THIOL-ENE BASED MICROFLUIDIC DEVICES FOR BIOANALYSIS

Sari Tähkä

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Pharmacy of the University of Helsinki, for public examination in Auditorium 2041, Viikki Biocenter 2, on January 18th 2019, at 12 o'clock noon.

Helsinki 2019
Miniaturized microfluidic systems hold huge potential for serving as biological and chemical research platforms. Miniaturization enables the use of small sample volumes and reagents, and microtechnology permits the integration of multiple operations on the same device, resulting in high-throughput analysis devices. Despite the substantial progress made in the past 30 years, the materials available for microfabrication still face certain challenges in terms of the assay performance, bonding, and chemical stability. Thiol-ene polymers have been proposed as new materials for microfluidic systems due to the possibilities for tuning their mechanical and surface properties and for processing by a variety of polymer microfabrication methods. Recent studies on the use of thiol-enes for fabrication of microfluidic devices have shown that these qualities are advantageous for both bonding as well facilitating for a variety of surface treatments.

The overall goal of this thesis was to study the feasibility of thiol-enes for fabrication of analytical microdevices by addressing the above-mentioned challenges. Another main aim of the work was to develop thiol-ene photopolymerization methods in the absence of the photoinitiator and to study its impact on the bulk and surface properties of crosslinked thiol-enes. To facilitate the microchip processing with standard laboratory instruments (a so-called non-cleanroom approach), the microfluidic chips were fabricated via UV replica molding. Three different types of thiol-ene compositions, including stoichiometric and allyl- or thiol-rich off-stoichiometric compositions, were compared with each other. The native surface and bulk properties were analyzed with a view to enzyme immobilization for proteolytic microreactors, to microchip electrophoresis, and to on-chip electrospray ionization mass spectrometry. The off-stoichiometric thiol- and allyl-rich compositions were mainly exploited to facilitate enzyme immobilization and tuning of the surface wettability, respectively, while stoichiometric compositions were mainly exploited to electrophoresis and mass spectrometry.

In this thesis, efficient enzyme immobilization was achieved in a straightforward way via the use of thiol-rich surfaces and gold nanoparticles facilitating enzyme binding via thiol-gold chemistry. Incorporation of a micropillar array provided a simple way to increase the surface area of the microreactor to maximize the number of thiol-gold interactions. In electrophoresis, stable and relatively high electroosmotic flow was obtained with all compositions tested, but the native allyl-rich thiol-ene composition was shown to be the most suitable material for elimination of nonspecific interactions. Owing to the inherently good solvent stability of thiol-enes, microchip electrophoresis separations could be conducted even in non-
aqueous media with promise of enhanced resolving power in peptide separations. The inherent optical clarity of thiol-enes and transparency even in the near UV range also allowed sensitive optical detection by laser-induced fluorescence. For mass spectrometry applications, two different on-chip electrospray emitter designs were fabricated from thiol-enes, namely an open-to-air (lidless) micropillar array chip and an integrated electrophoresis-electrospray chip. With optimized polymer composition, stable electrospray and good chip-to-chip reproducibility was achieved. To facilitate analysis of even 100 % aqueous biological solutions by using the lidless micropillar array chip, an allyl-rich composition was used and modified with polyethylene glycol to fully eliminate nonspecific surface interactions associated with complex biological samples.

The possibility to prepare microdevices with identical surface chemistry all over the channel is known to be important to the efficiency of electrophoretic separations and surface modifications. The most substantial benefit achieved via a photoinitiator-free curing of thiol-enes was the possibility to bond, not only surfaces with opposite excess of thiols and allyls, but also two alike surfaces. Even if cured without the photoinitiator, all thiol-ene microdevices produced in this study showed good structure fidelity. For replication of high aspect ratio and high resolution structures such as micropillars and on-chip electrospray emitter tips, respectively, the stiffness of the chosen polymer composition was also shown to be critical. The bulk properties were relatively similar between compositions cured with and without the photoinitiator. However, in the absence of the photoinitiator, the curing time (exposure dose) was shown to have a notable impact on the surface properties.

In all, this thesis work broadens our understanding of the material and surface properties of thiol-enes with a view to their use in mass spectrometry based bioanalysis as well as in other fields of microfluidics. The long-term impact of the work is envisioned to contribute to the adaptation of micro total analysis systems in routine chemical analysis by providing new, user-friendly approaches to low-cost microfabrication of customized devices. In most cases, the analytical setups developed in this work were the first of their kind in the field, laying the basis for other similar setups, which have since been reported in the literature.
This thesis work was carried out in the Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki during the years 2014-2018. I want to acknowledge the funders of the work: the Doctoral Programme of Chemistry and Molecular Sciences (CHEMS) and the European Research Council (grant no. 311705).

I want to thank my supervisors Docent Tiina Sikanen and Docent Susanne Wiedmer. I am grateful to Tiina for introducing me to the interesting field of microfluidics and giving me the opportunity to work under her supervision in the Chemical Microsystems research group. I am grateful for all the guidance she has given me during these years. I want to thank Susanne for all the support and valuable comments she has given me in research, particularly in analytical chemistry. I want to acknowledge the reviewers of this thesis, Professor Jörg Kutter (University of Copenhagen) and Professor Wouter van der Wijngaart (KTH Royal Institute of Technology) for the careful revision of the thesis, which led to improvement of the work.

Great thanks belong to all my coauthors for the collaboration and their contributions. I wish to thank Dr. Ville Jokinen, Dr. Anand Tatikonda, and Prof. Sami Fransson from the Department of Material Science and Engineering, Aalto University. I wish to thank people from the Division of Pharmaceutical Chemistry and Technology; Lauri Urvas, Maria-Elisa Nordberg, and Meeri Kanerva of their help in the laboratory, as well as Ashkan Bonabi, Dr. Katriina Lipponen, Riccardo Provenzani, Prof. Tapio Kotiaho, and Prof. Risto Kostiainen for their useful advice and comments. Special thanks belong to Ashkan and Ville for their work in cleanroom. I am also grateful for Dr. Jawad Sarfraz and Prof. Jouko Peltonen from the Laboratory of Physical Chemistry, Åbo Akademi University, for the help with AFM and XPS analyses.

I want to thank all the former and present personnel at the Division of Pharmaceutical Chemistry for the great work atmosphere! Especially, I warmly thank my “Chemisys family” for all the numerable fun moments, help, and encouragement!

My greatest gratitude belong to my dear friends and family. Especially Jenna, Mea, Anna, Tia, and the “Kemistit”, thank you for the happy, joyful and crazy moments. Without you this would have been much harder. I wish to thank my family and grandparents for their encouragement; you have always believed in me. Finally, I wish to express my gratitude to Peltsi for his love, understanding, and endless support on the way, thank you for standing beside me.

Sari Tähkä
Vantaa, December 2018
# CONTENTS

Abstract............................................................................................................... 2
Preface ............................................................................................................. 4
Contents........................................................................................................... 5
List of original publications ......................................................................... 7
Abbreviations ............................................................................................... 9

1  Introduction............................................................................................... 11

2  Review of the literature .......................................................................... 13
  2.1  Microfluidic total analysis systems...................................................... 13
      2.1.1  Common classes of microfabrication materials ............................ 14
      2.1.2  On-chip sample preparation....................................................... 17
      2.1.3  Microchip capillary electrophoresis .......................................... 19
      2.1.4  Detection of microfluidic separation systems .............................. 22
  2.2  Thiol-ene click chemistry for microfabrication...................................... 24
      2.2.1  Reaction mechanism................................................................. 24
      2.2.2  Off-stoichiometric thiol-enes................................................... 25
      2.2.3  Thiol-ene microfluidic devices in analytical applications .......... 28

3  Aims of the study .................................................................................... 31

4  Experimental ............................................................................................ 32
  4.1  Chemicals and materials..................................................................... 32
  4.2  Microchip fabrication materials and nomenclature ............................ 33
  4.3  Instrumentation.................................................................................... 34
  4.4  Microchip fabrication ......................................................................... 36
  4.5  Characterization of material properties ............................................ 38
      4.5.1  Bulk properties........................................................................... 38
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5.2</td>
<td>Surface properties</td>
<td>39</td>
</tr>
<tr>
<td>4.6</td>
<td>Functionalizations</td>
<td>40</td>
</tr>
<tr>
<td>4.7</td>
<td>Microchip designs used in the study</td>
<td>41</td>
</tr>
<tr>
<td>4.7.1</td>
<td>On-chip enzymatic digestion (I)</td>
<td>42</td>
</tr>
<tr>
<td>4.7.2</td>
<td>Microchip electrophoresis (II, III)</td>
<td>42</td>
</tr>
<tr>
<td>4.7.3</td>
<td>Fluorescence detection (II)</td>
<td>42</td>
</tr>
<tr>
<td>4.7.4</td>
<td>On-chip electrospray ionization emitters (III, IV)</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>Results and discussion</td>
<td>44</td>
</tr>
<tr>
<td>5.1</td>
<td>Thiol-ene microfabrication</td>
<td>44</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Feature resolution of thiol-enes cured without the photoinitiator</td>
<td>44</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Effect of photoinitiator and monomer composition on bulk properties</td>
<td>46</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Effect of photoinitiator on bonding</td>
<td>49</td>
</tr>
<tr>
<td>5.2</td>
<td>Thiol-enes’ native surface properties</td>
<td>50</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Wettability</td>
<td>50</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Functional groups</td>
<td>51</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Surface charge</td>
<td>51</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Storage stability</td>
<td>53</td>
</tr>
<tr>
<td>5.3</td>
<td>Thiol-ene microdevices for peptide research</td>
<td>54</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Enzymatic digestion (I)</td>
<td>54</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Separation of peptides in aqueous and non-aqueous modes (II, III)</td>
<td>56</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Mass spectrometry interfacing on-chip (III, IV)</td>
<td>60</td>
</tr>
<tr>
<td>5.4</td>
<td>Summary and critical review of the work</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>Conclusion</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>References</td>
<td>68</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This doctoral thesis is based on the following publications referred to in the text by their Roman numerals (I-IV):


These publications have been reprinted with the permission of their copyright holders. In addition, some unpublished material is presented.
Author’s contribution to the above publications:

**Publication I**
The microchip fabrication was carried out by the author, excluding the SU-8 cleanroom master fabrication, which was carried out by Dr. Ville Jokinen. The analytical research plan and the experimental work were executed by the author with contributions from others; titration of the surface thiols was carried out by Lauri Urvas, surface analyses by XPS and AFM by Dr. Jawad Sarfraz, and contact angle goniometry measurements by Dr. Ville Jokinen. The publication was written by the author with contributions from coauthors.

**Publication II**
The microchip fabrication was carried out by the author, excluding the SU-8 cleanroom master fabrication, which was carried out by Ashkan Bonabi. The analytical research plan and the experimental work were executed by the author with contributions from others; determination of the electroosmotic flow was carried out by Meeri Kanerva and detection and quantitation limits and MCE-LIF analyses (rhodamines) by Maria-Elisa Nordberg. The publication was written by the author with contributions from coauthors.

**Publication III**
The microchip fabrication was carried out by the author, excluding the SU-8 cleanroom master fabrication, which was carried out by Ashkan Bonabi. The analytical research plan and the experimental work were executed by the author. The publication was written by the author with contributions from coauthors.

