Biocompatible Ultrathin Coatings from Isocyanate Terminated Star PEG Prepolymers

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der Rheinisch-Westfälischen Technischen Hochschule Aachen zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

vorgelegt von

Diplom-Chemiker

Thomas Ameringer

aus Ulm (Donau)

Berichter: Universitätsprofessor Martin Möller
Universitätsprofessorin Doris Klee

Tag der mündlichen Prüfung: 14. 06. 2006

Die Natur ist unerbittlich und unveränderlich,
und es ist ihr gleichgültig,
ob die verborgenen Gründe und Arten ihres Handelns
dem Menschen verständlich sind oder nicht.

Galileo Galilei

Contents

List of Abbreviations

θ_{adv}	advancing contact angle
ΔG_m	free energy of mixing
ΔH_m	enthalpy of mixing
θ_{rec}	receding contact angle
ΔS_m	entropy of mixing
μ-star	mikto arm star polymer
A	area per molecule
Å	Angstroem
AA	acrylic acid
AAC	acrylic acid chloride
AFM/SFM	atomic/scanning force microscopy
ASTM	American Society for Testing and Materials
ATRP	atom transfer radical polymerization
$(AB)_x$	radial arm star polymer
A_xB_y	mikto arm star polymer (2 different arm types)
BO	butylene oxide
C_{60}	Buckminster Fullerene
D	polydispersity index ($D = M_w / M_n$)
DABCO	1,4-diazabicyclo[2.2.2]octane
DBTDL	dibutyl tin dilaurate
DSC	differential scanning calorimetry
DVB	divinyl benzene
EDA	ethylene diamine
ELISA	Enzyme-linked immunosorbent assays
EO	ethylene oxide
FITC	Fluorescein isothiocyanate
GC	Gas chromatography
GTP	group transfer polymerization
HDI	hexamethylene diisocyanate
HEUR	hydrophobically modified ethoxylated urethanes
HPLC	High Performance Liquid Chromatography

IPDI	5-isocyanatomethyl-3,3,5-trimethyl-1-cyclohexyl isocyanate
IR	infrared (spectroscopy)
KDL	short path distillation apparatus
LB	Langmuir Blodgett
M	monomer
MALDI-TOF MS	matrix assisted laser desorption by ionization - time of flight mass spectrometry
M_c	molecular weight between two crosslinks
M_n	number average molecular weight
M_w	weight average molecular weight
N	number of star arms
NCO	isocyanate endgroup
NIPAM	N-isopropyl acrylamide
NMR	nuclear magnetic resonance
OH	hydroxyl endgroup
P(L-Lac)	poly(L-lactide)
P2VP	poly(2-vinylpyridine)
PAMAM	poly(amido amine) dendrimer
PB	poly(butadiene)
PBO	poly(butylene oxide)
PBS	phosphate buffered saline
PDMS	poly(dimethyl siloxane)
PDVS	poly(divinyl sulfone)
PE	poly(ethylene)
PEG	poly(ethylene glycol)
PEO	poly(ethylene oxide)
PI	poly(isoprene)
pI	isoelectric point
PLL	poly(L-Lysine)
PMMA	Poly(methyl methacrylate)
PMMA	poly(methyl methacrylate)
pMU	primary monourethane
PO	propylene oxide
PPO	poly(propylene oxide)

PS	poly(styrene)
PS-*b*-PEO	poly(styrene)-*block*-poly(ethylene oxide)
PS_n-*b*-PEO_m	mikto-poly(styrene)-*block*-poly(ethylene oxide)
PU	polyurethane
PVC	poly(vinyl chloride)
QCM	quarz micro balance
RGD	arginine-glycine-aspartate
ROMP	Ring-opening metathesis polymerization
ROMP	controlled ring-opening metathesis polymerization
SAM	self assembled monolayer
SEC	Size Exclusion Chromatography
SFM	scanning force microscopy
sMU	secondary monourethane
T	temperature
TAC	“thiophilic” adsorption chromatography
TAEA	tris-aminoethyl amine
TDI	toluene diisocyanate
TEA	triethylamine
Tg	glass transition temperature
TGA	thermal gravimetric analysis
THF	tetrahydrofurane

Chapter 1

Introduction

1.1 Fabrication of DNA microarrays

The use of microarray-based technology is growing rapidly and has had considerable impact in genomic and proteomic research.[1,2] One crucial component of microarray technology is the surface chemistry of the substrate. The chemistry should be suitable for spotting and immobilizing a variety of biological active molecules (DNA, proteins, and cells) such that their biomolecular interactions may be evaluated. Therefore, strong emphasis is placed on developing innovative chemistries that provide high binding capacity, efficient hybridization, low background, good spot uniformity, and stability. A variety of surface chemistries have been described for DNA microarray fabrication. These include in situ synthesis of DNA directly on glass substrates by photolithography or ink-jet printing technology[3-5] and the immobilization of presynthesized DNA to the substrate surface by chemical or physical attachment.[6-13] The chemical attachment requires activation of the substrate surface with cross-linking reagents and modification of DNA probes with reactive groups.[6-11] While the covalent bonding of DNA on the slide surface usually provides good stability and reproducibility, surface derivatization and the use of cross-linker reagents involves the use of toxic chemicals. The modifications of DNA probes with active groups also add considerable expense. Physical attachment occurs through noncovalent interactions (i.e., hydrophobic interactions, electrostatic interactions, and entrapment in porous structures) between the DNA and the surface coatings of the substrate used for fabrication of DNA microarrays. The use of poly(L-lysine) (PLL) and aminosilane coatings are examples of this approach.[12-14] These methods do not require terminal modifications of DNA

probes and are easy to handle. However, it has been reported that these methods have low binding capacity and result in experimental inconsistencies that beget inconclusive data interpretation.[13] The thickness of the coating film deposited on the slide substrate is also an important factor for microarray performance. Two-dimensional (2-D) and three-dimensional (3-D) films have been used thus far for microarray fabrication. The 2-D coatings are usually monolayers of organic molecules containing active groups, such as thiol,[6] amine,[11-14] aldehyde, and epoxy[7-9,15] which bind DNA probes. These 2-D coatings are usually less than 10 nm thick. Here spacer arms longer than C12 are necessary in the oligonucleotide probes in order to improve the accessibility of target DNA.[7-9,15] The DNA microarrays fabricated using 2-D monolayer coatings have the advantages of good reproducibility and low background signal under fluorescent detection but have the disadvantages of low binding capacity and hybridization efficiency as well as narrow dynamic ranges. The 3-D coatings are usually constructed by depositing thick polymer films on slide supports. The 3-D platforms for microarray fabrication include acrylamide gel pads or gelatin pads structured by photolithography,[16,17] aldehyde activated agarose film,[18] hydrogel polymer,[19] and nitrocellulose film.[20] The thickness of these 3-D coatings is usually above the micrometers level. The thick polymeric films increase the number of coupling sites by introducing additional reactive groups through branched linker molecules, which can provide higher probe binding capacity, and thus give higher signal intensity and wider dynamic ranges. However, compared to 2-D coatings, the 3-D coatings have lower reproducibility and a higher background signal caused by autofluorescence of the polymer materials. The great demand for new chemistry which provides reliable attachment of DNA for various functional analyses, as well as the need for improved biocompatible layers, was the motivation for the development of multifunctional ethylene glycols supporting a densely packed network which easily can be derivated for further applications

1.2 Contents of this Thesis

This thesis concerns the synthesis and coatings of functionalized six arm star shaped poly(ethylene oxide-*co*-propylene oxide)s in order to control bioadhesion of proteins cells and oligonucleotides.

Chapter 2 gives an overview over existing literature concerning star polymer formation and the biopassivation of model substrates.

Chapter 3 shows the functionalization of the six arm star polymers with isophorone diisocyanate (IPDI) emphasizing the prevention of chain extension.

Chapter 4 describes the preparation of ultrathin hydrogel layers out of the polymer synthesized in chapter 3.

Chapter 5 deals with the swelling behavior of these hydrogels under aqueous conditions. The hydrogels were examined in bulk and covalently bound on model substrates.

Chapter 6 shows the end functionalization of the hydroxyl terminated star polymers with acrylic acid derivates. In a first series of experiments ultrathin hydrogel layers were prepared with these polymers.

Chapter 7 deals with synthesis of hydrogel layers via a layer-by-layer technique. IPDI terminated star polymers were bound to the surface and quenched with water. Further attempts to bind further layers of polymer onto these amino terminated layers were unsuccessful.

Chapter 8 describes the synthesis of vinyl sulfone terminated starpolymers. The hydroxyl terminated polymer was reacted with an excess of divinyl sulfone and the single stars purified by extraction with -20 °C cold divinyl ether.

Chapter 9 shows the hybridization of 20mer oligonucleotides spotted onto IPDI terminated star polymer layers. First amino terminated single strains were covalently attached to the NCO-terminated surface by spotting or stamping of a diluted solution. The proof for the hybridization of the fluorescein labeled complementary strain was confirmed by fluorescence measurements.

1.3 References

(1) Lander, E. S. *Nat. Genet. Suppl.* **1999**, *21*, 3-4.
(2) Lockhart, D. J.; Winterer, E. A. *Nature* **2000**, *405*, 827-836.
(3) Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science* **1991**, *251*, 767-773.
(4) Hacia, J. G.; Fan, J. B.; Ryder, O.; Jin, L.; Edgemon, K.; Ghandour, G.; Mayer, R. A.; Sun, B.; Hsie, L.; Robbins, C. M.; Brody, L. C.; Wang, D.; Lander, E. S.; Lipshutz, R.; Fodor, S. P. A.; Collins, F. S. *Nat. Genet*. **1999**, *22*, 164-167.

(5) Lipshutz, R.; Fodor, S. P. A.; Gingeras, T. R.; Lockhart, D. J. *Nat. Genet.* **1999**, *21*, 20-24.
(6) Rogers, Y. H.; Baucom, P. J.; Huang, Z. J.; Bogdanov, V.; Anderson, S.; Boyce-Jacino, M. T. *Anal. Biochem.* **1999**, *266*, 23-30.
(7) Dolan, P. L.; Wu, Y.; Ista, L. K.; Metzenberg, R. L.; Nelson, M. A.; Lopez, G. P. *Nucleic Acids Res.* **2001**, *29*, e107.
(8) Lindroos, K.; Liljedahl, U.; Raitio, M.; Syvänen, A. *Nucleic Acids Res.* **2001**, *29*, e69.
(9) Guo, Z.; Guilfoyle, R. A.; Thiel, A. J.; Wang, R.; Smith, L. M. *Nucleic Acids Res.* **1994**, *22*, 5456-5465.
(10) Lee, P. H.; Sawan, S. P.; Modrusan, Z.; Arnold, L. J.; Reynolds, M. A. *Bioconjugate Chem.* **2002**, *13*, 97-103.
(11) Podyminogin, M. A.; Lukhtanov, E. A.; Reed, M. W. *Nucleic Acids Res.* **2002**, *29*, 5090-5098.
(12) Belosludtsev, Y.; Iverson, B.; Lemeshko, S.; Eggers, R.; Wiese, R.; Lee, S.; Powdrill, T.; Hogan, M. *Anal. Biochem.* **2001**, *292*, 250-256.
(13) Lemeshko, S. V.; Powdrill, T.; Belosludtsev, Y. Y.; Hogan, M. *Nucleic Acids Res.* **2001**, *29*, 3051-3058.
(14) Benters, R.; Niemeyer, C. M.; Drutschmann, D.; Blohm, D.; Wöhrle, D. *Nucleic Acids Res.* **2002**, *30*, e10.
(15) Zhao, X. D.; Nampalli, S.; Serino, A. J.; Kumar, S. *Nucleic Acids Res.* **2001**, *29*, 955-959.
(16) Proudnikov, D.; Timofeev, E.; Mirzabekov, A. D. *Anal. Biochem.* **1998**, *159*, 34-41.
(17) Ermantraut, E.; Wohlfart, K.; Woelfl, S.; Schulz, T.; Koehler, M. In *Microreaction Technology- Proceedings of the First International Conference on Microreacion Technology*; Ehrenfeld, W., Ed.; Spring Verlag: Heidelberg, 1997; pp 332-339.
(18) Afanassiev, V.; Hanemann, V.; Wölfl, S. *Nucleic Acids Res.* **2000**, *28*, e66.
(19) Kodadek, T. *Chem. Biol.* **2001**, *8*, 105-115.
(20) Stillman, B. A.; Tonkinson, J. L. *Biotechniques* **2000**, *29*, 630-635.

Chapter 2

Star Polymers for Bioactive Films

2.1. Introduction

Branched polymers, polymer chains tethered to a central point or line, with their novel macromolecular, physical and chemical properties have led to a new variety of applications. Categorized by their number of arms and placement of the junctions they can be categorized into the following groups (Figure 2.1). Concerning the branched polymers, which form by further branching of the arms, the structural order increases form random branched to the dendrimers, which exhibit almost perfect structural homogeneity.

The first part of this literature review concerns the synthesis of branched polymers; the second part reports the formation of films, both with special focus on poly(alkylene oxide) polymers and their passive effects onto cells and proteins. Other branched architectures, i.e. the dendrimers and comb polymers are outlined shortly for comparative purposes.

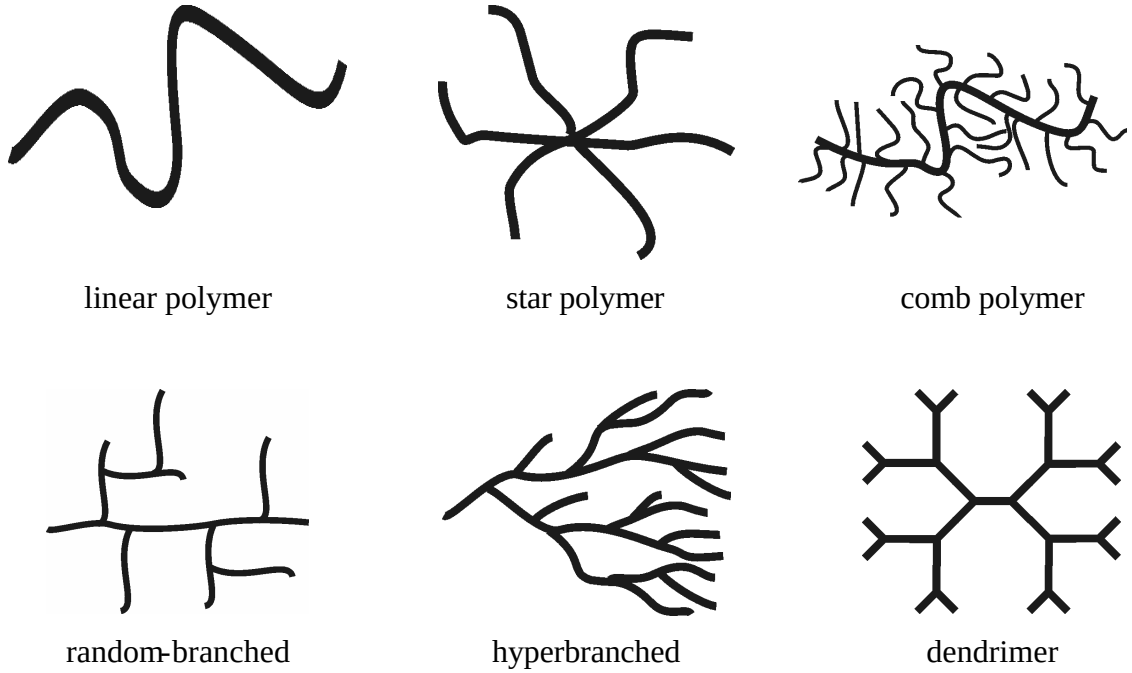


Figure 2.1: *Different shapes of macromolecules.*

2.2. Synthesis of Branched Polymer Structures

Branched Homo Polymers

Dendrimers are formed via so-called generations, where every further branching means a single reaction step leading to a globular structure with many reaction sides. The addition of the monomer units proceeds either from a multifunctional core (divergent approach) [1-3] or alternatively from the periphery where the structure is elaborated towards the core (convergent approach).[4,5] This type of reaction needs to be very selective, for only small amounts of side reaction products lead to considerable amounts of hyper branched stars in the higher generations. Furthermore, the dendrimers usually adopt a three-dimensional structure with backfolding of the arms. Several methods to shorten these syntheses have been reported[6] and include double stage, double exponential growth, hypermonomer, and orthogonal coupling strategies. Yamakawa et al. developed a one-pot synthesis of dendritic polyamides by a divergent method.[7] The density profile of dendrimers has been the subject of controversial discussions.[8]

Dendrimers are formed in two general ways. *(1)* In the divergent route branched repeat units are attached successively to a bi- or more functional core. *(2)* The convergent route prescribes the synthesis of the shell fragments (dendrons) which are connected to the core resulting in the desired product.

Without going into detail a few special products containing dendrimers can be mentioned. Except for different end functionalized dendrimers[9,10] there also exist dendrimers with different dendrons[11,12] or copolymers with blocks of dendrimers and linear polymers.[13,14] Dendrimers based on poly(alkylene oxide)s are well known in literature. They appear either as shell which surrounds other dendrimers made of poly(ether)s[15], poly(amido amine)[16] or poly(styrene)[17] or as cores with dendritic aromatic poly(ether) shells.[18] Gitsov et al.[19] have devised hydrophilic dendrimers from AB_3 monomers consisting of monobromo-functionalized oligo ethylene glycol as the A group and 1-methyl-2,6,7-trioxabicyclo[2.2.2]octane as the B group. Another working group relies on the transformation and doubling of the end-standing OH functions by introducing acetal rings and their subsequent cleavage.[20] Hawker et al.[21] on the other hand describes the synthesis of hyperbranched PEO´s on the basis of 3,5-dioxybenzoate derivatives of linear poly(ethylene glycol). This corresponds to an AB_2 monomer. Arborescent graft PS-*co*-PEO copolymers have been anionically polymerized with a PS-core / PEO-shell morphology.[22] PPO was used for synthesis of poly(urethane) dendrimers.[5]

Less perfect *multibranched structures*, the so called hyperbranched polymers are polymerized from AB_x monomers, where group A reacts selectively with group B.[23] Randomly branched polymers are obtained by random crosslinking of polymer chains.

Comb polymers are formed either by reaction of polymer arms along the backbone of the polymer chain or by linear reaction of macromonomers. To decrease the steric package of the side chains in two dimensional arrays, the polymer backbone undergoes a curvature which minimizes the side chain interactions and increases the volume available for each segment. By regulating the ratio of the chain lengths of

backbone to the side arms and by usage of different solvents the conformation of the comb polymers can be adjusted.[24]

The preparation of comb polymers proceeds by several pathways as depicted in Figure 2.2.[25] In the "grafting from" method, monomer is added to a linear polymer with initiating sites. In the "grafting to" method, linear end-functionalized polymer chains are connected to reactive sites along a polymer backbone. Finally, in the macromonomer method, polymer chains with polymerizable groups are either homo- or copolymerized with low molar mass monomers to form the comb polymer structure.[26,27]

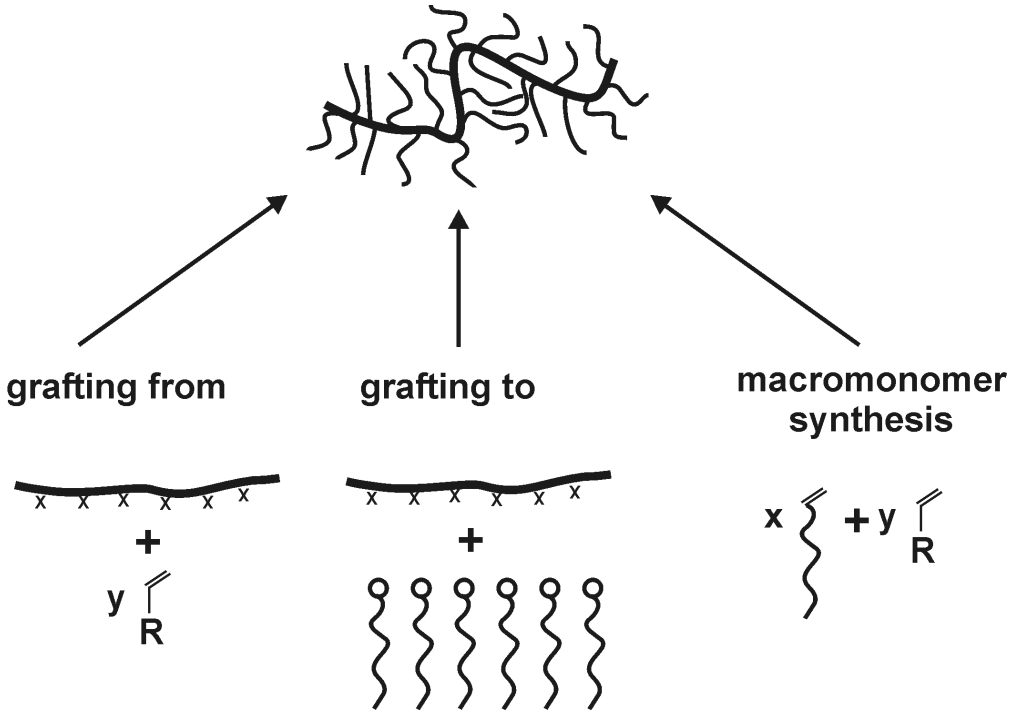


Figure 2.2: *Methods for the preparation of comb polymers*.

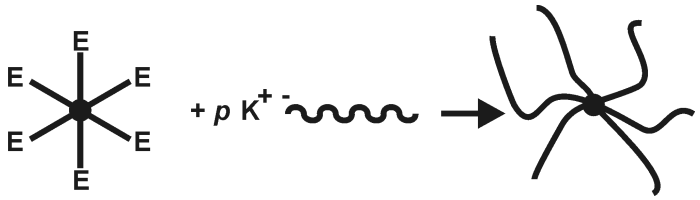
Comb polymers with a short backbone and large arms can also be considered as star polymers.

Star polymers on the other hand can be considered as a mixture between linear polymers and a small crosslinked network. Their density is increasing towards the core because the volume accessible to each arm is increasing with the distance from the core

which is in contrast to tethered linear polymers and comb polymers. Therefore the static and dynamic properties are different. Starpolymers can be described either by a defined core (even dendritic) with a known number of emanating arms, or an unknown number of arms formed by a crosslinking reaction forming the core.

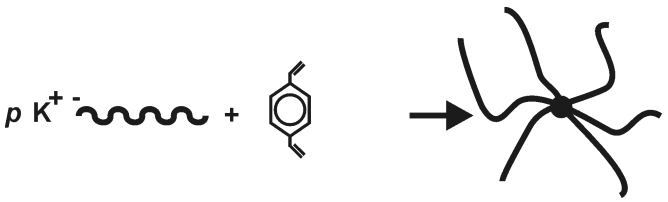
Early reports on the synthesis of *star polymers* relied on anionic polymerization methods and demonstrated the complications involved in star polymer synthesis.[28,29-31] The encountered problems differed according to their preparation method. The "arm-first" method describes the connection of a living monofunctional polymer to the core. The opposite "core-first" method results from the polymerization on a core with multiple sites for initiation. Rempp and co-workers[32] categorize the anionic synthesis procedures of star polymers in three classes:

a.) termination using a plurifunctional electrophilic deactivator (arm-first method),



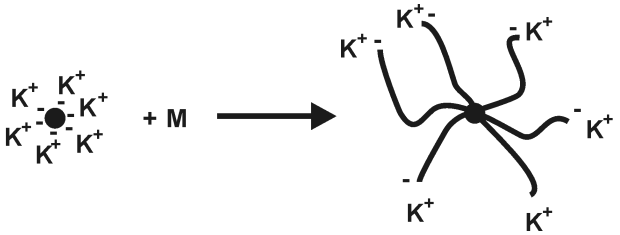
In this method the tedious removal of unreacted linear chains is unavoidable, however very well defined stars with comparatively few arms are produced.

b.) anionic block copolymerization of two monomers, the second being diunsaturated (either arm- or core-first method).



The introduction of a precise amount of diunsaturated compound is essential to avoid gelation. The core can be produced to accommodate many (e.g. 278 arms[33]) or even an almost unlimited amount of arm functionalities (e.g. 16-600 arms[34]). Stars with especially large cores and short arms lead to so-called "porcupine" polymers.[35] With this method, the star polymers are not as well-defined as in other procedures. Only an average number of arms can be provided.

c.) polymerizing with a plurifunctional initiator (core-first method).



For the preparation of uniform stars, equal reactivities of each initiating site are a prerequisite. The initiating sites should therefore not interact electronically, sterically or electrostatically with each other.

Because of its good control and selective reactions anionic polymerization has turned out to be the best way of star polymer formation.[36] Nevertheless more and more different polymerization methods were developed in the recent years. Maintaining the anionic character acrylate and methacrylate starpolymers were made by group transfer polymerization (GTP).[23,37] Other important methods (reviewed by Charleux et al.[38] and Ingrish et al.[39]) were living cationic polymerization of poly(vinyl ether)s[40-42], poly(alkoxy styrene)s[43] or poly(isobutylene)s[44]. Star polymers have also been synthesized by controlled ring-opening metathesis polymerization (ROMP),[45] living or controlled free radical polymerization[46] and atom transfer radical polymerization (ATRP).[47,48] Further preparation methods include star branched poly(styrene) by nitroxide living free-radical polymerization.[49,50]

Another method for the preparation of star polymers proceeds by immobilizing functional block copolymers.[51] Core shell type microspheres were obtained by crosslinking the micellar cores in solution or by crosslinking the spherical domains in solid state microphase-separated structures. An example are PS moieties of poly(isobutene)-*b*-poly(styrene)-*b*-poly(isobutene) block copolymers, which are crosslinked with $AlCl_3$ to form star polymers.[52] The same method was also applied for the preparation of Janus type micelles, which represent triblock copolymers, where the middle block is crosslinked.[53]

Some research groups report the successful synthesis of starpolymers by complexation of modified polymer chains as ligands to metal cores like iron or ruthenium. While Fraser et al. complexed Fe^{2+} or Ru^{2+} cores with bipyridyl terminated poly(caprolactones),[54] Sawamoto et al. used PMMA functionalized with triphenylphosphanes as ligands.[55]

Star polymers based on *alkylene oxides* are synthesized in similar ways.[56] The most common synthesis is done with plurifunctional alkoxides as initiators. So called "triols" like trimethylolpropane result in triarm stars, "hexols" like sorbitol are used to form six arm starpolymers. Therefore with this method the number of arms is limited to the functionality of the initiator. For stars with high amounts of arms other synthesis are more promising. Roovers et al. prescribed the synthesis of a PEO polymer with 16 arms using a carbosilane dendrimers as initiator.[57] Rempp et al. reported the utilization of Divinylbenzene (DVB) for the formation of multiarm cores with the following oxirane polymerization to form PEO star polymers with up to 100 arms.[58] With a similar method stars with up to 20 arms per molecule were synthesized by the utilization of diepoxides instead of DVB.[59,60]

The good developed synthesis of PEO starpolymers make them also of interest for industry. Examples include 3-4 arm star PEOs for non-flammable thickeners in aqueous hydraulic liquids,[61] or six arm PEG sorbitan monooleate (Polysorbate 80) which are used as surfactant, solubilizing agent or phase transfer catalyst,[62] allowing it to be used as a cheap alternative to crown ethers. Poly(propylene oxide)s[63] with three arms have been sold under the trade names Voranol (PPO, The Dow Chemical Co.), Niax Triol LG (Union Carbide Chemicals Co.) and Pluracol GP (Wyandotte Chemicals Co.). The Pluracol TP series from Wyandotte Chemicals Co. is prepared from trimethylolpropane instead of glycerol. The Niax Triol LHT series is based on hexanetriol-1,2,6 as three arm core (Union Carbide Chemicals Co.). Four armed stars have been sold under the trade names Tetronic (Atlas Chemical Ind., PPO / PEO block copolymer adducts of ethylene diamine) and Pluracol PeP (Wyandotte Chemicals Co., pentaerythritol initiator). Sorbitol based six armed star poly(propylene oxide)s were sold under the trade names Atlas G (Atlas Chemical Ind.), Pluracol SP (Wyandotte

Chemicals Co.) and Niax Hexol LS (Union Carbide Chemicals Co.). Analog to the star polyols, primary amine terminated PEO and PPO triamines are commercially available as Jeffamine ET.

Further examples of the wide variety of star PEOs include Fullerenol derived urethane connected PEO star polymers prepared by reaction of linear monoisocyanate PEO prepolymer to a multifunctional fullerenol core.[64] Monoisocyanate terminated PEOs have been attached to poly(ethylene imine)s to provide star and comb shaped PEOs with varying numbers of arms.[65] Similarly, nonylphenoxy poly(ethylene glycol)s have been connected to tri- and tetraisocyanates.[66] Recently, four arm star polymers were prepared from poly(propylene glycol) methacrylate macromonomers,[58] with the star polymer arms containing PPO side chains.

End-functionalized polymers

Because of their various modification possibilities, polymers with functional endgroups are gaining more and more interest for theoretical examinations[67,68] as well as for industrial applications.[69,70] The so called telechelics can serve as crosslinkers for polymer networks, as agents for the covalent binding of polymers onto solid surfaces or in a further step be the intermediate layer between substrates and different biological substances like oligonucleotides, proteins or cells.

The functionalization of the polymer endgroups is of great importance for the building of new block copolymers. They allow rather new physical or chemical properties of commonly known polymers. For example hyperbranched poly(glycerol)s can be made soluble in organic solvents by attaching a less polar propylene oxide block or other different functional groups.[71-73]

To introduce functional groups to polymers several methods are possible. First of all, the derivation of the polymer endgroups is to be mentioned. More elegant is the quenching of the polymerization by addition of suitable reactants. Further different endgroups can be achieved by controlled chain transfer.

In star polymer architectures, the endgroup effects are enhanced because of the larger number of chain ends. Thus, depending on the molecular weight of the polymer,

the incorporation of endgroups plays a more dominant role in the properties of the material.

Poly(ethylene oxide)s are as well end functionalized in many ways. In bulk they serve either as non-ionic surfactants, hydrogels,[74] phase transfer catalysts, associative thickeners or affinity chromatography, on surfaces they exhibit biocompatible functions.[75-79], Ringsdorf, in a theoretical study, suggested the possibility of pharmacological active PEG's with drug and a homing device connected to its termini to allow the drug being released only at the required place in the body.[80]

Functionalized star-shaped poly(ethylene oxide)s[36,56,81-83] have been prepared via the "core first" method with pre-crosslinked DVB as core. Anionic polymerization of the ethylene oxide and protonic quenching of the reaction led to hydroxyl terminated starpolymers, which easily can be crosslinked forming hydrogel networks[84,85] or, with adequate initiators, further PS arms can be attached via ATRP.[86] Another example of functionalized star PEO´s are thermoplastic biodegradable hydrogel spheres for peptide drug delivery.[87] These stars with 2 - 8 arms were composed of a hydrophilic PEO core with poly(L-lactide) endgroups. In another example of end-functionalized star poly(ethylene oxide), RGD peptide conjugates are attached to the star arm ends.[88] Also, one-, two- and three-branched PEG derivatives with each having only one carboxyl group per molecule[89] were obtained by reacting monomethoxy linear PEG with bromoacetic acid, protocatechnic acid, and gallic acid, respectively.

Taton et al.[90,91] reported the synthesis of C_{60} terminated three arm poly(ethylene oxide)s, which were investigated towards their organization at the air-water interface. This working group demonstrated the diversity of chemical functions in star polymers: six arm star poly(styrene)s were end-functionalized[92] at each arm terminus with allylic or azide groups. The allylic groups were then transformed into hydroxyl endgroups, which also served as initiation site for polymerization of ethylene oxide to form PS-*b*-PEO star block copolymers. The azide groups were subsequently reacted with fullerene to form C_{60}-terminated six armed star polystyrene polymers.

Physical properties of branched polymers

If polymers show branching, their physical properties can differ significantly from linear polymers with the same molecular weight. Because branched polymers cannot order themselves as easily as linear polymers for example their crystallinity is much lower. Whereas the relaxation processes for linear polymers proceed dominantly by the reptation mechanism, the introduction of a single long-chain branch point make other relaxation processes more observable such as the relaxation by an arm-retraction mechanism.[28,93,94] Short branched polymers also show a lower viscosity compared to linear polymers with the same molecular weight.[95] The self diffusion coefficient of star poly(ethylene oxide) decreases with star arm number (2, 3 and 4 arms).[96] In blends of star and linear poly(styrene), the regularly star-branched material segregates preferentially to the surface of the blend.[97] This exclusion phenomenon demonstrates the special properties of star compounds compared to their linear analogues. The entangled dynamics and melt flow of branched polymers have been reviewed by Milner et al.[98] The glass transition temperatures of star polymers can be correlated with those of linear polymers when the number of endgroups is taken to account.[99]

The star shaped conformation allows a high density of polymer units in the core. Therefore the solution properties of the star polymers can differ largely compared to the analogue linear polymers. The radius of gyration is decreasing with a growing amount of arms. J. Freire gave a good overview of the conformational properties of star shaped and other branched polymers.[100]

Star polymers behave like static unimolecular micelles in dilute solutions with the important exception that the number of chains per "micelle" does not vary with the thermal and preparative conditions of the macromolecular solution. This makes star polymers suitable as model compounds for polymeric micelles of end-associating polymers or block copolymers that are dissolved in a selective solvent.[99]

With their numerous arms, specific chemical functions can be introduced to their chain ends. For example, the density of star poly(ethylene oxide) layers increases with arm number, making them more effective in repelling proteins or cells.[101] Epoxy functional hydrogenated star poly(butadiene)s[102] have found application in adhesives

because of their reduced melt viscosity. Also, star branched poly(butadiene)s exhibit improved cold flow and stability.[103]

2.3 Adsorbed Polymers on Flat Surfaces

The adsorption of linear polymer chains is directed by their specific and non-specific interaction with the substrate. High positive interactions lead to a flat conformation of the polymer chain segments on the surface. This "train" conformation is frequently observed for polar polymers on polar substrates (Figure 2.3).

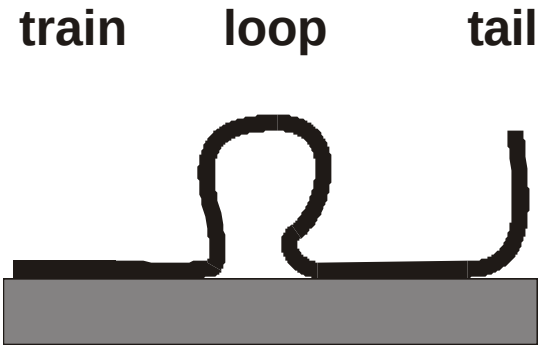


Figure 2.3*: Possible conformations of a surface adsorbed polymer chain*

With higher polymer concentrations, the surface is only partially covered. Consequently, free standing polymer segments ("loops") and chain ends ("tails") result.[104]

Thus end grafted linear polymers dissolved in a good solvent can form four major regimes as shown in Figure 2.4. Strong unattractive surface–polymer interactions combined with a low surface coverage results in isolated polymer chains (Figure 2.4a), whereas for high surface coverage the chains are stretched out of the surface forming the “brush” regime (Figure 2.4b). Strong attractive interactions between surface and polymer combined with low grafting density yields the so called “pancake” conformation (Figure 2.4c) with isolated chains that are adsorbed on the surface, whereas high polymer concentration leads to densely packed areas with the remaining units stretching out of the surface (Figure 2.4d).[105] Nevertheless the borders between these conformations are not sharp and that in practice their existence and their range of

surface coverage depends on the molecular weight of the polymer, surface interactions, solvents and other variables. For PEG it has been shown that a continuous range of structures from regime a to the brush regime can exist parallel.[106] Furthermore, PEO shows a somewhat amphiphilic character, since it is attracted to hydrophobic surfaces and to the water-air interface but not to lipid surfaces.[107-110] In addition, many experiments are carried out in the intermediate state between regime a and the brush regime.

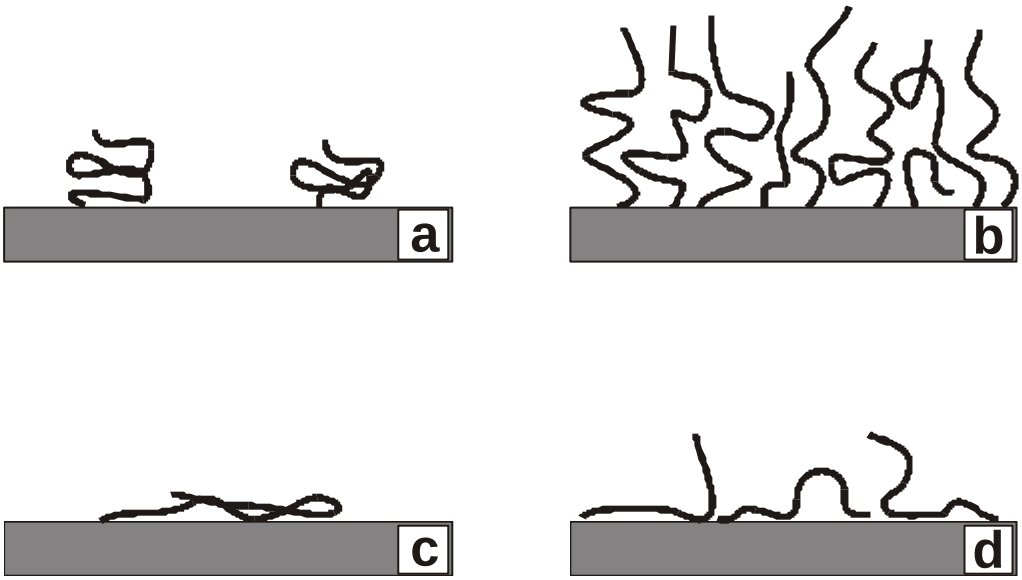


Figure 2.4: *Different conformations of end grafted linear polymer chains on a substrate for low (a,c) and high (b,d) surface coverage with repulsive (a,b) and attractive (c,d) forces between polymer and surface.*

Compared to the grafting of linear chains the overall situation is complicated by the presence of a focal point where the chains converge. Star polymers can bind to surfaces by one or more chain ends, or by a functional group at the center. The different possible conformations are displayed in Figure 2.5 according to Figure 2.4 for linear grafted chains.

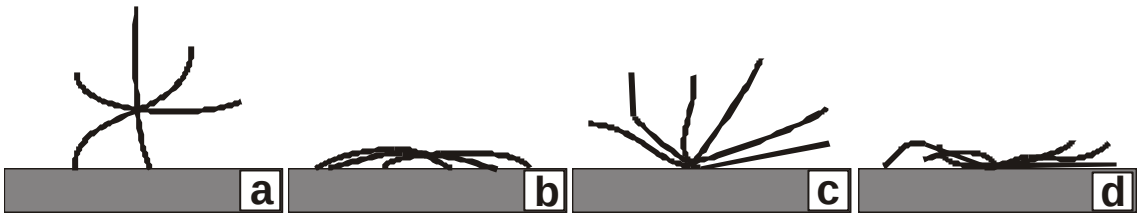


Figure 2.5*: Different conformations of end grafted star polymer chains on a substrate for repulsive (a,c) and attractive (b,d) forces between polymer and surface for end grafted (a,b) and center grafted (c,d) stars.*

Theoretic investigations have described the layer structure of three arm stars connected to the surface with one arm (single-chain mean-field theory)[111] for short chained star polymers (weighted-density functional method)[112] and long chained 3-12 arm stars (self-consistent field analysis)[113,114] within various parameters such as grafting density, arm number and star size. The volume fraction profile showed a discontinuity at the branching node for many armed stars. Thus the arms were partitioned either between the surface and the nodal point or beyond the branching node with monotonically decreasing volume fraction. It has been concluded that stars give a higher surface coverage at any grafting density due to their bulkier structure.[114] The star arms were reported to exhibit a higher "effective" grafting density stretching from the branch node into the top of the layer.

A star polymer connected to the surface by its center[115-117] in the sparse grafting regime does not change its spherical symmetry in the space above the surface. It can be envisioned as a half star with the double amount of branches. In the case of overlapping stars the difference between linear and star polymers arises from the new dependence of the brush thickness on the number of branches.

The chain ends of grafted star polymers are located in the outer regions due to the increased steric hindrance towards the center of the molecule. This is in sharp contrast to the properties of linear random coils, where the chain ends may be located anywhere within the volume of the molecule. Thus the grafting density is affected by the architecture of the polymer. With surface-grafted star polymers an attractive option is available to increase chain density in comparison to polymer brushes of linear chains and to increase long-term stability compared to self assembled monolayers.

