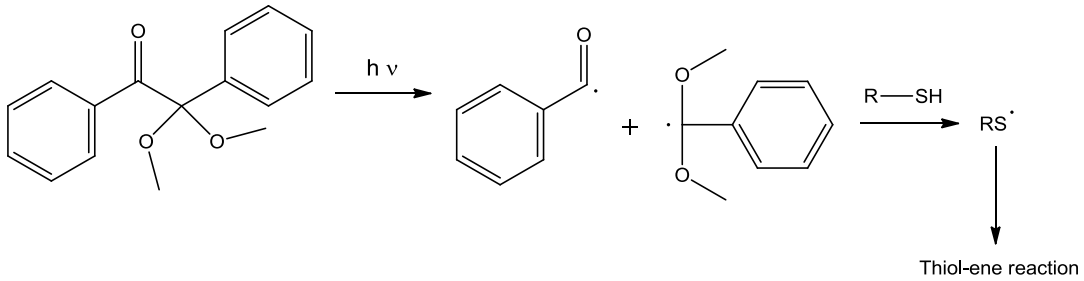
[18] Biermann, U.; Butte, W.; Koch, R.; Fokou, P. A.; Türünç, O.; Meier, M. A. R. M.; Metzger, J. O. *Chem Eur J* **2012**, *18*, 8201-8207.

supported by experimental evidence and differences in reactivity are reasoned by influences of polarity effects.[19] Soucek *et al*.[20] describes the reactivity of several aliphatic and aromatic dithiols in respect with the steric hindrance of the aliphatic backbone. However, very little information can be found on differences in the reactivity of thiols featuring additional functionalities.

1.1.5 Types of Initiation and Structures of Initiators

Basically, two ways of initiation are possible in terms of radical thiol-ene reactions, thermal and photochemical initiation. On the side of photochemical initiation, a further distinction between cleavage (type I) and H- abstraction (type II) initiators must be made. Additionally, direct lysis of the sulfur-hydrogen bond can initiate a free-radical reaction.

As for the type I initiators, the absorption of a photon cleaves the initiator molecule (e.g. 2,2-dimethoxy-2-phenylacetophenone **DMPA**) yielding a benzoyl and a tertiary carbon radical. A rearrangement of the tertiary carbon radical leads to the formation of a methyl radical as well as a methyl benzoate. Those species subsequently abstract a hydrogen atom from a present thiol group or insert into a carbon-carbon double bond directly. In any case, the free-radical chain reaction is initiated. Initiation with that type of initiators is more efficient than with hydrogen- abstraction photo initiators as higher quantum yields leading to the formation of active radicals are reached.[2,21]



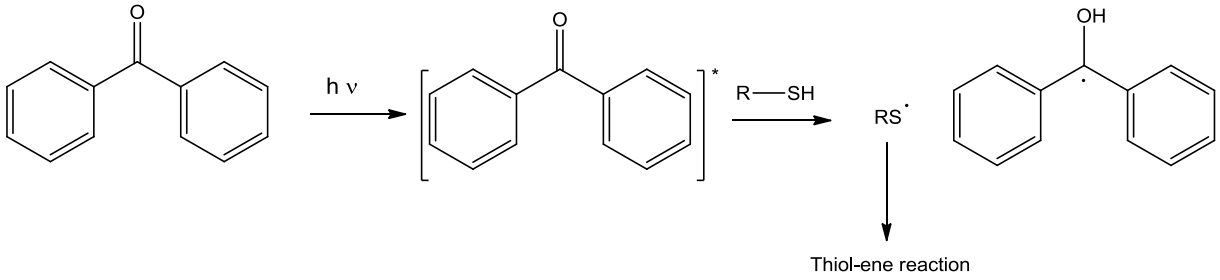
Scheme 1-5 Mode of action of a type I photo initiator (DMPA)[21]

[19] Stock, L. M. *J Chem Edu* **1972**, *49*, 400-404.

[20] Soucek, M. D.; Wutticharoenwong, K. *Macromol Mater Eng* **2008**, *293*, 45-56.

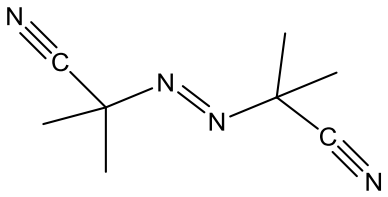
[21] Uygun, M.; Tasdelen, M. A.; Yagci, Y. *Macromol Chem Phys* **2010**, *211*, 103-110.

In case of type II initiators, a diarylketone (for example benzophenone) can be excited to its singlet state upon irradiation with UV light. A subsequent intersystem crossing leads to the excited triplet state. If thiols are present, a hydrogen transfer from the thiol to this excited state results in the formation of a ketyl radical as well as a thiyl radical which then acts along the described mechanism.[2]



Scheme 1-6 Mode of action of a type II photo initiator (benzophenone BP)[21]

A very well-known thermal radical starter is 2,2´- azoisobutyronitrile **AIBN**, which, upon thermal decomposition, yields cyanoisopropyl radicals. The driving force in that case is the formation of nitrogen.



Scheme 1-7 Structure of 2,2´- azoisobutyronitrile AIBN

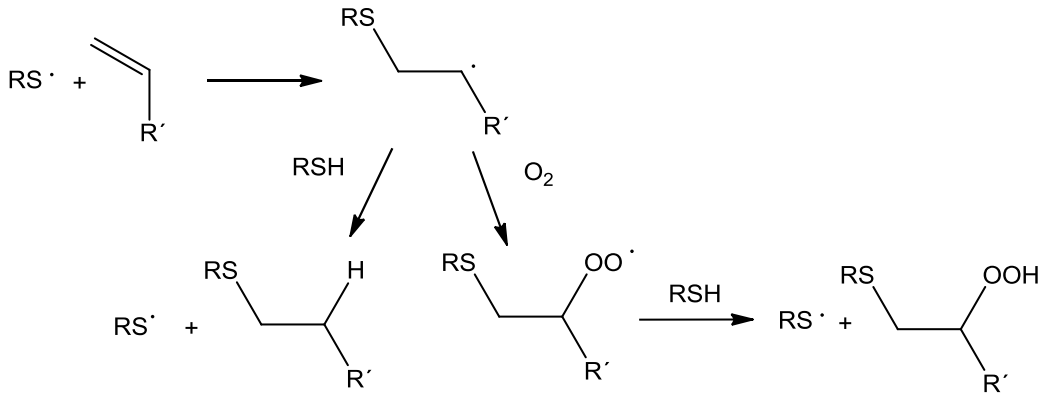
Although generally photochemically induced initiator systems exhibit faster conversions, thermal initiators are applied for large volume systems, where the photo initiation is hindered by flask form and light absorption such as bioorganic systems.[22]

[22] Triola, G.; Brunsveld, L.; Waldmann, H. *J Org Chem* **2008**, *73*, 3646-3649.

1.1.6 Influence of Oxygen on the Reaction

1.1.6.1 Oxygen inhibition

Contrary to other radical reactions, the thiol-ene reaction is relatively insensitive towards oxygen inhibition. The explanation lays in the formation of carbon-centered propagation radicals which react with oxygen to peroxy radicals which, in turn, abstract hydrogens from thiols. The thiyl radicals thus formed again add to carbon-carbon double bonds and continue the main propagation step.



Scheme 1-8 Oxygen scavenging mechanism for thiol-ene reaction in the presence of aliphatic thiols[2,23]

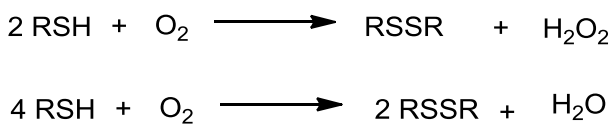
Therefore, thiol-ene reactions can be conducted under an ambient atmosphere.

1.1.6.2 Thiol Oxidation

However, what has to be kept in mind is the sensitivity of thiols to oxidize to the corresponding disulfide in aqueous solutions.[24]

[23] Beckwith, A. L. J.; Wagner, R. D. *J Org Chem* **1981**, *46*, 3638-3645.

[24] [a] Bagiyan, G. A.; Koroleva, I. K.; Soroka, N. V.; Ufimtsev, A. V. *Russ Chem Bull, Int Ed*. **2003**, *52*, 1135-1141. [b] Cavallini, D.; De Marko, C.; Dupre, S. *Arch Biochem Biophys* **1968**, *124*, 18-26.



The degree of oxidation depends on the pH of the reaction solution, whereas higher pH can also lead to sulfinic (RSO_2H) and sulfonic (RSO_3H) acids.

Additionally, traces of variable-valance metal chelates (*e.g*. ethylenediaminetetraacetic acid) lead to an increased oxidation behavior. Thiols containing additional functional groups (NH_2, COOH, OH) serve as chelating agents for metals such as Fe, Cu, Cr, Mn, Ni or Co.

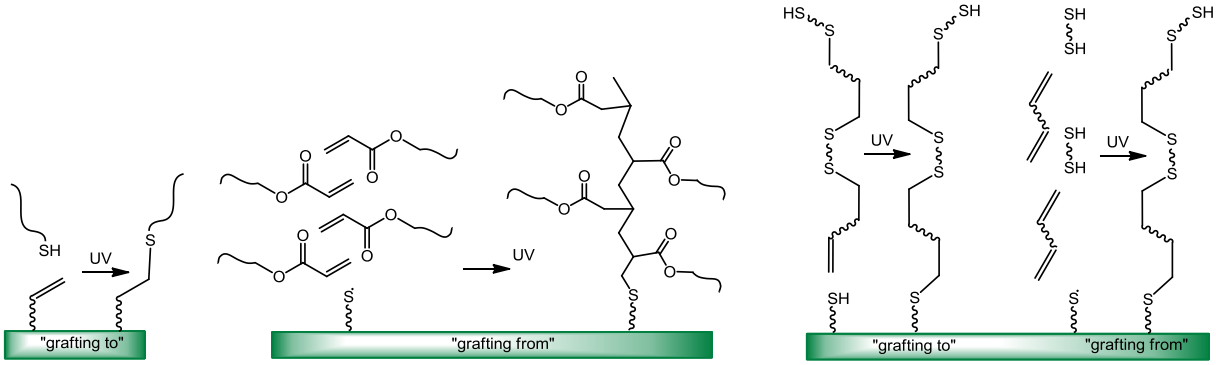
1.1.7 Applications of Thiol-Ene Reactions

The main application of thiol-ene reactions is the photo-polymerization yielding tight networks with very advantageous properties. Moreover, this type of reaction is used very frequently for the post- functionalization of polymers. Some of the most commonly used applications are described.

1.1.7.1 Thiol-ene Reactions for Surface Modification

There is a distinction between three types of surface modifications:

I) “grafting to” approaches use thiol-ene coupling reactions,
II) “grafting from” approaches utilize photolytically produced thiyl radicals on a surface to initiate acrylate polymerization and
III) combination of both processes: in an early stage of the reaction, groups on the surface react with monomeric species and oligomers and polymers at later stage of the reaction.



Scheme 1-9 Approaches for surface modification via photochemically induced thiol-ene click reactions (reproduced from Bowman and Hoyle[11])

"Grafting to" approaches are used to selectively functionalize surfaces to fulfill defined requirements by the incorporation of functional or biological active groups[25] or change surface polarity.[26] By "grafting from" processes, the polymerization from appropriate substrates is accomplished. By applying this method, lithographic patterning in a very precise manner can be done.[27] Additionally, combined methods are described, where thiyl radicals on a surface start polymerization of thiol and ene substrates.

1.1.7.2 Formation of Low-Stress Networks

1.1.7.2.1 Photolithographic Applications

As the thiol-ene photo polymerization features a step- growth mechanism, accompanied by a delayed gel point,[28] uniformity in network formation, reduced shrinkage and thereby

[25] [a] Kolb, N.; Meier, M. A. R. *Eur Polym J* **2013**, DOI: 10.1016/j.eurpolymj.2012.09.017. [b] Bertin, A.; Schlaad, H. *Chem Mater* **2009**, *21*, 5698-5700.

[26] Bexell, U.; Berger, R.; Olsson, M.; Grehk T. M.; Sundell, P.- E.; Johansson, M. *Thin Solid Films* **2006**, *515*, 838-841.

[27] Hagberg, E. C. Malkoch, M.; Ling, Y.; Hawker, C. J.; Arter, K. R. *Nano Lett* **2007**, *7*, 233-237.

[28] [a] Chiou, B.-S.; English, R. J.; Khan, S. A. *Macromolecules* **1996**, *29*, 5368-5374 .[b] Chiou, B.-S.; Khan, S. A. *Macromolecules* **1997**, *30*, 7322-7328.

shrinkage stress,[29] the thiol-ene reaction is an excellent candidate for photolithographic applications and the production of micro devices, energy-absorbing materials and glassy coverings. The formation and reproduction of very precise structures is possible. [30]

1.1.7.2.2 Optical Networks

One of the most frequent applications of thiol-ene polymerizations is the development of a separate liquid-crystalline phase that forms from an originally homogeneous single-phase mixture. The polymer-dispersed liquid-crystalline (PDLC) phases formed thereby are distinguished by electroactive liquid-crystalline phases as well as highly cross-linked phases, allowing an application as diffraction gratings as well as photonic crystals and lasers with excellent diffraction efficiencies, low switching voltages, high switching speed and many more.[31]

1.1.7.2.3 Dental Restorative Materials

In order to enlarge the scope of properties of thiol-ene polymers, mixed component photo reactions were investigated. Two systems were examined in this coherence: first, binary systems involving multifunctional thiols and conventional acrylates or methacrylates; second ternary processes including a thiol, an acrylate or methacrylate and an ene, incapable of homo-polymerization.[32] Those systems are distinguished by an increased acrylate conversion, equivalent or raised cure speed, a lowered oxygen inhibition and optimized glass transition temperatures as well as reduced shrinkage stress. Therefore, such material compounds are

[29] Bowmann, C. N.; Anseth, K. S. *Macromol Symp* **1995**, *93*, 269-276.

[30] [a] Cygan, Z. T.; Cabral, J. T.; Beers, K. L.; Amis, E. J. *Langmuir* **2005**, *21*, 3629-3634. [b] Schenk, V.; Ellmaier, L.; Rossegger, E; Edler, M.; Griesser, T.; Weidlinger, G.; Wiesbrock, F. *Macromol Rapid Commun* **2012**, *33*, 396-400.

[31] [a] White, T. J.; Natarajan, L. V.; Tondiglia, V. P.; Lloyd, P. F.; Bunning, T. J.; Guymon, C. A. *Polymer* **2007**, *48*, 5979-5987. [b] White, T. J.; Natarajan, L. V.; Tondiglia, V. P.; Bunning, T. J.; Guymon, C. A. *Macromolecules* **2007**, *40*, 1112-1120.

[32] Reddy, S. K.; Cramer, N. B.; Bowmann, C. N. *Macromolecules* **2006**, *39*, 3681-3687.

very promising candidates for dental restorative materials compared to conventional pure acrylate systems.[33]

Further applications of such polymer networks are hydrogels[34] or the functionalization of bioorganic systems, *e.g.* carbohydrates.[35]

The group of Wooley developed thiol-ene networks from amphiphilic fluoropolymers with anti-biofouling properties.[36] Moreover, thiol-ene reactions have been used to click poly(sulfobetaine) polymers on modified silica surfaces bearing acrylate groups.[37]

1.1.8 Use of Thiol-Ene Reactions for Post-Functionalization of Polymers

The post-modification of polymers is an excellent tool to fine-tune material properties. However, conventional post-functionalization tools suffer from undesired side products that are often hard to be removed. Therefore, thiol-ene reactions offer very good possibilities for fast modifications of polymers in high yields.

One polymer which is very often in the focus of examinations is poly(butadiene) with easily accessible double bonds, which are predominantly 1, 2- linked moieties. Schlaad *et al.*[38] performed several studies equipping this polymer with a range of thiols to tune properties very precisely. Amongst others, thiols featuring benzyl groups, primary or tertiary amines, hydrophilic acid groups, dihydroxy groups or fluorinated side groups were successfully clicked to poly(butadiene). However, limitations in the degree of functionalization are caused by

[33] Boulden, J. E.; Cramer, N. B.; Schreck, K. M.; Couch, C. L.; Bracho- Troconis, C.; Stansbury, J. W.; Bowman, C. N. *Dental Materials* **2011**, *27*, 267-272.

[34] Rydholm, A. E.; Reddy, S. K.; Anseth, K. S.; Bowman, C. N. *Biomacromolecules* **2006**, *7*, 2827-2836.

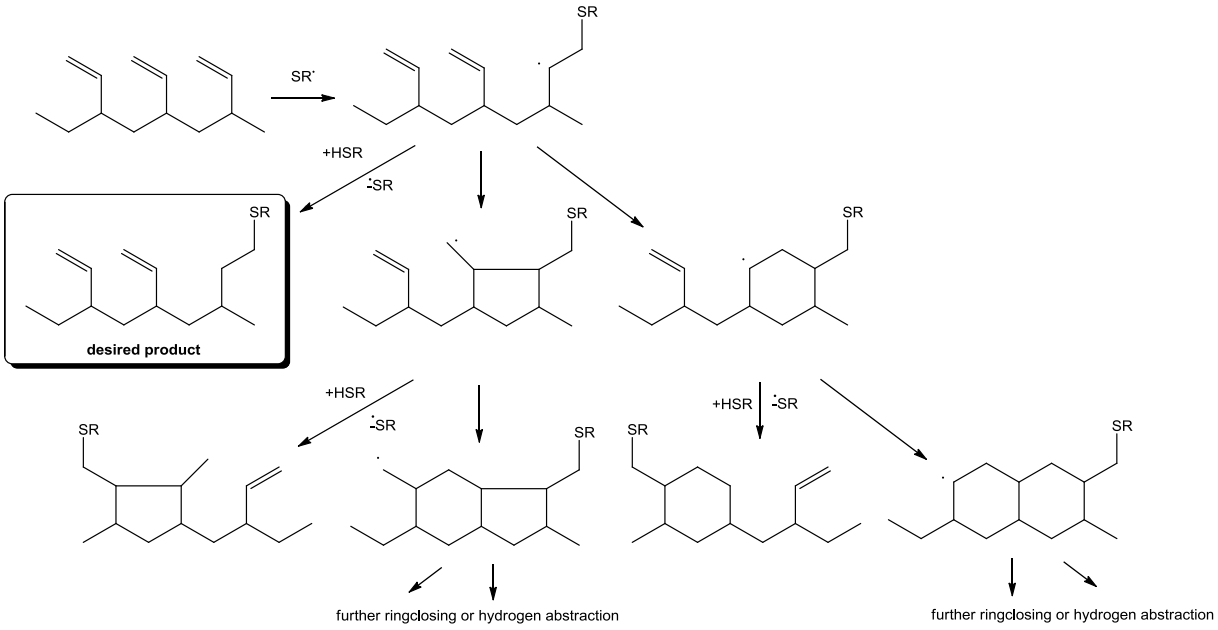
[35] Ortiz, R. A., Valdéz, A. E. G.; Aguilar, M. G. M.; Duarte, M. L. B. *Carbohydrate Polymer* **2009**, *78*, 282-286.

[36] Imbesi, P. M.; Raymond, J. E.; Tucker, B. S.; Wooley, K. S. *J Mat Chem* **2012**, *22*, 19462- 19473.

[37] Li, M.; Neoh, K. G.; Xu, L. Q.; Wang, R.; Kang, E.- T.; Lau, T.; Olszyna, D. P.; Chiong, E. *Langmuir* **2012**, *28*, 16408-16422.

[38] Justynska, J.; Hordyjewicz, Z.; Schlaad, H. *Polymer* **2005**, *46*, 12057-12064.

internal cyclization reactions of neighboring double bonds. Lodge and Hillmyer[39] first described such cyclization reactions for the free radical addition of perfluoroalkyl iodides to poly(butadiene) double bonds. Schlaad[38,40] and Kornfield[41] showed a comparable mechanism of the addition of mercaptans to 1,2-poly(butadiene) including ring-closing reactions. Thereby, the formation of 6-membered rings is more feasible than the formation of the five-membered analogues.



Scheme 1-10 Mechanism of thiol-ene radical reaction on poly(butadiene)[43]

To circumvent this problem, polymers with a geometry that does not allow reaction of carbon-centered radicals in the side groups with neighboring enes, were introduced. Those polymers include poly(oxazoline)s[42] or block copolymers.

[39] Ren, Y.; Lodge, T. P.; Hillmyer, M. A. *Macromolecules* **2001**, *34*, 4780-4787.

[40] ten Brummelhuis, N.; Diehl, C.; Schlaad, H. *Macromolecules* **2008**, *41*, 9946-9947.

[41] David, R. L. A.; Kornfield, J. A. *Macromolecules* **2008**, *41,* 1151-1161.

[42] Gress, A.; Völkel, A.; Schlaad, H. *Macromolecules* **2007**, *40*, 7928-7933.

A more recent work reports the successful incorporation of functional mercapants to poly(butadiene) nanoparticles from an aqueous solution.[43] Schlaad and coworkers[44] tethered poly(butadiene) on a self-assembled monolayer (SAM) of an *α,ω*-dithiol. Poly(butadiene) was functionalized with cysteamine in a thermal thiol-ene reaction and the obtained product used as hardener for epoxy resins.[45]

1.1.9 Network Formation via Thiol-ene Reactions

Beside the classical thiol-ene photo polymerization, network formation is also possible as post-functionalization step of readily polymers with an appropriate cross-linking agent. Thereby, unsaturated polyolefins are photocrosslinked with multifunctional thiols. Decker and coworkers intensively investigated crosslinking reactions of polystyrene-*block*-polybutadiene-*block*-polystyrene polymers with a trifunctional thiol already more than a decade ago.[46] The curing process was followed by infrared spectroscopy, insolubilization and hardness measurements, studying the influence of initiators used as well as thiol to ene content. Also in that case, thiyl radicals are formed by hydrogen abstraction on the thiyl by any of the secondary free radicals formed by the addition process. Decker thereby found, that crosslinking results from both the copolymerization of the butadiene groups with the multifunctional thiol as well as a homopolymerization of pendant vinyl groups.[46b]

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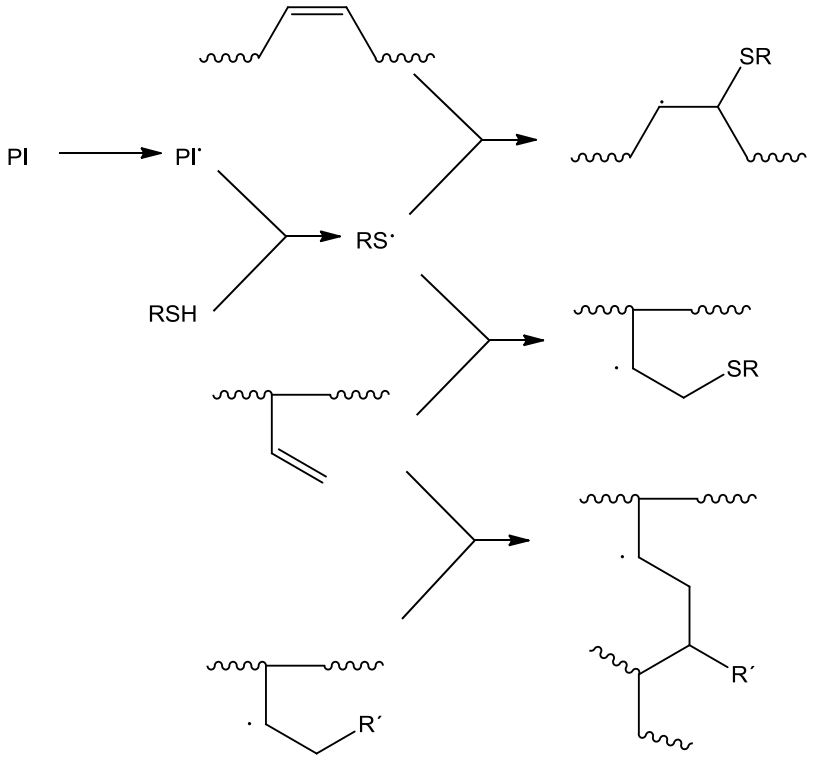
[43] Korthals, B.; Morant- Miñana M. C.; Schmid, M.; Mecking, S. *Macromolecules* **2010**, *43*, 8071-8078.

[44] Madaa, N.; Terry, A.; Harb, J.; Davis, R. C.; Schlaad, H.; Linford, M. R. *J Phys Chem C* **2011**, *115*, 22931-22938.

[45] Auvergne, R.; Desroches, M.; Clerc, S.; Carlotti, S.; Caillol, S. Boutevin, B. *React Funct Polym* **2012**, *72*, 393-401.

[46] [a] Decker, C.; Nguyen Thi Viet, T. *Polymer* **2000**, *41*, 3905-3912. [b] Decker, C.; Nguyen Thi Viet, T. *Macromol Chem Phys* **1999**, *200*, 1965-1974.



Scheme 1-11 Possible reaction pathways in thiol-ene photo crosslinking of high vinyl polyolefins

Applying natural rubber latex in a film falling reactor, Schlögl *et al.*[47] described a crosslinking reaction of the double bonds via a thiol-ene reaction with the aim to generate an allergen free surgical glove.

[47] [a] Schlögl, S.; Aust, N.; Schaller, R.; Holzner, A.; Kern W. *Monatsh Chem* **2010**, *141*, 1365-1372. [b] Schlögl, S.; Temel, A.; Holzner, A.; Kern, W. *J Appl Polym Sci* **2012**, *124*, 3478-3486.

1.2 ANTIBACTERIAL POLYMERS

1.2.1 INTRODUCTION

Since the French scientist Pasteur demonstrated that certain bacteria strains are crucial to fermentation and moreover the awareness that microbes are responsible for a high number of diseases took hold, microbiological hygiene started to be of interest. With the discovery of penicillin by Fleming, the successful fight against bacterial infections was possible. Nevertheless, antimicrobial contaminations are still an issue today, not only in hospital environment but also for water purification, food processing as well as sanitary and household equipment.

Conventional disinfectants are small molecules such as halogens, alcohols, phenols, acids or antibiotics.[48] However, those active agents are generally not covalently immobilized; therefore they suffer from disadvantageous leaching and accumulation effects with probable worst impact on the environment.[49] Additionally, bacteria cells are able to form resistances against low-molecular weight disinfectants.[50] Furthermore, because of the constant release of biocides, the antimicrobial activity of these materials will expire within time.[51] In that way, the alternative use of water insoluble macromolecular biocides is an attractive option. Besides, the increase in molecular weight and the accumulation of charges have a positive impact on the performance of the material.[52] Bacterial resistances are not known.

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[48] [a] Paulus, W. *Microbicides for the protection of materials*, Chapman & Hall: London- Glasgow- New York- Melbourne- Madras, **1993**. [b] Block, S.S. *Desinfection, Sterilization and Preservation*, Lea & Felbiger, Philadelphia, **1983**.

[49] Kenawy, E.-R.; Mahmoud, Y. A.-G. *Macromol Biosci* **2003**, *3*, 107–116.

[50] Tegos, G.; Stermitz, F. R.; Lomovskaya, O.; Lewis, K. *Antimicrob Agents Chemother* **2002**, *46*, 3133-3141.

[51] Cheng, G.; Xue, H.; Zhang, Z.; Chen, S.; Jiang, S. *Angew Chem* **2008**, *120*, 8963–8966.

[52] Kenawy, E.-R.; Worley, S. D.; Broughton, R. *Biomacromolecules* **2007**, *8*, 1359-1384.

1.2.2 THE BACTERIA CELL

Bacteria can be classified as prokaryotes. Contrary to eukaryotes, their cell nucleus is not separated from the rest of the cell by a cell wall, but is located in the cytoplasm in an irregular shape as a single circular chromosome, called nucleoid.

Further intracellular constituents are the cytoplasm and the ribosome, surrounded by a cell membrane, which serves as a barrier to hold nutrients. This lipid bilayer consists of two layers of phospholipids, featuring a hydrophilic head and two hydrophobic tails. The hydrophilic head groups consist of negatively charged phosphate groups, whereas the hydrophobic tail is composed of fatty acid hydrocarbon chains. This structure allows arrangement in water into a two-layered sheet (bilayer) with all the hydrophobic tails being orientated to the center.

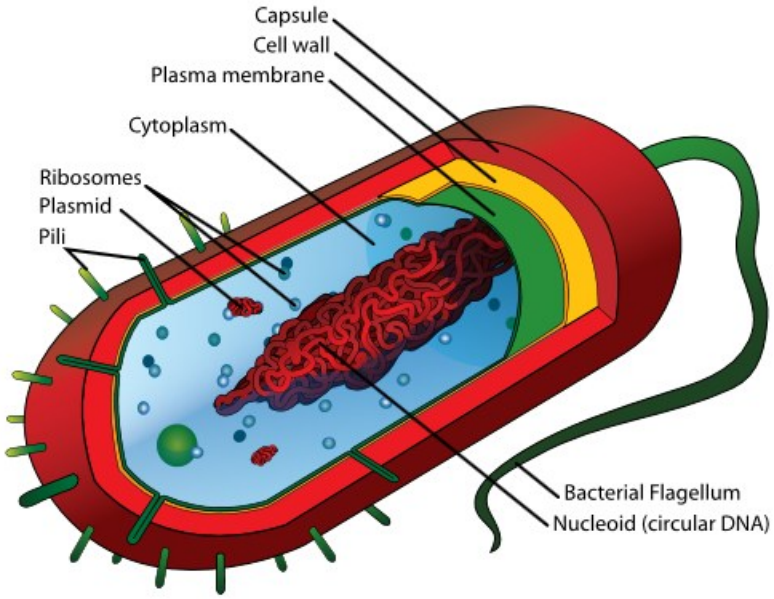


Figure 1-1 Structure and content of a typical Gram-positive bacteria cell[53]

The cytoplasmic membrane is surrounded by a bacteria cell wall, consisting of peptidoglycan, which is made from polysaccharide chains cross-linked by peptides. Generalized, there are two types of cell walls in bacteria, which allow a classification in Gram-negative and Gram-

[53] source: http://en.wikipedia.org/wiki/Bacteria, 04th December 2012

positive cells. Gram-positive bacteria (depicted in Figure 1-1) possess a thick cell wall (up to 50% of dry mass) containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall (approximately 10% of dry mass) consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins as shown in Figure 1-2. The naming results from a different behavior against staining with crystal violet and a subsequent decolorization of Gram-positive and negative cells which allows a very fast assignment. The differences in the composition of the cell wall lead to drastic differences in their stability against antimicrobial agents. Typical representatives for Gram-positive cells are *Staphylococcus* or *Listeria*. The proteobacteria are a major group of Gram-negative bacteria, including, amongst others, *Escherichia coli*, *Salmonella* or *Pseudomonas*. Moreover, the groups of cyanobacteria or green sulfur and non-sulfur bacteria belong to Gram-negative bacteria.[54]

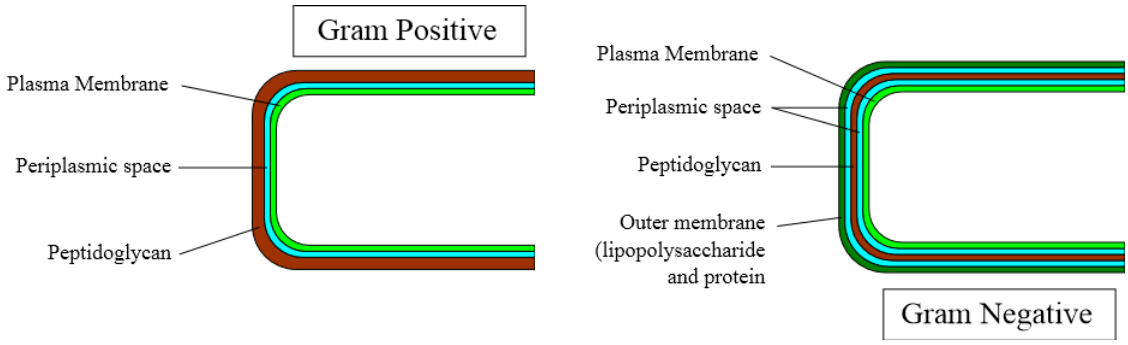


Figure 1-2 Gram-positive and -negative cell wall structure[55]

These structural features determine the main strategy for designing antibacterial polymers. The teichoic acid molecules of Gram-positive bacteria cell wall, the liposaccharides and phospholipids of Gram-negative outer membrane and the cytoplasm membrane itself, composed of a phospholipid bilayer with embedded essential functional proteins, provide a net negative charge of the bacteria cell, stabilized by the presence of cations such as Mg^{2+} or Ca^{2+}. The cytoplasmic membrane regulates the transfer of metabolites and nutrients in and

[54] source: http://en.wikipedia.org/wiki/Gram-negative_bacteria, 04th December 2012

[55] source: http://en.wikipedia.org/wiki/Gram-positive_bacteria; 04th December 2012

out the cell. Targeting this sensible system, most antibacterial polymers are designed as hydrophilic-hydrophobic macromolecules.[66]

1.2.3 Antibacterial Polymers

What makes polymers preferred candidates for usage in hygienic applications is their higher activity than exhibited by their molecular counterparts.[56] Additionally, they are generally non-volatile, do not penetrate skins and are therefore less vulnerable towards losses due to decomposition or transport.

Worley and Sun[57] demand several characteristics, that should be fulfilled by antibacterial polymers: first, an easy and inexpensive synthesis, stability for long-term applications, non-volatile and insolubility in water in case of application in aqueous media, no decomposition and release of toxic residues, non-toxicity towards those handling it and of course, high potency against a range of microbes in brief contact times.

In order to design antimicrobial polymers, several general approaches are possible. One method to obtain antibacterial activity is to add an organic or inorganic biocide to the polymers during or after processing.[57] Alternatively, the preparation of monomers equipped with antibacterial groups and the subsequent homo- or copolymerization of those is an often chosen approach.[58] Especially Ikeda and co-workers performed a lot of pioneering work on this topic in the early 80s.

[56] Ikeda, T.; Tazuke, S. *Makromol Chem* **1984**, *185*, 869-876.

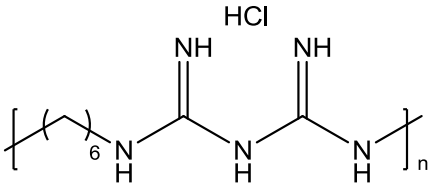
[57] Worley, S. D.; Sun, G. *Trends Polym Sci* **1996**, *4*, 364-370.

[58] [a] Ikeda, T.; Tazuke, S. *Makromol Chem, Rapid Comm* **1993**, *4*, 459-461. [b] Kreutzwiesner, E.; Noormofidi, N.; Wiesbrock, F.; Kern, W.; Rametsteiner, K.; Stelzer F., Slugovc, C. *J Poly Sci, Part A: Polym Chem* **2010**, *8*, 4504-4515.

1.2.3.1 Mode of Action

The major part of antimicrobial polymers is designed as so-called membrane-active agents. Thereby, hydrophobic and hydrophilic structural elements make up a macromolecular system, targeting the cytoplasmic membrane.

The cationic polyelectrolyte salt poly(hexamethylene biguanide) chloride **PHMB** was the first polycation, whose mechanism of interaction with Gram-negative bacteria cell *E. Coli* and model phospholipid membranes was studied by Broxton *et al*.[59] as well as Ikeda *et al*.[60] Generally, six elemental steps have been defined by Ikeda *et al*.[60] describing the elementary events leading to the lethal action: (i) adsorption onto the bacterial cell surface, (ii) diffusion through the cell wall, (iii) adsorption onto the cytoplasmic membrane, (iv) disruption of the cytoplasmic membrane, (v) leakage of the cytoplasmic constituents and (vi) death of the cell. Due to the high charge density along the polymer chains, step (i) is especially effective in case of macromolecular biocides. Figure 1-3 illustrates this mode schematically.



Scheme 1-12 Poly(hexamethylene biguanide) chloride PHMB

[59] Broxton, P.; Woodcock, P. M.; Heatley, M.; Gilbert, P. *J Appl Bacteriol* **1984**, *57*, 115-124.

[60] Ikeda, T.; Yamaguchi, H.; Tazuke, S. *Antimicrob Agents Chemother* **1984**, *26*, 139-144.

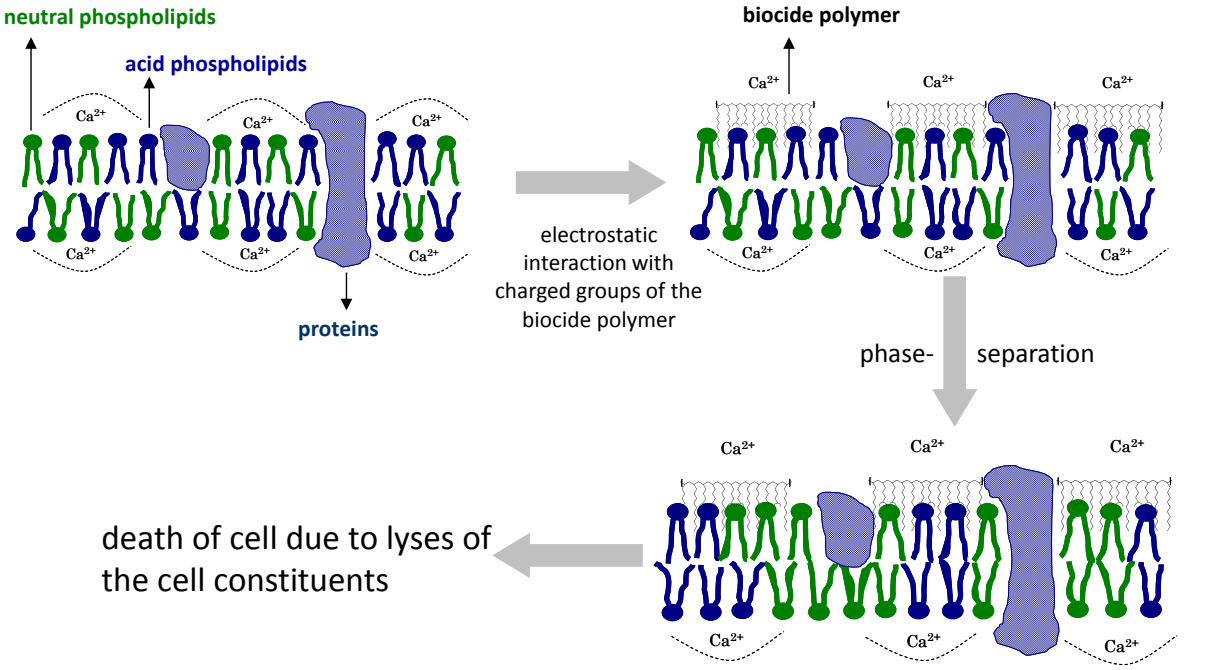


Figure 1-3 Mode of action of antibacterial polymers with the cytoplasm membrane leading to the lysis of cell constituents and the death of the cell

However, for contact biocides, diffusion through the cell wall is not possible. The adsorption of the bacteria cell on the biocide surface is required for an antimicrobial action. Therefore, several other models have been developed.