**Publication IV**
The microchip fabrication (thiol-ene replication) method was developed jointly by the author and Dr. Katriina Lipponen, apart from the silicon master fabrication, which was carried out by Dr. Anand Tatikonda. The chip characterization by the scanning electron microscopy was conducted by the author. The publication was written by Dr. Katriina Lipponen with contributions from the author and other coauthors.
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFM</td>
<td>atomic force microscope</td>
</tr>
<tr>
<td>BABE A</td>
<td>bisphenol A-based epoxy acrylate</td>
</tr>
<tr>
<td>BGE</td>
<td>background electrolyte</td>
</tr>
<tr>
<td>CCD</td>
<td>charged coupled device</td>
</tr>
<tr>
<td>CE</td>
<td>capillary electrophoresis</td>
</tr>
<tr>
<td>CHT</td>
<td>chymotrypsin</td>
</tr>
<tr>
<td>COC</td>
<td>cyclic olefin copolymer</td>
</tr>
<tr>
<td>COP</td>
<td>cyclic olefin polymer</td>
</tr>
<tr>
<td>EC</td>
<td>electrochemical</td>
</tr>
<tr>
<td>EOF</td>
<td>electroosmotic flow</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionization</td>
</tr>
<tr>
<td>FAS</td>
<td>field-amplified stacking</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>Flu</td>
<td>fluorescein</td>
</tr>
<tr>
<td>FDA</td>
<td>fluorescein diacetate</td>
</tr>
<tr>
<td>FDB</td>
<td>fluorescein dibutyrate</td>
</tr>
<tr>
<td>FDL</td>
<td>fluorescein dilaurate</td>
</tr>
<tr>
<td>GNP</td>
<td>gold nanoparticle</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>IEF</td>
<td>isoelectric focusing</td>
</tr>
<tr>
<td>IMER</td>
<td>immobilized enzyme reactor</td>
</tr>
<tr>
<td>ITP</td>
<td>isotachophoresis</td>
</tr>
<tr>
<td>LED</td>
<td>light-emitting diode</td>
</tr>
<tr>
<td>LIF</td>
<td>laser-induced fluorescence</td>
</tr>
<tr>
<td>LOC</td>
<td>lab on a chip</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>limit of quantification</td>
</tr>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption ionization</td>
</tr>
<tr>
<td>MCE</td>
<td>microchip capillary electrophoresis</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>NOA</td>
<td>Norland Optical Adhesives, Norland Products, Inc.</td>
</tr>
<tr>
<td>OSTE</td>
<td>off-stoichiometric thiol-ene</td>
</tr>
<tr>
<td>OSTE+</td>
<td>off-stoichiometric dual-cure thiol-ene-epoxy system</td>
</tr>
<tr>
<td>PC</td>
<td>polycarbonate</td>
</tr>
<tr>
<td>PDMS</td>
<td>poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PET</td>
<td>poly(ethylene terephthalate) toner</td>
</tr>
<tr>
<td>PI</td>
<td>photoinitiator</td>
</tr>
<tr>
<td>PMMA</td>
<td>poly(methylmethacrylate)</td>
</tr>
</tbody>
</table>
Abbreviations

PMT photomultiplier tube
PS polystyrene
PU polyurethane
SEM scanning electron microscope
SPE solid-phase extraction
Tg glass transition temperature
TPE thermoset polyester
UV ultraviolet
WCA water contact angle
XPS X-ray photoelectron spectroscopy
μTAS micro total analysis system
μPESI micropillar electrospray ionization
This chapter gives a brief introduction to the design and implementation of microfluidic total analysis systems (μTAS). Since its introduction in the 1990s [1], the field of microfluidic total analysis systems (μTAS) and miniaturization of bioanalytical assays has grown enormously. The first devices were aimed at miniaturized chromatographic [2] and electrophoretic [3-5] separations with a view to building a single device incorporating all of the analytical functions; thus, the concept was termed μTAS [6]. Various miniaturized devices and platforms covered under the concept of 'lab(orary) on a chip' (LOC) have since been introduced to chemical, biological, and medical fields, with applications ranging from drug discovery [7], point-of-care diagnostics [8], ‘omics’ [9, 10], to environmental analysis [11]. The high interest in and potential of microfluidic devices in various fields is related to the characteristics of miniaturization. As microfluidics deals with fluid and particle handling on a microscale, the small size leads to enhanced surface area-to-volume ratio and decreased analysis time. This means increased separation performance and throughput compared with bench-top instruments [12]. Miniaturization of the device size also enables reduced sample and reagent consumption, and portability, allowing the on-site testing needed, for example, for healthcare point-of-care devices [13] or environmental water analysis [14]. One of the major advantages of the microfluidic system over macroscale technologies, especially in analytical devices, is their good feasibility for integration and parallelization of multiple unit operations on the same device [15].

Despite the above-mentioned benefits, certain challenges remain with the fabrication and performance of the present-day microdevices. These are related to lack of reproducibility of the chip performance [16], the overall compatibility of materials for component integration [17] and the chip fabrication cost and/or mass production possibilities [18]. Most of these challenges are related to the properties of the materials available for microfabrication. Furthermore, with a view to integration of multiple chemical analysis steps on a single chip, the varying material requirements of each analytical unit must be considered.

For analytical systems, precise liquid dosing facilitated by good structure fidelity, compatibility with background electrolytes and pH, inert and stable surface and bulk properties are crucial for quantitative detection and repeatable sample loading and separation [19, 20]. In many cases, surface treatment is required after device fabrication to avoid biofouling and to enhance wettability [21, 22] or to be able to immobilize biomolecules on surfaces [23], and thus good control over surface properties is important. For
these reasons, glass and silicon have been the main materials of choice. However, their processing and bonding are relatively complex and require expensive cleanroom facilities. Polymers, in particular polydimethylsiloxane and a range of thermoplastics, have been used lower cost alternatives to glass and silicon [18]. Although polymers enable large volume fabrication and tuning of physico-chemical properties, many of them have issues with the stability and inertness of the surface chemistry (or the lack of appropriate functional groups) [17]. As none of the current materials can fulfill all requirements, compromises must be made between ease of chip processing and the assay performance. This has given rise to active materials research in quest of controlled surface chemistry and low-cost fabrication methods.

Thiol-ene thermosets have recently emerged as a promising alternative to fabrication of microfluidic devices with promise of resolving the common challenges associated with other materials [24]. The advantages of thiol-enes over other common microfabrication materials is the ability to tune the mechanical properties of the polymer ‘on-demand’ for each application simply by adjusting the monomer ratio. As the result, thiol-enes incorporate free thiol or allyl functional groups on the surface, which facilitate further (bio)coupling reactions via click chemistry reactions [25, 26]. Thus, thiol-enes have been increasingly used in fabrication of different kind of biological platforms, but before this thesis work, only a few studies have reported on implementation of thiol-ene based analytical microdevices.

In this thesis work, the suitability of thiol-enes for UV replica molding of bioanalytical microdevices was evaluated. Thiol-enes’ feasibility was demonstrated for a range of bioanalytical applications including an immobilized enzyme microreactor (IMER) (I), microchip electrophoresis (MCE) (II, III), and on-chip electrospray ionization mass spectrometric (ESI-MS) detection (III, IV). The performance of the IMER was evaluated by chymotrypsin-catalyzed peptide hydrolysis by ESI-MS detection (I). MCE of peptides was performed in aqueous and non-aqueous conditions using laser-induced fluorescence (LIF) (II) and ESI-MS detection (III), respectively. The performance of on-chip ESI chips was studied by using small drug molecule, peptide, and protein standards (III, IV). For each application, different thiol-ene compositions were characterized in terms of their bulk (stiffness, optical transparency) and surface properties (electroosmotic flow and surface chemistry) as well their suitability for replica-molding of the desired structure.
2 REVIEW OF THE LITERATURE

This chapter gives a brief overview to the common microfabrication materials used for implementation of microfluidic total analysis systems followed by discussion of the key technical aspects of their main functional units, i.e., sample preparation, separation, and detection (2.1). In addition, the reaction mechanisms of UV initiated thiol-ene click chemistry will be discussed followed by the benefits achieved via the use of off-stoichiometric thiol-ene compositions and an overview of the use of thiol-enes in microfluidic applications (2.2).

2.1 Microfluidic total analysis systems

Analytical chemical analysis comprises multiple steps, starting with sample preparation, followed by introduction (injection) and separation, and finally detection and data handling (Figure 1). An ideal μTAS system includes all of the sample pretreatment (protein digestion, labeling, and purification), separation/fluid transport, and detection steps on a single chip [27]. Many of these processes have been realized on-chip, and in recent years an increasing number of μTAS devices integrating all of the steps have been presented [28]. For protein and peptide analysis by miniaturized systems, the first step of sample preparation is often enzymatic digestion into smaller units. The main separation method for analytes in biological samples is microchip capillary electrophoresis (MCE) [29], although all macroscale separation systems can be miniaturized. For detecting the sample, optical and especially mass spectrometric detections are often preferred for bioanalysis. From a materials’ point of view, all of the steps have their own requirements for the chip, which determine the material (and fabrication) selection.

![Generalized schematic workflow of production of miniaturized devices. The first step is determining the requirements of the applications and related selection of material(s) and fabrication methods. This is followed by integration of analytical units suitable for fulfilling the application task.](image)

Figure 1. Generalized schematic workflow of production of miniaturized devices. The first step is determining the requirements of the applications and related selection of material(s) and fabrication methods. This is followed by integration of analytical units suitable for fulfilling the application task.
2.1.1 Common classes of microfabrication materials

Nowadays, multiple different materials exist for microsystem fabrication, all with special features and manufacturing methods as well as specific benefits and drawbacks. Table 1 presents common materials and their properties.

The first microfluidic analytical devices were made of silicon and glass by standard lithography and etching techniques adopted from the semiconductor industry [30]. Both materials have good thermal and solvent stability, have inherent surface functional groups modifiable via silane chemistry, and are extremely hard. In addition, glass is transparent and electrically insulating and has been the material of choice for MCE [31]. In contrast, silicon is opaque and cannot be used with high voltages, but it is well-suited for liquid chromatographic applications [32]. A common method to process glass and silicon is first patterning a mask by lithography, followed by wet-etching (often used for glass [33]) or plasma dry-etching (deep dry etching often used for silicon [34]) to remove the bulk material to obtain the microchannels. Although accurate channel quality [30] can be obtained and both glass and silicon have suitable material properties for analytical applications, the fabrication processes are relatively complex and require expensive cleanroom facilities. Recently, faster and less expensive methods to fabricate glass electrophoretic chips based on microcontact electrochemical etching [35] or laser ablation [36] have been proposed. Polymers are suitable low-cost alternatives to glass and silicon, and today they are the most used substrates due to their broad range of chemical, mechanical, optical, and electrical properties [18]. Based on physical (e.g. glass transition temperature, Tg) and material properties, polymers can be divided into thermoplastics, thermosets, and elastomers. Elastomeric poly(dimethylsiloxane) is the gold standard material for microdevices and is thus discussed separately.

Thermoplastics

Common thermoplastics used for analytical microchips are poly(methylmethacrylate) (PMMA), poly(carbonate) (PC) poly(styrene) (PS) poly(ethylene terephthalate) toner (PET), and cyclic olefin copolymer (COC). Most of the thermoplastics are already used in industry and have thus well-characterized properties. In general, thermoplastics are mechanically strong at room temperature, having high Tg values in the range of 70°C for PET to 150°C for PC and COC [37]. Many have fair to good resistance to alcohols and acid and base solutions, but not against organic solvent (except PET [38]), restricting their use to other than aqueous solutions. A common problem for thermoplastics is the inertness of the surface and native hydrophobicity, and for use in analytical purposes surface treatments are required. Thermoplastics are often processed by thermoforming, i.e. injection molding or hot embossing [39]. Both injection molding and hot embossing are based on heating the polymer above Tg value, after which the prepolymer is either injected into a
molding machine or pressed with a heated mold, respectively [40]. These techniques are designed for large-scale industrial processing and allow fast mass production with high accuracy. Separation chips with good performance have been fabricated by thermoforming of PMMA [41-44], PC [45, 46], PS [47, 48], and COC [49, 50]. The mass production enables lower cost per microchip, which is needed for disposable devices. PET [51, 52] and PMMA [53], for example, can be processed also by direct machining such as low-cost laser ablation based on material removal without the need for masks or molds [54]. However, this technique is relatively slow and has the drawbacks of poor microchannel resolution and rough surfaces, which affect, for example, the EOF stability, and thus, the overall analytical performance and reproducibility [52].