2.4 Protein Adsorption onto Artificial Surfaces

Proteins, containing both hydrophilic and hydrophobic amino acids in their primary structure, have a rather amphiphilic character and therefore show strong interactions with miscellaneous surfaces.[118-121] Although these interactions are used for several applications like biocatalysts, biosensors and biochips, protein chromatography, to stabilize emulsions or for colloidal drug delivery systems in medicine,[122-127] for the majority of applications they have to be prevented. Surfaces that suppress protein adsorption are important as substrates for enzyme-linked immunosorbent assays (ELISAs), sensors and contact lenses[128] tissue engineering,[129] microfluidic systems,[130] devices used for drug delivery[131,132] and systems for high-throughput screening using proteins[133] or cells.[134] Since cells interact with surface adsorbed proteins rather than with the material surface itself,[133,135] the biocompatibility of materials used in medical applications is strongly affected by the initial event of protein adsorption. Thus, the uncontrolled adhesion of biological material mainly originating from protein adsorption on synthetic materials results in many problems, ranging from life threatening to merely inconvenient consequences like acute and chronic inflammation,[136] fibrous encapsulation,[137] occlusion of small diameter artificial blood vessels,[138] complement activation[139] and biofouling of contact lenses.[140]

In order to design surfaces that prevent protein adsorption, the process of protein adsorption itself has to be examined more in detail. The kinetics of protein adsorption often exhibit remarkable differences between individual proteins[141,142] and will not be addressed further. Aside from kinetical aspects, the adsorption of globular proteins onto solid/aqueous interfaces is in most cases determined by hydrophobic interactions.[143,144] Hydrophobic surfaces interact with hydrophobic domains of the protein periphery what leads to protein adsorption and release of water molecules from the surface. This gain in entropy is the driving force of the adsorption process. Furthermore, proteins that show a small unfolding free energy can change their conformation to bring more hydrophobic side chains in contact with the substrate.[145-153]

In contrast to the general dependence of protein adsorption and hydrophobicity on the surface, the coherence of protein adsorption and electrostatic interactions

between surface and proteins is more complicated and may differ tremendously between individual proteins. For single protein systems, the adsorption can be minimized by adjusting the pH value of the solution and thereby changing the net charge of the protein. A reversible adsorption of lysozyme on oxidized silicon can be achieved by this method.[154] Nevertheless, in most cases the electrostatic interaction between protein and surface rather depends on charged areas on the periphery of the protein than on the net charge of the whole protein. In some cases proteins even adsorb on surfaces that bear the same charge.[155] In these cases, attractive hydrophobic forces compensate the electrostatic repulsion. Another important factor in this context is the ion strength of the solution. A high ion strength in the solution results in much weaker coulomb interactions. Adsorption can thereby be minimized for single molecule systems by adjusting the ion strength of the solution.[156] Lysozyme adsorption on oxidized silicon is decreasing with increasing ion strength at pH 7,[157] whereas serum albumin shows stronger adsorption with increasing ion strength.[158] This is due to the difference in the isoelectric point (pI) of the two proteins (lysozyme: pI = 11; serum albumin: pI = 5) and the resulting opposite net charge at pH 7. Generally, protein adsorption is very pronounced for pH values that equal the pI, since in such solutions the lateral repulsion between the adsorbed proteins is minimal.[159,160] These examples show that the influence of electrostatic forces on protein adsorption is protein specific and thus not suitable for the preparation of surfaces that resist protein adsorption in general. There are even reports that charged surfaces promote biofouling.[161-163]

Based on the phenomenological aspects of protein adsorption discussed here, surface modifications that resist protein adsorption have to be hydrophilic, uncharged and flexible. Several polymers meet these demands and are used for the creation of nonfouling surfaces such as poly(hydroxyethyl methacrylate), poly(acrylamide), poly(*N*,*N*-dimethyl acrylamide), dextran and poly(ethylene glycol) (PEG). PEG is nontoxic and nonimmunogenic and has been found to be the most effective of these polymers.[76] Surfaces modified with PEG exhibit excellent protein resistance.[164,165]

2.5 Bioresistance of PEG-covered Surfaces

Although this resistance still is not understood completely, PEG modified surfaces are the origin of widespread applications in biomaterial research.[76] With increasing grafting density and chain lengths the biological resistance of the PEGs is improving,[77,78,166] and such surfaces show reduced protein adsorption and platelet adhesion.[79,167] The tendency of PEG to form brush regimes, together with their high solubility in water, leads to a high grafting density. The densely grafted chains stretch out from the surface into the solution to be better dissolved and form a very effective steric barrier against protein adsorption. Exactly this steric barrier is on the other side the main problem for preparing such densely grafted brush surfaces, since during the preparation process, the polymer chains themselves have to overcome this barrier of already grafted chains to yield higher grafting density. It has been found that for chemisorption of endfunctionalized linear PEG, the best surface coverage was obtained when the grafting solution provided near phase separation conditions for the PEO chains (cloud point conditions). This can be achieved by adding K_2SO_4 which drives the lower critical solution point of PEO/water systems to lower temperatures and additionally moderately increasing the temperature.[82,168] This results in reduced chain repulsion during the grafting reaction and hence greater chain packing density at the interface. In contrast, the packing density of star polymers is already high in a good solvent, and only moderately improved by a poor solvent.[82,83]

The packing of PEO stars has been reported to depend on the molecular weight, i.e. PEO stars with 70 arms and a molecular weight of 5200 g/mol per arm have been reported to pack closely on the surface and inhibit adsorption of proteins.[169] Larger PEO stars were shown to pack poorly on the surface (Figure 2.6).

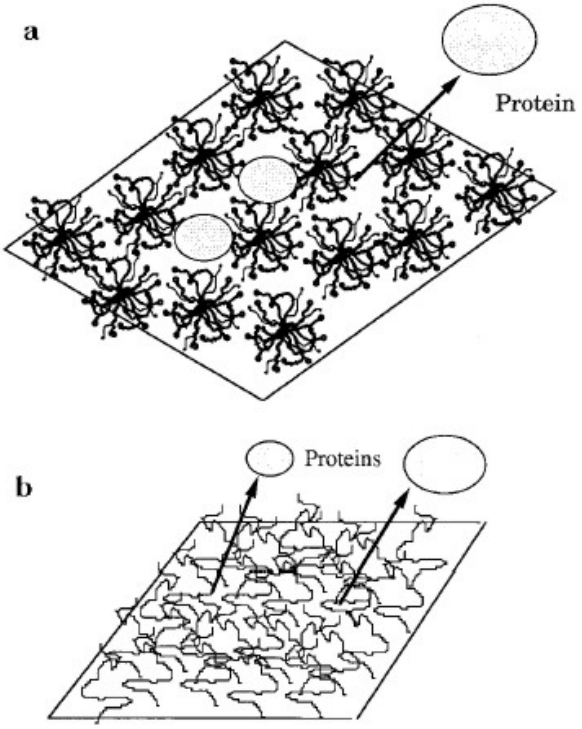


Figure 2.6*: Packing density of grafted PEO stars (a) and grafted linear PEG (b) on a surface and resulting protein repellant properties. Stars were grafted onto the substrate but not crosslinked and exhibited worse protein repellant properties than densely grafted linear PEG (scheme from ref.*[82]*).*

While the grafting probability of linear polymers decreases with increasing molecular weight, the star polymers in contrast retain their high initial grafting probability up to very high molecular weights. Furthermore the grafting density has been correlated with the critical chain overlap concentration in solution. Above this concentration the density of surface coupled linear polymer becomes high enough to resist the penetration of additional polymer chains to the surface. Because of the dense cores of PEO stars much higher concentrations were needed to achieve interpenetration of the star shaped molecules.[82] In a recent publication, the distribution of a RGD ligand bound to PEO stars before and after grafting them to a surface enabled control over nanoscale clustering of the RGD or random distribution on the surface (Figure 5).[88]

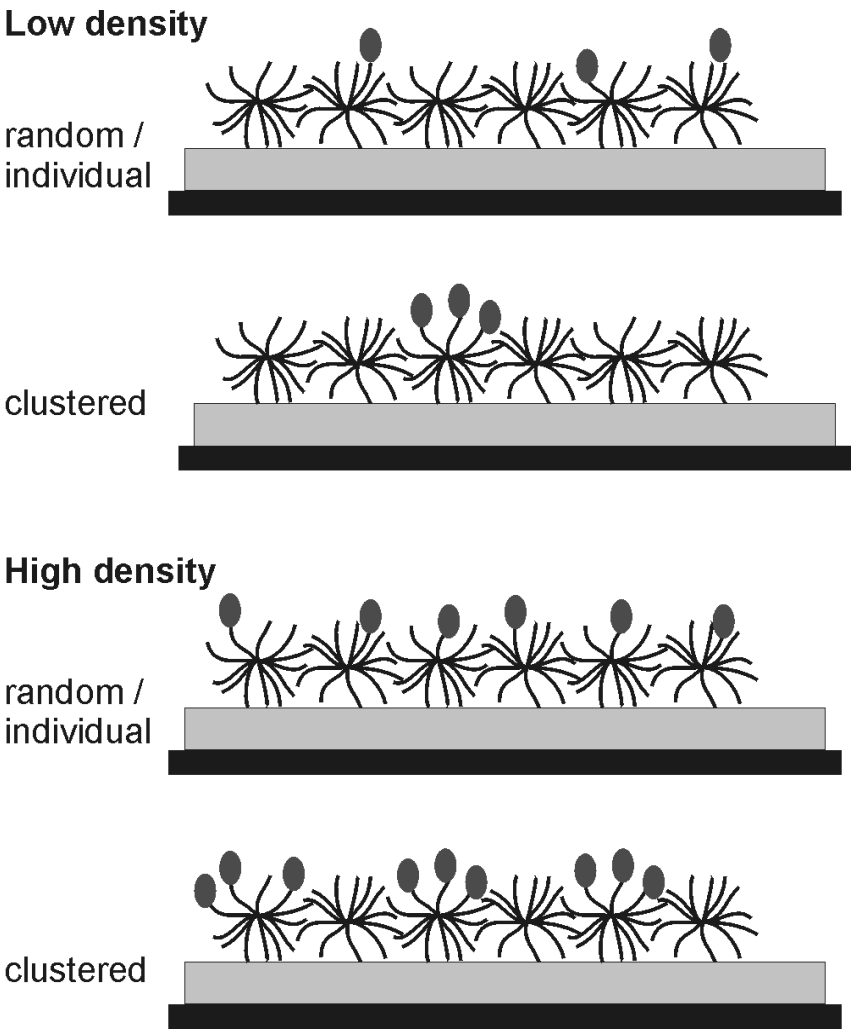


Figure 2.7*: Control over the nanoscale distribution of a RGD peptide linked to surface tethered PEO stars before (clustered distribution) and after (random distribution) the surface grafting (scheme redrawn from ref.*[88]*).*

Another attempt to achieve high grafting density is to use block copolymers of ethylene glycol with other blocks that serve as anchoring block.[170-175] The idea to use a block polymer as an anchoring group has the advantage that even small attractive forces between the segments of the polymer and the surface sum up according to the degree of polymerization of the whole block and may result a strong attachment. Recently a theoretical approach has been made that addresses especially such copolymer systems.[105] Nevertheless long term stability of these surfaces is worse than for covalently attached systems.

2.6 References

(1) De Brabander-van den Berg, E. M. M.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1308.
(2) Newkome, G. R.; Yao, Z.-Q.; Baker, G. R.; Gupta, K. J. *J. Org. Chem.* **1985**, *50*, 2003.
(3) Tomalia, D. A.; Baker, H.; Dewald, J. R.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Smith, P. *Polym. J. (Tokyo)* **1985**, *17*, 117.
(4) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 1638.
(5) Feast, J. W.; Rannard, S. P.; Stoddart, A. *Macromolecules* **2003**, *36*, 9704-9706.
(6) Grayson, M. S.; Frechet, J. M. *Chem Rev.* **2001**, *101*, 3819.
(7) Yamakawa, Y.; Ueda, M.; Nagahata, R.; Takeuchi, K.; Asai, M. J. *J. Chem. Soc. Perkin Trans. 1* **1998**, 4135.
(8) Zimmerman, S. C. *Curr. Op. Coll. Interf. Sci.* **1997**, *2*, 89.
(9) Hawker, C. J.; Fréchet, J. M. J. *Macromolecules* **1990**, *23*, 4726.
(10) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc. Perkin Trans.* **1991**, *1*, 1059.
(11) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1993**, *115*, 11496.
(12) Fréchet, J. M. J. *Science* **1994**, *263*, 1710.
(13) Van Hest, J. C. M.; Baars, M. W. P. L.; Elissen-Román, C.; van Genderen, M. H. P.; Meijer, E. W. *Macromolecules* **1995**, *28*, 6689.
(14) Chapman, T. M.; Hillyer, G. L.; Mahan, E. J.; Shaffer, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 11195.
(15) Liu, M.; Kono, K.; Fréchet, J. M. J. *J. Polym. Sci,Part A: Polym. Chem. Ed.* **1999**, *37*, 3492.
(16) Yen, D. R.; Merrill, E. W. *Polym. Prep. (Am. Chem. Soc. Div. Polym. Chem.)* **1997**, *38*, 531.
(17) Cloutet, E.; Six, J. L.; Taton, D.; Gnanou, Y. *Polym. Mat. Sci. Eng.* **1995**, *73*, 133.
(18) Gitsov, I.; Wooley, K. L.; Fréchet, J. M. J. *Angew. Chem.* **1992**, *104*, 1282.
(19) Gitsov, I.; Wu, S.; Ivanova, P. T. *Polym. Mat. Sci. Eng.* **1997**, *77*, 214.
(20) Bera, T. K.; Taton, D.; Gnanou, Y. *Polym. Mat. Sci. Eng.* **1997**, *77*, 126.
(21) Hawker, C. J.; Chu, F.; Pomery, P. J.; Hill, D. J. T. *Macromolecules* **1996**, *29*, 3831.
(22) Gauthier, M.; Tichagwa, L.; Downey, J. S.; Gao, S. *Macromolecules* **1996**, *29*, 519.
(23) Sivaram, S.; Lutz, P. J.; Mishra, M. K. In *Star and Hyperbranched Polymers*; Mishra, M. K., Kobayashi, S.,Ed., Marcel Dekker, Ed.: New York, 1999; pp 59-76.
(24) Potemkin, I.; Khokhlov, A. R.; Prokhorova, S.; Sheiko, S. S.; Möller, M.; Beers, K. L.; Matyjaszewski, K. *Macromolecules* **2004**, *37*, 3918-3923.
(25) Rempp, P. F.; Lutz, P. In *Encyclopedia of Advanced Materials*; Pergamon Press, 1994; p 934.
(26) Rempp, P. F.; Franta, E. *Adv. Polym. Sci.* **1984**, *142*, 129.
(27) Ito, K.; Kawaguchi, S. *Adv. Polym. Sci.* **1999**, *142*, 129.

(28) Bywater, S. *Adv. Polym. Sci.* **1978**, *30*, 89.
(29) Morton, M.; Helminiak, T. E.; Gadkary, S. D.; Bueche, F. *J. Polym. Sci. A. Polym. Chem. 2555* **1962**, *57*, 471.
(30) Hsieh, H. L.; Quirk, R. P. In *Anionic Polymerization, Principles and Practical Applications* pp 333-368.
(31) Worsfold, D. J.; Zilliox, J.-G.; Rempp, P. *Can. J. Chem.* **1969**, *47*, 3379.
(32) Lutz, P.; Rempp, P. *Makromol. Chem*. **1988**, *189*, 1051-1060.
(33) Roovers, J.; Toporowski, P.; Martin, J. *Macromolecules* **1989**, *22*, 1897.
(34) Eschwey, H.; Burchard, W. *Polymer* **1975**, *16*, 180.
(35) Taromi, F. A.; Rempp, P. *Makromol. Chem.* **1989**, *190*, 1791.
(36) Plentz Meneghetti, S.; Lutz, P. J.; Rein, D. In *Star and Hyperbranched Polymers*; Mishra, M. K., Kobayashi, S.,Ed., Marcel Dekker:, Ed.: New York, 1999; pp 27-57.
(37) Sogah, D. Y.; Hertler, W. R.; Webster, O. W.; Cohen, G. M. *Macromolecules* **1987**, *20*, 1473.
(38) Charleux, B.; Faust, R. *Adv. Polym. Sci.* **1999**, *142*, 1.
(39) Ingrish, S.; Nuyken, O.; Mishra, M. K. In *Star and Hyperbranched Polymers*; Mishra, M. K., Kobayashi, S.,Ed., Marcel Dekker:, Ed.: New York, 1999; pp 77-106.
(40) Kanaoka, S.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1991**, *24*, 2309.
(41) Kanaoka, S.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1991**, *24*, 5741.
(42) Kanaoka, S.; Omura, T.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1992**, *25*, 6407.
(43) Deng, H.; Kanaoka, S.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1996**, *29*, 1772.
(44) Jacob, S.; Kennedy, J. P. *Adv. Polym. Sci.* **1998**, *146*, 1.
(45) Bazan, G. C.; Schrock, R. R. *Macromolecules* **1991**, *24*, 817.
(46) Kuriyama, A.; Otsu, T. *Polym. J. (Tokyo)* **1984**, *16*, 511.
(47) Gaynar, S. G.; Matyjaszewski, K. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1997**, *38*, 758.
(48) Bosman, A. W.; Heumann, A.; Klaerner, G.; Benoit, D.; Frechet, J. M.; Hawker, C. J. *J. Am. Chem. Soc.* **2001**, *123*, 6461-6462.
(49) Pasquale, A. J.; Long, T. E. *J. Polym. Sci.: Polym. Chem. Ed.* **2001**, *39*, 216.
(50) Tsoukatos, T.; Pispas, S.; Hadjichristidis, N. *J. Polym. Sci.: Polym. Chem. Ed.* **2001**, *39*, 320.
(51) Ishizu, K. In *Star and Hyperbranched Polymers*; Mishra, M. K. K., S.,Ed. Marcel Dekker:, Ed.: New York, 1999; pp 135-178.
(52) Kéki, S.; Bogács, C.; Bogács, L.; Daróczi, L.; Zsuga, M. *Angew. Makromol. Chem.* **1997**, *245*, 183.
(53) Xu, H.; Erhardt, R.; Abetz, V.; Müller, A. H. E.; Goedel, W. A. *Langmuir* **2001**, *17*, 6787.
(54) Corbin, P. S.; Webb, M. P.; McAlvin, J. E.; Fraser, C. L. *Biomacromolecules* **2001**, *2*, 223-232.
(55) Terashima, T.; Kamigaito, M.; Baek, K.-Y.; Ando, T.; Sawamoto, M. *J. Am. Chem. Soc.* **2003**, *125*, 5288-5289.
(56) Gnanou, Y.; Lutz, P.; Rempp, P. *Makromol. Chem.* **1988**, *189*, 2885.

(57) Comanita, B.; Noren, B.; Roovers, J. *Macromolecules* **1999**, *32*, 1069.
(58) Shemper, B. S.; Acar, E.; Mathias, L. J. *J. Polym. Sci.: Polym. Chem. Ed.* **2002**, *40*, 334.
(59) Lapienis, G.; Penczek, S. *Macromolecules* **2000**, *33*, 6630.
(60) Lapienis, G.; Penczek, S. *Biomacromolecules* **2005**, *6*, 752-762.
(61) Rasp, C. *Triebologie + Schmierungstechnik* **1988**, *35*, 185.
(62) Thoman, C. J.; Habeeb, T. D.; Huhn, M.; Korpusik, M.; Slish, D. F. *J. Org. Chem.* **1989**, *54*, 4476.
(63) Frisch, K. C.; Davis, S. In *Polyethers, Pt. I. Polyalkylene Oxides and Other Polyethers*; Gaylord, N. G., Ed., Interscience Publ., Ed.: New York, 1963; pp 315-408.
(64) Chiang, L. Y.; Wang, L. Y.; Tseng, S.-M.; Wu, J. S.; Hsieh, K.-H. *J. Chem. Soc., Chem. Commun.* **1994**, 2675.
(65) Trzasko, P. T. *Star and Comb-Shaped Poly(oxyethylene), Ph.D. Thesis,* **1988**, *University of Connecticut*.
(66) Zhou, G.-B.; Smid, J. *Polymer* **1993**, *34*, 5128-5133.
(67) Joanny, J. F. *Polymer* **1980**, *21*, 71.
(68) Brédas, J. L.; Chance, R. R.; Silbey, R. *Macromolecules* **1988**, *21*, 1633.
(69) Nagata, N.; Kobatake, T.; Watanabe, H.; Veda, A.; Yoshioka, A. *Rubber Chem. Technol.* **1987**, *60*, 837.
(70) French, D. M. *Rubber Chem. Technol.* **1969**, *42*, 71.
(71) Sunder, A.; Mülhaupt, R.; Frey, H. *Macromolecules* **1999**, *32*, 4240.
(72) Haag, R.; Stumbé, J.-F.; Sunder, A.; Frey, H.; Hebel, A. *Macromolecules* **2000**, *33*, 8158.
(73) Knischka, R.; Lutz, P.; Sunder, A.; Mülhaupt, R.; Frey, H. *Macromolecules* **2000**, *33*, 315.
(74) Yong Lee, K.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869.
(75) Jones, M. N.; Weers, J. G. *Curr. Opin. Coll. Interf. Sci.* **1996**, *1*, 635.
(76) Harris, J. M. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York, 1992.
(77) Malmsten, M.; Emoto, K.; Van Alstine, J. M. *J. Colloid Interf. Sci.* **1998**, *202*, 507.
(78) McPherson, T.; Kidane, A.; Szleifer, I.; Park, K. *Langmuir* **1998**, *14*, 176.
(79) Jenney, C. R.; Anderson, J. M. *J. Biomed. Mater. Res.* **1999**, *44*, 206.
(80) Ringsdorf, H. *J. Polym. Sci. Symp.* **1975**, *51*, 135.
(81) Choi, Y. K.: World Patent No. WO99/29759, 1999.
(82) Sofia, S. J.; Premnath, V.; Merrill, E. W. *Macromolecules* **1998**, *31*, 5059.
(83) Irvine, D. J.; Mayes, A. M.; Satija, S. K.; Barker, J. G.; Sofia-Allgor, S. J.; Griffith, L. G. *J. Biomed. Mater. Res.* **1998**, *40*, 498.
(84) Graham, N. B. In *Hydrogels in Medicine and Pharmacy*; Peppas, N. A., Ed., CRC Press, Ed.: Boca Raton, 1987; Vol. 2, pp 95-113.
(85) Keys, K. B.; Andreopoulos, F. M.; Peppas, N. A. *Macromolecules* **1998**, *31*, 8149.
(86) Peleshanko, S.; Jeong, J.; Shevchenko, V. V.; Glenson, K. L.; Pikus, Y.; Ornatska, M.; Petrash, S.; Tsukruk, V. V. *Macromolecules* **2004**, *37*, 7497-7506.

(87) Choi, Y. K.; Kim, S. W.; Bae, Y. H. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1996**, *37*, 109.
(88) Maheshwari, G.; Brown, G.; Lauffenburger, D. A.; Wells, A.; Griffith, L. G. *J. Cell Sci.* **2000**, *113*, 1677.
(89) Fuke, I. *J. Controlled Release* **1994**, *30*, 27.
(90) Taton, D.; Angot, S.; Gnanou, Y.; Wolert, E.; Setz, S.; Duran, R. *Macromolecules* **1998**, *31*, 6030.
(91) Carino, S. R.; Liu, C.; Logan, J.; Gnanou, Y.; Taton, D.; Duran, R. S. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **2000**, *82*, 348.
(92) Cloutet, E.; Fillaut, J.-L.; Astruc, D.; Gnanou, Y. *Macromolecules* **1999**, *32*, 1043.
(93) Roovers, J. *TRIP* **1994**, *2*, 294.
(94) Milner, S. T.; McLeish, T. C. B. *Macromolecules* **1997**, *30*, 2159.
(95) Roovers, J. *Macromolecules* **1994**, *27*, 5359.
(96) Lee, S.-W.; von Meerwall, E. D.; Cheng, S. Z. D.; Hsiao, B. S. *Polym. Prepr. (Am. Chem. Soc. Div. Polym. Chem.)* **1995**, *36*, 259.
(97) Foster, M. D.; Greenberg, C. C.; Teale, D. M.; Turner, C. M.; Corona-Galvan, S.; Cloutet, E.; Butler, P. D.; Hammouda, B.; Quirk, R. P. *Macromol. Symp.* **2000**, *149*, 263.
(98) McLeish, T. C. B.; Milner, S. T. *Adv. Polym. Sci.* **1999**, *143*, 195.
(99) Roovers, J.; Toporowski, P. M. *J. Appl. Polym. Sci.* **1974**, *18*, 1685.
(100) Freire, J. *Adv. Polym. Sci.* **1999**, *143*, 35.
(101) Merrill, E. W. *J. Biomater. Sci. Polym. Ed.* **1993**, *5*, 1.
(102) Erickson, J. R.; Zimmermann, E. M.; Southwick, J. G.; Kiibler, K. S. *Adhesives Age* **1995**, *11*, 18.
(103) White, J. L. *Rubber Chem. Technol.* **1969**, *42*, 287.
(104) Fleer, G. J.; Cohen Stuart, M. A.; Scheutjens, J. M. H. M.; Cosgrove, T.; Vincent, B. In *Polymers at Interfaces*; Hall, C., Ed.: London, 1993.
(105) Szleifer, I.; Carignano, M. A. *Macromol. Rapid. Comm.* **2000**, *21*, 423.
(106) Szleifer, I. *Curr. Op. Coll. Interf. Sci.* **1996**, *1*, 416.
(107) Bijsterbosch, H. D.; Dehaan, V. O.; Degraaf, A. W.; Mellema, M.; Leermakers, F. A. M.; Stuart, M. A. C.; Vanwell, A. A. *Langmuir* **1995**, *11*, 4467.
(108) Faure, M. C.; Bassereau, P.; Carignano, M. A.; Szleifer, I.; Gallot, Y.; Andelman, D. *Eur. Phys. J. B.* **1998**, *3*, 365.
(109) Majewski, J.; Kuhl, T. L.; Gerstenberg, M. C.; Israelachvili, J. N.; Smith, G. S. *J. Phys. Chem. B* **1997**, *101*, 3122.
(110) Kuhl, T. L.; Majewski, J.; Wong, J. W.; Steinberg, S.; Leckband, D. E.; Israelachvili, J. N.; Smith, G. S. *Biophys. J.* **1988**, *75*, 2352.
(111) Carignano, M. A.; Szleifer, I. *Macromolecules* **1994**, *27*, 702.
(112) Cherepanova, T. A.; Stekolnikov, A. V. *Mol. Phys.* **1994**, *83*, 1065.
(113) Irvine, D. J.; Mayes, A. M.; Griffith-Cima, L. *Macromolecules* **1996**, *29*, 6037.
(114) Mayes, A. M.; Walton, D. G.; Irvine, D. J.; Griffith, L. *Amer. Chem. Soc. Proc. Div. Pol. Mat. Sci. Eng.* **1996**, *75*, 78.
(115) Ohno, K.; Binder, K. *J. Chem. Phys.* **1991**, *95*, 5444.
(116) Ohno, K.; Binder, K. *J. Chem. Phys.* **1991**, *95*, 5459.

(117) Zhulina, E. B.; Vilgis, T. A. *Macromolecules* **1995**, *28*, 1008.
(118) Malmsten, M. *Biopolymers at Interfaces*; Marcel Dekker: New York, 2003.
(119) Baszkin, A.; Norde, W. *Physical Chemistry of Biological Interfaces*; Marcel Dekker: New York, 2000.
(120) Horbett, T. A.; Brash, J. L. *Proteins at Interfaces II*; American Chemical Society: Washington, 1995.
(121) Andrade, J. D. *Surface and Interfacial Aspects of Biomedical Polymers*; Plenum Press: New York, 1985.
(122) Yang, L.; Alexandridis, P. *Curr. Op. Coll. Interf. Sci.* **2000**, *5*, 132.
(123) Uhrich, K. E.; Cannizzaro, S. M.; Langer, R. S.; Shakesheff, K. M. *Chem Rev.* **1999**, *99*.
(124) Willner, I.; Katz, E. *Angew. Chem.* **2000**, *112*, 1230.
(125) Weinberger, S. R.; Morris, T. S.; Pawlak, M. *Pharmacogenomics* **2000**, *1*, 395.
(126) Patel, N. G.; Meier, S.; Cammann, K.; Chemnitius, G.-C. *Sens. Actuators B* **2001**, *75*, 101.
(127) Homola, J.; Yee, S. S.; Gauglitz, G. *Sens. Actuators B* **1999**, *54*, 3.
(128) Ratner, B. D.; Hoffman, F. J.; Schoen, J. E.; Lemons, F. *Biomaterial Science. An Introduction to Materials in Medicine*; Academic Press: New York, 1996.
(129) Niklason, L. E.; Gao, J.; Abbott, W. M.; Hirschi, K. K.; Houser, S.; Marini, R.; Langer, R. *Science* **1999**, *284*, 489.
(130) Manz, A.; Becker, H. *Microsystem Technology in Chemistry and Life Sciences*; Springer Verlag: Berlin, 1998.
(131) Santini, J. T.; Cima, M. J.; Langer, R. *Nature* **1999**, *397*, 335.
(132) Fu, K.; Klibanov, A. M.; Langer, R. *Nat. Biotechnol.* **2000**, *18*, 24.
(133) Macbeath, G.; Schreiber, S. L. *Science* **2000**, *289*, 1760.
(134) Whitney, M.; Rockenstein, E.; Cantin, G.; Knapp, T.; Zlokarnik, G.; Sanders, P.; Durick, K.; Craig, F. F.; Negulescu, P. A. *Nat. Biotechnol.* **1998**, *16*, 1329.
(135) Cacciafesta, P.; Humphries, A. D. L.; Jandt, K. D.; Miles, M. J. *Langmuir* **2000**, *16*, 8167.
(136) Tang, L.; Jennings, T. A.; Eaton, J. W. *PNAS* **1998**, *95*, 8841.
(137) Johnson, R.; Harrison, D.; Tucci, M.; Tsao, A.; Lemos, M.; Puckett, A.; Hughes, J. L; Benghuzzi, H. *Biomed. Sci. Instrum.* **1997**, *34*, 47.
(138) Bos, G. W.; Scharenborg, N. M.; Poot, A. A.; Engbers, G. H.; Beugeling, T.; van Aken, W. G.; Feijen, J. *J. Biomed. Mater. Res.* **1999**, *44*, 330.
(139) Kidane, A.; Park, K. *J. Biomed. Mater. Res.* **1999**, *44*, 640.
(140) Kingshott, P.; St John, H. A.; Griesser, H. J. *Anal. Biochem.* **1999**, *273*, 156.
(141) Soderquist, M. E.; Walton, A. G. *J. Colloid Interf. Sci.* **1980**, *75*, 386.
(142) Hook, F.; Rodahl, M.; Kasemo, B.; Brzezinski, P. *PNAS* **1998**, *95*, 12271.
(143) Norde, W. *Macromol. Symp.* **1996**, *103*, 5.
(144) Norde, W.; Lyklema, J. *J. Colloid Interf. Sci.* **1979**, *71*, 350.
(145) Buijs, J.; Hlady, V. *J. Colloid Interf. Sci.* **1997**, *190*, 171.
(146) Bekos, E. J.; Ranieri, J. P.; Aebischer, P.; Gardella, J. A.; Bright, F. V. *Langmuir* **1995**, *11*, 984.
(147) Ball, A.; Jones, R. A. L. *Langmuir* **1995**, *11*, 3542.
(148) Horsley, D.; Herron, J. N.; Hlady, V.; Andrade, J. D. *Langmuir* **1991**, *7*, 218.
(149) Lensuns, L.; Smith, A.; Gee, M. L. *Langmuir* **2002**, *18*, 9924.

(150) Adams, S.; Higgins, A. M.; Jones, R. A. L. *Langmuir* **2002**, *18*, 4854.
(151) Zoungrana, T.; Findenegg, G. H.; Norde, W. *J. Colloid Interf. Sci.* **1997**, *190*, 437.
(152) Kondo, A.; Murakami, F.; Higashitani, K. *Biotechnol. Bioengin.* **1992**, *40*, 889.
(153) Czeslik, C.; Winter, R. *Phys. Chem. Chem. Phys* **2001**, *3*, 235.
(154) Su, T. J.; Lu, J. R.; Thomas, R. K.; Cui, Z. F.; Penfold, J. *Langmuir* **1998**, *14*, 438.
(155) Arai, T.; Norde, W. *Colloid. Surf.* **1990**, *51*, 1.
(156) Ladam, G.; Gergeley, C.; Senger, B.; Decher, G.; Voegel, J.-C.; Schaaf, P.; Cuisinier, F. J. G. *Biomacromolecules* **2000**, *1*, 674.
(157) Roth, C. M.; Lenhoff, A. M. *Langmuir* **1995**, *11*, 3500.
(158) Robinson, S.; Williams, P. A. *Langmuir* **2002**, *18*, 8743.
(159) Kondo, A.; Higashitani, K. *J. Colloid Interf. Sci.* **1992**, *150*, 344.
(160) Haynes, C. A.; Sliwinsky, E.; Norde, W. *J. Colloid Interf. Sci.* **1994**, *164*, 394.
(161) Turner, J. N.; Shain, W. *Neurol.* **1999**, *156*, 33.
(162) Schmidt, S.; Horch, K.; Normann, R. *J. Biomater. Res.* **1993**, *27*, 1393.
(163) Edell, D. J.; Toi, V. V.; McNeil, C. L. D. *IEEE Trans. Biomed. Eng.* **1992**, *39*, 635.
(164) Jeon, S. I.; Lee, J. H.; Andrade, J. D.; De Gennes, P. G. *J. Colloid Interf. Sci.* **1991**, *142*, 149.
(165) Sharma, S.; Johnson, R. W.; Desai, T. A. *Langmuir* **2004**, *^20*, 348.
(166) Jeon, S. I.; Lee, J. H.; Andrade, J. D.; de Gennes, P. G. *J. Coll. Interf. Sci.* **1991**, *142*, 159.
(167) Deible, C. R.; Petrosko, P.; Johnson, P. C.; Beckman, E. J.; Russell, A. J.; Wagner, W. R. *Biomaterials* **1998**, *19*, 1885.
(168) Kingshott, P.; Thissen, H.; Griesser, H. J. *Biomaterials* **2002**, *23*, 2043.
(169) Allgor, S. S.; Massachusetts Institute of Technology, 1996.
(170) Halperin, A.; Tirrell, M.; Lodge, T. P. *Adv. Polym. Sci.* **1991**, *100*, 31.
(171) Tirrell, M.; Patel, S.; Hadziioannou, G. *PNAS* **1987**, *84*, 4725.
(172) Kent, M. S.; Lee, L.-T.; Farnoux, B.; Rondelez, F. *Macromolecules* **1992**, *25*, 6240.
(173) Zhu, J.; Eisenberg, A.; Lennox, B. *J. Am. Chem. Soc.* **1991**, *113*, 5583.
(174) Kenausis, G. L.; Vörös, J.; Elbert, D. L.; Huang, N.; Hofer, R.; Ruiz-Taylor, L.; Textor, M.; Hubbell, J. A.; Spencer, N. D. *J. Phys. Chem. B* **2000**, *104*, 3298.
(175) Tosatti, S.; De Paul, S. M.; Askendal, A.; VandeVondele, S.; Hubbell, J. A.; Tengvall, P.; Textor, M. *Biomaterials* **2003**, *24*, 4949.

Chapter 3

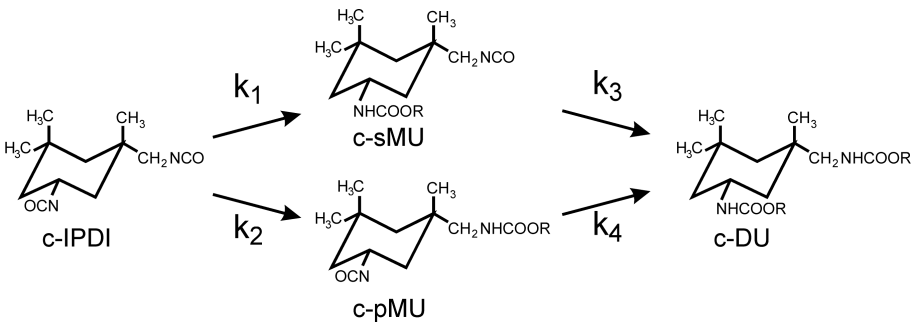
End-capping of 6-arm Star Polymers with Isophorone Diisocyanate

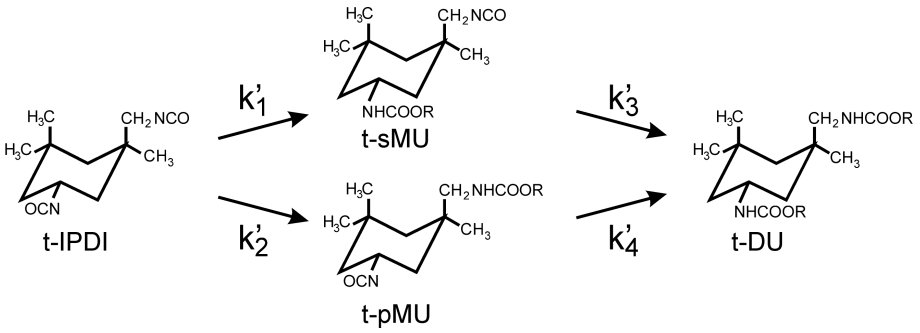
3.1 Introduction

Isocyanates are widespread in a variety of industrial applications. By reacting diisocyanates with diols polyaddition products are obtained, which are commercially used for polyurethane foams or thermoplastic urethanes.[1-4] Urethane prepolymers containing isocyanate endgroups are commonly used in moisture curing systems or urethane based coatings.[5-7] Because of their low melt- and solution viscosities, star shaped prepolymers are of particular advantage for curing systems. If they exhibit many functionalities such as star polymers, the reaction with polyols will result in highly crosslinked networks in short reaction times. Due to the high probability of occurring side products, well defined isocyanate terminated star polymers prove to be a synthetic challenge. Among these side products, the formation of diurethane products must be avoided, in order to obtain products with low viscosities for better handling.

In previous publications, star shaped hydroxy terminated polyethers were converted into isocyanate functionalized reactive polymers by bulk reaction with toluene diisocyanate (TDI)[8,9] or isophorone diisocyanate (IPDI).[10] The reaction procedure with TDI was optimized towards bw chain extension / crosslinking side reactions and yielded liquid products of low viscosity. Apart from their direct use as components in open-celled rigid[11] and flexible[12] polyurethane foams, a wide variety of structo-terminally functionalized adducts of these isocyanate prepolymers have been patented for use as metal quenchants,[13] lubricants or surfactant applications,[14] rubber lattices,[15] textile-[16] or solid surface coatings.[17] This high degree of versatility is a result of their high functionality incorporated in relatively low molecular weight compounds.

Isophorone diisocyanate (5-isocyanatomethyl-3,3,5-trimethyl-1-cyclohexyl isocyanate, IPDI) also contains two isocyanate groups, exhibiting different reactivities i.e. secondary and primary isocyanate group.[18-22] Its generally lower reactivity than the aromatic diisocyanates permits the preparation of water based coatings by deposition from aqueous solution.[23] The used poly(ethylene oxide) backbone can be used to achieve a high biocompatibility of the coatings, e.g. for application in medical, and laboratory devices.





Scheme 3.1: *Reaction Scheme of cis- and trans-IPDI with alcohols.*

Commercially available IPDI is a mixture of ~72% cis and ~28% trans isomers. Reactions with alcohols can thus yield four different monourethane structures (c-sMU, c-pMU, t-sMU, t-pMU) and two different diurethane moieties (c-DU, t-DU) (see Scheme 3.1).

Reactions of IPDI with different alcohols and catalysts are reported in literature, yielding four monourethane and two diurethane derivatives. The different reactivities of the isocyanate groups and the effect of catalysts such as dibutyltin dilaurate (DBTL) and 1,4-diazabicyclo[2.2.2]octane (DABCO) on the reactivity has been described. While IPDI without catalyst reacts mainly with the cycloaliphatic isocyanate group yielding around 83 % of secondary urethanes, Lewis acids, such as DBTL increase the selectivity up to 92 % of secondary urethanes. On the contrary, Lewis bases, like DABCO lead predominantly to primary urethanes (more than 50 %).

Kinetic data on the polycondensation of IPDI with poly(ethylene glycols) are reported in a recent publication.[24] Due to its poisonous effects on cells and proteins, the employment of DBTL was not possible in this work. Due to the interference of colored products in single molecule spectroscopic measurements, the use of DABCO was declined. In order to obtain well defined products without these catalysts, the reaction of IPDI with star polyols of different molecular weights is described, in particular to avoid crosslinking, chain extension and coloration of the products.

3.2 Experimental

Size Exclusion Chromatography (SEC): THF (HPLC grade) was used as the mobile phase. SEC columns were packed with a 5µm particle gel of nominal pore sizes 50, 100, 500 and 1000Å. The refractive index was used as the detection method (Waters RI 410). The prepolymers were reacted with methanol to inactivate the isocyanate groups. The SEC system has been calibrated by means of a mixture of poly(ethylene oxide-*co*-propylene oxide) and poly(propylene oxide) polymeric standards. The molar percentages of the chain extended products (bis-, ter- and quaterstars) have been obtained by numerical deconvolution of the SEC diagrams, applying Gauss type curves. Because of its asymmetry, the monostar peak was fitted by the sum of two Gaussian curves. The individual SEC-peaks of each discrete species have been normalized according to the fraction of isocyanate end groups which has been calculated from the hydroxyl content (OH-titration). The χ^2 fit parameter was determined to be smaller than 3×10^{-3}.

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer (operating frequencies 400 and 125 MHz, respectively) at room temperature. Chemical shifts refer to the $CDCl_3$ signal (7.24 ppm and 77.0 ppm, respectively).