1.2.3.1.1 Contact-Killing via the Polymeric Spacer Effect

This concept presumes that a surface immobilized biocide polymer is capable of penetrating the bacteria cell wall of an adherent bacteria cell. After reaching the cytoplasmic membrane and rupture of the phospholipid bilayer, death of the cell occurs (*cf*. Figure 1-4).[70]

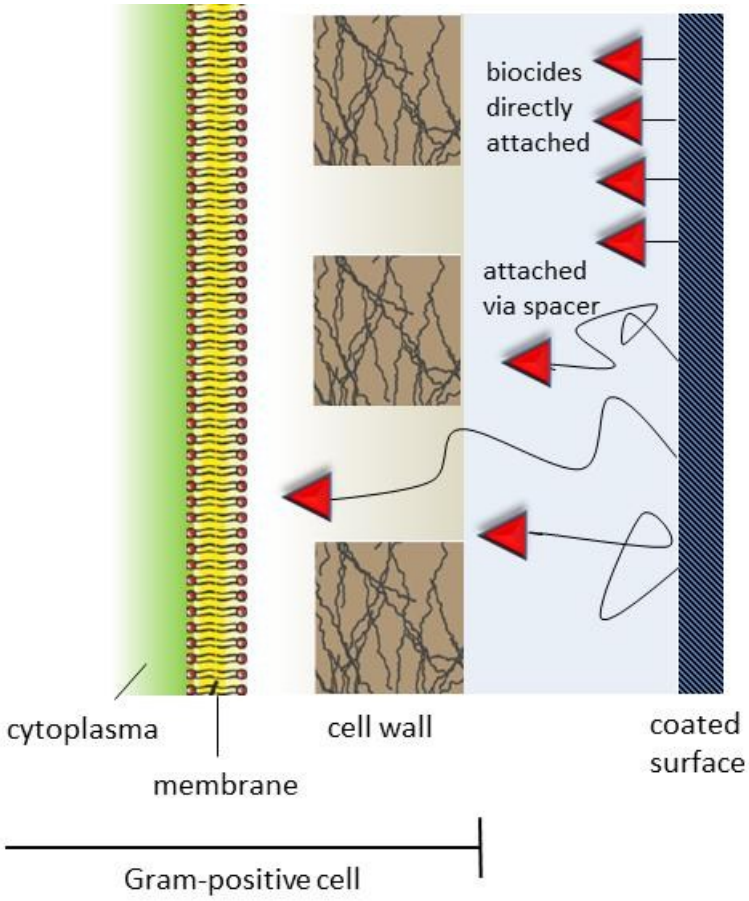


Figure 1-4 Contact-killing via the polymeric spacer effect[61]

1.2.3.1.2 Contact-Killing via Phospholipid Sponge Effect

The aforementioned concept, however, did not succeed in explaining the antimicrobial activity of several short-spacer featuring cellulose polymers.[62] Therefore, an alternative model was developed, based on an adsorption of negatively charged phospholipids, leading to the rupture of the cell. Yet, this concept still suffers from the unexplained point of how the water-insoluble phospholipids travel through the cell wall and reach the antimicrobial surface.[63]

[61] source: Siedenbiedel, F.; Tiller, J. *Polymer* **2012**, *4*, 46-71.

[62] Bieser, A. M.; Thomann, Y.; Tiller, J. C. *Macromol Biosci* **2011**, *11*, 111-121.

[63] Bieser, A. M.; Tiller, J. C. *Macromol Biosci* **2011**, *11*, 526-534.

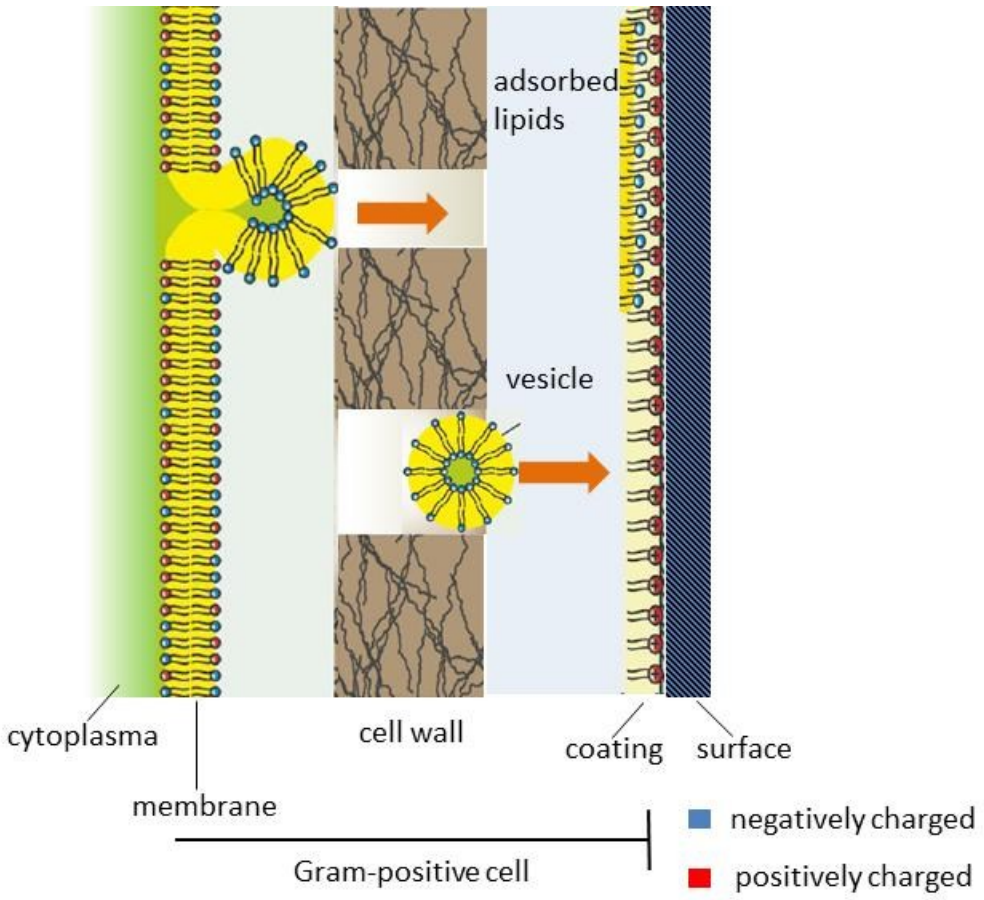


Figure 1-5 Contact killing via phospholipid sponge effect[61]

1.2.3.2 Factors Affecting Antimicrobial Activity of Macromolecules

For the subsequent considerations, a distinct differentiation between water-soluble and water-insoluble polymers has to be made, as the activity of those differs tremendously.

1.2.3.2.1 Effect of Molecular Weight

It was found that a molecular weight of 1.6 x 10^4 to 1.2 x 10^5 Da is optimal for biocidal activity against Gram-positive bacteria.[60,64] In the case of grafted polymers, even higher polymer weights are necessary.[65] It is reasonable, that the enhancement of hydrophobic mass, an enlargement of the polymer coil and an increase of the overall charge and thereby

[64] Kanazawa, A.; Ikeda, T., Endo, T. *J Polym Sci, Part A: Polym Chem* **1993**, *31*, 1441-1447.

[65] Klibanov, A. M. *J Mater Chem* **2007**, *17*, 2479-2482.

number of active sites strengthen their adsorption ability, binding affinity and destructive interaction with the cell of a microbe.[66]

1.2.3.2.2 Effect of Counterion

The activity of antimicrobial polymers is insofar dependent on the counter ion, as it is low for counterions forming tight ion pairs with the phosphonium ion, while high activity was found for those facilitating ionic dissociation to free ions. However, an accurate ordering seems to be difficult, as works by Chen *et al*.[67] as well as Panarin *et al*.[68] led to contradictory results. The reason for differences in the activity might be explained by solubility differences.

1.2.3.2.3 Effect of Spacer Length

A change in spacer length between the active groups and the polymer backbone leads to differences in conformation and charge density of this macromolecule. It is reasonable, that the mode of interaction with the cytoplasmic membrane is affected and hence different activities are obtained. Although the absolute spacer length for the maximum lethal action could not be determined doubtlessly, several works found higher activity with longer spacers.[69]

1.2.3.2.4 Effect of Alkylation

The effect of alkylation of the active site was investigated by several groups. Chen and co-workers discovered a parabolic relationship between antibacterial activity and hydrophobic

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[66] Timofeeva, L.; Kleshcheva, N. *Appl Microbiol Biotechnol* **2011**, *89*, 475-492.

[67] Chen, C. Z.; Beck-Tan, N. C.; Dhurjati, P.; Van Dyk, T. K.; LaRossa, R. A; Cooper, S. L. *Biomacromolecules* **2000**, 1, 473-480.

[68] Panarin, E. F.; Solovaskii, M. V.; Zaikina, N. A.; Afinogenov, G. E. *Makromol Chem Suppl* **1985**, *9*, 25-33.

[69] [a] Ikeda, T.; Hirayama, H.; Suzuki, K.; Yamaguchi, H.; Tazuke, S. *Makromol Chem* **1986**, *187*, 333-340. [b] Nonaka, T.; Hua, L.; Ogata, T.; Kurihara, S. *J Appl Polym Sci* **2003**, *87*, 386-393. [c] Semenov, V. E.; Voloshina, A. D.; Toroptzova, E. M.; Kulik, N. V.; Zobov, V. V.; Giniyatullin, R. K.; Mikhailov, A. S.; Nikolaev, A. E.; Akamsin, V. D.; Reznik V. S. *Eur J Med Chem* **2006**, *41*, 1093-1101.

chain length, whereas a maximum activity was found for C_{10} alkyl chains.[67] Considering the changes of several characteristics and parameters of the polymer with increasing side chain length, the very sensitive balance between hydrophobicity and hydrophilicity, influencing the killing ability, can be altered. Thereby, changes in the macromolecular aggregation and conformation behavior of the polymer on the cell surface have to be considered. Therefore, the optimal alkyl chain length depends a lot on the macromolecular system that should be equipped. For grafted polymers, shorter chain lengths have shown to be more effective.[70]

1.2.3.3 Preparation of Antibacterial Contact Biocides

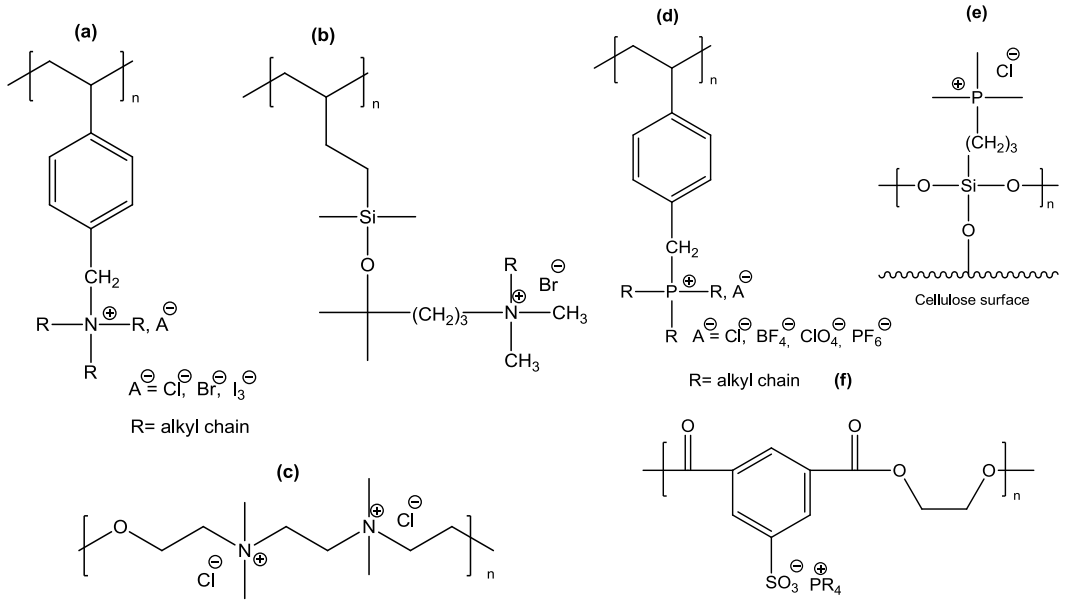
Generally, any method for the functionalization of polymers can be used to implement antimicrobial functionalities into macromolecules. However, a high number of polymers described in literature are water-soluble and can therefore not be considered as contact biocides. Descriptions of those can be found in comprehensive review articles of Tashiro[71] and Kenawy.[52] Yet, in the subsequent chapter, development of water-insoluble polymers and the applied biocide groups are illustrated.

For water-insoluble polymers, active groups used are primarily polymeric quaternary ammonium or phosphonium materials.

This class, featuring quaternary ammonium groups as active species- so called “poly QUAT”s- is probably the group gaining the most interest within the last years. In Scheme 1-13, several general types of typical structural elements are depicted.

[70] Tiller, J. C.; Liao, C. J.; Lewis, K.; Klibanov, A. M. *Proc Natl Acad Sci* **2001**, *98*, 5981-5985.

[71] Tashiro, T. *Macromol Mater Eng* **2001**, *286*, 63-87.



Scheme 1-13 left: possible structures of poly QUATs in the main (c) or side chain (a, b); right: possible structures of polymeric phosphonium biocidal material with the functional group in the main (f) or side chain (d,e)

1.2.3.3.1 Polymerization of Antimicrobial Monomers

Polymeric biocides are polymers that consist of bioactive repeating units, in other words, a potentially biocide monomer is polymerized. However, there are still several restrictions to overcome, such as an altered solubility of the polymers compared with the monomers.

Imazato and co-workers reported the polymerization of methacryloyloxydodecylpyridinum bromide **MDPD** in a water-insoluble form. Thereby, a little bactericidal activity has been determined, decreased compared to the water-soluble monomer.[72]

Kreutzwiesner *et al*. designed norbornene monomers bearing potentially antimicrobial groups, which were polymerized via ring-opening metathesis polymerization.[58b]

Another possibility to generate antimicrobial polymers is the defined structuring along the polymer strain. Thereby, also the polymerization of *per se* non-antimicrobial structural units might lead to a biocide macromolecule due to a defined sectioning in a hydrophobic and a hydrophilic compartment. This concept is summarized as mimics for antimicrobial peptides (AMP) such as magainin, a class of membrane-active biocide compounds. Such molecules

[72] Imazato, S.; Russell, R. R. B.; McCabe, J. F. *J Dent* **1995**, *23*, 177-181.

efficiently disrupt a microbial cell by insertion in the backbone.[73] Polymers based thereon are for example poly(phenylene ethynylene)-based conjugated polymers with amino side groups, but also polymers exhibiting random peptide sequences prepared by ring-opening of beta-lactames.[74] However, there it has to be kept in mind, that this very promising group of biocides is water-soluble and can therefore not be considered as a contact biocide, though, this class should not be unmentioned in this chapter.

1.2.3.3.2 Surface Bound Antimicrobial Polymers

A grafting-to process as described in 1.2.3.2.4 marks one example. There, poly(4-vinyl-N-hexylpyridinium) was surface-grafted and subsequently alkylated on glass slides treated with an aminosilane and acryloyl chloride.[70] Alternatively a grafting-from method allows the attachment of cationic polymers on various surfaces, *e.g.* the growth of cationic poly(acrylate)s via atom transfer radical polymerization on glass or paper.[75] However, although those strategies exhibit excellent antibacterial activity, an industrial exploitation is not possible.

A more practicable example of bioactive surfaces has been given by Wynne and co-workers.[76] By synthesizing polyurethanes having random copolymer 1,3-propylene oxide soft blocks with alkylammonium and either trifluoroethoxy or PEGlyted side chains as polymer-surface modifier, good antibacterial activity in 2 wt% coatings with poly(urethane) were obtained. Thereby, the surface modifier is concentrated on the surface by a combination of differing hard block/soft block solubility parameters and entropic effects and subsequently

[73] Tew, G. N.; Scott, R. W.; Klein, M. l.; De Grado, W. F. *Account Chem Res* **2010**, *43*, 30-39.

[74] Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J Am Chem Soc* **2007**, *129*, 15474-15476.

[75] Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y. J.; Russell, A. J. *Biomacromolecules* **2004**, *5*, 877-882.

[76] Kurt, P.; Wood, L.; Ohman, D. E.; Wynne, K. J. *Langmuir* **2007**, *23*, 4719-4723. Wynne, J. H.; Fulmer, P. A.; McCluskey, D. M.; Mackey, N. M.; Buchanan, J. P. *ACS Appl Mater Interfaces* **2011**, 2005-2011.

determines the surface properties whereas the matrix material maintains the bulk polymer properties. Comparable work is delivered by Tiller.[77]

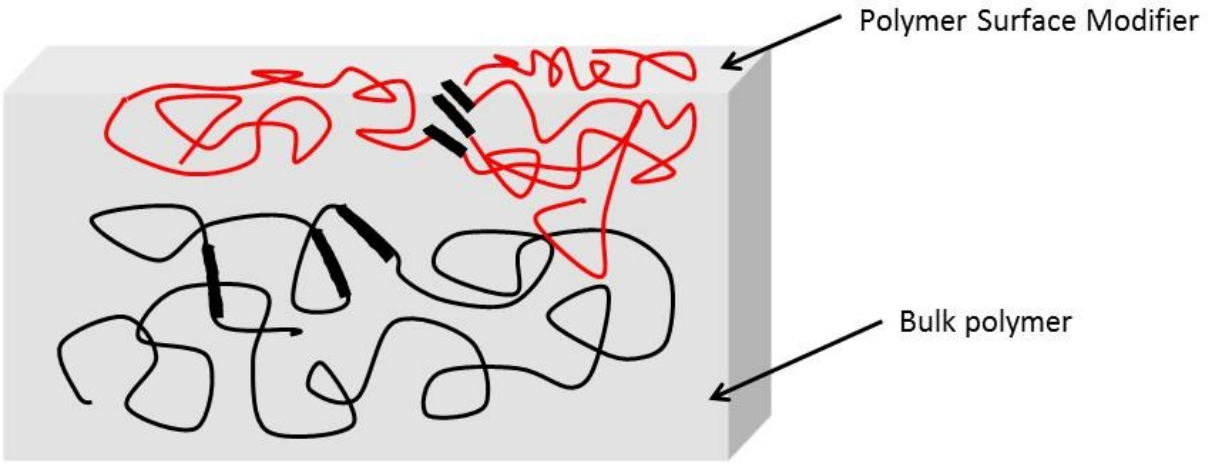


Figure 1-6 Polymer surface modifier concept

However, that concept suffers from the disadvantage that abrasion of the surface leads to the loss of the assembled antimicrobial polymer. Therefore, antimicrobial additives that are distributed continuously within the polymer matrix lead to a permanent and stable antimicrobial product. Wiesbrock *et al*. developed partly hydrolyzed poly(2-nonyl-2-oxazoline)s contact biocides, which exhibit antimicrobial activity in ≤5 wt% compounds.[78]

1.2.3.3.3 Combined Concepts

A very recent field of investigations are surfaces, which combine different strategies to obtain microbial hygiene. Contact-active surfaces are often considered as self-deactivating, as killed microbes might adhere and serve as a growing layer for following cells, leading to the formation of biofilm.[79] The innovative surfaces thereby combine either “repelling and

[77] Waschinski, C. J.; Zimmermann, J.; Salz, U.; Hutzler, R.; Sadowski, G.; Tiller, J. C. *Adv Mater* **2008**, *20*, 104-108.

[78] Kelly, A. M.; Kaltenhauser, V.; Mühlbacher, I.; Rametsteiner, K.; Kern, H.; Slugovc, C.; Stelzer, F.; Wiesbrock, F. *Macromol Biosci* **2012**, *13*, 116-125.

[79] O´Toole, G.; Kaplan, H. B.; Kolter, R. *Annu Rev Microbiol* **2000**, *54*, 49–79.

releasing", "releasing and contact-killing" or "contact-killing and repelling". The latter one is based on a change of surface energy upon a certain trigger.

Chen and co-workers have designed a contact-killing and repelling surface based on a hydrolytic cleavage of the ethyl ester next to quaternary ammonium groups by continuous contact with aqueous media. The resulting zwitterionic structure very effectively repeals microbes, however the effect is not reversible.[80] Thermoresponsive surfaces have been developed by grafting oligo(ethylene glycol) methacrylates, that are stretched at room temperature- acting as antibacterials- and collapse at 35°C, presenting the poly(ethylene glycol) structure and bacterial remains are detached.[81]

Recently Zhao and co-workers[82] have developed a coating based on Ni-P-PTFE in combination with an antimicrobial poly(norbornene)[58b], which very efficiently inhibits the detachment of bacteria.

[80] Cheng, G.; Xue, H.; Zhang, Z.; Chen, S.; Jiang, S. *Angew Chem Int Ed* **2008**, *47*, 8831-8834.

[81] Laloyaux, X.; Fautre, E.; Blin, T.; Purohit, V.; Leprince, J.; Jouenne, T.; Jonas, A. M.; Glinel, K. *Adv Mater* **2010**, *22*, 5024-5028.

[82] Zhao, Q.; Wang, S.; Xueju, S.; Slugovc, C.; Kienberger, J. **2012**: EP 2530126. Coating comprising Ni-P-PTFE in combination with a polycationic polymer.

2 RESULTS AND DISCUSSION

Within this work, the synthesis of new antimicrobial, water insoluble macromolecules should be accomplished. In previous work performed within the institute poly(norbornene)s were designed, exhibiting all necessary functionalities allowing antimicrobial activity.[83,58b] However, those polymers suffered from the disadvantage of bearing ester functionalities, prone to degradation due to hydrolysis. Therefore, the thiol-ene reaction was chosen to post-modify unsaturated polymers. However, to gain insights in the mechanism and additionally learn about reaction conditions, several test reactions using small molecule models were conducted in a first step.

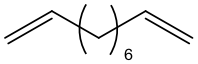
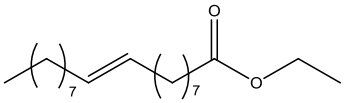
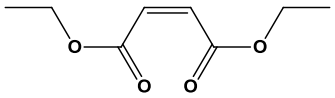
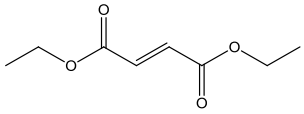
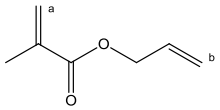
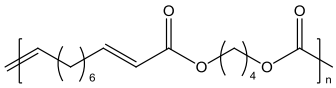
2.1 MODEL THERMAL THIOL-ENE REACTIONS

As there is a clear discrimination against certain types of double bonds as described in literature,[2] several small molecules were investigated using two different thiols. On the one hand, dodecanthiol **C_{12}SH**, an aliphatic, long-chain mercaptan with very low polarity, on the other hand, cysteamine **cys**, an aminothiol, were the two thiols under investigation in a first attempt. Regarding the range of unsaturated species, strained molecules as well as α,β unsaturated esters, so called Michael acceptors,[84] but also vinylic species were tested. The reaction was initiated by the addition of 2,2´-azobisisobutyronitrile **AIBN** and the reactions were allowed to stir overnight in refluxing toluene. Conversion was calculated from reduction of integrals of the double bond signals in ^{1}H NMR.

[83] Seyfriedsberger, G.; Rametsteiner, K.; Kern, W. *Eur Polym J* **2006**, *42*, 3383-3389.

[84] Michael, A. *Am Chem J* **1887**, *9*, 115.

Table 2-1 Thermal thiol-ene reaction with model substances

entry	educt	cys	$C_{12}SH$
1		16.0 %	100 %
2		0 % [i]	96.5 % [i]
3		27.0 %	92.5 %
4		6.0 %	89.0 %
5		a: 100 % b: 0 %	a: 100 % b: 100 %
6		50.0 %	0%

Conversion declared by percentage reduction of integral of double bonds compared to uninfected reference peak; [i] applying 5 eq of thiol.

From that data, it can be clearly seen, that not only the double bond offered to the radical attack influences the conversion rate, but also the nature of the thiol has a tremendous influence on reaction effectiveness. Especially interesting is entry 5, as the two double bonds exhibit completely different reaction behavior against cysteamine. The methyl methacrylic double bond was consumed entirely, whereas the allyl side does not show any reactivity at all. As the former double bond is a Michael acceptor due to its electron withdrawing character, it was tested whether these reactivity differences could be traced back to a nucleophilic reaction of the nitrogen instead of the thiol. This could not be ruled out distinctively from ^{1}H NMR spectra, as a direct correlation of peaks is hardly possible, due to the similar shift of CH_2-S and CH_2-N. For that reason allylmethacrylate was reacted with ethylenediamine under radical conditions, which did not lead to conversion of the amine, but to a polymerization of the acrylatic species. However, it could be concluded, that the amine did not interfere with the thiol-ene reaction in a noteworthy manner. Instead, the basic character of the thioamine might lead to a nucleophilic reaction pathway beside the radical one. Although nucleophilic *thio*-Michael reactions are often conducted using an *e.g.* phosphine catalysts, the basic character of the cysteamine might be sufficient for a

pronounced conversion.[85,86] Despite that, a more pronounced reactivity of the long-chain dodecanthiol towards internal double bonds can be found. There, the better miscibility of thiols with the unsaturated ester can be reasoned (compare entry 2).

To summarize, thermal activated thiol-ene reactions were possible with two different mercaptans. On the one hand, cysteamine, an amine-bearing ethylthiol and on the other hand, dodecanthiol, an aliphatic thiol, were reacted with several unsaturated small molecules. Dodecanthiol showed a good reactivity against internal double bonds, whereas cysteamine reacted pronounced with $\alpha,\beta-$ unsaturated esters. Maybe, there the basic nature of the primary amine led to a nucleophilic reaction to some extent.

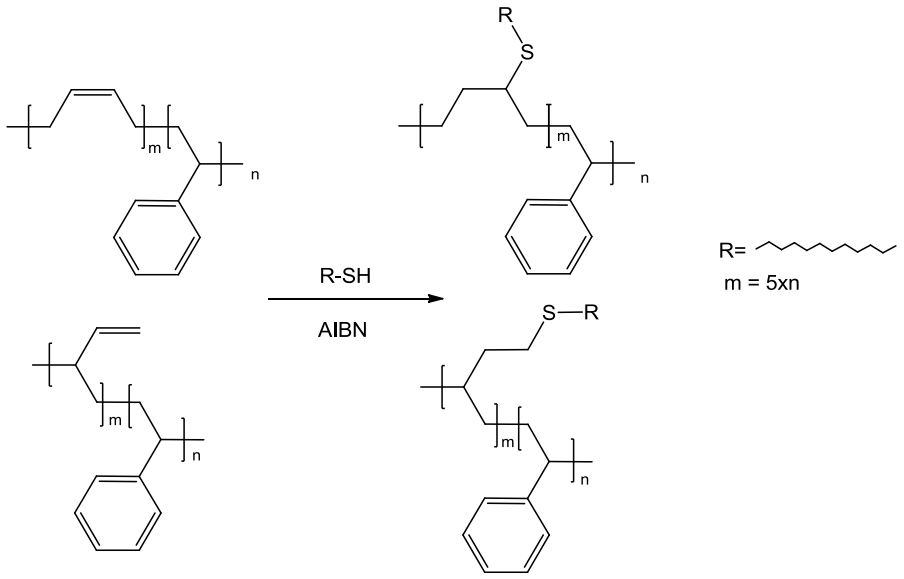
Those findings were subsequently transferred to a macromolecular system. As the aim was the introduction of antimicrobial active groups, cysteamine and derivatives were chosen as the thiol species to use. Although the tendency of the model reactions showed a preferred conversion of α,β- unsaturated esters, it was decided to use polyolefins to avoid hydrolysis sensitive bonds.

[85] Mather, B. D.; Viswanathan, K.; Miller, K. M.; Long, T. E. *Prog Poly Sci* **2006**, *31,* 487-531.

[86] Lutolf, M. P.; Hubbell, J. A. *Biomacromolecules* **2003**, *4*, 713-722.

2.2 Thermal Thiol-ene Reactions on Industrial Samples

In a first attempt it was decided to use styrene-butadiene rubber (**SBR**) as unsaturated macromolecule. Again, the already used two standard thiols were selected to learn about the reaction behavior. SBR featured a styrene to butadiene ration of approximately 1:2, whereas the proportion within the butadiene content was found to be roughly 80% of 1,4- linked monomer units and 20% of vinylic units. A distinction between *cis* and *trans* linked moieties was not performed. This allocation was based on the peaks at 7.29-7.02 ppm (m, 5H, styrene), 5.56 ppm (bs, 0.72 H, C*H*=CH_2) and 4.96 ppm (t, 1.54 H, CH=CH_2) as well as 5.40 ppm (bs, 9.60 H, CH=CH *cis* and *trans*).[87]



Scheme 2-1 Thiol-ene reaction on styrene-butadiene rubber

The reaction with dodecanthiol (**C_{12}SH**) was performed three times, once with a 10fold molar excess of double bonds, once in equimolar ratio and finally with a 10fold excess of thiol. Integration of aromatic peaks as internal immune standards exhibited no conversion for the former set-ups. As for the equimolar reaction as well as the latter one, only a weak up to no reduction of the ^{1}H NMR signal could be detected (max. 16% with high excess of thiol). However, better conversion was found for the vinyl double bonds. In the case of equimolar

[87] [a] Zhang, Z.; Zhang, L.; Li, Y.; Xu, H. *Polymer* **2005**, *46*, 129-136. [b] Sardelist, K.; Michels, H. J.; Allen, G. *Polymer* **1984**, *25*, 1011-1019.

addition of reactants, a reduction of 20% and in the thiol excess experiment a reduction of 80% was detected. In the region of 2.48 ppm in ^{1}H NMR, a new broad peak appeared which might be assigned to methyl groups neighboring thioether functionalities. Since the purification of modified SBR from unreacted dodecanthiol turned out to be difficult even after repeated precipitation in stirred ethanol, it was refrained from integration of the respective peak and hence stating a conversion rate. In the case of reaction with cysteamine, however, in any case a reaction could be determined.

A second polymer, that was subjected to thiol-ene modification was 1,4- poly(butadiene) **1,4-PB**. ATR-IR measurements of an industrial samples revealed predominantly *cis*- content, as assignment of characteristic peaks revealed (732 cm^{-1}, C-H out of plane bending, *cis*-CH=CH-; 1655 cm^{-1}, C=C str. *cis* –CH=CH-).[88] A minor content of vinylic double bonds resulting from 1,2- linked moieties can be assumed due to weak bands at 995 and 913 cm^{-1}, assigned to C-H out of plane bending of vinyl R-CH=CH_2. Again, small quantities of that polymer were reacted with **cys** and **C_{12}SH** in CH_2Cl_2 using **AIBN** as radical initiator. For quantification of conversion, again proton NMR spectra were recorded. There, the pristine peak at 5.38 ppm (double bonds *cis* **PB**) was diminished and a new peak at 5.41 ppm (double bonds *trans* **PB**) arose in both cases, suggesting that an isomerization as described in 1.1.3 occurred. This could be also approved by ATR-IR analysis of the modified samples, where a strong peak at 967 cm^{-1}, indicative for C-H out of plane bending of *trans* R-CH=CH-R´, gave incidence of a rearrangement. The minor content of vinylic double bonds underwent a modification, as could be found from ^{1}H NMR.[89] Peaks at 4.99 and 5.60 ppm, respectively, were slightly reduced (~ 10% for modification with **cys**, ~ 20% for modification with **C_{12}SH**). However, as that only sums up to 1- 2% of modification related to the entire double bond content, this was assumed as too low for further work.

Nevertheless, from those findings, the conclusion could be drawn, that vinyl double bonds in polymers exhibit a higher reactivity. In literature, modifications of 1,2- poly(butadiene) via thiol-ene reactions are described already.[7,12,41] Therefore, anionically polymerized poly(isoprene) was chosen as material of choice for thermally induced thiol-ene reactions with amine featuring mercaptans.

[88] Nallasamy, P.; Anbarasan, P. M.; Mohan, S. *Turk J Chem* **2002**, *26*, 105-111.

[89] Hung, N. Q.; Sanglar, C.; Grenier-Loustalot, M. F.; Huong, P. V.; Cuong, H. N. *Polymer Degradation and Stability* **2011**, *96*, 1255-1260.

2.3 Thermal Thiol-ene Reactions on Tailor-made Polymers

2.3.1 Preparation of High Vinyl Content poly(Isoprene)

It was decided to use anionically polymerized isoprene, as this polymerization method allows a certain control over the microstructure of the formed polymer. Especially if using polar solvents as for example THF, the formation of 1,2- and 3,4- linked repeating units is preferred.[90] In the precise case, freshly distilled isoprene was polymerized by initiation with butyl lithium in dry THF as solvent at room temperature. Analysis via ^{1}H NMR spectroscopy was done in accordance to Bywater *et al.*[91] and revealed a microstructure as follows: 25±2% of 1,2-, 15±4% of 1,4- and 60±3% of 3,4- linked repeating units. For that assignment, the olefinic signals at 5.72 ppm (-C*H*=CH_2; i.e. 1,2-linked repeating units), 5.02 ppm (-C*H*=C(Me)-; 1,4-linked repeating units) and 4.85-4.65 ppm (-CH=CH_2 and –C(Me)=CH_2; 1,2- and 3,4-linked repeating units) were used. The assignment of ^{13}C{^{1}H} NMR spectra was made according to Rozentsvet *et al*.[92] and beard no special features. From gel permeation chromatography (GPC) against poly(styrene) standards in THF a number molecular weight M_n of 10500 g mol^{-1} and a polydispersity index (PDI) of 1.1 was found.

2.3.2 Thermal Thiol-ene Modification of poly(Isoprene)[93]

Following a protocol by the group of Aglietto[94], high vinyl content poly(isoprene) was diluted in toluene. A two-fold molar excess of cysteamine in respect to double bonds was added,

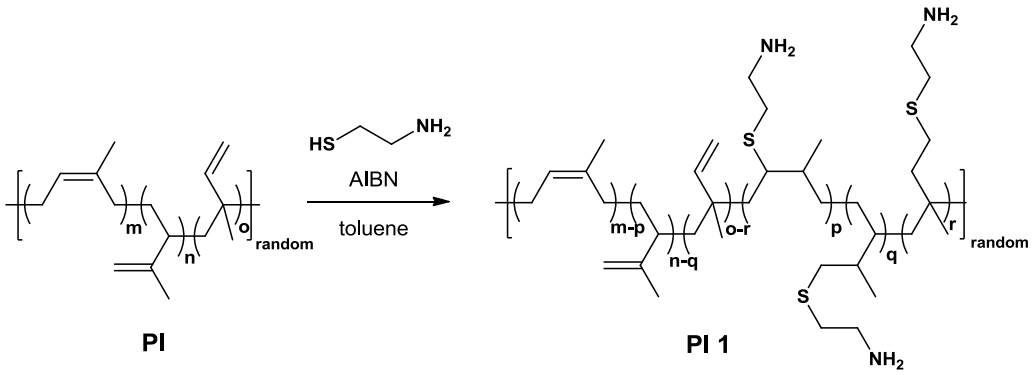
[90] Worsfold, D.J.; Bywater, S. *Can J Chem* **1964**, *42*, 2884-2892.

[91] Brès, P.; Viguir, M.; Sledz, J.; Schué, F.; Balck, P.E.; Worsfold, D.J.; Bywater, S. *Macromolecules* **1986**, *19*, 1325-1328.

[92] Rozentsvet, V.A.; Khachaturov, A.S.; Ivanova, V.P. *Polymer Science, Ser A* **2009**, *51*, 870-876.

[93] [a] Kienberger, J.; Kreutzwiesner, E.; Noormofidi, N.; Klarholz, I.; Harms, C.; Slugovc, C. *Macromol Symp* **2012**, *311*, 98-102. [b] Kienberger, J.; Noormofidi, N.; Mühlbacher, I.; Klarholz, I.; Harms, C.; Slugovc, C. *J Polym Sci Pol Chem* **2012**, *50*, 2236-2243.

followed by 1 mol-% of **AIBN**. The reaction mixture was allowed to stir for 20 h at 80°C, afterwards the solvent was distilled off and the residue resolved in a small amount of chloroform. The polymer was purified from unreacted thiol upon precipitation in vigorously stirred methanol. Repeating this precipitation procedure three times yielded 66% of a brittle, white polymer (**PI 1**).



Scheme 2-2 Thiol-ene modification of poly(isoprene)

This polymer was characterized by standard analytical methods, thus elemental analysis, IR and NMR spectroscopy as well as GPC. From elemental analysis, an average functionalization of approximately every fifth (*i.e*. 20%) repeating unit could be determined. Regarding ^{1}H NMR spectra, two new peaks were detected at 2.78 ppm and 2.50 ppm, which could be assigned to methylene groups next to nitrogen and sulfur. In parallel, the olefinic signals were distinctly diminished compared to aliphatic signals. Careful integration of the corresponding peaks revealed similar results for conversion rate. Evaluation of the signals for the unreacted double bonds revealed a ratio of 1 : 1 : 4 of 1,2-, 1,4- and 3,4- linked repeating units, suggesting that- as expected from experiments with SBR- predominantly 1,2- linked repeating units have been functionalized. Using poly(butadiene) as a starting material for this kind of modification, the formation of six- and five-member cyclic units as a side reaction under radical conditions has been described very detailed.[40] However, in that case, the reaction could not be unequivocally proven. Having a closer look at the aliphatic region of the polymer spectrum revealed the broadening of the peak at 0.94 ppm, indicating methyl groups connected to an aliphatic carbon atom in the mother polymer. This change in shape

[94] Romani, F.; Passaglia, E.; Aglietto, M.; Ruggeri, G. *Macromol Chem Phys* **1999**, *200*, 524-530.

and intensity by a factor of 2 might be traced back to either a formation of cyclic structures or a functionalization of 3,4- linked repeating units occurred, too.

From $^{13}C\{^{1}H\}$ NMR spectra a differentiation between thiol-ene modification and cyclization is not possible neither, however, several new peaks between 41.5 ppm and 38.0 ppm indicated again the successful functionalization of double bonds.

As no defined structure could be found from mere NMR spectroscopy measurements, infrared spectra of thin films of the polymer were recorded. As for the 3,4- linked moieties, a signal at 888 cm^{-1} could be assigned to a CH_2 vinylidene out-of-plane bending.[95] Compared to the spectrum of the pristine species, a clear diminution of the latter peak was observed. As for the alkene C-H out-of-plane deformation vibration, peaks at 1003 cm^{-1} and 906 cm^{-1} are indicative for vinyl groups, in that case resulting from vinyls from a 1,2- linkage.[96] Again, for those peaks, a clear reduction compared to the spectrum of unmodified poly(isoprene) is visible.

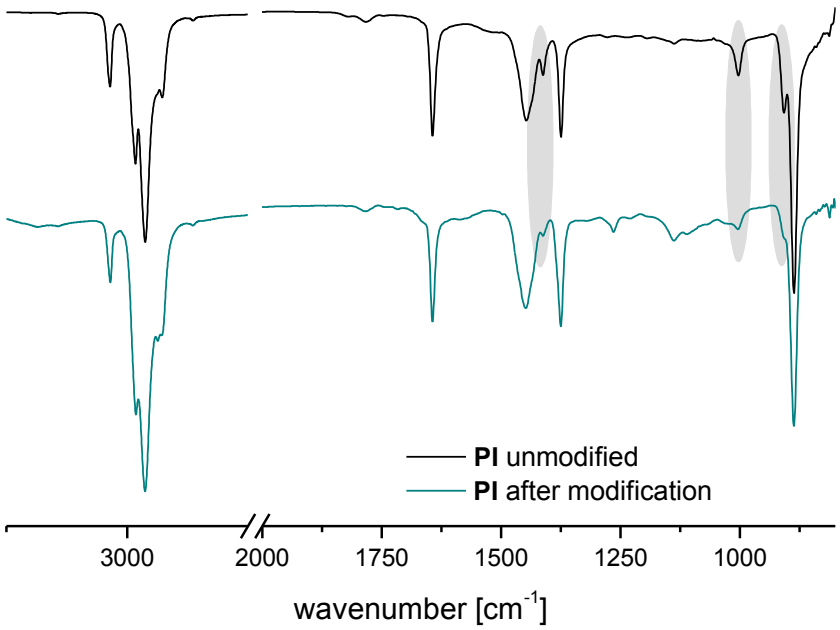


Figure 2-1 IR- spectra of poly(isoprene) and the thiol-ene modification thereof

[95] Assiana, F.; Cornibert, J.; Marchal, J. *Cr Acad Sci II C*, **1987**, *265*, 1023-1023.