**Thermosets**
Thermoset polymers cross-link irreversibly into a 3-dimensional network when the prepolymer (called resin) is exposed to heat or UV light [18], and thermosets are often processed by replication or lithography methods. A well-known thermoset material is epoxy photoresist SU-8. It is widely used as a master substrate for other polymers [55], but due to high mechanical, thermal, and chemical stability similar to that for glass and silicon, it has also been used for free-standing separation devices [56, 57]. SU-8 processing relies, however, on UV lithography and thus on cleanroom instruments. Also poly(dimethylsiloxane) (PDMS) is a thermoset, but due to its different polymeric properties, it is explained in the next section. To find complementary material to PDMS with simple replication but with better mechanical properties, other thermosets such as polyurethane (PU) [58] and thermoset polyester (TPE) [59, 60] have been proposed for microchip devices. Also another novel thermoset bisphenol A epoxy acrylate (BABEA) [61] has recently been introduced as a microchip electrophoresis substrate, although the fabrication is based on UV lithography similar to SU-8. These thermosets have similar mechanical properties (Young’s modulus values) and surface hydrophobicity as thermoplastics (values ranging from 61°C for TPE [62] to 101°C for PU [19], but in general better organic solvent compatibility and thermostability. The BABEA copolymer was also intrinsically hydrophilic, with water contact angle (WCA) of 43° similar to glass.

**Polydimethylsiloxane**
PDMS polymer has remained the main low-cost substrate material for microfluidic devices from its introduction in the 1990s [63]. PDMS is composed of a physically entangled polymer network, with an elastic and reverse stretch when external force is applied. The elasticity (Young’s modulus of 0.0005 GPa) of PDMS makes it highly desirable for flexible on-chip pneumatic valves or pumps [64]. The elasticity also produces a gas
permeability not obtained with other rigid polymers, glass, or silicon, and hence, PDMS has been the main material for cell applications [65]. The elasticity causes, however, monomer leaching [66] and together with high hydrophobicity (WCA of 100°) PDMS is vulnerable to, for example, organic solvent [67], protein [68] or small molecule [69] absorption, limiting its use in native form. To serve as an analytical chip substrate, PDMS requires surface treatment [70]. The reason for ongoing popularity of PDMS is the low-cost casting method suitable for simple chip prototyping, and PDMS is often the material utilized in academic research [71]. In casting, also called replica molding or soft lithography, a prepolymer mixture is cast against a master, followed by production of negative replica. Replica-molding allows production of tens to hundreds of replicas, and the quality of the final device is dependent on master quality [18]. Although suitable for rapid prototyping, PDMS is not preferred for large-scale manufacturing due to manual molding as well as hours of thermal curing.

Table 1. Summary of different material properties used for microfluidic devices

<table>
<thead>
<tr>
<th>Property</th>
<th>Silicon/glass</th>
<th>Thermoplastics</th>
<th>Thermosets</th>
<th>PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young’s modulus (GPa)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130–180/50–90</td>
<td>1.4–4.1</td>
<td>2.0–2.7</td>
<td>~0.0005</td>
</tr>
<tr>
<td>Microfabrication method&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lithography, etching</td>
<td>Thermoforming</td>
<td>Casting, photolithography</td>
<td>Casting</td>
</tr>
<tr>
<td>Smallest channel dimension&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;100 nm</td>
<td>~100 nm</td>
<td>&lt;100 nm</td>
<td>&lt;1 μm</td>
</tr>
<tr>
<td>Thermostatbility&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Very high</td>
<td>Medium to high</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Organic solvent resistance&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Very high</td>
<td>Medium to high</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Acid/base resistance&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Very high</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Hydrophobicity&lt;sup&gt;a&lt;/sup&gt;*</td>
<td>Hydrophilic</td>
<td>Hydrophobic</td>
<td>Hydrophilic-hydrophobic</td>
<td>Hydrophobic</td>
</tr>
<tr>
<td>Optical transparency&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Opaque/good</td>
<td>Medium to high</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Material price&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Medium to high</td>
<td>Low to medium</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>EOF measured by current monitoring method (× 10⁻⁴ cm² V⁻¹ s⁻¹)</td>
<td>4.21 for glass (5.0 mM Tris–HCl, pH 9.2) [19]</td>
<td>2.07 for PMMA and 1.54 for PS (5.0 mM Tris–HCl pH 9.2) [19] 1.1 for oxidized COC (20 mM phosphate, pH 7.0) [70]</td>
<td>2.6 for PU(20 mM potassium-phosphate buffer pH 7.0) [71] 5.8 for SU-8 (10 mM sodium phosphate buffer pH 7.0) [54]</td>
<td>4.1 for native PDMS and 7.8 for PDMS oxidized (20 mM TES, pH 7.0) [72]</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data obtained from reference [69].  <sup>b</sup>Data obtained from reference [17]  
<sup>a</sup>Here, the classification of material to hydrophobic or hydrophilic is based on water contact angle (WCA) on solid surface; if the WCA is under 90° the material is considered hydrophilic, if over 90° the material is hydrophobic.
**Bonding possibilities of microfluidic devices**
For most analytical separations, the device needs to be sealed to obtain fluid movement inside a chip. Although hybrid systems consisting of two different materials are commonly used, for analytical purposes the different top and bottom surfaces may lead to band broadening, and hence, reduced performance [72]. Besides surface modifications, bonding is often the problematic step in device production. Although glass and silicon have reactive surface groups, their mechanical hardness requires bonding with cleanroom conditions and tools and with high temperatures (e.g. 500°C for fusion bonding). By contrast, thermoplastics lack surface functional groups and the bonding of two sheets requires additive layers or surface modifications to obtain high bonding strength. Common methods to bond thermoplastics are thermal fusion bonding, solvent- or adhesive-based bonding, and bonding after surface modifications [73]. Thermal bonding involves heating the sealable layers near or above Tg, and with applied pressure the layers are connected. Strong bonding with lower temperatures can be obtained with solvent or adhesives, and especially adhesives of uncured PDMS or UV-curable resins [74] are suitable for thermoplastics. The problem with adhesives or glues is possible clogging of the microchannels and non-uniformity of the layer. Various surface treatments, including plasma [75], UV, or UV/ozone [76], are also used for thermoplastics. The clogging problem can be prevented by surface modification, but extra chip processing steps to introduce functional groups onto the substrates are needed. Thermosets are also connected by adhesive bonding, and often the material itself can be used as adhesive to seal the device. For example, for TPE [59] a layer of uncured sticky resin or for SU-8 a partial curing (softbaking) [77] can be used to seal the layers before finalizing by UV. PDMS, on the other hand, allows extremely simple sealing just by laminating two sheets together without a need for special equipment or adhesives [78]. The bonding is, however, reversible and cannot withstand high pressures. For higher bonding strength, surface treatments by plasma activation [79] or adhesive gluing [80] similar to thermoplastics are needed.

### 2.1.2 On-chip sample preparation
Most of the real-life samples of environmental or biological origin require some sort of pretreatment or preparation steps before analysis such as extraction, concentration, and biochemical treatment. For conventional “macroscale” systems, sample preparation is often the most time-consuming part of the analysis due to multiple (manual) processing steps [81]. To reduce reagent consumption and analysis time, miniaturization of sample preparation to a microscale has been extensively studied [82]. Microtechnology enables integration of even multiple sample preparation
steps on-chip [83], which results in reduced time, sample handling, and contamination. Especially solid-phase extraction (SPE) capable of simultaneous sample purification and extraction [84, 85] has been used for on-chip pretreatments. If the samples are present in low amounts, preconcentration is needed to obtain high sensitivity, and often this step is integrated with MCE chips [86]. For fluorescence applications, the samples may also require derivatization with a chromophore label, and due to wide use of MCE with fluorescence detection, numerous on-chip labeling methods are available. Most of the preconcentration and derivatization steps are based on electrokinetic fluid controlling and are briefly discussed in the next sections. Biochemical sample pretreatment is needed for DNA and protein analyses, both of which are performed clearly faster in on-chip format (minutes vs. hours) [87, 88].

The most common pretreatment step for complex protein (or peptide) samples is enzymatic digestion by trypsin, chymotrypsin, or pepsin [86]. Due to numerous advantages over solution-based systems, the enzymes are commonly used in an immobilized format known as immobilized enzyme reactors (IMERs). Immobilization of enzymes facilitates the purification of products from the reaction solution, thus allowing reuse and prolonged lifetime of the enzymes [90]. Immobilization can also enhance the enzyme stability and activity, leading to increased conversion rates [91], and the immobilization strategy has an important role for the function of the IMER [92]. The predominant method used is covalent bonding of the enzyme via carbodiimide activation [93] or cross-linking by glutaraldehyde [94]. These covalent methods require, however, aldehyde, carboxyl, or amine functional groups on the immobilization target. For inert surfaces, adsorption [95, 96] or encapsulation into a support [97, 98] is a suitable alternative method. To perform efficient on-chip digestion, the enzymes are commonly immobilized onto a stationary support structure and the reaction solution is in a liquid phase [99]. Similar to macroscale column or capillary IMERs, the most common stationary phases are based on particles/beads [100] and monolithic structures [101].

Various nanoparticles, such as silica [83, 102] and gold [96, 103], and microparticles, such as magnetic [104-106] and polymer- and agarose-based [107, 108] beads, have been demonstrated as enzyme support structures in microchips. For retaining the support onto the chip, nanoparticles are often retained by adsorption [100] or in the case of gold via thiol-gold chemistry [109], whereas for microparticles mechanical barrier structures [102] or external magnets [104] are used. Due to simple replication with various geometries, PDMS is often the material of choice for particle-based IMERs [100], despite suspected adsorption issues.

Continuous phase porous monolithic structures are more appealing alternatives to particles due to straightforward in situ polymerization by UV or
heat into the chip [110]. Especially UV polymerization allows monolith polymerization in well-defined areas of the microchannel by masking [111, 112] (Figure 2), and the porosity can be tuned from nanometer scale to large micrometer scale by selecting optimal monomers, initiators, and porogen solvent reagents. Monoliths are made of inorganic silica or organic polymers, of which acrylate- and methacrylate-based polymers [113, 114] are most often used due to versatile surface coupling chemistry and a simple polymerization process compared with inorganic silica. To prevent void formation at the interfaces and to obtain good integration, monoliths need to be anchored covalently on the chip surfaces. For inert polymers, such as thermoplastics, surface treatments are needed to introduce grafting functional groups on the surface [115, 116], although, for example, PMMA thermoplastics have been used without prior treatment with methacrylate monomers due to similar chemistry [117, 118]. Anchoring of the monolith inside a PDMS chip is difficult due to its tendency to adsorb monolith reagents [119]. Although glass has native silanol groups on its surface, silanization reactions are commonly needed to introduce suitable anchoring groups for covalent monolith arrangement [114]. In general, enzyme linking on common IMERs requires multiple steps since both surfaces of the chip (for the support) and the support (for the enzyme) need to be modified. Because most of the on-chip IMERs function by hydrodynamic actuation, the device material and bonding need to withstand high pressures [20]. In the case of electrokinetic actuation, the chip material needs to generate electroosmotic flow.