Elemental Analysis was performed using a Heraeus CHNO Rapid Analyzer.

NCO titration: The isocyanate content was determined by reaction with excess dibutylamine and subsequent titration with 1 N HCl solution according to ASTM Standard Test Method D 5155-91, Test Method A.

Hydroxy titration: The hydroxy group content was determined with phthalic acid and imidazole as catalyst according to ASTM Standard Test Method D 4274-94, Test Method D.

Short path distillation was done on a KDL1 unit from UIC GmbH.

Materials: Isophorone diisocyanate (Aldrich, 98 %) containing 72% cis-, and 28% trans-isomer) was distilled twice under reduced pressure ($2*10^{-2}$ mbar). The polyols (Table 3.2.1) were made at DOW Benelux N.V., Terneuzen, The Netherlands. Before use, they were degassed and dried under reduced pressure ($2*10^{-2}$ mbar) at 80°C

for 2 hours. The water content was determined by Karl-Fischer-Titration to be lower than 0.05%.

Table 3.2.1: *Polyols employed for prepolymer preparation.*

Polyol	backbone composition [a)]	number of arms	experimental OH content / mol%	M_n [g/mol] [b)]	M_n(arm) [g/mol]
A	$EO_{80\%}$-co-$PO_{20\%}$	6	3.30	2.914	486
B	$EO_{80\%}$-co-$PO_{20\%}$	6	0.85	12.000	2000
C	$EO_{80\%}$-co-$PO_{20\%}$	6	0,57	17895	2983

[a)] *EO = ethylene oxide, PO = propylene oxide*

[b)] *determined by endgroup analysis (%OH)*

Synthesis of IPDI based prepolymers: The typical preparation of NCO-prepolymers is described at the example of polymer **B**.

In a 250 ml three neck flask with contact thermometer, dropping funnel and argon inlet 85 g of freshly distilled IPDI were heated to 50 °C under argon atmosphere. To the liquid 25 mg of benzoylchloride were added. At constant stirring 50 g of the dried polyol **B** were introduced through the dropping funnel at a rate of approximately 25 g per hour. The mixture was then stirred for 5 days at 50 °C, which remained colorless throughout the reaction. After cooling down to room temperature, the excess of IPDI was removed via thin film distillation. The polymer was distilled at 70 °C under reduced pressure (10^{-2} mbar) at an addition rate of ~40 ml/h yielding the desired product as an almost colorless viscous oil.

Ageing experiments: In a glove box ~100 mg of the product were filled in a in small airtight glass vials and stored under inert gas 7 days at 60 °C in an oven. The isocyanate terminated polymers where then quenched in 5 ml of dry methanol and stirred for 1 day at 50 °C. After evaporating the methanol under reduced pressure the amount of formed bistar was detected by SEC.

3.3 Results and Discussion

During this work 6 arm star polymers have been successfully end capped with IPDI. The reactions were carried out in bulk with an excess of IPDI (mixture of 72 : 28 cis : trans). The excess diisocyanate was removed almost completely from the product by thin film distillation. The parameters of 70°C distillation temperature at $1*10^{-2}$ mbar pressure turned out to be the mildest conditions for a sufficient removal of the free IPDI. By this procedure, the polymer exhibited ~0,06 weight % of free IPDI molecules (~4 % compared to reacted IPDI).

The prepolymers are numbered in following manner: The capital letter stands for the molecular weight of the original polyol (Table 3.2.1) and the number means the excess of IPDI with respect to the number of hydroxyl groups; eg.: the prepolymer **A_4** is the reaction product of the star polyol with the molecular weight of 3000 g/mol and IPDI in a four-fold excess.

The extent of chain extension can be evaluated from size exclusion chromatography (SEC) analysis. Since SEC does not distinguish between the two isomeric c-DU and t-DU moieties, only the overall diurethane content was determined. As a representative example the SEC elugram of prepolymer **A_4** is shown in with the main peak corresponding to the lowest molecular weight particles, the IPDI terminated monostars. The two shoulders in the higher molecular region show the bistar and terstar molecules respectively. The presence of quatermers or higher "xmers" could not be resolved by Gauss fitting. The Gauss fit for peak 1 has been added additionally due to the tailing of SEC curves at the lower molecular weight side of the signal.

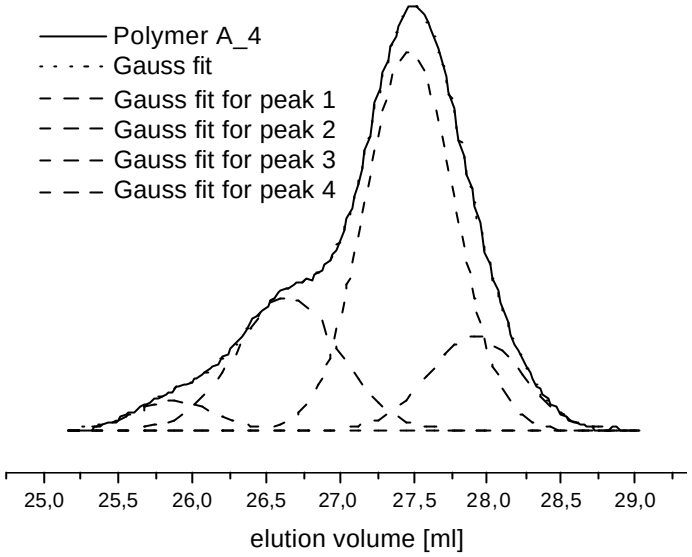


Figure 3.3.1: *Gaussian fit of the SEC diagram of polymer **A_4***

The quantity of the different adducts was calculated by fitting the SEC diagram with Gauss type curves. The abundance h(x) of each molecular species was obtained by numerical deconvolution of the SEC traces by fitting Gauss type curves. The integral intensity of each peak was divided by the degree of polymerization of the respective oligo-star, and subsequently normalized to 100%.

Thus the fraction f_c of chain extension reactions compared to the total number of formed urethane groups could be evaluated. This value is identical to the molar fraction of diurethane linkages:

$$f_C = \frac{N_{DU}}{N_{DU} + N_{MU}} \cdot 100\% \qquad \text{eq. 1)}$$

(f_C = fraction of urethane forming reactions that lead to chain extension = molar fraction of diurethane units, N_{DU} = number of diurethane linkages per macromolecule, N_{MU} = number of monourethane units per macromolecule).

The number of urethane units per molecule follows from the number of arms per star molecule, n, and the number of coupled star molecules, x:

$$N_U = n \cdot x \qquad \text{eq. 2)}$$

(N_U = number of urethane groups per macromolecule, n = number of arms per monostar = 6, x = number of coupled monostar molecules).

Since only $N_C = x - 1$ chain extension reactions are necessary to couple x monostars to one x-mer, the coupling fraction f_C of each individual macromolecule is given by eq. 3:

$$f_C(x) = \frac{x-1}{n \cdot x} \cdot 100\% \qquad \text{eq. 3)}$$

($f_C(x)$ = fraction of urethane forming reactions that lead to chain extension, n = number of arms per monostar = 6, x = number of coupled monostar molecules).

The average of $f_C(x)$ from an arbitrary mixture of x-mers is then obtained from eq. 4:

$$< f_C > = 100\% \cdot \sum_{x=1}^{\infty} \frac{x-1}{n \cdot x} \cdot h(x) \qquad \text{eq. 4)}$$

($< f_C >$ = average fraction of urethane forming reactions that lead to chain extension, n = number of arms per monostar = 6, x = number of coupled monostar molecules, h(x) = numerical abundance of an x-mer).

These equations were used for all of the following multistar calculations.

Chain extension, i.e. coupling of two or more star polymer molecules, could be largely avoided by adding a large excess of IPDI. In the following SEC diagrams and table (,

Table 3.3.1) the decrease of bi-(or multi-) star molecules with the increase of excess IPDI is shown.

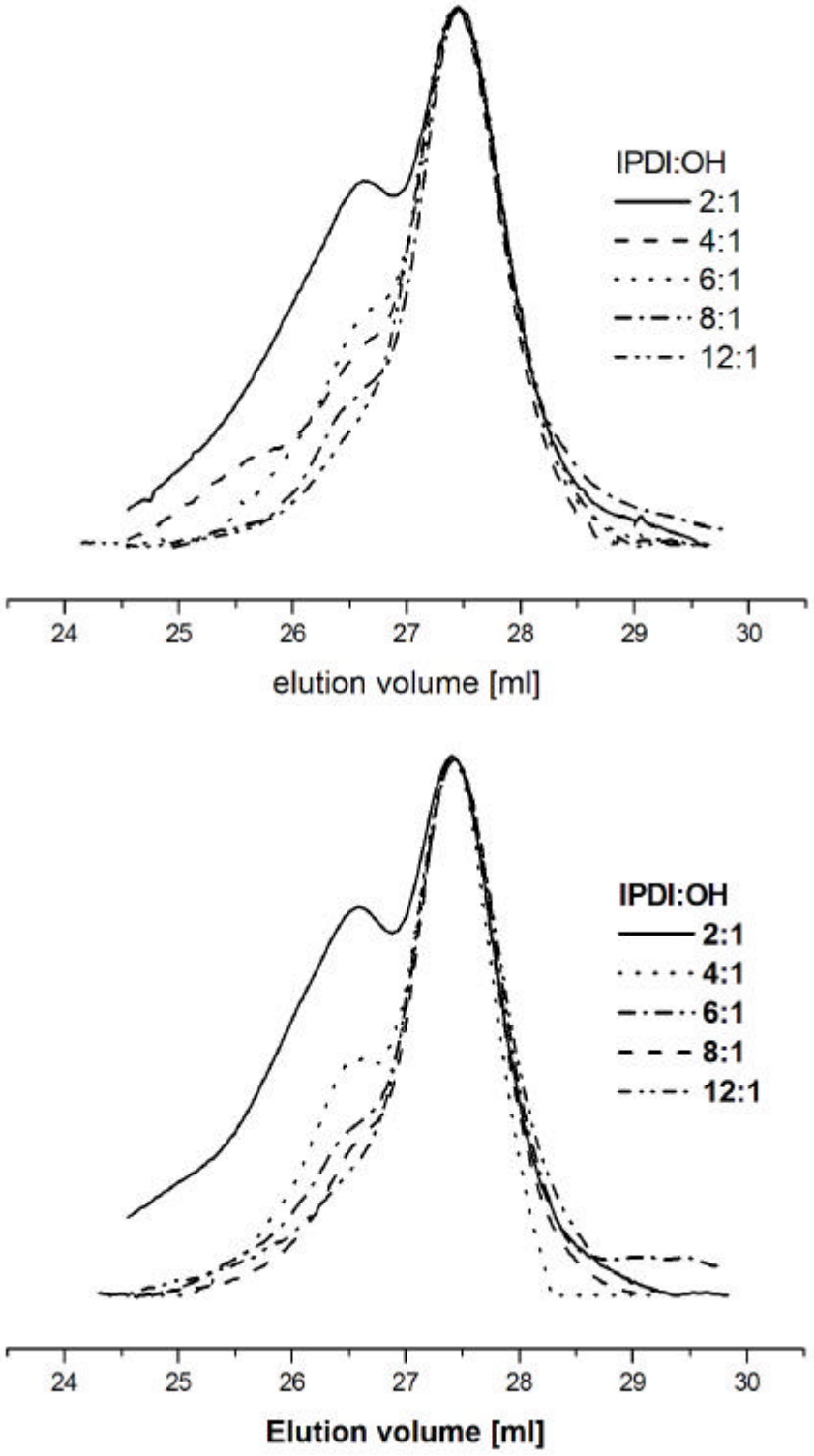


Figure 3.3.2: *SEC diagrams of polymer **A** before and after distillation.*

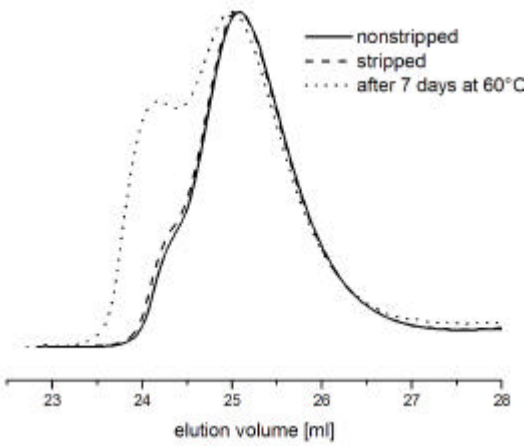
Table 3.3.1: *Bistar ratios of polymer A at different ratios of IPDI to hydroxyl end groups of the polymer*

Polymer name	[IPDI] : [OH] (Polymer	multistar ratio nonstripped [%]		multistar ratio stripped [%]	
		bistar	terstar	bistar	terstar
A_2	2:1	23,9	20	21,7	21,6
A_4	4:1	17,5	12,7	20,7	13,3
A_6	6:1	18,4	9,4	18,2	9,0
A_8	8:1	8,8	5,3	6,8	5,6
A_12	12:1	9,0	0	5,9	0
A_15	15:1	n.d.	n.d.	5,8	0

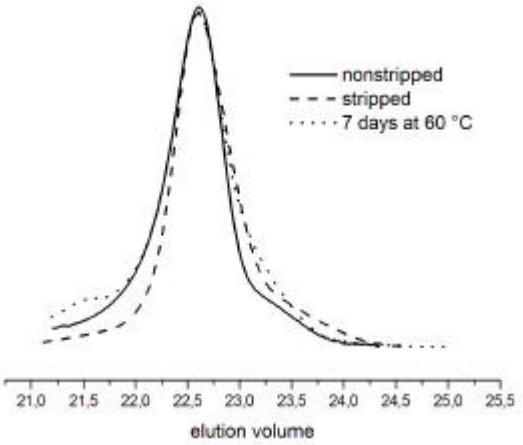
n.d.: not determined

The percentage of reacted polymer hydroxyl end groups was determined by ageing experiments. The resulting curves of the SEC were fitted by Gauss type curves (**Table 3.3.2**).

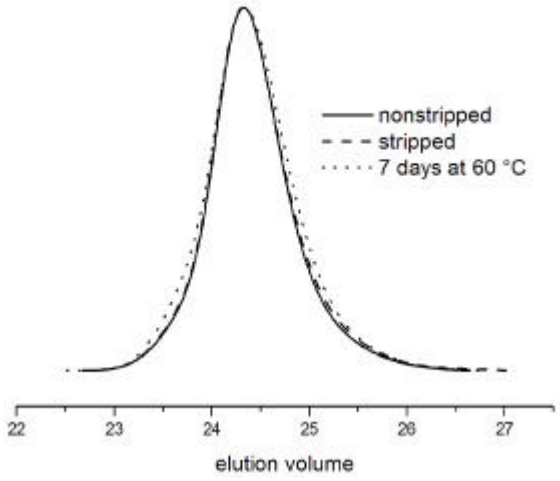
It turned out that the functionalization reaction worked better for the higher molecular weight polymers. The polymer with the molecular weight of ~18,000 g/mol exhibited no bistar formation during the reaction and only 4 % of bistar after ageing of the polymer at 60 °C. The 12,000 g/mol polymer showed similar values (Table 3.3.1). Only the 3,000 g/mol polymer exhibited a comparatively large amount of bistar molecules after the ageing process.



A_15



B_15



C_15

Figure 3.3.3**:** *SEC diagram of prepolymers **A_15**, **B_15**, **C_15** nonstripped, after stripping and after ageing at 60°C for 7 days*

Table 3.3.2: *Bistar ratio of IPDI terminated polyols*

Polymer (Mn/1000 g/mol))	DABCO	Bistar ratio; stripped (<f_c>) [mol %]	Bistar ratio after 7 days at 60°C (<f_c>) [mol %]
A_15	No	2 (0,17)	12,5 (1,0)
A_15	Yes	2 (0,17)	11 (0,88)
B_15	No	2 (0,17)	4 (0,32)
B_15	No	3 (0,25)	5 (0,40)
C_15	No	0	4 (0,32)
C_15	No	0,5 (0,041)	4 (0,32)

(<f_C> = average fraction of urethane forming reactions that lead to chain extension)

Unexpectedly the use of DABCO as a catalytic converter has not improved the selectivity of the reaction. On the contrary it increased the unwanted discoloration of the products.

^{1}H-NMR analysis was also used to verify the extent of nonreacted hydroxyl groups. However, as a result of the relatively high molecular weight of the investigated star polymer, the NMR signals concerning the hydroxyl groups and the IPDI moieties

exhibited a comparatively low intensity. Therefore the obtained values proved to be too imprecise.

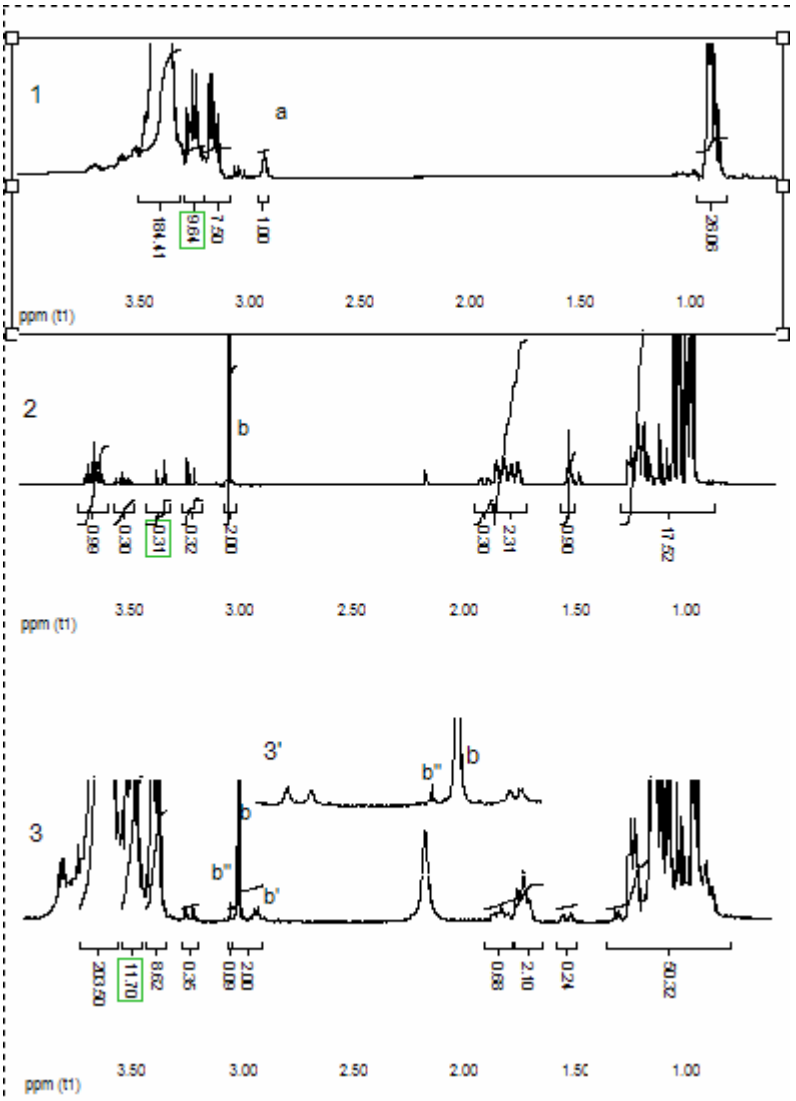


Figure 3.3.4: *^{1}H-NMR of the unreacted starpolymer (1), free IPDI (2), the IPDI terminated polymer (3) and the area from 3.4 to 2.8 of spectrum 3 (3')*

Taking the standard margin of error for ^{1}H-NMR of 2% into account, the disappearance of the hydroxyl peak of the crude polymer (**a**) after the reaction indicates the complete conversation of the hydroxyl groups. The integrals of the methylene group connected to the primary isocyanate group (**b**) and to the equivalent urethane group (**b'**), respectively, fit well with the expected values for the reacted isocyanate

prepolymer. According to eq. 3.3.5 and eq. 3.3.6 the degree of polymerization per arm (P) was calculated to be 42.

$$M_{(EO/PO)} = 0.8 * M_{(EO)} + 0.2 * M_{(PO)} = 46.8 \text{ g/mol} \qquad \text{eq. 3.3.5)}$$

$$P = \frac{M_{w(polyol)} - M_{(sorbitol)}}{M_{(EO/PO)} * N} \qquad \text{eq. 3.3.6)}$$

with $M_{(EO)}$ = 44 g/mol; $M_{(PO)}$ = 58 g/mol, $M_{(sorbitol)}$ = 182 g/mol, $M_{w(polyol)}$ = 12,000 g/mol and the number of arms N = 6.

Assuming 100 % of end group conversion the ratio of the integrals of the methyl group (**b**) to the aliphatic peaks must be 2 (methyl protons) to 187 (protons per arm + 2 core protons) eq. 3.3.8)

$$H_{(EO/PO)} = 0.8* H_{(EO)} + 0.2 * H_{(PO)} = 4.4 \qquad \text{eq. 3.3.7)}$$

$$H_{(arm)} = H_{(EO/PO)} * P \qquad \text{eq. 3.3.8)}$$

with H = number of protons; $H_{(EO)}$ = 4, $H_{(PO)}$ = 6, $H_{(EO/PO)}$ = average number of protons per chain unit and $H_{(arm)}$ = number of protons per arm

According to this calculation the endgroup conversion with IPDI would lie around 80%. Concerning the ageing experiments and the results of the elemental analysis (following) this value is too low. The error of the integration of the small methyl peak of the IPDI group can result in a larger error when compared to the integrals of the polymer signals. Therefore this calculation is only taken for a rough estimation of the end group conversation.

Regarding the signal **b''** (-C**H$_2$**-NCO of the free IPDI) it can be seen, that the polymer still exhibits a small amount of free IPDI. The amount is so low with respect to

the polymer with 0,06 wt%, that it can not be detected by SEC and also should not affect the further reactions done with these polymers.

In addition to NMR analysis, eelemental analysis confirmed the good conversion towards isocyanate terminated star prepolymers (Table 3.3. 3).

Table 3.3. 3 *Elemental analysis of IPDI terminated star polymers*

polymer	Calculated* C/H/N	found C/H/N
A_15	58.3/8.9/3.9	58.16/8.88/4.09
B_15	57.0/9.3/1.3	56.93/9.22/1.29
C_15	56.8/9.3/0.9	56.65/9.28/0,89

** calculated for100 % end group conversion*

3.4 Conclusions

Six arm star prepolymers with the molecular weights of 3.000, 12.000 and 18.000 g/mol with IPDI end caps can be prepared with good control of chain extension by using a large excess of IPDI. Short path distillation to remove the IPDI, in the presence of benzoic acid chloride resulted in a colorless to pale yellow, viscous prepolymer liquid corresponding to a good end group conversation. Storage stability was excellent: When stored in a glove box or at 5 °C under argon the product kept stable over months. Although usually applied to improve isocyanate reaction efficiency, the catalysis with DABCO has not resulted in a decrease of the crosslinking products. On the contrary it led to increased discoloration of the product, which negatively affects subsequent fluorescence measurements[25]. Catalysis with DBTL was not possible due to its poisonous effect on cells, also employed in further experiments[25].

NMR analysis and ageing experiments have shown the good conversation of the hydroxyl groups of the star polymers. By SEC it was demonstrated that no chain extension occurred during the functionalization of the star polymers.

3.5 References

(1) Dearth, R. S.; Bock, M.; Mertes, H. In *3rd Congress for New Tecnologies for Coatings and Inks*; Bayer, Ed., 1995.

(2) Kahl, L.; Bock, M.; Laas, H. J.; Klinksiek, B. In *I-Lack*; Bayer, Ed.; Vincentz Verlag: Hannover, 95; Vol. 12/95

2/96.

(3) Laas, H. J.; Halpaap, R.; Pedain, J. In *Farbe + Lack*, 1994; Vol. 5.

(4) Laas, H. J.; Halpaap, R.; Pedain, J. *J. prakt. Chem.* **1994**, *336*, 185-200.

(5) Graham, N. B.; Zulfiqar. *Polymer* **1989**, *30*, 2130.

(6) Peterson, S. *Justus Liebig's Ann. Chem.* **1949**, *562*, 205.

(7) Smith, T. L.; Magnusson, A. B. *J. Polym. Sci.* **1960**, *42*, 391.

(8) Grünbauer, H. J. M.; Bartelink, C. F.; Elwell, M. J.; Mills, R. In *Proc. Polyurethanes World Congress, Amsterdam,*, 1997; Vol. 1, p 347.

(9) Bartelink, C. F.; de Pooter, M.; Grünbauer, H. J. M.; Beginn, U.; Möller, M. *J. Polym. Sci. A. Polym. Chem. 2555* **2000**, *38*, 2555.

(10) Götz, H.; Beginn, U.; F., B. C.; Gruenbauer, H. J. M.; Möller, M. *Macromol. Mater. Eng.* **2002**, *287*, 223-230.

(11) Smits, G. F.; Cini, G.; Gruenbauer, H. J. M.; Broos, J. A. F. In *U.S. Patent No. 5,856,678*, 1999.

(12) Elwell, R. J.; Sewell, R. A.; Lidy, W. A.; Thoen, J. A. *U.S. Patent No. 5,114,989* **1992**.

(13) Bartelink, C. F.; Van Voorst, R.; Grünbauer, H. J. M.; Möller, M.; Critchley, D. In *World Patent No. WO98/06770*, 1998.

(14) Grünbauer, H. J. M.; Bartelink, C. F.; Möller, M. In *U.S. Patent No. 5,808,131*, 1998.

(15) Elwell, M. J.; van der Wal, H. R.; Bartelink, C. F.; Grünbauer, H. J. M. In *World Patent No. WO98/37113*, 1998.

(16) Bartelink, C. F.; Grünbauer, H. J. M.; Götz, H.; Möller, M. In *World Patent No. WO99/16800*, 1999.

(17) Grünbauer, H. J. M.; Bartelink, C. F.; Elwell, M. J.; Möller, M. In *World Patent No. WO98/20060*, 1998.

(18) Lorenz, O.; Decker, H.; Rose, G. *Angew. Makromol. Chem.* **1984**, *122*, 83.

(19) Bialas, N.; Höcker, H.; Marschner, M.; Ritter, W. *Makromol. Chem.* **1990**, *191*, 1843.

(20) Ono, H.-K.; Jones, F. N.; Pappas, S. P. *J. Polym. Sci.: Polym. Lett. Ed.* **1985**, *23*, 509.

(21) Hatada, K.; Ute, K.; Oka, K.-I. *J. Polym. Sci.: Part A: Polym. Chem.* **1990**, *28*, 3019.

(22) Hatada, K.; Ute, K.; Pappas, P. *J. Polym. Sci.: Part C: Polym. Lett.* **1987**, *25*, 477.

(23) Okkema, A. T.; Braatz, J. A.; Curtin, C.; Custer, L. M.; Gunther, V. J.; Heifetz, A. H. In *World Patent No. WO94/23771*, 1994.

(24) Rochery, M.; Vroman, I.; Lam, T. M. *J.M.S. - Pure Appl. Chem.* **2000**, *A37*, 259.

(25) Groll, J; *PhD. Thesis*, Deutsches Wollforschungsinstitut, RWTH: Aachen, 2004.

Chapter 4

Preparation and Characterization of IPDI-terminated Star Polymer Coatings

4.1 Introduction

Biocompability is of growing interest for the biological and medical scientists. As well as for medical implants also for the controlled tissue engineering inside the human body biocompatible products are of great interest. Therefore the surface of these implants must be unrecognizable to proteins and cells. To achieve this three different main technologies were of biocompatible surface coverage were developed in the recent years. First the self assembly of monolayers onto model substrates like silicon[1], glass[2], gold[3-5], titanium and titanium oxide[6,7] and modifications thereof[8,9] had been developed. The second possibility is to adsorb polyelectrolyte multilayer films[10-13]. The third, probably most promising method is the formation of hydrogels[14], which contents can be mainly divided into natural polymers, e.g. collagene[14], gelatine[14] or polysaccharides[15,16], and synthetic polymers like ethylene glycol[17,18], acrylic acid derivative based systems[19-21] or polypeptides[22,23].

While tailoring of the hydrogels for specific interactions with proteins or cells had been made, still problems like mechanical stability, ageing of the substrates, low network density, patterning of functional areas are not satisfyingly solved.

Due to their high network density and number of functional end groups, star polymers are very promising to solve these problems. Especially the orientation of the end groups towards the surface, which was shown by Griffith-Cima et al.[24] is very desirable for biological applications.

Hydrogels have been formed from PEO star molecules by radiation crosslinking reaction,[25-27] but the resulting networks were non-functional. Lately, nitrocinnamate terminated PEO star molecules with eight arms were photocrosslinked and modified with a fluorescent peptide to design a highly selective hydrogel membrane for copper ions[28]. For applications as layers for biomaterial substrates, a comparison between linear and photo cross linked star PEO was done,[29] but the stars in this study did not form a network by crosslinking. Furthermore, a radiation cross linked PEO star hydrogel was modified with galactose to enhance interaction with liver cells,[30] and the group of Jeffrey A. Hubbell recently used 4 arm star derived PEO hydrogels created by Michael-type addition for cell experiments[31-33]. Nevertheless these studies were rather dealing with cells in three dimensional hydrogels than with thin surface coatings.

In this chapter, the formation of thin and ultrathin layers from six arm star shaped polyethers is presented. The polymer backbone consists of a statistical copolymer of 80% ethylene oxide and 20% propylene oxide. Each star molecule bears 6 reactive isocyanate end groups (star PEG). Films were obtained by spin coating of star polymer solutions from different solvents in various concentrations. Crosslinking of the system leads to a dense network of PEG chains connected by biocompatible urea groups. The coatings were studied regarding thickness, homogeneity and wetting behaviour.

4.2 Experimental

Materials: Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Glass substrates (50.8x50.8x0.175mm) were purchased from Schott Desag. High refractive index glass (LaSFN 9) was purchased by Hellma Germany. Acetone, isopropanol and ethanol

(Merck, selectipur) were stored in the clean room and used as received. THF and toluene were dried over $LiAlH_4$, distilled under argon and transferred into a glove box. N-[3-(trimethoxysilyl) propyl] ethylenediamine (Aldrich, 97%) was stored in the glove box and filtered before usage. The polyols were made at DOW Benelux N.V., Terneuzen, The Netherlands. Syringe filters with pore size 0.02 µm were purchased from Whatman.

Methods: Silicon and glass substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath. Oxygen plasma was generated by a TePla 100-E system with 100 W at a process gas pressure of 0.5 mbar. Samples were treated with UV/ozone using a 40 W UV lamp (main emission 185 nm; UV-Technik Speziallampen GmbH) in an oxygen stream of 350 ml/min with a sample distance of 5 mm to the lamp. Films were generated with a CONVAC ST 146 spin coater. Layer thicknesses were examined using a MM-SPEL-VIS ellipsometer (OMT). Contact angles were measured using the sessile drop method with a G 40 contact angle measuring device (Krüss GmbH). Lower detection limit of the method is around 10 degrees. Light microscopy and fluorescence microscopy were performed by means of an Axioplan2 Imaging microscope from Zeiss. Light microscopy pictures were taken with a Zeiss AxioCam HR camera. Scanning force microscopy (SFM) investigations were performed with a Nanoscope IIIa (Digital Instruments) operating in tapping ModeTM. The oscillation frequency for Tapping ModeTM was set in the range of 320 – 360 kHz depending on the Si cantilever (k ~ 50 N/m, Nanosensors). The evaluation of the results was done with the 5.12r3 software version of Nanoscope III. In every following image the large picture shows the topography of the surface with the phase in the upper left frame showing the contrast in smoothness of the surface. The frame below shows a cross section of the height image.

Substrate preparation: Cutting and cleaning of the substrates was performed in a class 100 clean room. Silicon wafers and glass plates were cut into pieces of 14x14 mm. The samples were then cleaned by sonication in acetone, water and isopropanol for one minute each followed by drying in a stream of prefiltered nitrogen. Activation of the surface can either be achieved by treatment with UV/ozone for 12 min or by exposure to oxygen plasma

for 10 min. After both processes, the contact angle of the substrates with water was below the detection limit. Partially the substrates were used immediately for aminofunctionalization.

Aminosilylation of the substrates: After activation, the substrates were transferred into an Unilab glove box (MBraun) and immersed into a solution of 0.3 ml N-[3-(trimethoxysilyl)propyl] ethylenediamine in 50 ml dry toluene for 2 hours. Then the samples were washed several times with dry toluene and stored under dry toluene until further usage.

Sample preparation: Different amounts of the polymers made in chapter three were dissolved in the glove box in THF or toluene, respectively. The concentration varied from 0.1 to 100 g/mol. These solutions were transferred to the clean room and spin coated through a 0.02 µm syringe filter onto the prepared substrates at different rotation speeds. For spin coating, the substrates were placed on the spin coater, covered by the corresponding solution and then accelerated within 5 seconds to the final rotation speed, either 1500 rpm, 2500 rpm or 5000 rpm, and kept rotating for 40 sec. The resulting films were stored for several days in ambient atmosphere or under a water saturated atmosphere so that crosslinking could take place.

For spin coating of chain extended polymers to the polymer solutions different concentrations of ethylene diamine or triaminoethyl amine were added and spin coated through a syringe filter after varying times. The molar ratios of IPDI groups to the amine groups of the cross linker were in the range of 3:1 to 1:3. Time between the addition of cross linker and spin coating varied between 10s to 30 min.

If only a monolayer of the polymer had to be adsorbed the substrates were immersed in the glove box into the respective polymer solution, stored for 5 to 30 minutes and then washed thoroughly with the equivalent solvent. For the case of quenching the isocyanate surface the substrates were immersed again into 5 ml THF, taken out of the glove box and the THF diluted with 5 ml of Millipore water. In this mixture the samples were kept for 1 hour, taken out and rinsed again with THF.

For a better wetability one weight percent of the surfactant hexafluoro propyleneoxide diglycol dimethylether was added to some of the polymer solutions before spin coating.

Layer thickness determination: The silicon substrates were examined by ellipsometry with a spectral method in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15 degrees; the integration time was dependent on the layer thickness and the resulting signal intensity. In order to minimize systematic errors in the data collection, in a series of experiments always one substrate was just cleaned and activated and one was just cleaned, activated and aminosilylated. These two substrates were measured as references, and thicknesses of the hydrogel films were obtained as relative values to the aminosilylated substrate. Each sample was measured at 5 different places, and the presented data are the average values of each sample. Errors were determined through evaluation of the standard deviation of the measurements.

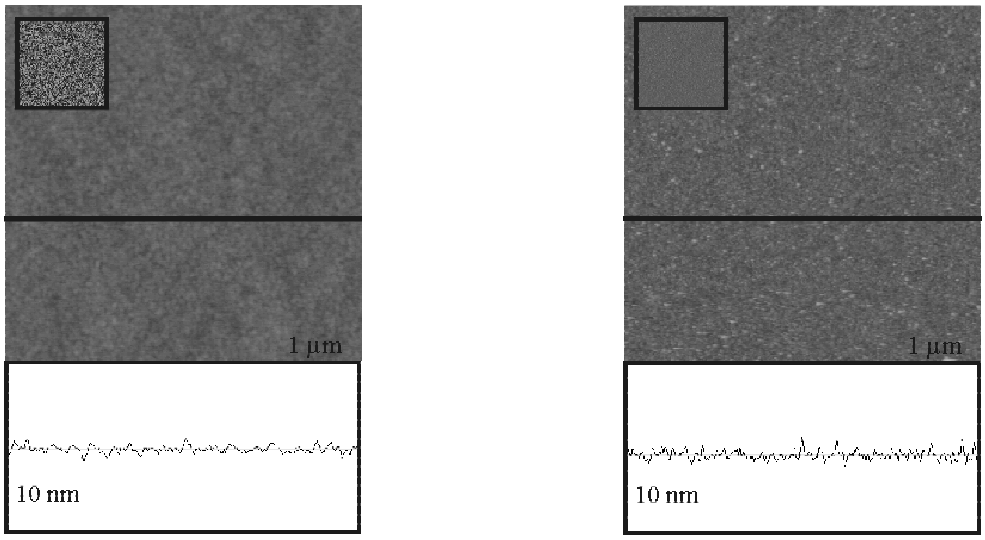
Contact angle measurements: On each substrate four droplets of Milipore water were measured on different places of the sample. For each drop ten measurements were performed with time intervals of 1 sec between the measurements. The resulting value of each single measurement is the average value of left and right contact angle. The presented data are the average values of 4 measurements per substrate. Special care was taken that the routine of the measurements was always the same so that the time between droplet deposition and first measurement was the same for all measurements. Errors were determined through evaluation of the standard deviation of the measurements.

4.3 Results and Discussion

During this work silicon and glass substrates were coated with the IPDI terminated star polymers synthesized in chapter three. The coating of the substrates occurred by spin coating of PEG solutions in THF or toluene, respectively. Layer thickness of the coatings was varied by different solution concentration and/or rotation speeds. In order to quench the observed dewetting processes the polymers were treated before and after the spin coating process in several different ways.

In order to obtain strong covalently bound coatings the substrates were first covered with an aminosilane (in long time measurements in water the Si-O-CO bond could be

hydrolyzed and the IPDI-PEG's were removed partially from the plain surface). This silylation worked in good reproducibility resulting in a smooth amino terminated surface at a layer thickness of around 1.5 nm and an average roughness (rms) of 0.4 nm (Figure 4.3.1). Combined with 2.6 nm oxygen layer of the activated surface of the silicon wafer reference substrates exhibited an average layer thickness of 4.1 nm. Variation of the time immersion into the amino-solution from 1 to 16 hours didn't improve the smoothness of the substrates. Also the contact angles stayed with 45 ° ± 5 ° rather the same.



a) rms = 0,2 nm b) rms = 0,4 nm

Figure 4.3.1: *AFM images of activated (a) and aminosilylated (b) silicon wafer.*

Due to the fact that silicon oxide, the aminosilane and the polymers exhibit the same refractive index, ellipsometric measurements can not distinguish between the different layers. Therefore at estimated layer thicknesses larger than 20 nm the average reference value was subtracted from the result. For layers with an expected thickness below 20 nm each substrate was measured separately before coating.

To cover the substrates solutions of varying concentrations of the prepolymers in THF or toluene were prepared. By varying the concentrations and the rotation speed of the spin coating table, the average layer thickness of the substrates could be well controlled (Figure 4.3.2).

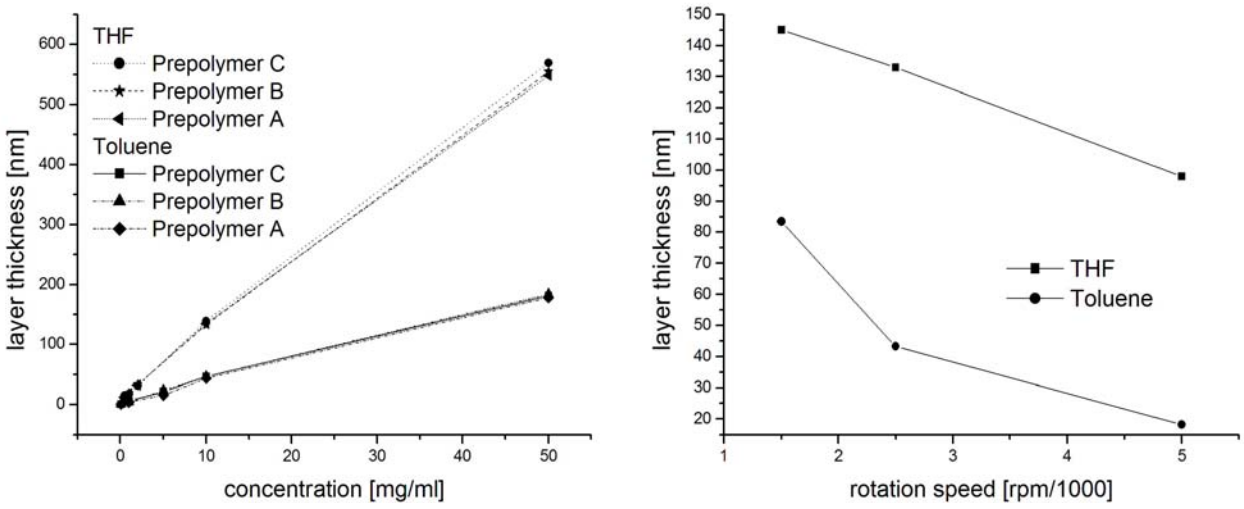


Figure 4.3.2: *Dependency of the layer thickness to: the concentration(at), rotation speed (b); layers were made by spin coating of the prepolymers A-C out of THF or toluene, respectively. Due to the uniformity of the different prepolymers in figure b) only the prepolymer B was applied.*

While with higher concentrations of the prepolymer solutions thicker layers were obtained, the polymer weight didn't show any influence on it. The increase of the layer thickness with the concentration is almost linear. Due to the interactions with the surface with higher concentrations a small decrease in the slope can be observed. With a higher rotation speed the layer thicknesses were decreasing due to following reason. In the beginning of the spin coating a thin solvent film is formed on the substrate which evaporates during further spinning leaving the prepolymer layer behind. The thicker the starting film is, the thicker also the resulting layer is. Higher rotation speeds result in a thinner solvent film and therefore thinner prepolymer layers. Further could be observed that in every experiment the layers spin coated out of THF were thicker than the respective toluene layers. THF evaporates faster than toluene, so that the concentration in the evaporating solvent film is becoming higher for the THF film. The resulting polymer layers are bigger. Therefore also the effect of the rotation speed is lower for the THF solutions. In the work of J. Groll[34] this dependency is also shown for different THF/water solutions.