[96] Assiana, F.; Cornibert, J.; Marchal, J. *Cr Acad Sci II C*, **1988**, *266*, 1563-1566.

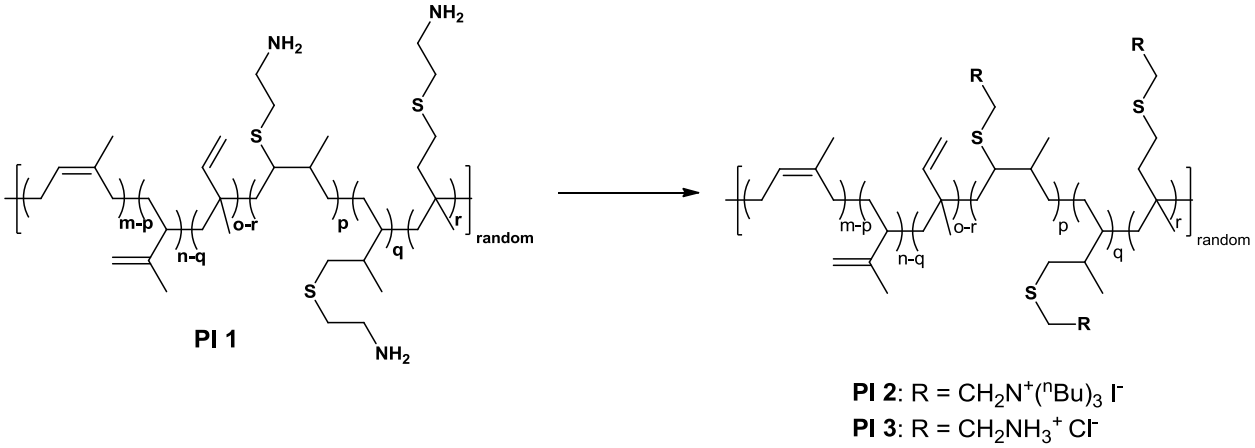
However, also IR spectra did not shed light on that issue, as the reaction on 1,2- and 3,4-linked repeating units could be equally traced back to cyclization reaction as well as a thiol-ene modification. For complementation of analysis, GPC measurements were performed and revealed only a slight increase in number molecular weight. Polydispersity index PDI increased from 1.1 for poly(isoprene) to 1.8 for the modified polymer.

Nevertheless, from elemental analysis, a successful functionalization can be approved. The functionalization degree can be estimated as approximately 20%- in other words, every fifth repeating unit underwent the reaction and is now bearing a terminal, primary amino group.

2.3.3 Antimicrobial Modifications and Characterizations of Modified Polymers

2.3.3.1 Antimicrobial modifications of amine-bearing **PI 1**

As already mentioned, amino functionalities are described as very active biocides if they are charged. As the aim is the antimicrobial modification of a polyolefine, the primary amine bearing poly(isoprene) was subsequently transferred in a quaternary amine salt. This was achieved on the one hand via irreversible alkylation, on the other hand by protonation yielding a pH- dependent polymer.



Scheme 2-3 Synthesis of quaternary amines

Alkylation of the primary amine bearing poly(isoprene) **PI 1** was achieved by treatment with butyl iodide in a solvent mixture of chloroform and acetonitrile under reflux for a reaction time of 20 h.[97] The pure polymer was obtained by precipitation from *n*-pentane, yielding 75% of a brownish, brittle polymer **PI 2**.

For protonation, **PI 1** was reacted with a mixture of acetic acid and 10% HCl at room temperature for 2 h, resulting in an insoluble residue, which was separated by decanting the solution. After drying to constant weight, a yield of 66% **PI 3** was obtained.

For characterization of those new polymers, ^{1}H NMR spectra were recorded in a first step. New peaks in the region of 3.50 ppm in the case of **PI 2** and 3.11 ppm for **PI 3** are especially indicative for quaternary amines.98 Spectra were recorded in MeOD in the latter case, as solubility in CDCl3 was not achievable any longer, indicating charged groups within the polymer. 13C{1H} NMR exhibited new peaks at the region of 53.2 ppm, again allowing conclusion of a successful quaternization.99 A comparison of spectra of PI- PI 2 can be seen in Figure 2-2. It has to be noted, that solvent peaks are omitted for clarity (marked with *). Characterization of polymers was completed by GPC, FT-IR as well as elemental analysis. Detailed data for each polymer can be found in 4.3.4- 4.3.6; an overview is summarized in Table 2-2.

[97] Seyfriedsberger, G. PhD thesis "Kontaktbiozide auf Polymerbasis: Herstellung und Charakterisierung" Graz, **2007**.

[98] Sajomsang, W.; Gonil, P.; Tantayanon S. *Int J Biol Macromol* **2009**, *44,* 419-427.

[99] [a] Kowalczyk, I. *Molecules* **2008,** *13*, 379-390. [b] Brycki, B.; Szule, A.; Kowalczyk, I. *Molecules* **2010**, *15*, 5644-5657. [c] Lu, G.; Wu, D.; Fu, R. *Reactive* & *Functional Polymers* **2007**, *67*, 355-366.

Table 2-2 Characterization data for polymer under investigation

entry	sample	yield (%)	Mn, GPC [a] / g mol^{-1}	PDI [a]	N (%) [b]
1	PI	87	10500	1.1	-
2	PI 1	66	11900	1.9	2.06 ± 0.1
3	PI 2	75	12500	1.5	2.00 ± 0.2
4	PI 3	89	10900	1.8	n.d.

[a] Determined by GPC (solvents: $CHCl_3$:Et_3N:isopropanol = 94:4:2; relative to poly(styrene) standards).

[b] Determined by elemental analysis

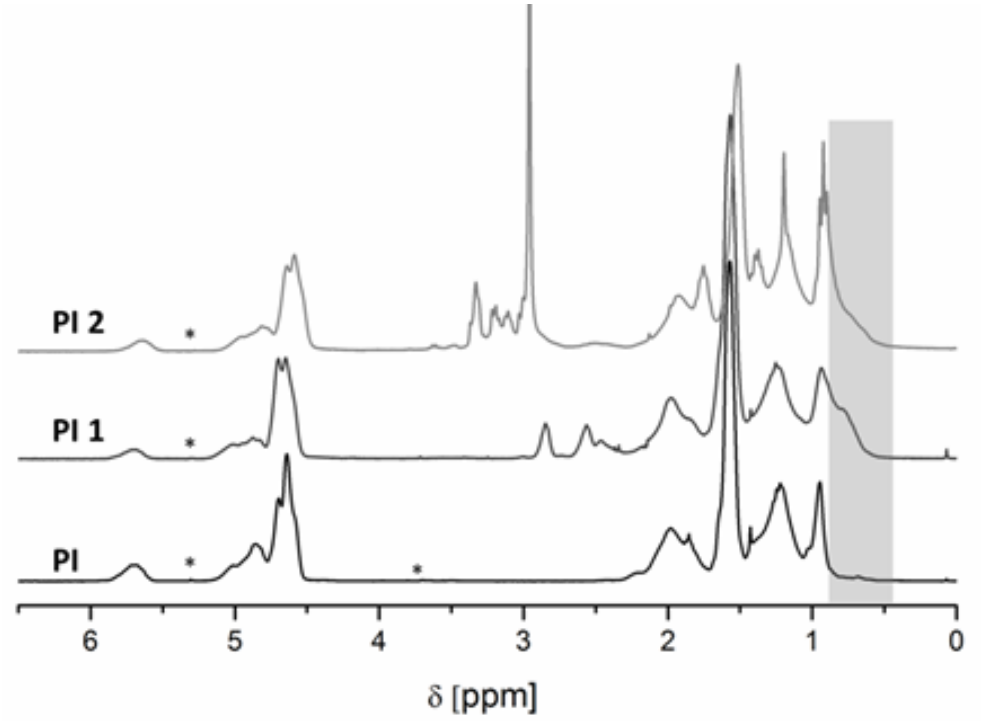


Figure 2-2 ^{1}H NMR spectra of polymers PI - PI 2 recorded in $CDCl_3$

2.3.3.2 THERMAL PROPERTIES

As it was aimed at compounding the polymer with commodity matrix materials such as poly(propylene), thermal stability had to be ensured. For that purpose, thermo gravimetric measurements were performed. As compounding temperature was 190°C, a mass loss of less than 3% at a temperature of 200°C was defined as a criterion. The unmodified **PI** was stable up to a temperature of 327°C according to that criterion. Modifications lowered this temperature in all cases; nevertheless at 200°C all of the test polymers met the criterion. In detail, values were as follows:

Table 2-3 Criterion temperature for polymers under investigation

entry	sample	criterion temperature (°C)
1	PI	327
2	PI 1	209
3	PI 2	232
4	PI 3	196

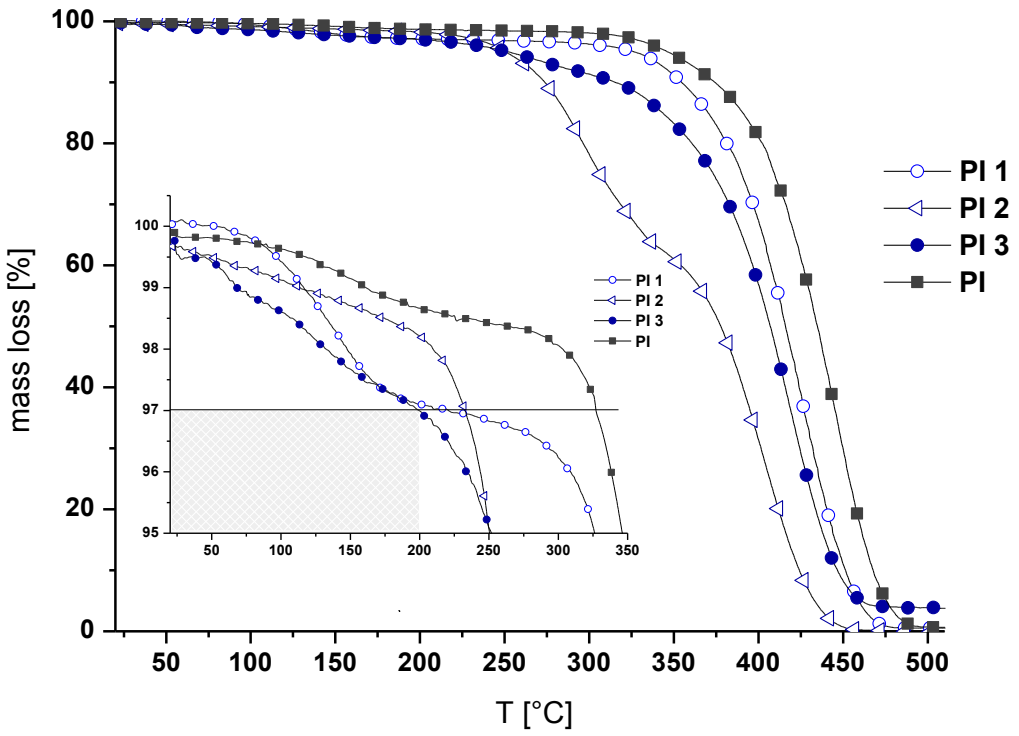


Figure 2-3 TGA of polymers PI- PI 3

2.3.3.3 Surface Characterization

Charges on surfaces alter surface polarity and thereby the measureable quantity of surface energy delivers important information. If quaternary amine functionalities can be found on the surface, the contact angle against a polar liquid such as water should be lowered; simultaneously the surface energy should increase. In the context of antibacterial activity, an increased surface energy might implement a higher activity, as electrostatic forces between a charged surface and charged bacteria cells can lead to accelerated interaction. In contrast to that is an approach to prevent microbiological affection by a lowered surface energy, which inhibits the attachment of cells. Surface energies of below γ = 30 mN m^{-1} can reduce

biofouling significantly. An example for such surfaces is poly(tetrafluoroethylene) PTFE, exhibiting a high contact angle with polar liquids and thus a low cell adhesion.[100]

Samples were prepared by spin coating solutions of **PI**- **PI 2** in CH_2Cl_2 and **PI 3** in MeOH on glass substrates. Surface energies were determined from measurement of the contact angles of diiodomethane and water[101] on the corresponding films employing the Owens-Wendt computation.[102] Thereby contact angles were determined fivefold and average values have been taken.

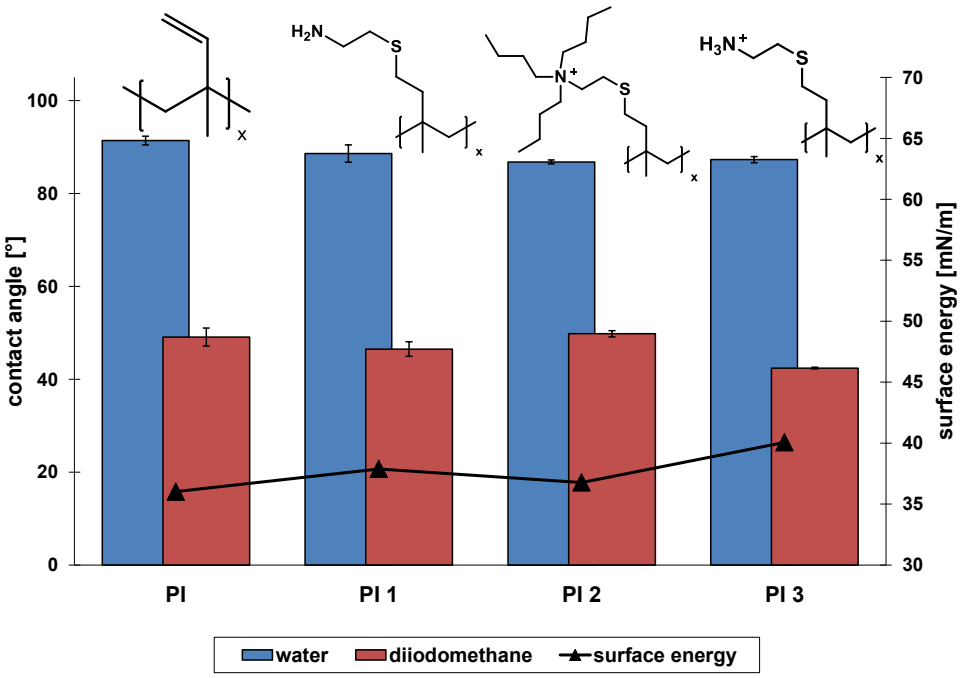


Figure 2-4 Surface properties of polymers under investigation

Detailed values can be found in Table 2-4. Generally, higher surface energies γ were found for the modified films. Unmodified poly(isoprene) was used as reference and exhibited a surface energy of 36 mN m^{-1}. No subsequent modifications- neither reaction with cysteamine

[100] [a] Baier, R. E.; Meyer, A. E. *Biofouling* **1992**, *6*, 165-180. [b] Allion, A.; Baron, J.- P.; Boulange-Petermann, L. *Biofouling* **2006**, *22*, 269-278.

[101] Ström, G.; Fredriksson, M.; Stenius, P. *J Colloid Interf Sci* **1987**, *119*, 352-361.

[102] Owens, D. K.; Wendt, R. C. *J Appl Polym Sci* **1969**, *13*, 1741-1747.

nor alkylation or protonation- did alter the surface tremendously. All surface energy values of the modified films are in the range from 37-40 mN m^{-1}.

Table 2-4 Contact angles and surface energy values of PI- PI 3

entry	sample	Θ_{H2O}	Θ_{CH2I2}	surface energy γ/ mN m^{-1}	$\gamma_{dispers}$	γ_{polar}
1	PI	91.4 ± 0.9	49.1 ± 1.9	36.0 ± 0.1	34.8 ± 0.1	1.2 ± 0.0
2	PI 1	88.6 ± 1.9	46.5 ± 1.6	37.9 ± 0.3	36.2 ± 0.3	1.7 ± 0.1
3	PI 2	86.8 ± 0.4	49.8 ± 0.7	36.8 ± 0.2	34.4 ± 0.1	2.4 ± 0.0
4	PI 3	87.3 ± 0.6	42.4 ± 0.2	40.1 ± 0.1	38.4 ± 0.0	1.7 ± 0.1

The further aim was using new polymers as additives for standard commodity materials such as poly(ethylene) PE or poly(propylene) PP. For a sufficient antibacterial activity, it is necessary to locate charged ammonium groups on the surface of the material. However, as both PE and PP exhibit surface energy values around 30 mN m^{-1},[103] a detection of modified poly(isoprene)s with this method does not lead to significant results.

2.3.3.4 Antibacterial Test Results

Of special interest was, of course, the antibacterial performance of those probes. For that purpose, TTZ Bremerhaven determined biocide activity according to a modified Japanese Industrial Standard (JIZ) Z2801:2000 protocol.[104] This standard specifies the testing methods to evaluate antimicrobial activity and antimicrobial efficacy on bacteria on the surface of antimicrobial products. It is a film based methodology that is mainly applied for duroplasts, thermoplastic materials and lacquers in the field of organic macromolecules; however, it can

[103] Source: http://www.surface-tension.de/solid-surface-energy.htm, 12th October 2012

[104] Suzuki, S.; Imai, S.; Kourai, H. *Biocontrol Science* **2006**, *11*, 135-145.

be also applied to metal or ceramic products. One of the strengths of this method is the possibility to differ between two terms - microbiocides and microbiostads.[48a] A microbiocide is defined as a bacteria killing agent, whereas the latter one inhibits the growth of microorganism. The distinction between these two species underlies the range of reduction of the logarithmic values of absolute colony forming unit numbers. If the number of cells, counted after 24 h, is reduced more than two log levels compared to the number of cells on an untreated reference material, a material is defined as a microbiocide. However, if a stagnation or slight diminution between the starting concentration and biocide behavior is stated in absolute cell numbers, the behavior is called bacteriostatic.

To prepare adequate surfaces for the test series, polymers were diluted in CH_2Cl_2 (**PI**- **PI 2**) and methanol, respectively (**PI 3**). Solutions with a concentration of 10 mg mL^{-1} were prepared and spin coated on cleaned glass substrates (4x4 cm). Again, unmodified poly(isoprene) was used as reference material.

The test procedure is condensed as follows: an inoculated working culture with a starting concentration of approximately 10^5 cells mL^{-1} is used to coat the glass slides. The glass slides are capped with a sterile cover glass and incubated for 24 h at 35°C. Samples were transferred in a casein peptone- soya meal peptone nutrient solution and eluted. Dilutions were dripped on a culture medium and again incubated for 44 h. Then colony forming units (CFU) were counted and reduction compared to unmodified polymer sample is stated.

Tests were performed against Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) as well as Gram-negative strains (*Escherichia coli* and *Pseudomonas fluorescens*). The choice of those bacteria strains was made by an evaluation of onsite probes of food processing companies. *Listeria monocytogenes* was chosen as test organism, as it was of special interest for a cheese producing company. This work was performed in advance of this thesis within the framework of the BIOSURF project.

In the depicted graphic (*cf*. Figure 2-5) absolute colony forming unit (CFU) numbers were normalized against CFUs of reference **PI** at t= 24 h and the logarithmic value is plotted. The dotted line illustrates the respective boundary values indicating an antimicrobial behavior.

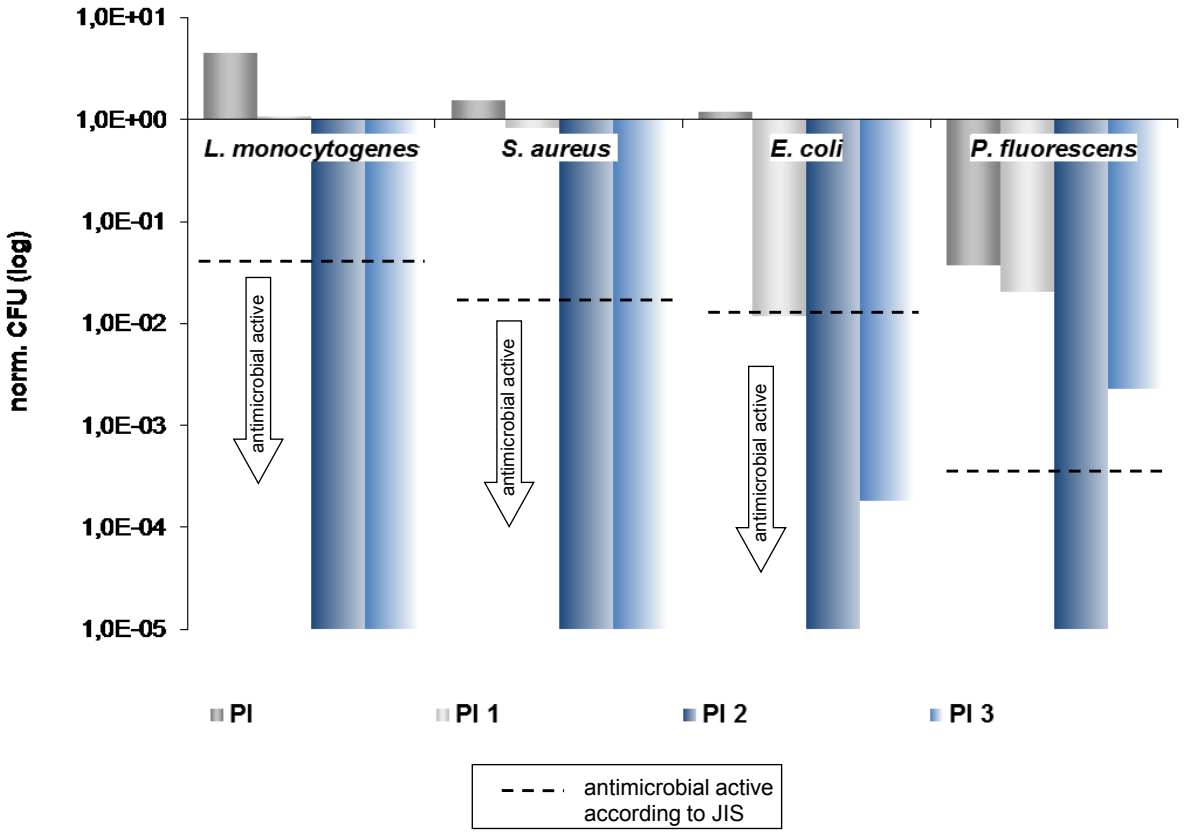


Figure 2-5 Reduction of CFU after 24 h

On untreated poly(isoprene) reference sample **PI** only a slight growth of bacteria strains *L. monocytogenes*, *E. coli* and *S. aureus* was found, whereas colony forming units of *P. fluorescence* were reduced already for 1.43 logarithmic values.

The first modification yielding cysteamine modified **PI 1** did not render the material biocide, so the mere attachment of uncharged amines does not lead to the desired result. Solely CFU numbers of *E. coli* bacteria were reduced by 1.92 log values. For all other tested strains, reduction was in the range of 0.26-0.62 logarithmic units.

However, after quaternization excellent bacteria killing properties were obtained. Both **PI 2** and **PI 3** exhibit antimicrobial activity against *L. monocytogenes*, *E. coli* and *S. aureus*. As for *P. fluorescens* **PI 3** showed a bacteriostatic effect, revealing a reduction of only 1.21 logarithmic units compared to the cell number on the unmodified poly(isoprene). Against *E. coli* CFU decreased 3.81 logarithmic units, which satisfies the demand of two logarithmic

units. However, **PI 2** turned out to be a potent biocide against all kind of microbes tested. After 24 hours of contact, no living bacteria cells of any of the four bacteria strains were counted.

So, the quaternization step leads to a remarkable increase of antibacterial activity. Neither Gram-positive nor Gram-negative bacteria cells were found to survive 24 h of contact time. The butylated **PI 2** thereby exhibited the best results. As for **PI 3** the pH sensibility might explain the reduced activity against *E. coli* and *P. fluorescens*. Therefore, permanent alkylation seems to be the method of choice to generate antibacterial polymers by post-functionalization of unsaturated polymers.

Additionally, thermal stability at 190°C of **PI 2** allows compounding processes (*cf*. chapter 2.3.3.2). Therefore polymer **PI 2** was chosen to use it as active additive for a commodity material poly(propylene) PP.

2.3.4 Preparation of Compounds

2.3.4.1 Motivation

As the aforementioned modifications are rather time and money consuming, it is not possible to manufacture complete components from the active material. Therefore it was decided to add the active polymer in additive amounts. In industrial processing, additives are typically added in ranges not exceeding 5 wt% to low-cost commodity materials such as poly(ethylene), poly(propylene) or poly(styrene).

2.3.4.2 Compound Fabrication

As described, **PI 2** fulfilled all requirements that allow compounding of the material. It was stable at the compounding temperature of 190°C and exhibited remarkable activity against all four target organisms. Therefore, it was decided to compound **PI 2** in 2.5 and 5.0 wt% with

the commodity material poly(propylene) PP by extrusion molding. Higher mass fractions would not be feasible any more, as they would increase manufacturing costs and efforts.

The compounding process itself was conducted at 190°C for 2 min. Thereby different additives can be added or the thermoplastic material can also be mixed with other thermoplastic or non-thermoplastic materials, moldable at elevated temperatures.

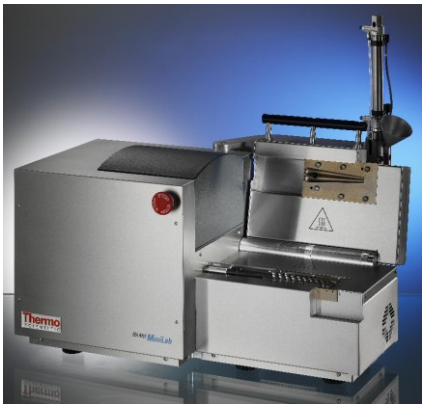


Figure 2-6 Thermo-Haake mini-extruder [105]

Afterwards the extrudate was pressed into plates (16x16 cm), from that test specimen in 4x4 cm were cut.

Prior to antimicrobial activity measurements, plates were characterized via ATR-IR spectroscopy, zeta-potential measurements and elementary analysis.

2.3.4.3 CHARACTERIZATION OF PLATES

Surface characterization tests were performed, as it is of special interest to qualify and, if possible, quantify active polymer on the surface of the probes, as only there contact with bacteria cells is possible. By visual examination the plates revealed a brownish color compared to the white PP plate, traced back to the already slightly brownish **PI 2**. The texture, which is visible, comes off the pressing process.

[105] source: http://www.thermo.com/eThermo/CMA/Images/Product/productImg_10815.jpg.

Figure 2-7 Reference plate PP (left), PP+2.5 wt% PI 2 (middle) and PP+5.0 wt% PI 2 (right)

2.3.4.3.1 Elemental Analysis

In order to verify, that no polymer has been degraded during the compounding and molding process and to define the actual concentration of **PI 2** in the matrix material, elemental analysis was made. It revealed a good accordance of calculated and found values for sulfur, suggesting that the actual concentration corresponds with the calculated one.

Table 2-5 Elemental analysis of compound materials

entry	PP+ 2.5 wt% PI 2				PP+ 5.0 wt% PI 2			
	%N	%C	%S	%H	%N	%C	%S	%H
experimental	<0.1	85.15	**0.26**	14.89	< 0.1	84.90	**0.52**	14.55
theoretical	-	-	**0.26**	-	-	-	**0.51**	-

2.3.4.3.2 ATR-IR

Regarding ATR-IR spectra (*cf*. Figure 2-8), a sharp peak can be seen at 667 cm^{-1}, indicative for the S-C stretch vibration. As this peak could be found in spectra of both plates, the presence of the active species on the surface of the plates could be confirmed.

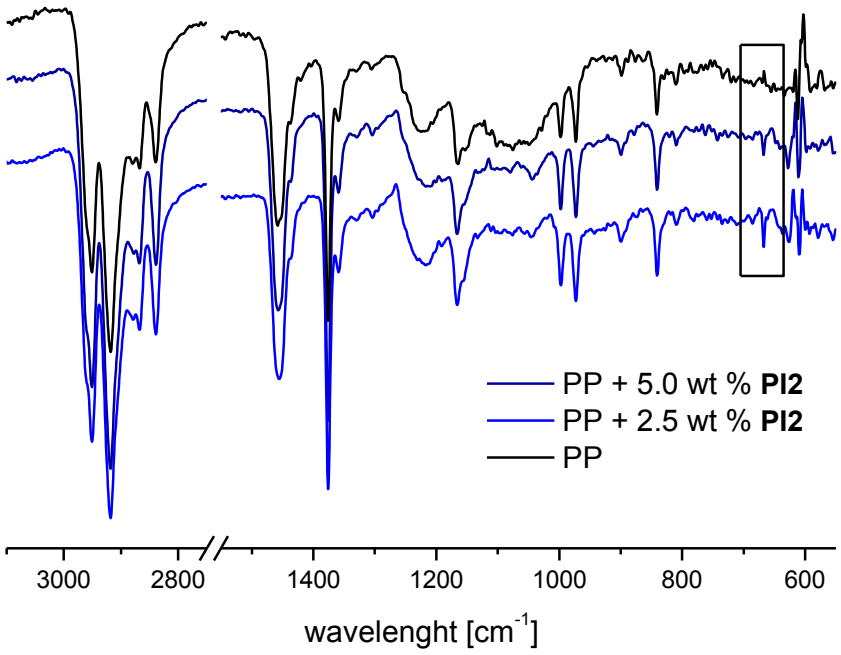


Figure 2-8 ATR-IR spectra of poly(propylene) and compounds

2.3.4.3.3 Zeta Potential Measurements

The ζ potential describes a surface charge that is build up in an aqueous solution, if functional groups of a hydrophilic surface dissociate or negatively charged ions on a hydrophobic surface adsorb. If the pH value of the surface is varied, the balance between dissociation and absorption is affected and thereby allows conclusion about the chemical behavior of the surface. It is determined by the mobility of charged particles in an electrical field.[106] The isoelectric point IEP, in that context, is the specific pH value with a ζ potential of zero.

In that definite case, it should be possible to detect positive charges on the surface of the plates if factually **PI 2** is present on the outer surface.

[106] source: Luxbacher, T. *Zetapotenzial als Indikator für Oberflächeneigenschaften*, Anton Paar, **2007**, http://www.laborpraxis.vogel.de/index.cfm?pid=4035&pk=105140&p=1, 16.10.2012

Pure **PI 2** exhibited a potential of ζ= + 40 mV at approximately pH= 6.0. This confirmed the positive charges which are possessed by the polymer. The measured value for the zeta-potential is in good accordance with literature values for comparable polymers.[107] The isoelectric point of **PI 2** lies at approximately pH= 8.0.

The finished plates were again subjected to zeta potential measurements. The starting IEP of the reference material poly(propylene) PP was determined at pH= 3.65. With an increasing content of **PI 2** in the compound, this IEP was shifted to higher values. For compound comprising 2.5 wt% of **PI 2** the IEP was detected at pH= 3.75 and at pH= 4.0 for the compound with 5.0 wt% of **PI 2**, respectively. That, once again, gave strong hint for positive charges on the surface of the compound plates.

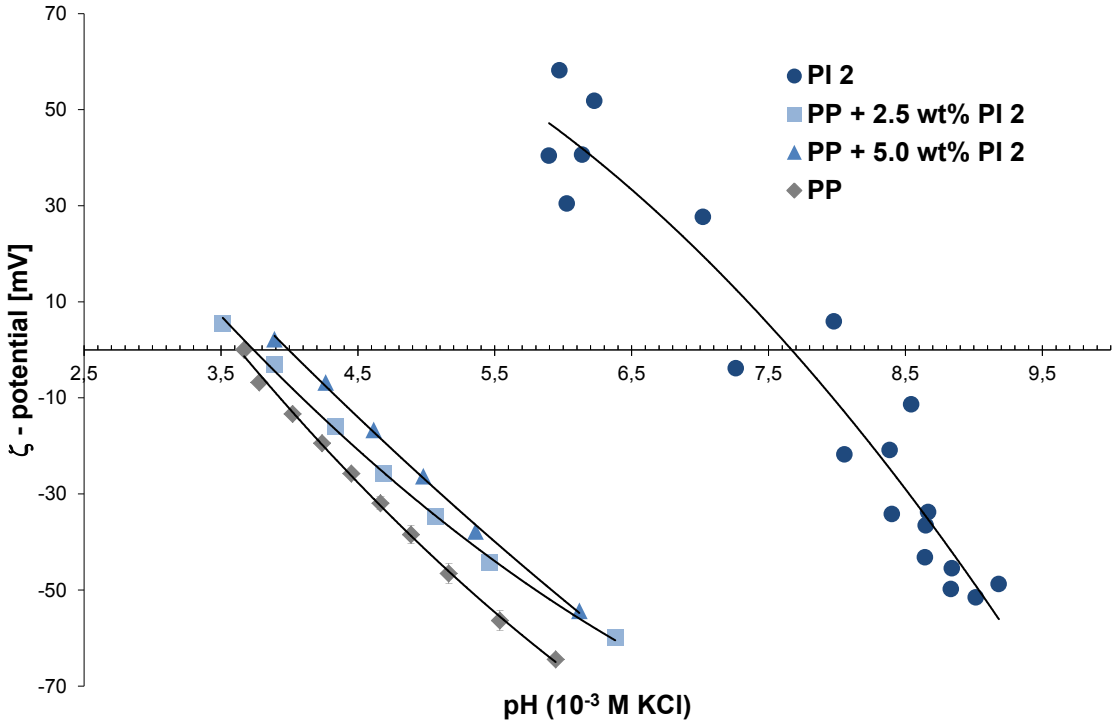


Figure 2-9 ζ – potential measurements of PI 2, PP and compounds

[107] [a] Elshafeey A. H.; Kamel, A. O.; Awad, G. A.S. *Colloids and Surfaces B: Biointerfaces* **2010**, *75*, 398-404. [b] Li, R.; Wei, L.; Hu, C.; Xu, C.; Wang, J. *J Phys Chem B* **2010**, *114*, 12449-12454.

2.3.4.3.4 Antibacterial Activity

Activity of compounds was determined in the same manner as described for pure polymers (*cf*. 2.3.3.4). Compound plates (4x4 cm) were covered with inoculation media, incubated and transferred in a nutrient solution. Dilutions from that were again incubated and colony forming units (CFU) are counted. The reduction of cells compared to unmodified polymer sample is stated. In that case, poly(propylene) plates were used as reference material.

What was striking was that cells on unmodified blank reference material were already reduced after 24 h for 1- 2 logarithmic values. As it is defined within the Japanese Industrial Standard, comparison of the cell concentration after 24 h on the reference plate is stated. Therefore, the activity of the compounds seems weaker. If the CFU numbers are compared with the actual starting cell concentration, a much higher reduction has happened. However, it was decided to stick to the JIS protocol.

For compounds, a dependence of amount of active material on the antimicrobial behavior was found. The compound containing 2.5 wt% of active polymer **PI 2** did not reveal antimicrobial activity. Against the Gram-positive bacteria *S. aureus* and *L. monocytogenes* the starting cell number was preserved, whereas against Gram-negative *E. coli* and *P. fluorescence* a reduction of 1.18 and 1.16 logarithmic values was determined after 24 h what would be called a bacteriostatic activity. However, compared to the reference material after 24 h, it appears as if CFUs have increased up to 1.29 logarithmic values as it is the case against *S. aureus* due to the strong reduction on PP itself.

As for the compound with the higher ratio of active polymer, a biocide activity against all four strains was found. The reduction was in the range of 3.15-3.20 logarithmic values for *S. aureus* and *E. coli* and slightly weaker against *L. monocytogenes* and *P. fluorescence* (2.56 and 2.16 logarithmic values) compared to the initial cell concentration. Nevertheless, the JIS´s demand, a reduction of CFUs in the range of 2 log levels, is not given, as the number of cells was reduced after 24 h by the reference material itself. Hence, a bacteriostatic activity was found according to the JIS demand with reductions ranging from 1.22-1.94 logarithmic values. A detailed chart with measured logarithmic CFU numbers after 24 h is provided in Table 2-6 (Δ refers to reduction of cell numbers compared to the cell number on PP after 24 h).

Table 2-6 CFUs after 24 h reduction in logarithmic values

entry	sample	*L. monocytogenes*		*S. aureus*		*E. coli*		*P. fluorescence*	
		24 h	Δ	24 h	Δ	24 h	Δ	24 h	Δ
1	PP	4.77	*-0.90*	4.59	*-1.27*	4.36	*-1.96*	5.00	*-0.94*
2	2.5 wt% PI 2	5.62	*+0.85*	5.88	*+1.29*	5.15	*+0.78*	4.78	*-0.22*
3	5.0 wt% PI 2	3.11	*-1.66*	2.65	*-1.94*	3.18	*-1.19*	3.78	*-1.22*

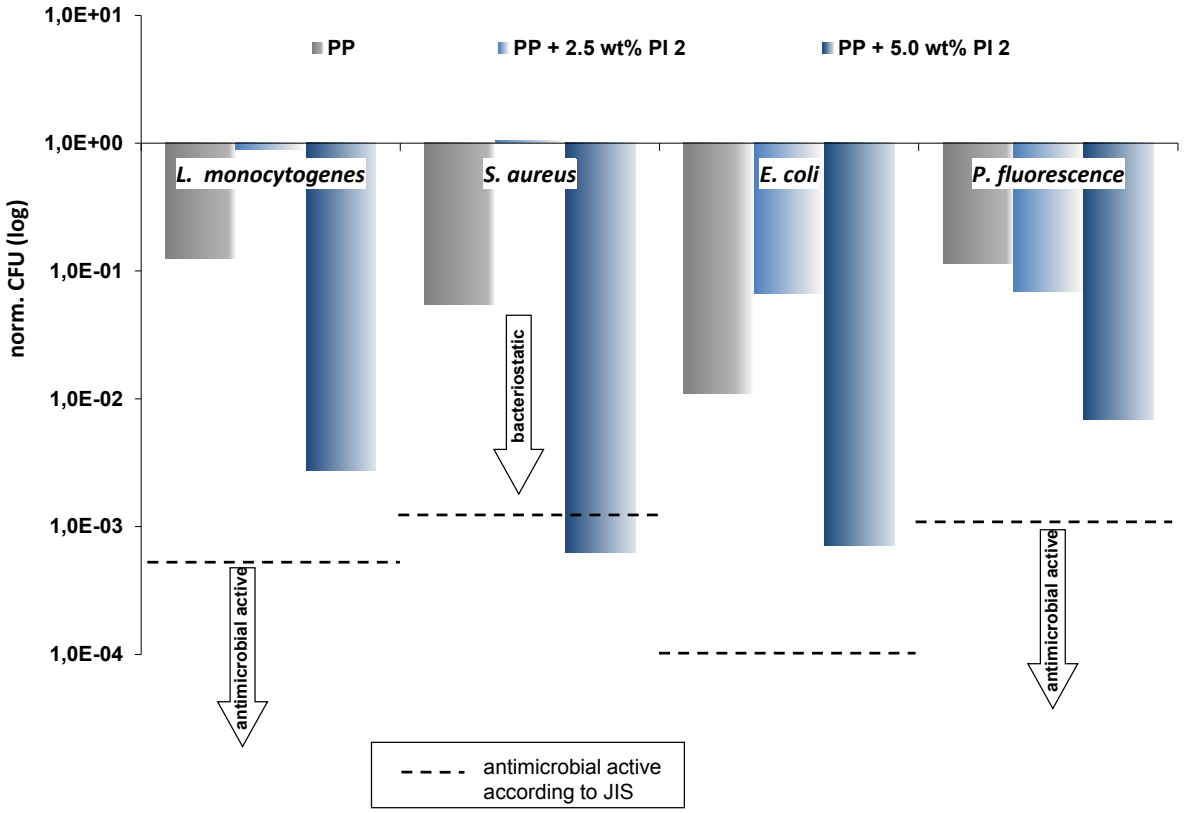


Figure 2-10 CFU reduction after 24 h on compound plates

Those results were not entirely satisfying, as the JIS´s demand for an antibacterial material could not be fulfilled. Yet, those results should be regarded conspicuously, as the strong

diminution of bacteria cells on the reference material is unexpected and might lead to a misinterpretation of data.