**Figure 2.** SEM images of methacrylate polymer monolith patterned inside COC device. Reproduced from reference [111] with permission from the Royal Society of Chemistry.

### 2.1.3 Microchip capillary electrophoresis

Capillary electrophoresis (CE) is the most often implemented separation mode in miniaturized analytical systems due to a relatively easy setup and convenient electrokinetic liquid handling [10]. In CE and MCE, charged analytes are moved by their electrophoretic mobilities and by the bulk EOF
under the influence of an electric field. A key difference and a benefit of MCE is the sample introduction [120]. In MCE, the sample introduction (injection) channel is incorporated on-chip with the separation channel, enabling higher complexity of the device design. These two channels can be connected with various intersecting geometries, the most commonly used being injection offset (double-T) or cross designs (Figure 3). For CE, both electrokinetic and hydrodynamic injections are used, whereas for MCE mainly electrokinetic injection is used due to simplicity of applying voltages to inlets. Additionally, by controlling the voltages applied to the sample and buffer inlets, the sample introduction to the main channel can be pinched (Figure 3), which prevents sample leaking and results in highly controlled and reproducible sample volume (based on the intersection geometry). Small sample volumes (pL to nL) also improve heat and mass transfer, allowing use of high voltages during separation. The microchip separation channels are normally only a few centimeters in length and separations within seconds can be obtained with MCE.

For MCE, the surface properties of the microchip are highly important since the surface charge of the material is the source for bulk EOF. Almost all of the common microfabrication materials have been used for MCE, and even with inert thermoplastics native EOF is observed (depending on pH and concentration of background electrolyte (BGE)) (Table 1). Glass has well-characterized surface properties and high, stable EOF [19], therefore being the main material employed for MCE separations. The fabrication process of glass requires cleanroom techniques, and thus, the lower cost alternatives of PDMS [121], PMMA [41, 42], and COC [49] are also widely used in MCE. In general, polymer materials have lower EOF mobilities than glass. On relatively inert thermoplastics, such as PMMA and PS lacking ionizable groups, the surface charge is assumed to be from the bulk additives, photochemical alterations, or
hydroxide ion adsorption [122, 123]. Native COC has a zero surface charge, but when the surface is oxidized COC has been shown to support EOF [124].

Although polymers allow cheaper fabrication of the separation devices, they are relatively hydrophobic, which can cause nonspecific interaction between analytes and the channel wall. PDMS, in particular, is prone to nonspecific adsorption of proteins [68] and small molecules [69]. Plasma treatments are often used with hydrophobic polymers to increase their hydrophilicity [125], but are not well suited for PDMS due to fast recovery, within a few hours [126]. To overcome this problem, much effort has been dedicated to finding suitable surface modification methods, and various organic molecules and polymer coating materials have been used for PDMS [127, 128], COC [49], and PMMA [43, 44] to improve biomolecule separations. Alongside improving the coatings with common polymers, materials with inherently hydrophilic nature have been presented in MCE; these include photoresists SU-8 [57], organically modified ceramics [129], and UV-curable photoresist copolymers [61]. With these inherently hydrophilic materials combined with (coated) thermoplastics, separation efficiency of theoretical plate numbers of $10^5$-$10^6$ /m can be reached, comparable to conventional CE.

The possibility to implement multiple channels on-chip and the various materials available have enabled higher integration of analytical procedures. Due to simple fluid manipulation by voltages, electrokinetic on-chip preconcentration steps, such as field-amplified stacking (FAS) [130], isotachophoresis (ITP) [131], or isoelectric focusing (IEF) [132], can be relatively easily integrated with sample introduction, separation, and detection. Labeling of analytes can be integrated on-chip by additional channels for label solution introduction [133] and/or it is often performed as a part of sample preconcentration on SPE while the analytes are retained on the support [134, 135]. An example of a truly integrated microchip system incorporating on-chip pumps, sample introduction, monolithic SPE, labeling, and MCE and LIF detection has been demonstrated for analysis of birth biomarkers on the PDMS-COC device with a total analysis time of only 1 h [134] (Figure 4). Other recent works of fully automated and MCE detection integrated microchips for bioanalysis have been shown with on-chip PCR on coated cyclic olefin polymer (COP) chips [136], IEF on SU-8 and PDMS/SU-8/quartz hybrid microchips [137, 138], micellar electrokinetic chromatography on PDMS-glass microchip [139], protein digestion on coated glass chip [108], and SPE on SU-8 [112]. Most of the chips were made by connecting different materials together [134, 138, 140], which can make the integration complex if the bonding and adhesion between components are not performed well.
2.1.4 Detection of microfluidic separation systems

Microsystems are implemented with all of the optical, electrochemical, and mass spectrometric detection modes. Optical detection is widely used on a macroscale, but on a microscale, especially absorbance faces problems. This is due to reduced optical path lengths, according to Beer-Lambert’s law [141]. Laser-based fluorescence detection is, however, suitable for micron-size channels, thus being the main detection method for biological samples in miniaturized systems [142]. Laser-induced fluorescence (LIF) combined with photon detection by photomultiplier tube (PMT) or charge-coupled device (CCD) results in low background signals and detection limits in the nM [143] to sub-nM ranges [144], allowing detection of even a single molecule [145]. As most biological samples are not natively fluorescent, often analyte labeling is required prior to fluorescence detection. Absorbance detection has been achieved with channel design to extend the detection cells [146], with optical fibers [147] or microlenses [148], resulting in over 10-fold detection sensitivity, reaching the low-μM range. Light-emitting diode (LED) fluorescence systems have been increasingly used as lower cost alternatives to LIF. These provide on-chip integration [149] and the low detection sensitivity can be overcome by integrated optical elements such as microlenses [150] or photodiode detection [151]. Detection of low analyte concentrations can also

![Automated microfluidic device integrating hydrodynamic controls, solid-phase extraction (SPE), fluorescent labeling and microchip electrophoresis (μCE) for preterm birth biomarker analysis. Eight reservoirs were used (1 sample, 2 fluorescent label, 3 rinse, 4 label eluent, 5 labeled sample eluent, B buffer, BW buffer waste, W waste). Color scheme: blue fluidic channels, gray control channels, and green monolith. Reproduced from reference [134] with permission.](image-url)
be improved by implementing sample concentration on-chip prior to MCE [152].

For optical detection, the optical clarity of microchip materials is the most critical parameter. Many thermoplastics, such as PMMA, PC, and PS, give gradual autofluorescence in the visible range and are nontransparent in the UV range [153, 154], which complicates their use in these biologically important wavelength ranges. COC thermoplastic (depending on the brand), PDMS, and glass have low autofluorescence and are transparent up to the 200-330 nm range, which make them suitable for optical purposes and even for native deep UV fluorescence detection [155, 156]. Contrary to optical detection, electrochemical (EC) detection benefits from transfer to the microscale since both the electrodes and the control system can be easily miniaturized [157]. EC setup enables also portability, making it suitable for on-site testing [158].

MS is often the detection method for bioanalytical and protein analyses in MCE [159]. Especially with IMERs, the most desirable detection of the digested protein fragments is MS, and many projects aim to integrate ionization step on-chip [47, 106-108]. Currently, electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are the most used ionization modes in microfluidic systems. Especially ESI is well suited for online integration with MS owing to compatible flow rates [160]. The main issue is a proper coupling between microchip and ionization interface. Methods to implement ESI are direct spraying from the chip edge, inserting an external emitter or monolithic integration of tip on-chip [160]. In the first reports of on-chip ESI, direct spray from the blunt channel was used, but these suffered from sample spreading on the edge, causing large Taylor cone (drop size), and thus, deteriorated ionization performance [161, 162]. To obtain stable and efficient electrospray, the end of the channel must have a sharp point. To ensure this, external emitters have been made from, for instance, fused silica capillaries [107, 163-165] and nanospray needles and tips [166-169] (Figure 5A). Although high performance is demonstrated by inserted silica capillaries on PDMS for cell studies [170] or on PMMA for protein digestion [107], the manual insertion of the emitter can be complicated. To overcome these problems, the most stable method is monolithically integrated ESI-tip on the end of the microchip channel.

Integrated on-chip emitters have been realized from glass by manual pulling [171], edge sawing [31, 172], and standard microfabrication techniques [173]. Standard lithography techniques have also been used for silicon [174] or silicon-glass hybrid materials [32]. These cleanroom-based techniques produce sharp tips, but the processing is time-consuming. Silicon is also semiconductive, which prevents its use with CE applications; it is mostly developed for liquid chromatography electrospray chips. Microtechnology enables also fabrication of parallel multi-emitter devices. Recently, arrays with multiple ESI emitters have been developed from silicon [175, 176], showing
increased MS sensitivity and high throughput analysis performance. Also polymers have been processed by common microfabrication techniques, such as photolithography of SU-8 [56], edge sawing of organic ceramics [177], laser micromachining of PC [178] or PMMA [179], and hot embossing of COC [180]. However, fabrication and/or bonding of the above-mentioned materials can be complex, and thus, PDMS has been tested for on-chip emitter replication [181, 182]. However, due to elasticity of PDMS, replication of a sharp and thin tip is difficult. Alternative approaches based on PDMS/glass hybrids [183] or multilayer chip with PDMS membrane [184] (Figure 5B) have been introduced to form thin (<200 μm) PDMS emitters, reaching tip thicknesses similar to cleanroom-produced glass [31]. A drawback of polymeric substrates is that they can suffer from high chemical background caused by leaching of bulk material to MS due to poor solvent compatibility. This has been reported for PMMA [185] and in particular for PDMS-based ESI chips [66, 167]. Monomer leaching can be reduced to some extent by increasing the curing time to obtain a higher cross-linked polymer [66, 167] or by extracting the substrate with multiple organic solvents [182].

![A) Photograph of the integrated nanospray emitter, reprinted with permission [169] and B) photographs of a PDMS membrane-based microfluidic ESI emitter, adapted with permission [184]. Copyright (2011) American Chemical Society.](image)

**Figure 5.** A) Photograph of the integrated nanospray emitter, reprinted with permission [169] and B) photographs of a PDMS membrane-based microfluidic ESI emitter, adapted with permission [184]. Copyright (2011) American Chemical Society.

## 2.2 Thiol-ene click chemistry for microfabrication

### 2.2.1 Reaction mechanism

Thiol-ene reactions belong to the class of efficient click chemistry reactions, which proceed rapidly by high conversion rates and in a region- and stereoselective manner [186]. Thiol-ene click reactions can be performed at mild solventless or aqueous conditions [187] and are insensitive to oxygen. The reaction can occur between any thiol and alkene functional group, meaning various cross-linked polymer structures can be obtained [188]. Thiols can react with carbon-carbon double bonds (“allyls/enes”) by nucleophilic or free-radical additions, where the latter is termed the thiol-ene reaction. There
are two radical-mediated thiol-ene polymerizations, thiol-ene [186] and thiol-yne [189] reactions. Both of these follow a step-growth radical mechanism, where a thiyl radical is formed from a thiol functional group by a thermal initiator or the more commonly used photoinitiator. The thiyl radical reacts with the double or triple bond, followed by a chain transfer of the radical to another thiol group, creating a new thiyl radical (propagation step), and the polymerization process starts again (Figure 6). The polymerization terminates by radical-radical recombination. The thiyl radical formation can also be self-initiated by UV light [190]. This ‘initiator-free’ polymerization proceeds upon exposure to a light wavelength of 254 nm, whereas with a photoinitiator (depending on the absorbance maximum) higher wavelengths, such as 365 nm for benzophenone or ethyl(2,4,6-trimethylbenzoyl “TPO-L”, are used. Without the initiator, the polymerization proceeds more slowly [191]. However, initiator-free polymerization has multiple beneficial effects on the polymer, which are discussed in more detail in the results section. The exceptional advantages of thiol-ene click chemistry reactions have made them preferred choices for polymer and macromolecular synthesis [192] and for functionalization of (bio)molecules and polymers [193].