Contact angles were measured within the first ten seconds after the droplet was put on the surface. A rather fast decrease of the values made it necessary to keep a precise routine at

every measurement. This decrease is also the reason for the rather high standard deviations. In Figure 4.3.3 the dependency of the contact angle to the layer thickness of the prepolymer layer is shown.

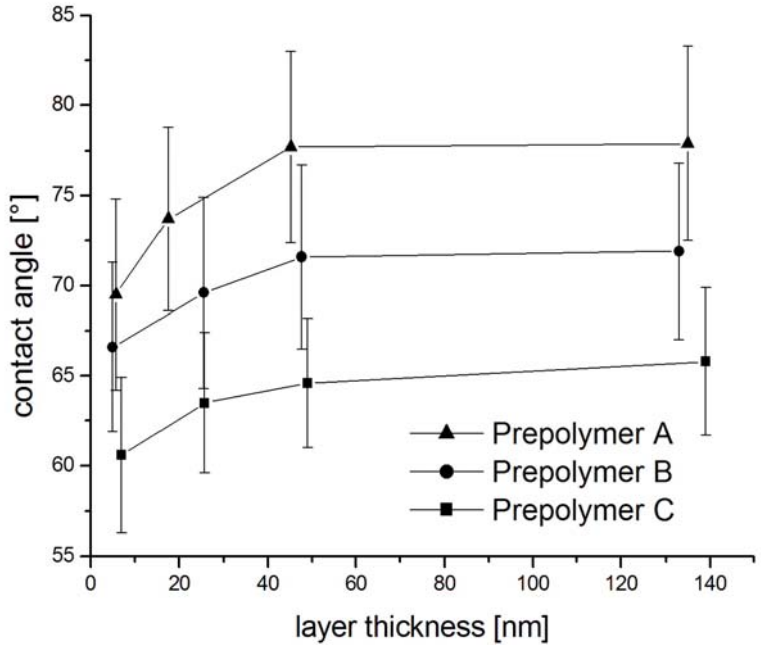


Figure 4.3.3: *Dependency of contact angle of water droplets to the thickness of the prepolymer layers.*

With increasing weight the contact angles are decreasing. The reason therefore lies in the higher IPDI concentration in the prepolymers with the lower molecular weight. IPDI and the resulting amine are less polar than the polymer backbone, therefore the higher amine concentration which also occurs on the surface results in a lower surface tension towards the water droplets. The contact angle is increasing. Another phenomenon could be observed for all three prepolymer layers. With increasing layer thickness also the contact angle is increasing until a limit value is reached. The precise explanation for this phenomena is given elsewhere[34]. Since the IPDI end groups are rather hydrophobic, the isocyanate terminated stars have an amphiphilic character that was expected to have a big influence on structure and morphology of films formed by them (Figure 4.3.4).

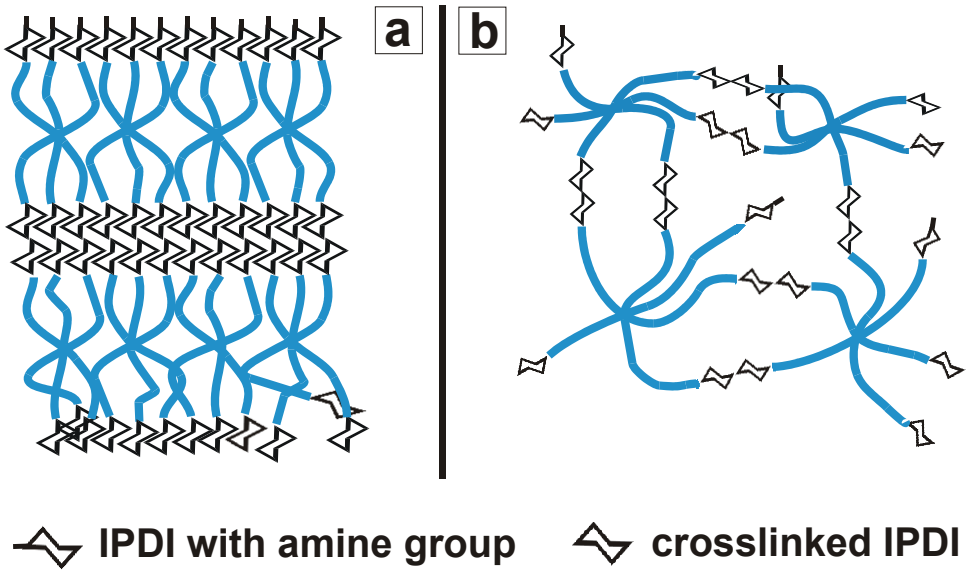


Figure 4.3.4: *Possible layer morphologies due to the amphiphilic character of the star shaped prepolymers. In thermodynamic equilibrium, the layers are expected to exhibit a layer-by-layer morphology (a), whereas the formation of this morphology can be quenched by fast chemical crosslinking what results in an unordered network structure (b).*

If the system has enough time to come into thermodynamic equilibrium prior to crosslinking, a layer like structure that shows minimal surface energy towards air will result. This can be achieved by working with solutions in water free organic solvents, since the crosslinking reaction that leads to a dense network structure starts with the hydrolysis of isocyanate groups to amine groups. Amines are much more reactive than water or alcohols towards isocyanate groups and react with those to form stable and biocompatible urea groups. Thereby, a dense network of PEO/PPO stars linked via urea groups is formed.

Another proof for this concept is given, when the prepolymers are spin coated without solvent. After storage in humid atmosphere, the several micron thick layers exhibit contact angles from 85 ° to 114 ° which lies close to the values of alkyl terminated surfaces.

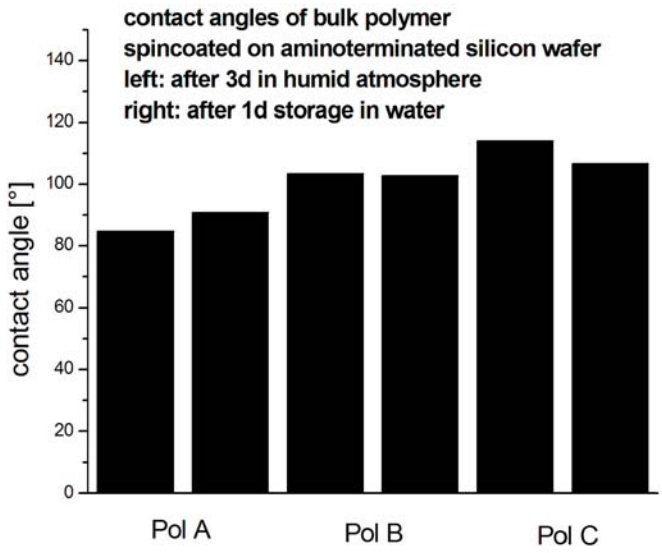
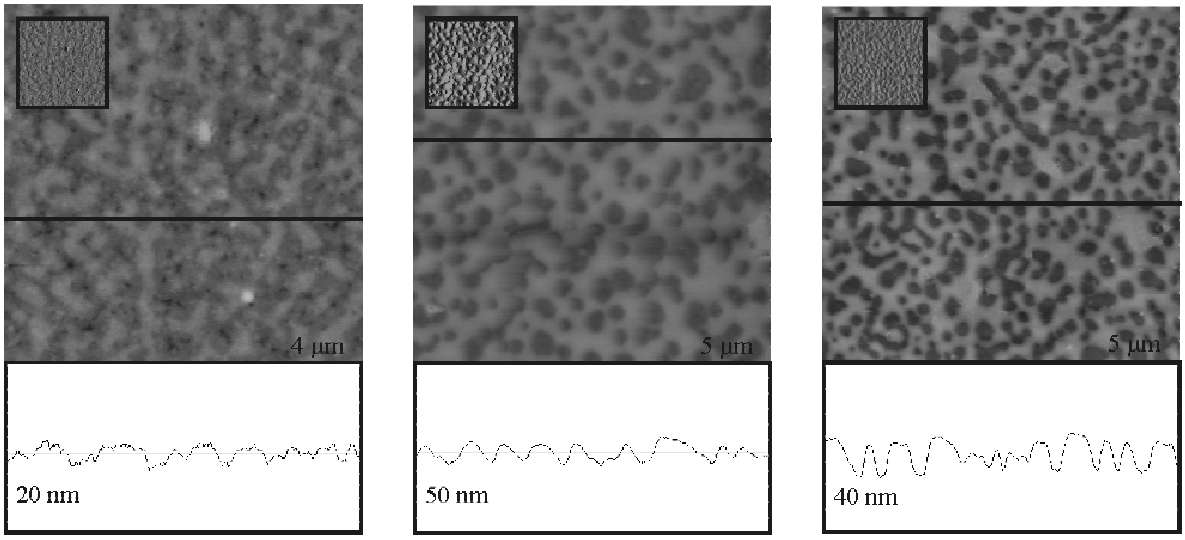


Figure 4.3.5: *Contact angles of the prepolymers spin coated in bulk at a rotation speed of 5000 rpm.*

In spite of the higher IPDI concentration the lower molecular weight prepolymers here exhibit the lower contact angles. This lies in the flexibility of the single polymer arms. The longer arms are more flexible and can therefore arrange themselves in the film. The incorporated water helps the shorter chains to rearrange, resulting in an increase of the contact angle for the prepolymer A.

Covering the substrates with different concentrated solutions of the IPDI terminated prepolymers **A-C** (**A:** 3000 g/mol; **B**: 12000 g/mol; **C**: 18000 g/mol) a strong dewetting process could be detected.



A) rms = 0.8 nm **B**) rms = 1.7 nm **C**) rms = 2.6 nm

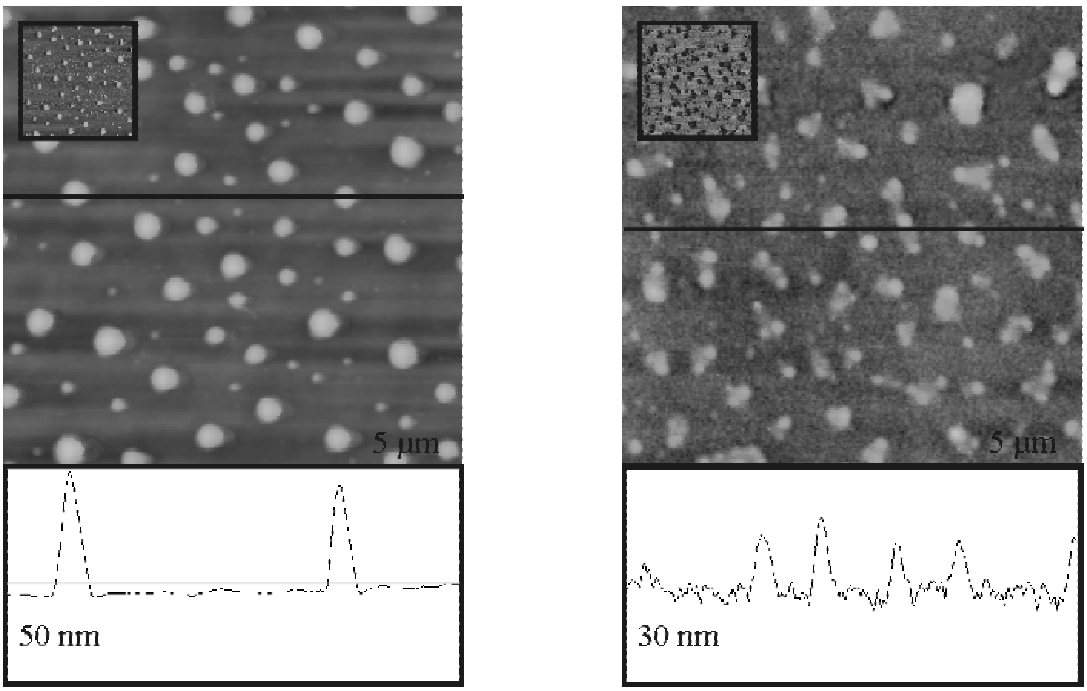
Figure 4.3.6: *AFM images of prepolymers **A-C** spin coated onto aminosilylated substrates at a rotation speed of 2500 rpm with a solution of 1.0 g/l prepolymer in toluene*

Regarding the mean roughness values (rms) a tendency to stronger dewetting with higher molecular weight could be observed. This can be explained with the increasing flexibility of the polymer arms. The longer the chains are the more they tend to coagulate. The driving force for this phenomena is supposed to be the 'autophobic dewetting', which means that during the coating process a first monolayer adsorb to the surface and reject all following polymer units. The reaction of the first layer takes place still in the liquid and when the solvent is evaporated the residue polymer tries to minimize its surface tension by building small droplets. In the case of the IPDI terminated star polymers in a first step the unpolar IPDI end groups attach and react with the amine or hydroxyl terminated surface driving the rather polar backbone to the surface. If this process occurs with all six arms of the stars further polymer units have no free binding sites on the surface and therefore are only adsorbed on the first monolayer. But the surface energy of the ethylene oxide backbone is too low to allow stable layers of the further adsorbed polymer.

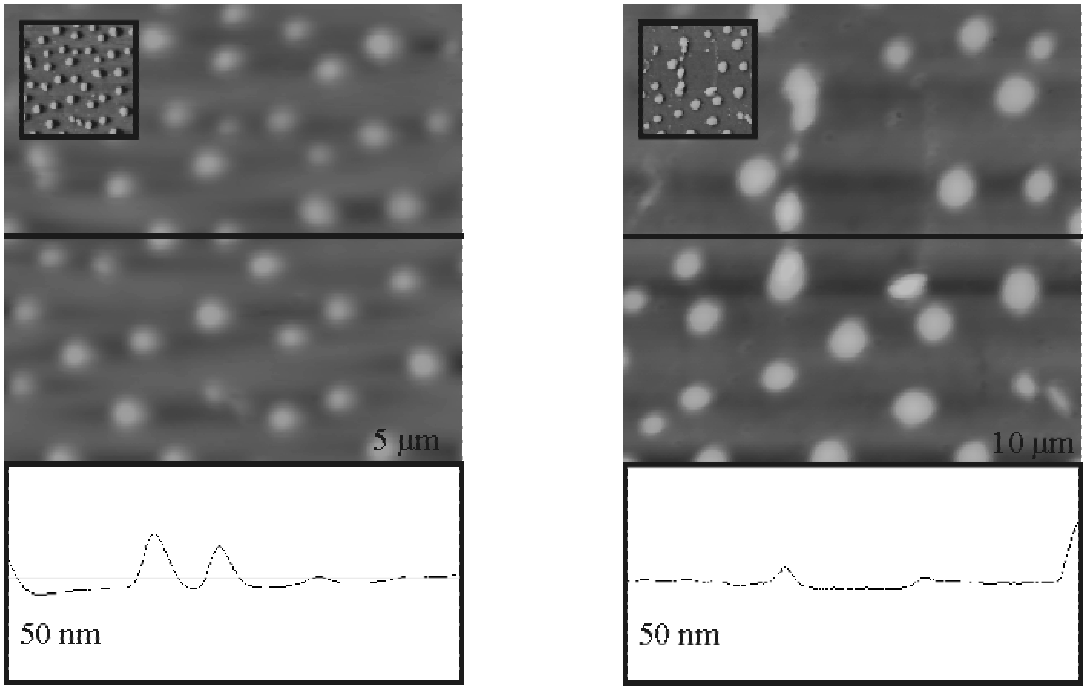
The proof of this concept is given in the following experiments in which it was tried to quench the dewetting process. This was tried either by varying the surface tension by different surfaces (a), partially chain extension of the polymers before the spin coating process (b), exposing the freshly coated layers to different environments (c) or combination of this three

methods. In these experiments the three different prepolymers **A-C** were spin coated out of solutions in THF and toluene in concentrations from 0.1 to 10 mg/ml.

a) Variation of the surface / surface tension: In order to examine the influence of the surface onto the layer formation different substrates were examined. Due to the expected high reactivity of the amine terminated surface, which is supposed to support the binding of all six arms of the prepolymers to the surface, only plain, ozone activated substrates were prepared. Except for plain silicon wafers, also flat glass and glass with a higher refractive index (LaSFN 9) were applied. Due to the higher refractive index of 1.85 the polymer should wet this kind of glass better, resulting in a more stable film. But with none of the applied substrates a smooth polymer layer could be obtained. Examined were the prepolymers **A** and **B.** (Figure 4.3.7, Figure 4.3.8)

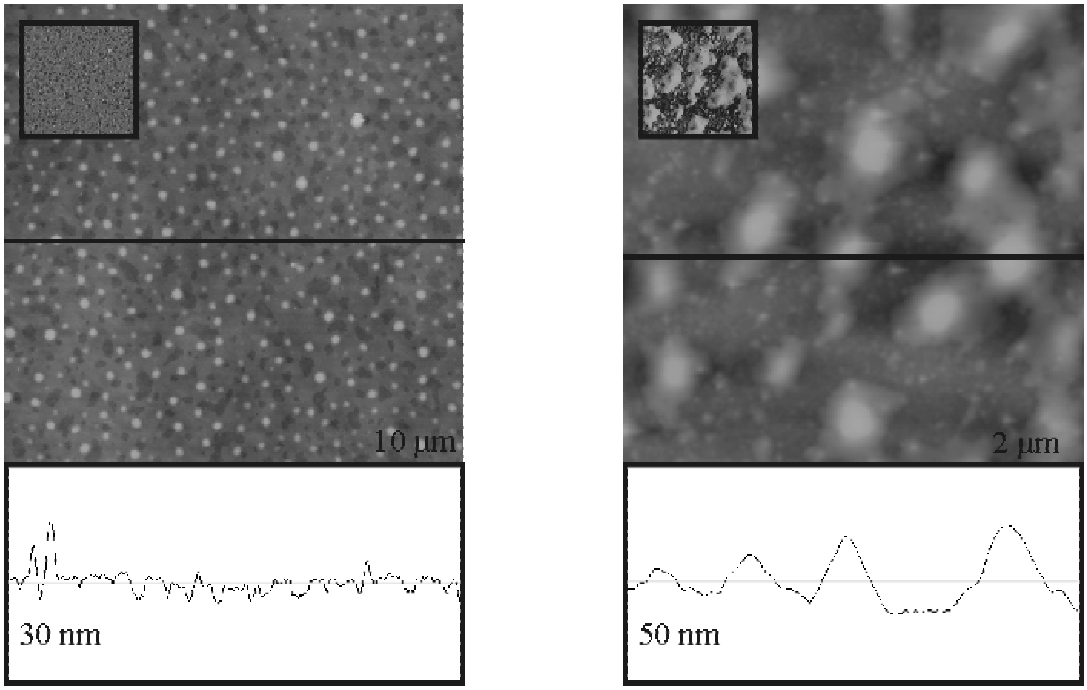


a) b)

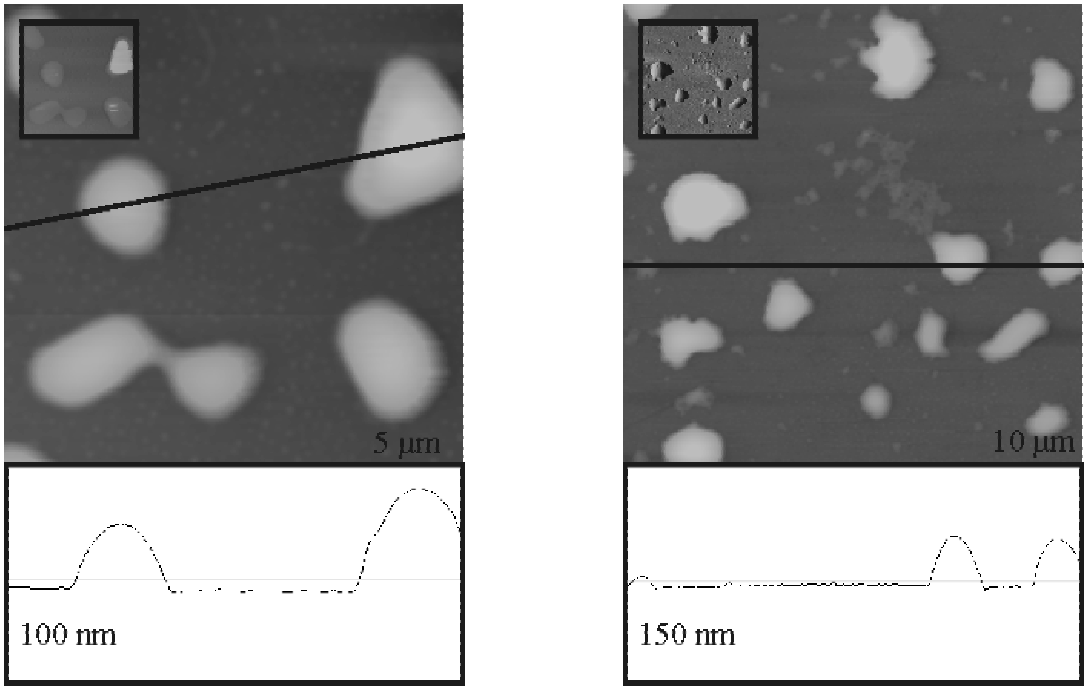


c) d)

Figure 4.3. 7 *AFM pictures of 0.1 mg/ml solution of prepolymer A in THF, spin coated onto four different ozone activated substrates; a) silicon, b) aminosilylated silicon, c) flat glass d) LaSFN 9 glass*



a) b)



c) d)

Figure 4.3.8: AFM pictures of 0.1 mg/ml solution of prepolymer B in THF, spin coated onto four different ozone activated substrates; a) silicon, b) aminosilylated silicon, c) flat glass d) LaSFN 9 glass

For both polymers it can be seen that spin coating on aminosilylated substrates result in the most smooth layers which can be a matter of the increased roughness of the applied surface. Still they also exhibited a strong dewetting of the surface. Applying high refractive index glass didn't improve the stability of the coated layers. They dewetted in the same degree. Table 4.3.1 gives the values of the droplets resulted from the dewetting.

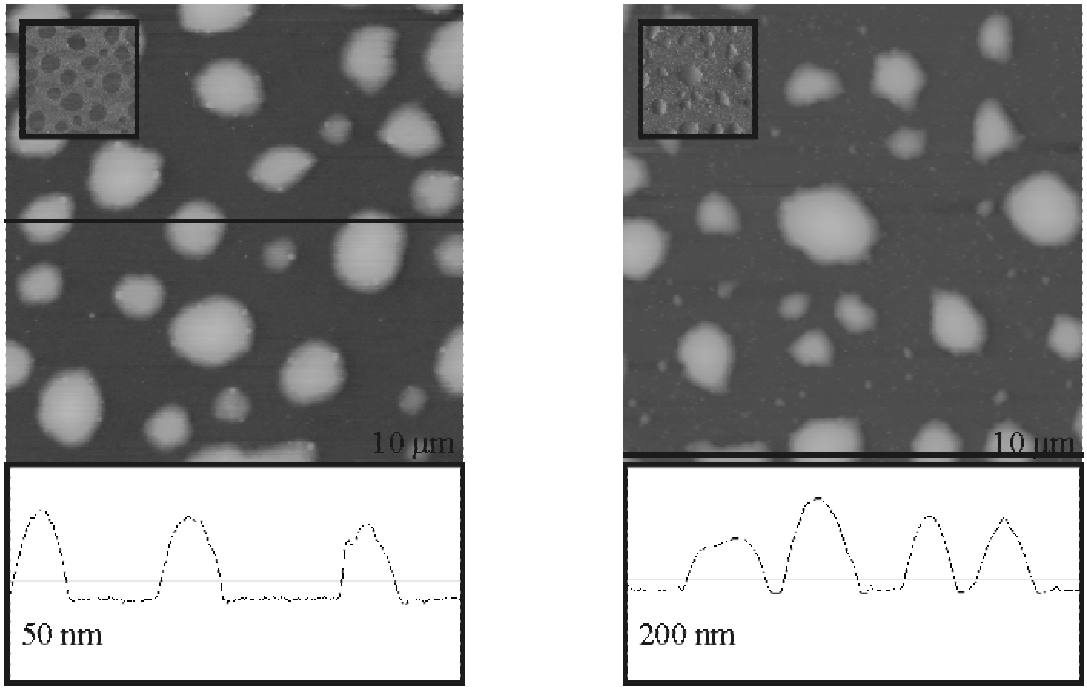
Table 4.3.1: *Particle size of the prepolymers A and B spincoated onto different substrates.*

Polymer	Substrate	Rms [nm]	Average droplet height [nm]	Average droplet width [nm]
A	silicon	4.8	17.6	284
A	aminosilyl.	3.5	9.8	238
A	flat glass	3.5	11.7	415
A	LaSFN 9	3.5	13.4	275
B	silicon	1.2	5.9	217
B	aminosilyl.	3.6	8.8	177
B	flat glass	14.3	38	970/ 100
B	LaSFN 9	15	48	760

Regarding this values again it can be seen that the polymer with the higher molecular weight shower a stronger dewetting of the surface. The particles of prepolymer **B** are much larger compared to those of prepolymer **A.**

Regarding image d) (Figure 4.3.7) a square area of 1 μm^2 can be seen. In this area the sample was scratched during the AFM measurement. From the small walls on two sides of the square it can be seen, that a small amount of polymer could be scratched from the surface. The height difference between the scratched area and the polymer covered sides is only some angstrom. The polymer between the bigger clusters therefore can only be a wide stretched monolayer. Thus can be another proof for the assumption of the autophobic dewetting.

Another indication therefore is given by a further experiment. Therefore amino terminated substrates were primarily covered with a monolayer of the prepolymer **B** (description is given in chapter 8). Onto these samples the respective polymer solutions were spin coated. Again the polymer showed a strong dewetting from the surface (Figure 4.3.9).

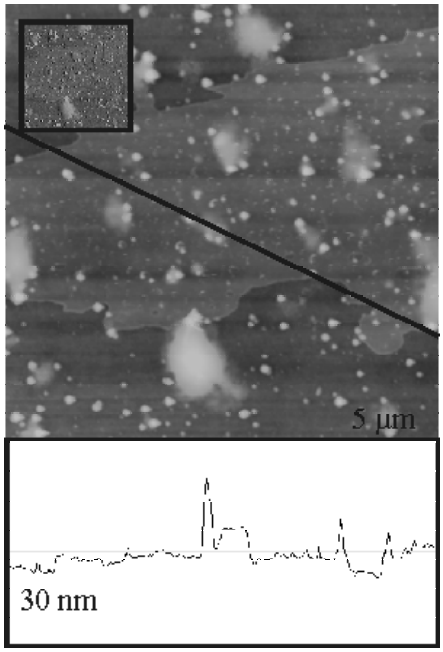


a) rms = 6.5 b) rms = 14,8

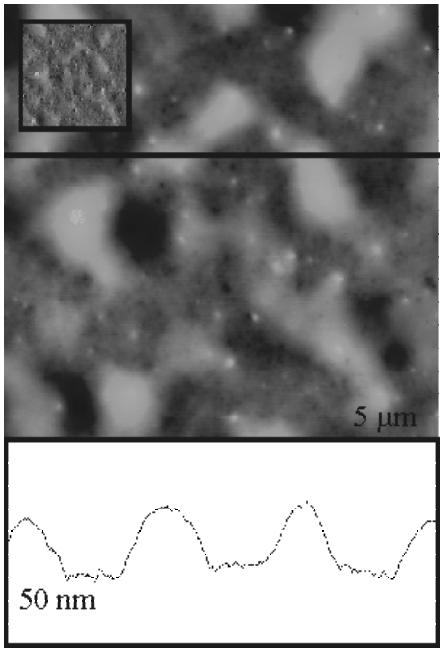
Figure 4.3.9: *AFM images of prepolymer B spin coated onto substrates covered with a monolayer of the respective polymer; a) 0.5 mg/ml polymer in THF, b) 1.0 mg/ml in THF*

b) *Chain extension of the prepolymers:* Although the prepolymers exhibit already six functional end groups it seemed to be not enough. Due to their flexibility of the arms the star polymers had the possibility to arrange all six arms toward the surface leaving no binding sites for further reactions. With controlled chain extension it was tried to gain larger polymer units with a bigger amount of reactive sites. Therefore small bifunctional or trifunctional amine compounds were added in different concentrations to the polymer solutions and allowed to react before spin coating. Due to the high reactivity of IPDI towards amines the polymers were enlarged within some seconds. This could be seen when higher concentrations of the trimanine was added, as within some seconds a white milky precipitation was formed.

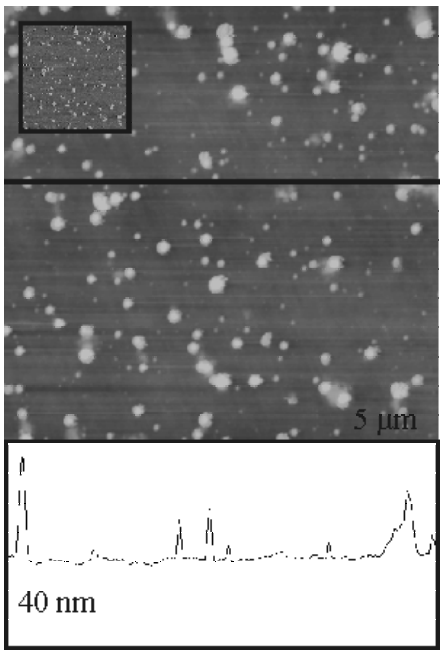
First the chain extensions were carried out with ethylene diamine. Following molar ratios of prepolymer to diamine were applied: 2:1, 1:1 and 1:2. The former two concentrations showed no improve in the surface topography. Dewetting occurred as in the untreated layers. The latter solutions resulted in layers with minor dewetting structures (Figure 4.3.10).



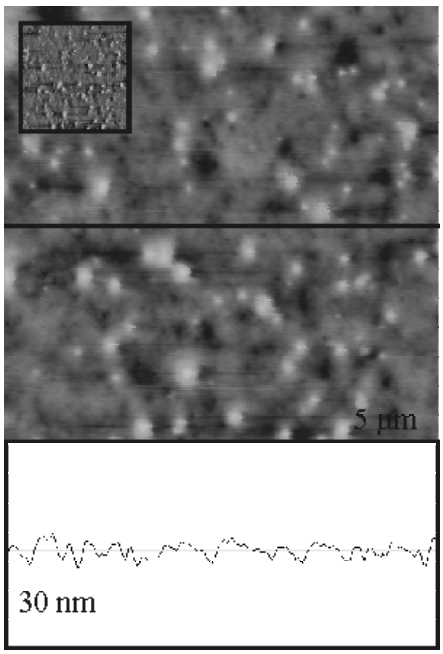
a) rms = 3.6 nm



b) rms = 5.2 nm



c) rms = 3.9 nm



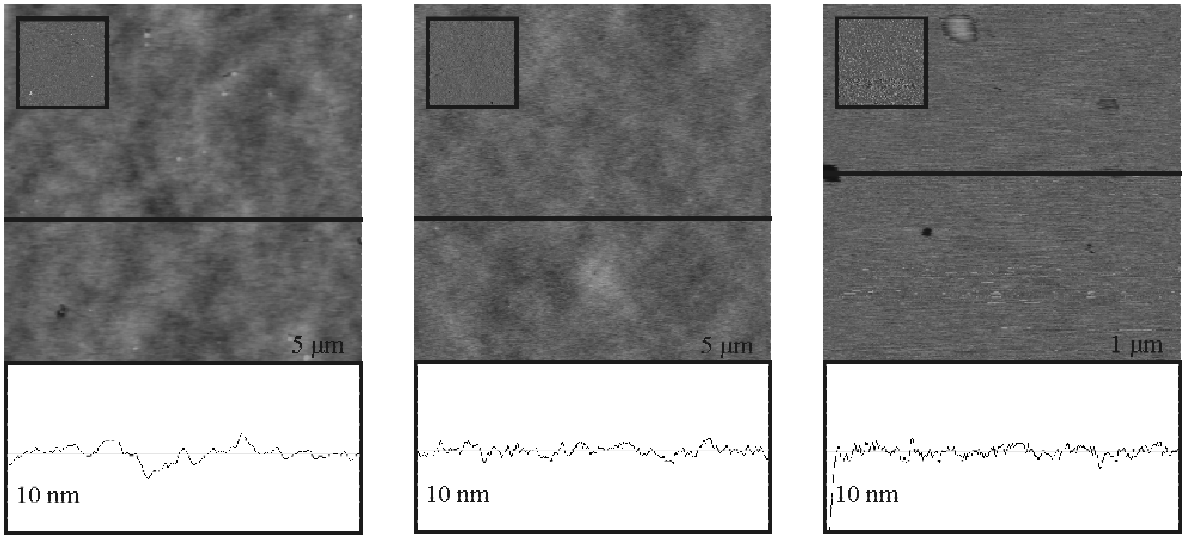
d) rms = 2.8 nm

Figure 4.3.10: *AFM images of EDA modified prepolymer layers spin coated out of THF solutions. With molar ratio of EDA: prepolymer B/C (a,b/c,d) 1:2. The concentration of the solutions was 0.1 mg/ml (left) or 1.0 mg/ml (right), respectively.*

The substrates covered with the 0.1 mg/ml solution (a/c) showed rather poor surface structures. While the substrate covered with prepolymer C (c) only exhibited undefined polymer clusters on the surface of the other sample an area covered with a thin polymer layer could be observed. But also large undefined polymer clusters are stuck on the surface. Better results delivered the coatings with the 1.0 mg/ml solution. In both cases only a small droplet formation was observable Also the rms values show a significant decrease compared to the plain prepolymer samples. Together with the low phase contrast a complete coverage of the surface could be confirmed. But still the prepolymers tended to dewet from the surface.

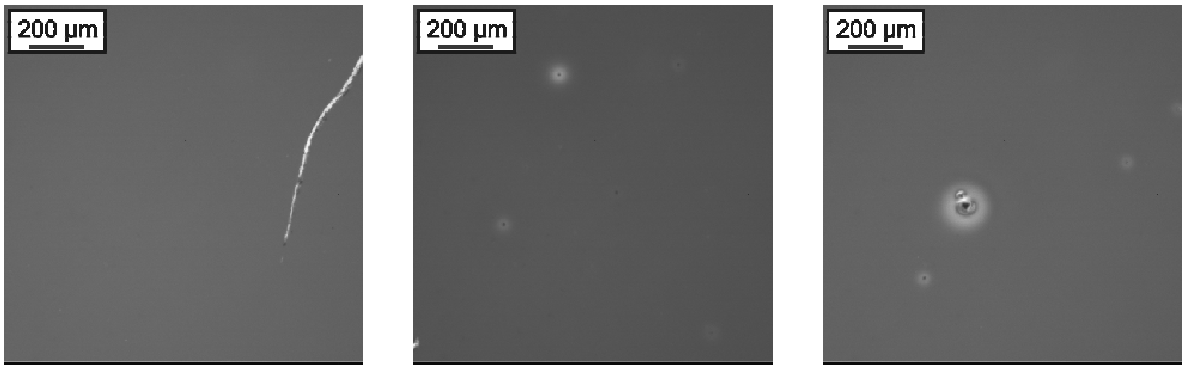
To test the stability of these films they were half dipped several times into deionised water and both sites were examined by ellipsometry. Unfortunately except for a small layer the polymer could be washed away. This indicates that the addition of EDA caused a chain extension, which partially quenched the dewetting. But still the first covalently bound layer exhibited no further reactive sites to attach further polymer units. At least the crosslinking of the prepolymer layers in humid atmosphere for 7 days had a further stabilization as a result. Then only small amounts of the layer could be washed away. A higher concentration of EDA in the spin coating solution as well as different reaction times before spin coating didn't smooth the layer roughness.

The rather promising results of the experiments with EDA lead to further experiments with pre-crosslinking agents. In another set of experiments similar to the above, tris-aminoethyl amine (TAEA) was applied as chain extender. The molar ratio of prepolymer to TAEA was set to 1:1, leaving still reactive NCO-groups behind. Coating and examination were performed according to the former experiments. Figure 4.3.11 and Figure 4.3.12 show the smooth surfaces of the prepolymer coatings spin coated out of solutions of 1.0 or 10.0 mg/ml, respectively.



Prepolymer C Prepolymer B Prepolymer A

Figure 4.3.11: *AFM images of TAEA modified prepolymer layers spin coated out of THF solutions with a polymer concentration of 1.0 mg/ml*



Prepolymer C Prepolymer B Prepolymer A

Figure 4.3.12: *Optical microscope images of TAEA modified prepolymer layers spin coated out of THF solutions with a polymer concentration of 10.0 mg/ml. The defect in the left image was caused by a scratch accidentally.*

In all experiments a well defined smooth surface was obtained. Neither the concentration nor the polymer weight showed any significant influence onto the surface topography. Rinsing the samples with water also didn't result in the complete removal of the layer. Still some amount of prepolymer could be washed away. This amount varied with the

experiments and was not hundred percent reproducible. The most promising results are shown in Figure 4.3.13.

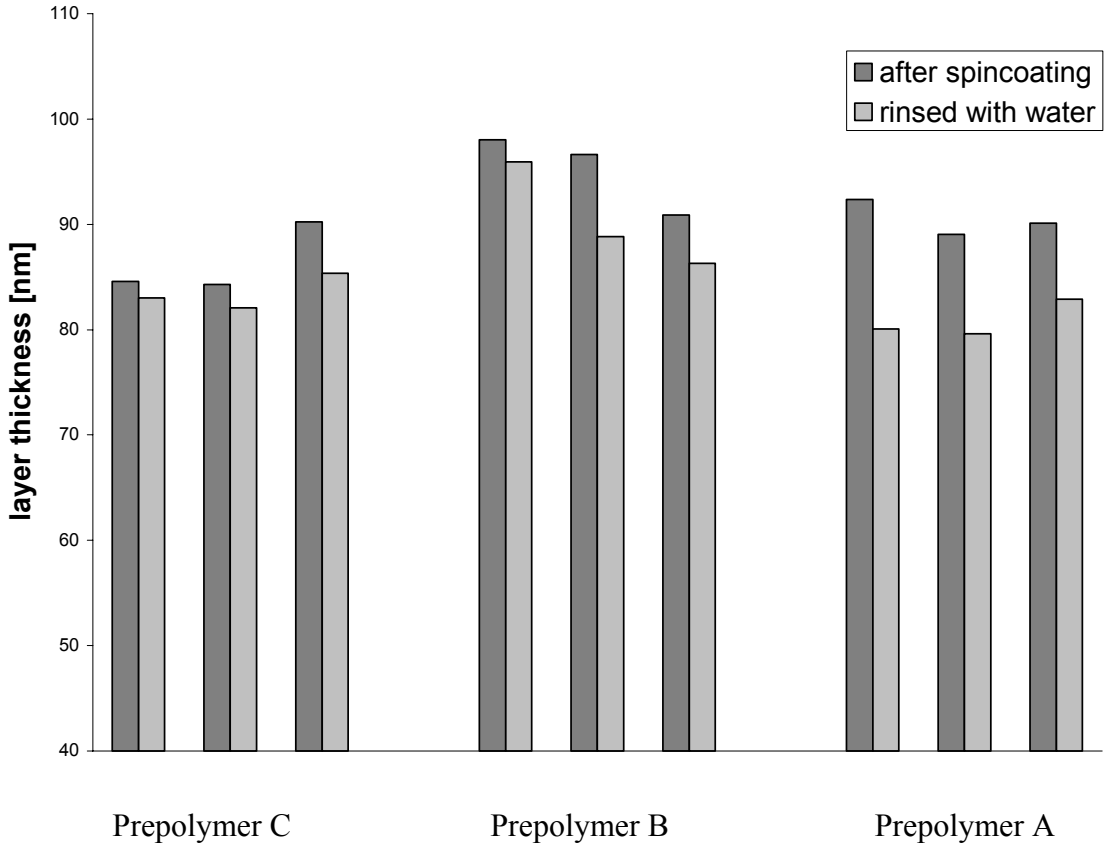


Figure 4.3.13: *Layer thickness of TAEA modified prepolymer layers before and after washing.* Molar ratio of prepolymer to TAEA = 1:1

Nether the less the loss of polymer could reach 90 percent of the freshly prepared layer. Crosslinking of the prepolymer in humid atmosphere again had a further stabilizing effect onto the layers.

c) Exposing the freshly coated layers to different environments: To quench the dewetting process in the former two methods changes in the reaction mechanism were only made before the spin coating process. This had the benefit that theoretically still some NCO-groups remained after the spin coating process. In this third method the dewetting was tried to be quenched by crosslinking the ready coated layer. By exposing the IPDI-terminated prepolymer layers to a water or ammonia saturated atmosphere this crosslinking could be

achieved. The exposure to humidity was already prescribed above and was performed to stabilize the rather smooth films against removal by washing with water. Although this worked rather well, it didn't work for the freshly spin coated, plain prepolymer layers. As prescribed in chapter 5 the water uptake of the thin prepolymer layers is rather poor and slow. In the time till enough water is absorbed and has reacted with the NCO-groups to form a cross linked network, the fresh prepolymer layer already started to dewet. So the exposure to a humid atmosphere only had little effect on the topography of the samples. In order to increase the reaction speed some plain prepolymer layers were exposed to an ammonia saturated atmosphere. Therefore in the bottom of an exsiccator a small beaker filled with 30 % ammonia solution was placed. After the samples were put in the exsiccator it was closed and slightly evaporated. Storage of the samples over night turned out to be sufficient enough to quench the dewetting.