One other way that might lead to antibacterial surfaces is the implementation of higher amounts of the active polymer. As this would increase production costs disproportionally, it was refrained from further efforts in that direction.

However, the outcome of this development was taken as prove of principle, that it is possible to implement antibacterial activity on unsaturated double bonds via a radical thiol-ene reaction.

Additionally, a re-use of the antimicrobial polymer surface can be of interest, therefore investigations in that directions were conducted. Compound plates were subjected to additional tests in order to learn about the re-use potential of these materials. The plastic compounds, already used for antimicrobial tests on *E. coli*, were incubated in 70% ethanol for 5 min each and were subsequently treated in a similar manner as described (*cf*. 2.3.3.4). However, already the first test series using *P. fluorescens* as test bacteria strain showed a strong proliferation of cells on the modified surfaces. The cell number increased 2.14 logarithmic units on compound containing 2.5 wt% and 2.25 logarithmic units on the compound plate with 5.0 wt% of active polymer **PI 2**. However, the same rather unusual behavior of cells on the unmodified poly(propylene) was determined again, resulting in a reduction of cells in 0.92 logarithmic values. Yet, it could not be clarified, why the plates lost their antibacterial activity. Due to its polarity, ethanol 70% is not a suitable solvent for the polymer and should therefore not lead to a leaching of the active material if the compound plates are incubated. Another possible explanation could be that bacteria strains are bound quite tightly on the surfaces due to electrostatic interactions of the negatively charged bacteria cell wall and the positively charged antimicrobial polymer, making more intense rinsing processes to clean the surface from bacteria cell fragments, serving as a growing layer, necessary.

2.3.5 Summary and Outlook

With the illustrated studies, it was possible to show, that the functionalization of polyolefins with amine featuring thiols is possible by means of thiol-ene chemistry. Via further post-

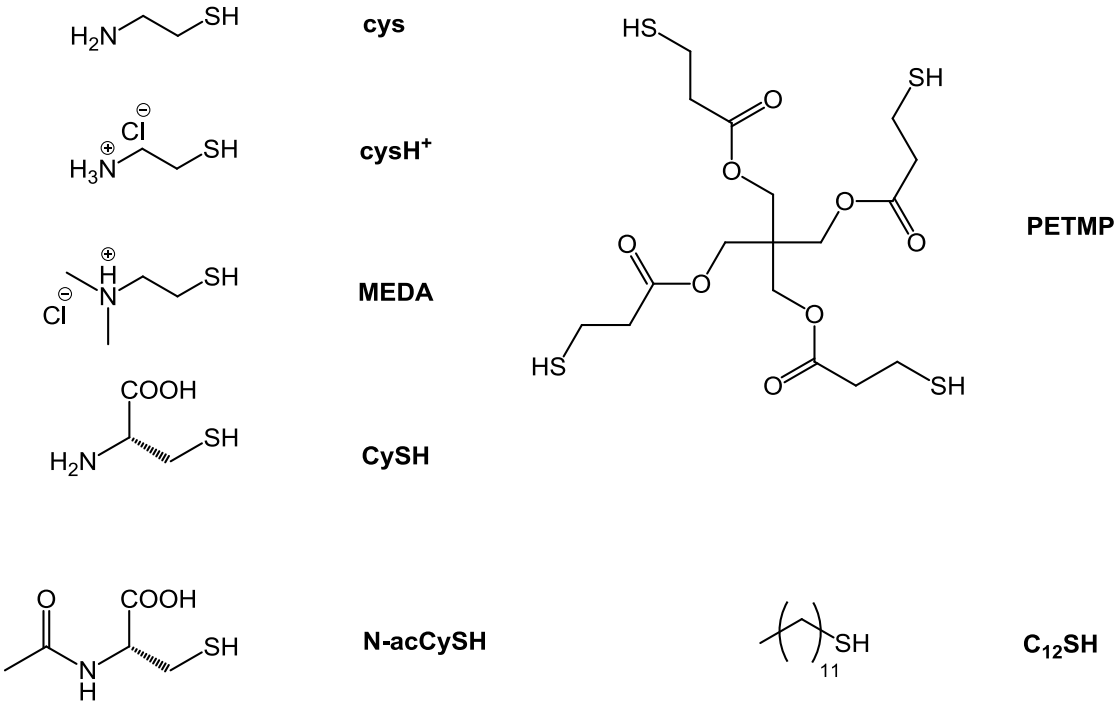
functionalization steps, the implementation of antibacterial activity is possible as well. The developed materials were suitable for compounding processes and the antibacterial activity was preserved within these.

However, the developed method still suffered from several drawbacks:

Due to the wet chemical synthesis and purification methods, high amounts of solvents are necessary, making the materials expensive and very unsatisfying from an ecological point of view. Therefore, keeping the obtained results in mind, the development of methods using less or environmentally benign solvents (e.g. water, ethanol) was aimed at. Additionally, two post-modification steps raise the required amounts of solvents even more. Therefore, the use of already quaternary ammonium salts is promoted.

2.4 Solvent-free Thiol-ene Reactions on Macromolecules

In first trials, several polyolefins with natural and industrial origin were kneaded in a Brabender® mixer by addition of diverse thiols and **AIBN** as radical initiator. It was assumed that within the kneading process, temperature should raise enough to activate the radical initiator without extra external heating. The polymers used were natural rubber (**NR**), styrene-butadiene rubber (**SBR**), 1,4- poly(butadiene) (**1,4-PB**) and poly(octene) (**PO**). Those polymers were chosen due to their easy accessibility on the market. On the other side, thiols used were mainly derivatives of the amino acid cysteine, namely cysteamine (**cys**), cysteamine hydrochloride (**cysH$^+$**), N, N- Dimethylcysteamine hydrochloride (**MEDA**), L-cysteine (**CySH**) and L-N-acetyl cysteine (**N-acCySH**). Additionally, pentaerythritol tetrakis(β-thiopropionate) (**PETMP**), known for its ability to crosslink polymers very easily and fast, and dodecanthiol (**C_{12}SH**) as aliphatic thiol were used in some cases as well.



Scheme 2-4 Thiols used for thiol-ene reaction

Generally procedure for trials was as follows: 50 g polymer was masticated for seven minutes at a rotation speed of 50 r min^{-1}. After that time, thiol (1 g) and **AIBN** (0.5 g) (premixed) were

added at once and the whole mass kneaded for further 7-12 min, depending on the homogenization speed. After that, the polymer was removed from the mixer and NMR were recorded of soluble parts in $CDCl_3$ (filtration through glass wool). If a change in the spectra was detectable, a part was subjected to Soxhlet extraction, using methanol as solvent suitable for thioamines.

Table 2-7 Conversion of unsaturated polymers with thiols in the Brabender®

entry	thiol	NR	SBR	1,4-PB	PO
1	cys	no conversion [b,c]	not soluble [b,c]	no conversion	-
2	$cysH^+$	no conversion [b,c]	no conversion [c]	not soluble	-
3	MEDA	no conversion [a,b,c]	no conversion	no conversion [b]	no conversion
4	CySH	no conversion [b]	-	-	-
5	N-acCySH	no conversion [b]	-	not soluble	-
6	$C_{12}SH$	no conversion	-	no conversion	-
7	PETMP	not soluble	not soluble	not soluble	-

[a] amount of thiol increased in 1 g steps from 1-5 g,; [b] experiment retried without **AIBN**, [c] thiol additionally added in a solution of CH_2Cl_2/MeOH

As can be seen clearly from Table 2-7, solvent-free conversion of double bonds by mere kneading did not lead to the desired aim. In the case of an insoluble polymer, it was further subjected to ATR-IR measurements. Yet, no prove for network formation via thiol-ene reaction could be found and it can be assumed, that crosslinking resulted from oxidative reactions, accelerated by the relatively high amount of radical initiator added.[108] A procedure of Passaglia and Donati[109] was adopted, stirring **SBR** at high temperatures (130°C) in toluene, as this solvent is component of **SBR** oil, adding either **cys** or **MEDA**, yet no reaction occurred. Therefore it was decided to change the system to an UV light induced radical reaction. From that, higher conversion rates with shorter reaction times were expected.

[108] Tillet, G.; Boutevin, B.; Ameduri, B. *Progress in Polymer Science* **2011**, *36*, 191-217. [b] Tobolsky, A. V.; Mercurio, A. *J Am Chem Soc* **1959**, *81*, 5539-5540.

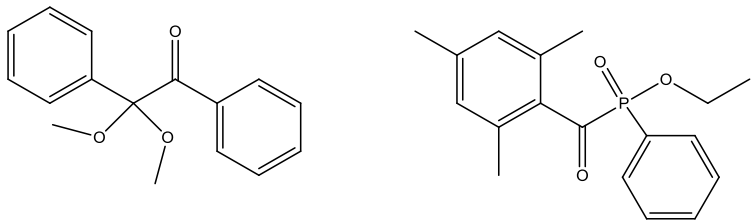
[109] Passaglia, E.; Donati, F. *Polymer* **2007**, *48*, 35-42.

2.5 UV-Light induced Thiol-ene Reactions

2.5.1 Motivation

As functionalization of unsaturated polyolefins without solvent was not possible with thermal radical initiators, it was decided to switch to a photo- chemically activated system. From that, higher conversion rates and faster reaction times were expected.

The initiators used were either 2,2- Dimethoxy-2-phenylacetophenone (Irgacure 651®, **DMPA**) or Ethyl-(2,4,6- trimethylbenzoyl)phenylphosphinate (Lucirin® TPO-L, **TPO-L**).

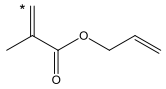
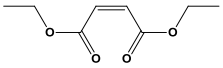
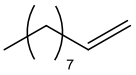


Scheme 2-5 Irgacure® 651 (left) and Lucirin® TPO-L (right)

2.5.2 UV-Light induced Thiol-ene Reactions on Small Molecules

In order to find optimized reaction conditions small molecules were - comparable to 2.1 - subjected to model reactions. It was found that reaction times of 15 min led to good conversion rates. The thiol used was **MEDA**, as the aim was the direct implementation of a potent biocide reagent. For the sake of comparison, **C_{12}SH** was reacted, too. Reactions were conducted in solvents (CH_2Cl_2/ MeOH) as the salt like structure of **MEDA** did not allow a neat reaction. Initiator concentration was 0.5 eq in that case.

Table 2-8 Thiol-ene reaction of thiols with model small molecules; percent conversion rates of double bonds are depicted

entry	-en	MEDA	$C_{12}SH$
1		15 %	>99 %
2		51 % [*49* %][a]	33 % [*67* %][a]
3		>99 %	>99 %

* only methacrylate double bond considered; [a] rearrangement from *cis* to *trans* detected [*trans ratio*]

Table 2-8 shows conversion rates as calculated from conversion of the corresponding double bond peaks in ^{1}H NMR after UV illumination under thiol-ene reaction conditions. Although it can be assumed that the reaction occurred preliminary is the desired thiol-ene conversion, it cannot be excluded, that side reactions caused by the harsh radical conditions occurred. In case of dimethyl maleate, isomerization to the dimethyl fumarate was monitored, explainable by the observations made by Metzger.[18]

2.5.3 UV-Light Induced Thiol-ene Reaction on Macromolecules

Again, styrene-butadiene-rubber was chosen as first test sample. Probes were diluted in $CHCl_3$ and reacted with **cys** (5 eq with regard to vinylic double bonds- *cf*. 2.2) and **DMPA** (0.5 eq). The reaction was illuminated at 9400 mW cm^{-2} for 15 min. However, the polymer cross-linked, resulting in insoluble parts. As it was assumed, that again oxygen influence led to this result, the reaction was repeated in degassed solvents under nitrogen flushing, shorter illumination times (1- 5min) and reduced amount of initiator (0.05 eq), yet leading to the very same result. Therefore, investigations in that direction were not further conducted.

In a next step, poly(isoprene) **PI** was used to perform the reaction neat. The polymer (100 mg) and 0.5 eq **cys** were mixed with 0.01 eq **DMPA** and stirred with a magnetic bar. Again the probes were illuminated (10 min, 8300 mW cm^{-2}) without addition of solvent. This set-up imitated the "brabender" trials in a smaller scale under UV-illumination. The probes were purified from unreacted thiol by precipitation in methanol. ^{1}H NMR analysis revealed a reduction of approximately 3- 4 % as calculated from integration. New peaks in the region of

2.80 ppm as well as 2.58-2.46 ppm, comparable to the peaks assigned for **PI 1**, could be detected. Yet, a relatively strong broadening of the methylene peak at 0.94 ppm might be indicative for intramolecular reactions as described.[40]

This reaction set-up was extended to **Lithene AL**, a short chain poly(butadiene), which is a product of SYNTHOMER Limited, United Kingdom, featuring a high number of vinylic double bonds.[110] Due to that and the low viscosity of this polymer, good conversions were expected. However, integration of the corresponding peaks in ^{1}H NMR revealed comparable conversions of double bonds as obtained for **PI 1**. Increasing the amount of **cys** to 0.5 eq or the amount of **DMPA** to 0.05 eq did not raise the conversion rate. Nevertheless, it was possible to perform this reaction solvent-free. However, two reasons made this accomplishment not entirely satisfying: first, as shown in chapter 2.3.3.4, the primary amine alone will not exhibit antibacterial activity; therefore an additional quaternization step would be necessary. Second, the degree of functionalization might be sufficient for an antibacterial activity in its pure form, but will most probably be inadequate if compounded. Therefore, hydrochloride salts of the respective thiols were used.

Solvent-free test trials were conducted using **MEDA** (0.1 eq) and **DMPA** (0.01 mol%, equates to 5 wt%), though not leading to a reaction. Alternatively, **MEDA** was exchanged with **cysH**$^{+}$, which was neither converted.

As solvent free reactions implementing charged thiols were not possible at that point, it was decided to perform the synthesis wet-chemically using **Lithene AL** and **MEDA**.

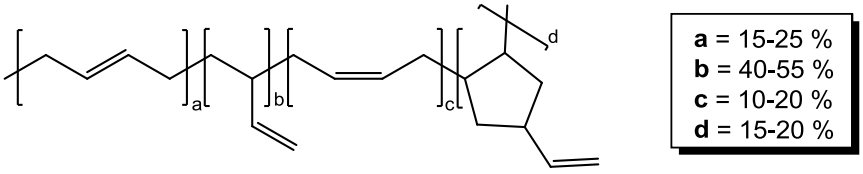
[110] source:

http://www.synthomer.com/pkt/pdf_3.php?ProdId=2073&TdsId=12073&ProdBez=LITHENE%20AL; 22.10.2012

2.5.3.1 **MEDA**- FUNCTIONALIZED LITHENE

MEDA as active antibacterial side group is of interest, as water-soluble copolymers based on methacrylate featuring comparable protonated primary, tertiary of quaternary ammonium functionalities, exhibited high antimicrobial activity. [111]

Lithene AL exhibits the following microstructure as declared by the producer (40-55% 1,2; 15-25% *trans* 1,4; 10-20% *cis* 1,4; 15-20% cyclic structures), which was in good accordance with NMR data: 61.7±2% vinyl double bonds (peaks at 5.80 ppm, 5.58 ppm, 4.96 ppm) and 38.1±2% are assigned to internal double bonds.[45] A distinction between *cis* and *trans* ratio was not performed in that case due to a strong overlapping of the corresponding peaks at 5.42- 5.38 ppm. The origin of peaks at 7.18 and 7.26 ppm- partly hidden by the residual solvent peak- was to some extend unexplainable in a first attempt, but could be traced back to the preparation via telomerization in toluene with an organo-lithium initiator.[112] The peak at 7.18 ppm (*o*, *p* protons in toluene) was subsequently used as immune standard with an integral of 3. From gel permeation chromatography (GPC) against poly(styrene) standards in THF a number molecular weight M_n of 1700 g mol^{-1} and a polydispersity index (PDI) of 2.1 was found, again confirming the oligomeric character of this product.



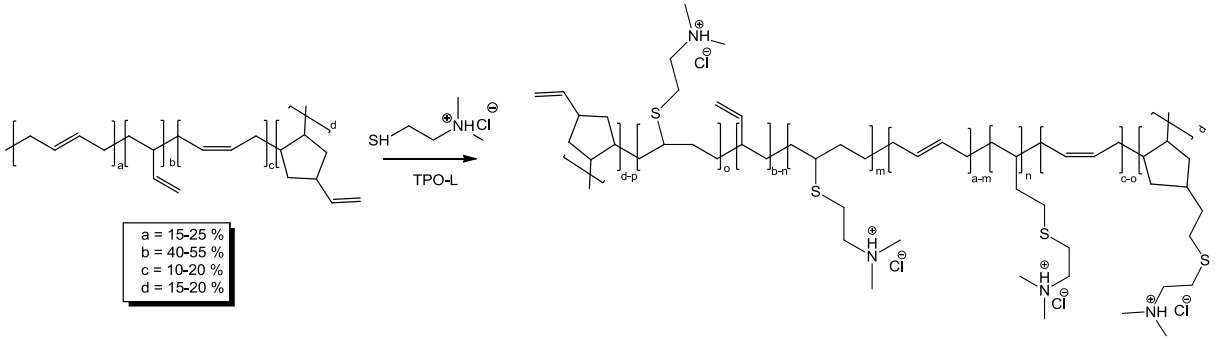
Scheme 2-6 Structure of Lithene AL as proposed by the producer

Lithene AL was diluted in a minimal amount of CH_2Cl_2 to solubilize. **MEDA** (0.2 eq) was prepared separately and diluted in MeOH. For these conversions, photo initiator **TPO-L** was used in 2 wt% calculated on the entire mass and added to the polymer solution. Separately prepared fractions were combined and sufficient amounts of CH_2Cl_2 were added to ensure

[111] Palermo, E. F.; Kuroda, K. *Biomacromolecules* **2009**, *10*, 1416-1428.

[112] Kume, S.; Saka, H.; Takahashi, A.; Nishikawa, G.; Hatano, M.; Kambara, S. *Die Makromolekulare Chemie* **1966**, *98*, 109-119.

persistent dissolution within the illumination time (no precaution against vaporization of solvent were conducted, reactions were carried out under an ambient atmosphere). Illumination time was optimized with 15 min; illumination intensity was between 4500-6500 mW cm^{-2}. The polymer was purified by precipitation in *n*-pentane, followed by careful resolution in CH_2Cl_2 and filtration. ^1H NMR as well as $^{13}C\{^1H\}$ NMR spectra of the modified oligomer were recorded, revealing reduction of the double bond peaks and simultaneously occurrence of new peaks, indicating a successful conversion. Overall, double bond peaks were reduced about 20%, whereas vinylic signals sustain the strongest diminution. Four broad singlets (3.20 ppm, 7.34 H; 3.00 ppm, 5.74 H; 2.86 ppm, 20.60 H; 2.59 ppm, 8.99 H) arise from the **MEDA** functionalization. From $^{13}C\{^1H\}$ NMR similar conclusions could be drawn. Double bond peaks in the downfield region at 142- 112 ppm are assignable to the vinylic and internal olefinic carbons as well as the toluene moieties. Peaks at 57.3 ppm and a sharp peak at 42.8 ppm are indicative for carbons surrounding a protonated amine (CH_2 and CH_3 respectively).



Scheme 2-7 MEDA- functionalized Lithene (PB 1)

Elemental analysis revealed a nitrogen content of 3.01% and a sulfur content of 6.90%, respectively. This suggests a functionalization of approximately every fifth repeating unit, what is in good accordance with the employed amount of thiol and the calculated conversion rate from ^1H NMR.

Characterization was completed by gel permeation chromatography, revealing a PDI of 2.2 and a M_n of 2300 g mol^{-1} against a poly(styrene) standard in $CHCl_3$:Et_3N:isopropanol = 94:4:2. Higher amounts of thiol did not lead to further conversion, but to mere dimerization of the thiol to the disulfide. The same counts for higher amounts of initiator or longer illumination times.

So, the thiol-ene functionalization via UV light of a high vinyl polymer in solution is possible. The obtained product is of interest, as only one wet-chemical step is possible for the synthesis, which is an improvement compared to the aforementioned results (*cf*. 2.3.3), as no additional quaternization step was necessary. However, it has to be kept in mind that the amine functionality is only protonated and not irreversible alkylated.

From these results several statements were made:

- UV light initiation allows shorter reaction times for thiol-ene reactions (factor 40)
- amine implementation to commercially available polymers such as **SBR** is not possible by means of thiol-ene reaction with the at hand ratio of internal: vinylic double bonds
- solvent free addition of primary amine featuring thiols is possible to high vinyl content polymers
- solvent free addition of hydrochloric salts of the respective thiols is not possible

So, for further research a compromise had to be found. Solvent-free functionalization is possible to a small extend, however, only uncharged mercaptamines and derivatives are capable to perform this reaction. Alternatively, protonated and therewith potentially biocide thioamines require at least a minor amount of solvent to ensure adequate mixing of all components. However, as this step still is environmentally more justifiable than an additional wet-chemical reaction step, as it would be required by a subsequent quaternization reaction, it was decided to track that pathway. Besides, the idea arose to investigate not solely protonated tertiary thioamines, but also alkylated structures. There, increasing alkyl chains should lead to better solubility of the thiol in the polymer. Efforts going in this direction are described in the subsequent chapter. In that context, a possible influence of the amine on the oxidation behavior of the thiol species is of certain interest.

2.6 Development of Long Chain Mercaptamines for Solvent-Free Thiol-ene Reaction

2.6.1 Motivation

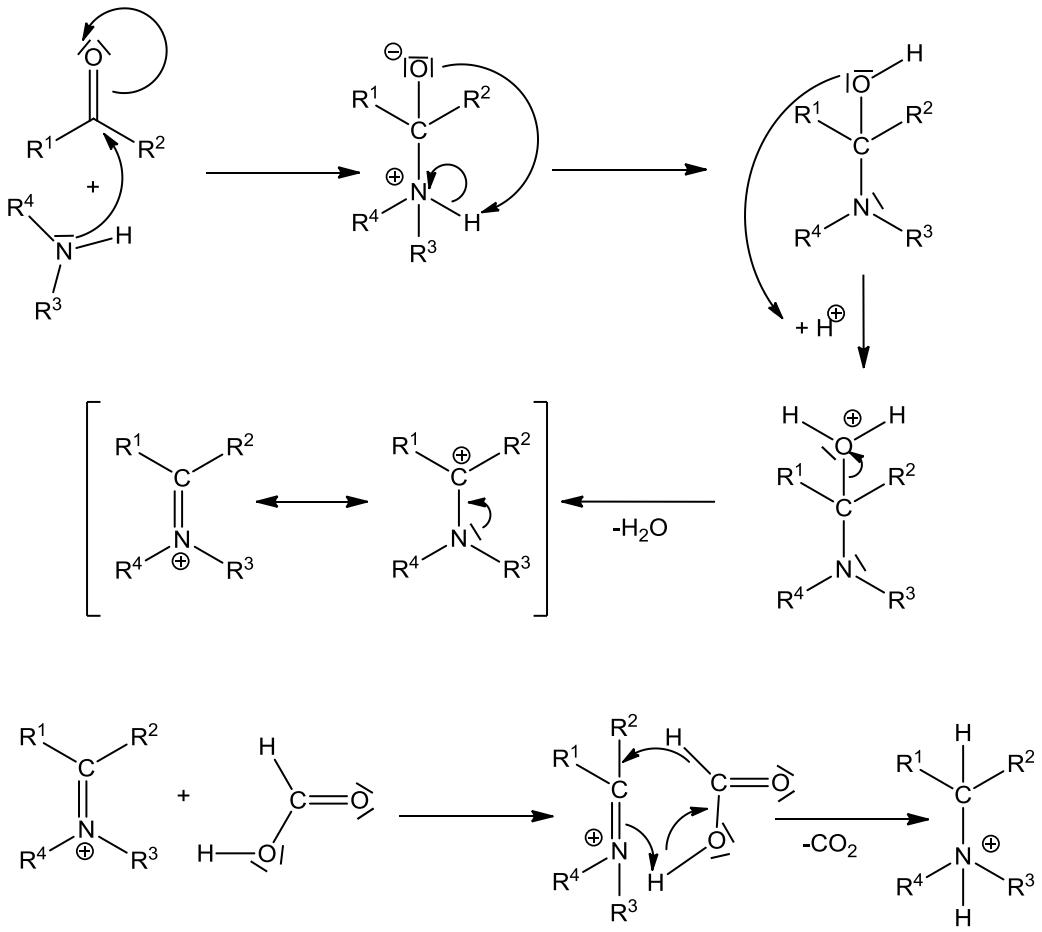
As described, the direct implementation of a protonated aminothiol to an unsaturated polymer via a thiol-ene reaction is not possible. Therefore- and as no suitable substrates are commercially available- it was decided to synthesize thioamines with quaternary ammonium functionalities, which can, in the optimum case, be reacted with an appropriate polyolefin without addition of solvent.

2.6.2 Synthesis of Thiols from Cystamine Dihydrochloride

2.6.2.1 The Leuckart- Wallach Reaction

As solvent-free functionalization of polyolefins was not possible with salts of mercaptamine and its derivatives, it was decided to synthesize a series of N, N-substituted cysteamines with preferentially long-chain alkyl side groups. It was decided to apply Leuckart-Wallach type reactions, which allow the reductive alkylation of ammonia, primary or secondary amines by aldehydes and ketones using formic acid or derivatives.[113] In a first step, one molecule of amine adds to the carbonyl group with transferring one proton from the amine to the oxygen atom. Formic acid then protonates this intermediate, leading to the elimination of one molecule of water and the formation of a resonance- stabilized carbenium- immonium ion. The subsequent reaction with formic acid via a cyclic intermediate state leads to the oxidation of the formic acid carbon and the formation of carbon dioxide, forcing the reaction to the product side.

[113] [a] Leuckart, R. *Ber Dtsch Chem Ges* **1885**, *18*, 2341-2344. [b] Wallach, O. *Justus Liebigs Annalen der Chemie* **1892**, *272*, 99-122. [c] Moore, M. L. *Organic Reactions* **1949**, *5*, 301-330.



Scheme 2-8 Reaction mechanism of the Leuckart-Wallach Reaction[114]

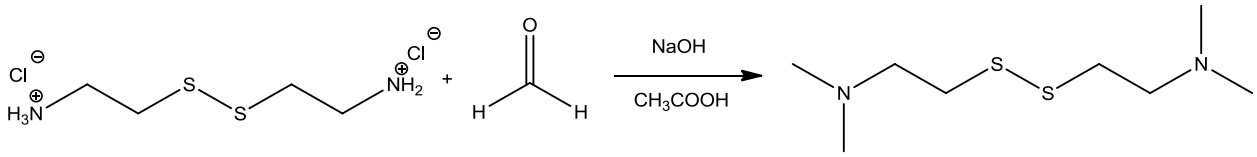
If the simplest aldehyde, formaldehyde, is used for the conversion, it is addressed as Eschweiler-Clarke reaction.[115]

[114] source: http://de.wikipedia.org/wiki/Leuckart-Wallach-Reaktion; 24th October 2012

[115] [a] Eschweiler, W. *Ber Dtsch Chem Ges* **1905**, *38*, 880. [b] Clarke, H. T.; Gillespie, H. B.; Weisshaus, S. Z. *J Am Chem Soc* **1933**, *55*, 4571-4587.

2.6.2.1.1 Synthesis of **Me_2CySS**

In a first attempt, a protocol by Thebault *et al*.[116] was adopted to react cystamine dihydrochloride to the corresponding tetra-methylated derivative. This reaction proceeded smoothly, using sodium hydroxide as base to deprotonate the amines. Then the aforementioned Eschweiler-Clarke reaction using formaldehyde (4 eq to ensure reaction to the tertiary amines) and formic acid (huge excess) was conducted. Upon addition of potassium hydroxide, extraction of the aqueous phase with diethyl ether was possible, yielding a pale yellow liquid (65% yield).

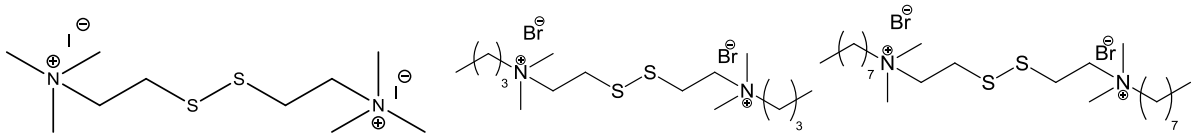


Scheme 2-9 Conversion of cystamine dihydrochloride, following an Eschweiler-Clarke reaction (Me_2CySS (1))

Characterization was done via ^{1}H NMR spectroscopy and was in accordance with literature data.[117]

2.6.2.1.2 Quaternization of **Me_2CySS**

The obtained compound was further reacted with different alkyl halogenides with increasing chain length. The following derivatives featuring quaternary ammonium functionalities could be obtained:



Scheme 2-10 Derivatives of N,N,N´,N´- Tetramethylcystamine with quaternized amine functionalities (left: Me_2CySS^+ (2); middle: Me_2CySS^+ (3); right: Me_2CySS^+ (4))

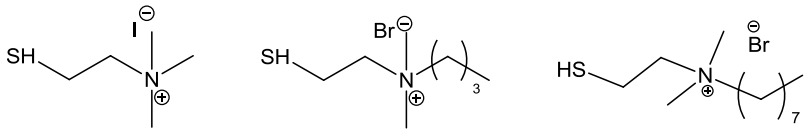
[116] Thebault, P.; Taffin de Givenchy, E.; Guittard, F.; Guimon, C.; Géribaldi, S. *Thin Solid Films* **2008**, *516*, 1765-1772.

[117] Zhang, J.; Jing, B.; Janout, V.; Regen, S. L. *Langmuir*, **2007**, *23*, 8709- 8712.

The reactions were conducted in refluxing acetonitrile overnight, resulting in a precipitate which was filtered and washed with *n*-pentane. Due to the bad solubility of the methyl and butyl derivatives, the corresponding NMR spectra were recorded in D_2O and MeOD, respectively. Apparently, the quaternization of the amine was achieved, refering to the remarkable shift of peaks in the ^1H NMR. In all three cases, new peaks at 3.68-3.77 ppm arose, allocated to the CH_2 neighboring the N^+ groups. Integrals of all peaks were in aggreement with the proposed structures. Of special interest is the peak at 29.7-30.0 ppm in $^{13}C\{^1H\}$ NMR spectra, appearing in all strucures, indicative for the CH_2 adjacent to the disulfide functionality as this peak is used to track the conversion to the free thiol as described subsequently.

2.6.2.1.3 Reduction of **Me_2CySS^+**

Adopting a procedure of Robinson *et al*.[118] using zinc powder and acetic acid for the *in-situ* generation of hydrogen gas as reducing agent, those species were subsequently reduced to the free thiol. The reaction was performed in ethanol at room temperature over 7- 14 h. Solids were removed by filtration and the residue dried, leading to the following products:



Scheme 2-11 Reduced derivatives of N,N,N´,N´- Tetramethylcysteamine with quaternized amine functionalities (left: Me_2CySH^+ (5), middle: Me_2CySH^+ (6), right: Me_2CySH^+ (7))

^1H NMR spectra were recorded again, Figure 2-11 shows exemplarily the conversion of **Me_2CySS(1)** to **Me_2CySS^+(3)** and **Me_2CySH^+(6)**. Yields were not isolated, conversion was approved via NMR.

[118] Robinson, C.; Hartmann, R. F.; Rose, S. D. *Bioorg Chem* **2008**, *36*, 265-270.

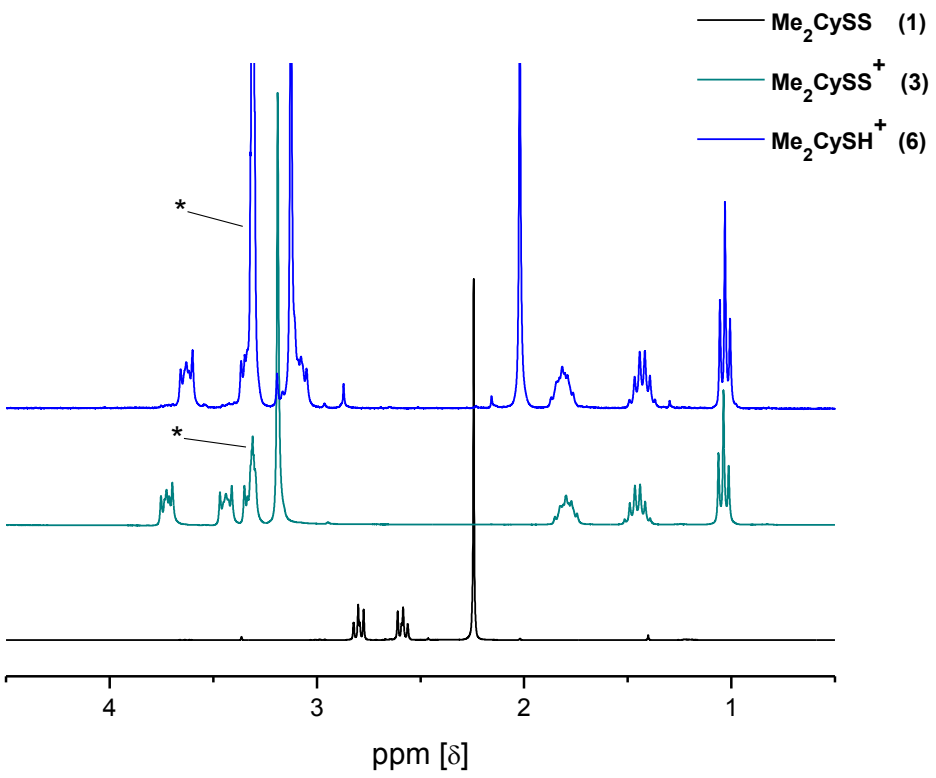


Figure 2-11 ^{1}H NMR spectra of Me_2CySS (1) in $CDCl_3$, Me_2CySS^+ (3) and Me_2CySH^+ (6) in MeOD (solvent residual peak marked with *)

The singlet peak at 2.02 ppm in the spectra of **Me_2CySH^+** (**6**) results from the formed zinc acetate, which remained in the product after filtration. Of special interest is, of course, the successful conversion to the free thiol species, necessary for the subsequent thiol-ene reaction. Yet as the differences in the shift of methylene groups adjacent to –SH groups or S-S is not remarkably large in ^{1}H NMR (3.17 ppm for the oxidized form, 3.02 ppm, respectively, for the reduced form), $^{13}C\{^{1}H\}$ NMR were recorded to shed light on this issue. There, a shift of approximately 15 ppm is indicative for the reduction from the disulfide to the thiol. Consideration of spectra of the pure compounds cysteamine hydrochloride and cystamine dihydrochloride as well as literature data confirmed that assumption.[119] In case of cysteamine hydrochloride (**$cysH^+$**) and the respective oxidized form (**$cysSS^+$**), the peak of the β-CH_2 atom lies at 22.4 ppm for the former one, whereas the signal arising from the same atom shifts to 39.3 ppm for the oxidized dimeric form. There, it has to be noted, that a

[119] Guo, W.; Pleasants, J.; Rabenstein, D. L. *J Org Chem* **1990**, *55*, 373-376.

protonation of the nitrogen atom does not influence this shift remarkably, aliphatic substitution further reduces this effect.

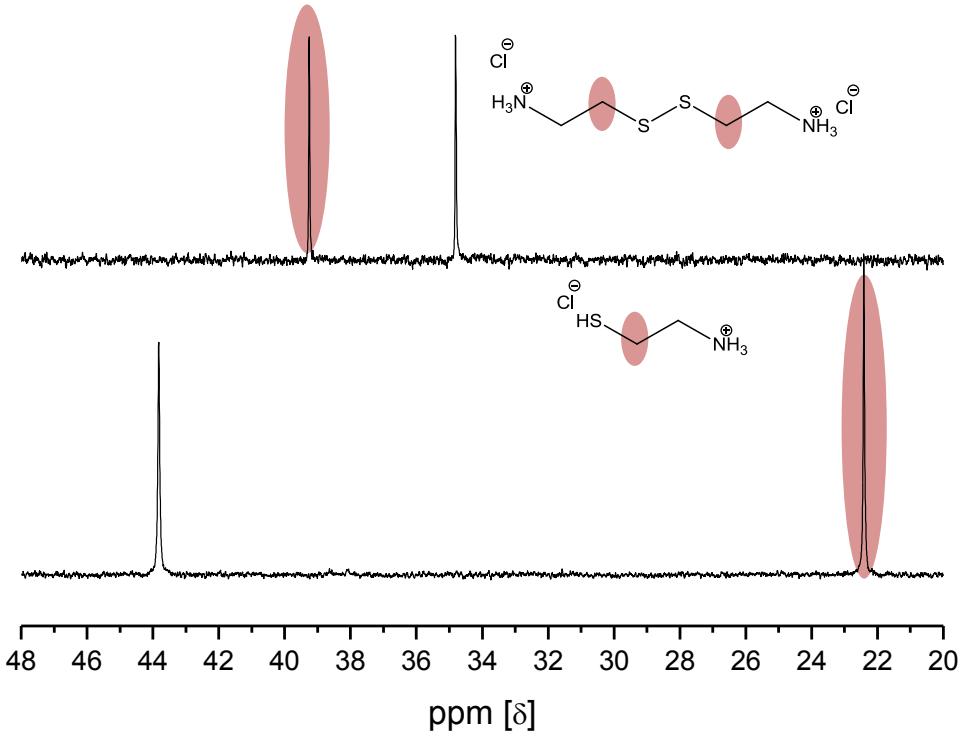


Figure 2-12 $^{13}C\{^{1}H\}$ NMR spectra of cysSH^{+} and cysSS^{+} (spectra recorded in D_2O)

As for the reaction of **Me_2CySS^{+}** to **Me_2CySH^{+}**, the described shift is reduced to 10.6 ppm. The oxidized form **Me_2CySS^{+}** exhibits a shift of the β-CH_2 to 31.7 ppm, whereas the same atom of the reduced form can be assigned to the peak at 21.1 ppm. This shift was considered as strong hint for a successful reduction.