![Thiol-ene photopolymerization process](image)

Figure 6. Thiol-ene photopolymerization process.

### 2.2.2 Off-stoichiometric thiol-enes
Thiol-enes are processable by replica-molding [24, 25], and thus, well suited for fast prototyping. Thiol-enes are well suited also for high-throughput industrial imprinting [194] and reaction injection molding [195] and even for mass production by roll-to-plate fabrication [196]. The step-growth mechanism together with delayed gelation of thiol-ene polymerization results in a uniform and low shrinkage network [191]. This enables good thickness control of microchannel features and even sub-100-nm features can be achieved by nanolithography [197, 198]. Due to the thermosetting nature of thiol-enes, they are well suited also for photolithography [26, 199], with much better structure quality than acrylate analogs [200].
The first presented thiol-ene microfluidic devices were made of commercially available UV-curable adhesive NOA formulations (Norland Optical Adhesive, Norland Products, NJ, USA) already used in microfluidics as suitable UV adhesive for bonding other polymers. In 2004, Harrison et al. presented the first NOA devices fabricated by standard photolithography [201], after which molding methods, such as imprinting [194] and replica-molding [202], became common for NOA structuring. However, similar to acrylates NOAs suffer from oxygen inhibition [194], which may cause issues with surface reproducibility. At the same time, custom-made thiol-ene formulations became popular due to the exceptional material tuning, which cannot be achieved with commercial NOA adhesives or other thermoplastics or thermosets. The Bowman group demonstrated that the rigidity of stoichiometric molar amount thiol-ene microdevices were tunable only by selection of monomer precursor and functionality [203]. Thiol-ene step-growth mechanism enables, however, also polymerization in off-stoichiometric ratios, which creates interesting properties for the polymer network and surfaces [24, 198]. In 2011, Carlborg et al. [24] introduced the concept of “off-stoichiometric thiol-enes” OSTE, which emphasized the use of large off-stoichiometric monomer ratios in a multifunctional thiol-ene mixture. Since then, OSTE have served in multiple application fields, and as an indication of their suitability as an alternative material for LOCs the company ‘OSTEMERs’ was founded.

OSTEs provide even further polymer bulk and surface tuning thanks to the reactivity of the excess of one monomer. Now, only by tuning the stoichiometric ratio of monomers, polymers with properties similar to thermoplastics or PDMS were realized [24]. For thiol-rich OSTE, elastic structures with Young’s modulus of 10 MPa and Tg of 35°C comparable to PDMS were shown, whereas for allyl-rich OSTE stiffness of 1750 MPa and Tg of 68°C similar to PMMA were obtained. OSTE’s mechanical flexibility for LOC systems has been demonstrated by, for example, production of pneumatic valves of thiol-OSTE [24], rigid pressure-tolerable microreactors on allyl-OSTE [204], and hydrogel networks on both OSTE [205]. The use of off-stoichiometry introduces also free functional groups on the surface, which are beneficial for surface modifications and bonding. A few years after OSTE was introduced, the same group launched another OSTE system, a ternary dual-cure thiol-ene-epoxy system, OSTE+ [206]. The OSTE platform is based on purely thiol and ene click reaction (with initiator), whereas OSTE+ is based on reactions between thiol and allyl and between thiol and epoxy. The reactions are controlled by separate radical and anionic initiators [207]. Now, due to the additional epoxy precursor, highly rigid structures are obtained after the second curing step (Elastic modulus of 5 GPa [207]), and with this material fabrication of micropillars with an aspect ratio as high as 10 can be obtained [208]. In one study, off-stoichiometry was noted to affect also
photolithography structuring. By increasing the off-stoichiometric ratio in the bulk a decrease in feature broadening was noticed [199].

**Surface chemistry**

OSTEs’ inherent surface chemistry (thiol and allyl functional groups) can be utilized in a straightforward manner as linkers via thiol-ene click reactions for further modifications [25, 26]. Wettability is an important parameter for microfluidic devices since it affects the handling of the fluid inside the channels, but also the amount of (usually unwanted) interactions between the surface and the analytes. Native thiol-enes are reported to be slightly hydrophilic with water contact angles (WCA) ranging from 68° for allyl-rich to 76° for thiol-rich OSTEis [24, 26, 209], and these are in general comparable to other commonly used polymer materials such as PMMA, PC, PS [125], and COC [210]. For higher hydrophobicity or hydrophilicity, OSTEis can be processed in the straightforward manner of photolithographically defined patterns [26, 211]. WCAs ranging from 18-35° (by hydroxylated methacrylates [26, 212] and PEG [24, 205]) to 102-118° (by fluorinated methacrylates and 2-methoxy(polyethylenoxy) propyltrichlorosilane (PTS) [209]) have been demonstrated. Also surface plasma treatments carried out on NOA have shown higher stability than PDMS over time [213].

**Bonding of thiol-enes**

Similar to PDMS, thiol-enes can be directly bonded without any adhesives or glues [24]. Furthermore, the reactive surface groups of OSTE and OSTE+ allow click chemistry-based covalent bonding [24, 195] (Figure 7), which has been utilized for joining thiol-ene with silicon, alumina [214, 215], and gold [216, 217], and bonding strength as high as 200 bars has been reported with glass [218]. A drawback of pure OSTE is that only opposite surface chemistries are bondable together (Figure 7A). This can be solved by OSTE+ and the thiol-ene-epoxy linking to finalize the bonding of two layers [195] (Figure 7B). OSTE+ dual curing can also be done in reversed polymerization order, which facilitates bonding to heat-sensitive bio-functionalized surfaces such as protein microarrays [219]. Another approach to bond two similar thiol-enes together is by omitting a photoinitiator in a thiol-ene mixture. The use of a photoinitiator enables fully polymerized and inert thiol-ene surfaces [220], but is not useful for bonding two similar surfaces. Without initiator, the UV curing produces fully cured bulk polymer, but holds reactive functional groups on the surface that are capable of further bonding [221]. The most critical factor for initiator-free bonding of two similar OSTEis is the cross-linked polymer bulk rigidity, resulting in fairly high bonding strengths [221].
Figure 7. Illustrations of A) thiol-ene OSTE bonding between two different surface chemistries, B) thiol-ene OSTE+ bonding between thiol and epoxy, and C) OSTE bonding between two similar surfaces (here thiol-rich) without an initiator.

2.2.3 Thiol-ene microfluidic devices in analytical applications

Thiol-enes’ inherent surface chemistry, mechanical tunability, and simple fabrication by molding are useful in multiple analytical bioapplications (Table 2). Thiol groups for surface grafting have been used for decades [193], and also in thiol-ene microdevices the linking is mainly done via thiol-rich surfaces. Thiol-enes have low autofluorescence at the biologically important UV and visible ranges ([195], Study II), which make them ideal for optical detection. The optical properties are independent of the monomer stoichiometric ratio [222], and thus, different thiol-ene compositions can be combined to form the microdevice. Good optical clarity together with the possibility to selectively and spatially modify surfaces by UV click reactions have proven useful in optical biochips [25, 222]. Thiol-rich OSTEs were grafted with biotin-streptavidin via simple 1-step modifications and the functionalization protocol was performed both before and after bonding [25]. OSTEs’ good bondability against various substrates in an adhesive-free manner even at RT make them suitable for biofunctionalized microarrays. Thiol-rich OSTE [223] and OSTE+ [219] have been demonstrated as biostickers for protein and DNA arrays. OSTE+ devices were fabricated by large-scale suitable injection molding, making these approaches possible for low-cost healthcare applications [219]. In addition, NOA81-based thiol-enes bound on glass were shown to be suitable for soft-imprinted biomicrostickers for cell patterning without aging or thermal defects [194, 224]. NOA63 thiol-enes have also been used as immunoassays, where antibody-immobilized beads were retained on an NOA device [225]. However, for both of the above NOA devices the bonding was not as simple as with pure OSTE and incorporated use of ‘uncured residual’ thiolene layers [194] or required surface plasma treatments [225], respectively.
Recently, thiol-enes have been revealed to be suitable material for enzymatic microreactors and separation devices. Both thiol-OSTE and allyl-OSTEs were demonstrated as monolithic microreactors for immobilized galactose oxidase and peptide-N-glycosidase enzymes [204]. In that study, various thiol-ene based monoliths were synthesized and anchored inside a microreactor by simple click chemistry photopolymerization. As the monolith and the chip were made of the same material, they formed a strong seamless interface, not easily achieved with other polymers. Based on the detailed optimization, the best monolith forming a highly regular continuous bead-like structure was obtained by allyl-rich OSTE, which was further used in other studies of immobilized pepsin [226] and trypsin [227] microreactors. In another work by Hoffmann et al. [228], thiol-rich OSTE was used as an enzyme immobilization screening platform. The utility of the platform was the simple click chemistry reactions of thiol groups by different allyl, vinyl, or acrylic group linked monomers with various hydrophilic, hydrophobic, and ionic functionalities. The system was tested with horseradish peroxidase (HRP) for which allyl-glycidyl ether was the best immobilization linker. In their further studies, the efficiency of this HRP-IMER on an OSTE+ device was compared with computational models, resulting in good agreement on the immobilization strategy [229].

For MCE coupled with LIF detection, stoichiometric [221] and coated thiol-rich OSTEs [230] have been used. For analysis of fluorescent dyes, native stoichiometric thiol-ene was shown to be a suitable material, with good baseline resolution and high migration time repeatability [221]. Positively charged protein samples are known to cause unwanted adsorption on surfaces, and for this reason thiol surface groups were utilized for covalent grafting with neutral copolymer. This straightforward covalent surface modification showed stable hydrophilic coating (WCA reduced from native 60° to 34° of coated surface), and basic and acidic proteins were separated in less than 10 s [230]. Also NOAs have been demonstrated for MCE-LIF of Rhodamine B dye [231]. Comparison of optical properties of different NOAs showed NOA81 to be the best material for MCE-LIF, but low auto-fluorescence needed for optical measurements was obtained only at 540 nm, which makes NOA an unsuitable substrate for visible and low UV ranges [231]. Allyl-rich and stoichiometric OSTEs provide the strong mechanical strength needed for fabrication of thin structures such as ESI emitters. Besides the ESI emitters demonstrated in this thesis (III, IV), fully integrated on-chip emitters have been molded from thiol-enes [232]. 3D emitter was prepared by PDMS molding and high precision milling, and as a result of the sharpness of the tip a stable and highly repeatable electrospray was achieved (RSD of 8 ± 3% for total ion current n= 3 chips). A monolithic SPE channel (fabricated by the protocol of [204]) was integrated prior to the ESI tip. Although the recovery of SPE was only half of what can be
Review of the literature

achieved with conventional methods, this was the first demonstration of sample clean-up and MS detection integrated on a thiol-ene device.

Table 2. List of selected thiol-ene microchips used in bioanalytical applications.