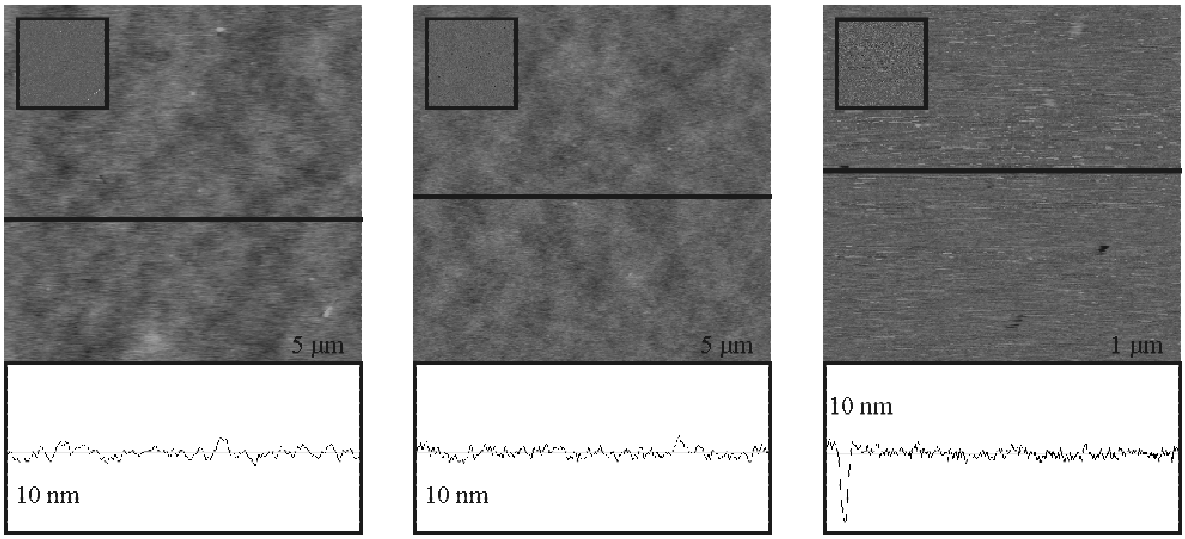
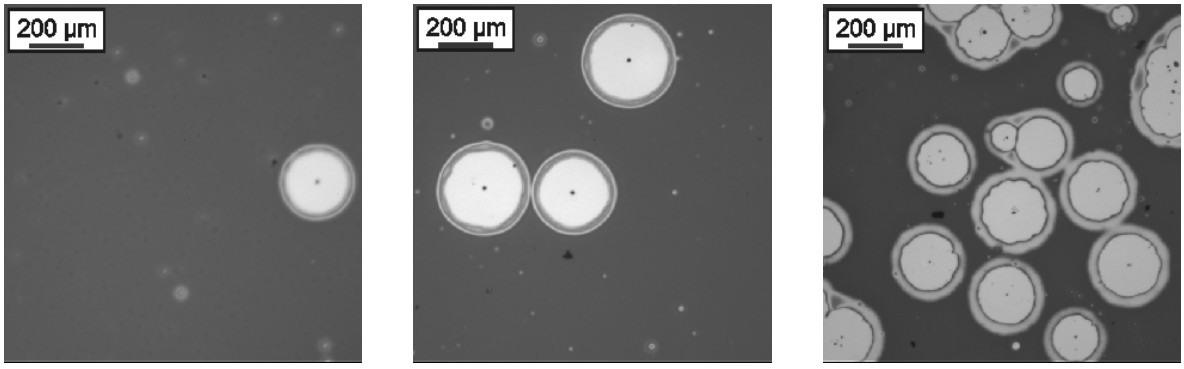


Figure 4.3.14: *AFM images of prepolymer layers exposed to ammonia atmosphere directly after spin coating out of 1.0 mg/ml solution in THF.*



Prepolymer C Prepolymer B Prepolymer A

Figure 4.3.15: *Optical microscope images of prepolymer layers exposed to ammonia atmosphere directly after spin coating out of 10.0 mg/ml solution in THF.*

As easily can be seen the dewetting could not be fully quenched. In Figure 4.3.15 some areas are detectable, were it already started. Due to the fact that the dewetting starts directly after the spin coating, in the beginning the amount of adsorbed ammonia is too low to quench this process. Still it slowed down the dewetting till it ceases completely. Also a tendency is observable, that the higher the molecular weight is, the better the quenching worked. This is believed to be the matter of the more flexible chains of the higher molecular weight polymers. After reaction with ammonia they can find more easily another NCO-terminated chain end for the urea formation.

As examined by ellipsometry the formed prepolymer layers could be partially washed away when rinsed with water. Unlike in other experiments not only a polymer monolayer remained on the surface. The amount of remaining polymer on the surface seemed to be statistically. It wasn't possible to detect any relation to molecular weights of the polymer or the layer thicknesses. Long time exposal could probably solve this problem, but due to lack of time this experiments haven't been carried out.

4.4 Conclusions

During this work three different IPDI-terminated star polymers were covalently bound to alternating surfaces by means of spin coating out of solutions in THF or toluene, respectively. The average height of the prepolymer layers could be well controlled by varying the concentration of the solutions or by adjusting the rotation speed of the spin coating table.

All three prepolymers showed a strong dewetting if spin coated onto aminosilylated surfaces. Due to the fact, that the polymer layers were removable except for one ultrathin layer, the conclusion could be made, that this process could be specified to be an 'autophobic dewetting' which means, that only the first polymeric monolayer is covalently bound to the surface, and all further polymer units are repelled by the polymeric backbone of this monolayer. Several efforts were made to quench this process.

a) Applying different samples like plain activated silicon or several different glass types had no positive effect on the dewetting, which is another indication for the autophic dewetting.

b) By chain extension of the prepolymers with bi or trifunctional amines this process could be quenched. While ethylene diamine could only moderate this effect, tris-aminoethyl amine fully quenched the dewetting process. But still the polymer layers were partially removable by rinsing with water

c) The third method was to expose the freshly spin coated layers to a NCO reactive atmosphere. Water worked only poorly to quench the dewetting. Further it was only applied to stabilize already modified prepolymer layers. Exposure of the layers to an ammonia saturated atmosphere slowed down rapidly the dewetting and quenched it completely after some time. But like in part (b) the polymer could be washed off.

All in all it can be said that it was possible either to obtain NCO-containing prepolymer layers with a more or less rough surface topography, or to obtain smooth stable prepolymer layers without any NCO-groups left. Water stable smooth prepolymer layers which still exhibit NCO-groups wasn't possible in any case.

4.5 References

(1) Petrash, S.; Cregger, T.; Zhao, B.; Pokidysheva, E.; Foster, M. D.; Brittain, W. J.; Sevastianov, V.; Majkrzak, C. F. *Langmuir* **2001**, *17*, 7645.
(2) Yang, Z.; Galloway, J. A. *Langmuir* **1999**, *15*, 8405.
(3) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.
(4) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *J. Phys. Chem. B* **1998**, *102*, 426.
(5) Schwendel, D.; Dahint, R.; Herrwerth, S.; Schloerholz, M.; Eck, W.; Grunze, M. *Langmuir* **2001**, *17*, 5717.
(6) Tosatti, S.; Michel, R.; extor, M.; Spencer, N. D. *Langmuir* **2002**, *18*, 3537.
(7) Michel, R.; Lussi, J. W.; Csucs, G.; Reviakine, I.; Danuser, G.; Ketterer, B.; Hubbell, J. A.; Textor, M.; Spencer, N. D. *Langmuir* **2002**, *18*, 3281.
(8) Chapman, T. M.; Hillyer, G. L.; Mahan, E. J.; Shaffer, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 11195.
(9) Chapman, R. G.; Ostuni, E.; Yan, L.; Whitesides, G. M. *Langmuir* **2000**, *16*, 6927.
(10) Serizawa, T.; Yamaguchi, M.; Matsuyama, T.; Akashi, M. *Biomacromolecules* **2000**, *1*, 306.
(11) Richert, L.; Lavalle, P.; Vautier, D.; Senger, B.; Stotz, J.-F.; Schaaf, P.; Voegel, J.-C.; Picart, C. *Biomacromolecules* **2002**, *3*, 1170.
(12) Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. *Langmuir* **1999**, *15*, 5355.
(13) Chluba, J.; Voegel, J.-C.; Decher, G.; Erbacher, P.; Schaaf, P.; Ogier, J. *Biomacromolecules* **2001**, *2*, 800.
(14) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101(7)*, 1869.
(15) Zacher, T.; Wischerhoff, E. *Langmuir* **2002**, *18*, 1748.
(16) Shu, X. Z.; Liu, Y.; Luo, Y.; Roberts, M. C.; Prestwich, G. D. *Biomacromolecules* **2002**, *3*, 1304.
(17) Lee, B. P.; Dalsin, J. L.; Messersmith, P. B. *Biomacromolecules* **2002**, *3*, 1038.
(18) Halstenberg, S.; Panitch, A.; Rizzi, S.; Hall, H.; Hubbell, J. A. *Biomacromolecules* **2002**, *3*, 710.
(19) Delgado, M.; Spanka, C.; Kerwin, L. D.; Wentworth, P., Jr.; Janda, K. *Biomacromolecules* **2002**, *3*, 262.
(20) Liang, L.; Rieke, P. C.; Liu, J.; Fryxell, G. E.; Young, J. S.; Engelhard, M. H.; Alford, K. L. *Langmuir* **2000**, *16*, 8016.
(21) Stile, R. A.; Healy, K. E. *Biomacromolecules* **2001**, *2*, 185.
(22) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424.
(23) Collier, J. H.; Hu, B.-H.; Ruberti, J. W.; Zhang, J.; Shum, P.; Thompson, D. H.; Messersmith, P. B. *J. Am. Chem. Soc.* **2001**, *123*, 9463.
(24) Irvine, D. J.; Mayes, A. M.; Griffith-Cima, L. *Macromolecules* **1996**, *29*, 6037.
(25) Griffith-Cima, L.; Lopina, S. T. *Macromolecules* **1995**, *28*, 6787.
(26) Keys, K. B.; Andreopoulos, F. M.; Peppas, N. A. *Macromolecules* **1998**, *31*, 8149.
(27) Zheng, Y.; Micic, M.; Mello, S. V.; Mabrouki, M.; Andreopoulos, F. M.; Konka, V.; Pham, S. M.; Leblanc, R. M. *Macromolecules* **2002**, *35*, 5228.

(28) Zheng, Y.; Gattas-Asfura, K. M.; Li, C.; Andreopoulos, F. M.; Pham, S. M.; Leblanc, R. M. *J. Phys. Chem. B* **2003**, *107*, 483.
(29) Irvine, D. J.; Mayes, A. M.; Satija, S. K.; Barker, J. G.; Sofia-Allgor, S. J.; Griffith, L. G. *J. Biomed. Mater. Res.* **1998**, *40*, 498.
(30) Lopina, S. T.; Wu, G.; Merrill, E. W.; Griffith-Cima, L. *Biomaterials* **1996**, 559.
(31) Lutolf, M. P.; Hubbell, J. A. *Biomacromolecules* **2003**, *4*, 713-722.
(32) Lutolf, M. P.; Weber, F. E.; Schmoekel, H. G.; Schense, J. C.; Kohler, T.; Müller, R.; Hubbell, J. A. *Nature Biotechnology* **2001**, *21*, 513.
(33) Lutolf, M. P.; Raeber, G. P.; Zisch, A. H.; Tirelli, N.; Hubbell, J. A. *Advanced Materials* **2003**, *15*, 888.
(34) Groll, J. In *Deutsches Wollforschungsinstitut*; RWTH: Aachen, 2004.
b) Groll, J.; Ameringer, T.; Spatz, J. P. ; Moeller, M.; *Langmuir* **2005**, *21*, 1991-1999

Chapter 5

Swelling Behavior of IPDI-terminated Star Polymer Hydrogels in Bulk and on Surfaces

5.1 Introduction

Thin layers of surface-attached polymer networks offer a simple method to fabricate soft surfaces with well defined mechanical, physical and biochemical properties.[1-4] First, polymer networks provide a soft 3-dimensional scaffold capable of hosting a wide array of functionalities, ranging from proteins to inorganic nanoparticles. Second, polymer networks exhibit substantial swelling and contraction in response to specific stimuli,[5-8] making them excellent candidates for "smart" surfaces with sensing and actuating characteristics. While macroscopic gels have been hindered by slow response times, the collective diffusion of the network is the rate limiting step; therefore, reducing their dimensions to the micro-nanoscale should significantly enhance performance, making them especially attractive in microsystems technology. [9,10]

A critical issue for understanding and predicting the properties of surface-attached polymer networks is the influence of confinement on their swelling behavior.[11] Chemical linkage of the network to a surface prevents swelling parallel to the substrate, effectively confining the volume change to one dimension, or normal to the surface. Such an effect will

impact important properties such as the structure, mechanical properties, dynamics, and permeability of the network. It has already been observed that confinement of *N*-isopropylacrylamide (NIPAM) networks raises the volume phase transition temperature in comparison to that with unconfined networks.[7,12] Furthermore, it was recently reported that surface-attached NIPAM gels have a total volume change around 15-fold while the corresponding bulk gels have experienced a volume change as large as 100-fold.[13,14] For isotropic, neutral networks, the widely applied Flory-Rehner theory has met with some success in describing the relationship between the cross-link density and the equilibrium swelling in a good solvent.[15,16] For sufficiently low cross-link densities, the Flory-Rehner theory predicts that the equilibrium swelling of a gel relates to the cross-link density to the power of -3/5.[17] However, as surface attachment restricts the swelling to one dimension, a smaller dependence is expected on the cross-link density. In this chapter the dependence of the swelling ratio to layer thickness is investigated. This was performed by measuring the weight or layer thickness of various hydrogels in the bulk state or attached to surfaces.

5.2 Experimental

Materials: Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Glas substrates (50,8x50,8x0,175mm) were purchased from Schott Desag. Acetone, isopropanol and ethanol (Merck, selectipur) were stored in the clean room and used as received. THF and toluene were dried over $LiAlH_4$, distilled under argon and transferred into a glove box. N-[3-(trimethoxysilyl) propyl] ethylenediamine (Aldrich, 97%) was stored in the glove box and filtered before use. The polyols were made at DOW Benelux N.V., Terneuzen, The Netherlands. Applied were isocyanate prepolymers with the molecular weights of 3000 g/mol (prepolymer **A**), 12000 g/mol (prepolymer **B**) and 18000 g/mol (prepolymer **C**). Syringe filters with pore size 0.02 µm were purchased from Whatman.

Methods: Silicon and glass substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath. Oxygen plasma was generated by a TePla 100-E system with 100 W at a process gas pressure of 0.5 mbar. Samples were treated with UV/ozone using a 40 W UV lamp (main emission 185

nm; UV-Technik Speziallampen GmbH) in an oxygen stream of 350 ml/min with a sample distance of 5 mm to the lamp. Films were generated with a CONVAC ST 146 spincoater. Layer thicknesses were examined using a MM-SPEL-VIS ellipsometer (OMT). Contact angles were measured using the sessile drop method with a G 40 contact angle measuring device (Krüss GmbH). Lower detection limit of the method is around 10 degrees. Scanning force microscopy (SFM) investigations were performed with a Nanoscope IIIa (Digital Instruments) operating in tapping Mode TM. The oscillation frequency for Tapping Mode TM was set in the range of 320–360 kHz depending on the Si cantilever (k ~ 50 N/m, Nanosensors).

Substrate preparation: Cutting and cleaning of the substrates was performed in a class 100 clean room. Silicon wafers were cut into pieces of 14x14 mm. The samples were then cleaned by sonication in acetone, water and isopropanol for one minute each followed by drying in a stream of prefiltered nitrogen. Activation of the surface was achieved by treatment with UV/ozone for 12 min. The substrates were then used immediately for amino-functionalization.

Aminosilylation of the substrates: After activation, the substrates were transferred into an Unilab glove box (MBraun) and immersed into a solution of 0.3 ml N-[3-(trimethoxysilyl)propyl] ethylenediamine in 50 ml dry toluene for 2 hours. Then the samples were washed several times with dry toluene and stored under dry toluene until further usage.

Preparation of thin films: In a glove box different amounts of the prepolymers synthesized in chapter three were dissolved in toluene. The concentration varied from 1.0 to 10 g/l. These solutions were transferred to the clean room and the filtered solutions (0.02 µm syringe filter) were spin-coated on the prepared substrates at different rotation speeds. For spin-coating, the substrates were placed on the spin-coater, covered by the corresponding solution, accelerated within 5 seconds to the final rotation speed of 2500 rpm and kept rotating for 40 sec. The layer thickness of the resulting films was measured immediately by ellipsometry. Then the substrates were either stored several days in a humid atmosphere (~94%) or directly immersed in deionized water, respectively.

Preparation of thick films: After aminosilylation, the dry substrates were weighed and spin-coated with the bulk polymers. For each sample, three droplets of the liquid polymer were applied on the substrates and accelerated within 5 seconds to a rotation speed of 5000 rpm. The rotation speed was kept for 40 seconds. Excess polymer on the side and back of the substrate was carefully removed with class 100 clean room paper and Millipore acetone. Then the weight of substrates was measured again. The amount of applied polymer could be determined by subtracting the reference value.

Preparation of bulk polymer samples: In a glove box approximately 0.5 g of the prepolymers were weighed into glass vials. Outside the glove box the open vials were either stored in a humid atmosphere or directly filled with 40 ml of deionized water. For the humid atmosphere the bottom of an exsiccator was filled with deionized water and kept on a 50 °C warm plate. After the samples were put in the lid was closed and the exsiccator kept under a slight vacuum.

Determination of layer thickness: The silicon substrates were examined by ellipsometry with a spectral method in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15 degrees, the integration time was dependent on the layer thickness and the resulting signal intensity. In order to minimize systematic errors in the data collection, in a series of experiments always one substrate was just cleaned and activated and one was just cleaned, activated and aminosilylated. These two substrates were measured as references, and thicknesses of the hydrogel films were obtained as relative values to the aminosilylated substrate. Each sample was measured at 4 different places, and the presented data are the average values of each sample. Errors were determined through evaluation of the standard deviation of the measurements.

Detection of water uptake: The amount of water taken up by the hydrogels were either determined by weighing of the polymers or substrates or in the case of thin films by ellipsometric measurements. The samples immersed directly in water were taken out of the water and residue water carefully removed with dust free Kimwipe papers. After the measurements the samples were put back into renewed deionized water. The samples stored

under humid atmosphere were taken out, directly measured and put back into the humid chamber. After 14 days they were dried for one day under vacuum and then stored under deionized water. Then the thus precrosslinked samples were treated according to the other samples.

5.3 Results and Discussion

In this chapter the swelling behavior of isophorone diisocyanate (IPDI) terminated 6 arm star polymers is described. To achieve this they were either treated in bulk with water or an humid atmosphere or spin-coated onto aminosilylated silicon surfaces and treated afterwards with water or a humid atmosphere, respectively. By subjugating the samples to a humid atmosphere, two separate steps were fulfilled: 1. the water uptake of air humidity could be measured and 2. the prepolymers could be cross-linked to a more dense network compared to polymers cross-linked in water atmosphere. Thus, the differently treated polymers showed large differences in swelling degrees.

The difference in the equilibrium swelling degree was most significant . for the bulk polymers. The rather low water uptake in an humid atmosphere (Figure 5.3.1) turned out to be sufficient enough for the cross-linking of the IPDI chain ends.

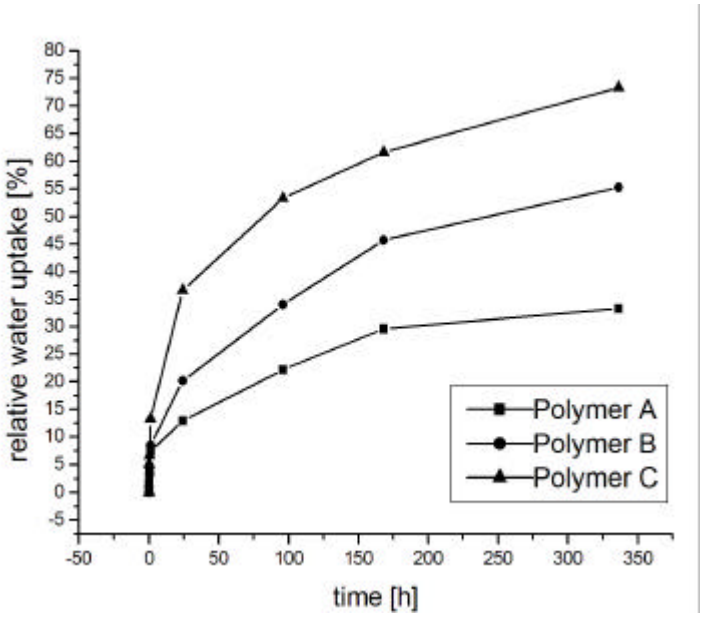
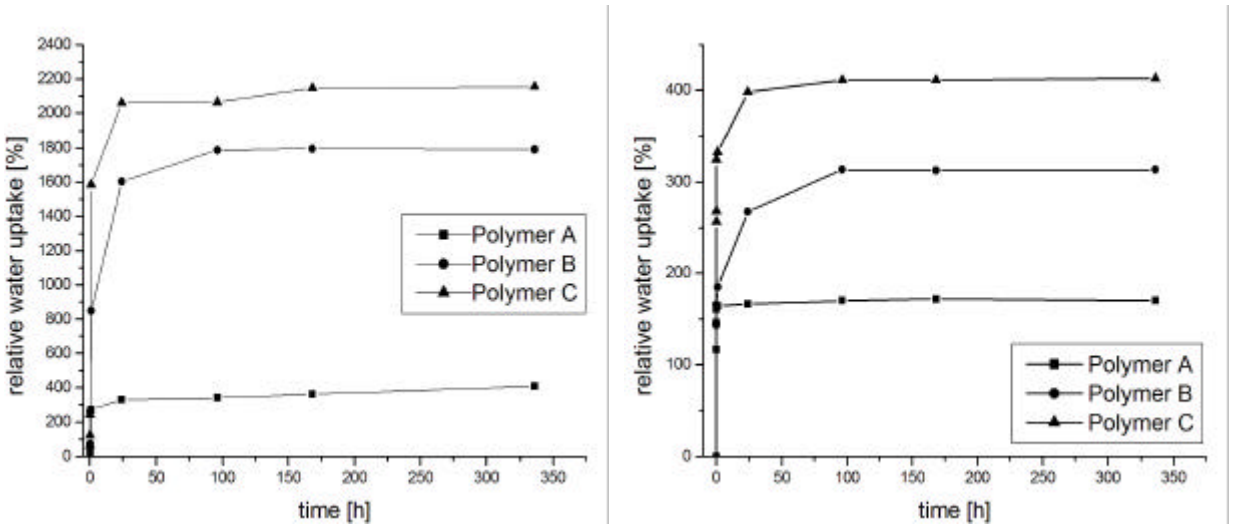


Figure 5.3.1: *Swelling of bulk polymers in humid atmosphere*

According to Figure 5.3.1 it seems that the equilibrium swelling degree is not reached. On the other hand the weight increase of the polymers within the second week did not exceed 0.02 gwhich lies within the error of the balance. Therefore this experiment was stopped after 2 weeks.

In Figure 5.3.2 and Table 5.3.1 the different water uptake of untreated and precrosslinked polymers with different molecular weights is shown.



a) b)

Figure 5.3.2: *Relative water uptake of bulk prepolymers in water: a) non treated polymer b) stored 2 weeks in humid atmosphere*

Table 5.3.1: *Water uptake of prepolymers with different molecular weights in bulk and previously stored in humid atmosphere (94% humidity)*

Prepolymers	Mol. Weight [g/mol]	Weight of bulk polymer [g]		Water uptake [g] (equilibrium swelling degree [%])	
		untreated	Precross-linked	untreated	Precross-linked
A	**(**3000)	0.52	0.54	2.13 (410)	0.92 (170)
B	(12000)	0.58	0.94	10.39 (1791)	2.95 (314)
C	(18000)	0.5	0.6	10.78 (2156)	2.48 (413)

** polymers were separately prepared and weighed before storage in humid atmosphere*

In this diagrams both the differences between precross-linked and bulk polymer and the influence of the molecular weight can be observed. The water uptake decreased with an increasing degree of cross-linking and a decreasing in molecular weight of the applied polymers. Both is in accordance with literature and can be explained as follows: With an increase in the degree of cross-linking the chain ends become less and less flexible. Also the amount of chain units between two cross-linked chain positions is decreasing. Therefore the possibility of the polymer network to increase its volume itself is getting smaller. The amount of absorbable solvent is decreasing. On the contrary a higher molecular weight of the polymer means a higher chain flexibility, leading to better swelling conditions. The polymers can absorb a lager amount of solvents.

In a work Sukumar and Lopina[18] developed an equation to determine the molecular weight between cross-links (M_c) which based upon the theory of Flory[16].

$$\frac{1}{M_c} = \left(\frac{F}{F-2}\right)\frac{1}{M_n} - \frac{\left[\ln(1 - v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2\right]\frac{\bar{v}}{V_1}}{\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{2}{F}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]v_{2,r}} \qquad \text{eq. 1)}$$

With: F = chains emanating from a crosslink, M_n = number average molecular weight of a single polymer strand in the cross-linked network, V_1 = molar volume of the solvent, $v_{2,r}$ and $v_{2,s}$ = volume fractions of polymer in the solution during cross-link formation and following equilibrium swelling, ? polymer-solvent interaction parameter and ? the specific volume of the polymer.

According to this equation they determined that for 6 arm starpolmers with a molecular weight of 10500 g/ mol and a M_c of 3060 the degree of swelling lies around 92 %. Extrapolating this values to the values measured during this experiments, the cross-linking density is much lower.

What is not affected by cross-linking is the kinetics of the swelling. In all cases the main amount of water is taken up within a few hours while the equilibrium swelling is reached within several days.

The results of the bulk polymers were comparable to those of the several microns thick polymer films spin-coated onto aminosilylated silicon wafers. During the treatment in humid atmosphere (94%) no increase in the weight of the samples (water uptake) could be detected.

At this rather low weight of applied polymer the amount of water which can be absorbed in the layers lied below the detection limit. The tendency of increased water uptake with higher molecular weight and a lower degree of cross-linking is shown in Table 5.3.2

Table 5.3.2: *Water uptake of prepolymers spin-coated onto aminosilylated silicon wafers.*

Prepolymers	Mol. weight [g/mol]	Weight of bulk polymer [mg]		Water uptake [mg] (equilibrium swelling degree [%])	
		untreated	precrosslinked	untreated	precrosslinked
A	(3000)	32.52	49.38	45.7 (141)	23.3 (47)
B	(12000)	16.83	16.02	159 (945)	47.9 (299)
C	(18000)	22.93	28.00	224 (980)	85.3 (305)

** polymers were separately prepared and weighed before storage in humid atmosphere*

The values of the equilibrium swelling degree are similar to those of the bulk polymers. This can be explained with the fact that, with the exception of the hydrogels made of prepolymer **A**, all layers were detached from the surface during the swelling procedure. They consequently swelled in all directions, whereas a surface bound polymer only swells mainly perpendicular to the surface. Therefore the layers have to be regarded as small "bulk" hydrogels which explains the rather high amounts of incorporated water. Only for the layers made of prepolymer **A** the osmotic pressure was not big enough to cleave the layers totally from the surface. Still the layers exhibited some areas, where they were not bound to the surface. Therefore they also could partially swell in "x" and "y" direction. The degree of swelling is still higher than expected, but in comparison to the other polymers it is much lower.

In going to thin hydrogel layers, the following results were gained. By spin-coating three different concentrations (10.0 g/l , 5.0 g/l and 1.0 g/l) of the three prepolymers **A**, **B** and **C** nine different polymer layers were obtained. As shown in Figure 5.3.3 none of the layers showed a significant increase of the thickness during 12 days of storage in humid atmosphere (94 %). However, after the storage for one day under deionized water, the layer thickness slightly decreased. Drying the samples afterwards under reduced pressure ($2*10^{-2}$ mbar) resulted in a significant decrease of the layer thicknesses.

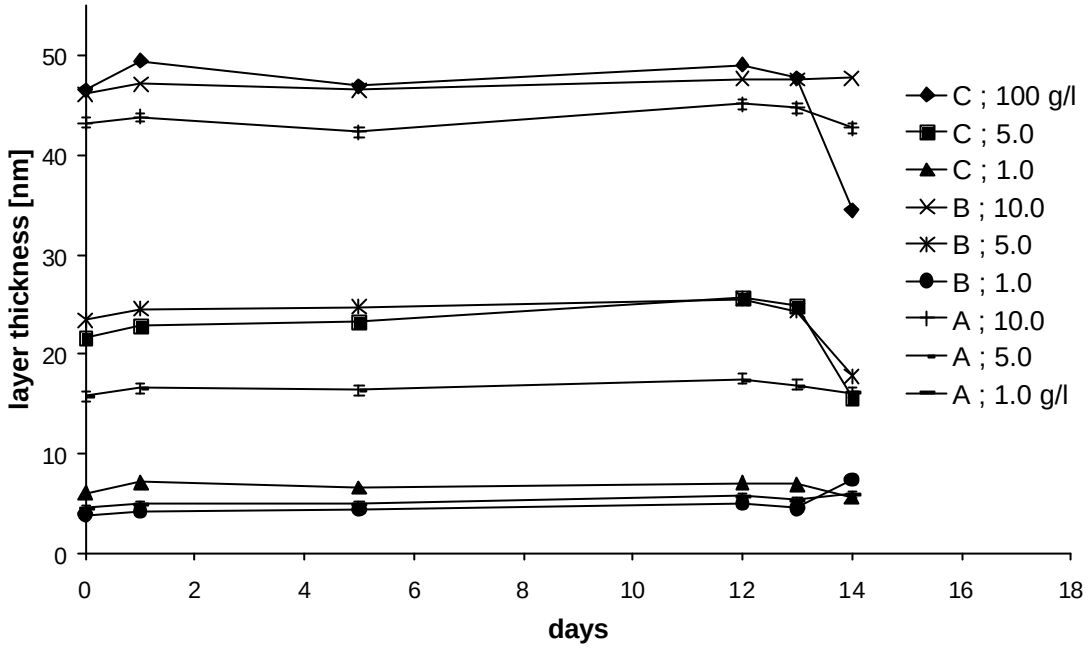


Figure 5.3.3: *Dependence of layer thickness of prepolymer films stored under different conditions; 1-12 days: storage under 94 % humid atmosphere; day 13: after 1 day storage under deionized water; day 14: after 1 day under vacuum (2*10^{-2} mbar). Error bars stand for standard deviation of the values measured for layers spin-coated with prepolymer* ***C****.*

Like the thick films no water uptake of the thin layers could be detected due to the low amount of incorporated water. During storage under water the slight swelling of the layers could not be detected, because some amount of the still non-crosslinked polymer was dissolved in the water. This might explain the decrease of the layer thickness under vacuum below the starting values. This was especially the case for the layers of prepolymer **C**. The layers of prepolymer **A** exhibit no significant changes meaning that neither water was taken up nor parts of the layer were dissolved. For prepolymer **B** no clear statement can be made. While the thickest layer showed no significant changes, the layer spin-coated with the 5.0 g/l solution decreased significantly with time. Additionally the thinnest layer is increasing its thickness during evaporation which is not possible.

5.4 Conclusions

During this work the swelling properties of hydrogels made out of three different isocyanate terminated starpolymers were examined. Therefore the prepolymers with the molecular weights of 3000, 12000 and 18000 g/mol were treated with water in different ways either in bulk or as layers with varying thickness onto silicon surfaces. Measured were the samples either by weighing, or in the case of thin films by ellipsometry.

Only the bulk prepolymer samples showed an weight increase during the treatment in a humid atmosphere. The amount of incorporated water in the polymers attached to the surface was below the detection limit of both methods, weighing and ellipsometry.

Regarding the bulk prepolymers in all experiments a relation between the molecular weight and degree of crosslinking to the amount of uptaken water can be seen. The longer the chains are, the more water is incorporated whereas with increasing degree of crosslinking the amount of incorporated water is decreasing. This is also the fact for the thick polymer films attached to the aminosilylated surface. Although no water uptake could be detected during the treatment in humid atmosphere, this procedure had a big influence to the crosslinking density.

Due to the fact, that most of the covalent attached layers were ruptured from the surface, the water uptake was higher than expected.

For both methods it also can be said, that the main water uptake takes place within a few hours whereas the equilibrium swelling degree is reached after a few days.

Due to experimental problems for the ultrathin polymer layers no clear statement can be made.

5.4 References

(1) Revzin, A.; Russell, R. J.; Yadavalli, V. K.; Koh, W.-G.; Deister, C.; Hile, D. D.; Mellott, M. B.; Pishko, M. V. *Langmuir* **2001**, *17*, 5440.
(2) Sanford, M. S.; Charles, P. T.; Commisso, S. M.; Roberts, C.; Conrad, D. W. *Chem. Mater.* **1998**, *10*, 1510.
(3) Tang, Y.; Lu, J. R.; Lewis, A. L.; Vick, T. A.; Stratford, P. W. *Macromolecules* **2001**, *34*, 8768.
(4) Kuckling, D.; Harmon, M. E.; Frank, C. W. *Macromolecules* **2002**, *35*, 6377.
(5) Tanaka, T.; Nishio, I.; Sun, S.-T.; Ueno-Nishio, S. *Science* **1982**, *218*, 467.
(6) Siegel, R. A. *Adv. Polym. Sci.* **1993**, *109*, 233.
(7) Suzuki, A.; Kojima, S. *J. Chem. Phys.* **1994**, *101*, 10003.
(8) Qui, Y.; Park, K. *Adv. Drug Delivery Rev.* **2001**, *53*, 321.
(9) Zhao, B.; Moore, J. S. *Langmuir* **2001**, *17*, 4758.
(10) Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B.-H. *Nature (London)* **2000**, *404*, 588.
(11) Onuki, A. *Adv. Polym. Sci.* **1993**, *109*, 63.
(12) Harmon, M. E.; Kuckling, D.; Frank, C. W. *Macromolecules* **2003**, *36*, 162.
(13) Seelenmeyer, S.; Deike, I.; Rosenfeldt, S.; Norhausen, C.; Dingenouts, N.; Ballauf, M.; Narayanan, T.; Linder, P. J. *J. Chem. Phys.* **2001**, *114*, 10471.
(14) Harmon, M. E.; Jakob, T. A. M.; Knoll, W.; Frank, C. W. *Macromolecules* **2002**, *35*, 5999.
(15) Flory, P. J.; Rehner, J. *J. Chem. Phys.* **1943**, *11*, 521.
(16) Flory, P. J. *J. Chem. Phys.* **1950**, *18*, 108.
(17) Sukumaran, S. K.; Beaucage, G. *Europhys. Lett.* **2002**, *59*, 714.
(18) Sukumar, V. S.; Lopina, S. T. *Macromolecules* **2002**, *35*, 10189.

Chapter 6

Synthesis and Coating of Acrylate-terminated Star Polymers

6.1 Introduction

Poly(ethylene glycol), PEG is a polymeric molecule of vital biological importance. It has characteristic properties such as high hydrophility, good tissue biocompatibility, lack of toxicity and availability of reactive sites for chemical modification. A very important property is its resistance against protein and cell adsorption and against recognition fro the immune system.[1] Hydrogels derived from the crosslinking of functionalized PEG/ PEO derivatives have been investigated in a number of biotechnical applications, ranging from controlled drug delivery systems and enzyme immobilization matrices to size-selective biomembranes and wound healing dressings.[1-3]

Photopolymerization provides an effective and benign method of in situ hydrogelation. Facile photogelation exhibits considerable advantages compared to the conventional methods of physical or chemical hydrogel formation: mild reaction conditions, minimum byproduct formation, absence of potential toxic catalysts and initiators and easily controlled processing.

Consequently, in recent years there has been an increasing interest in utilizing photogelation as a means of biomaterial preparation in the field of medicinal and biomedical science.[4-6]

A common strategy for photoinduced hydrogel formation involves a polymerization system of water-soluble macromers that have pendant acrylate groups along the polymeric chain. [6-8] In this method, the photosensitive macromers are polymerized in the presence of a photoinitiator (such as benzophenone and xanthene dyes), and optionally a co-catalyst or an accelerator upon exposure to long-wavelength ultraviolet radiation (UV) or visible light. Another approach involves the intermolecular photodimerization of photosensitive groups such as cinnamate,[4,9] , nitrocimmanate,[10] coumarin,[11] or benzyl diethyldithiocarbamate[12] attached as terminal functional groups to hydrophilic polymers. Although all the previous work demonstrated photopolymerization, most systems are associated with inherent drawbacks, such as slow gelation rate, need for potentially toxic initiators, and thermal or storage instability. In addition, a limited number of these photoinduced systems demonstrate photoreversibility.[4,5,13,14]

6.2 Experimental

Reagents and Materials: Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Glass substrates (50,8x50,8x0,175mm) were purchased from Schott Desag. Acrylic acid (Aldrich, 96% stab. with 400ppm phenothiazine) and acrylicoylchloride (Aldrich, 96% stab. with 400ppm phenothiazine) were distilled under reduced pressure (~$5*10^{-1}$ mbar) and stored under argon at 4°C. The polyols were purchased by DOW Benelux N.V., Terneuzen, The Netherlands. Before use they were degassed and dried under reduced pressure ($2*10^{-2}$ mbar) at 80°C for 2 hours. THF, chloroform and toluene (technical) were dried over lithium aluminum hydride, distilled and stored under argon atmosphere. Triethyl amine (TEA) was dried over calcium hydride and distilled. The $HfCl_4*(THF)_2$ catalyst (Aldrich 98%) was used as received. Molecular sieve (4 Å) was washed thoroughly with water and methanol and dried at 250 °C under reduced pressure of 15 mbar. Calcium hydrogen carbonate and ammonia (30 % solution in water) were used as received. Acetone and isopropanol (Merck, selectipur) were stored in the clean room and used as received. N-[3-(trimethoxysilyl) propyl] ethylenediamine (Aldrich, 97%) was stored in the glove box and filtered before usage. Syringe filters with pore size 0.02 μm were purchased from Whatman.

Methods: Silicon and glass substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath.

Oxygen plasma was generated by a TePla 100-E system with 100 W at a process gas pressure of 0.5 mbar. Samples were treated with UV/ozone using a 40 W UV lamp (main emission 185 nm; UV-Technik Speziallampen GmbH) in an oxygen stream of 350 ml/min with a sample distance of 5 mm to the lamp. Films were generated with a CONVAC ST 146 spincoater. Layer thicknesses were examined using a MM-SPEL-VIS ellipsometer (OMT). Contact angles were measured using the sessile drop method with a G 40 contact angle measuring device (Krüss GmbH). Lower detection limit of the method is around 10 degrees. Light microscopy was performed by means of an Axioplan2 Imaging microscope from Zeiss. Pictures were taken with a Zeiss AxioCam HR camera. Atomic force microscopy (AFM) investigations were performed with a Nanoscope IIIa (Digital Instruments) operating in tapping ModeTM. The oscillation frequency for Tapping ModeTM was set in the range of 320 – 360 kHz depending on the Si cantilever (k ~ 50 N/m, Nanosensors).

Size Exclusion Chromatography (SEC): THF (HPLC grade) was used as the mobile phase. SEC columns were packed with a 5 μm particle gel of nominal pore sizes 50, 100, 500 and 1000 Å. The refractive index was used as the detection method (Waters RI 410). The prepolymers were reacted with methanol to inactivate the isocyanate groups. The SEC system has been calibrated by means of a mixture of poly(ethylene oxide-*co*-propylene oxide) and poly(propylene oxide) polymeric standards. The molar percentages of the chain extended products (bis-, ter- and quaterstars) have been obtained by numerical deconvolution of the SEC diagrams, applying Gauss type curves. Because of its asymmetry, the monostar peak was fitted by the sum of two Gaussian curves. The individual SEC-peaks of each discrete species have been normalized according to the fraction of isocyanate end groups which has been calculated from the hydroxyl content (OH-titration). The χ^2 fit parameter was determined to be smaller than 3×10^{-3}.

^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer (operating frequencies 400 and 125 MHz, respectively) at room temperature. Chemical shifts refer to the $CDCl_3$ signal (7.24 ppm and 77.0 ppm, respectively).

Elemental Analysis was performed using a Heraeus CHNO Rapid Analyzer.

Synthesis of acrylate based Prepolymers with hafnium catalyzed reaction of acrylic acid: The typical preparation of acrylate based prepolymers is described at the example of polymer **B** (chapter 3). In a 100 ml three neck flask fitted with a pressure-equalized addition funnel (containing a septum and ~3 g of 4 Å molecular sieves), reflux condenser, argon inlet and septum 5 g (0.4 mmol) of the dried polymer **B** were dissolved in 30 ml of dry toluene. 0.4 mg (~1 mol%) of the hafnium catalyst were added. After the injection of 0.1 ml (1.5 mmol) acrylic acid through the septum the mixture was refluxed for two hours. After cooling to room temperature the mixture was filtered and the solvent removed under reduced pressure ($2*10^{-2}$) the product was analyzed with a ^{1}H-NMR spectrometer.