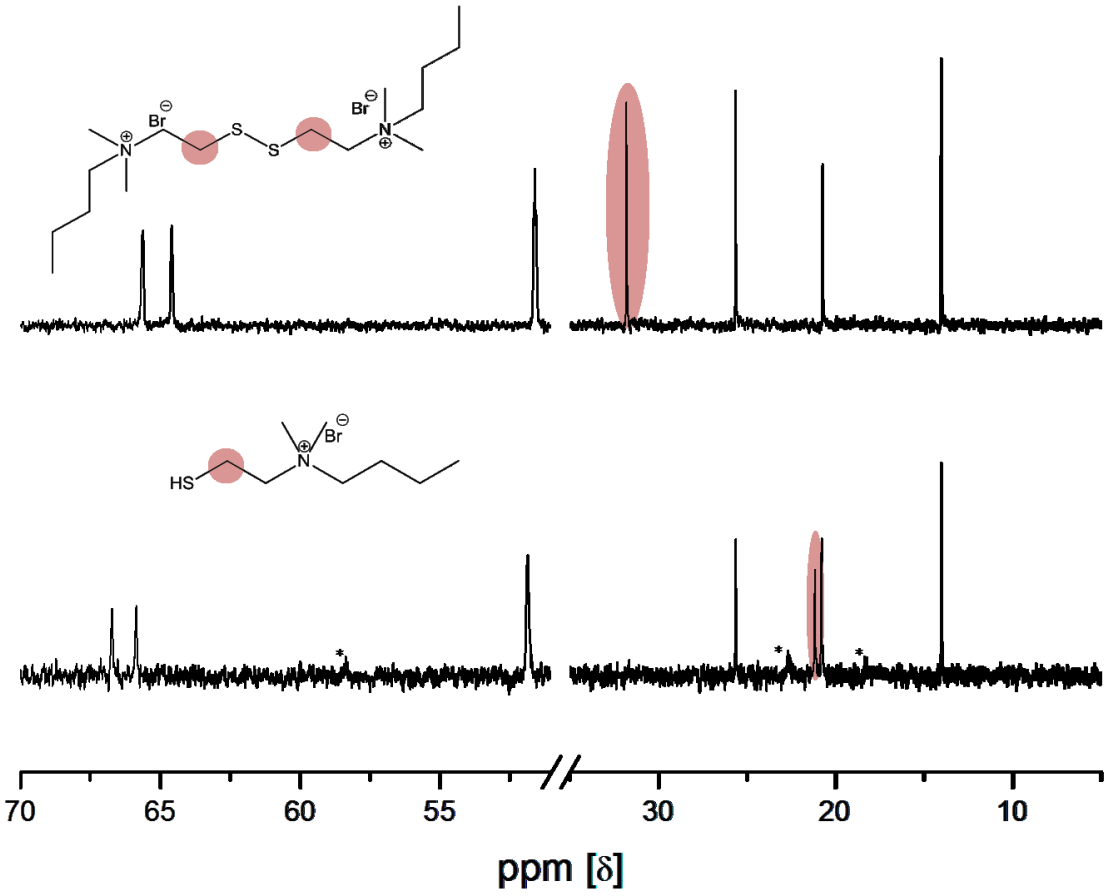


Figure 2-13 $^{13}C\{^{1}H\}$ NMR of Me_2CySS^+ (3) (up) and Me_2CySH^+ (6) (down) in MeOD; solvent peaks are reduced for clarity and denoted with *

2.6.2.2 Synthesis of higher Analogues

However, it was also investigated, if the used protocol for cystamine functionalization could also be extended to further aldehydes to equip the thiols not only with one aliphatic side chain, but with at least two of them. The aim was to extend the methylation of the nitrogen to a higher number of carbons. A review article by Moore[113c] describes the conversion of a wide spread number of aldehydes and ketones, also featuring additional functionalities or aromatic rings. However, the amines used in the cases described are composed of relatively simple structures, generally ammonia, ammonium formate or other salts as well as (substituted) formamides but also aromatic amines (benzylamine) are used. Generally, either

higher analogues of formaldehyde are used with simple amines or vice versa, formaldehyde is reacted with more sophisticated amine derivatives.[120]

The reaction was conducted under retention of the used conditions, using cystamine dihydrochloride and a 10 fold excess of *iso*-valeraldehyde in formic acid as solvent.[121] The evolvement of CO_2 was monitored by dissipation of the gas in a beaker with water and the development of bubbles. At 90°C, bubbles evolved for 20 min, then the bubbling ceased and the reaction was further stirred for 2 hours without additional heating. The reaction mixture was extracted with diethyl ether, followed by basic extraction and repeated extraction with diethyl ether. The crude product **7** was investigated with NMR spectroscopy, revealing one major product and negligible amounts of one or more side products.

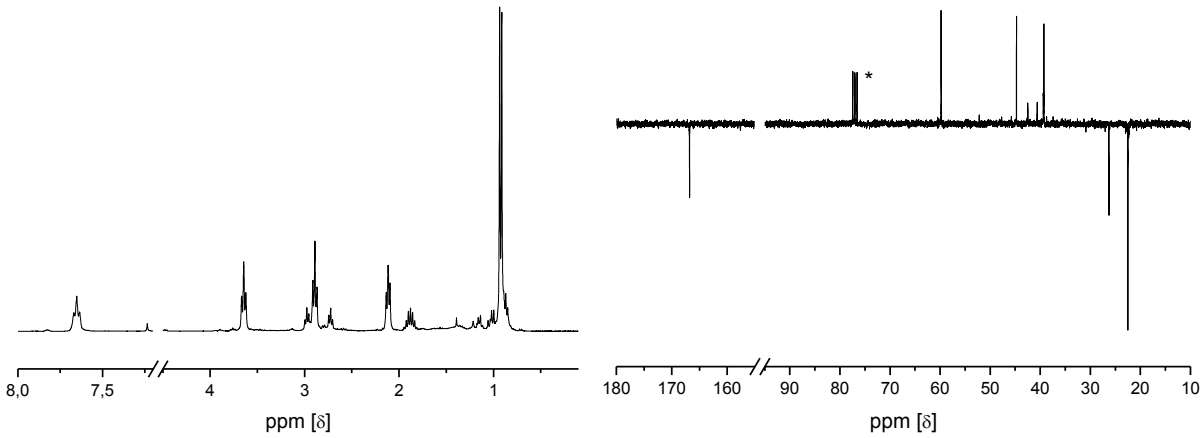
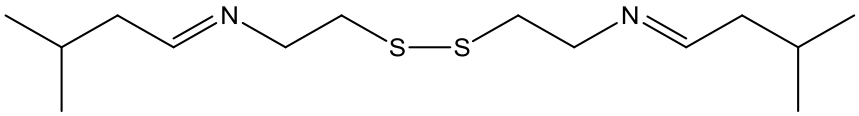


Figure 2-14 ^{1}H and ^{13}C APT-NMR spectra of crude product 7 (solvent residual peak marked with *)

Those spectra, especially the very pronounced peak at 7.65 ppm in ^{1}H NMR and at 166.8 ppm in ^{13}C APT spectra, respectively, lead to the assumption, that an unsaturated species was formed during the conversion. From the ratio of integrals, di-substitution could be excluded and the following structure is proposed:

[120] [a] Baltzly, R.; Buck, J. S. *J Am Chem Soc* **1940**, *62*, 161-164. [b] Baltzly, R.; Buck, J. S. *J Am Chem Soc* **1941**, *63*, 1964-1966. [c] Baltzly, R.; Buck, J. S. *J Am Chem Soc* **1942**, *64*, 2263-2264.

[121] Wallach, O. *Justus Liebigs Annalen der Chemie* **1905**, *343*, 54-74.



Scheme 2-12 Proposed structure of 7

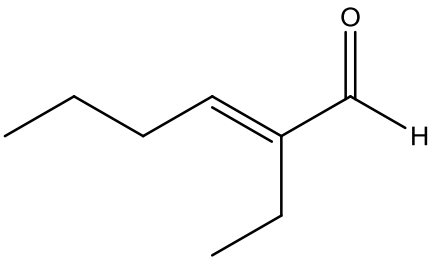
The formation of a structure as depicted is very feasible, caused by an incomplete conversion. It can be assumed that the reduction force of formic acid is not sufficient in that case, as longer reaction times or higher temperatures did not improve the result and the conversion could not be shifted to the desired product.

In a further step, the same reactants were used for a conversion following Forsee and Pollard,[122] using hydrogen as the reducing agent, comparable to the reaction described in 2.6.2.1.3. Although the conversion of secondary amines is described, it was hoped to obtain the desired products or at least the mono-substituted derivative. Additionally, it was assumed, that the reduction of the disulfide to the free thiol is possible under the particular conditions. However, the reaction with acetaldehyde did not lead to any isolable product, whereas from the reaction with *iso*-valeraldehyde the corresponding alcohol was obtained. So, in that case, the reduction of the aldehyde to the alcohol was faster than the alkylation of the amine.

Alternatively, work by Giovenzana *et al*.[123] describes the formation of secondary and tertiary amines by the reaction of primary amines with aldehydes in 5% KOH solution in water and zinc dust. This was a promising approach, as the base should deprotonate the cystamine dihydrochloride, facilitating the subsequent alkylation and the oxidation of zinc in water is slow enough to prevent mere reduction of the aldehyde. Following this procedure, an aldol addition followed by dehydration was observed instead of the desired alkylation, leading to the following product, as obtained from ^{1}H NMR spectra. Characteristic peaks at 9.36 ppm (aldehyde) and 6.42 ppm (double bond) led to the proposed structure, which is the aldol condensation product of butanal.

[122] Forsee, W. T.; Pollard, C. B. *J Am Chem Soc* **1935**, *57*, 1788-1789.

[123] Giovenzana, G. B.; Imperio, D.; Penoni, A.; Palmisano, G. *Beilstein J Org Chem* **2011**, *7*, 1095-1099.



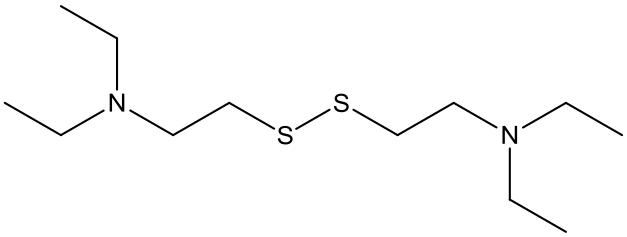
Scheme 2-13 Proposed product due to aldol condensation

To summarize, formic acid and zinc dust in a basic aqueous media turned out to be too weak reducing agents, whereas zinc dust and hydrochloric acid reduced the aldehyde before the imine could be formed.

Therefore, $NaBH_4$ was examined in regard to its performance in the desired reaction, keeping in mind the less environmental bengin character of this reducing agent. However, due to its beneficial reducing force and the positive atomic balance (4 protons per molecule) this reactant was taken into considerations.

After a modified protocol by Tajbakhsh *et al*.[124] cystamine dihydrochloride was diluted in methanol and 10 eq of the aldehyde were added, followed by the slow and careful addition of 5 eq of $NaBH_4$. The white precipitate formed after overnight reaction was filtered and the filtrate concentrated under reduced pressure. During that, a precipitate formed again, which was separated by filtration once more and the filtrate finally yielded a slightly yellow oil.

Using acetaldeyde, the diethyl compound **Et_2CySS (8)** was yielded, whereof the following spectra were obtained in $CDCl_3$.



Scheme 2-14 Structure of compound 8 Et_2CySS

[124] Tajbakhsh, M.; Hosseinzadeh, R.; Alinezhad, H.; Ghahari, S.; Heydari, A., Khaksar, S. *Synthesis* **2011**, *3*, 490-496.

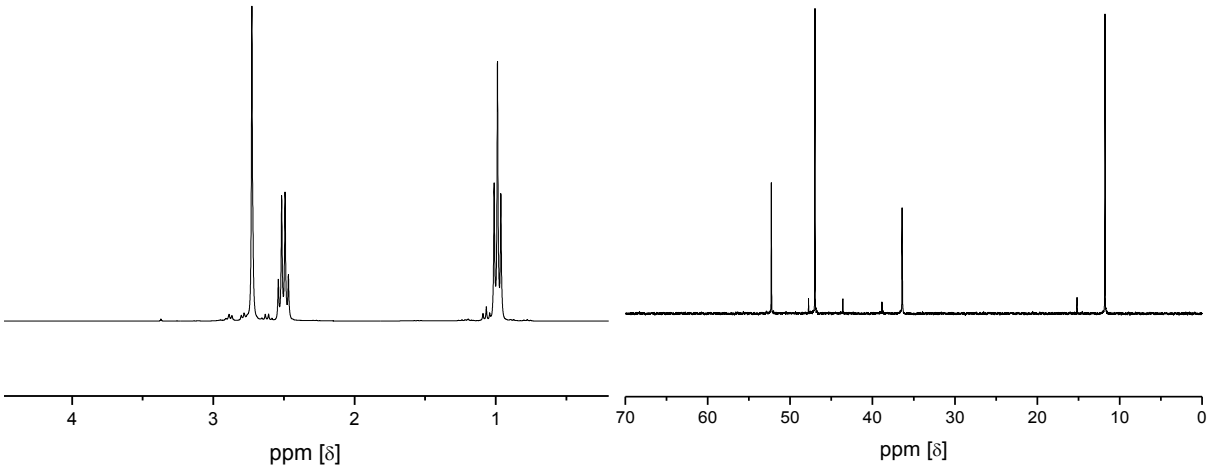
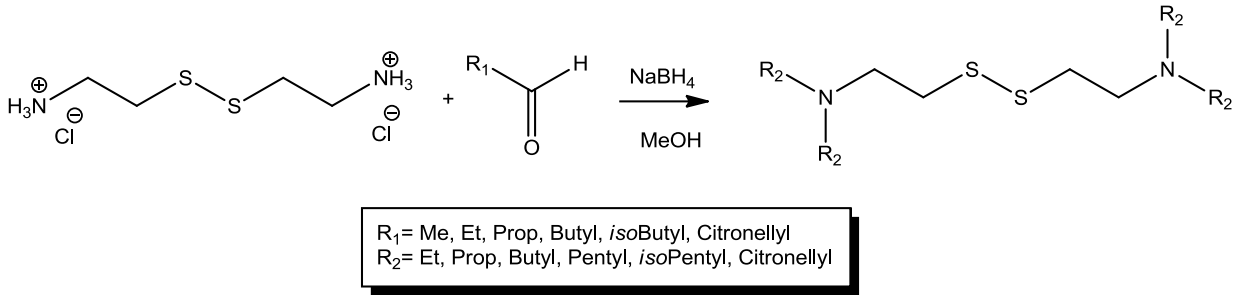


Figure 2-15 ^{1}H and ^{13}C{^{1}H} NMR spectra of 8

From ^{1}H NMR, 2 significant peaks, one singlet at 2.72 ppm and one quadruplet at 2.50 ppm with an integral of eight each, could be assigned to the protons dedicated to the carbons adjacent to the nitrogen and sulfur. The triplet at 0.99 ppm is indicative for the methyl groups. Due to the integral ratio of 8: 8: 12 of the signals, an exhaustive functionalization yielding the tertiary amine is feasible. Regarding the ^{13}C{^{1}H} NMR, peaks at 52.3 and 47.0 ppm correlate with carbons neighboring the nitrogen atom, whereas the peak at 36.4 again indicates the carbon adjacent to the disulfide bonding. Finally, the signal at 11.8 ppm marks the methyl group of both ethyl side groups. Minor signals at 2.87 (t), 2.77 (t) and 2.62 (q) in the ^{1}H and 47.8, 43.6, 38.9 and 15.2 ppm in the ^{13}C{^{1}H} NMR spectrum, respectively, indicate the mono-substituted analogue. Longer reaction times resulted exclusively in the desired product.

So, although the conversion using $NaBH_4$ is not entirely environmentally harmless due to the toxicity of the boride, using this protocol, the successful formation of the Leuckhart product with longer side chains on the nitrogen was found. The protocol was further adopted in terms of reaction time and equivalents. So, the used amount of aldehyde was reduced to 5 eq, whereas 2.5 eq of $NaBH_4$ were added. The reaction temperature was increased to 40°C and finally 70°C (reflux conditions); however, raising the temperature above 40°C did not accelerate the reaction significantly. In the case that after approximately 15 h no full conversion was achieved, additional amounts of aldehyde and $NaBH_4$ were added and the

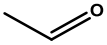
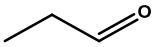
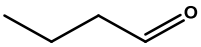
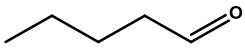
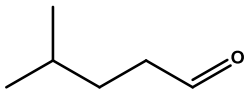
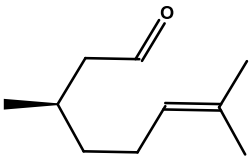
reaction allowed stirring for further 24 h. After that time, complete conversion was obtained in all cases.



Scheme 2-15 Conversion of cystamine dihydrochloride with several aldehydes

Systematically, aldehydes ranging from two (ethyl) up to ten (citronellal) carbon atoms were reacted under the reported conditions, yielding the corresponding N,N,N´,N´ tetra-alkylated cystamine derivatives in all cases. Reduction of the aldehyde to the respective alcohol could be neglected up to a chain length of four carbon atoms, for higher analogues, purification was achieved by acidic and basic extraction. Solely, the conversion with citronellal, yielding citronellol as side product necessitated purification via column chromatography using CH_2Cl_2: MeOH 100:1, R_f= 0.12. The ratio of alcohol formed was determined by integration of the corresponding peak at 3.61 ppm.

Table 2-9 Yields of the conversion of cystamine dihydrochloride with several aldehydes

number	name	educt	yield [%]	ratio alcohol [b]	purification method
8	Et_2CySS		80.1	0	-
9	Pr_2CySS		67.9	0	-
10	Bu_2CySS		n.d.[a]	0	-
11	Pe_2CySS		65.0	0.52	extraction
12	*iso*Pe_2CySS		63.6	0.50	extraction
13	Cit_2CySS		15.0	0.53	column chromatography

[a] full conversion determined via NMR; [b] %product: %alcohol as determined via NMR

An overview over all conversions done and the yields obtained is provided in Table 2-9. Yields were moderate in all cases, except for **13**, which can be rationalized by the purification via column chromatography, leading to the loss of product.

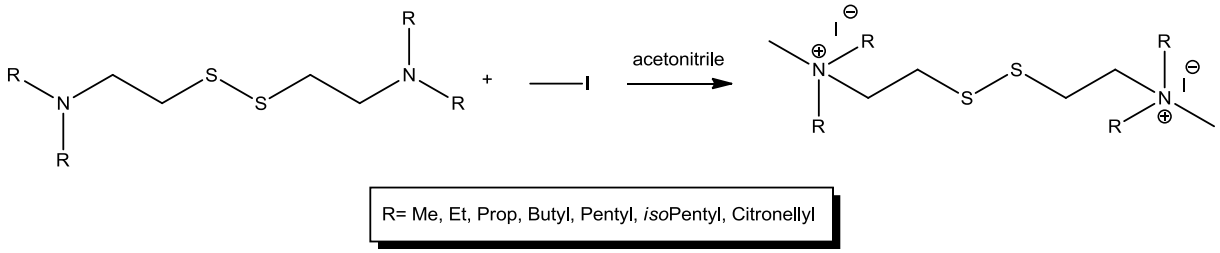
Citronellal as educt was introduced into this study as the feedstock is renewable. The material is a monoterpenoid, naturally occurring in the oil from citrus leaves or fruits. Commercially, it is used as insect repellent.[125] For this study, it is of special interest, as the structural relationship with natural rubber might facilitate sufficient mixing to lead to a reaction without the addition of extra solvent. Additionally, its renewable origin makes it very attractive from an ecological point of view.

2.6.2.2.1 Quaternization of the obtained Derivatives Et_2CySS- Cit_2CySS

Again, the obtained derivatives were subjected to quaternization reactions of the amines. As described previously, several haloalkanes were reacted with the smallest derivatives of the

[125] source: http://en.wikipedia.org/wiki/Citronellal, 29th October 2012

series, **8**. All conversions were conducted in acetonitrile under reflux conditions. However, no conversion was detected for butyl and octyl bromide respectively. In addition, the conversion was not successful with butyl chloride or iodide. Solely the reaction using methyl iodide did lead to the desired product, visible by the formation of a precipitate, which again exhibits the characteristic shift to 3.61 ppm (m, 4H, $SCH_2CH_2N^+$) in the ^1H NMR. Therefore it was decided to extend this route and to methylate all derivatives, resulting in a series **Et_2CySS^+**- **Cit_2CySS^+**, according to Scheme 2-16.



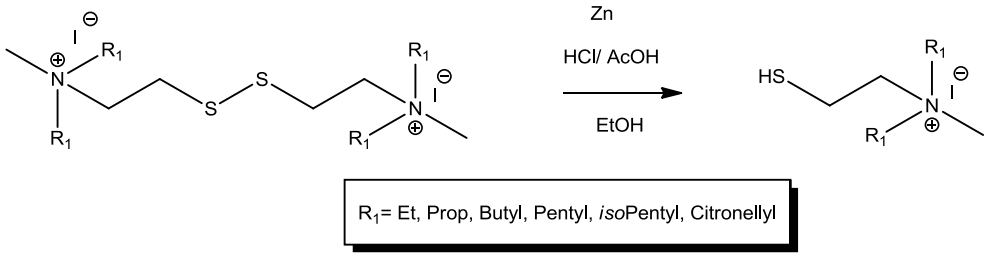
Scheme 2-16 Quaternization of Et_2CySS- Cit_2CySS

In all cases almost quantitative conversion was obtained. Due to its toxicity and high price, methyl iodide was replaced by methyl sulfate, still obtaining the same results. **Pr_2CySS^+** and **Bu_2CySS^+** were not prepared.

2.6.2.2.2 Reduction of obtained Derivatives Et_2CySS^+- Cit_2CySS^+

As described previously (2.6.2.1.3), all derivatives were subjected to reducing reactions.[118] As the solubility of the methylated derivatives increased with increasing chain length, removal of the formed zinc acetate was possible for **23**- **25** with aqueous extraction. Additionally, an alternative protocol was used, applying hydrochloric acid additionally to the formerly used acetic acid.[126]

[126] Hartman, R.F.; Rose, S.D. *J Org Chem* **2006**, *71*, 6342-6350.



Scheme 2-17 Reduction reaction of methylated derivatives

In general, educts were diluted in ethanol and a minor amount of acetic acid. Then zinc dust and hydrochloric acid were added alternatingly. After 5 h, the solvent was evaporated to 1.5 mL and $CHCl_3$ was added. The organic phase was washed with 10% NaCl solution. After drying of the organic phase, products in a brittle to highly viscous state were obtained. For characterization, NMR spectra were recorded. However, as partly relative low amounts of product were obtained, $^{13}C\{^1H\}$ NMR spectra could not be obtained in all cases. An overview of the obtained yields is given in Table 2-10.

Table 2-10 Overview of obtained derivatives after quaternization (14-19) and reduction (20-25)

quaternization			reduction		
number	name	yield [%]	number	name	yield [%]
14	Et_2CySS^+	99	20	Et_2CySH^+	61
15	Pr_2CySS^+	-[a]	21	Pr_2CySH^+	-[a]
16	Bu_2CySS^+	-[a]	22	Bu_2CySH^+	-[a]
17	Pe_2CySS^+	89	23	Pe_2CySH^+	55
18	$isoPe_2CySS^+$	99	24	$isoPe_2CySH^+$	65
19	Cit_2CySS^+	97	25	Cit_2CySH^+	n.d.[b]

[a] reaction not performed; [b] conversion confirmed via ^1H NMR

Alternatively, it was investigated whether the sole pass of hydrogen would suffice for the reduction. If the reaction was conducted in one phase (water) with zinc and hydrochloric acid, the reduction proceeded within one hour; yet, the product could only be isolated from the aqueous phase, requiring a high amount of energy. If both reactions were separated (oxidation of zinc, reduction of protons in one flask and oxidation of hydrogen gas and reduction of the thiol in a second flask) where the through-flow of the *in-situ* generated hydrogen could be ensured in the thiol containing system, no reaction occurred. If the reaction was conducted in one flask with a two-phase system (water and ether), allowing the evolvement of nascent hydrogen in the below aqueous phase and subsequent streaming of the gas through the etheric phase, a reaction could be detected neither.

One question, that could not be clarified in that context concerns the counter ion of the respective derivatives. After the methylation process, it is feasible, that the iodine remains as counter ion; however, after the reduction process an exchange of this counter ion with chloride or acetate is conceivable. For calculation of the obtained yield it was assumed, that still the iodine remained as counter ion. Due to the contamination with residual zinc acetate it was refrained from further analysis, leading to the identification of the counter ion.

2.6.2.3 SUMMARY

As a solvent free synthesis route to a modified poly(olefin) was not successful with commercially available mercaptamines, bearing a charged amine functionality, a systematic series of N,N,N- tri-alkyl cysteamine derivatives was created. Thereby, two classes of molecules were designed, on the one hand, a tetra-methyl cystamine was developed, which was subsequently methylated, butylated and octylated. On the other hand, cystamine dihydrochloride was reacted with C_2- C_{10} aldehydes with subsequent methylation. All those derivatives were subsequently reduced to the free thiol species by reduction using acid (hydrochloric acid or acetic acid) and zinc.

So, the species obtained consists either of two short and one longer alkyl chain or of two longer and one short alkyl chain.

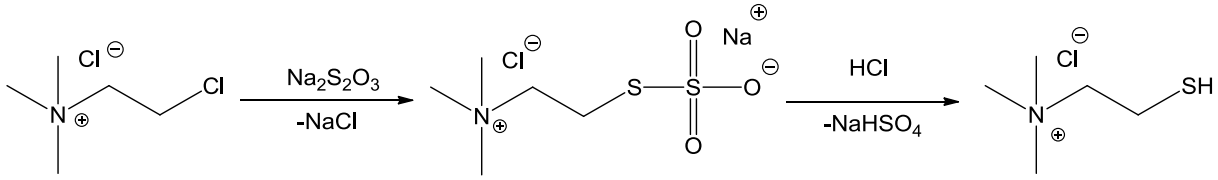
From the range of obtained products, several were selected and subsequently reacted in a thiol-ene reaction (**Me_2CySH^+** (**5**, **6**), ***iso*Pe_2CySH^+** (**24**), *cf*. 2.6.4).

2.6.3 Thiols obtained by alternative Reaction Pathways

2.6.3.1 Conversion with Sodium Thiosulfate Pentahydrate

Davis *et al*.[127] report the formation of thiols by reaction of sodium thiosulfate pentahydrate with appropriate halogen amines. The intermediate Bunte salt is further converted with 6M hydrochloric acid. As starting product in those cases, chlorcholine chloride (chlormequat chloride) was used, as this chloro-derivative of choline chloride is an industrially used raw material as phytohormone in the growing of grain.

1 eq of chlormequat chloride was reacted with 1 eq of sodium thiosulfate pentahydrate in water under reflux conditions for 15 h. After removal of the solvent, the white salt was diluted in 6M HCl and further refluxed for 2 h. After drying the product, a white precipitate was obtained. Although product peaks were detected in the respective ^{1}H NMR spectrum of the raw material, recrystallization as described in the literature only yielded the dimeric product.



Scheme 2-18 Conversion of chlormequat chloride via an intermediate Bunte salt to the free thiol

2.6.3.2 Conversion with Thiourea

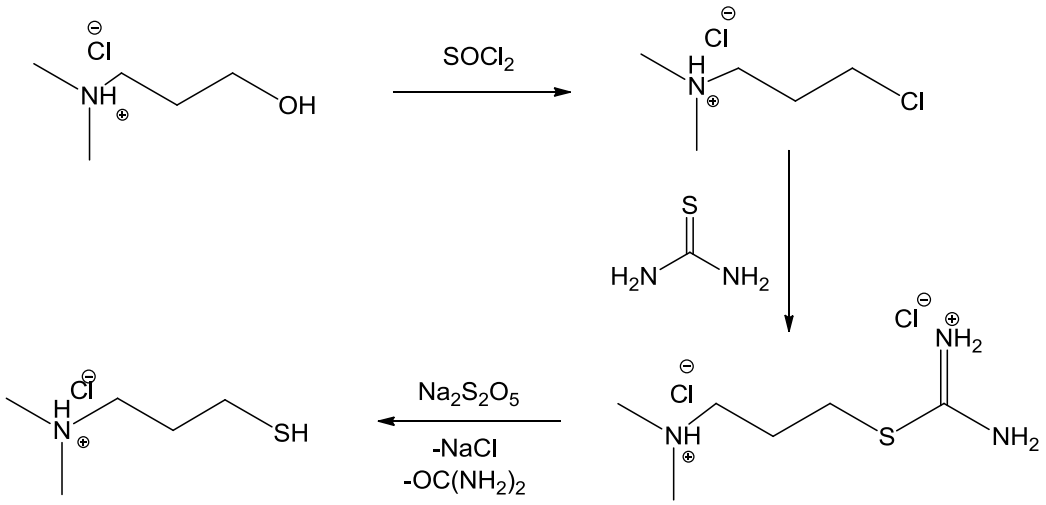
Following a procedure of Cankař,[128] thiourea was reacted with appropriate halogen amines, yielding the isothiouronium salt, which is decomposed to the free thiol by addition of base.

[127] Bernardes, G. J. L.; Chalker, J. M.; Errey, J. C.; Davis, B. G. *J Am Chem Soc* **2008**, *130*, 5052-5053.

[128] Stýskala, J.; Cankař, P.; Soural, M.; Bednář, P.; Lemrb, K. *Arkivoc* **2007**, *15*, 171-180.

The intermediate isothiouronium salt can be isolated and its successful formation can be proved via $^{13}C\{^{1}H\}$ NMR. This intermediate is than further converted with base.

In the present case, the reaction was started with 3-dimethylaminopropanol, which was reacted with thionyl chloride to obtain the required halogenid. This reaction proceeds very smooth, yielding 90% of the pure product. In that context, it has to be marked, that basic extraction, aiming at deprotonating the ammonium salt and enabling conversion in organic solvents led to the substitution of the halogenide with a hydroxyl group.



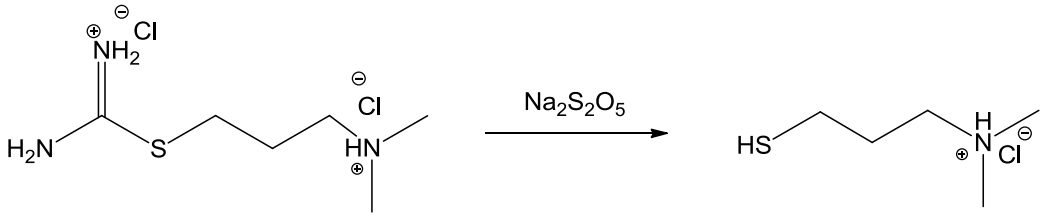
Scheme 2-19 Reaction pathway using thiourea via an intermediate isothiouronium salt

Further conversion with thiourea yielded the isothiouronium salt, a white crystalline powder. The salt was converted to the free thiol by addition of two mol of sodium hydroxide. However, as only the dimer could be isolated after contact with base, an alternative route had to be found.

As described in a procedure by Sureshbabu,[129] the aforementioned harsh conditions can lead to the dialkyl sulfides. By using sodium pyrosulfite instead, a mild method is described, allowing hydrolysis under neutral conditions.

[129] [a] Sureshbabu, V. V.; Vishwanatha, T. M.; Vasantha, B. *Synlett* **2010**, *7*, 1037-1042. [b] Bernardes, G. J. L.; Gamblin, D. P.; Davis, B. G. *Angew Chem Int Ed* **2006**, *45*, 4007-4011.

So, following the reaction pathway as depicted in Scheme 2-20, the last step, neutral hydrolysis of the isothiouronium salt was performed with $Na_2S_2O_5$ in water.



Scheme 2-20 Hydrolysis of isothiouronium salt using sodium pyrosulfite

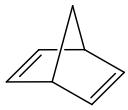
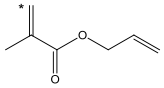
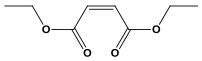
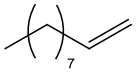
Purification was done via recrystallization from ethanol. The product (**Me_2PropSH**, **26**) was obtained as white crystalline powder and characterized via ^{1}H and ^{13}C{^{1}H} NMR spectroscopy. ^{1}H NMR spectra did not reveal any peculiarities, from ^{13}C{^{1}H} NMR, a peak at 24.1 ppm gives incidence, that indeed the free thiol was obtained. Yet, elementary analysis of the obtained product gave strong incidence that inorganic salts remained in the product. Nevertheless, this product was included in the subsequent study (Table 2-11).

2.6.4 Thiol-ene Reactions with Synthesized Small Molecules

As previously, the reactivity of the prospective thiols was first evaluated using several unsaturated small molecules. For thiol-ene reactions the respective mercaptammonium salts were diluted in 1 mL MeOD (10 mg of the thiol). Then the unsaturated species was added (1 eq of thiol for each double bond). For initiation of the reaction, 5 mg of **TPO-L** was added and the reaction irradiated for 10 min at 9000 mW cm^{-1}. After that, the conversion of the double bonds was determined by integration of the respective peaks in the ^{1}H NMR spectra.

The model substances used were norbornadiene, allyl methacrylate, maleic acid diethyl ester and 1-decene.

Table 2-11 Thiol-ene reactions of synthesized thiols with model small molecules

entry	-ene	Me_2CySH^+ (5)	Me_2CySH^+ (6)	$isoPe_2CySH^+$ (24)	$Me_2PropSH$ (26)
1		< 5 %	< 5 %	12 %	24 %
2		11 %	18 %	<5 %	<5 %
3		6 % [*50 %*] [a]	< 5 % [> *95 %*] [a]	<5 %	<5 % [> *95%*] [a]
4		24 %	26 %	-	25 %

[a] rearrangement from *cis* to *trans* detected [*trans ratio*]; [*] only methacrylate double bond considered.

It has to be stressed, that the obtained results are approximations as none of the used thiols were entirely pure, either contaminated with residual zinc acetate or other inorganic salts. Therefore stoichiometry could not be adjusted properly.

As can be seen from Table 2-11, the reactivity of the obtained thiols is moderate. Highest conversion of double bonds was obtained by reaction with 1-decene, where approximately one fourth of the available double bonds reacted with **5**, **6** and **26**, respectively.

Additionally, cysteamine derivatives featuring longer alkyl chains suffered from an unidentified decomposition reaction, which occurred by mere storage under ambient environment, yet under exclusion of light. This reaction was accelerated under UV irradiation and might be a reason for the poor performance. Exemplary, the degradation of **Cit_2CySH^+** is depicted:

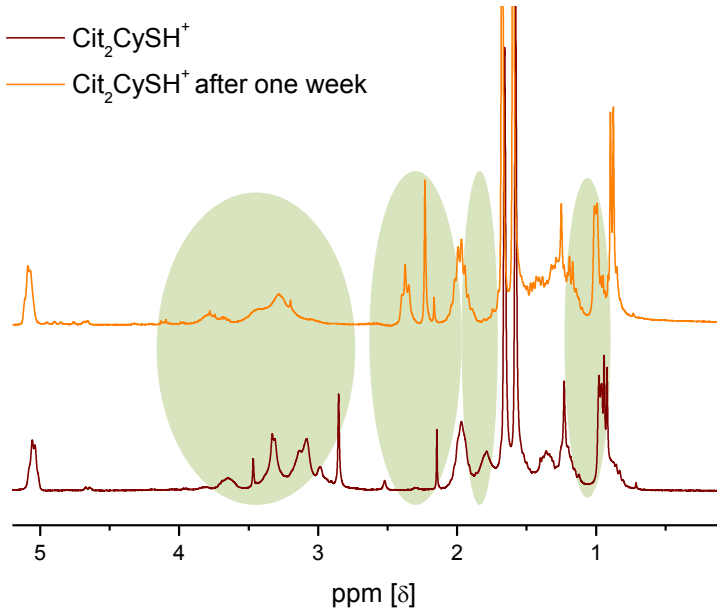


Figure 2-16 Degradation of Cit_2CySH^+ under ambient conditions after one week

This degradation could be rationalized by a Hofmann elimination, which occurs if an exhaustively methylated quaternary amine is treated thermally. The iodine counter ion is replaced by a hydroxyl anion, followed by an elimination of an alkene under reduced pressure.[130] However, no direct prove for this could be found so far.

So, the original intention to functionalize polymers with quaternized derivatives of cysteamine with long chain side chains was not successful. Yet, the question remained, whether the degradation reaction inhibits a thiol-ene conversion or the reduction to the free thiol was unsuccessful and no reactive species is available at all. Therefore, **Cit_2CySS** was directly reduced to obtain **Cit_2CySH**, which features a protonated amine instead of a methylated one. Again, this derivative was reacted with several unsaturated small molecules; however, it could not be shown, that abstaining from quaternization of the amine leads to improved conversion rates of the thiol species or to an improvement in terms of enhanced oxidation behavior.

[130] [a] von Hofmann, A. W. *Justus Liebigs Annalen der Chemie* **1851**, *78*, 253-286. [b] Cope, A. C.; Bach, R. D. *Org Synth* **1969**, *49*, 39.

As for **26**, prepared by an alternative method, moderate conversion rates were found as well. However, it has to be stressed again, that products were not isolated and purified, therefore, it cannot be excluded, that double bonds were converted by side reactions. One side reaction that is highly feasible in that context is the back reaction to the disulfide species, leading to the removal of active thiol from the reaction mixture.

Consistently with that **Lithene AL** could not be converted with any of the designed mercaptamines, despite longer illumination times or higher amounts of radical initiator added. Therefore, no more efforts in that direction were undertaken.

2.6.5 SUMMARY

The aim of this work was the development of thiols featuring quaternary ammonium functionalities and the ability to react in a thiol-ene reaction with an appropriate polymer.

Altogether, 26 new derivatives were synthesized and characterized by means of ^{1}H, $^{13}C\{^{1}H\}$ NMR, IR and elementary analysis. As required, all those thiols feature quaternary ammonium groups, generally obtained by an alkylation reaction. The derivatives were either obtained by reduction of converted cystamine dihydrochloride or by alternative ways (thiourea). Several selected representatives were reacted with a choice of small unsaturated molecules. However, conversion rates were remarkably low and no conversion with **Lithene AL** was possible. Thiols synthesized by alternative ways thereby performed better, yet no reaction with the desired target polymer was possible. Derivatives featuring long side chains (>4 C atoms) underwent an unexplained decomposition reaction.

2.7 Development of an Antibacterial Lacquer

2.7.1 Motivation

The aim of the FFG project KONZID, within that a large part of this work was performed, was the development of a water-insoluble antibacterial lacquer formulated from renewable raw materials. As a matrix material for the formulation it was decided to use natural rubber. From previous studies (*cf.* 2.4) it is known, that a direct modification of this polymer via a thiol-ene type of reaction without solvent is not possible. Therefore, it was decided to combine the readily obtained **PB 1** with **NR** and to crosslink those two materials with **PETMP**. There a photochemical thiol-ene conversion shall be applied.

2.7.2 Cross Linking of Natural Rubber

In a first attempt, the performance of natural rubber itself under exposure of UV light was investigated. For that purpose, solutions of **NR** in CH_2Cl_2 (10 mg mL^{-1}) were spin coated on CaF_2 substrates (5 times 200 µL, 800 U min^{-1}, 30 s) and the probes illuminated with UV-light for defined time periods under an ambient atmosphere. FT-IR measurements were performed after each illumination circle. This experiment was repeated upon addition of 2 wt% of radical starter **DMPA**.

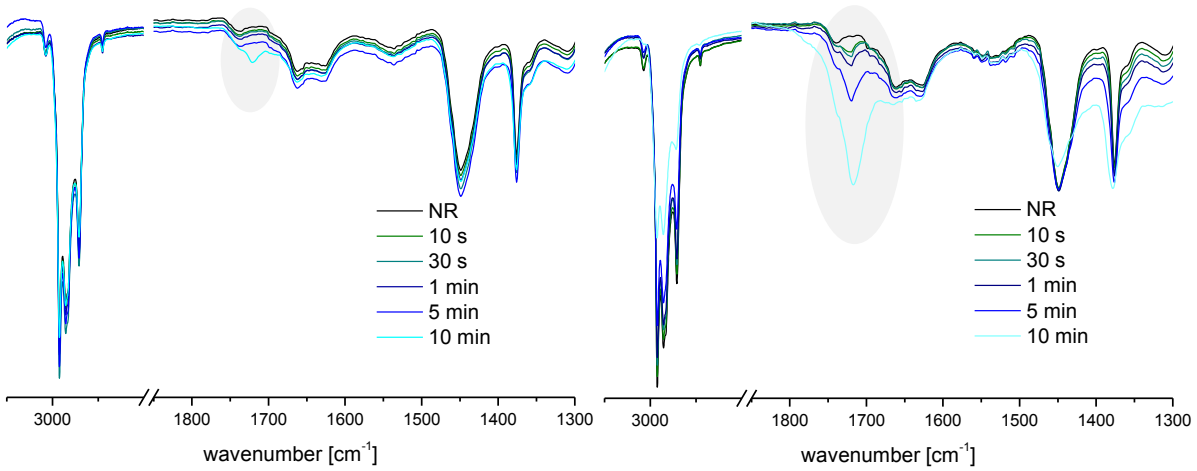


Figure 2-17 Oxidation of natural rubber (left: without radical initiator, right: with radical initiator)

In the figure depicted above, the strong evolvement of a peak at 1720 cm^{-1} is visible, indicating the formation of carbonyl moieties by the impact of UV light and oxygen on the unprotected rubber. This peak arises more pronounced if radical conditions were provided. From that, a maximum illumination time of 10 min was determined.