<table>
<thead>
<tr>
<th>Application</th>
<th>Composition</th>
<th>Fabrication</th>
<th>Analytes/use</th>
<th>Initiator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochips</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optical waveguides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiol-rich OSTE</td>
<td>Molding</td>
<td></td>
<td>Evanescent wave-induced fluorescence</td>
<td>No</td>
<td>[25]</td>
</tr>
<tr>
<td>Thiol-rich OSTE</td>
<td>Molding</td>
<td></td>
<td>Fluorescence waveguide</td>
<td>Yes</td>
<td>[222]</td>
</tr>
<tr>
<td>Bio-microstickers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiol-rich OSTE</td>
<td>Molding</td>
<td></td>
<td>Protein assay and the DNA hybridization</td>
<td>No</td>
<td>[223]</td>
</tr>
<tr>
<td>OSTEMER 327*</td>
<td>Injection molding</td>
<td></td>
<td>Protein assay</td>
<td>Yes</td>
<td>[219]</td>
</tr>
<tr>
<td>NOA81*</td>
<td>Soft imprinting</td>
<td></td>
<td>UV-assisted cell patterning</td>
<td>N/A</td>
<td>[224]</td>
</tr>
<tr>
<td>Microreactors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOA63*</td>
<td>Molding</td>
<td></td>
<td>Immunoassays</td>
<td>-</td>
<td>[225]</td>
</tr>
<tr>
<td>Thiol-OSTE and allyl-OSTE</td>
<td>Molding</td>
<td></td>
<td>Monolithic galactose oxidase and peptide-N-glycosidase F enzyme reactor</td>
<td>No</td>
<td>[264]</td>
</tr>
<tr>
<td>Allyl-OSTE</td>
<td>Molding</td>
<td></td>
<td>Monolithic pepsin microreactor</td>
<td>No</td>
<td>[226]</td>
</tr>
<tr>
<td>Allyl-OSTE</td>
<td>Molding</td>
<td></td>
<td>Monolithic trypsin microreactor</td>
<td>No</td>
<td>[227]</td>
</tr>
<tr>
<td>Thiol-OSTE and thiol-OSTE+</td>
<td>Molding</td>
<td></td>
<td>Immobilized horseradish peroxidase on microplate enzyme screening platform</td>
<td>Yes</td>
<td>[228, 229]</td>
</tr>
<tr>
<td>Separation devices</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOA63*</td>
<td>Molding</td>
<td></td>
<td>MCE-LIF of Rho B</td>
<td>-</td>
<td>[231]</td>
</tr>
<tr>
<td>Stoichiometric thiol-ene</td>
<td>Molding</td>
<td></td>
<td>MCE-LIF of fluorescent model compounds</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Thiol-OSTE</td>
<td>Molding</td>
<td></td>
<td>MCE-LIF of acidic and basic proteins</td>
<td>No</td>
<td>[230]</td>
</tr>
<tr>
<td>Stoichiometric</td>
<td>Molding</td>
<td></td>
<td>SPE-ESI-MS of progesterone</td>
<td>No</td>
<td>[232]</td>
</tr>
</tbody>
</table>

*Commercial OSTE+ and NOA adhesive thiol-enes
3 AIMS OF THE STUDY

The overall aim of this work was to evaluate the feasibility of thiol-enes for the implementation of bioanalytical microdevices, with controlled and customized surface and bulk properties achieved with the use of photoinitiator-free compositions. The devices were primarily intended for mass spectrometry based bioanalysis and thus a lot of emphasis was put on the material stability and structure fidelity facilitating their seamless integration with electrospray ionization. Another main goal of the work was to develop out-of-cleanroom microfabrication concepts for simple and low cost manufacturing of μTAS devices in order to promote adaptation of microfluidics in routine bioanalysis.

More detailed aims of the research were:

- To study replica molding of thiol-enes without an initiator or other additives (I-IV) with a focus on straightforward bonding
- To develop replication methods facilitating high feature resolution structures such as electrospray tip (III, IV) and high aspect ratio micropillars (I, IV)
- To determine how the lack of photoinitiator affects the microdevice feature resolution, bulk, and surface properties (I-IV)
- To evaluate the effects of the stoichiometry of photoinitiator-free thiol-ene compositions and their fabrication conditions on surface chemistry (I), wettability (I), topography (I), charge (II) and aging of the substrate (II)
- To investigate the effect of the surface properties of native thiol-ene compositions on the performance of analytical devices in bioanalysis (I-IV)
- To develop rapid and simple surface functionalization methods for enzyme immobilization (I) and elimination of nonspecific surface interactions (IV)
4 EXPERIMENTAL

This chapter describes the experimental settings used, including (bio)chemicals and materials (4.1), chip fabrication materials (4.2), and instrumentation (4.3), as well as microfabrication (4.4), characterization (4.5), functionalization (4.6) and analytical (4.7) methods.

4.1 Chemicals and materials

Chemicals, reagents, and materials used in this work are listed in Table 3. All aqueous separation buffers and the BGE solutions were prepared in deionized Milli-Q water (Millipore, Bedford, MA, USA) and filtered through a 0.2 μm filter. The stock solutions of fluorescein dyes, drug molecules, peptides, and proteins were prepared in Milli-Q water, methanol, or dimethyl sulfoxide and diluted to appropriate buffers and electrolyte solutions as stated in the original publications (I-IV).

Table 3. Chemicals and reagents used in this work. N/A= not available.

<table>
<thead>
<tr>
<th>Reagent/ solvent/ standard</th>
<th>Grade</th>
<th>Supplier</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>HPLC</td>
<td>*Sigma-Aldrich, Steinheim, Germany</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Acetone</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>I, IV</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>III</td>
</tr>
<tr>
<td>Angiotensin I human acetate salt hydrate</td>
<td>≥90%</td>
<td>Sigma-Aldrich</td>
<td>II, III</td>
</tr>
<tr>
<td>Angiotensin II acetate salt</td>
<td>96.2%</td>
<td>Bachem, Bupendorf, Switzerland</td>
<td>II, III, IV</td>
</tr>
<tr>
<td>Angiotensin III</td>
<td>98%</td>
<td>Sigma-Aldrich</td>
<td>III</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>HPLC</td>
<td>Riedel-de-Haen, Seelze, Germany</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Ammonium bicarbonate</td>
<td>HPLC</td>
<td>Fluka, Steinheim, Germany</td>
<td>IV</td>
</tr>
<tr>
<td>Ammonium hydroxide</td>
<td>LC-MS</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Boric acid</td>
<td>HPLC</td>
<td>Riedel-de-Haen, Seelze, Germany</td>
<td>II</td>
</tr>
<tr>
<td>Bradykinin (fragment 1-9)</td>
<td>≥98%</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>α-chymotrypsin (type II, 40 units/ mg protein)</td>
<td>N/A</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Cytochrome c from bovine heart</td>
<td>95%</td>
<td>Sigma-Aldrich</td>
<td>III, IV</td>
</tr>
<tr>
<td>Dibasic sodium phosphate</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>LC-MS</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>5,5-dithiobis(2-nitrobenzoic acid) DTNB</td>
<td>≥98%</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
</tbody>
</table>
### Reagent/ solvent/ standard

<table>
<thead>
<tr>
<th>Reagent/ solvent/ standard</th>
<th>Grade</th>
<th>Supplier</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL-dithiothreitol</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>I, IV</td>
</tr>
<tr>
<td>3-6 nm dodecanethiol functionalized gold nanoparticle</td>
<td>N/A</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Ethanol</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>III</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>≥99%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Fluorescein diacetate</td>
<td>≥99%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Fluorescein dibutyrate</td>
<td>≥98%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Fluorescein dilaurate</td>
<td>97%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Fluorescein isothiocyanate</td>
<td>≥90%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Formic acid</td>
<td>HPLC</td>
<td>Merch Millipore, Darmstadt, Germany</td>
<td>III, IV</td>
</tr>
<tr>
<td>10 nm gold nanoparticles (stabilized in 0.1 mM PBS)</td>
<td>N/A</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>HPLC</td>
<td>Riedel-de-Haen, Seelze, Germany</td>
<td>II</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>HPLC</td>
<td>Fluka, Steinhem, Germany</td>
<td>IV</td>
</tr>
<tr>
<td>Methanol</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>I, III, IV</td>
</tr>
<tr>
<td>Monobasic sodium phosphate</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Phosphate-buffered saline</td>
<td>BioPerform ance Certified</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Propanol</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>III</td>
</tr>
<tr>
<td>Rhodamine 123</td>
<td>≥85%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Sodium hydrogen carbonate</td>
<td>HPLC</td>
<td>VWR, Leuven, Belgium</td>
<td>II</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>HPLC</td>
<td>Riedel-de-Haen, Seelze, Germany</td>
<td>II</td>
</tr>
<tr>
<td>Substance P (6-11)</td>
<td>98%</td>
<td>Bachem, Bupendorf, Switzerland</td>
<td>II</td>
</tr>
<tr>
<td>Sulforhodamine B</td>
<td>99%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Trypsin</td>
<td>Sequencing grade</td>
<td>Promega, Madison, WI, USA</td>
<td>IV</td>
</tr>
<tr>
<td>Toluene</td>
<td>HPLC</td>
<td>Sigma-Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>99%</td>
<td>Sigma-Aldrich</td>
<td>II</td>
</tr>
<tr>
<td>Verapamil</td>
<td>99%</td>
<td>ICN Biomedicals, Aurora, OH, USA</td>
<td>III, IV</td>
</tr>
</tbody>
</table>

### 4.2 Microchip fabrication materials and nomenclature

The thiol-ene compositions studied in this thesis are named as triallyl-rich, 1:1 stoichiometric and tetra- or trithiol-rich thiol-enes. These are related to 50 mol-% excess of functional groups, either allyl or thiol, on the polymer or equal 1:1 stoichiometric molar ratio. In Publication II, the different thiol-ene compositions were named as 2:3, 1:1, or 3:2, referring to molar stoichiometric...
ratio in the thiol-ene (trithiol: triallyl monomer). Figure 8 presents the chemical structures of the thiol-ene monomers used. The microchip fabrication materials used in this work are listed in Table 4.

![Molecular structures of the thiol-ene monomers used in this work.](image)

**Figure 8.** Molecular structures of the thiol-ene monomers used in this work.

<table>
<thead>
<tr>
<th>Material/ monomer (purity)</th>
<th>Supplier</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3,5-Triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (≥98.0%) (triallyl)</td>
<td>Sigma-Aldrich Saint Louis, MO, USA</td>
<td>I-IV</td>
</tr>
<tr>
<td>Trimethylolpropane tri(3-mercaptopropionate) (≥95.0%) (trithiol)</td>
<td>Sigma-Aldrich Saint Louis, MO, USA</td>
<td>I-IV</td>
</tr>
<tr>
<td>Pentaerythritol tetrakis(3-mercaptopropionate) (≥95.0%) (tetrathiol)</td>
<td>Sigma-Aldrich Saint Louis, MO, USA</td>
<td>I, III</td>
</tr>
<tr>
<td>Sylgard 184 base elastomer and curing agent</td>
<td>Down Corning Corporation, Midland, MI, USA</td>
<td>I-IV</td>
</tr>
<tr>
<td>SU-8 100 photoresist</td>
<td>Microchem Corporation Newton, MA, USA</td>
<td>I, II</td>
</tr>
<tr>
<td>SU-8 50 photoresist</td>
<td>Microchem Corporation Newton, MA, USA</td>
<td>II, III</td>
</tr>
<tr>
<td>AZ 5214E photoresist</td>
<td>MicroChemicals, Germany</td>
<td>IV</td>
</tr>
<tr>
<td>PEG sulphhydryl (mPEG-SH) Mw 1000</td>
<td>Creative PEGWorks Chapel Hill, NC, USA</td>
<td>IV</td>
</tr>
</tbody>
</table>

**4.3 Instrumentation**

Commercial instruments used in the study are listed in Table 5. In addition, some standard laboratory equipment and devices, not listed here, such as electric resistors and vacuum pumps, were used in the experiments.
### Experimental