Synthesis of acrylate based prepolymers with acrylic acid chloride (AAC): (The whole synthesis was carried out in brown, UV absorbing flasks). In a 100 ml three neck flask fitted with a reflux condenser, argon inlet and septum 5g (0.4 mmol) of the dried polymer **B** were dissolved either in 50 ml of THF or chloroform respectively. The solution was treated with triethyl amine and cooled to 0 °C. Then the AAC was added slowly through the septum (strong formation of smoke). The mixture was stirred for further 12 hours while turning the color to yellow or brown respectively. Efforts of removing the precipitated ammonium salt were not successful due to the blocking of the filters with the salt. If THF was applied as solvent it now was removed under reduced pressure and the residue dissolved in chloroform. All of the products were then washed three times each with 50 ml of hydrochloric acid (5 % in water) and deionized water respectively. The phase separation occurred very slowly (1 hour each). The organic phase was then dried over sodium sulfate and the solvent removed under reduced pressure ($1*10^{-2)}$ mbar).

Aminosilylation of the substrates: After activation, the substrates were transferred into an Unilab glove box (MBraun) and immersed into a solution of 0.3 ml N-[3-(trimethoxysilyl)propyl] ethylenediamine in 50 ml dry toluene for 2 hours. Then the samples were washed several times with dry toluene and stored under dry toluene until further usage.

*Sample preparation: The p*repolymers A-C were dissolved in Toluene or THF in concentrations from 0.5 to 10.0 mg/ml. These solutions were either spincoated directly onto

aminosilylated silicon wafers or different amounts of ethylene diamine were added before the coating process. All solutions were filtered by a syringe filter. Former substrates were separately irradiated for 2 hours with UV–light. After examination by ellipsometry, AFM or optical microscopy all substrates were half dipped in deionized water for several times and again examined by ellipsometry and with the optical microscope.

Some freshly spincoated substrates were exposed to an ammonia atmosphere. Therefore they were put directly after spincoating into an exsiccator provided with an ammonia solution in the lower chamber.

6.3 Results and Discussion

During this work 6 arm star polymers have been successfully end capped with acrylic acid chloride. The reaction was carried out in different solvents in good conversion. The product proofed to be highly reactive. Polymerization of the crude and the pure product could not always be prevented. Working under inert gas conditions with excluding of UV-light was inevitable.

The conversion of the end groups, determined by NMR, differed by the application of the added base. Without base the maximum conversion laid around 95 %. With the addition of triethyl amine it could be raised to around 99 %. The calculation with NMR was done as followed:

According to eq. 3.3.5 and eq. 3.3.6 the degree of polymerization per arm (P) was calculated to be 42.

$$M_{(EO/PO)} = 0.8 * M_{(EO)} + 0.2 * M_{(PO)} = 46.8 \text{ g/mol} \qquad \text{eq. 3.3.5)}$$

$$P = \frac{M_{w(polyol)} - M_{(sorbitol))}}{M_{(EO/PO)} * N} \qquad \text{eq. 3.3.6)}$$

with $M_{(EO)}$ = 44 g/mol; $M_{(PO)}$ = 58 g/mol, $M_{(sorbitol)}$ = 182 g/mol, $M_{w(polyol)}$ = 12,000 g/mol and the number of arms N = 6.

Assuming 100 % of end group conversion the ratio of the integral of the acrylic peak (**b**) (Figure 6.3.1 and Figure 6.3.2) to the sum of the aliphatic peaks must be 1 to 187 (aliphatic protons per arm + 2 core protons) eq. 2)

$$H_{(EO/PO)} = 0.8* H_{(EO)} + 0.2 * H_{(PO)} = 4.4 \qquad \text{eq. 1)}$$

$$H_{(arm)} = H_{(EO/PO)} * P \qquad \text{eq. 2)}$$

With H = number of protons; $H_{(EO)}$ = 4, $H_{(PO)}$ = 6, $H_{(EO/PO)}$ = average number of protons per chain unit and $H_{(arm)}$ = number of protons per arm

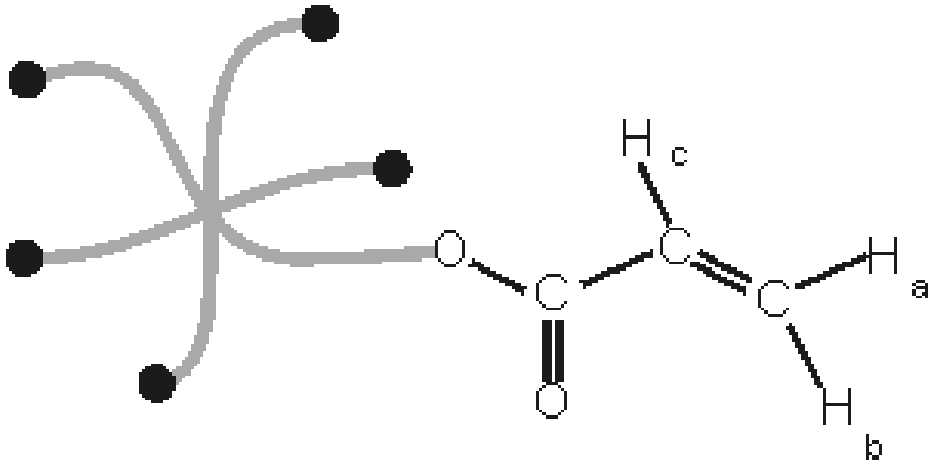


Figure 6.3.1: *Scheme of the acryl terminated prepolymer (PEG-AA)*

The proof of the reaction of AAC with the polyol is shown in the shift of the three acrylic peaks (**a** 5.84 ppm; **b** 6.37 ppm; **c** 6.11 ppm) correspond exactly with the shifts of acrylic acid esters in literature.[15] Due to the different possible endgroups of the polymer backbone (ethylene- propylene oxide) the doublets **a** and **b** are split in two partially eclipsed doublets. Signal **c** results therefore in a multiplet. The significant signal of the peak **b'** of acrylic acid chloride (6.63 ppm) and acrylic acid (6.53 ppm) could not be detected. Their occurrences can be excluded.

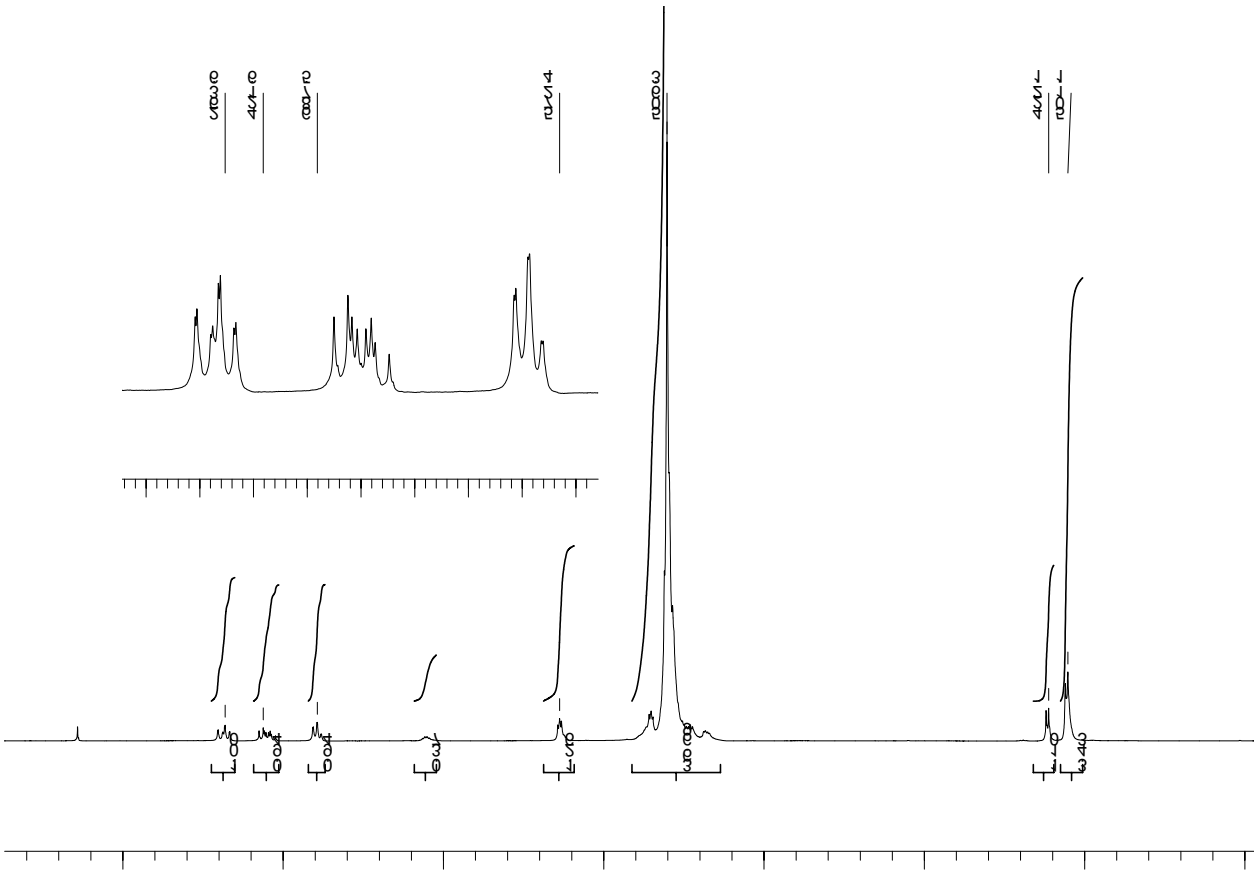


Figure 6.3.2: *^{1}H-NMR of AA terminated prepolymer*

Table 6.3.1: *Conversion of the starpolymers **A, B** with acryloyl chloride at different reaction conditions*

Polymer [mmol]	solvent	AAC [mmol] (Excess (AAC:OH)	TEA [mmol]	Conversion [%]	Color*
A 3.3	$CHCl_3$	44 (2:1)	116	90	dark brown
A 3.3	$CHCl_3$	44 (2:1)	116	90	dark yellow
A 1.67	$CHCl_3$	44 (4:1)	116	95	dark yellow
A 3.3	THF	22 (1:1)	58	100	pale brown
A 1.67	$CHCl_3$	55 (5:1)	---	65	pale yellow
A 1.67	THF	44 (4:1)	---	70	pale yellow
A 1.67	THF	88 (8:1)	---	80	pale yellow
A 1.67	THF	176 (18:1)	---	90	pale yellow
A 1.67	---	209 (20:1)	---	80	pale yellow
B 0.83	$CHCl_3$	11 (2:1)	30	34	pale yellow
B 0.83	THF	11 (2:1)	30	86	brown
B 0.83	Toluene	11 (2:1)	30	85	brown
B 0.83	$CHCl_3$	55 (10:1)	---	95	pale yellow

** Color of the resulting product*

According to Table 6.3.1 the reaction of the star polymers with acryloyl chloride worked in good conversion with or without addition of triethyl amine. With triethyl amine as base for scavenging the developing hydrochloric acid the excess of AAC could be kept rather low. Unfortunately the resulting products exhibited a rather dark color. Without triethyl amine a large excess of AAC had to be applied, while the products remained quite colorless.

The employment of sodium hydrogen carbonate as an inhomogeneous scavenger of the hydrochloric acid didn't result in an improvement of the end group conversion. Further the attempt to remove the hydrochloric acid with a continuous argon stream didn't enhance the conversion.

Unlike to chapter three with the employment of AAC no chain extension by inter molecular reactions could occur. But due to the high reactivity of the acrylic group still chain extension / crosslinking was possible by polymerization. Therefore for each product the

molecular weight distribution had to be checked by SEC. It turned out, that either the products remained as monostars, or if chain extension occurred it happened so fast, that the polymers were almost fully cross linked and could not be used any more.

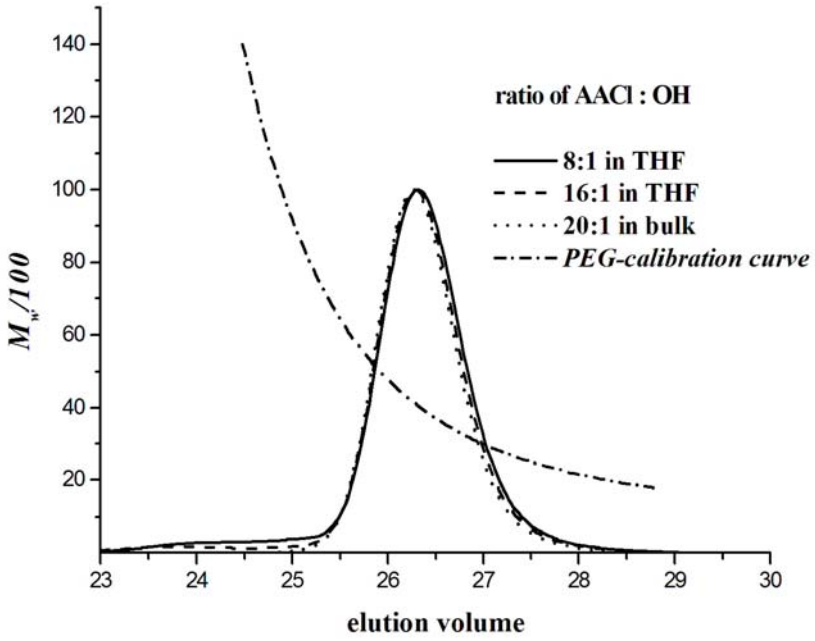
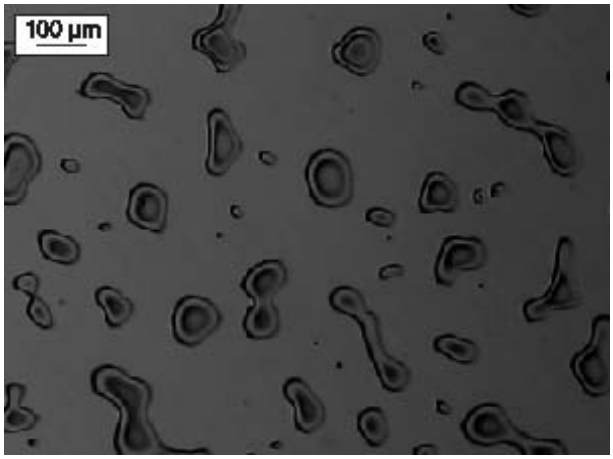


Figure 6.3.3: *GPC curves of polymer **A** reacted with three different excesses of AAC*

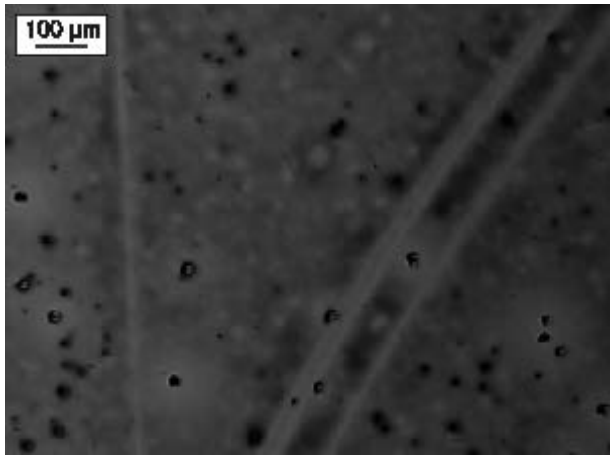
During this work also the functionalization of the starpolymers with acrylic acid was tried. Yamamoto et al. prescribed in their work the reaction of carbon acids with alcohols via highly active hafnium catalysts [16]. The $HfCl_4$*2(THF) catalyst seemed to be the most efficient for reacting the polyols with acrylic acid. Unfortunately the reaction never exceeded 5 % endgroup conversion. Varying the excess of acrylic acid didn't result in an improvement of the reaction. This phenomenon can occur because of the probable poisonous effect of the vinyl group to the hafnium catalyst. The double bond of the acrylic acid can coordinate to the free binding site of the catalyst and therefore block further reactions. Regarding the publication of Yamamoto this can be confirmed. The only allylic compound they employed was 1-phenyl-3-hydroxyl-prop-1-en. This compound probably worked only because of the

steric hindrance of the bulky phenyl group. Coordination of the vinyl group could be prevented like this. Other reactions of allylic compounds were not reported.

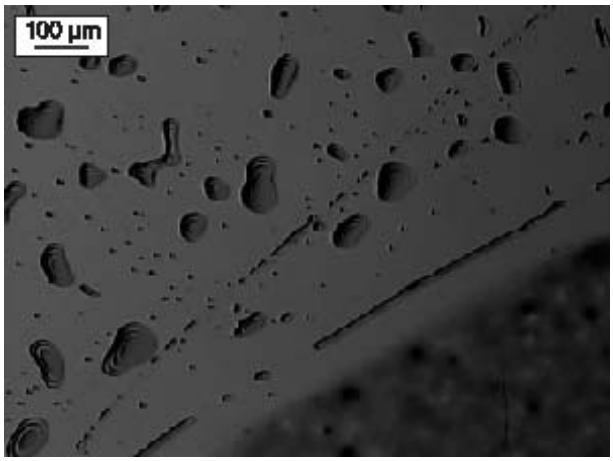
In further steps the synthesized prepolymers were coated onto silicon substrates. For the spincoating process the prepolymers were solved in either in THF or toluene in concentrations of 0.5, 1.0 and 10.0 mg/ml. These solutions were then spincoated onto the aminosilylated substrates. In order to quench the fast occurring dewetting of the polymer the substrates were irradiated by UV-light. To obtain a good contrast for optical microscopy the substrates were half covered by an aluminum sheet. After 10 minutes a strong dewetting on the dark side of the substrate could be observed while the irradiated site stayed rather smooth (Figure 6.3.4).



a)



b)



c)

Figure 6.3.4: *Optical images of prepolymer B spincoated out of a 10.0 mg/ml solution in THF onto aminosilylated silicon wafers; a) stored in darkness, b) irradiated 2 hours by UV-light, c) border between dark and irradiated side.*

According to the border the stabilizing effect of the UV irradiation can be seen. Via polymerization of the acrylic endgroups the polymer is crosslinked and therefore immobilized on the surface. No further dewetting could occur.

Spincoating the different concentrations of the prepolymers with THF and toluene resulted in layer thicknesses according to those spincoated with NCO terminated prepolymers reported in chapter 4 (Figure 6.3.4).

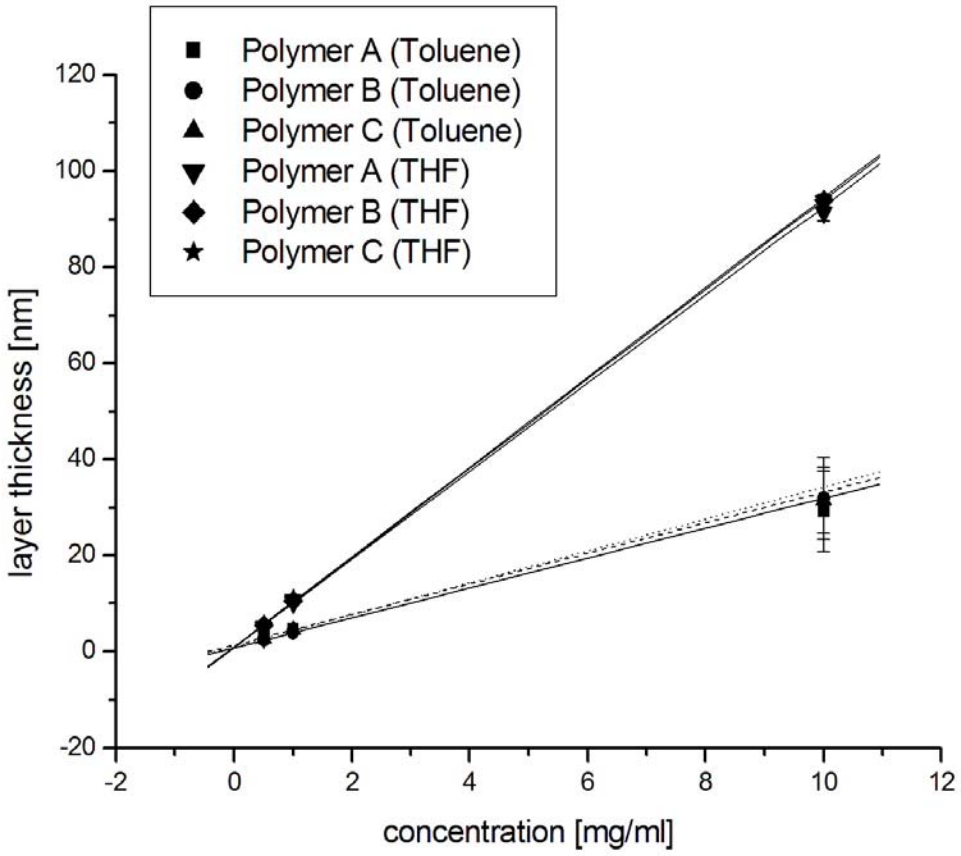
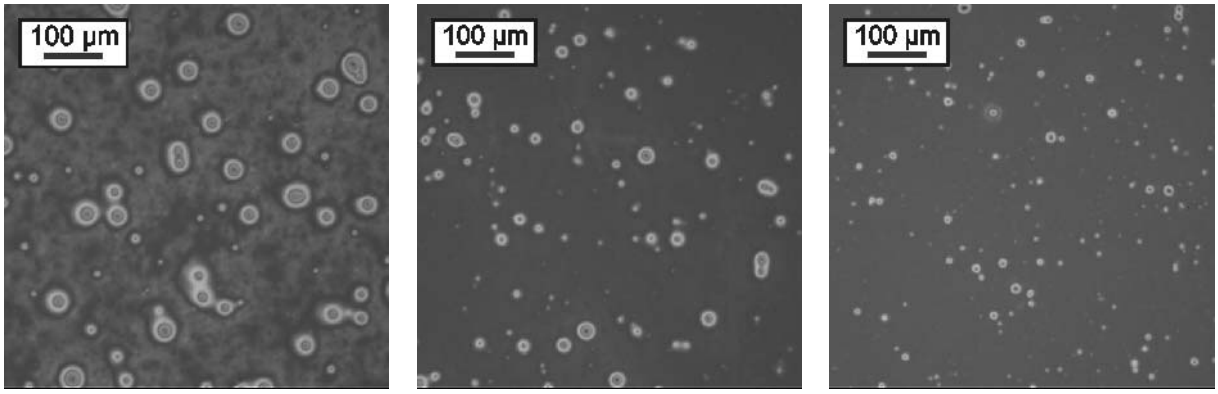


Figure 6.3.5: *Dependency of the thickness of polymer layers to the concentration of the solution in THF or toluene.*

Unfortunately the irradiation of the layers with UV-light could only quench the dewetting process. Rinsing the substrates with deionized water resulted in a strong removal of the polymer from the surface. In every case only a 4-6 nanometer thick monolayer remained on the surface. Like in chapter 4 all six arms of the prepolymer units reacted with the amino terminated surface. Any further acryl terminated polymers could be crosslinked but the layer not be attached covalently to the surface and therefore be washed away. Contact angle measurements gave another hint for this theory. While freshly spincoated and irradiated layers showed small contact angles around 10 degrees (± 1 °), after washing they rose to a value of 49 ° (± 1.5 °). This indicates, that first the acrylic endgroups had a strong influence onto the

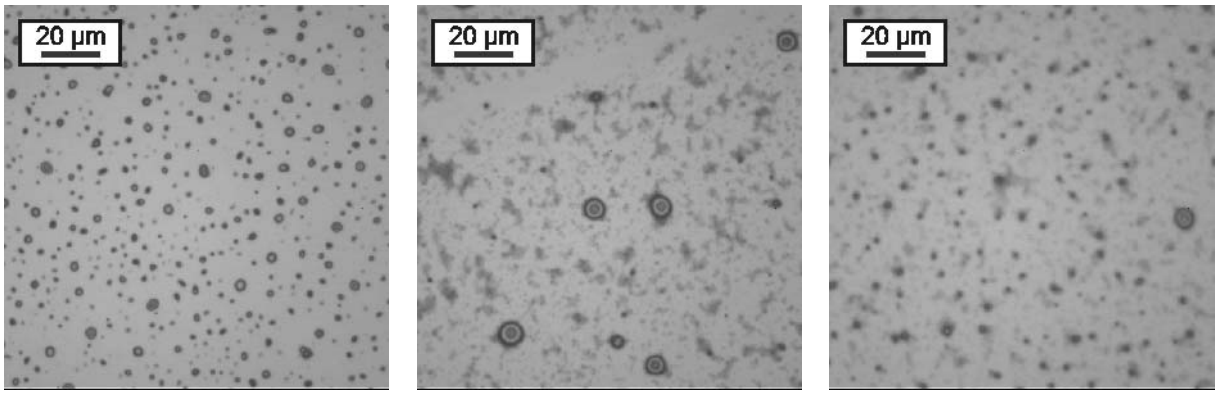
surface tension whereas after washing only the polymer backbone reaches towards the surface.

To prevent this phenomenon, the prepolymer solutions were treated with different amounts of triaminoethyl amine. This solution was allowed to react from 1 to 60 minutes before spincoating. The ratios of primary amine groups to acrylic endgroups were ¼, ½ and 1/1. In the case of the latter two concentrations within some seconds the solutions turned cloudy. Filtration before spincoating became more difficult due to increasing blockage of the filters.



a) b) c)

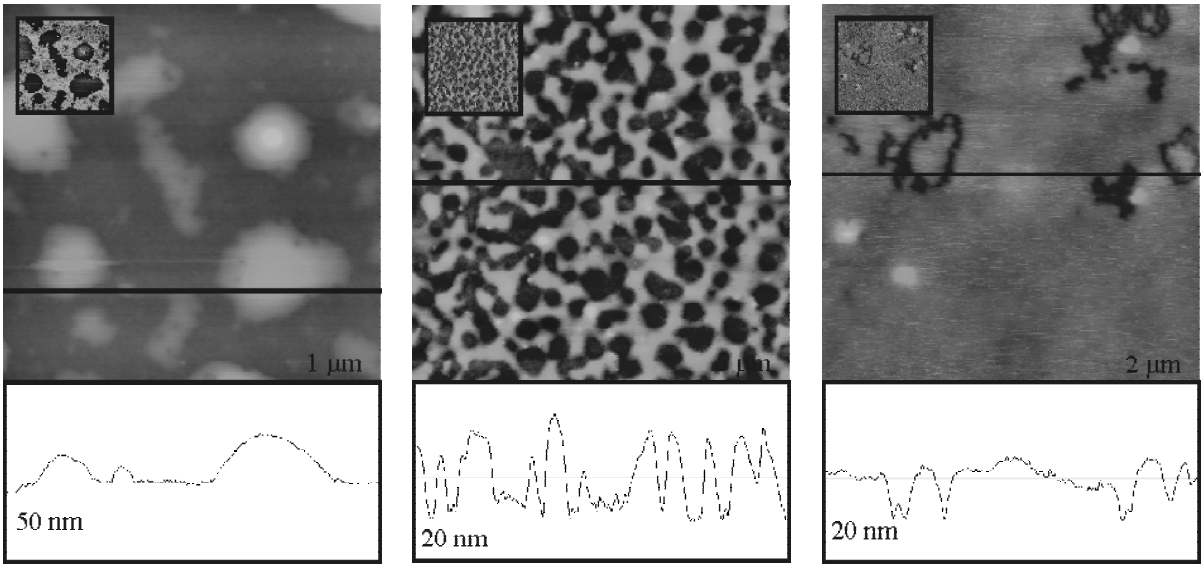
After spincoating



d) e) f)

Rinsed with water

Figure 6.3.6: *Optical images of layers of prepolymers spincoated out of 10.0 g/l solution in THF with different amounts of added triamine before and after washing: ratio of amine to acrylic endgroups: a and d = ¼; b and e = ½; c and f = 1/1*



a) b) c)

Figure 6.3.7: *AFM images of layers of prepolymers spincoated out of 1.0 g/l solution in THF with different amounts of added triamine before and after washing: ratio of amine to acrylic endgroups: a = ¼; b = ½; c = 1/1*

Like the irradiation with UV the treatment with the triamine could quench the dewetting process. The higher the amine concentration was, the smoother resulting layers became (Figure 6.3.6, Figure 6.3.1). But when rinsed with water the polymer layer was removed from the surface except a thin monolayer and in the case of thicker polymer layers some areas of clustered polymer remained (Figure 6.3.6 d-f). Different reaction times didn't have any significant influence on the thickness of the remaining layer while with a ratio of 1 to 1 primary amine groups to acrylic endgroups even at lower layer thicknesses small clusters of polymer could be detected. A concentration of amine groups higher than 1/1 didn't improve this matter.

In an other experiment the dewetting was tried to be suppressed by exposing the freshly spincoated layers to an ammonia atmosphere. The ammonia was supposed to penetrate into the polymer layers and crosslink the acrylic endgroups via "Michael addition". But during this process the dewetting of the polymer layer could not be suppressed. Whether the penetration of the ammonia occurred too slowly or the crosslinking reaction didn't work could not be detected.

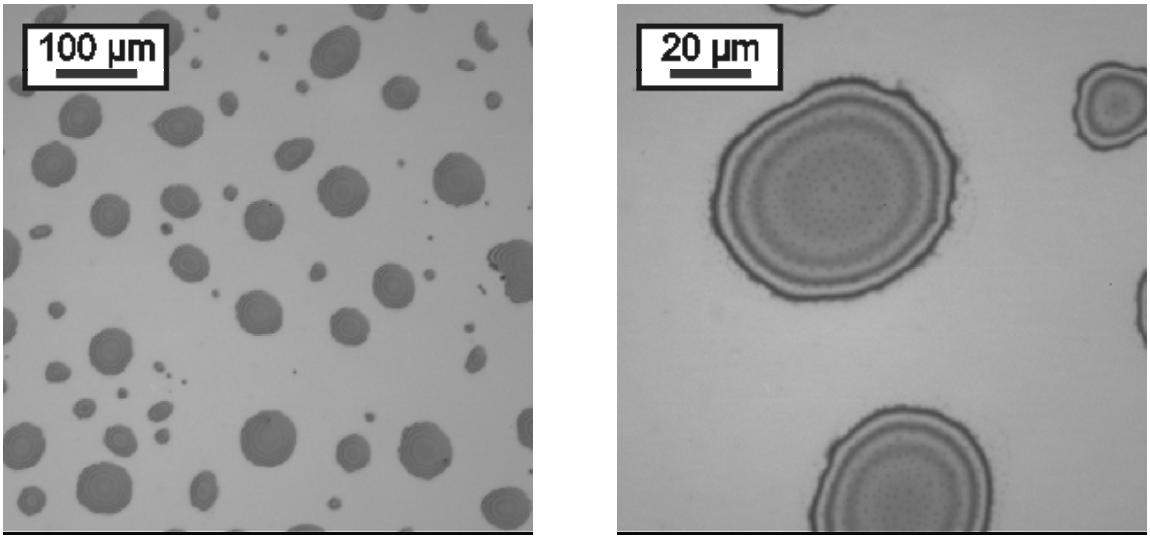


Figure 6.3.8: *Optical images of polymer layer spincoated out of a 10.0 mg/ml solution in THF and stored under ammonia atmosphere*

6.4 Conclusions

During this work it was possible to functionalize the hydroxyl terminated star polymer with acrylic acid in high conversion. The employment of triethyl amine reduced the amount of necessary excess of AAC but resulted in an unwanted coloring of the product. Other possibilities of scavenging the developing hydrochloric acid, like calcium hydrogen carbonate or a continuous argon stream, didn't improve the end group conversion. The more elegant way of synthesis with a hafnium catalyst did not work due to the poisonous effect of the allylic group to the catalyst.

The reactions had to be carried out under very strict conditions. Oxygen, UV-radiation and higher temperatures (<30°C) had to be prevented in order to minimize the polymerization (crosslinking) of the products. The functionalized polymers had to be kept at a temperature below 4 °C.

Coatings of the polymers onto aminosilylated substrates didn't work properly. Layer thicknesses could be controlled by varying the concentration of the prepolymer solutions. The dewetting process already known from the IPDI terminated starpolymers could be suppressed by UV-irradiation or addition of a trifunctional amine, but the polymer layers were not stable against rinsing processes with water. Biocompatible experiments were not taken through so far.

6.5 References

(1) Harris, J. M. In *Poly(ethylene glycol) Chemistry:*; Harris, J. M., Ed.; Plenum Press: New York, 1992; pp 1-14.
(2) Hubbell, J. A. *J. Controlled Release* **1996**, *39*, 305.
(3) Peppas, N. A.; Keys, K. B.; Torres-Lugo, M.; Lowman, A. M. *J. Controlled Release* **1999**, *62*, 81.
(4) Nakayama, Y.; Matsuda, T. *J. Polym. Sci, Part A: Polym. Chem.* **1992**, *50*, 2451.
(5) Andreopoulos, F. M.; Christopher, R. D.; Stauffer, M. T.; Weber, S. G.; Wagner, W. R.; Beckman, E. J.; Russell, R. J. *J. Am. Chem. Soc.* **1996**, *118*, 6235.
(6) Nakayama, Y.; Matsuda, T. *J. Biomed. Mater. Res.* **1999**, *48*, 511.
(7) Lyman, M. D.; Malanson, D.; Sawhney, A. S. *Biomaterials* **1996**, *17*, 359.
(8) Hubbell, J. A.; Chandrashekhar, P.; Amarpreet, S.; Desai, N. P.; Syed, F. A. In *US Patent 5 529914*, 1996.
(9) Nakayama, Y.; Matsuda, T. *J. Polym. Sci, Part A: Polym. Chem.* **1993**, *31*, 977.

(10) Zheng, Y.; Andreopoulos, F. M.; Micic, M.; Huo, Q.; Pham, S. M.; Leblanc, R. M. *Advanced Functional Materials* **2001**, *11*, 37.
(11) Chujo, Y.; Sada, K.; Saegusa, T. *Macromolecules* **1990**, *23*, 2693.
(12) Nakayama, Y.; Miyamura, M.; Hirano, Y.; Goto, K.; Matsuda, T. *Biomaterials* **1999**, *20*, 963.
(13) Chen, Y.; Chen, K. *J. Polym. Sci, Part A: Polym. Chem.* **1997**, *35*, 613.
(14) Andreopoulos, F. M.; Beckman, E. J.; Russell, R. J. *J. Polym. Sci, Part A: Polym. Chem.* **2000**, *38*, 1466.
(15) *SDBS*, 1424; 1931.
(16) Ishihara, K.; Ohara, S.; Yamamoto, H. *Science* **2000**, *290*, 1140-1142.

Chapter 7

Layer-by-layer Growth of Isocyanate/ Acrylate-terminated Star Polymers on Silicon Substrates.

7.1 Introduction

In various scientific and biomedical fields, the deposition of ultrathin polymer films on material surfaces is important for the modification of the surface properties. These can be tailored specifically to various environments, such as biological systems. The coating of biomedical materials with polymers may serve to drastically change their biological affinity. The chemical composition of the surface is of decisive importance for the material characteristics. Layer-by-layer assembly, which can be achieved by the alternate immersion of a certain substrate into oppositely charged water-soluble polymers, is a promised methodology that uses electrostatic interaction, e.g. polyionic complexes, to fabricate polyelectrolyte multilayers on substrates[1-3]. Other polymeric interactions have also been utilized for layer-by-layer assembly, including hydrogen bonds[4], charge transfer[5-7], and stereocomplex[8,9] formation. On the basis of this concept, we have fabricated ultrathin polymer films using a process involving repetitive adsorption/drying of a substrate[10-12]. These assembly-coated substrates have considerable potential in biomedical applications. The layer-

by-layer assembly process creates a monolayer of adsorbed polymer material with each assembly step. It is important that the outermost monolayer shows any specific properties similar to that of the bulk film against a given biochemical environment. If possible, alternating biological activities of the layers in the assembly should be constructed. Hubbell et al.[13] prepared polyelectrolyte multilayers on biological surfaces to obtain bioinert surfaces, in which cell interactions with the underlying surface were suppressed. In one paper Akashi el al. present their work on ultrathin polymer films prepared by the layer-by-layer assembly technique and their studies towards their alternating anti-vs procoagulant activity against human blood. The introduction of anti- and procoagulant activities onto material surfaces is an important topic in the biomedical field. Dextran sulfate[14,15] and chitosan[16] were described as polymers with anti- and procoagulant activities, respectively. The layer-by-layer assembly of these polymers was quantitatively analyzed by a quartz crystal microbalance (QCM), which had been utilized extensively for investigating the process of assembly[8-12]. The films were applied to a cell disk, which were the studied towards anti- vs procoagulant activity.

In this work it was tried to functionalize silicon substrates with isocyanate terminated starpolymers by the layer by layer technique. The starpolymers were used, because of their high functionality which should result in a dense crosslinked network improving the already mentioned properties of hydrogel layers.

7.2 Experimental

Materials: Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Acetone, isopropanol and ethanol (Merck, Selectipur) were stored in the clean room and used as received. THF and toluene were dried over $LiAlH_4$, distilled under argon and transferred into a glove box. N-[3-(trimethoxysilyl) propyl] ethylenediamine (Aldrich, 97%) was stored in the glove box and filtered before usage. Aminofluorescein /Aldrich; p.a. for fluorescence >90%) was used as received. The polyols were made at DOW Benelux N.V., Terneuzen, The Netherlands. Syringe filters with pore size 0.45 µm were purchased from VWR. Glass vials (VWR 40 ml) were cleaned in piranha acid (33 % sulphuric acid (96 % p.a. Merck) and 66% hydrogen peroxide (30 % p.a. Merck)), rinsed with millipore water until the pH value of the water was higher than 5 and allowed to dry for at least one day. They were covered with clean room suitable paper to prevent contamination with dust.

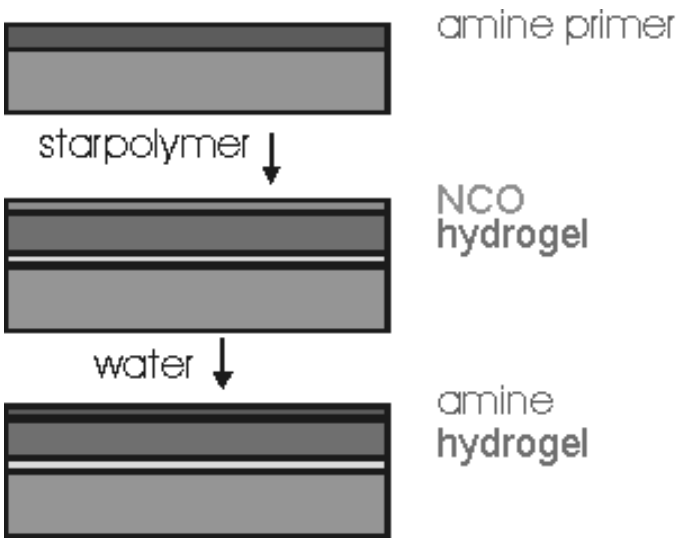
Methods: Silicon and substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath. Samples were treated with UV/ozone using a 40 W UV lamp (main emission at 185 nm; UV-Technik Speziallampen GmbH) in an oxygen stream of 350 ml/min with a sample distance of 5 mm to the lamp. Layer thicknesses were examined using a MM-SPEL-VIS ellipsometer (OMT). Contact angles were measured using the sessile drop method with a G 40 contact angle measuring device (Krüss GmbH). Lower detection limit of the method is around 10 degrees. Scanning force microscopy (SFM) investigations were performed with a Nanoscope IIIa (Digital Instruments) operating in tapping ModeTM. The oscillation frequency for Tapping ModeTM was set in the range of 320 – 360 kHz depending on the Si cantilever (k ~ 50 N/m, Nanosensors).

Substrate preparation: Cutting and cleaning of the substrates was performed in a class 100 clean room. Silicon wafers were cut into pieces of 14x14 mm. The samples were then cleaned by sonication in acetone, water and isopropanol for one minute each followed by drying in a stream of prefiltered nitrogen. Activation of the surface was achieved by treatment with UV/ozone for 12 min resulting in a contact angle below the detection limit. The substrates were used immediately for amino functionalization or for hydrogel coatings.

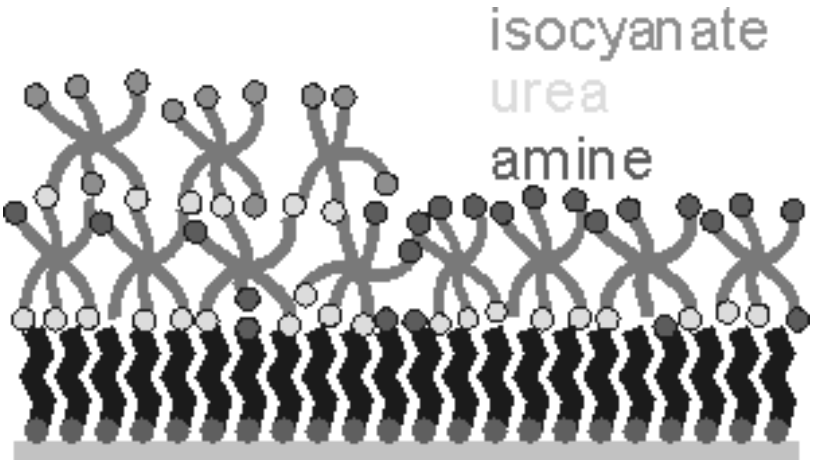
Aminosilylation of the substrates: After activation, the substrates were transferred into a Unilab glovebox (MBraun) and immersed into a solution of 0.3 ml N-[3-(trimethoxysilyl)propyl] ethylenediamine in 50 ml dry toluene for 2 hours. Then the samples were washed several times with dry toluene and stored under dry toluene until further usage.