2.7.3 Thin Films

Several formulations were prepared, mixing **NR**, **PB 1**, **PETMP** and **TPO-L**. From those formulations, films were prepared, either by spin coating or by drop coating. Those preliminary tests served for the development of a general protocol.

Typical compositions used were as followed:

NR	0.25- 0.50 eq	} 1 eq
PB 1	0.50- 0.75 eq	
PETMP	0.03- 0.50 eq	
TPO-L	0- 2.0 wt%	to amount of thiol

Other parameters varied were illumination time and distance between light source and probe.

Generally, after illumination continuous, non-soluble films were obtained. Blind probes, containing all aforementioned ingredients, but not illuminated, could be easily dissolved again, suggesting that no or only to a minor extend reactions occurred without irradiation.

It can be expected, that preliminary vinylic double bonds react. To confirm that assumption, FT-IR measurements of thin films with the aforementioned concentration of components were spin coated on CaF_2 plates and- as described for mere natural rubber- illuminated for defined time periods under an ambient atmosphere. In the precise case, the following lacquer formulation was used (**COAT 1**):

COAT 1	**NR**	100 phr	0.50 eq	} 1 eq
	PB 1	122 phr	0.50 eq	
	PETMP	36 phr	0.03 eq	
	TPO-L	7 phr	20 wt%	to amount of thiol

The distance between light source and probe was set with 7 cm. After each illumination circle (10 s, 30 s, 1 min, 2 min, 10 min), FT-IR measurements were made. For better comparison, the curves were normalized to the peak at 2927 cm^{-1}, indicating the asymmetric C-H stretching vibration of the CH_3 group. One characteristic curve is depicted in Figure 2-18. Notably informative peaks are highlighted.

Although not much difference can be found at a first glance, a more detailed look reveals several alterations. As expected, the reactivity of the vinylic peaks of **PB 1** is higher compared to the internal double bonds of **NR**. This can be illustrated very vivid by consideration of the peaks at 1664 cm^{-1} (C=C stretching vibration *cis*) and 1637 cm^{-1} (C=C stretching vibration vinylenes). The resonance vibration of the latter ones is significantly reduced with proceeding illumination times. Corresponding to that is the reduction of the peak at 906 cm^{-1} and- not explicitly marked in the graphic- at 969 cm^{-1}, attributed to the C-H out of plane bending of vinyls R-CH=CH_2. The peak at 837 cm^{-1}, resulting from the C-H out of plane bending of the internal double bonds of **NR**, was not affected at all, again confirming the unequal reactivity. However, as only one occurring reaction along one polymer backbone chain is sufficient for

the formation of a tight network, already minor and therefore unquantifiable conversion rates might be sufficient for film formation.

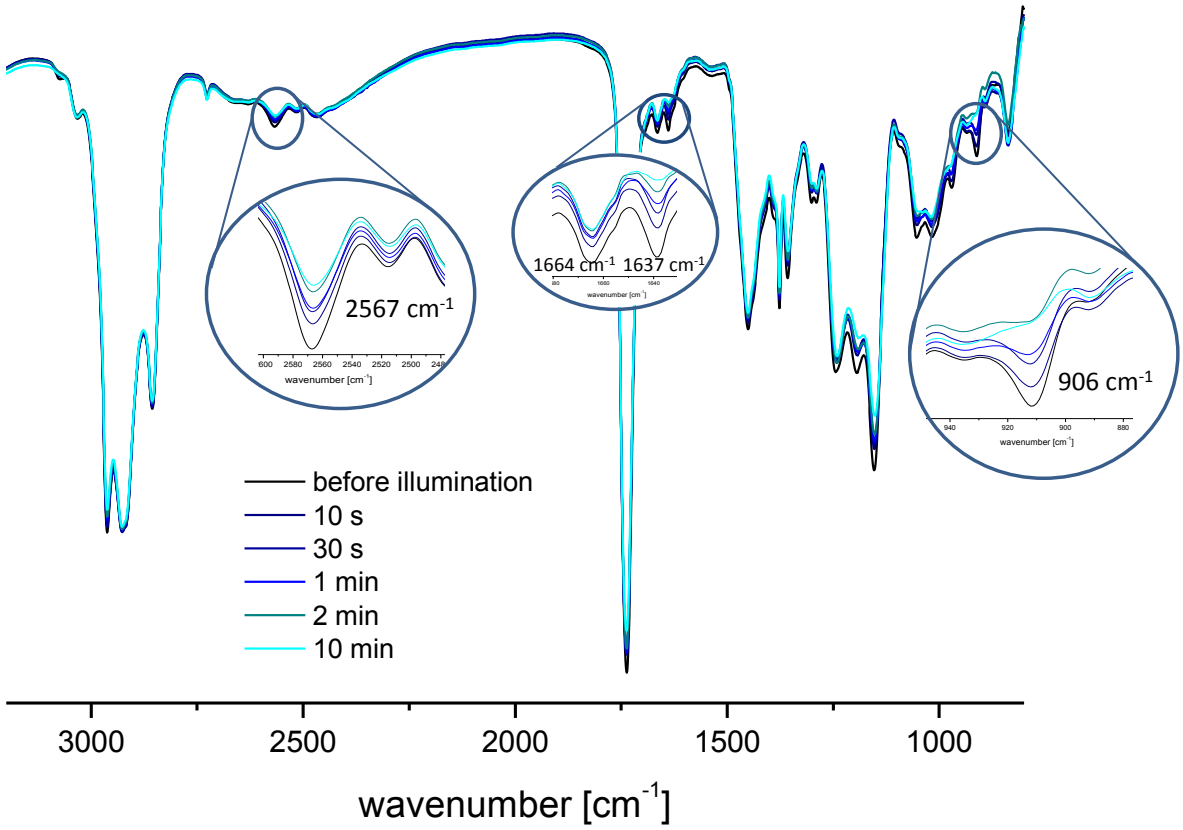


Figure 2-18 FT-IR spectra after periodic illumination circles of COAT 1

The peak at 2567 cm^{-1} indicates the SH stretching vibration of free thiols. The reduction of this peak is consistent with the ongoing thiol-ene conversion. As the final curve after 10 minutes is not entirely plane, it can be assumed, that still unreacted thiol moieties are within the network.

Finally, it has to be marked, that the peak at 1737 cm^{-1}, resulting from the ester moieties of **PETMP** is not increased within the conversion, leading to the assumption, that the impact of undesired oxidation reactions of double bonds as observed for the **NR** smaple occur only to a small extend.

So, the formation of a network occurred and as expected, mainly the vinyl double bonds of **PB 1** showed pronounced activity.

2.7.3.1 Surface Characterization

For contact angle measurements of the polymers under discussion, several probes were prepared. To obtain an inherent reference sample, natural rubber **NR** together with **PETMP** was diluted in toluene (accordingly to **COAT 1**, **NR** content counts for 1 eq of double bonds) and subsequently spin coated. In order to ensure good crosslinking 25 wt% to thiol amount of **TPO-L** were added for radical initiation. Probes were illuminated under UV light for 15 min in order to ensure comparable surface conditions.

The active reference probe was prepared analogously by spin coating solutions containing 1 eq of **PB 1**, 0.03 eq of **PETMP** and 25 wt% of **TPO-L** (again to thiol amount) and treated accordingly.

In order to obtain hybrid films from **NR** and **PB 1**, coatings with following compositions were spin coated from 10 mg mL^{-1} solutions in toluene (**COAT 2**).

COAT 2	**NR**	100 phr	0.80 eq	} 1 eq
	PB 1	25 phr	0.20 eq	
	PETMP	25 phr	0.04 eq	
	TPO-L	6 phr	25 wt%	to amount of thiol

Table 2-12 Surface energies and contact angles of films under investigation

entry	sample	Θ_{H2O}	Θ_{CH2I2}	surface energy γ / mN m^{-1}
1	$NR_{unexposed}$	77.7 ± 0.4	93.9 ± 0.4	26.8 ± 0.1
2	$NR_{exposed}$	80.9 ± 0.3	51.2 ± 2.1	35.6 ± 0.6
3	COAT 2	76.4 ± 0.7	42.5 ± 0.1	43.4 ± 0.3
4	PB 1	60.0 ± 1.5	32.9 ± 2.7	54.3 ± 0.8

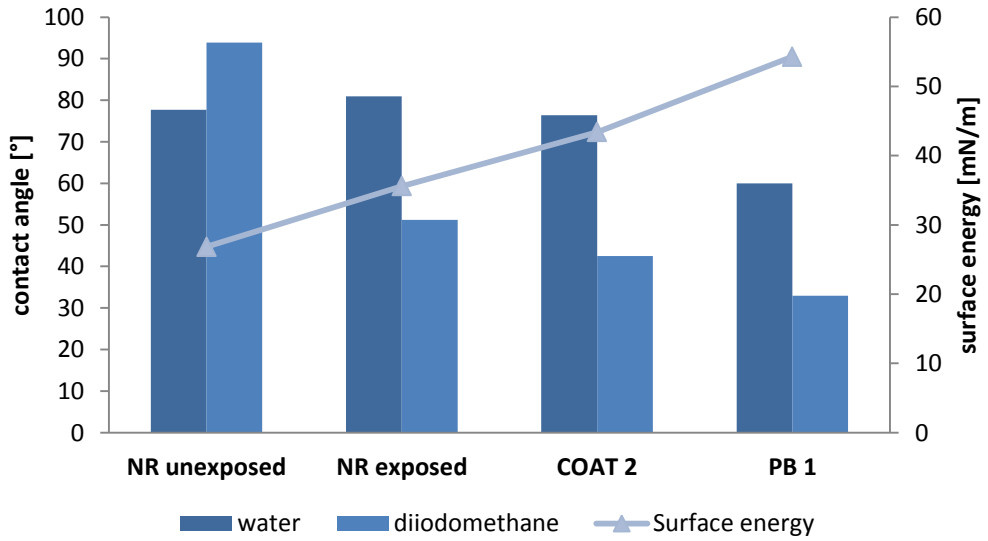


Figure 2-19 Surface properties of films under investigation

For a better comparability, also unexposed **NR** was subjected to contact angle measurements. The strong increase of surface energy again indicates an alteration of the surface due to the UV- treatment. Cross-linked **PB 1** reveals the highest surface energy values. Regarding the hybrid material **COAT 2** the value for surface energy sums up from the surface energy values for $\mathbf{NR_{exposed}}$ and **PB 1**. Remarkably is the increase of the surface energy for cross-linked **PB 1**, which is significantly higher than the value for **PI 3**, which is- to some

extent- a comparable material. Summarizing, it can be assumed, that charged groups can be found on the surface.

2.7.3.2 Antimicrobial Activity Tests

Again, probes from the type **COAT 2** were prepared and tested against the following microbes: *Candida albicans*, *E. coli*, *S. aureus*, *P. aerigunosa* and *L. pneumophila* according to a JIS Z 2801 protocol (at Hbicon GmbH, Bielefeld).

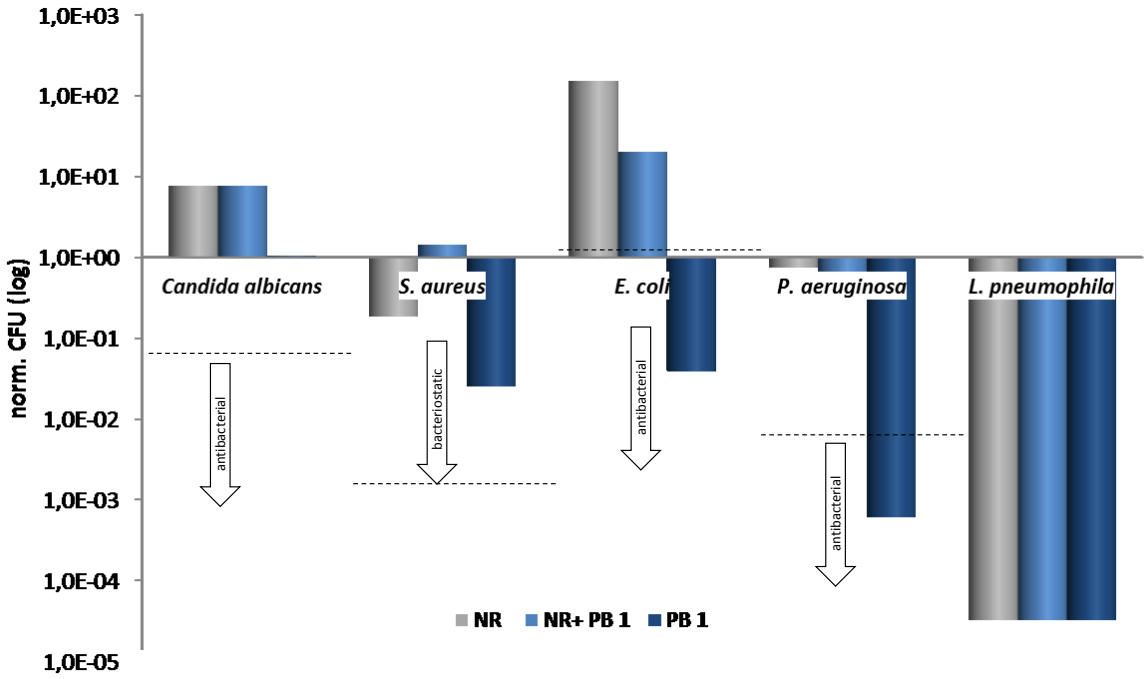


Figure 2-20 Reduction of CFU after 24h, dotted lines indicate the border line for antimicrobial behavior

In that case, one fungus (*Candida albicans*), one Gram-positive bacterium (*Staphylococcus aureus*) and three Gram-negative strains (*Escherichia coli*, *Pseudomonas aerigunosa Legionella pneumophila*) were used as test microbes. Again, the absolute colony forming

numbers were normalized against CFUs on the reference **NR** after 24h and the logarithmic value is plotted.

What is a striking fact at a first glance is the very strong activity of all probes against *Legionella pneumophila*; there no bacteria could be found after 24 h on any probe. In that case, *Legionella* strains seem to be inoperative as test strain, maybe due to acidic groups on the surface after the illumination process.[131] Therefore it was refrained from further discussion of this particular probe.

Generally, cross-linked **PB 1** exhibits activity compared to the reference plate. Against *E. coli* and *P. aerigunosa* an antibacterial activity is found as the reduction is higher than 2 log units (3.57 log units for *E. coli* and 3.08 logarithmic values for the latter one), whereas against the fungus and the Gram-positive strain, a bacteriostatic activity is found with reductions of 0.86 logarithmic values for both microbes. These results are in good agreement with previous findings (*cf*. 2.3.3.1), that sole protonation of an amine leads to biocide activity, even though slightly reduced compared to an alkylated one as featured by **PI 2**. Additionally, Gram-negative bacteria are more prone towards an antimicrobial attack; a fact, that is mirrored well by those results.

However, after cross linking of this polymer **PB 1** with a matrix material **NR**, activity drops remarkably. Only against *E. coli* and *P. aerigunosa*, a slight bacteriostatic effect is detectable. So, the preservation of the activity within the crosslinking process seems not possible in a first attempt. An explanation therefore might be a too low content of ammonium groups, or more likely, the sole protonation of a tertiary amine is not sufficient for antimicrobial activity under the respective conditions.

2.7.4 Solid Films

Consistently with the aforementioned efforts, thicker films were developed to investigate recovery after swelling in a solvent. The formulation developed was used again; however this

[131] [a] Karanika- Kouma, A.; Dioysolpoulos, P.; Koliniotou- Koubia, E.; Kolokotronis, A. *Journal of Oral Rehabilitation* **2001**, *28*, 157-160. [b] Lüscher- Mattli, M. *Antiviral Chemistry & Chemotherapy* **2000**, *11*, 249-259.

time, spin coating was conducted at lower rates (500 rpm, 30 s). From a stock solution (equivalent to **COAT 1**) containing 100 mg mL^{-1} polymer (**NR** and **PB 1**; 0.5 eq each), 2 x 300 µL were used for coating. To investigate the influence of **TPO-L** to achieve complete cross-linking, films with higher content of **TPO-L** were produced, too. The obtained films **COAT 3** and **COAT 4** were illuminated at 9000 mW cm^{-2}, detached, weighed, covered with 1 mL CH_2Cl_2 and soaked overnight. After that, the solvent was removed, the film dried and again weighed. The recovery of insoluble fractions is given (*cf.* Figure 2-23). After 30 s, a recovery rate of 55 % is obtained for **COAT 3** and 75 % for **COAT 4**, respectively. NMR analysis of the soluble share after 10 s, 1 min and 10 min obtained from extraction revealed predominantly natural rubber, followed by minor fractions of **PB 1** and **PETMP**, which is consistent with FT-IR measurements described in 2.7.3. After 10 s and 1 min, minor parts of **PETMP** were detected, too. The relative numbers were obtained by integration of the respective peaks in ^1H NMR. Additionally, identical samples of the very same films were soaked with acidic D_2O; however, any soluble shares could be found there, which excludes that active antimicrobial polymer leaches and can therefore indeed be considered as a contact biocide.

COAT 3	**NR**	100 phr	0.50 eq	} 1 eq
	PB 1	122 phr	0.50 eq	
	PETMP	36 phr	0.03 eq	
	TPO-L	0.7 phr	2 wt%	to amount of thiol
COAT 4	**NR**	100 phr	0.50 eq	} 1 eq
	PB 1	122 phr	0.50 eq	
	PETMP	36 phr	0.03 eq	
	TPO-L	7 phr	20 wt%	to amount of thiol

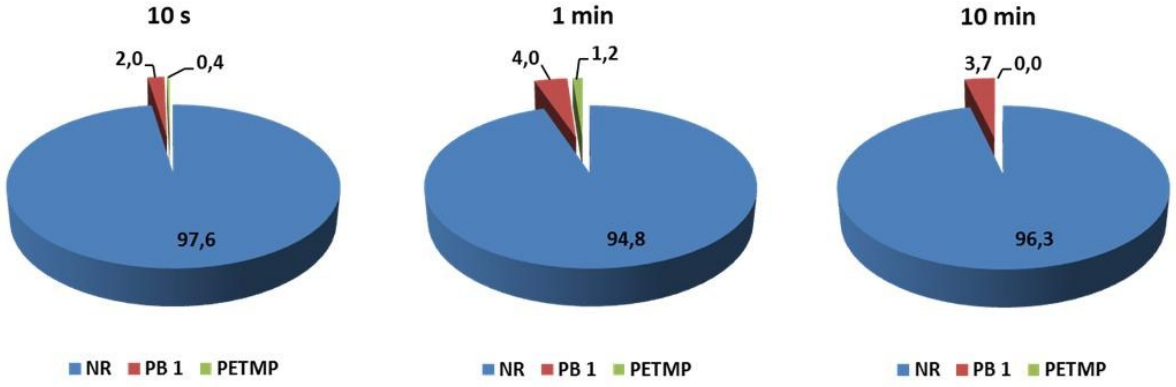


Figure 2-21 Apportionment of soluble parts after soaking for 20 h in $CDCl_3$ (COAT 3)

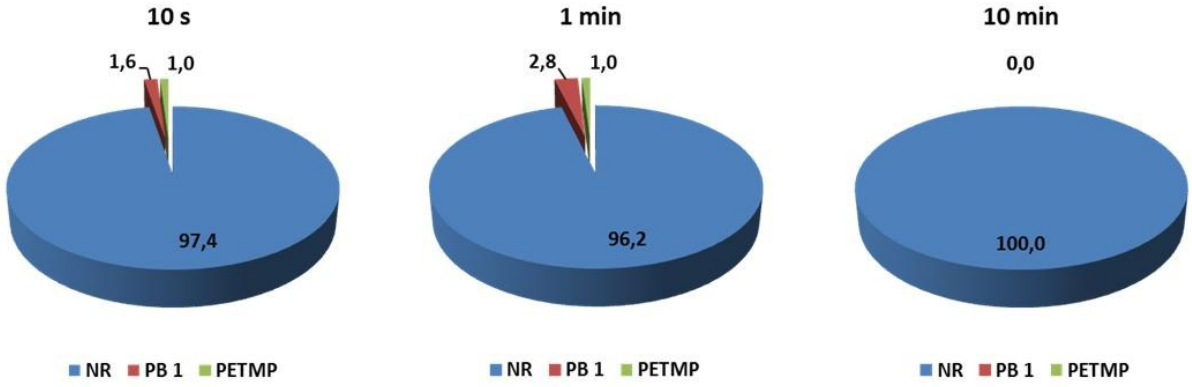


Figure 2-22 Apportionment of soluble parts after soaking for 20 h in $CDCl_3$ (COAT 4)

From those pie charts of the soluble fractions of **COAT 3** and **COAT 4**, it is obvious, that predominantly **NR** is not covalently incorporated in the networks. After 10 min, no free **PETMP** could be found in any sample. Although the equivalent amount of **PB 1** is higher of a factor of ten compared to **PETMP**, the relative amounts of free species found after illumination are comparable. This is in good accordance with Decker´s findings that a significant amount of vinyl double bonds additionally undergoes homopolymerization.[46]

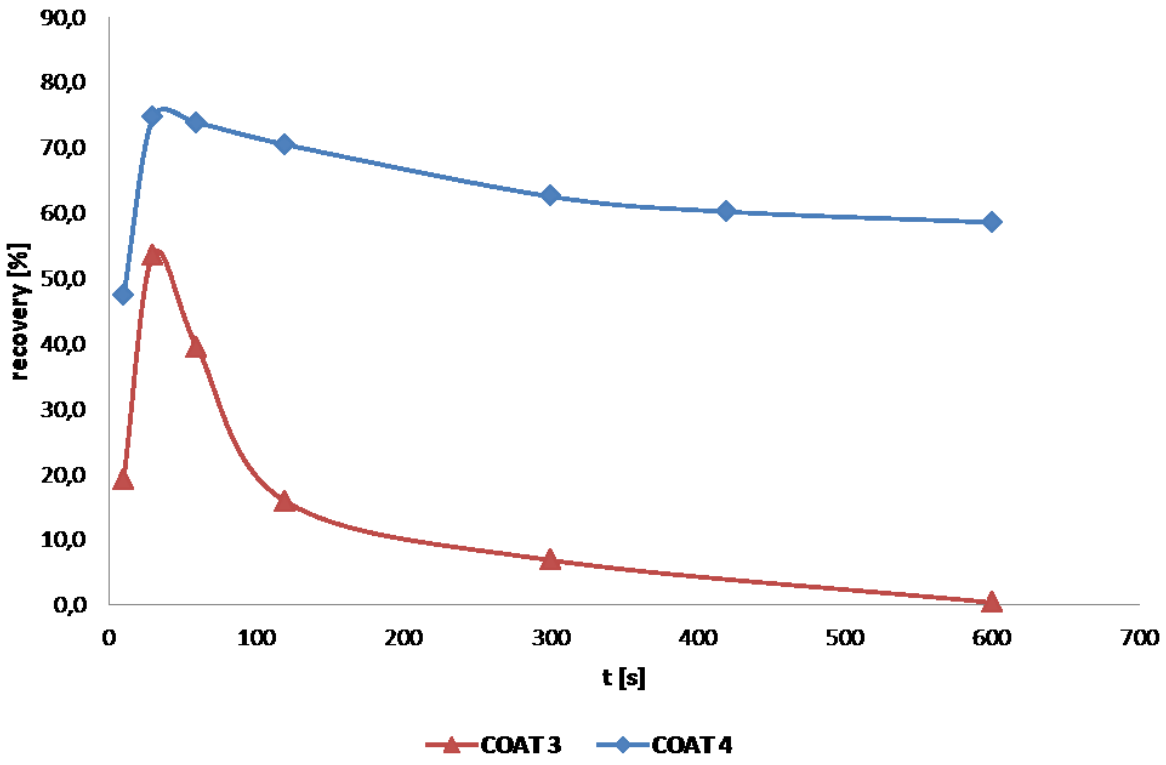


Figure 2-23 Recovery of insoluble fractions of COAT 3 and COAT 4 (colored lines serve as guide for the eye)

From Figure 2-23 it can be seen, that the ratio of radical initiator used has a tremendous impact on the stability of the obtained films. After 30 s, the highest amount of solid recoveries could be obtained in both cases, after that a strong decrease in the stability of the films was found for the coating containing less photo initiator. In case of **COAT 4** a plateau at approximately 70 % was obtained and the films maintained their shape after the soaking process. For **COAT 3** not one continuous film, but very thin fragments were recovered after 5 min. From these results, an optimized illumination time of 30 s was determined.

2.7.5 BULK MATERIALS

Finally, it was tried to develop bulk biocide materials. Therefore, **NR** was swollen in CH_2Cl_2 under stirring. **PETMP** and **TPO-L** were added accordingly to obtain a mixture as described for

COAT 2. That mixture was distributed in a 15x15 cm mold, dried overnight and finally illuminated for 15 min. By doing so, a dense film (**COAT 5**) with approximately 1 mm strength was obtained.

COAT 5	**NR**	100 phr	0.80 eq	} 1 eq
	PB 1	25 phr	0.20 eq	
	PETMP	25 phr	0.04 eq	
	TPO-L	6 phr	25 wt%	to amount of thiol

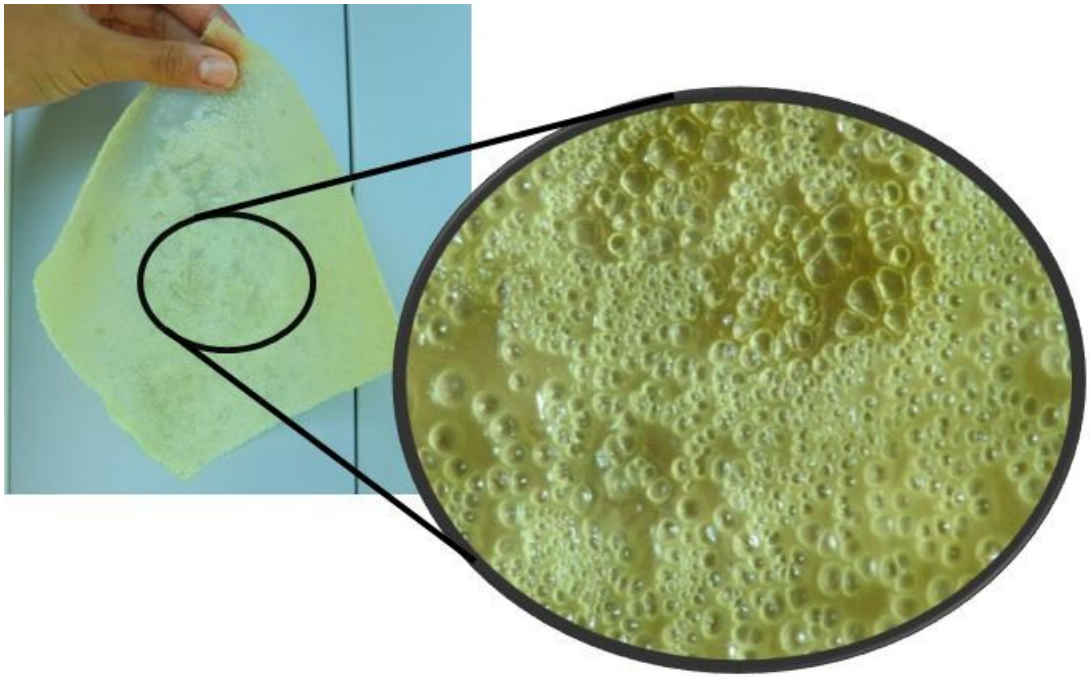


Figure 2-24 Illustration of COAT 5

As clearly visible from Figure 2-24, the surface of the obtained coating is very rough and covered with blisters, resulting from the evaporation of the solvent. Therefore, it was refrained from conducting antimicrobial tests of those films, as the result is expectable to be comparable to **COAT 2**. Nevertheless, this particular film was subjected to ATR-IR measurements.

2.7.5.1 ATR-IR Measurements

From Figure 2-25, several peculiarities indicate a successful cross-linking, although the film was thicker than the previously described coatings. First, regarding the region at 2560 cm^{-1} the peak indicating the free SH vibration of unbound **PETMP** is clearly reduced, alike behaves the sharp peak at 838 cm^{-1}, which originates from a C=C-H out of plane vibration of **NR**. In conjunction with that, a new signal arises at 699 cm^{-1}, indicating the formation of S-C bonds.

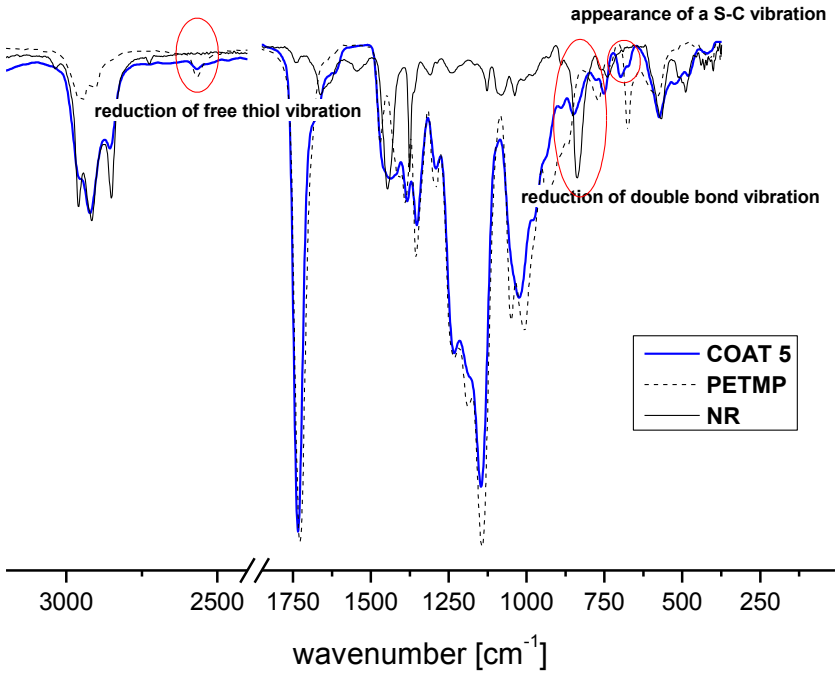


Figure 2-25 ATR-IR measurements of dense film COAT 5

From swelling experiments as described, a recovery of insoluble parts of 88 % was possible, suggesting a higher degree of cross linking as already obtained. Analysis of the soluble parts was performed as described in 2.7.4.

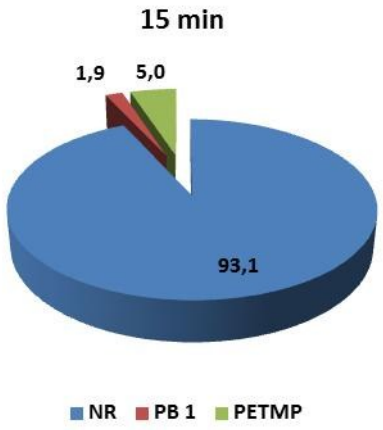


Figure 2-26 Apportionment of soluble parts after soaking for 20 h in $CDCl_3$ (COAT 5)

Again, the largest share consists of natural rubber, however in this case, higher amounts of **PETMP** were found compared to **PB 1**, which is consistent with the composition of the formulation. Again after soaking in D_2O at pH 1 or deuterated methanol, respectively, no polymeric residues or unbound **PETMP** were found.

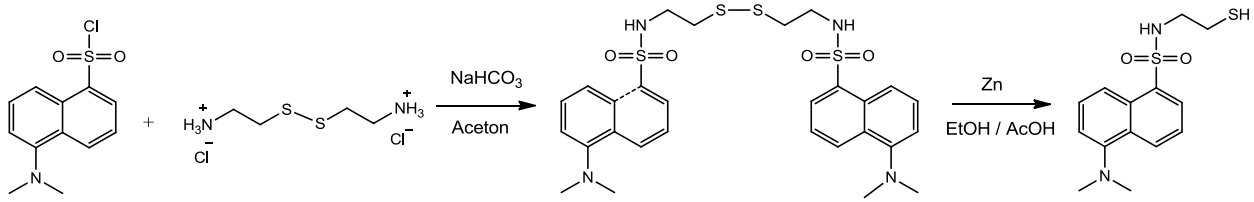
2.7.6 SUMMARY

Within this chapter, the development of an antibacterial lacquer is presented. Several coating formulations, containing **NR**, **PB 1**, **PETMP** and **Lucirin TPO-L** as radical initiator were spin coated, illuminated and subjected to FT-IR measurements, contact angle measurements, recovery tests as well as biocide activity tests. From FT-IR measurements, a predominant reaction of vinyl double bonds is reasonable, which was further approved by swelling experiments, where recovery rates of approximately 55-75 % were found after an illumination time of 30 s. Surface energy increased, suggesting that charged groups are present on the surface. Investigations of the soluble residue showed that prevalently **NR** was not covalently bond into the film. Antibacterial activity tests of cross-linked **PB 1** revealed a bacteriostatic activity against a fungus and a Gram-positive bacteria strain and antibacterial activity against Gram-negative strains. However, this activity could not be preserved within a spin coated lacquer formulation, additionally containing **NR**. Although it was possible to generate a tense foil with the developed formulation, it was refrained from conducting biocide tests due to the very inhomogeneous surface state.

2.8 Development of a "Click-able" Leaching Control

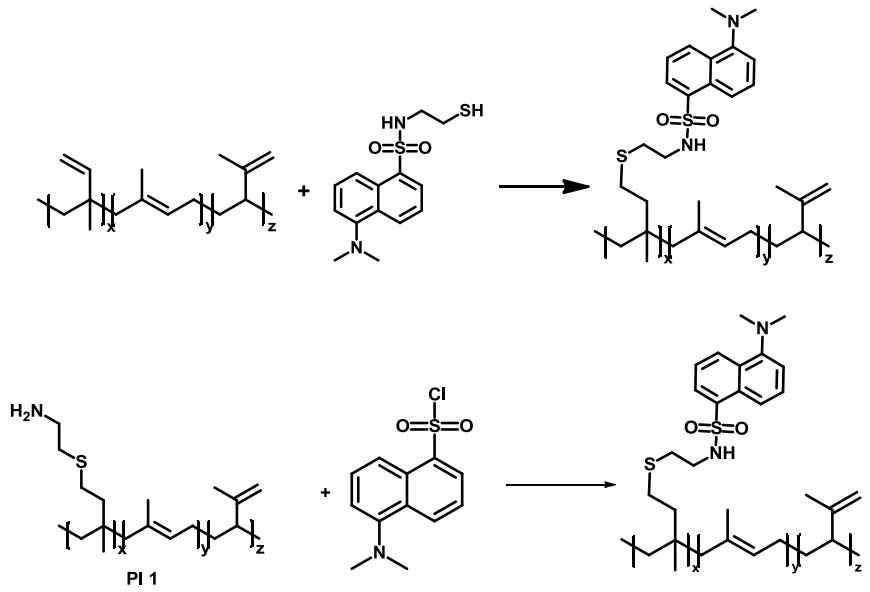
As it is of crucial interest to obtain polymers that stay within the polymer matrix even after long-run applications and if not, to have a tool to measure the amount of leached polymer, a tailor-made leaching control was developed. The recommendations for such a control cover an easy and doubtless detection as well as properties that do not alter the characteristics of the polymer under investigation.

Therefore it was decided to synthesize a thiol bearing a dansyl side group (**27**) according to the following scheme.



Scheme 2-21 Synthesis of dansyl chloride featuring thiol (27)[118]

This thiol was subsequently reacted with **PI** accordingly to the described protocol.[118] Additionally, **PI 1** was reacted with dansyl chloride, leading to the equivalent product **PI 4**, which emitted in a blue- greenish color upon illumination with UV light at 385 nm. The successful conversion of double bonds was confirmed by ^{1}H NMR measurements.



Scheme 2-22 Reaction of PI with 20 (up), reaction of PI 1 with danysl chloride (down)

Figure 2-27 Polymer PI 4 under irradiation with UV light at a wavelength of 385 nm

Photo physical properties were determined by fluorescence spectroscopic measurements of solutions in $CHCl_3$ containing **PI 4** obtained by reaction of **PI** with **27** ($c = 5 \cdot 10^{-10}$ g mL^{-1}).

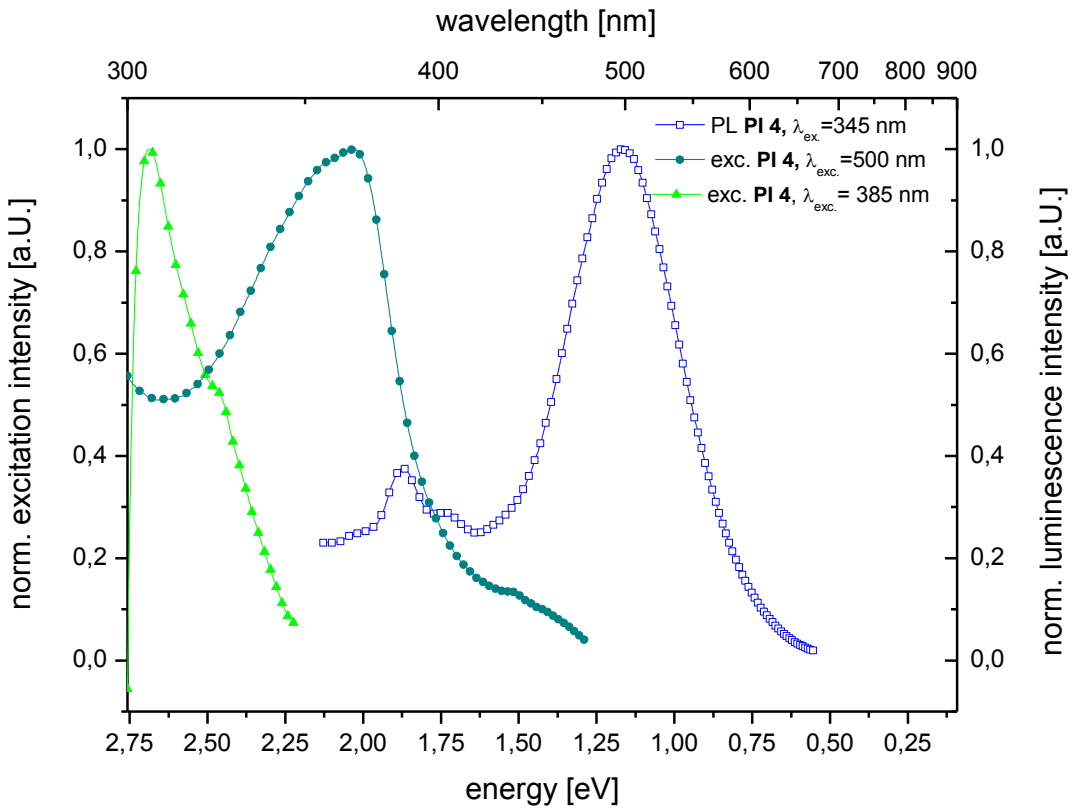


Figure 2-28 Photo physical properties of PI 4

In Figure 2-28 the excitation and luminescence spectra of **PI 4** are depicted. The photo luminescence curve exhibits two maxima upon irradiation at 345 nm, one at 385 nm and the second one at 500 nm. The first peak can be assigned to the excitation of **PI** itself, whereas the latter maximum refers to the maximum fluorescence of the dansyl chloride, which is in good agreement with literature values.[132]

So, a detectable leaching control was synthesized and can be used for long-term leaching tests.