**Table 5.** Commercial instruments used in this work.

<table>
<thead>
<tr>
<th>Instrumentation</th>
<th>Manufacturer/Supplier</th>
<th>Note</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For microfabrication</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA-6 mask aligner</td>
<td>Süss Microtec, Garching, Germany</td>
<td>SU-8 master fabrication</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Plasma Enhanced Chemical Vapor Deposition (PECVD)</td>
<td>Plasmalab 80+, Oxford Instruments, UK</td>
<td>SU-8 and silicon master fabrication</td>
<td>I-IV</td>
</tr>
<tr>
<td>Dymax 5000-EC Series UV flood exposure lamp</td>
<td>Dymax Corporation, Torrington, CT, USA</td>
<td>Thiol-ene curing</td>
<td>I-IV</td>
</tr>
<tr>
<td><strong>For microchip electrophoresis and EOF measurements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADC100 converter and PicoLog software</td>
<td>Pico Technology, St. Neots, UK</td>
<td>Signal recording</td>
<td>II</td>
</tr>
<tr>
<td>Microfluidic ToolKit, computer-controlled (Labview)</td>
<td>Micralyne, Edmonton, Canada</td>
<td>High voltage power supply</td>
<td>II, III</td>
</tr>
<tr>
<td><strong>For microscopy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leica DMIL inverted microscope equipped with</td>
<td>Leica Nilomark, Espoo, Finland</td>
<td>Fluorescence detection</td>
<td>II</td>
</tr>
<tr>
<td>Zeiss Axioscope A1 upright epifluorescence microscope equipped with</td>
<td>Carl Zeiss, Espoo, Finland</td>
<td>Fluorescence detection</td>
<td>II</td>
</tr>
<tr>
<td>• Continuous wave argon laser (488 nm, 20 mW, JDS Uniphase, Cheos, Espoo, Finland)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Pulsed UV laser (355 nm, 15 μJ at 1 kHz, Team Photonics, Cheos, Espoo, Finland)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>For mass spectrometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agilent 6330 ion trap MS</td>
<td>Agilent Technologies, Santa Clara, CA, USA</td>
<td>Mass spectrometer</td>
<td>I, III, IV</td>
</tr>
<tr>
<td>Harvard PHD 2000 Advanced syringe pump</td>
<td>Harvard Apparatus, South Natick, MA, USA</td>
<td>Pressure-driven flow</td>
<td>I</td>
</tr>
<tr>
<td>Modified nanospray source with xyz stage and CCD camera</td>
<td>Proxeon Biosystems, Odense, Denmark</td>
<td>Chip holder</td>
<td>III, IV</td>
</tr>
<tr>
<td><strong>For thiol-ene characterization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanning electron microscope</td>
<td>FEI Quanta™ FEG, Hillsboro, OR, USA</td>
<td>Surface analysis</td>
<td>I, III, IV</td>
</tr>
<tr>
<td>Quorum Q150TS, turbomolecular-pumped high resolution coater</td>
<td>Quorum Technologies, UK</td>
<td>SEM sample preparation</td>
<td>I, III, IV</td>
</tr>
<tr>
<td>X-ray photoelectron spectroscope</td>
<td>PHI Quantum 2000 scanning spectrometer</td>
<td>Surface analysis</td>
<td>I</td>
</tr>
<tr>
<td>Atomic force microscope</td>
<td>NTEGRA PRIMA, NT-MDT, Russia</td>
<td>Surface analysis</td>
<td>I</td>
</tr>
<tr>
<td>Contact angle goniometry</td>
<td>Theta Attension, Espoo, Finland</td>
<td>Wetting</td>
<td>I</td>
</tr>
<tr>
<td>Instron tensile testing machine, Model 4204</td>
<td>Instron, Buckinghamshire, UK</td>
<td>Tensile strength</td>
<td>III</td>
</tr>
</tbody>
</table>
Experimental

<table>
<thead>
<tr>
<th>Instrumentation</th>
<th>Manufacturer/Supplier</th>
<th>Note</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential scanning calorimetry DSC 823e</td>
<td>Mettler-Toledo, Switzerland</td>
<td>Glass transition</td>
<td>Unpublished</td>
</tr>
<tr>
<td></td>
<td></td>
<td>temperatures</td>
<td></td>
</tr>
<tr>
<td>Fourier-transform infrared spectroscope</td>
<td>Vertex 70, Bruker, MA, USA</td>
<td>Surface analysis</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Varioskan LUX Multimode Microplate Reader</td>
<td>ThermoScientific, Vantaa, Finland</td>
<td>Absorbance detection</td>
<td>I</td>
</tr>
</tbody>
</table>

Other

<table>
<thead>
<tr>
<th>In-house built gas delivery system with electronic regulator and solenoid valves</th>
<th>SMC Pneumatics Finland, Espoo, Finland</th>
<th>Pressure tolerance</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistive heater equipped with DC power supply and controller</td>
<td>Iso-Tech IPS-603, RS Components Ltd., Northants, UK, OMEGA Engineering, Manchester, UK</td>
<td>Heating element</td>
<td>I</td>
</tr>
<tr>
<td>3D-printer</td>
<td>Minifactory 3, Seinäjoki, Finland</td>
<td>Fabrication of</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chip holders</td>
<td></td>
</tr>
</tbody>
</table>

4.4 Microchip fabrication

All microchips presented in this work were fabricated by a replica-molding process. The chip fabrication process comprised a) SU-8 master fabrication in the cleanroom, b) casting of a PDMS negative mold with the help of the SU-8 master (non-cleanroom), c) fabrication of a thiol-ene from the PDMS, and d) bonding by additional UV curing. The schematic fabrication process is presented in Figure 9.

The masters for thiol-ene microchannels used in I-III were fabricated out of SU-8 negative photoresist by UV photolithography in cleanroom conditions. The SU-8 masters were separately prepared for the microchannel and the inlet and cover layers. The more detailed spin coating, softbake, and hardbake parameters can be found in the original articles and the supporting materials. Briefly, SU-8 50 or SU-8 100 photoresist was spin coated on a silicon wafer (varying rpm and time parameters) to obtain the first SU-8 layer. After softbake on a hotplate, the outer chip features were patterned by UV exposure on a mask aligner, followed by hardbake and development in mr-Dev 600 (I-III). Finally a fluoropolymer coating (CHF₃) was applied using PECVD to act as an anti-adhesion layer. For microchannel masters holding also the inlet structures or in the case of thick SU-8 structures (500 μm height), the SU-8 layer was applied in two spin coating steps. The master for the μPESI chips (IV) was fabricated out of silicon by inductively coupled plasma reactive ion etching as explained in detail in the original publication IV.

The PDMS molds were prepared by mixing the elastomer and the curing agent in a ratio of 10:1 (w/w). After degassing in vacuum for 30 min, the PDMS was cast onto the SU-8 or silicon master and cured at 80°C for at least 3 h.
Figure 9. Schematic presentation of the chip fabrication process used for MCE chips: (a) SU-8 master fabrication in the cleanroom, separate masters for both the channel wafer and the bonding layer comprising the inlets, (b) casting of PDMS molds with help of the SU-8 masters (non-cleanroom), (c) mixing of the thiol and allyl components and replication of the thiol-ene channel by UV, and (d) bonding of the thiol-ene by lamination, followed by additional UV curing.

The thiol-ene fabrication was done by mixing commercially available trithiol or tetrathiol monomers with triallyl monomer in varying functional group molar ratios (stoichiometric or 50 mol-% excess off-stoichiometric). No photoinitiator or other additives were used during the thiol-ene crosslinking process. First, thiol-ene solution was poured into a PDMS mold, featuring the microstructures and cured without any cover plate by exposing to UV for 5-20 min from a UV flood exposure lamp (nominal power of 225 mW/cm²).

The bonding protocol as such was adopted from elsewhere [221]. For bonded thiol-ene chips (I, II, IV), the cover layer featuring inlet structures or only the outer edges of the chip was prepared in a similar manner as above. The cured top and bottom layers were laminated and the bonding was finalized with additional UV exposure of 2-5 min. The thiol-ene layers were preheated to 70°C before bonding to soften the polymer and to obtain a uniform seal between the layers. The ready-made, cured (and bonded) chips were stored at RT in the dark and under atmospheric pressure.
4.5 Characterization of material properties

The material and physico-chemical properties of different thiol-ene compositions were characterized in detail for each application. The wettability and surface chemistry were evaluated by water contact angle, electroosmotic flow, and surface thiol density measurements. Also the solvent compatibility and mechanical strength were analyzed for applications requiring special solvent and pressure tolerance. To study in more detail the resolution of structures and surface topographical aspects, scanning electron- and atomic force microscopes as well as x-ray photoelectron spectroscopy were used.

4.5.1 Bulk properties

Optical properties
Optical properties of different thiol-ene compositions were studied by molding thin thiol-ene sheets (height 0.8-1.5 mm) with and without photoinitiator (0.17% TPO-L) and absorbance measurement by Varioskan LUX well plate reader. The obtained absorbance values were normalized to 1 mm height. For more detailed optical evaluation, the limit of detection (LOD) and limit of quantification (LOQ) determinations were performed for stoichiometric and triallyl-rich thiol-enes according to International Council for Harmonisation (ICH) validation guidelines using umbelliferone (near-UV) and fluorescein (Vis) as model compounds for MCE-LIF experiments (n=4 run-to-run). The LOD (signal-to-noise ratio S/N = 3) and LOQ (signal-to-noise ratio S/N = 10) were calculated based on the residual standard deviations of the response of the analytes and the slope of the linear fitting.

Glass transition
Glass transition values of different thiol-ene compositions cured with and without photoinitiator (0.17% TPO-L) (n=2 each) were analyzed by differential scanning calorimetry (DSC) with a three-segments method: 1) cooling to -35°C, 2) isothermal sequence for 3 min, and 3) heating from -35°C to 100°C at a rate of 20°C/min with nitrogen as a purge gas. The glass transition temperatures were determined by STARe software.

Tensile strength
The tensile strength of different thiol-enes was determined at RT using an Instron tensile testing machine by using test specimens of 1×13×76 mm³ according to the ISO 527-3 test conditions.
Experimental

Solvent compatibility tests
The solvent compatibility of different thiol-ene compositions were studied by immersing a test piece (average h=0.8-0.9 mm, A=1 cm²) into 1 mL of the solvent for 1 h and then for 4 days, followed by visual monitoring for any swelling, degradation, or defects.

4.5.2 Surface properties

Contact angle measurements
The wetting properties of different native thiol-ene compositions and gold nanoparticle functionalized surfaces were characterized by advancing and receding water contact angle measurements (I) and by sessile water drop contact angle method (II) by contact angle goniometer and optical contact angle and surface tension meter, respectively (n=3-5 spots). AttensionTheta or OneAttension softwares were used to extract the contact angles by Young-Laplace fitting. The advancing contact angle was measured by increasing the droplet volume from 2 μL to 8 μL and the receding angle from 8 μL to 0 μL, both at a rate of 0.1 μL/s.

Surface charge and surface chemistry
The surface charge of thiol-ene chips were analyzed by two methods: EOF determination by the current monitoring method [233] and indirectly by MCE-LIF monitoring of migration times of selected fluorescein dyes holding identical electrophoretic mobility, but different lipophilicity (logD ranging between -1.94 and 8.58) (II).

For EOF determination (n=4 run-to-run), the microchannel was filled with high and low conduction buffers, 20 mM and 15 mM, respectively, and under applied electric field (typically between 220 and 450 V/cm) the electrical current drop between the buffers was recorded by a PicoScope 2203 AD converter and PicoLog software. The EOF was determined based on the electrokinetic filling time relative to the applied electric field strength by the equation:

$$\mu_{eo} = \frac{L}{t \times E},$$

where L is the length of the microchannel (in centimeters), t is the time elapsed (in seconds), and E is the applied electric field strength (in V/cm).