Sample preparation: Different amounts of the polymers described in chapter three and six, respectively, were dissolved in THF or toluene under inert atmosphere in a glove box. The concentration varied from 1 to 100 g/mol. Into this solution both the activated and aminosilylated substrates were immersed from 30 seconds to 1 hour. The substrates were washed with the respective solvent, stored in 5 ml of this solvent and transferred out of the glove box. To the NCO-polymer covered substrates, 5 ml of Millipore water was added and allowed to react for one hour. Afterwards the substrates were rinsed with THF and evacuated

at $1*10^{-2}$ mbar for 10 min. to remove residual solvents. After measuring the layer thicknesses and contact angles, the substrates were immersed into the equivalent solvent, transferred back to the glove box where the next coating step was performed. The principle of this technique involves the repeated formation of an amino-terminated surface, which reacts with the next layer of isocyanate star polymers as shown in Scheme 7.2.1 and Scheme 7.2.2



Scheme 7.2.1: *Layer-by-layer growth of NCO terminated starpolymers onto amino terminated substrates*



Scheme 7.2.2: *Composition of a layer-by-layer assembled substrate involving functional star polymers.*

In the same manner the samples were coated with precrosslinked polymers. Therefore the polymer solutions were treated with different amounts of ethylene diamine and stored for 5 min before the immersion of the substrates. The further steps were implemented according to the above described coatings.

The layer by layer growth with acrylate terminated polymers was performed in a similar way. Dip-coating of the substrates was performed as described above for the NCO polymers. The substrates were taken out of the glove box and then immersed for 1 hour in 10 ml of a 5 weight % solution of ethylene diamine in THF. The substrates were rinsed with THF and dried under reduced pressure at $1*10^{-2}$ mbar. After measuring the layer thicknesses and contact angles the substrates were transferred back into the glove box, where the next coating step was performed.

Detection of NCO groups by fluorescence: In a glove box aminosilylated substrates were half dipped into a 10 mg/ml solution of isocyanate terminated star polymer (12000 g/mol) in THF for 30 seconds. The substrates were rinsed with THF. In this process the THF was dripped on the non-covered upper side so that it could run down and wash away the excess polymer on the lower half. It was prevented that the polymer loaded THF came in contact with the non-covered side of the substrates. The substrates were then immersed into a

solution of 10 % amino fluorescein in THF for 30 minutes. The substrates were washed thoroughly with THF and investigated by fluorescence microscopy.

Layer thickness determination: The silicon substrates were examined by ellipsometry with a spectral method in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15 degrees; the integration time was dependent on the layer thickness and the resulting signal intensity. In order to minimize systematic errors in the data collection, in a series of experiments always one substrate was just cleaned and activated and one was just cleaned, activated and aminosilylated. These two substrates were measured as references, and thicknesses of the hydrogel films were obtained as relative values to the aminosilylated substrate. Each sample was measured at 5 different places, and the presented data are the average values of each sample. Errors were determined through evaluation of the standard deviation of the measurements.

Contact angle measurements: Time dependent contact angle measurements were done with Millipore water. Ten measurements were performed with time intervals of 1 sec between the measurements. The resulting value of each single measurement is the average value of left and right contact angles. For each substrate, 5 droplets were measured at different places on the sample. The presented data are the average values of 5 measurements per substrate. Special care was taken that the routine of the measurements was always the same so that the time between droplet deposition and first measurement was the same for all measurements. Errors were determined through evaluation of the standard deviation of the measurements.

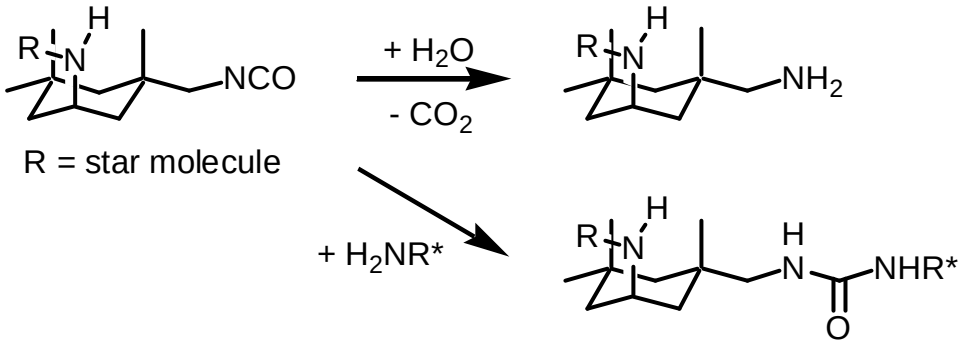
7.3 Results and Discussion

In this chapter it was attempted to grow an ultra thin polymer coating in a layer-by-layer procedure. This method promised to result in a very precise method to control layer thicknesses and morphologies of the coatings. In addition, the integration of specific foreign reactants like proteins into the layer would have been possible.

Never the less it was not possible to cover the substrates with more than one layer. This layer could be varied in the thickness within several Angstroms. It was not possible to attach further layers to the first one.

In order to prevent cleavage of the layer during the reaction of the substrates with water the substrates were aminosilylated. The results of this process are shown in chapter 4. Due to the expected small difference in layer thicknesses the reference value of each substrate was measured separately.

Then the first layer was coated onto the surface. This was done with NCO-polymers with average molecular weights of 3000 (3k-NCO), 12000 (12k-NCO) and 18000 g/mol (18k-NCO). In this step the isocyanate groups of the prepolymer should react with the amine groups of the surface to form stable urea groups. The remaining NCO-groups on top of the layer where converted to amino groups by reacting them with water outside the glove box.



Scheme 7.3.1: *Crosslinking reaction of the system. Isocyanate reacts with water to carbaminic acid which decarboxylates to a primary amine. Other isocyanate groups react with these amines to form biocompatible urea groups.*

The first experiments were done with different concentrations of the polymers in THF (**Table 7.3.1**). In every given value of the layer thickness, the respective reference value of the silicon and aminosilane layer is already subtracted.

Table 7.3.1: *Layer thickness of monolayers dip-coated at different polymer solution concentrations (all values are given in nm)*

Polymer	Polymer concentration [mg/ml]			
	1	5	10	100
3k-NCO	0,55	0,68	0,73	0,9
12k-NCO	0,58	0,75	0,88	1,05
18k-NCO	0,53	0,7	0,8	0,93

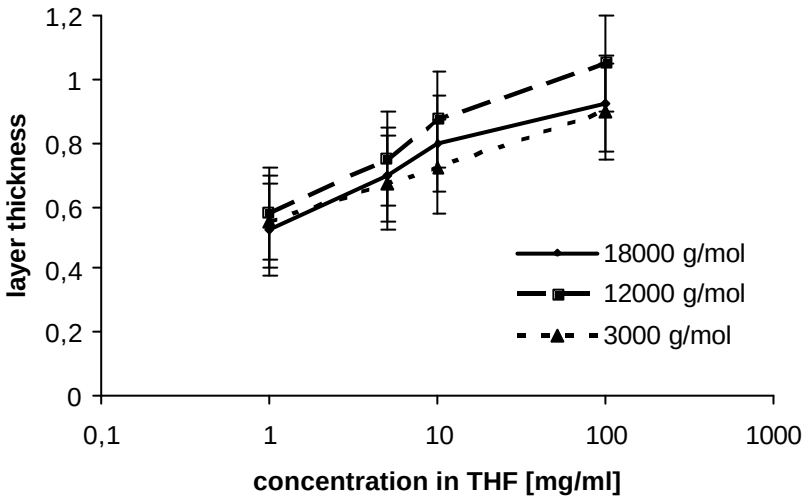


Figure 7.3.1: *Layer thickness of monolayers dip-coated from THF solution*

The dependence of the layer thickness on the solution concentration is evident. At low polymer concentration the star polymer molecules are spread flat on the substrate surface. At increasing concentration, the polymer molecules sterically interfere with each other and the segments are more forced away from the substrate surface (Scheme 7.3.2).

Scheme 7.3.2: *Concentration dependant conformations of star polymers on the amino terminated surface*

Different molecular weights have not affected the layer thicknesses. Polymers with a higher molecular weight and therefore longer chains are expected to form thicker layers however they are more flexible to spread more easily on the substrate surface. As a result the layer thicknesses for star polymers of varying different molecular weights did not differ significantly.

It was tried to bind further layers onto this first layer using the same reaction method. With neither of the three molecular weights and different concentrations it was possible to add a second or more layers to the monolayer. In no case a significant increase of the layer thickness was detectable.

Table 7.3.2: *Layer by layer deposition of three star polymers onto aminosilylated silicon substrates. Each step describes one complete functionalization process.*

Polymer	concentration	1. step	2. step	3. step
3k-NCO	1	0,55	0,575	0,625
	5	0,675	0,675	0,675
	10	0,725	0,775	0,725
	100	0,9	0,95	1
12k-NCO	1	0,575	0,6	0,6
	5	0,75	0,75	0,7
	10	0,875	0,95	0,975
	100	1,05	1,05	1,1
18k-NCO	1	0,525	0,55	0,525
	5	0,7	**0,675**	**0,7**
	10	0,8	0,825	0,825
	100	0,925	0,9	0,925

Although various variations were made in order to obtain a layer growth, all of the following efforts to functionalize the substrates with multiple layers were unsuccessful. Consequently, layer thicknesses are not listed. In every case, the attempt of functionalization was ceased after the third step without layer growth. The variations involve:

- Concentrations of 0,1 and 500 mg/ml
- substitution of THF with toluene since sufficient water is soluble in toluene to quench the isocyanate groups on the coated surfaces
- chain extension of the isocyanate polymers with ethylene diamine (ratios of hydroxyl : amine groups 2:1 ; 1:1 and 1:2) prior to surface adsorption
- variation of the immersion time into the polymer solution (30 sec., 5 min., 30 min. and 3h)

Significant differences in layer thicknesses of the first layer were not detectable. One possible explanation for this might be the ordering of the star polymers to the surface. Due to the flexibility of the chains and the high reactivity of the NCO groups towards the amine groups it is possible that all six polymer chain ends reacted with the surface. Therefore no more binding sites existed for further reactions with subsequent layers. Further adsorbed polymers would then easily be washed away. In contrast to Scheme 7.3.2 the following conformation of the star polymer molecules on the substrate surface is proposed (Scheme 7.3.3).

Scheme 7.3.3: *Revised orientation of NCO-starpolymers on an aminosilylated surface.*

As a consequence, the observed small increase of the layer thickness with increasing polymer concentration is explainable. The conformational flexibility of the polymer arms is low since all end groups are bound to the surface.

To avoid this problem the polymers were also coated onto plain activated silicon wafers. The reactivity of the isocyanate groups towards Si-OH groups is much lower so that the probability to obtain non-reacted end groups is higher. Due to the lower stability of the binding sites the quenching time in water was reduced to 10 min.

Again the covalent bonding of the first layer to the substrate surface was possible. Within 10 minutes quenching time, the monolayer was not cleaved from the surface. Subsequent efforts to attach further layers were not successful.

To prove this concept, aminosilylated substrates were half dipped into a polymer solution. Then the samples were treated with an amino fluorescein solution to allow the NCO groups to react with the amine group of the fluorescein. By fluorescence microscopy a border should be visible between the polymer-covered and the non-covered side of the substrate. In none of the experiments this border could be detected. Assuming that all excess fluorescein was washed from the surface, this observation provides evidence that no or not enough isocyanate groups were left on the polymer coated side of the substrates.

In a further series of experiments acrylate terminated star polymer (prepolymer B –AA terminated) was employed. In this case the acrylic endgroups are applied to react in a Michael-type reaction to form covalent bonds with the surface. Excess acrylic groups are left to react with ethylene diamine to supply the surface again with amine end groups. This modified surface is suitable for the attachment of the next polymer layer.

However, as in the experiments with NCO polymers, it was not possible to coat the substrates with more than one polymer layer. Layer thicknesses were comparable to the upper experiments. In conclusion, also the acrylate terminated starpolymers tended to attach with all end groups to the amino surface.

7.4 Conclusions

In this part of the thesis an effort was made to build up a well defined, ultrathin, polymeric hydrogel by utilizing a layer-by-layer methodology. One monolayer of different

star polymers was covalently bound to altering surfaces. With a short reaction step this layer was activated for further coating steps with the same polymer.

In the experiments, it was observed that all six arms of the polymers reacted with the surface and therefore no more binding sites for further reactions were left. Further adsorbed polymer layers were easily washed away.

To avoid this phenomenon in further efforts, either shorter chain lengths or polymers with a higher functionality (>12 end groups) should be applied. Due to its increased stiffness the former product shouldn't react with all six arms with the substrate. The latter must have such a high density of reactive groups that not every end group can attach to the surface.

7.5 References

(1) Hong, J.-D.; Decher, G. *Ber. Bunsen-Ges. Phys. Chem.* **1991**, *95*, 1430.
(2) Decher, G. *Science* **1997**, *277*, 1232.
(3) Decher, G. *Compr. Supramol. Chem.* **1996**, *9*, 507.
(4) Stockton, W. B.; Rubner, M. F. *Macromolecules* **1997**, *30*, 2717.
(5) Shimazaki, Y.; Mitsuishi, M.; Ito, S.; Yamamoto, M. *Langmuir* **1997**, *13*, 1385.
(6) Shimazaki, Y.; Mitsuishi, M.; Ito, S.; Yamamoto, M. *Langmuir* **1998**, *14*, 2768.
(7) Shimazaki, Y.; Mitsuishi, M.; Ito, S.; Yamamoto, M. *Macromolecules* **1999**, *32*, 8220.
(8) Serizawa, T.; Hamada, K.; Kitayama, T.; Katsukawa, K.; Hatada, K.; Akashi, M. *Langmuir* **2000**, in press.
(9) Serizawa, T.; Hamada, K.; Kitayama, T.; Fujimoto, N.; Hatada, K.; Akashi, M. *J. Am. Chem. Soc.* **2000**, *9*, 1891.
(10) Serizawa, T.; Akashi, M. *J. Polym. Sci, Part A: Polym. Chem.* **1999**, *37*, 1903.
(11) Serizawa, T.; Hashiguchi, S.; Akashi, M. *Langmuir* **1999**, *15*, 5363.
(12) Serizawa, T.; Yamamoto, K.; Akashi, M. *Langmuir* **1999**, *15*, 4682.
(13) Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. *Langmuir* **1999**, *15*, 5355.
(14) Akashi, M.; Sakamoto, N.; Suzuki, K.; Kishida, A. *Bioconjugate Chem.* **1996**, *7*, 393.
(15) Sakamoto, N.; Shioya, T.; Serizawa, T.; Akashi, M. *Bioconjugate Chem.* **1999**, *10*, 538.
(16) Muzzarelli, R. A. A. *Chitin*; Pergamon Press: Oxford, 1997.

Chapter 8

Endcapping of 6 arm Star Polymers with Divinyl Sulfone

8.1 Introduction

Divinyl sulfone (DVS) is an extremely reactive compound. Its Michael-like addition with amines, thiols and hydroxyl groups is prescribed early in literature[1-3]. Under alkaline conditions it reacts with amines[4-14], thiols[12,14-18], phosphanes[19] and sodium peroxide[20]. For reactions with alcohols[12,17] the hydroxyl group has to be deprotonated with stronger reactants like sodium and sodium hydride. Vinyl sulfones have become generally accepted as useful intermediates in organic synthesis[21-23]. They serve efficiently as both Michael acceptors and as 2π partners in cycloaddition reactions. They are also important substances for the formation of macrocycles[24].

Nowadays DVS is often used in polymer chemistry. The polymerization with diamines[4,25-27] is described as well as chain extension of polysulfides[28] or polyglycols[29]. An important feature is the possibility of cross linking polymers like proteins, hyaluronic acid, cellulose derivates[30] and polyglycols[31] with DVS. The reaction with proteins is of special interest to biologists. The mild reaction conditions with amines allow for an enhanced detection possibility of the a fragment ion in MS/MS analysis.

The high affinity of the amine group of proteins and immunoglobulins to DVS enables their purification and immobilization. This is normally performed by DVS functionalized agarose beads. By implementing a second reaction of these beads with mercapto ethanol, the so called "thiophilic" adsorption chromatography (TAC) is possible[32-34], which serves a frequently used purification method for proteins. The term "thiophilic" refers to the affinity to sulfone groups that lie in close proximity to thioether groups.

8.2 Experimental

Size Exclusion Chromatography (SEC), ^{1}H-NMR and ^{13}C-NMR spectra and Elemental Analysis: The parameters of the analysis are described in Chapter 3.

Materials: Divinyl sulfone (Aldrich, 97 %) was distilled under reduced pressure ($2*10^{-2}$ mbar) and stored under argon at 4 °C. The polyol was purchased by DOW Benelux N.V., Terneuzen, The Netherlands. Before use, it was degassed and dried under reduced pressure ($2*10^{-2}$ mbar) at 80°C for 2 hours. Toluene (technical) was dried over lithium aluminum hydride, distilled and stored under argon atmosphere. Chloroform and diethyl ether were distilled once and the ether cooled to -25 °C. Sodium hydroxide powder (Merck, 98%) was dried at 80 °C under reduced pressure (~15 mbar).

Synthesis of DVS based Prepolymers: The typical preparation of DVS based prepolymers is described for the example of polymer **B** (cf. Chapter 3).

In a 100 ml flask 5 g of the dried polymer were dissolved in 30 ml of dry toluene. 0.2 g sodium was added and the mixture stirred for 20 h at room temperature. After removal of the excess sodium the solution was added drop wise to a solution of 10 ml DVS in 20 ml dry toluene. During the addition the originally colorless solution turned slightly yellow but became clear after further reaction. The reaction was carried out for additional 24 hours at room temperature. After acidifying the mixture with acetic acid, the toluene was evaporated at reduced pressure. The remaining oil was dissolved in 50 ml of chloroform and washed twice with 30 ml of an aqueous sodium chloride solution. After the separation the chloroform of the organic phase was removed at reduced pressure. The oil was then precipitated in 50 ml of

cold diethyl ether (-25°C). The mixture was stored for 1 hour at -25°C. The ether was decanted leaving a colorless high viscous oil behind. For purification the oil was dissolved in chloroform and precipitated in cold ether for four times. The remaining ether was removed at reduced pressure ($2*10^{-2}$ mbar).

Reaction of DVS with aqueous sodium hydroxide: 0,5 g of sodium hydroxide (0,013 mol) was dissolved in 10 ml of deionized water and poured into a solution of 1 ml DVS (0,75 mol) in 50 ml methylene chloride. The mixture was stirred vigorously for 16 hours at room temperature. The white precipitate was removed by filtration. The remaining solution was evaporated at reduced pressure ($2*10^{-2}$) leaving a pale viscous oil behind. The product was dissolved in deuterated chloroform and characterized by NMR and MALDI-TOF.

Reaction of DVS with dry sodium hydroxide: 0.5 g of dried sodium hydroxide was added to a solution of 1 ml DVS in 50 ml dry methylene chloride. The mixture was vigorously stirred for 16 hours at room temperature. The mixture was filtered and evaporated at reduced pressure ($2*10^{-2}$).

8.3 Results and Discussion

In these studies, 6 arm star polymers have been successfully end-capped with DVS. The reactions were carried out in toluene with an excess of DVS. In contrast to the publication of Hubbell et al.[29], the reaction with sodium hydride in dichloromethane was observed to lead to a low conversion. In addition, chain extension, i.e. coupling of several star polymer molecules could not be fully avoided. During the purification the vinyl-functionalized polymer showed a high tendency to polymerize. The crude product was observed to polymerize within a few hours when stored at room temperature whereas the purified product polymerizes already at room temperature within a few minutes.

The conversion of the end groups was determined by ^{1}H-NMR to be approximately 85-90% of functionalized hydroxyl groups. The calculation with NMR was done as followed:

According to eq. 1) and eq. 2) the degree of polymerization per arm (P) was calculated to be 42.

$$M_{(EO/PO)} = 0.8 * M_{(EO)} + 0.2 * M_{(PO)} = 46.8 \text{ g/mol} \qquad \text{eq. 1)}$$

$$P = \frac{M_{w(polyol)} - M_{(sorbitol))}}{M_{(EO/PO)} * N} \qquad \text{eq. 2)}$$

with $M_{(EO)}$ = 44 g/mol; $M_{(PO)}$ = 58 g/mol, $M_{(sorbitol)}$ = 182 g/mol, $M_{w(polyol)}$ = 12,000 g/mol and the number of arms N = 6.

Assuming 100 % of end group conversion the ratio of the integral of the vinylic peak (**b**) (Figure 8.3.1 and Figure 8.3.2) to the sum of the aliphatic peaks must be 1 to 191 (aliphatic protons per arm + 4 protons (d+e) + 2 core protons) (eq. 4)

$$H_{(EO/PO)} = 0.8* H_{(EO)} + 0.2 * H_{(PO)} = 4.4 \qquad \text{eq. 3)}$$

$$H_{(arm)} = H_{(EO/PO)} * P \qquad \text{eq. 4)}$$

with H = number of protons; $H_{(EO)}$ = 4, $H_{(PO)}$ = 6, $H_{(EO/PO)}$ = average number of protons per chain unit and $H_{(arm)}$ = number of protons per arm

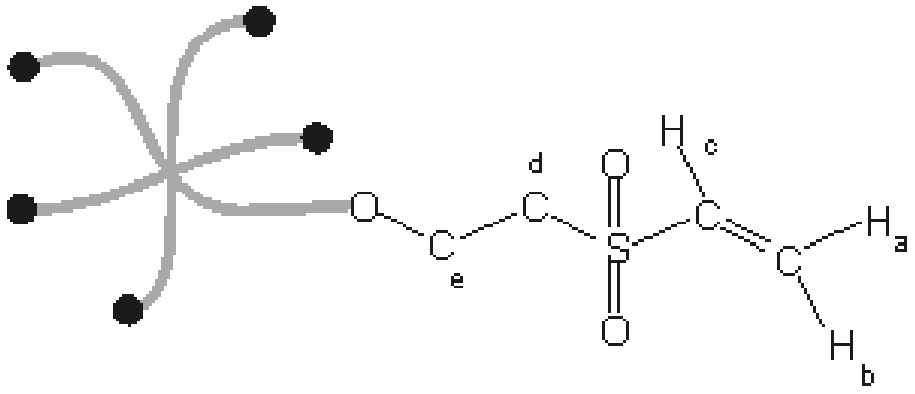


Figure 8.3.1: *Scheme of the VS terminated prepolymer (PEG-VS)*

The NMR spectra to demonstrate the reaction of DVS with the polyol is shown in Figure 8.3.2. The three vinyl peaks after reaction (**a** 6.05 ppm; **b** 6.37 ppm; **c** 6.82 ppm) are significantly shifted to those of remaining free DVS (**a'** 6.1 ppm; **b'** 6.45 ppm; **c'** 6.55 ppm). The polymerization of DVS, which was observed in former reactions using DVS in the presence of aqueous sodium hydroxide, can be excluded due to the small difference in the described peaks.

a

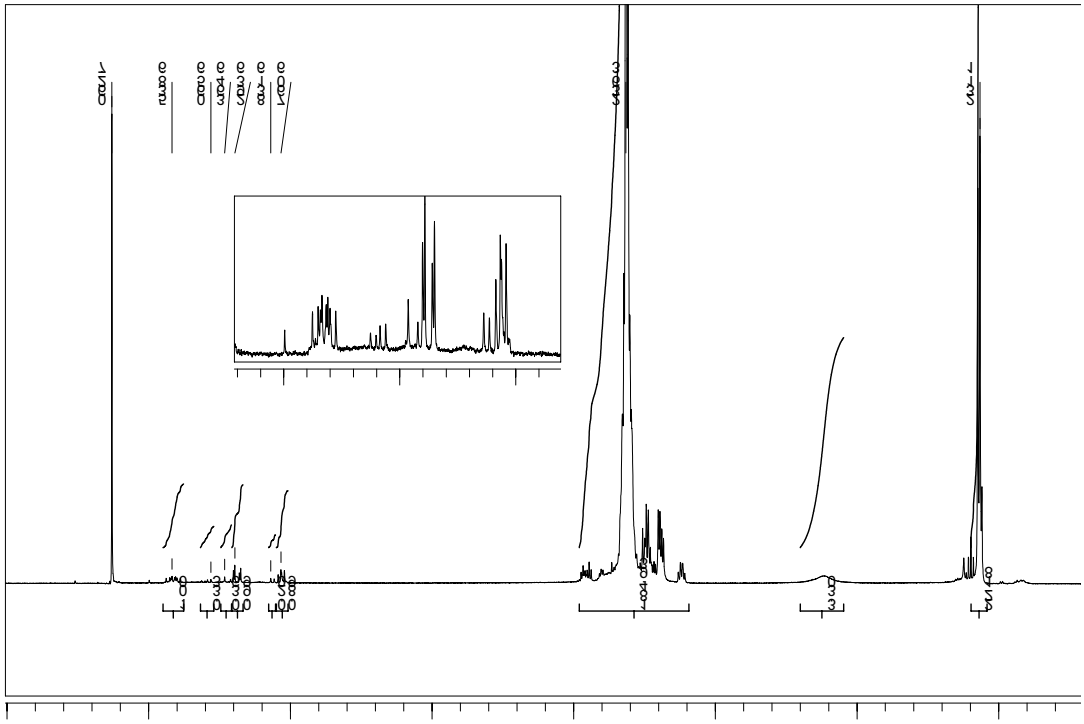


Figure 8.3.2: *^{1}H-NMR of VS-terminated prepolymers with a remaining amount of free DVS*

b

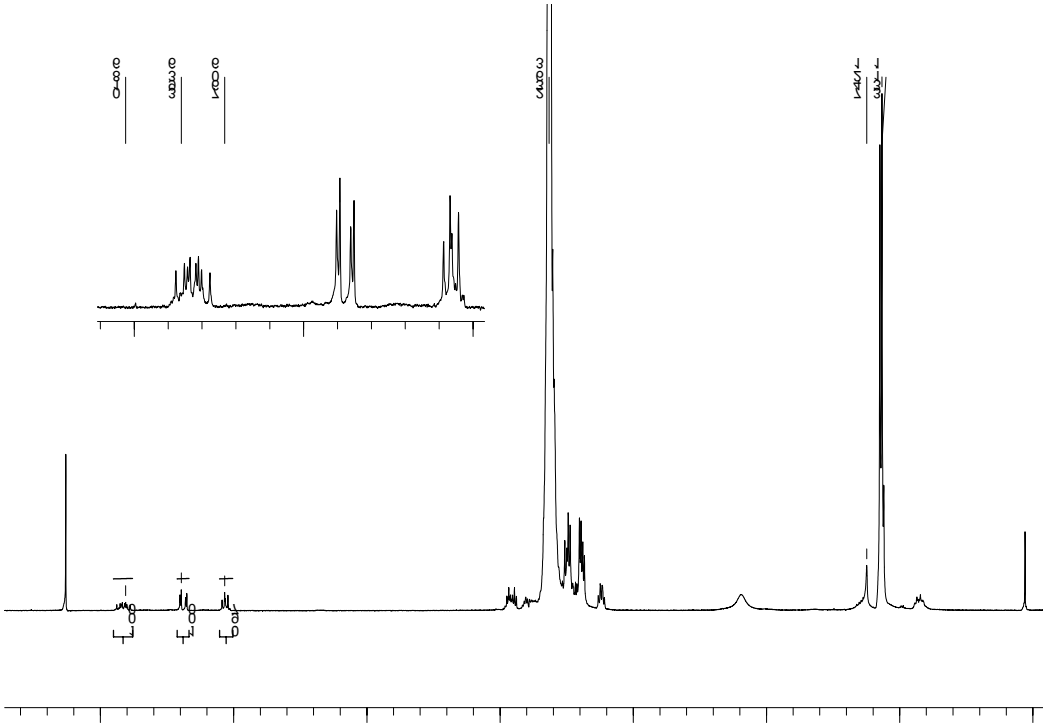


Figure 8.3.3 *^{1}H-NMR of VS terminated prepolymers without traces of free DVS*

To exclude that side reactions like DVS polymerization occurred, SEC and MALDI-TOF measurements were carried out. SEC exhibited only signals for polymers with a molecular weight of ~13000 g/mol, 26000 g/mol and some higher molecular weight analogues, which indicates that only bi- and multi- stars were formed (Figure 8.3.4). Nonetheless with approximately 42 % was their amount relatively high.

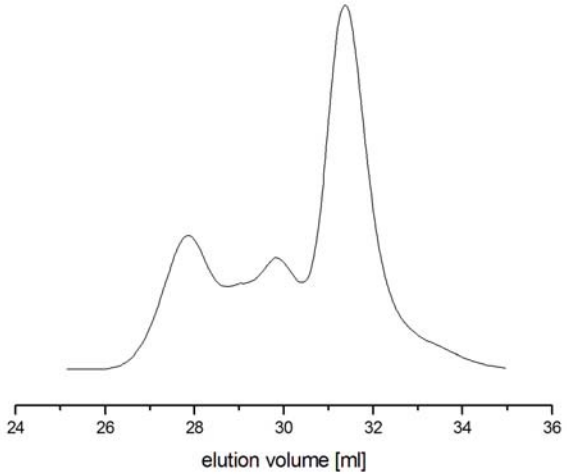


Figure 8.3.4: *GPC of VS-terminated starpolymer with chain extensions*

Polymers with lower molecular weight, which should occur if DVS was polymerized, couldn't be detected. MALDI experiments only showed broad signals with a shift in the molecular weight from ~10500 g/mol (educt) to ~12200 g/mol (product). In contrast, 12000 g/mol were calculated by end group titration. To explain this, the determination of the molecular weights by MALDI-TOF MS usually leads to lower values due to decreased desorption of the high molecular weight fractions.

As seen in Figure 8.3.2 the removal of DVS is not complete. This is due to the precipitation conditions. In order to get a reasonable yield of product both the ether and the beaker used for precipitation had to be cooled down to -25°C. After precipitation, the mixture had to be stored for at least 1h at 25°C. At these conditions the product was a solid and could be separated from the solution. But the DVS could not be completely removed from the polymer. After four or five precipitation steps, the amount of free DVS remained constant at about 3 % (~30% free DVS to reacted DVS). The remaining DVS seems to be encapsulated in the quickly precipitating polymer. The yield for this procedure was around 50 %. If the ether is cooled to ~20 °C and the beaker is at room temperature, the DVS could be removed quantitatively after four precipitation steps at the cost of the yield, which sank to below 5 %.

In an other method it was tried to purify the product via centrifugation. 200 mg of the washed and dried crude product was dissolved in 2 ml chloroform. This solution was pressed through a filter (pore size 3 kDa) in an eppendorf centrifuge (14000 rpm). The filter was filled with chloroform and centrifuged. The residue in the filter was then dissolved in 0.6 ml of deuterated chloroform. This solution was measured by NMR. It was not possible to extract enough of the product for a clear spectrum.

In the reaction of DVS with an aqueous sodium hydroxide solution a white insoluble precipitate was formed. It is assumed to be high molecular weight poly(divinyl sulfone ether) (PDVS). The ^{1}H-NMR and MALDI-TOF data show that the residue oil of this reaction was a low molecular weight fraction of PDVS (Figure 6.3.3). According to the two main peaks in the MALDI-TOF spectrum at 550 and 690, the number of repeat units (**n**) is around 2-3. The difference in the integrals of the end group carbons ***c*** and ***i*** can be explained by the three possible end group combinations: (**1**) both ends vinyl terminated, (**2**) one vinyl and one hydroxyl end group or (**3**) both ends hydroxyl terminated. According to the integrals of ***c*** and ***i*** combination (**1**) can be excluded. Therefore, the ratio of (**2**) to (**3**) is about 1 to 0.4.

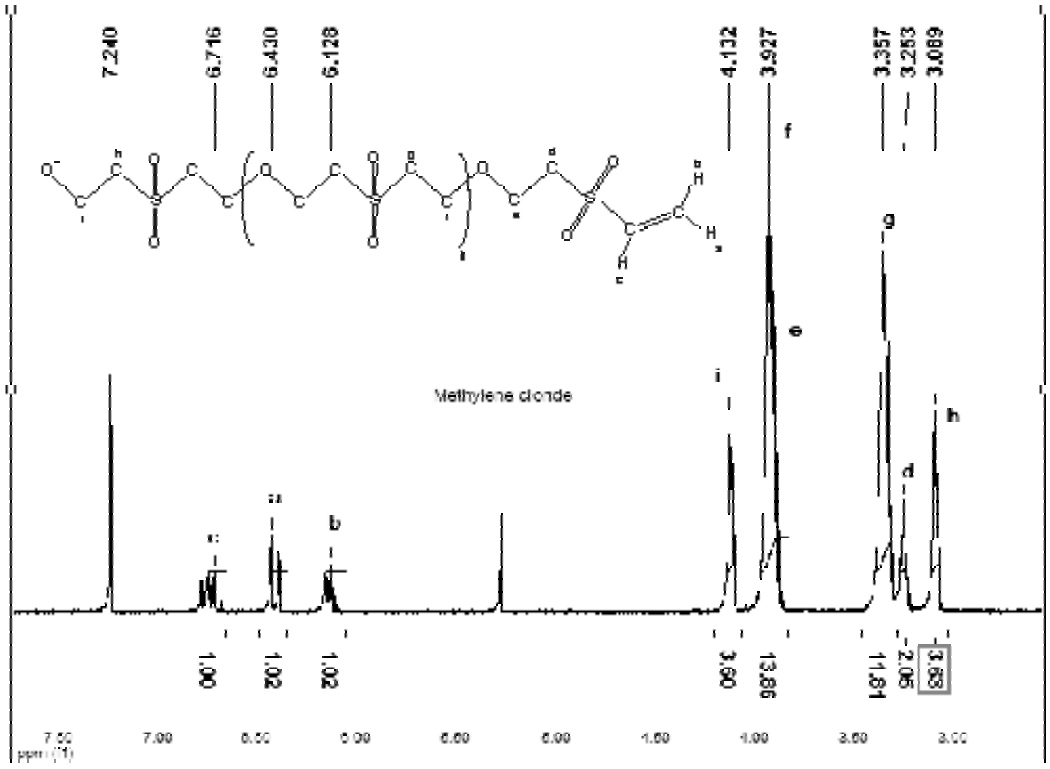


Figure 8.3.5: *^{1}H-NMR of Poly(divinyl sulfone ether) (PDVS)*

No reaction occurred when using a base without any present water. In the ^{1}H-NMR only the three DVS-signals were detectable. For the polymerization an abstractable proton must therefore be present.

8.4 Conclusions

During this work it was possible to functionalize the hydroxyl terminated star polymer with divinyl sulfone. The purification of the crude product proved to be a compromise between yield and purity. By variation of the precipitation conditions it was possible either to remove the excess of DVS completely at cost of high losses in yield, or to get good yields of about 50 % with around 3 % of free DVS. Further optimization of the procedure should however allow for better yields of pure product.

As shown by SEC, during the removal of solvents, the product tended to form bi- and multi stars. This unwanted polymerization occurred at room temperature within a few

minutes. The drying of the product should therefore be performed in a freeze drying process. Afterwards the product should be kept at low temperatures prior to use.

As shown in additional reactions of DVS in the presence of sodium hydroxide, it is important to dry the employed reactants and solvents to avoid side reactions such as the formation of poly(divinyl sulfone). It was not possible to remove this side product from desired product.

8.5 References

(1) Alexander; McCombie. *J.Chem. Soc.* **1931**, 1913.
(2) Ford-Moore, A. H. *Unpublished data obtained in Great Britain* **1940**.
(3) Stahmann, M. A.; CGolumbic, C.; Stein, W. H.; Fruton, J. S. *J. Org. Chem.* **1946**, *11*, 719-735.
(4) Gao, C.; Yan, D. *Chem. Commun.* **2001**, 107-108.
(5) Hallas, G.; Marsden, R.; Heytworth, J. D.; Mason, D. *J. Chem. Soc., Perkin Trans. 2* **1986**, 123-126.
(6) Minbaev, B. U.; Shostakovskii, M. F.; Kagarlistskii, A. D. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1983**, *19*, 957-959.
(7) Nagarajan, K.; Arya, V. P.; George, T.; Sudarsaman, V.; Shah, R. K.; Goud, A. N.; Shenoy, S. J.; Hakan, V.; Kulkarni, Y. S.; Rao, M. K. *Indian J. Chem.* **1982**, *21B*, 928-940.
(8) Arya, V. P. *Indian J. Chem.* **1975**, *13*, 1262-1266.
(9) Arbuzov, B. A.; Batenko, G. G.; Remizov, A. B.; Klimovitskii, E. N. *Chem. Heterocycl. Compd. (Engl. Transl.)* **1972**, *8*, 375-379.
(10) Maillard, J.; Vincent, M.; Delaunay, P.; Rapin, M.; Vo-Van-Tri; Remond, G. *Chim. Ther.* **1969**, *4*, 80-88.
(11) Yoda, K.; Toda, T. *Bull. Chem. Soc. Jpn.* **1968**, *41*, 2519-2521.
(12) Prilezhaeva, E. N.; Shapiro, E. S. *Dokl. Chem. (Engl. Transl.)* **1967**, *579-582*.
(13) Bellaart, A. C. *Recl. Trav. Chim. Pays Bas* **1967**, *81*, 156-159.
(14) Reppe, W.; al., e. *Liebigs Ann. Chem.* **1956**, *601*, 111-128.
(15) Li, L.; Tsai, S. W.; Anderson, A.-L.; Keire, D. A.; Ravbitschek, A. A.; Shively, J. E. *Bioconjugate Chem.* **2002**, *13*, 110-115.
(16) Black, R. M.; Brewster, K.; Clarke, R. J.; Harrison, J. M. *Phosphorus, Sulfur, Silicon* **1992**, *71*, 49-58.
(17) Ford-Moore, A. H. *J. Chem. Soc.* **1949**, *2433-2440*.
(18) Morpurgo, M.; Veronese, F. M.; Kachensky, D.; Harris, J. M. *Bioconjugate Chem.* **1996**, *7*, 363-368.
(19) Nagao, Y.; Sakurai, H. *Chem. Lett.* **1976**, 379-380.

(20) Kropf, H.; Ball, M. *Tetrahedron* **1972**, *28*, 1391-1401.
(21) Kuroki, Y.; Lett, R. *Tetrahedron Lett.* **1984**, *25*, 197-200.
(22) Enders, D.; Müller, S. F.; Raabe, G.; Runsink, J. *Eur. J. Org. Chem.* **2000**, 879-892.
(23) Simpkins, N. S. *Tetrahedron* **1990**, *46*, 6951-6984.
(24) Teyssot, M.-L.; Fayolle, M.; Philouze, C.; Dupuy, C. *Eur. J. Org. Chem.* **2003**, 54-62.
(25) Gao, C.; Yan, D.; Zhu, X.; Huang, W. *Polymer* **2001**, *42*, 7603-7610.
(26) Yan, D.; Gao, C. *Macromolecules* **2000**, *33*, 7693-7699.
(27) Gao, C.; Yan, D.; Tang, W. *Macromol. Chem. Phys.* **2001**, *202*, 2623-2629.
(28) Rehor, A.; Tirrelli, N.; Hubbell, J. A. *Macromolecules* **2002**, *35*, 8688-8693.
(29) Lutolf, M. P.; Hubbell, J. A. *Biomacromolecules* **2003**, *4*, 713-722.
(30) Sannino, A.; Madaghiele, M.; Conversano, F.; Mele, G.; Maffezzioli, A.; Netti, P. A.; Ambrosio, L.; Nicolais, L. *Biomacromolecules* **2004**, *5*, 92-96.
(31) Rizzi, S. C.; Halstenberg, S.; Hubbell, J. A. In *European Cells and Materials*, 2001; Vol. 2, pp 82-83.
(32) Porath, J.; Belew, M.; Maisano, F.; Olin, B. In *Physical Chemistry of Colloids and Macromolecules*; Ranby, B., Ed.: Uppsala, Sweden, 1984; pp 137-142.
(33) Porath, J.; Maisano, F.; Belew, M. *FEBS Letters* **1985**, *185*, 306-310.
(34) Hutchens, T. W.; J., P. *Anal. Biochem.* **1986**, *159*, 217-226.