[132] Holmes- Farley, S. R.; Whiteside, G. M. *Langmuir* **1985**, *2*, 266-281.

3 CONCLUDING REMARKS

In this work, the antimicrobial equipment of polymers is discussed. Thereby, thiol-ene reactions are used for connecting antimicrobial groups to a polymer backbone. The resulting polymers are characterized regarding their chemical properties and their antimicrobial performance. Additionally, new strategies in the development of antibacterial monomers as well as biocide coverings are presented.

In the first part, the functionalization of anionically polymerized isoprene with cysteamine via a thiol-ene reaction is reported. The polymers were rendered antimicrobial by quaternization of the amino functionality by either alkylation or protonation. Characterization of the new obtained macromolecules included ^{1}H, $^{13}C\{^{1}H\}$ NMR and infrared spectroscopy, GPC as well as elemental analysis. Additionally, surface energies and thermal stabilities were determined. Antibacterial test results of the pure polymers according to a JIZ Z2801:2000 protocol revealed excellent antimicrobial activity of the quaternary ammonium featuring species. Thermal stability up to 200°C allowed extrusion processing of the functionalized poly(isoprene)s. The best performing polymer, i.e. bearing butylated ammonium-groups, was compounded with the commodity material poly(propylene). The compound bearing 5 wt% of the antibacterial polymer exhibited satisfactory biocidal properties. So, it could be shown, that the antimicrobial finishing of poly(isoprene) via thiol-ene chemistry is possible and the obtained polymers preserve their antibacterial activity after compounding with a matrix material. In order to obtain environmentally and ecologically more benign systems, it was tried to develop a solvent free reaction pathway. However, the direct solvent-free addition of amino-bearing thiols was only possible to a very low extend and for uncharged amine groups. Therefore, two strategies were developed: on the one hand, a new series of thiols, featuring potentially antimicrobial groups for the direct implementation to unsaturated polymers was developed, on the other hand a poly(butadiene) was functionalized with a tertiary protonated amine and the obtained polymer cross-linked with natural rubber in a thiol-ene reaction, yielding a potentially antimicrobial lacquer.

Following the first mentioned strategy, series of alkylated cysteamine derivatives were produced. Thereby, either a Leuckart-Wallach reaction yielding dimethylation of each nitrogen atom or a reductive amination with $NaBH_4$ to synthesize higher analogues were applied. A series covering one to ten carbon atoms in the alkyl chain was achieved. All of the

obtained tertiary amine species were quaternized and subsequently subjected to reduction reactions, yielding mercaptamines with potentially biocide activity. Alternatively, thiourea was used to synthesize thiols and from intermediate isothiouronium salt the thiol was obtained. Model reactions were conducted, however only very low conversions with the synthesized thiols were observed. Accordingly, the functionalization of a polymer with the new compounds was not possible.

In order to develop an antimicrobial finishing, poly(butadiene) was functionalized with (dimethylamino)ethanethiol hydrochloride and the obtained polymer cross-linked with natural rubber in a photochemical thiol-ene reaction. From FT-IR measurements, a clear favoritism of the vinyl double bonds compared to the internal ones was observed. According to that, insolubilization measurements reveled that predominantly natural rubber was not incorporated in the network. Antimicrobial activity tests of the pure polymer (cross-linked) and a lacquer formulation revealed a bacteriostatic to antibacterial activity of the pure compound, which could not be preserved in the lacquer formulation.

Finally, a tool was developed, allowing for the monitoring of leaching of functionalized polymer in practical applications. Therefore, dansyl chloride was used as fluorescent moiety and either clicked to a polymer featuring primary amine groups or a thiol bearing moiety was synthesized and reacted accordingly. The photo physical properties were determined. In that way, a fluorescent leaching control was developed.

Although several new findings were achieved within this work, some problems still remained unsolved. It could be shown, that the equipment with antibacterial groups via a thiol-ene reaction is possible on polymers, yet, the reaction pathways are not environmentally friendly enough and rather money-consuming. A series of new thiols was developed. Long-chain derivatives, using cystamine dihydrochloride as starting material, suffered from an unidentified decomposition reaction which makes them inoperative for practical applications. However, the developed idea of designing thiols, bearing long aliphatic chains to increase the miscibility with unsaturated polymers, will be perpetuated, yet, the aliphatic region shall move in between the nitrogen and sulfur atom. In other words, a local separation of charged nitrogen and the thiol functionality shall be achieved and lead to reactive thiols. First trials in that direction were conducted and already led to very promising results. Such developed compounds will further be used for equipping Lithenes. The obtained

polymers might serve as starting material for coverings as described. The antimicrobial activity, which was not preserved in the experiments conducted so far, might be accelerated by using alkylated quaternary ammonium salts for functionalization. Additionally, the surfactant-like character of the described species might allow the functionalization from aqueous solutions, such as latex emulsions. Then an industrial realization of the developed materials is feasible.

4 EXPERIMENTAL

4.1 MATERIALS

If not mentioned otherwise, all chemicals were used as purchased from commercial sources (Aldrich, ABCR…). If necessary, solvents were dried according to standard procedures and degassed using argon. Poly(isoprene) was freshly prepared (c*f.* 4.3.3). Poly(butadiene) Lithenes were obtained from Synthomer Limited, United Kingdom in industrial purity. Photoinitiator Lucirin **TPO-L** (Ethyl - 2,4,6- Trimethylbenzoylphenylphosphinate) was obtained from BASF. Thermal radical starter **AIBN** (2,2´- Azobisisobutyronitrile) was recrystallized from ethanol prior to use.

4.2 INSTRUMENTATION

4.2.1 INFRARED SPECTRA

Infrared-spectra of soluble polymers **PI**- **PI 3** were recorded with a Perkin Elmer Spectrum One with a DTGS detector, ν_{max} in cm^{-1}. Samples were measured on calcium fluoride plates as thin films. Plates were characterized with an ATR-IR support on the named instrument, whereas **PB 1**, **PI 4** as well as oils were measured on a Bruker Alpha FT-IR spectrometer.

4.2.2 NUCLEAR MAGNETIC RESONANCE (NMR) MEASUREMENTS

NMR-measurements were performed on a VARIAN INOVA 300MHz spectrometer. Deuterated solvents were purchased from Cambridge Isotope Laboratories and peak

referencing was done according to literature.[133] ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C{^{1}H} spectra were recorded at 75 MHz. Multiplicity and peak shapes are indicated with s (singlet), d (doublet), dd (doublet of doublets), t (triplet), m (multiplet), b (broad), bs (broad singlet). For polymer samples, the number of scan was set to 64 for ^{1}H NMR spectra and to 10000 for ^{13}C{^{1}H} NMR spectra and the relaxation delay for ^{1}H NMR spectra was set to 2 s in order to guarantee complete relaxation.

4.2.3 Thin Layer Chromatography

Reactions were monitored by TLC (silica gel 60 F254 on aluminum, Merck). Detection was carried out under UV-light (254 nm and 365 nm for luminescent compounds) and staining with potassium permanganate (2 wt% in H_2O dest. for unsaturated and reducing compounds).

4.2.4 Gel Permeation Chromatography

Gel permeation chromatography was used to determine the weight and number average molecular weights (M_w and M_n) as well as the polydispersity index (PDI) using THF as eluent. In case of analysis of polymers containing amine functionalities a mixture of chloroform, triethylamine and *iso*-propanol ($CHCl_3$: Et_3N : *iso*-propanol = 94 : 4 : 2) was used. The instrumentations consists of a Merck Hitachi L6000A pump (delivery volume: 1 mL min^{-1}, membrane pulse attenuator between pump and injector), STV-gel-columns from Polymer Standards Service with 5 µm particle size (pre column: 8x50 mm, 100 Å pore diameter, separation column: 8 x 300 mm, 10^6 Å, 10^4 Å, 10^3 Å pore diameter), refractive index detector from Wyatt Technology, model Optilab DSP Interferometric Refractometer. Polystyrene standards purchased from Polymer Standard Service were used for calibration.

[133] Gottlieb H.E., Kotylar A., Nudelman A. *J Org Chem* **1997**, *62*, 7512-7515.

4.2.5 Contact Angle Measurements

Contact angle measurements were performed with a Drop Shape Analysis System DSA 100 (Krüss GmbH, Hamburg, Germany) on spin coated polymer surfaces. The mode used was the sessile drop method and measured immediately after deposition of the droplet. Water and diiodomethane were used as test liquids (drop volume ~ 3 µL, literature values for test liquids: 50.8 mN m^{-1} for diiodomethane and 72.7 mN m^{-1} for water). Based on the Owens-Wendt equitation, surface energy was calculated.[102]

4.2.6 TGA measurements

TGA measurements were performed with a Netzsch Simultaneous Thermal Analyzer STA 449C (crucibles: aluminum from Netzsch). A helium flow of 50 mL min^{-1} was used in combination with a protective flow of 8 mL min^{-1}.

4.2.7 Zeta-Potential Measurements

Zeta-potential vs. pH curves were recorded by streaming current measurements of plane polymer samples using an electrokinetic analyzer (SurPASS, Anton Paar GmbH, Graz, Austria). Using the "Adjustable Gap Cell" (AGC), two samples with 20 mm x 10 mm were fixed on the sample holders using double-sided adhesive tape. The channel height was adjusted to about 100 µm. At this distance between adjacent sample surfaces, a strong contribution of interfacial conductance can be ruled out. The measurements were performed with KCl electrolyte solution (10^{-03} mol L^{-1}, 500 mL), additional a nitrogen flow streamed through the electrolyte solution, in order to exclude CO_2. Starting at neutral pH the pH was then changed stepwise (0.3 – 0.4 units) by titration with HCl (0.05 mol L^{-1}) and NaOH (0.05 mol L^{-1}), respectively. Using the "Adjustable Gap Cell" a pressure ramp from 0 to 300 mbar was performed to force the electrolyte solution through the cell. The zeta-potential (ζ) was calculated from the recorded streaming current according to the method of Helmholtz-

Smoluchowski.[134,135] All reported zeta potential data are averaged over four individual measurements.

4.2.8 ANTIMICROBIAL TESTS

Antimicrobial tests were performed by the Technologie Transfer Zentrum TTZ Bremerhaven or the Hbicon GmbH. The polymer test and reference samples of uncured polymers were spin coated from $CHCl_3$ and MeOH solutions on glass substrates (4 x 4 cm) in a 1 mg mL^{-1} concentration. At TTZ Bremerhaven the procedure chosen was a modified JIS Z 2801:2000 protocol.[136] Bacteria strains tested were *Escherichia coli* DSM 10290, *Listeria monocytogenes* DSM 20600, *Pseudomonas fluorescen*s DSM 6147 and *Staphylococcus aureus* DSM 799. 50 µL of the inoculated working culture were diluted in a 1: 10 ratio, until a final concentration of approximately 5.0 x 10^5 cells mL^{-1} was reached. Glass slides were coated three times with 75 µL of the diluted bacteria suspension, capped with sterile cover glasses and incubated for 24 h at 35°C. The use of glass cover instead of poly(ethylene) films marks a minor deviation of the JIS Z 2801:2000 procedure, but still meets its requirements. Samples were transferred in a casein peptone- soya meal peptone nutrient solution and eluted. Dilutions were dripped on a culture medium and again incubated for 44h. Then colony forming units (CFU) were counted and reduction compared to unmodified polymer sample is stated.

For cured test samples (*cf*. 2.7.3.2) formulations as described were prepared, spin-coated from 10 mg mL^{-1} solution and illuminated for 15 min. Those probes were tested at Hbicon GmbH according to the JIS Z 2801:2000 protocol without any deviations (protocol as described, poly(ethylene) films were used). The test strains used in this case were *Escherichia coli* ATCC25922, *Legionella pneumophila* (in-house strain- isolated probes) *Pseudomonas aeruginosa* ATCC27853, *Candida albicans* ATCC14053 and *Staphylococcus aureus* ATCC29213.

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[134] Bukŝek, H.; Luxbacher, T.; Petrinić, I. *Acta Chim Slov* **2010**, *57*, 700-706.

[135] Luxbacher, T. *Desalination* **2006**, *199*, 376-377.

[136] Ström, G.; Fredriksson, M.; Stenius, *P. J Colloid Interf Sci* **1987**, *119*, 352–361.

4.2.9 COMPOUND SPECIES

Compounds were prepared with a Thermo Haake Minilab double screw extruder. Extrusion temperature was 190°C, extrusion time 2 min. Plates (200 x 200 mm) were pressed at the University of Leoben. Alternatively, test species were prepared by swelling a polymer in an appropriate solvent and applying it to molds (150 x 150 mm) and cure them via photo-induced or thermal reaction.

4.2.10 UV REACTIONS

UV reactions were conducted using the following lamps:

The UV-illumination for photochemical thiol-ene reactions with small molecules and solid film illuminations were carried out by using an ozone-free mercury lowpressure lamp (EXFO EFOS Novacure), with an irradiation dose between 7000-9000 mW cm^{-2}. For synthesis of **PB 1** and for bulk illuminations, an Oriel Research Lamp Housing Model 66921 450 to 1000 Watt Hg, Xe and Hg(Xe) DC arc lamp with a spectral range from 200-2500 nm was used.

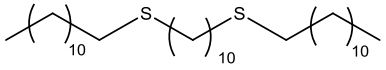
4.3 SYNTHESIS

4.3.1 THERMAL THIOL-ENE REACTIONS WITH SMALL MOLECULES

Generally, 1 eq of alkene was reacted with 5 eq of thiol and 0.5 eq of **AIBN** in degassed toluene or toluene: methanol= 9:1 in case of **SH2**. The reaction mixture was allowed to stir overnight at 80°C in an inert atmosphere of nitrogen according to a protocol by Campos *et al.*[137] After 22 h the solvent was removed under reduced pressure and the product dried under vacuum. Generally, no further cleaning measures were taken and the conversion was determined via ^{1}H NMR spectroscopy. Compounds exhibiting complete conversion were further purified by flash column chromatography and characterized via ^{1}H NMR.

4.3.1.1 1,10-BIS(DODECYLTHIO)DECANE **SH2**

(Table 2-1 Thermal thiol-ene reaction with model substances, entry 1, reaction with **C_{12}SH**)

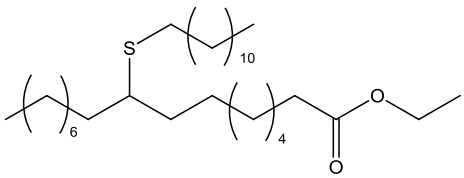


^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2,49 (t, 8H, CH_2-S-), 1,94-1,51 (m, 8H, -CH_2-CH_2-S-), 1,25 (s, 44H, -CH_2-), 0,86 (t, 6H, -CH_3).

4.3.1.2 ETHYL 10-(DODECYLTHIO)OCTADECANOATE

(Table 2-1 Thermal thiol-ene reaction with model substances, entry 2, reaction with **C_{12}SH**)

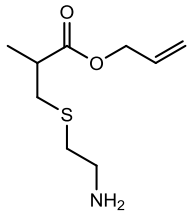
[137] Campos, L. M.; Killops, K. L.; Sakai, R.; Paulusse, J. M. J.; Damiron, D.; Drockenmuller, E.; Messmore, B., W.; Hawker, C.J. *Macromolecules* **2008**, *41*, 7063-7070.



^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 4.11 (q, 2H, *C*H_2-COOEt), 2.50 (q, 2H, S-C*H*$_2$-), 2.34 (s. 1H, C^{tert}*H*-S-), 1.61 (m, 8H, S-CH_2-C*H*$_2$-), 1.26 (m,

4.3.1.3 1-(2-AMINOETHYLTHIO)-2-METHYLHEPT-6-EN-3-ONE **SH3**

(Table 2-1 Thermal thiol-ene reaction with model substances, entry 5, reaction with **cys**)



^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.92- 5.76 (m, 1H, -C*H*=CH_2), 5.24 (q, 2H, -CH=C*H*$_2$), 4.54 (d, 2H, -O-C*H*$_2$-CH=), 2.83-2.45 (m, 7H, -C*H*$_2$-NH_2, -C*H*$_2$-S-CH_2-, CH_3-C*H*-CH_2-S-), 1.26 (bs, 2H, NH_2), 1.20 (d, 3H, -CH-C*H*$_3$).

4.3.2 PHOTOCHEMICAL THIOL-ENE REACTION WITH SMALL MOLECULES

For model reactions, 1 eq of ene was reacted with 1 eq of thiol and 50 wt% of radical initiator **TPO-L** in respect to the thiol. Generally, 10 mg of the thiol was used. Illumination time was 10 min using the EXFO EFOS Novacure lamp (9000 mW cm^{-1}). No precautions against evaporation of solvent were taken. Conversion was calculated from the corresponding ^{1}H NMR signals.

4.3.3 POLY(ISOPRENE) PI

In a three necked flask, 7.5 mL (5.1 g, 75.0 mmol) of freshly distilled isoprene were diluted with 15 mL of dry THF under an inert atmosphere. *n*-BuLi (468 µL of a 1.6 M solution, 0.75 mmol) was slowly added under maintained stirring. After 3 h, the reaction solution is concentrated under reduced pressure and the polymerization mixture was slowly added to stirred ethanol (50 mL). A white viscous oil was formed, which was separated from the supernatant by decantation and dried in vacuum. Yield: 4.5 g (88%).

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.88-5.51 (bs, 1.02H, -C*H*=CH_2), 5.23-4.97 (bs, 0.61H, -C*H*=C(Me)-), 4.97-4.77 (bs, 2.03H, -CH=C*H*$_2$), 4.75-4.39 (d, 5.81H, -C(Me)=C*H*$_2$), 2.45-1.72 (bs,

5.76H, CH and -CH_2-), 1.71-1.46 (bs, 10.72H, -CH_3), 1.46-1.06 (bs, 8.09H, -CH_2-), 1.05-0.73 (bs, 3.51H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 149.6-146.6 (-*C*H=CH_2, -*C*H=*C*(Me)-, -*C*(Me)=CH_2), 112.4-09.8 (-CH=*C*H_2, -C(Me)=*C*H_2), 48.1-46.1 (-CH-, 3,4 backbone), 45.5-43.6 (-CH_2- 1,2 backbone), 42.4-40.9 (-C-, 1,2 backbone), 40.8-39.5 (-CH_2- 1,4), 23,8-23.2 (-CH_2- 3,4), 21.5-20.8 (-CH_3 1,2), 19.0-17.5 (-CH_3 1,4 and 3,4 unresolved).

FT-IR (film on CaF_2, cm^{-1}): 3076, 2970, 2930, 2850, 1642, 1440, 1410, 1378, 886.

GPC: M_n (g mol^{-1})= 10500, PDI= 1.1

4.3.4 CYSTEAMINE MODIFIED **PI** (**PI 1**)

poly(Isoprene) (2.0 g, 29.4 mmol of double bonds) and cysteamine (4.54 g, 58.8 mmol, 2 eq in respect to double bonds) were dissolved in 53 mL of toluene at 80°C. **AIBN** (117.3 mg, 1 mol-% in respect to cysteamine) was added and the reaction mixture was stirred over night at 80°C. Afterwards, the solvent was evaporated under reduced pressure. The product was repeatedly precipitated in vigorously stirred cooled methanol yielding a white solid. The product was dried under vacuum and 2.88 g (66 %) of **PI 1** were obtained.

Anal. Calc. for **PI 1** and complete functionalization: C: 56.68; H: 10.70; N: 9.92. Found: C: 80.50; H: 11.16, N: 2.06.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.88-5.51 (bs, 0.29H, -C*H*=CH_2), 5.23-4.97 (bs, 0.36H, -C*H*=C(Me)-), 4.97-4.77 (bs, 0.62H, -CH=CH_2), 4.75-4.43 (d, 2.93H, -C(Me)=CH_2), 3.07-2.75 (bs, 0.49H, NH_2CH_2-), 2.71-2.39 (bs, 0.92H, CH_2-S-CH_2- and CH_2-S-C*H*), 2.32-1.74 (bs, 3.34H, CH and -CH_2-), 1.71-1.46 (bs, 7.35H, -CH_3), 1.46-1.06 (bs, 4.73H, -CH_2-), 1.05-0.55 (bs, 3.84H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 149.6-146.6 (-*C*H=CH_2, -*C*H=*C*(Me)-, -*C*(Me)=CH_2), 113.6-109.6 (-CH=*C*H_2, -C(Me)=*C*H_2), 45.5-43.6 (-CH_2- 1,2), 42.4-41.6 (-C-, 1,2), 41.4-41.0 (C-S-C), 38.8-38.3 (NH_2-C), 23.7-23.3 (-CH_2- 3,4), 19.0-18.3 (-CH_3 1,4 and 3,4 unresolved).

FT-IR (film on CaF_2, cm^{-1}): 3350, 3076, 2970, 2930, 2850, 1642, 1440, 1410, 1378, 1262, 1142, 1003, 886.

GPC: M_n (g mol^{-1})= 11900, PDI= 1.8

4.3.5 BUTYLATED **PI 1** (**PI 2**)

PI 1 (300 mg) was dissolved in 50 mL of a mixture of chloroform and acetonitrile (v/v = 1:1). Butyl iodide (784.98 µL, 6.87 mmol) was added and the reaction mixture was allowed to stir overnight. After 20 h reaction time, the solvent was evaporated under reduced pressure and the product was dissolved in methanol (1 mL). The polymer was purified by repeated precipitation of methanol solutions in *n*-pentane (100 mL) and dried in vacuum. Yield: 375 mg (74 %).

Anal. Calc. for **PI 2**: C: 59.41; H: 10.92; Br: 18.82; N: 3.30; S: 7.55 for complete conversion. Found: C: 65.37; H: 9.56; Br: 18.11, N: 2.00; S: 4.96.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 8.43 (bs, 0.42H), 5.82-5.52 (bs, 0.35H, C*H*=CH_2), 5.19-4.94 (bs, 0.16H, -C*H*=C(Me)-), 4.93-4.72 (bs, 0.47H, -CH=C*H*$_2$), 4.72-4.36 (d, 2.74H, -C(Me)=C*H*$_2$), 3.42-3.26 (m, 0.95H, cys-C*H*$_2$-N^+), 3.26-3.03 (m, 1.40H, cys-C*H*$_2$-S, C*H*$_2$-S-C*H*$_2$ and C*H*$_2$-S-C*H*) , 3.02-2.85 (bs, 2.59H, butyl-C*H*$_2$-N^+), 2.85-2.25 (bs, 0.51H, C*H*$_2$-S-C*H*$_2$ and C*H*$_2$-S-C*H*), 2.25-1.65 (bs, 4.67H, -C*H* and -CH_2- 1,4, 3,4 functionalized and butyl), 1.65-1.43 (bs, 6.26H, -CH=C(C*H*$_3$) and –C(C*H*$_3$)=CH_2), 1.43-1.30 (bs, 1.95H) 1.30-1.01 (bs, 5.38H, C*H*$_2$-C(Me)-), 1.02-0.45 (bs, 5.58H, -CH_2-C(C*H*$_3$)-, functionalized and butyl -C*H*$_3$).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 149.6-146.6 (*C*H=CH_2, -*C*H=*C*(Me)-, -*C*(Me)=CH_2), 113.8-110.2 (-CH=*C*H_2, -C(Me)=*C*H_2), 53.5-53.1 (N^+-*C*H_2), 48.0-46.7 (CH, aliphatic), 46.0-43.8 (-CH_2-C(Me)), 42.4-41.6 (CH_2-*C*(Me)-), 27.8-27.4 (*C*H_2-S), 24.3-23.1 (-C(Me)=*C*H_2), 19.2-16.9 (CH=C(*C*H_3) and –C(*C*H_3)=CH_2)), 13.8-13.3 (butyl-CH_3).

FT-IR (film on CaF_2, cm^{-1}): 3391, 3214, 3076, 2970, 2930, 2850, 1642, 1440, 1410, 1378, 1003, 886.

GPC: M_n (g mol^{-1})= 12500, PDI= 1.6

4.3.6 PROTONATED **PI 1** (**PI 3**)

PI 1 (200 mg) was dissolved in acetic acid (10 mL) and HCl (10%, 6 mL) was added. A precipitated was formed, which was separated by decanting the solution and dried to constant weight. Yield: 180mg (66 %).

^{1}H-NMR (δ, 20°C, MeOD, 300 MHz): 6.05-5. 57 (bs, 0.25H, C*H*=CH_2), 5.38-5.01 (bs, 0.75H, -C*H*=C(Me)-), 4.99-4.50 (d, 2.76H , -CH=C*H*$_2$, C(Me)=C*H*$_2$), 3.24-2.98 (bs, 0.77H, C*H*$_2$-NH_3^+), 2.90-2.68 (bs, 0.86H, C*H*$_2$-S-), 2.68-2.35 (bs, 0.76H, C*H*-S-), 2.38-1.83 (bs, 2.67H , CH=C(C*H*$_3$) and –C(C*H*$_3$)=$CH_2$and 3,4 functionalized), 1.77-1.49 (bs, 5.47H,CH=C(C*H*$_3$) and –C(C*H*$_3$)=CH_2), 1.43-1.09 (bs, 3.12H ,C*H*$_2$-C(Me)), 1.10-0.61 (bs, 3.85H, -CH_2-C(C*H*$_3$)-).

^{13}C-NMR not recorded due to bad solubility of the polymer.

FT-IR (film on CaF_2, cm^{-1}): 3380, 3076, 2970, 2930, 2850, 1642, 1604, 1510, 1440, 1410, 1378, 1262, 1142, 1003, 886.

GPC: M_n (g mol^{-1})= 10900, PDI= 1.8

4.3.7 N,N- DIMETHYL CYSTEAMINE HYDROCHLORIDE MODIFIED POLY(BUTADIENE) (**PB 1**)

1000 mg of poly(butadiene) (18,5 mmol, 1 eq) were diluted in approx. 2 mL of CH_2Cl_2, in parallel N, N- Dimethylcysteamine hydrochloride (524,7 mg, 0,2 eq, 3.7 mmol) was diluted in 2-3 mL MeOH and added in small portions to the solution of the polymer. Adequate amounts of CH_2Cl_2 were added to keep the polymer solution clear. Finally, Lucirin **TPO-L** (30.49 mg, 2 wt%) were added as solution in CH_2Cl_2 and the reaction was initiated by illumination using NovaCure Hg lamp (illumination time: 10 min, 6500 mW cm^{-2}). Unreacted thiol can be easily removed by dilution in CH_2Cl_2 and filtration.

Anal. Calc. for **PB 1**: C: 51.78; H: 9.17; Cl: 16.68; N: 6.71; S: 15.36 for complete conversion. Found: C: 65.91; H: 9.52; N: 3.01; S: 9.52

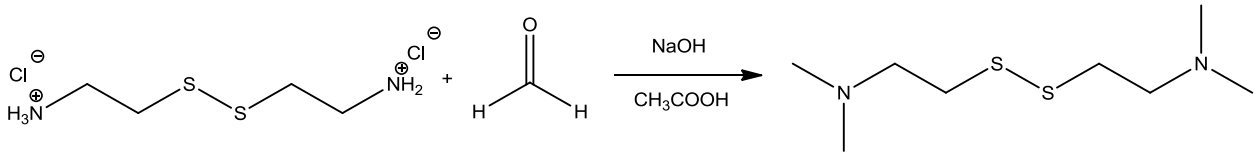
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 7.17 (bs, 1H, toluene) 5.80 (bs, 0.88H, cyclic C*H*=CH_2), 5.58 (bs, 0.39H, 1,2 C*H*=CH_2), 5.37 (bs, 3.07H, *cis* C*H*=C*H*), 4.96 (bs, 3.79H, CH=CH_2), 3.21 (bs, 2.19H, CH_2N^+), 3.00 (bs, 1.78H, $N^+CH_2CH_2S$), 2.85 (s, 6.74H, $(CH_3)_2N^+$), 2.66 (m, 2.67H, 1,2 C*H*-CH=CH_2 and -CH_2-S), 2.36 (bs, 6.39H, C*H*-CH=CH_2), 2.03 (s, 13.92H, CH_2-CH=CH), 1.78-1.03 (m, 2.98H, CH_{cyclic}, C*H*-CH_2, CH-CH_2), 0.88, 0.62 (s, H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 144.2- 142.6 (*C*H=CH_2), 131.6, 131.1, 130.8, 129.9, 128.3, 125.5 (*C*H_2=CH_2, CH_2=*C*H_2, arom.CH_2=CH_2 toluene), 114.5, 113.8, 111.7 (CH=*C*H_2), 57.3 (N^+-CH_2-), 43.5, 43.3, , 42.8, 41.3 (-*C*H-S-CH_2, -*C*H-CH=CH_2), CH, CH_2 cyclic) 43.2 ($N^+(CH_3)_2$), 38.0, 36.7, 36.0, 34.6, 33.9, 32.6, 31.1, 30.0 (CH_2 backbone, *C*H_2-S-CH_2, CH_2-S-*C*H_2), 27.3, 26.5, 25.8 (*C*H-S-CH_2, CH_2 backbone), 17.9, 15.0 (-CH_3).

ATR-IR (cm^{-1}): 3072, 2912, 2850, 2443, 1637, 1452, 1376, 1310, 1240, 1164, 1130, 1017, 993, 963, 908, 748, 698, 470.

GPC: M_n (g mol^{-1})= 2300, PDI= 2.2

4.3.8 Synthesis of N, N, N´, N´- Tetramethyl Cystamine **1**

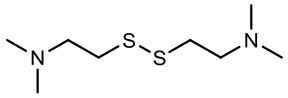


Scheme 4-1 Synthesis of N,N, N´, N´- tetramethyl cystamine

5.0 g (1 eq, 22 mmol) of cystamine dihydrochloride are dissolved in 100 mL of a 1:1 mixture of EtOH and water and cooled in an ice bath. 1.9 g of sodium hydroxide (2 eq, 44 mmol) are added slowly, followed by 4.2 mL acetic acid (5 eq, 110 mmol) drop wise. The ice bath is removed and formaldehyde is added drop wise again (1.9 mL, 3 eq, 66 mmol). The reaction mixture is stirred overnight at an oil bath temperature of 90°C. After cooling the reaction to room temperature, 100 mL of a 10% KOH solution are added and organic constituents are extracted with diethyl ether. The combined organic layers are washed with Brine, water and

are dried with Na_2SO_4, filtered and the solvent evaporated. The yellowish product is dried under vacuum.

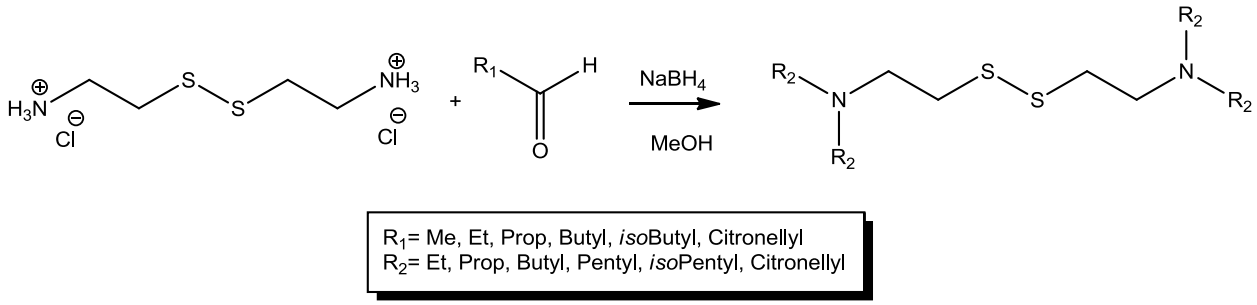
yield: 75.0%



^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.78 (dt, 4H, S-CH_2-), 2.57 (t, 4H, N-CH_2-), 2.22 (s, 6H, N(CH_3)$_2$).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 58.8 (2C, N-*C*H_2-), 45.4 (4C, N(*C*H_3)$_2$), 37.0 (2C, S-*C*H_2-).

4.3.9 Synthesis of Higher N, N, N´, N´-Dialkyl Thioamines

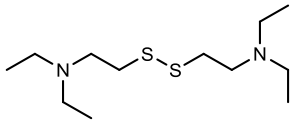


Scheme 4-2 Synthesis of higher N,N,N´,N´-dialkyl thioamines

Generally 1 eq of cystamine dihydrochloride was diluted in methanol, 2,5 eq of aldehyde component and 5 eq of $NaBH_4$ were added carefully. After approximately 15 h of stirring under reflux the same amount of aldehyde and $NaBH_4$ was added once again and the reaction allowed stirring for further 15 h under reflux. Subsequently, the reaction mixture was filtered, the solvent evaporated and the oily residue was dried under vacuum. For valeraldehyde and *iso*-valeraldehyde the amine species was separated from the formed alcohol by basic extraction, followed by acidic extraction. In the case of citronellal-substituted thioamine, purification was performed applying column chromatography using dichloromethane/ methanol in a ration 100:1 (TLC: solvent CH_2Cl_2: MeOH= 20:1, R_f= 0.3).

4.3.9.1 N,N, N´, N´- TETRAETHYL CYSTAMINE **8**

yield: 80.1%



Anal. Calc. for $C_{12}H_{28}N_2S_2$ (264.49): C: 54.49; H: 10.67; N: 10.59; S: 24.25. Found: C: 56.31; H: 10.13; N: 8.89; S: 20.90.

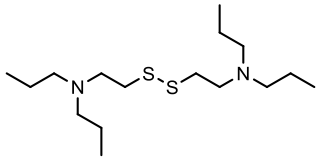
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.72 (s, 8H, N-(CH_2)$_2$-), 2.50 (q, 8H, N-CH_2-, CH_2-S), 0.98 (t, 12H, CH_2-CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 52.3 (2C, N-CH_2-), 47.0 (4C, N-(CH_2)$_2$), 36.4 (2C, S-CH_2-), 11.8 (4C, -CH_2-CH_3).

IR (cm^{-1}): 2965, 2926, 2875, 2809, 1454, 1375, 1292, 1199, 1137, 1066, 991, 915, 780, 736, 505.

4.3.9.2 N,N, N´, N´- TETRAPROPYL CYSTAMINE **9**

yield: 67.9%



Anal. Calc. for $C_{16}H_{36}N_2S_2$ (320.60): C: 59.94; H: 11.32; N: 8.74; S: 20.00. Found: C: 59.50; H: 10.95; N: 8.33; S: 18.92.

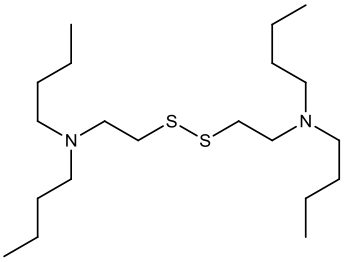
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.77 (s, 8H, N-(CH_2)$_2$-), 2.44 (t, 8H, N-CH_2-, -CH_2-S), 1.45 (h, 8H, CH_2-CH_3), 0.88 (t, 12H, CH_2-CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 56.3 (4C, N-(CH_2-)$_2$), 53.7 (2C, N-CH_2-CH_2-S-), 36.9 (2C, S-CH_2-), 20.5 (4C, N-CH_2-CH_2-CH_3), 11.9 (4C, -CH_2-CH_3).

IR (cm^{-1}): 2958, 2931, 2870, 2805, 1459, 1375, 1289, 1190, 1075, 980, 886, 842, 743, 510.

4.3.9.3 N,N, N´, N´- TETRABUTYL CYSTAMINE **10**

yield: n.d.



Anal. Calc. for $C_{20}H_{44}N_2S_2$ (376.71): C: 63.77; H: 11.77; N: 7.44; S: 17.02. Found: C: 63.32; H: 10.71; N: 7.24; S: 17.08.

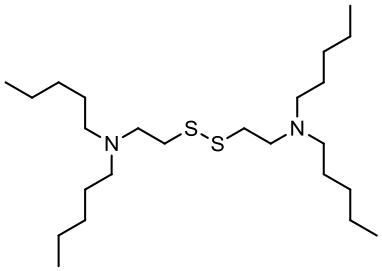
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.77 (s, 8H, N-(CH_2)$_2$-), 2.44 (t, 8H, N-CH_2-, -CH_2-S), 1.55- 1.28 (m, 16H, CH_2-CH_2-CH_3, -CH_2-CH_2-CH_3) 0.88 (t, 12H, CH_2-CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 54.0 (4C, N-(CH_2-CH_2-)$_2$), 53.6 (2C, N-CH_2-CH_2-S-) 36.8 (2C, S-CH_2-), 29.4 (4C, N-CH_2-CH_2-CH_2-), 20.7 (4C, -CH_2-CH_3), 14.1 (4C, -CH_2-CH_3).

IR (cm^{-1}): 2954, 2928, 2865, 2802, 1650, 1459, 1373, 1295, 1177, 1083, 990, 942, 899, 799, 732, 511.

4.3.9.4 N,N, N´, N´- TETRAPENTYL CYSTAMINE **11**

yield: 65.0%



Anal. Calc. for $C_{24}H_{52}N_2S_2$ (432.81): n.d.

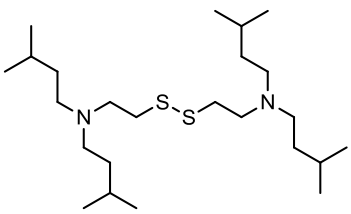
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.78 (s, 8H, N-(CH_2)$_2$), 2.44 (t, 8H, N-CH_2-, -CH_2-S), 1.45- 1.25 (m, 16H, -CH_2-CH_2-CH_3, -CH_2-CH_2-CH_3), 0.89 (t, 12H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): n.d.

IR (cm^{-1}): n.d.

4.3.9.5 N,N, N´, N´- TETRA*ISO*PENTYL CYSTAMINE **12**

yield: 63.6%



Anal. Calc. for $C_{24}H_{52}N_2S_2$ (432.81): C: 66.60; H: 12.11; N: 6.47; S: 14.82. Found: C: 66.41; H: 11.20; N: 6.41; S: 14.90.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 2.78 (s, 8H, N-(CH_2)$_2$-), 2.45 (bs, 8H, N-CH_2-, CH_2-S), 1.65 (h, 4H, -CH(CH_3)$_2$), 1.36 (bs, 8H, -CH_2-CH(CH_3)$_2$), 0.89 (d, 24H, -CH_3).

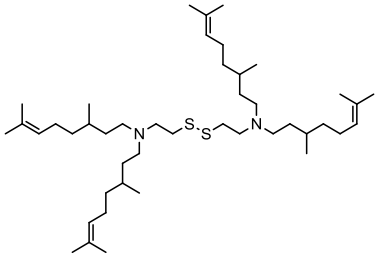
^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 53.5 (2C, -S-CH_2-CH_2-N-), 52.3 (4C, N(CH_2-*iso*pent)$_2$), 36.6 (2C, -S-CH_2-), 36.1 (4C, -CH_2-CH-), 26.4 (4C, -CH(CH_3)$_2$), 22.8 (8C, -CH_3).

IR (cm^{-1}): 2952, 2924, 2867, 2805, 1685, 1462, 1369, 1287, 1173, 1131, 1089, 1034, 980, 939, 757, 511.