Chapter 9

Ultrathin Functional Star PEG Coatings for DNA Microarrays

9.1 Introduction

The development of so called DNA chips resulted in a rapid increase of gene expression analysis, thereby accelerating the human genome project dramatically[1,2]. Today, biochips consist of microarrays of more than 400,000 different oligonucleotides on a single array or 40 sequences for each of 10,000 genes[3]. For example the detection of single nucleotide polymorphism (snp) is performed by this technique[4,5]. Advanced technologies to generate microarrays are based on spotting oligomer solutions onto substrates. Alternatively, the spot structures can also be prepared by means of ink jet techniques[6], photolithography[7], dip pen nanolithography[8] or microcontact printing which is an easy method to gain square centimeter big areas of structured surfaces[9]. Nevertheless, spotting is the method of choice for the generation of microarrays. In contrast to the technology of arraying, the surface chemistry varies considerably for different approaches and applications,[10] and the solid support materials of microarrays is still developping[11]. One key issue is that in order to attach the oligonucleotides covalently to the surface, the oligomers have to be functionalized with a

spacer to prevent steric hindrance during hybridization, and the surfaces have to be activated by introducing complementary reactive groups. This surface activation, e.g. active ester groups, is often not stable over extended times. Hence samples vary in their reactivity upon storing and the reproducibility of results suffers[12]. Although some approaches show good results to regenerate full surface reactivity[12,13], this always requires additional chemical reaction steps. Furthermore, after spotting of the oligonucleotides on the reactive substrate the remaining active groups have to be deactivated by a so called blocking agent. In order to prevent these extra steps, it has been proposed to use supramolecular chemistry concepts or physisorption of oligonucleotides to electrolyte covered surfaces[14]. It was reported before that ultrathin Star PEG layers prevent unspecific interaction with biological systems, but can easily be modified towards specific protein immobilization and cell adhesion[15-17].

In this chapter the binding and hybridization of oligonucleotides to hydrogels is prescribed. The hydrogel layers were prepared according to chapter 4. Aminoterminated oligonucleotides have been covalently bound to this layers by microcontact printing and by spotting on freshly prepared layers that still contain isocyanate groups. Thus, this method does not require any chemical crosslinker or other additional surface activation. After crosslinking of the layer, nonreacted isocyanate groups are completely hydrolyzed by air humidity and no blocking agent is needed. Besides covalent binding of the oligonucleotides, the Star PEG films prevent unspecific adsorption of target molecules. Within the spotted substrate area, hybridization of a 20mer oligonucleotide was achieved with hybridization efficiencies of between 30 and 80%, depending on film thickness and preparation.

9.2 Experimental

Materials: Silicon wafers (100) were purchased from CrysTec GmbH/Berlin. Glass substrates (50.8x50.8x0.175mm) were purchased from Schott Desag. Acetone, isopropanol and ethanol (Merck, selectipur) were stored in the clean room and used as received. THF and toluene were distilled over $LiAlH_4$ and stored in a glove box. N-[3-(trimethoxysilyl)propyl] ethylenediamine (Aldrich, 97%) was stored in a glove box and filtered before use. Isophorone diisocyanate (IPDI) was purchased from Fluka and double distilled before use. Syringe filters with pore size 0.02 µm were purchased from Whatman. Phosphate buffered saline tablets

(Sigma) were dissolved in 200 mL deionized water each to obtain 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C. For the washing solution, the PBS concentration was doubled (2xPBS). Three different oligonucleotides have been synthesized: O_1, 3'-amino terminated-5'-fluorescein labelled oligonucleotide FITC-GTA-AAA-CGA-CGG-CCA-GTT-TT-$(CH_2)_7$-NH_2; O_2, 3'-amino terminated oligonucleotide GTA-AAA-CGA-CGG-CCA-GTT-TT-$(CH_2)_7$-NH_2; O_3, 3'-fluorescein labelled complementary oligonucleotide to O_2. All Oligonucleotides were dissolved at a concentration of 100 pmol/µl in a PBS buffered water and stored at -20 °C. Before use the solutions were carefully warmed up to room temperature.

Methods: Silicon and glass substrates were cut with a RV-125 diamond cutting device from ATV Technologie GmbH. Samples were sonicated using a TK 52H ultrasonic bath. Oxygen plasma was generated by a TePla 100-E system with 100 W at a process gas pressure of 0.5 mbar. Samples were treated with UV/ozone using a 40 W UV lamp (main emission 185 nm; UV-Technik Speziallampen GmbH) in an oxygen stream of 350 ml/min with a sample distance of 5 mm to the lamp. Films were generated with a CONVAC ST 146 spincoater. Layer thicknesses were examined using a spectral MM-SPEL-VIS ellipsometer (OMT/Ulm, Germany). Measurements were performed in the wavelength range from 450 to 900 nm. The azimuthal angle was kept at 15 degrees, the integration time was 300sec. Light microscopy and fluorescence microscopy were performed by means of an Axioplan2 Imaging microscope from Zeiss. Light microscopy pictures were taken with a Zeiss AxioCam HR camera, pictures for fluorescence microscopy were obtained using a Princeton Instruments NTE/CCD 512EBFT camera. A N XBO 75 lamp from Zeiss was used as light source for fluorescence microscopy. The samples were spotted by a Omnigrid 2, Genmachines using a SMP 2.5B (ArrayIt^{TM}) micro spotting pin.

Substrate preparation: Cutting and cleaning of the substrates was performed in a class 100 cleanroom. Silicon wafers were cut into pieces of 14x14 mm. The samples were then cleaned by sonication in acetone, water and isopropanol for one minute each step followed by drying in a stream of prefiltered nitrogen. Activation of the surface can either be achieved by

treatment with UV/ozone in humid atmosphere for 12 min or by exposure to oxygen plasma for 10 min. The substrates were used immediately for aminofunctionalization.

Aminosilylation of the substrates: After activation, the substrates were transferred into a Unilab glove box (MBraun) and immersed into a solution of 0.3 ml N-[3-(trimethoxysilyl) propyl] ethylenediamine in 50 ml dry toluene for 2 hours. Then the samples were washed several times with dry toluene and stored under dry toluene until further use.

Preparation of the isocyanate-terminated Star PEG: The preparation of the star polymers has been described elsewhere[18]. Briefly, OH teminated star polyols (M_n = 12,000 g/mol; PD = 1.15) were functionalized through reaction with 12 times excess isophorone diisocyanate (IPDI) in a solvent free process at 50°C for 5 days under an inert gas atmosphere. The excess of IPDI was removed by short path distillation. Size exclusion chromatography of the product (Star PEG) proved that no dimer or trimer formation takes place.

Sample preparation: Different amounts of Star PEG were dissolved in the glove box in THF. After removal of the samples from the glove box, the solutions were either used as prepared or the same amount of water was added. The freshly prepared solutions of 0.1, 1.0 or 10.0 mg/ml were filtered through a 0.02 µm syringe filter and within 5 min after water addition used for spincoating. For spincoating, the substrates were placed on the spincoater, covered by the corresponding solution and then accelerated within 5 seconds to the final rotation speed of 2500 rpm and kept rotating for 40 sec. Onto this substrates the oligonucleotides were brought either by spotting or stamping.

Microcontact printing: PDMS stamps were prepared as described in ref. 9, cleaned by sonication in ethanol for 2 min and dried in a stream of nitrogen. Then they were activated in UV/ozone for 15 minutes. Directly after the activation the solution of oligonucleotide O_1 was distributed homogeneously on the stamp and allowed to dry in a nitrogen stream. After complete drying, the stamp was pressed onto the freshly prepared Star PEG coated substrates. After 30 minutes in contact with the substrate the stamp was peeled off and the substrate

stored over night in a humid atmosphere at slightly elevated temperature to allow proper crosslinking of the layer. The sample was then washed several times, dried and examined by fluorescence microscopy.

Spotting of the oligonucleotides: The solutions O_1 and O_2 were either spotted by a glass capillary or a metal needle in a spotting apparatus. Former resulted in spots with diameter between 2 and 3 mm, latter in about 50 µm wide spots. The spotted substrates were then kept over night under humid atmosphere at slightly elevated temperature to allow proper crosslinking of the layer. For hybridization, the substrates were washed several times with 2xPBS solution and demineralised water. Then, the spotted area of the samples was covered with a solution of 5.5 ml 2xPBS, 500 µL O_3 and 10 µL Tween20 (concentration O_3: 10 pmol/µl) and stored at 4°C over night. The substrates were then washed with 2xPBS, dried in a stream of nitrogen and examined by fluorescence microscopy.

9.3 Results and Discussion

The preparation of Star PEG coatings by spincoating aqueous solutions of the reactive prepolymer **B** onto aminofunctional substrates has been reported before[15]. In aqueous environment the isocyanate groups hydrolyze to amino groups. These are more reactive towards isocyanate groups than water, so that a crosslinking reaction between the molecules takes place with resulting urea groups as connecting unit between two star shaped molecules. At the end of this process, a dense polymer network results on the substrate that, due to the high functionality of the prepolymer, contains free amino groups. It was demonstrated earlier that such films prevent unspecific protein adsorption and cell adhesion but can, due to their high functionality, easily be modified towards specific biological recognition.[16,17] The superior performance as well as the versatility of the system is most probably due to the combination of several things like the well known and often used properties of poly (ethylene glycol), the small amount of hydrophobic motives, the content of biocompatible urea and amino functional groups and the high surface coverage of the system due to the molecular architecture of the Star PEG prepolymer.

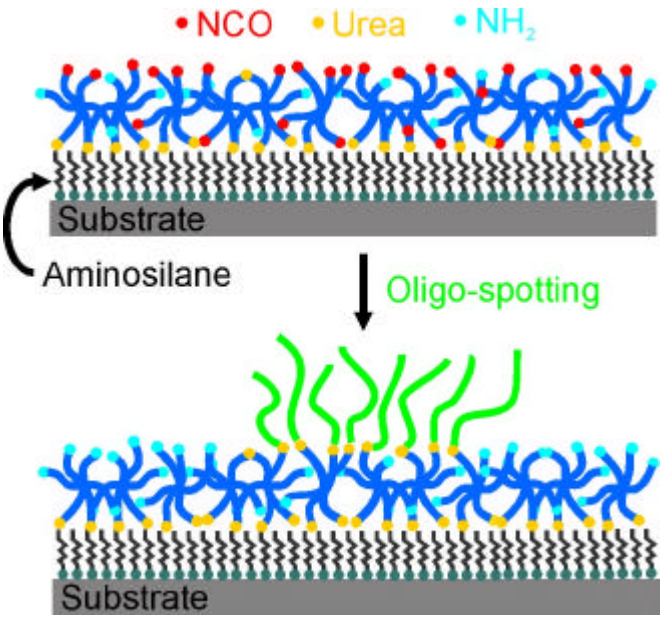


Figure 9.3.1: *Scheme of the immobilization strategy of aminoterminal oligonucleotides (green). Freshly prepared Star PEG layers contain isocyanate groups (red) that covalently bind the printed or spotted oligonucleotides. After hydrolysis and crosslinking, the reactive isocyanate groups are gone, and the nonfunctionalized parts of the Star PEG coating are nonadhesive.*

In this study these ultrathin intrinsically nonadhesive surface coatings were used for DNA microarrays. A scheme of this process is displayed in Figure 9.3.1. For the immobilization of aminoterminated oligonucleotides, Star PEG coatings were prepared by spincoating solutions in THF or in 50% aqueous THF with concentrations of 1 mg/ml and 10 mg/ml onto aminofunctional glass substrates. This yielded films with layer thicknesses between 5.6 and 106 nm. Freshly prepared Star PEG coatings, especially when prepared from mainly organic solvent mixtures, contain a high number of isocyanate groups that are very reactive towards the aminofunction of the spotted oligonucleotides. Thus, the DNA molecules react with the isocyanate groups directly after the spotting and bind covalently to the coating. This has the advantage that the immobilization of the DNA does neither require a chemical crosslinker or other surface activation or reactivation step, nor a blocking agent for the remaining reactive groups after DNA immobilization since the isocyanate groups hydrolyze and either react with other isocyanates to form urea groups during the crosslinking process or remain as amino groups in the layer.

The immobilization of aminoterminated and fluorescently labelled oligonucleotides was achieved by different techniques. Figure 9.3.2 shows a fluorescence microscopy image of a pattern of oligonucleotides that were patterned onto a Star PEG coating prepared from THF with 17.7 nm layer thickness by microcontact printing.

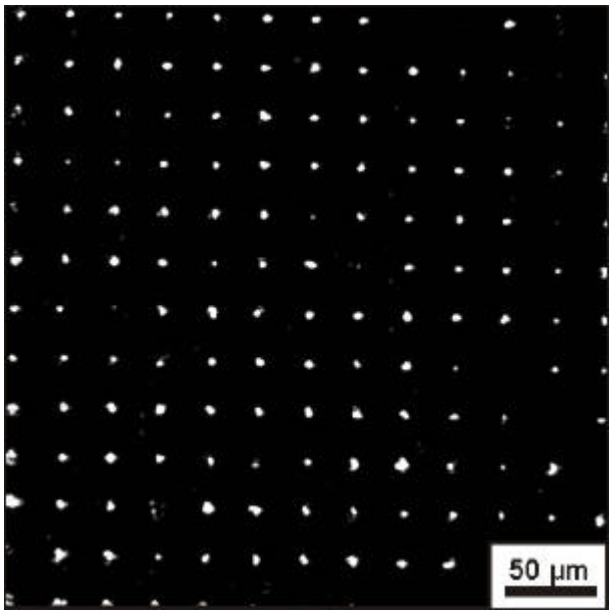


Figure 9.3.2: *Fluorescence microscope image of oligonucleotide O_1 stamped onto a Star PEG layer prepared from a solution in THF (17.7 nm thickness) by microcontact printing.*

As alternative immobilization technique, spotting onto Star PEG modified silicon substrates with a micro litre glass pipette resulted in spots with the size of around 2-3 mm. Spotting onto Star PEG coatings prepared from THF and 50% aqueous THF in different concentrations was done to check the effect of different layer preparation techniques and different layer thicknesses on the hybridization efficiency. The spotting was done directly after spincoating of the Star PEG layer. Hybridization of a spotted aminofunctional non-fluorescent 20mer oligonucleotides has been performed by the fluorescently labelled complementary oligonucleotide. Figure 9.3.3 presents the fluorescence microscopy pictures of the resulting samples: (i) thicker films result in higher fluorescence intensities, (ii) thinner films result in better hybridization efficiencies and (iii) solutions in non-aqueous THF result in higher fluorescence intensities but lower hybridization efficiencies. These results are in

accordance with both the reactivity and the amphiphilic character of the Star PEG prepolymers consisting of hydrophilic backbones and hydrophobic endgroups[15].

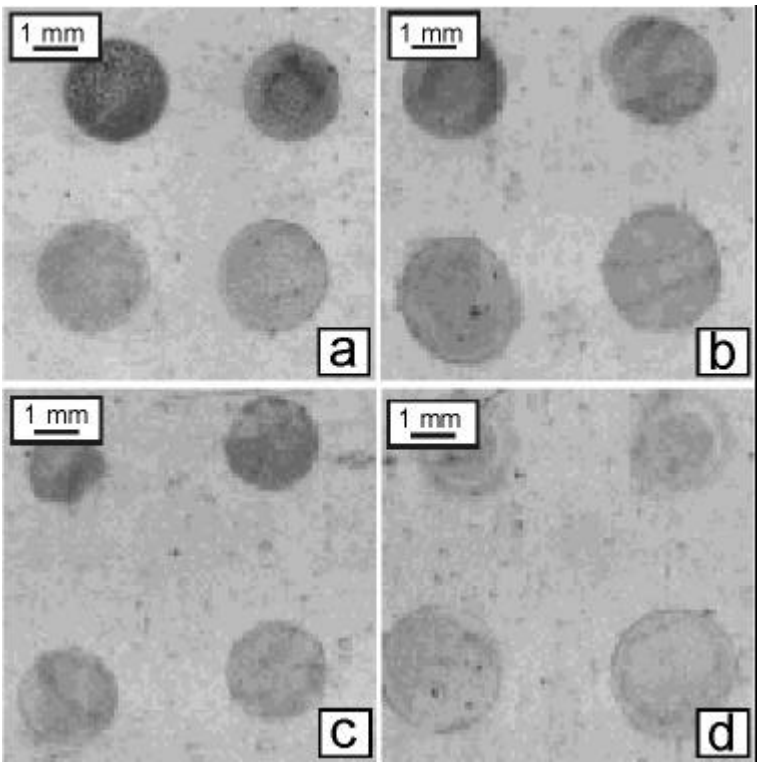


Figure 9.3.3: *Fluorescence images of oligonucleotides spotted onto different Star PEG coatings. The hydrogels were prepared by spincoating solutions in THF (a, b) and THF : water = 1:1 (c, d) onto aminosilanized glass slides in different concentrations to result in different layer thicknesses (a: 106 nm; b: 17.7 nm; c: 51 nm; d: 10.5 nm). Upper spots: fluorescently marked oligomer O_1. Lower spots: nonfluorescent oligomer O_2 hybridized with fluorescently labelled complementary oligomer O_3. Comparison of the fluorescence intensities shows a hybridization efficiency of 30% (a), 50 % (b,c) and 80% (d). The absolute fluorescence intensity values decrease from a) to d) to about one fourth.*

The water content of the aqueous THF solutions results in hydrolysis of isocyanate groups and reaction of the resulting amino groups with other isocyanate groups in the solution, so that di- and trimer formation takes place. This results in the fact that the Star PEG layers from aqueous THF contain less isocyanate groups but are better crosslinked than coatings prepared from THF with comparable layer thickness. Therefore, layers prepared from THF allow the immobilization of more DNA molecules what results in higher

fluorescence intensities, but due to the less crosslinked character compared to coatings prepared from aqueous solutions, the spotted oligonucleotides may sterically interact stronger with the coating itself what results in lower hybridization efficiencies. Independent from the layer preparation, layer thickness has an effect on the layer morphology. In thicker layers, the crosslinking procedure is slower, and the system has more time for self organization. This organization is driven by interfacial energy, since a surface segregation of the hydrophobic end groups of the Star PEG results in a low energy surface at air compared to the hydrophilic backbones[15]. Therefore, thicker layers display a higher number of functional groups at the interface what allows for a higher immobilization density of DNA. Nevertheless, the lower crosslinking of the thicker films reduces the hybridization efficiency in the same way as described above for the layers prepared from THF.

In order to demonstrate the compatibility of the Star PEG coatings with the method of choice for the preparation of microarrays, spotting, oligonucleotides were spotted onto Star PEG layers with a Omnigrid 2 spotting device using a SMP 2.5B (ArrayIt^{TM}) micro spotting pin. Based on the results obtained with the microcapillary spotting on differently prepared layers presented above, we chose Star PEG films from THF with a layer thickness of 5.6 nm as optimum. Since the layers are prepared from THF, many isocyanate groups are present for DNA immobilization. At the same time the layers are thin enough to ensure good hybridization efficiency. The result of the spotting and hybridization experiments are displayed in Figure 9.3.4. Although the hybridization efficiency of 35% is a good value for a 20mer oligonucleotide when compared to values reported in literature[19], it was less than expected from the results of the microcapillary spotting presented above. This value can still be optimized by variation of the molecular weight of the Star PEG system and thereby changing the length of the arms of each star shaped molecule and by optimizing the layer preparation step. In addition, a comparison to other systems used as microarray platforms is in preparation.

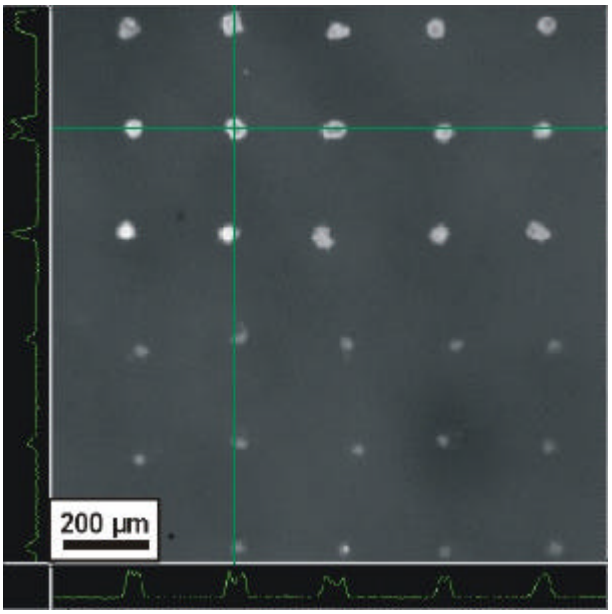


Figure 9.3.4: *Fluorescence microscope images of oligonucleotides spotted onto Star PEG layers prepared from THF with 5.6nm layer thickness. The brighter upper spots refer to spotted oligonucleotide O_1, the dimmer lower spots refer to oligonucleotide O_2 hybridized with oligonucleotide O_3. The hybridization efficiency was 35%.*

9.4 Conclusions

In this chapter it is prescribed how amino terminated single strained oligomers can be covalently attached to a NCO-terminated hydrogel layer. It was shown that these oligomers are still accessible and can be hybridized by a complementary strain. By labelling the oligomers with FITC the concept could be proofed by fluorescence microscopy. Further it was possible to created well ordered patterns of the oligomers either by spotting or micro contact printing with PDMS stamps. The efficency of the hybridization was shown to be correlated to the thickness and the preparation method of the hydrogel layers.

9.5 References

(1) Lander, E.S. *Nat. Genet. Suppl.* **1999**, *21*, 3.

(2) Lockhart, D.J.; Winterer, E. A. *Nature* **2000**, *405*, 827.

(3) Jain, K.K. *Science* **2001**, *294*, 621.

(4) Fan, J.B.; Chen, X.; Halushka, M.K.; Berno, A.; Huang, X.; Ryder, T.; Lipshutz, R. J.; Lockhart, D. J.; Chakravarti, A. *Genome Res.* **2000**, *10*, 853.

(5) Wang, D.G.; Fan, J.B.; Siao, C.J.; Berno, A.; Young, P.; Sapolsky, R.; Ghandour, G.; Perkins, N.; Winchester, E.; Spencer, J.; Kruglyak, L.; Stein, L.; Hsieh, H.L.; Topaloglou, T.; Hubbell, E.; Robinson, E.; Mittmann, M.; Morris, M.S.; Shen, N.; Kilburn, D.; Rioux, J.; Nussbaum, C.; Rozen, S.; Hudson, T.J.; Lipshutz, R.J.; Chee, M.; Lander, E.S. *Science* **1998**, *280*, 1077.

(6) Pardo, L.; Wilson, W.C., Jr.; Boland, T. *Langmuir* **2003**, *19*, 1462.

(7) Sorribas, H.; Padeste, C.; Tiefenauer, L. *Biomaterials* **2002**, *23*, 893.

(8) Lim, J.-H.; Ginger, D.S.; Lee, K.-B.; Heo, J.; Nam, J.-M.; Mirkin, C.A. *Angew. Chem.* **2003**, *115*, 2411.

(9) Kumar, A.; Whitesides, G.M. *Appl. Phys. Lett.* **1993**, *63*, 2002.

(10) Pirrung, M.C. *Angew. Chem. Int. Ed.* **2002**, *41*, 1276.

(11) Kusnezow, W.; Hoheisel, J.D. *J. Mol. Recognit.* **2003**, *16*, 165.

(12) Grainger, D.W.; Gong, P.; Lochhead, M.; Metzger, S.; Greef, C.; *European Cells and Materials* **2001**, *6*, 13.

(13) Benters, R.; Niemeyer, C.M.; Drutschmann, D.; Blohm, D.; Wöhrle, D. *Nucleic Acid Research* **2002**, *30(2)*, e10.

(14) Zhou, X.; Wu, L.; Zhou, J. *Langmuir* **2004**, *20*, 8877.

(15) Groll, J.; Ameringer, T.; Spatz, J.P.; Moeller, M.; *Langmuir*, **2005**, *21*, 1991.

(16) Groll, J.; Ademovic, Z.; Ameringer, T.; Klee, D.; Moeller, M.; *Biomacromolecules*, **2005**, *6*, 956.

(17) Groll, J.; Haubensak, W.; Ameringer, T.; Moeller, M.; *Langmuir* **2005**, *21*, 3076.

(18) Goetz, H.; Beginn, U.; Bartelink, C.F.; Gruenbauer, H.J.M.; Moeller, M.; *Macromol. Mater. Eng.* **2002**, *287*, 223.

(19) Demers, L.M.; Mirkin, C.A.; Mucic, R.C.; Reynolds III, R.A.; Letsinger, R.L.; Elghanian, R.; Viswanadham, G.; *Anal. Chem.* **2000**, *72*, 5535.

Summary

The main topic of this thesis was the functionalization of 6 arm starpolymers with different amino reactive end groups and homogeneous coatings of them on aminosilanized substrates. The starpolymers consisted of an ethylene oxide propylene oxide mixture randomly distributed in the ratio of 80:20. The hydroxyl terminated starpolymers were reacted with three different main compounds yielding either isocyanate, acrylate or vinyl sulfone endgroups.

1. To obtain isocyanate endgroups the starpolymers were reacted in bulk with an excess of Isophorone diisocyanate (IPDI). Reaction conditions were optimized to prevent chain extension and the coloring of the products. The products remained colorless by drying the starpolymer (containing less than 0.05% water) and double distilling the IPDI at reduced pressure. Chain extension ceased above an twelve fold molar excess of IPDI to the starpolymer. The products were purified by short path distillation. An oil bath temperature of 70 °C at a pressure below $1x10^{-2}$ turned out to be sufficient for a complete removal of the excess IPDI. After purification the product was stable under inert gas for several months.

2. Acrylic acid chloride (AAC) was used to obtain acrylic endgroups. The reaction was carried out in different solvents. Although an over 20 times molar excess of AAC was used, the conversion of the hydroxyl groups never exceeded 90 %. The addition of tertiary amines improved the conversion but had an unwanted coloring of the product as a side effect. Other HCl scavenging methods didn't show better results. The purified product had to be stored under inert gas in a fridge and to be used within several weeks.

3. Divinyl sulfone (DVS) was used to gain starpolymers with a vinyl sulfone endgroup. Although a high excess of DVS was applied, during the purification process

chain extension could not be prevented. The pure product was only obtained with a high loss in yield. Great care had to be taken during purification and storage to prevent polymerization of the product. Storage under inert gas and at temperatures below 4 °C was inevitable.

The upper two prepolymers were then coated onto silicon substrates. To covalently bind the prepolymers the silicon wafers were first functionalized via an aminosilanization. The prepolymers then were dissolved in THF and brought on the surface via spincoating. The resulting layers were examined by ellipsometry, optical microscopy and AFM.

The layer thickness could be well controlled by varying concentration of the solution and / or the rotation speed. AFM images and, for thick layers (> 50 nm), optical microscope images showed strong dewetted structures for all polymers. Whereas polymers with lower molecular weight (3000 g/mol) showed less dewetting than higher molecular weight products (12000 / 18000 g/mol).

To quench these phenomena the prepolymers were treated with amines either before or after the spincoating process. Chain extension before spincoating resulted in more or less stable polymer films. The dewetting could be quenched but still the films could be removed, except for a small layer, by rinsing the substrates with water. Exposing the films to an ammonia saturated atmosphere after spincoating had the same effect.

Applying different substrates like flat glass or high refractive index glass also had no significant influence on the layer properties.

In another series of experiments it was tried to build up films layer by layer. Therefore the aminosilanized substrates were dipped into IPDI-prepolymer solutions. The non reacted prepolymers were then rinsed off with dry THF. After reacting the isocyanate groups with water the next layer was attached.

In any case of the experiments it was possible to attach more than one layer. Every further layer could be washed away.

The same results were obtained in similar experiments with the acrylate terminated prepolymers.

As a first biological application aminoterminated 20mer oligonucleotides were attached to the prepolymer layers via spotting or microcontact printing. To these oligomers a fluorescein terminated complementary strain could be hybridized. The efficiency of hybridization was related to the layer thickness and the preparation method of the polymers and reached the value of about 30 %.

Zusammenfassung

Das grundlegende Thema dieser Arbeit bestand in der Funktionalisierung von 6 Arm Sternpolymeren mit unterschiedlichen, aminoreaktiven Endgruppen. Bei den Sternpolymeren handelte es sich dabei aus statistischen Copolymeren bestehend aus 80% Ethylenoxid und 20% Propylenoxid. Diese hydroxy terminierten Sternpolymere wurden mit drei verschiedenen Komponenten umgesetzt. Daraus resultierten Sternpolymere mit entweder Isocyanat-, Acrylat- oder Vinylsulfonendgruppen.

1. Um Isocyanat-Endgruppen zu erhalten wurden die Sternpolymere mit einem Überschuss von Isophorondiisocyanat umgesetzt. Dabei wurden die Reaktionsbedingungen soweit optimiert, dass Kettenverlängerungen der Prepolymere verhindert und deren unerwünschte Färbung eliminiert wurde. Dies wurde durch Trocknung der Sternpolymere (Wassergehalt < 0,05%) und zweifachem Destillieren des IPDI's erreicht. Kettenverlängerung konnte ab einem zwölffachem IPDI Überschuss unterbunden werden. Überschüssiges IPDI wurde durch Kurzwegdestillation wieder abgetrennt. Eine Ölbadtemperatur von 70 °C bei einem Druck von $1x10^{-2}$ mbar genügte um das IPDI vollständig abzutrennen. Unter Inertgas waren die Produkte nach der Reinigung über Monate stabil.

2. Acrylsäurechlorid wurde eingesetzt um die Sternpolymere mit Acrylendgruppen zu derivatisieren. Obwohl ein bis zu 20-facher Überschuss an Acrylsäurechlorid eingesetzt wurde überstieg die Umsetzung der Hydroxy-Endgruppen nie 90 %. Die Zugabe von tertiären Aminen verbesserte zwar die Umsetzung, allerdings hatte sie auch ein ungewollte Färbung der Produkte zur Folge. Andere Methoden um das entstehende HCl abzufangen brachten keine Verbesserung der Ergebnisse. Das Aufgereinigte Produkt musste unter Inertgas bei 4 °C aufbewahrt und innerhalb weniger Wochen verwendet werden.

3. Vinylsulfon terminierte Präpolymere wurden durch die Umsetzung der Sterne mit Divinylsulfon synthetisiert. Trotz eines bis zu 200 fachen Überschusses an DVS konnte die Kettenverlängerung der Sternpolymere nicht verhindert werden. Da bei der Aufreinigung das Produkt leicht vernetzte ist anzunehmen, dass auch die Kettenverlängerung während des Reinigungsprozesses stattfand. Zudem brachte die Aufreinigung auch einen hohen Verlust bei der Ausbeute mit sich. Trotz Lagerung unter Schutzgas bei 4 °C vernetzte das Produkt innerhalb weniger Tage.

Die zwei erstgenannten Präpolymere wurden anschließend auf Siliziumsubstrate aufgebracht. Diese wurden zuvor aminosilanisiert um eine stabile kovalente Anbindung der Präpolymere zu gewährleisten. Diese wurden in unterschiedlichen Konzentrationen in THF gelöst und mittels spincoating auf die Substrate aufgebracht. Die daraus resultierenden Schichten wurden mittels Ellipsometrie, dem optischem und dem Rasterkraftmikroskop (AFM) untersucht.

Die Schichtdicke konnte durch Variation der Konzentration und /oder der Rotationsgeschwindigkeit gut kontrolliert werden. AFM Aufnahmen und , bei Schichtdicken >50 nm, optische Mikroskopaufnahmen zeigten starke Entnetzungsstrukturen der Polymerfilme. Diese wirkten sich bei Präpolymeren mit niedrigeren Molekulargewichten (3000 g/mol) nicht so stark aus, wie bei den höher molekularen (12000 bzw. 18000 g/mol).

Um diesen Prozess zu Unterdrücken wurden die Präpolymere vor oder nach dem Spincoaten mit Aminen behandelt. Eine Kettenverlängerung vor dem Spincoaten konnte die anschließende Entnetzung unterdrücken. Allerdings konnten die nun homogenen Schichten bis auf eine dünne Monoschicht mit Wasser abgewaschen werden. Wurden die fertigen Filme in eine mit Ammoniak gesättigte Atmosphäre gebracht, erhielt man ähnliche Resultate.

Die Verwendung von Substarten wie Flachglas oder Glas mit hohem Brechungsindex ergab auch keine signifikante Veränderung in der Schichtbildung.

In einer weiteren Versuchsreihe wurde versucht die Präpolymere Schicht für Schicht auf die Substrate aufzubringen. Dabei wurden die aminosilanisierten Substrate

in eine Lösung der IPDI-Präpolymere in THF gedippt. Anschließend wurde das ungeundene Präpolymer wieder abgewaschen und die terminalen Isocyanatgruppen des gebundenen Polymers mit Wasser zu primären Aminen umgesetzt. Auf diese Schicht wurde nun versucht mit der gleichen Methode die nächste Schicht aufzubringen. Allerdings konnte mit dieser Methode nie mehr als eine Schicht aufgebracht werden. Jede weitere Schicht konnte vollständig abgewaschen werden. Ähnliche Resultate lieferten die Versuche mit den Acrylat-Sternen.

Als eine erste biologische Anwendung wurden aminoterminierte 20mer Oligomere auf die IPDI-Sterne mittels tüpfeln oder „microcontact printing“ aufgebracht. An diese Oligomere wurde der Fluorescein terminierte Gegenstrang hybridisiert. Die Effiktivität der Hybridisierung korrelierte dabei mit der Dicke und der Präparierungsart der Präpolymer Schichten. Alles in allem blieb sie geringer als 30 %.

Danksagung

Die vorliegende Arbeit wurde in der Zeit von März 2000 bis Mai 2005 in der Abteilung Organische Chemie III / Makromolekulare Chemie der Universität Ulm angefertigt.

An dieser Stelle möchte ich namentlich diejenigen Personen erwähnen, die wesentlich zu der Fertigstellung dieser Arbeit beigetragen haben und mich somit auf das Herzlichste bedanken.

Herrn Prof. Dr. Martin Möller möchte ich für die interessante Themenstellung, seine Anregungen und Diskussionsbereitschaft danken.

Frau Prof. Dr. Katharina Landfester für die Erlaubnis, auch nach ihrer Übernahme des Lehrstuhls der Makromolekularen Chemie, meine praktische Arbeit fertigzustellen.

Herrn Dr. Ulrich Ziener für die organisatorische Unterstützung und Diskussionsbereitschaft insbesondere nach dem Umzug der Abteilung nach Aachen.

Mike Wendel für die Durchführung von zahlreichen GPC Messungen. Elvira Kaltenecker für die Aufnahme von IR-Spektren. Marlies Fritz für TGA und DSC. Ulli Ziegler und weiteren OC1 Mitgliedern für die Aufnahme der NMR-Spektren. Dr. Schmidtberg und Markus Wunderlin für die Aufnahme der Massenspektren.

Marat Galliamov, Bernd Tartsch, Ahmed Mourran und Krystyna Albrecht für die Hilfestellungen bei AFM-Messungen.

Herrn Dr. Michael Hinz für die Synthese der Oligonucleotide sowie Frau Dr. Cludia Mourran bei den Tips für die Anwendung selbiger.

Herrn Dr. Camiel Bartelink für die problemlose Bereitstellung der Sternpolymere sowie den guten Tips zur deren Verarbeitung.

Herrn Dr. Haitao Rong für die gute Zusammenarbeit bei der Synthese der Sternpolymere.

Hans sowohl für die gute Vorarbeit und zahlreichen Hilfestellungen die zur Fertigstellung dieser Arbeit wesentlich beitrugen, als auch für die vielen Exkursionen außerhalb der Dienstzeit.

Jürgen (04) nicht nur für die hervorragende Zusammenarbeit während und nach unserer Zeit in Ulm sondern auch für die Teilung einiger meiner nicht immer ganz zeitgemäßen Hobbys.

Peter für die Einführung in knifflige Aufgaben auch neben der Arbeit

Silke, Claudia, Olli, Marcell, Stefan, Jürgen, Christoph, Reza, Manne, Vanessa, Marc, Michael, Sascha, Yayun und Blazej von der „alten OC3“, sowie Verena, Andi, Andreas, Daniel, Jean-Baptiste, Anna, Christoph, Myriam und Stefan von der „neuen OC3“ möchte ich stellvertretend für alle Mitglieder der OC3 für die gute Teamarbeit, die hervorragende Atmosphäre und die zahlreichen Abteilungsfeste zur Streßbewältigung danken.

Nicht zu vergessen sei das Volleyball-Team, das den unerwarteten Titelgewinn im Böttinger Turnier ermöglichte.

Meinen Eltern gilt mein besonderer Dank für die lebenslange Unterstützung in allen Bereichen, ohne die mein Studium sicherlich nicht möglich gewesen wäre.

Abschließend danke ich meiner Freundin und Verlobten dafür, daß sie mich stets motiviert und angetrieben hat. Ramona, ohne deine Liebe und gelegentlichen „Hiebe“ hätte die Fertigstellung dieser Arbeit wohl noch viel länger gedauert.

Lebenslauf

	Thomas Ameringer * 06. Juli 1974 in Ulm ledig
	Schulbildung
1980-1984	Grundschule Dornstadt
1984-1993	Schubart Gymnasium, Ulm;
	Naturwissenschaftliches Profil
Mai 1993	Allgemeine Hochschulreife
	Notendurchschnitt: 3,0
Okt. 1993 - April 1994	Ableistung des Wehrdienstes; Grundwehrdienst im 3. Transportbataillon in Günzburg Weitere Stationierung im 2. Transportbataillon in Dornstadt
	Hochschulstudium
1994-1999	Studium der Chemie an der Universität Ulm Schwerpunkte: Makromolekulare Chemie, Organische Chemie, bioorganische Chemie
Mai 1999	**Diplom** (gut)
Juni 1999– Februar 2000	Diplomarbeit bei Prof. Dr. M. Möller in der Abteilung Organische Chemie III/Makromolekulare Chemie, Thema: *Photoinduziertes Grafting von Polymeren auf nanostrukturierten Goldoberflächen*
seit März 2000	**Promotion** bei Prof. Dr. M. Möller in der Abteilung Organische Chemie III/Makromolekulare Chemie der Universität Ulm, Thema: *Synthesis of biocompatible ultrathin hydrogel layers on silicon substrates*
	Berufstätigkeit
März 2000 – Juni 2004	Wissenschaftlicher Mitarbeiter in der Abteilung Organische Chemie III/Makromolekulare Chemie der Universität Ulm
Seit August 2005	Wissenschaftlicher Mitarbeiter am Institut für Mikrosystemtechnik (IMTEK) in Freiburg

Veröffentlichungen

J. Groll, E. Amirgoulova, T. Ameringer, C. D. Heyes, C. Röcker, G. U. Nienhaus and M. Möller, *Biofunctionalized, Ultrathin Coatings of Crosslinked Star-shaped Poly(ethylene oxide) allow reversible folding of immobilized proteins*, JACS 2004, 126, 4234-4239.

E. V. Amirgoulova, J. Groll, C. D. Heyes, T. Ameringer, C. Röcker, Martin Möller and G. U. Nienhaus, *Biofunctionalized polymer surfaces exhibiting minimal interaction towards immobilized proteins*, ChemPhysChem 2004, 5, 552-555.

J. Groll, T. Ameringer, J.P. Spatz, M. Moeller; *Biocompatible Ultrathin Coatings from Isocyanate Terminated Star Prepolymers*, Langmuir 2004, 20, 7439.

J. Groll, T. Ameringer, J.P. Spatz, M. Moeller; *Ultrathin Coatings from Isocyanate Terminated Star PEG Prepolymers: Layer formation and characterization,* Langmuir 2005, 21, 1991-1999.

J. Groll, W. Haubensak, T. Ameringer, M. Möller; *Ultrathin Coatings from Isocyanate Terminated Star PEG Prepolymers: Patterning of Proteins on the Layers*, Langmuir 2005, 21, 3076-3083.

J. Groll, Z. Ademovic, T. Ameringer, D. Klee, M. Moeller; *Comparison of Coatings from Reactive Star Shaped PEG-stat-PPG Prepolymers and Grafted Linear PEG for Biological and Medical Applications,* Biomacromolecules 2005, 6, 956-962.

J. Groll, J. Fiedler, E. Engelhardt, T. Ameringer, S. Tugulu, H.-A. Klok, R. E. Brenner, M. Moeller, *A Novel PEG based Surface Coating for Specific Cell Adhesion*, submitted.
T. Ameringer, J. Groll, M. Hinz, C. Mourran, H. Seliger, M. Moeller; *Ultrathin Functional Star PEG Coatings for DNA Microarrays*, Biomacromolecules, accepted

T. Ameringer, C. Mourran, U. Begin, M. Möller; *Nanocomposits*, Literaturübersicht für Daimler Chrysler; 2001

Patente

Prof. M. Moeller, T. Ameringer, Dr. H. Rong, Dr. S. Levi; S*ternförmige Präpolymere für die bakteriostatische Ausrüstung von Oberflächen* **DE 10330560.2**.

Prof. M. Möller, J. Groll, T. Ameringer, Dr. C. Mourran, Dr. H. Rong, Dr. S. Levi; Arrays immobilisierter Biomoleküle auf Hydrogel-bildenden Oberflächen, deren Herstellung und deren Verwendung **DE 10332849.1**.

Poster

Feb. 2002: *Ultrathin Bioactive Hydrogel Layers*, T. Ameringer, J. Groll, H. Rong, C. Mourran, M. Möller; *Makromolekulares Kolloquium*, Februar 2002, Freiburg.

Hobbys

Sport, Jugendarbeit, Reisen, Puzzeln, Rätsel des SZ-Magazins