4.3.9.6 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N-(3,7-DIMETHYLOCT-6-EN-1-YL)-3,7-DIMETHYLOCT-6-EN-1-AMINE) **13**

yield: 15 %

Anal. Calc. for $C_{44}H_{84}N_2S_2$ (705.28): C: 74.93; H: 12.00; N: 3.97; S: 9.09. Found: C: 75.01; H: 11.38; N: 3.66; S: 9.43.

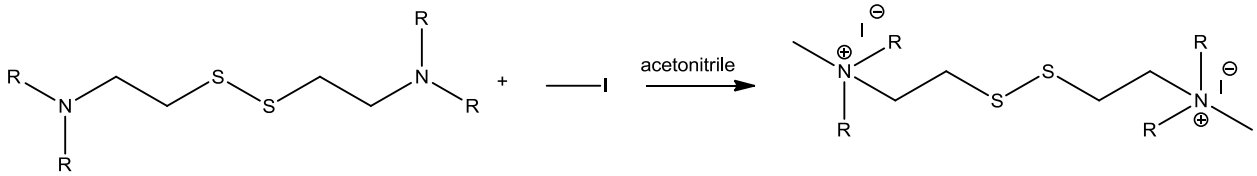


^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.09 (t, 4H, C*H*=C(CH_3)$_2$), 2.78 (s, 8H, -N-(C*H*$_2$)$_2$), 2.44 (bs, 8H, -N-C*H*$_2$-, C*H*$_2$-S), 1.90 (p, 8H, -C*H*$_2$-CH=C), 1.68 (s, 12H, CH(C*H*$_3$)), 1.60 (s, 12H, CH(C*H*$_3$)), 1.46- 1.13 (m, 20H, N-(CH_2-C*H*$_2$)$_2$, -C*H*(CH_3)CH_2, CH-C*H*$_2$-), 0.88 (d, 12H, -CH-C*H*$_3$).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 130.9 (4C, -CH=*C*(CH_3)$_2$), 124.8 (4C, -*C*H=C(CH_3)$_2$), 53.6 (2C, S-CH_2-*C*H_2-N-), 52.1 (4C, -N(*C*H_2-CH_2-)$_2$), 37.3 (4C, -CH(CH_3)-*C*H_2-CH_2CH=), 36.6 (2C, -S-*C*H_2-), 34.1 (4C, N-CH_2-*C*H_2-CH-), 30.8 (4C, -*C*H(CH_3)-), 25.7 (4C, =C(*C*H_3)), 25.5 (4C, -*C*H_2-CH=C), 19.7 (4C, =C(*C*H_3)), 17.6 (4C, -CH(CH_3)).

IR (cm^{-1}): 2956, 2918, 2856, 2806, 2727, 1731, 1675, 1453, 1375, 1289, 1184, 1112, 1084, 1029, 981, 882, 828, 742, 555, 509, 442.

4.3.10 Methylation of Thioamines



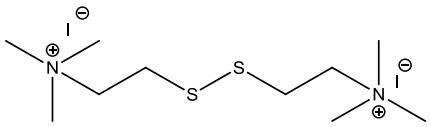
Scheme 4-3 Methylation of thioamines

In a flask 1 eq of N, N, N´, N´- tetraalkyl cystamine was prepared in acetonitrile and cooled with an ice bath. Alkyliodid (2 eq) was diluted in Et_2O and added drop wise via a septum.

Immediately a white precipitate was formed. Nevertheless the solution was allowed to stir overnight. The precipitate was isolated by filtration and dried.

4.3.10.1 2,2'-DISULFANEDIYLBIS(N,N,N-TRIMETHYLETHANAMINIUM) IODIDE **2**

yield: n.d.



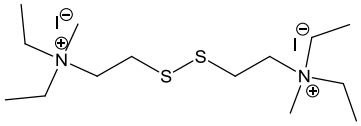
^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.77-3.72 (m, 4H, -C*H*$_2$N$^+$), 3.22 (t, 4H, -C*H*$_2$-S-S-), 3.20 (s, 12H, (C*H*$_3$)$_3$N$^+$).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): 65.3 (2C, CH_2N^+), 53.3 (6C, $(CH_3)_3N^+$), 30.0 (2C, CH_2-S-S).

4.3.10.2 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N-ISOPENTYL-N,3-DIMETHYLBUTAN-1-AMINIUM) IODIDE **14**

yield: 99%

Anal. Calc. for $C_{14}H_{34}I_2N_2S_2$ (548.37): C: 30.66; H: 6.25; I: 46.28; N: 5.11; S: 11.69. Found: C: 31.42; H: 5.87; N: 5.12; S: 11.24.



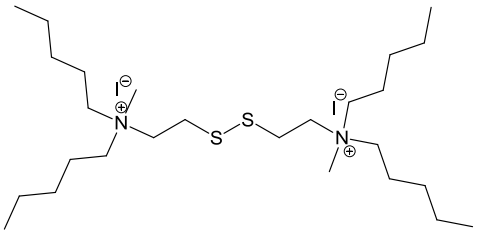
^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.61 (m, 4H, - N^+-C*H*$_2$-CH_2S), 3.38 (q, 8H, N(C*H*$_2$CH$_3$)$_2$), 3.10 (m, 4H, -$SCH_2CH_2N^+$), 3.01 (s, 6H, N^+(C*H*$_3$), 1.36 (t, 12H, (CH_2C*H*$_3$)$_2$).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): 59.3 (4C, N(*C*H$_2$CH$_3$)$_2$), 56.8 (2C, $SCH_2CH_2N^+$), 46.9 (2C, $N(CH_3)$), 29.3 (2C, *C*H$_2$-S-S), 7.1 (4C, N(CH_2*C*H$_3$)$_2$).

IR (cm^{-1}): 2970, 1479, 1449, 1403, 1316, 1250, 1178, 1122, 1090, 1022, 963, 920, 816, 767, 721, 549.

4.3.10.3 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N-METHYL-N-PENTYLPENTAN-1-AMINIUM) IODIDE **17**

yield: 89%



Anal. Calc. for $C_{26}H_{58}I_2N_2S_2$ (716.69): C: 43.57; H: 8.16; I: 35.41; N: 3.91; S: 8.95. Found: C: 40.99; H: 7.35; N: 3.85; S: 8.50.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 3.92 (bs, 4H, N^+-C*H*$_2$-CH_2S), 3.53 (bs, 12H, pent-C*H*$_2$-N^+, -C*H*$_2$-S-S-), 3.36 (s, 6H, N(CH_3)), 1.73 (s, 8H, N-CH_2-C*H*$_2$-), 1.39 (bs, 16H, (C*H*$_2$)$_3$-CH_3), 0.85 (t, 12H, CH_3).

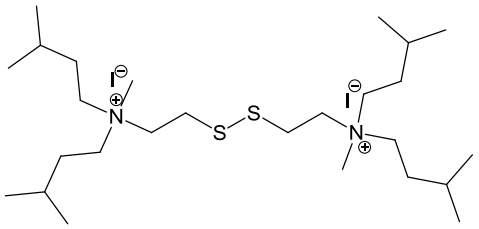
^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 62.0 (4C, pent-*C*H_2-N^+), 61.9 (2C, N^+-*C*H_2-CH_2-S-S-), 49.3 (2C, N^+(CH_3)), 32.3 (2C, -S-S-CH_2-), 28.2 (4C, N^+(CH_2)$_2$*C*H_2-), 22.4 (4C, N^+-CH_2-*C*H_2-), 22.3 (4C, -*C*H_2-CH_3), 13.8 (4C, CH_2-*C*H_3).

IR (cm^{-1}): 2955, 2929, 2865, 2193, 1462, 1379, 1253, 1052, 913, 851, 723, 641.

4.3.10.4 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N-ISOPENTYL-N,3-DIMETHYLBUTAN-1-AMINIUM) IODIDE **18**

yield: 99%

Anal. Calc. for $C_{26}H_{58}I_2N_2S_2$ (716.69): C: 43.57; H: 8.16; I: 35.41; N: 3.91; S: 8.95. Found: C: 39.90; H: 6.71; N: 3.52; S: 7.61.



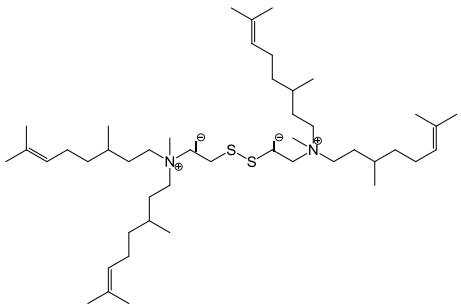
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 3.95 (t, 4H, N^+-CH_2-CH_2S), 3.54 (bs, 12H, *iso*pent-CH_2-N^+, -CH_2-S-S-), 3.36 (s, 6H, N(CH_3)), 1.79-1.68 (m, 4H, N-(CH_2)$_2$-C*H*-), 1.58 (t, 8H, -CH_2-CH), 0.85 (d, 24H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 61.8 (4C, *iso*pent-*C*H_2-N^+), 60.7 (2C, N^+-*C*H_2-CH_2-S-S-), 49.4 (2C, N^+(CH_3)), 32.4 (2C, -S-S-CH_2-), 30.8 (4C, -*C*H_2-CH-), 26.1 (4C, *C*H-(CH_3)$_2$), 22.6 (8C, -CH_3).

IR (cm^{-1}): 2953, 2871, 2190, 1464, 1371, 1256, 1171, 1129, 1054, 998, 916, 885, 769, 726, 641.

4.3.10.5 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N-(3,7-DIMETHYLOCT-6-EN-1-YL)-N,3,7-TRIMETHYLOCT-6-EN-1-AMINIUM) IODIDE **19**

yield: 97%



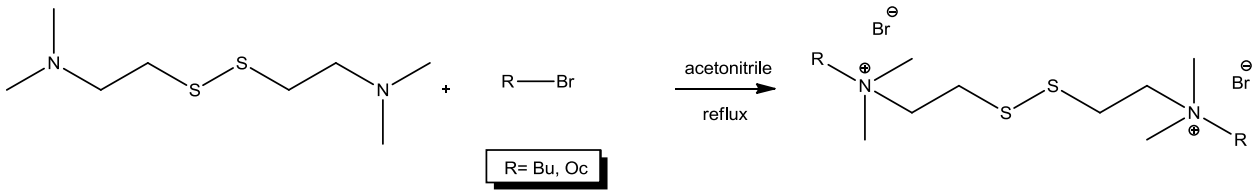
Anal. Calc. for $C_{46}H_{90}I_2N_2S_2$ (989.16): C: 55.58; H: 9.17; I: 25.66; N: 2.83; S: 6.48. Found: C: 56.14; H: 8.55; N: 2.29; S: 5.54.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.06 (t, 4H, C*H*=C(CH_3)$_2$), 3.99 (bs, 4H, N-CH_2-CH_2-S), 3.55- 3.47 (m, 12 H, N^+(CH_2-)$_2$, S-CH_2), 3.40 (s, 6H, N^+(CH_3)), 1.98 (bs, 8H, -CH_2-CH=C), 1.68 (s, 12H, CH(CH_3)), 1.60 (s, 12H, CH(CH_3)), 1.46- 1.13 (m, 20H, N-(CH_2-CH_2-)$_2$, -C*H*(CH_3)CH_2, CH-CH_2-), 0.88 (d, 12H, -CH-CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 131.8 (4C, CH=*C*(CH_3)$_2$), 123.8 (4C, -*C*H=C), 61.8 (4C, N^+(*C*H_2-)$_2$), 60.3 (2C, N^+-*C*H_2-CH_2-S), 49.3 (2C, N^+(*C*H_3)), 36.7 (4C, CH-*C*H_2-), 32.1 (4C, *C*H(CH_3)CH_2), 30.2 (4C, N-(CH_2-*C*H_2-)$_2$), 29.0 (2C, -S-S-*C*H_2-), 25.5 (4C, -CH=C(*C*H_3)), 25.0 (4C, -*C*H_2-CH=C), 19.3 (4C, CH(*C*H_3)), 17.6 (4C, CH=C(*C*H_3).

IR (cm^{-1}): 2959, 2918, 2857, 2728, 1724, 1675, 1455, 1378, 1259, 1189, 1159, 1116, 1054, 987, 948, 884, 827, 769, 547, 510, 443.

4.3.11 Alkylation of N, N, N´, N´- Tetramethyl Cystamine



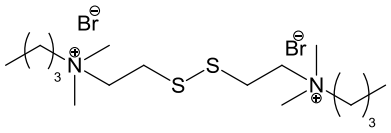
Scheme 4-4 Alkylation of N,N,N´,N´-tetramethyl cystamine

In a flask, 1 eq of educt was diluted in acetonitrile and 2.6 eq of alkyliodid was added slowly. The reaction mixture was allowed to stir at reflux temperature overnight. After that time, a precipitate was formed in the dark reaction mixture, which was filtered, washed with n-pentane and dried.

4.3.11.1 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(N,N-dimethylbutan-1-aminium) Iodide **3**

yield: 41%

Anal. Calc. for $C_{16}H_{38}Br_2N_2S_2$ (482.42): C: 39.83; H: 7.94; Br: 33.13; N: 5.81; S: 13.29. Found: C: 39.42; H: 7.09; N: 5.76; S: 12.79.



^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.68 (dt, 4H, N^+-C*H*$_2$-CH_2S), 3.36 (dt, 4H, but-C*H*$_2$-N^+), 3.17 (dt, 4H, -C*H*$_2$-S-S-), 3.12 (s, 12H, $(CH_3)_2N^+$), 1.77 (p, 4 H, NCH_2-C*H*$_2$-), 1.38 (h, 4H, CH_2-CH_3), 0.95 (t, 6H, CH_3).

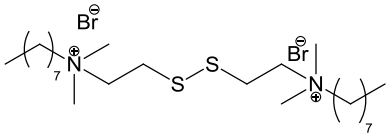
^{13}C-NMR (δ, 20°C, MeOD, 75 MHz): 65.6 (2C, but-*C*H_2-N^+), 64.6 (2C, N^+-*C*H_2-CH_2S), 51.6 (4C, $(CH_3)_2N^+$), 31.7 (2C, -*C*H_2-S-S-), 25.6 (2C, NCH_2-*C*H_2-), 20.7 (2C, *C*H_2-CH_3), 14.0 (2C, CH_2*C*H_3).

IR (cm^{-1}): 3001, 2955, 2870, 1663, 1627, 1462, 1418, 1260, 1174, 1130, 1097, 1057, 1026, 992, 954, 904, 791, 747, 713, 506.

4.3.11.2 N,N'-(DISULFANEDIYLBIS(ETHANE-2,1-DIYL))BIS(N,N-DIMETHYLOCTAN-1-AMINIUM) BROMIDE **4**

yield: 14%

Anal. Calc. for $C_{24}H_{54}Br_2N_2S_2$ (594.64): C: 48.48; H: 9.15; Br: 26.87; N: 4.71; S: 10.78. Found: C: 47.94; H: 8.41; N: 4.64; S: 10.56.



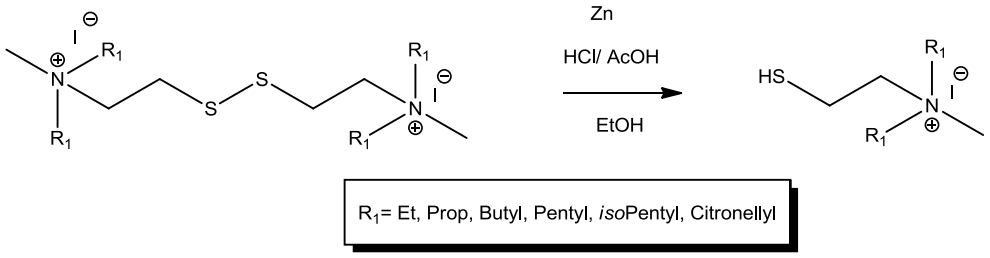
^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 3.68 (dt, 4H, N^+-C*H*$_2$-CH_2S), 3.36 (dt, 4H, oct-C*H*$_2$-N^+), 3.17 (dt, 4H, -C*H*$_2$-S-S-), 3.12 (s, 12H, $(CH_3)_2N^+$), 1.77 (p, 4 H, NCH_2-C*H*$_2$-), 1.35 (bd, 20H, $(CH_2)_5$-CH_3), 0.85 (t, 6H, CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 64.3 (2C, oct-*C*H_2-N^+), 62.8 (2C, N^+-*C*H_2-CH_2S), 50.8 (4C, $N^+(CH_3)_2$), 31.0 (2C, -*C*H_2-S-S-), 29.7 (2C, -*C*H_2-CH_2-CH_3), 28.2 (4C, N^+-$(CH_2)_3$-*C*H_2-$(CH_2)_4$), N^+-$(CH_2)_4$-*C*H_2-$(CH_2)_3$), 25.4 (2C, N^+-

CH_2-*C*H_2-), 22.0 (2C, -*C*H_2-CH_3), 21.9 (2C, $N^+(CH_2)_2CH_2(CH_2)_5$), 13.4 (2C, -CH_3).

IR (cm^{-1}): n.d.

4.3.12 Reduction of quaternary Aminothiols



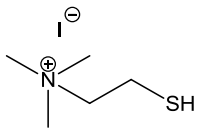
Scheme 4-5 Reduction of quaternary aminothiols

Procedure according to Robinson *et al*.[118]: Typically, 250 mg of the quaternary thioamine were diluted in 10 mL EtOH. Alternating 4 g of zinc and 6 mL of acetic acid were added in small portions and stirred overnight. Zinc and zinc salts were removed by filtration and the solvent evaporated. The precipitate formed during that was again filtered off and the filtrate evaporated to dryness and additionally dried at the vacuum line.

Procedure according to Rose *et al.*[126]: 250 mg of the quaternary thioamine were diluted in 7 mL EtOH and 50 µL of acetic acid were added drop wise, followed by 750 mg zinc and 250 µL HCl 38% alternating and again in small portions. After 5 h of reaction time, the solvent is evaporated to 1.5 mL, diluted with 40 mL $CHCl_3$ and extracted with 10% NaCl solution once. The organic layer was dried with Na_2SO_4, filtrated and the solvent evaporated.

4.3.12.1 2-MERCAPTO-N,N,N-TRIMETHYLETHANAMINIUM IODIDE **5**

yield: n.d.

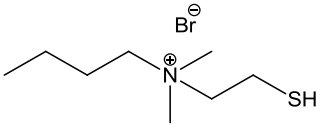


^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.39 (dt, 2H, -CH_2N), 3.07 (s, 6H, $(CH_3)_3N^+$), 2.85 (dt, 2H, -CH_2-SH).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): 52.8 (1C, CH_2N^+), 22.3 (3C, $(CH_3)_3N^+$), 17.5 (1C, CH_2-SH).

4.3.12.2 N-(2-MERCAPTOETHYL)-N,N-DIMETHYLBUTAN-1-AMINIUM IODIDE **6**

yield: n.d. due to acetat content

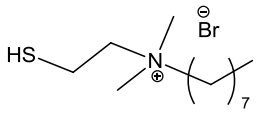


^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.31 (t, 2H, N^+-C*H*$_2$-CH_2S), 3.21 (t, 2H, but-C*H*$_2$-N^+), 2.99 (s, 6H, $(CH_3)_2N^+$), 2.76 (t, 2H, -C*H*$_2$-SH), 1.67 (p, 2 H, NCH_2-C*H*$_2$-), 1.34 (h, 2H, C*H*$_2$-CH_3), 0.93 (t, 3H, CH_3).

^{13}C-NMR (δ, 20°C, MeOD, 75 MHz): 66.7 (1C, N^+-*C*H_2-CH_2SH), 65.9 (1C, butyl-*C*H_2-N^+), 51.6 (2C, $N^+(Me)_2$), 25.6 (2C, $N^+CH_2CH_2CH_2$), 21.1 (1C, SH-*C*H_2-), 20.8 (2C, -*C*H_2CH_3), 14.0 (2C, CH_2CH_3).

4.3.12.3 N-(2-MERCAPTOETHYL)-N,N-DIMETHYLOCTAN-1-AMINIUM BROMIDE **7**

yield: only for NMR

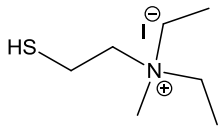


^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.69 (dt, 4H, N^+-C*H*$_2$-CH_2SH), 3.23 (dt, 2H, but-C*H*$_2$-N^+), 3.10 (s, 6H, $(CH_3)_2N^+$), 3.02 (dt, 2H, -C*H*$_2$-SH), 1.73 (bs, 2 H, NCH_2-C*H*$_2$-), 1.27 (bd, 10H, (C*H*$_2$)$_5$-CH_3), 0.83 (t, 3H, CH_3).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): n.d.

4.3.12.4 N,N-DIETHYL-2-MERCAPTO-N-METHYLETHANAMINIUM IODIDE **20**

yield: 61%

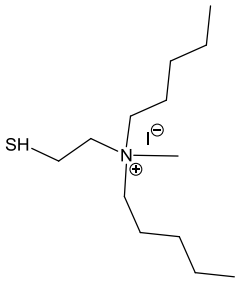


^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.36 (dt, 2H, $N^+C\mathit{H}_2$-CH_2SH), 3.27 (q, 4H, $N^+(C\mathit{H}_2$-$CH_3)_2$), 2.90 (s, 3H, N^+-CH_3), 2.82 (dt, 2H, $C\mathit{H}_2$-SH), 1.22 (t, 6H, -CH_2-$C\mathit{H}_3$).

^{13}C-NMR: n.d.

4.3.12.5 N-(2-MERCAPTOETHYL)-N-METHYL-N-PENTYLPENTAN-1-AMINIUM IODIDE **23**

yield: 55%

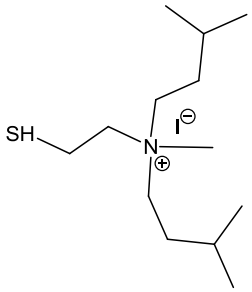


^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 3.69 (bs, 2H, N^+-$C\mathit{H}_2$-CH_2SH), 3.33 (bs, 4H, pent-$C\mathit{H}_2$-N^+), 3.21 (s, 3H, $(CH_3)_2N^+$), 3.10 (bs, 2H, -$C\mathit{H}_2$-SH), 1.73 (bs, 4H, NCH_2-$C\mathit{H}_2$-), 1.35 (bs, 12H, $(C\mathit{H}_2)_3$-CH_3), 0.90 (t, 6H, CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 62.3 (1C, N^+-$\mathit{C}H_2$-CH_2S), 56.2 (2C, pent-$\mathit{C}H_2$-N^+), 49.5 (N^+CH_3), 28.4 (2C, $N^+CH_2\mathit{C}H_2$), 22.4 (1C, SH-CH_2), 22.3 (4C, N^+-CH_2-$\mathit{C}H_2$-, -$\mathit{C}H_2CH_3$), 13.9 (2C,-$\mathit{C}H_3$).

4.3.12.6 N-ISOPENTYL-N-(2-MERCAPTOETHYL)-N,3-DIMETHYLBUTAN-1-AMINIUM IODIDE **24**

yield: 65%

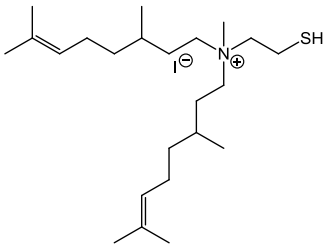


^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 3.70 (bs, 2H, N^+-C*H*$_2$-CH_2SH), 3.43 (bs, 4H, *iso*pent-C*H*$_2$-N^+), 3.28 (s, 3H, (CH_3)N^+), 3.10 (bs, 2H, -C*H*$_2$-SH), 1.72-1.60 (m, 2H, N^+-(CH_2)$_2$-C*H*-), 1.60 (t, 4H, -C*H*$_2$-CH-), 0.99 (d, 12H, -CH_3).

^{13}C-NMR (δ, 20°C, $CDCl_3$, 75 MHz): 60.6 (1C, N^+-*C*H_2-CH_2SH), 55.5 (2C, *iso*pent-*C*H_2-N^+), 49.6 (N^+(CH_3)), 30.6 (2C, -*C*H_2-CH-), 26.4 (1C, -*C*H_2-SH), 26.2 (2C, N^+-(CH_2)$_2$-*C*H-), 22.6 (4C, -CH_3).

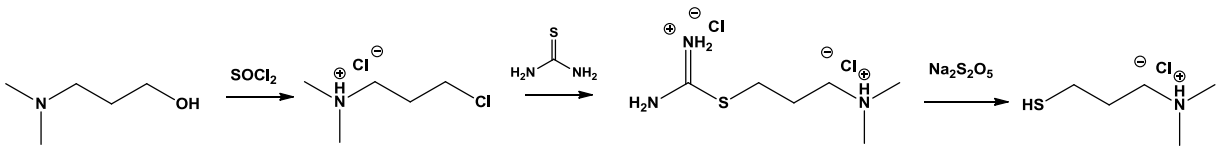
4.3.12.7 N-(3,7-DIMETHYLOCT-6-EN-1-YL)-N-(2-MERCAPTOETHYL)-N,3,7-TRIMETHYLOCT-6-EN-1-AMINIUM IODIDE **25**

yield: n.d.



^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 5.07 (t, 2H, C*H*=C(CH_3)$_2$), 3.66-2.87 (m, 12H, N-C*H*$_2$-CH_2-S, N^+(C*H*$_2$-)$_2$, S-C*H*$_2$, N^+(C*H*$_3$)), 1.98 (bs, 4H, -C*H*$_2$-CH=C), 1.68 (s, 6H, CH(C*H*$_3$)), 1.60 (s, 6H, CH(C*H*$_3$)), 1.46- 1.13 (m, 20H, N-(CH_2-C*H*$_2$-)$_2$, -C*H*(CH_3)CH_2, CH-C*H*$_2$-), 0.88 (d, 6H, -CH-C*H*$_3$).

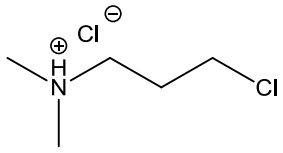
4.3.13 Synthesis of 3-Mercapto-N,N-dimethylpropan-1-aminium Chloride **28**



Scheme 4-6 Synthesis of 3-mercapto-N,N-dimethylpropan-1-aminium chloride

3-chloro-N,N-dimethylpropan-1-aminium chloride: A solution of 3.00 g of 3-dimethylaminopropanol (3.44 mL, 29.08 mmol, 1 eq) in 45 mL $CHCl_3$ was prepared and cooled with an ice bath. Thionyl chloride (4.84 g, 2.94 mL, 40.7 mmol, 1.4 eq) was added drop wise under stirring. Upon complete addition, the reaction mixture was refluxed for 2.5 h, dissipating the developed HCl. After complete reaction, 30 mL MeOH were added and again stirred for 15 min and the solvent removed, obtaining a yellow precipitate, which was recrystallized from 50 mL Et_2O.

yield: 4.14 g (90.2 %)



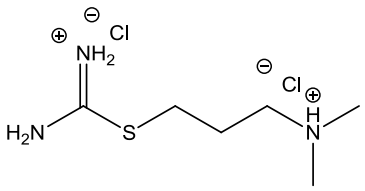
Anal. Calc. for $C_5H_{13}Cl_2N$ (158.07): C: 37.99; H: 8.29; Cl: 44.86; N: 8.86. Found: C: 38.13; H: 8.22; N: 8.86.

^{1}H-NMR (δ, 20°C, MeOD, 300 MHz): 3.68 (t, 2H, Cl-C*H*$_2$-), 3.29- 3.16 (m, 2H, N^+-C*H*$_2$-), 2.83 (d, 6H, $N^+(CH_3)_2$). 2.42- 2.38 (m, 2H, -CH_2-).

^{13}C-NMR (δ, 20°C, MeOD, 75 MHz): 56.1 (1C, N^+-*C*H_2), 43.2 (2C, $N^+(CH_3)_2$), 41.8 (1C, Cl-CH_2-), 27.2 (1C, -CH_2-).

2-(3-(dimethylammonio)propyl)isothiouronium chloride: 500 mg (3.16 mmol, 1 eq) of the previously synthesized 3-chloro-N,N-dimethylpropan-1-aminium chloride was diluted in 2 mL H_2O together with 240.8 mg (3.16 mmol, 1 eq) of thiourea under reflux conditions. After stirring overnight, the solvent was removed and the product recrystallized from 2 mL ethanol upon addition of 4 mL Et_2O.

yield: 439.9 mg (59.4 %)



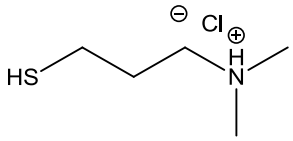
Anal. Calc. for $C_6H_{17}Cl_2N_3S$ (234.19): C: 30.77; H: 7.32; Cl: 30.28; N: 17.94, S: 13.69 Found: C: 31.45; H: 7.34; N: 17.98; S: 14.59.

^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.36- 3.25 (m, 4H, S-CH_2-, N^+-CH_2-), 2.95 (s, 6H, $N^+(CH_3)_2$), 2.27-2.17 (m, 2H, -CH_2-).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): 182.0 (1C, $NH_2C(NH_2^+)S$-), 55.8 (1C, N^+-CH_2-), 43.0 (2C, $N^+(CH_3)_2$), 27.6 (1C, -CH_2-), 23.6 (1C, CH_2S-).

3-mercapto-N,N-dimethylpropan-1-aminium chloride: 2-(3-(dimethylammonio)propyl) isothiouronium chloride was used (400 mg, 1 eq, 1,71 mmol) and reacted with 389.6 g (2.04 mmol, 1.2 eq) of sodium pyrosulfite in 5 mL H_2O overnight under reflux conditions. The solvent was removed and the raw product was suspended in methanol, heated and insoluble parts removed by filtration and the filtrate precipitated with Et_2O. After crystallization, a white, fine powder was obtained.

yield: 116.5 mg (43.8 %)



^{1}H-NMR (δ, 20°C, D_2O, 300 MHz): 3.31 (dt, 2H, SH-CH_2-), 3.18 (t, 2H, N^+CH_2), 2.92 (s, 6H, $N^+(CH_3)_2$), 2.25 (p, 2H, -CH_2-).

^{13}C-NMR (δ, 20°C, D_2O, 75 MHz): 56.7 (1C, N^+-CH_2-), 43.2 (2C, $N^+(CH_3)_2$), 31.8 (1C, -CH_2-), 24.1 (1C, SH-CH_2-).

4.3.14 Preparation of antibacterial lacquers

4.3.14.1 Preparation of Natural Rubber Films

10 mg mL^{-1} solutions of natural rubber were prepared and spincoated on CaF_2 discs (2x 200 µL, 800 rpm, 30 s) and illuminated using an Oriel Research Lamp Housing Model 66921.

4.3.14.2 PREPARATION OF FILM COATING **COAT 1**- **COAT 5**

Formulations were prepared according to the respective recipes. All coatings were prepared in CH_2Cl_2, except for **COAT 2**, which was prepared in toluene.

Table 4-1 Coating formulations of COAT 1- COAT 5

COAT 1	**NR**	100 phr	0.5 eq	50.0 mg
	PB 1	122 phr	0.5 eq	60.8 mg
	PETMP	36 phr	0.03 eq	18.0 mg
	TPO-L	7 phr	20 wt%	3.6 mg
COAT 2	**NR**	100 phr	0.8 eq	2.00 g
	PB 1	25 phr	0.2 eq	0.50 g
	PETMP	25 phr	0.04 eq	0.50 g
	TPO-L	6 phr	25 wt%	0.13 mg
COAT 3	**NR**	100 phr	0.5 eq	500.0 mg
	PB 1	122 phr	0.5 eq	608.1 mg
	PETMP	36 phr	0.03 eq	179.9 mg
	TPO-L	0.7 phr	2 wt%	3.6 mg
COAT 4	**NR**	100 phr	0.5 eq	500.0 mg
	PB 1	122 phr	0.5 eq	608.1 mg
	PETMP	36 phr	0.03 eq	179.9 mg
	TPO-L	7 phr	20 wt%	36.3 mg
COAT 5	**NR**	100 phr	0.9 eq	10.00 g
	PB 1	20 phr	0.1 eq	2.00 g
	PETMP	27 phr	0.03 eq	2.68 g
	TPO-L	5 phr	20 wt%	0.54 g

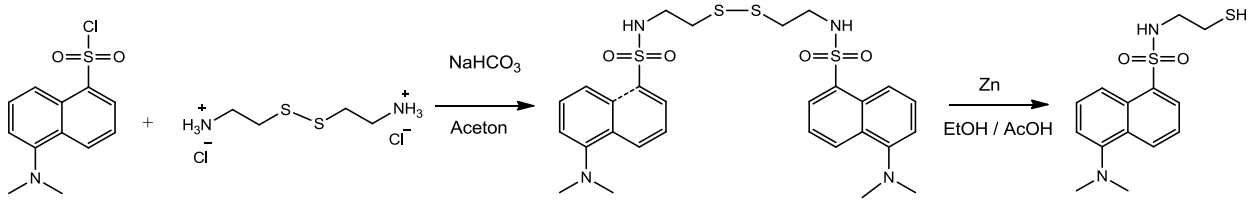
COAT 1 was spin coated on CaF_2 discs (2x 200 µL, 800 rpm, 30 s) and illuminated using an Oriel Research Lamp Housing Model 66921.

COAT 2 was spin coated on 4x4 cm glass substrates (300 µL, 1500 rpm, 30 s) and illuminated using an Oriel Research Lamp Housing Model 66921 for 15 min.

COAT 3 and **COAT 4** were spin coated on 2x2 cm glass substrates (2x 300 µL, 500 rpm, 30 s) and illuminated using an ozone-free mercury lowpressure lamp (EXFO EFOS Novacure) with an irradiation dose of 9000 mW cm^{-2}. All films were prepared and measured as duplicates, mean values were used for curves.

COAT 5 was prepared by soaking natural rubber in CH_2Cl_2, adding the other components and pouring the mixture in a 15x15 cm mold. The coating was predried at 60°C for 20 min and then illuminated using an Oriel Research Lamp Housing Model 66921 for 15 min.

4.3.15 Synthesis of Dansyl-Cysteamine

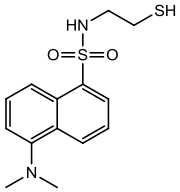


Scheme 4-7 Synthesis of dansyl-cysteamine

994.8 mg of dansyl chloride (3.7 mmol, 1 eq) were dissolved in 275 mL acetone and 7.5 mL H_2O. Cystamine dihydrochloride (418.4 mg, 1.8 mmol, 0.5 eq) was dissolved in 25 mL $NaHCO_3$ and added drop wise whereas pH value was kept to 7.5- 8 by adding NaOH solution (controlled via pH indicator stripes). Reaction progress was monitored via TLC in CH_2Cl_2 : MeOH = 20:1 (R_f = 0,75) until no further dansyl chloride was detected. After 2.5 hours, 100 mL $CHCl_3$ were added and the solution was extracted with saturated $NaHCO_3$ solution 4 times, followed by distilled water. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining product was dried, yielding 855.1 mg (74.8%) of a fluffy, crispy yellow solid.

Dansyl cystamine was diluted in 200 mL EtOH. The yellowish solution turned greenish- blue after the alternating addition of 6.1 g Zn and 12.5 mL acetic acid. Again the reaction process was monitored via TLC in acetone : cyclohexane = 3+1. As educt was still present after 4 hours, the reaction was stirred overnight. Afterwards solids were removed by filtration and the solvent evaporated. The residue was redissolved in 100 mL $CHCl_3$ and extracted with H_2O dist. three times, followed by saturated $NaHCO_3$ solution and brine. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a yellowish-greenish film.

yield: 745.4 mg (86 %)



^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 8.57 (d, 1H, *arom*), 8.26 (dt, 2H, *arom*), 7.55 (p, 2H, *arom*), 7.20 (d, 1H, *arom*), 5.13 (t, 1H, N*H*), 3.08 (q, 2H, CH_2N), 2.89 (s, 6H, N(CH_3)$_2$), 2.53 (dq, 2H, CH_2S), 1.20 (t, 1H, S*H*).

4.3.16 DANSYL- FUNCTIONALIZED POLY(ISOPRENE) **PI 4**

50 mg (1 eq, 0.74 mmol) of poly(isoprene) were reacted with 114.1 mg of dansyl-cysteamine (0.5 eq, 0.37 mmol) in 2 mL of $CHCl_3$. The reaction was initiated upon addition of 0.6 mg (0.1 mol% in respect to thiol) **AIBN** and allowed to stir for 72 h. After that time, the solvent was evaporated and the polymer purified upon precipitation in MeOH, filtered and dried.

yield: n.d.

^{1}H-NMR (δ, 20°C, $CDCl_3$, 300 MHz): 8.54 (dd, 1H, *arom*), 8.26 (t, 1.84H, *arom*), 7.53 (q, 2.24H, *arom*), 7.17 (dd, 1.15H, *arom*), 5.88-5.51 (bs, 0.50H, -C*H*=CH_2), 5.23 (bs, 0.65H, NH), 5.23-4.77 (m, 0.97H, -C*H*=C(Me)-, -CH=CH_2), 4.75-4.39 (d, 5.25H, -C(Me)=CH_2), 3.03 (bs, 1.73H, CH_2N-dansyl), 2.88 (s, 5.82H, N(CH_3)$_2$), 2.48 (bs, 1.81H, CH_2S-dansyl, CH_2-S-CH_2 and CH_2-S-C*H*), 2.36-1.72 (bs, 9.60H, CH and -CH_2- 1,4 and 3,4 functionalized), 1.71-1.46 (bs, 22.11H, -CH=C(CH_3) and –C(CH_3)=CH_2), 1.46-1.06 (bs, 16.94H, CH_2-C(Me)-), 1.05-0.73 (bs, 22.53H, -CH_2-C(CH_3)-).

IR (cm^{-1}): 3307, 3070, 2925, 2864, 2782, 1642, 1578, 1450, 1402, 1377, 1324, 1260, 1198, 1144, 1071, 1020, 943, 882, 791, 683, 625, 570, 536, 496, 470.

GPC: M_n (g mol^{-1})= 30900, PDI= 1.9

5 APPENDIX

5.1 LIST OF ABBREVIATIONS

AIBN	2,2´- Azobisisobutyronitrile
APT	Attached Proton Test
ATR-IR	Attenuated total reflectance
bs	broad singlet
C12SH	Dodecanthiol
CFU	Colony Forming Unit
CH_2Cl_2	Dichloromethane
COAT	Coating
cys	Cysteamine
$cysH^+$	Cysteamine hydrochloride
d	doublet
Da	Dalton
DMPA	2,2-Dimethoxy-2-phenylacetophenone
eq	equivalent
Et	Ethyl
FT-IR	Fourier Transform Infrared Spectroscopy
GPC	Gel Permeation Chromatography
IEP	Isoelectric Point
JIS	Japanese Industrial Standard
m	multiplet
MEDA	N, N- Dimethylcysteamine hydrochloride
MeOH/ MeOD	Methanol/ deuterated Methanol
M_n	Number Average Molecular Weight
M_w	Mass-average Molecular Weight
NMR	Nuclear Magnetic Resonance
NR	Natural Rubber
PB	Poly(Butadiene)
PDI	Polydispersity Index
PE	Poly(ethylene)
PETMP	Pentaerythritol tetrakis(β-thiopropionate)
phr	Parts per Hundred Rubber
PI	Poly(Isoprene)
PP	Poly(Propylene)

ppm	parts per million
q	quadruplet
QUAT	Quaternary Ammonium Cation
RT	Room Temperature
SBR	Styrene-Butadiene Rubber
STA	Simultaneous Thermo Analysis
t	triplet
TGA	Thermal Gravimetric Analysis
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TPO-L	Ethyl-(2,4,6- trimethylbenzoyl)phenylphosphinate (Lucirin® TPO-L)
wt%	Weight Percent

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