For MCE-LIF based surface charge analysis, the migration times (the apparent net mobilities) of four fluorescein conjugates were monitored (n=5 run-to-run). The MCE analyses were performed in 20 mM sodium borate buffer (pH 10.0) containing 5% of 2-propanol at 650 V/cm.
In addition, the surface chemistry, mainly thiol functional group density, was evaluated (n=2-3 chips). The amount of thiol groups on the fabricated thiol-ene chip was quantitated by a solution of Ellman's reagent (DTNB) [234] run through the micropillar chip at 5 μL/min. The collected yellow-colored product 2-nitro-5-thiobenzoate (TNB) was analyzed by UV absorbance (412 nm) detection by Varioskan LUX Multimode Microplate Reader. The number of free (reacted) thiols was determined using molar extinction coefficient of ε= 14 150 M⁻¹cm⁻¹[235].

The surface functional groups (evaluated by thiol peak at 2550 cm⁻¹ and =CH₂ at 3080 cm⁻¹) of different thiol-enes were analyzed by Fourier transform IR spectroscopy (FTIR) instrument with attenuated total reflectance (ATR) accessory. The spectra were recorded at 4000–650 cm⁻¹ with a resolution of 4 cm⁻¹ using OPUS 5.5 software (n= 3 test pieces).

Surface topographical analysis
SEM was used for imaging and to confirm the micrometer-range structure quality for each chip design. The thiol-ene samples were attached onto the sample stage with a carbon-coated double-sided tape and ~5-nm-thick coating was obtained by platinum sputtering for 25 s (by 30 mA).

The native thiol-ene and gold-nanoparticle functionalized surfaces were analyzed by atomic force microscopes (AFM) and X-ray photoelectron spectroscopy (XPS) elemental analysis (n=2 spots each). AFM images (1024 × 1024 pixels) were captured using gold-coated silicon cantilever (tip radius of 10 nm) and were analyzed by SPIP software. XPS spectra were captured by using monochromatic A1 Kα x-ray source excitation with pass energies of 187.85 eV for survey and 29.35 eV for high-resolution spectra. The atomic concentration of the different elements was calculated by the area of the peaks and corrected for the sensitivity factors using MultiPak v6.1A software. The details of AFM and XPS measurement parameters can be found in the original publication I.

4.6 Functionalizations

Off-stoichiometric thiol-ene compositions were utilized for surface functionalizations. Thiol-rich thiol-enes were used for an immobilized enzyme microreactor study, where the enzyme was linked on a thiol-rich surface via gold-thiol chemistry (I). The immobilization of chymotrypsin (CHT) on the thiol-rich surfaces was achieved by thiol-gold interactions and included two steps: 1) micropillar array filled with the gold nanoparticle suspension and incubation at 4°C overnight and 2) incubation with CHT (1 mg/mL) at 4°C overnight. Between functionalization steps, the microchip was rinsed with fresh buffer solution. Before enzyme incubation, the disulfide bridges of CHT were reduced using 5 mM dithiothreitol (in buffer). Allyl-rich thiol-enes were
used for covalent linking of PEG to obtain inert and hydrophilic surface for bioanalysis in ESI-MS (IV). Thiol-linked PEG was grafted on allyl-rich μPESI channel covalently by UV. First, 1 μL of PEG-SH solution (1000 Mw, 1 mg/mL at each step) in 100% MeOH was pipetted into the sample introduction spot and the chip was exposed to UV-light for 5 min. The procedure was repeated with PEG-SH in 90% MeOH (twice), in 80% MeOH (once), and in 70% MeOH (twice). After the PEG-SH treatment, any excess of PEG was removed by sonicating the chip in 100% MeOH for 15 min.

4.7 Microchip designs used in the study

Depending on the final applications, different microchip designs (Figure 10) and dimensions (listed in Table 6) were used in this study.

![Figure 10](image)

**Figure 10.** Schematic of chip designs used in this work: 1) Micropillar array chip used for IMER study, 2) MCE-LIF chip, 3) MCE-ESI-MS chip, and 4) μPESI chip. SI sample inlet, SW sample waste, BI buffer inlet, BW buffer waste, and SLI sheath liquid inlet. Dimensions not to scale.

**Table 6.** Channel dimensions for microchip designs used in this study (the design numbers indicate the different microchips presented in Figure 10).

<table>
<thead>
<tr>
<th>Microchip design</th>
<th>Channel width × height × length</th>
<th>Note</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 mm × 200 μm × 30 mm</td>
<td>Micropillar diameter of 60 μm</td>
<td>IMER (I)</td>
</tr>
<tr>
<td>2</td>
<td>50 μm × 20 μm × 55 / 85 mm</td>
<td>Cross or double-T inj. (length 100 μm)</td>
<td>MCE-LIF (II)</td>
</tr>
<tr>
<td>3</td>
<td>50 μm × 50 μm × 20 mm</td>
<td>Cross inj., SLI of 200 μm × 50 μm width × height</td>
<td>MCE-ESI-MS (III)</td>
</tr>
<tr>
<td>4</td>
<td>1 mm × 20 μm × 5.6 or 7.6 mm</td>
<td>Micropillar diameter of 20 μm</td>
<td>μPESI (IV)</td>
</tr>
</tbody>
</table>
4.7.1 On-chip enzymatic digestion (I)

The performance of microreactor was studied by chymotrypsin catalyzed hydrolysis reaction of a nine amino acid peptide bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and by mass spectrometric analysis. Thiol-ene microreactors were operated by coupling with a syringe pump, followed by fraction collection and off-line analysis. Microreactor activity was tested also at physiological temperature (37°C) by heating the chip with a resistive heater placed on the bottom of the chip. Mass spectrometric analysis of the enzymatic product was performed by direct infusion of normal ESI-MS mode by an Agilent 6330 ion trap. The product fractions were diluted 1:1 with sheath liquid (methanol-water 90:10% containing 0.2% acetic acid) and analyzed at a flow rate of 5 μL/min. The ion trap was operated in positive ion mode with a capillary voltage set at -3500 V and end plate offset at -500 V. The flow rate of nitrogen drying gas was 4.0 L/min at 325°C. The MS data were acquired by averaging 5 cycles over a mass range of m/z 100–2000 with maximum accumulation time of 300 ms.

4.7.2 Microchip electrophoresis (II, III)

For MCE (II, III), a standard electrophoresis chip design was used, featuring separation channel of 25-85 mm and simple cross or double-T (100 μm) injector. The effective separation length was 40 mm and 20 mm for MCE-LIF and MCE-ESI-MS analyses, respectively. Before use, thin PDMS sheets with inlet holes were attached on top of the inlets to increase the sample volume and to limit spreading of the sample and buffer aliquots over the chip surface. Before analysis, thiol-ene microchannels were sequentially rinsed with deionized Milli-Q water and the separation buffer or electrolyte solution for 2-5 min. The samples were introduced by applying a voltage difference between the sample inlet and sample waste, and when pinched injection was used a small focusing voltage was applied on the buffer inlet. During injection the sample outlet was floating. The MCE analyses were performed by applying a high electric field (250-800 V/cm) between the buffer inlet and outlet (II) or between the buffer inlet and sheath liquid inlet (III). To prevent sample leakage during separation, small push-back voltages were applied to the sample inlet and sample outlet during separation. A computer-controlled external high voltage power supply was used for the voltages.

4.7.3 Fluorescence detection (II)

Optical fluorescence detection was accomplished by LIF in the visible range (ex 488 nm) with an inverted epifluorescence microscope equipped with an N-PLAN 20×/0.25 objective and a continuous wave 488 nm argon laser. For detection in the UV range, an upright epifluorescence microscope equipped
with a Plan Neofluar 10×/0.30 and a pulsed UV laser was used. Both microscopes were equipped with a Hamamatsu R5929 PMT with integrated housing and a signal amplifier module and a PicoScope 2203 AD converter. PicoLog software was used for recording the signal from the PMT. The fluorescence emission was collected perpendicular from the microchip by detection window of 50 μm×200 μm (visible) or 50 μm×50 μm (UV range) using emission filters of 500-700 nm (ex 488 nm) or 400-700 nm (ex 355 nm). For fluorescence measurements, the peptides were labeled overnight with 2-fold molar excess of FITC solution.

4.7.4 On-chip electrospray ionization emitters (III, IV)
For chip ESI-MS, the microchips were incorporated with an integrated electrospray emitter (III, IV), and for MCE-ESI-MS chip (design III) an on-chip sheath liquid channel was connected to a separation channel. The microchips were placed on an xyz aligning stage and coupled to an Agilent 6330 iontrap mass spectrometer. The MS was operated in positive ion mode with nitrogen used as a drying gas and produced from compressed air by a Parker nitrogen generator. Data Analysis 3.4 was used for data acquisition and processing. For direct infusion, capillary voltage was set at -1200 V or -1500 V (III) or -1600 V (IV) and end plate offset at -500 V. The ESI voltage, typically 2–3.5 kV for design III and 1.5–2.5 kV for design IV, was obtained from an external power supply through a platinum electrode placed on the inlet. The electrospray current was typically ~60–200 nA, and the distance between the tip and the MS orifice was between 5 and 10 mm. The recorded m/z range was 100–2200. Other MS parameters can be found in the original publications. For MCE-ESI-MS (III), the separation voltages and their operations are explained in Section 4.7.2. The separation current (typically 30–40 μA) was divided at sheath liquid intersection and the excess current was led to ground through a 50 MΩ resistor. The electrospray performance of MCE-ESI-MS chips was studied by small drug molecule verapamil, orexin neuropeptides, and protein sample cytochrome c. For analytical performance, angiotensin peptides were analyzed in aqueous and non-aqueous MCE. For micropillar ESI-chip, the sensitivity of the chip was analyzed by angiotensin II peptide and verapamil, and the total performance was evaluated by cytochrome c protein, trypsin digested cytochrome c protein, and human foreskin fibroblast cell lysate. The MS analysis of the μPESI chip is excluded from this thesis.
5 RESULTS AND DISCUSSION

This chapter discusses the main observations and results related to thiol-ene microfabrication (5.1) followed by physico-chemical characterization of native thiol-ene compositions with a view to bioanalytical applications (5.2) and performance characterization of the developed thiol-ene microdevices in their intended use (5.3). Last, the main results of this thesis work are critically reviewed in comparison to the state-of-the-art analytical microsystems (5.4).

5.1 Thiol-ene microfabrication

Thiol-ene based microchips were fabricated by a non-cleanroom replica-molding method. The fabrication process of thiol-enes starts with a lithographically produced SU-8 master, but thereafter all of the steps from the PDMS mold to the final bonded thiol-ene device can be processed under standard laboratory conditions. The SU-8 masters were produced in the cleanroom to obtain the high μm resolution needed for the separation chips. However, the masters can also be made outside of the cleanroom [236] if needed. The infrastructure for thiol-ene molding includes only standard equipment such as a UV illumination source and oven. Another advantage of using molding for chip preparation is that each SU-8 master can be re-used for the PDMS mold at least 5-10 times and each PDMS mold for thiol-ene fabrication at least 5-10 times, thus reducing the cost of the final device. For example, with MCE and MCE-ESI masters featuring 9 (II) and 12 (III) chip units, respectively, over 900 chips can be produced from a single SU-8 master.

A difference between this work and previously published research is the additive- and initiator-free UV polymerization. Thiol-ene photopolymerization occurs even without initiators [220], although higher curing doses (dependent on the UV curing power) may be needed to polymerize the thiol-ene layers. For instance, the curing times used in this work were 5-20 min compared with tens of seconds by initiator-catalyzed thiol-ene systems [24, 199].

5.1.1 Feature resolution of thiol-enes cured without the photoinitiator

Thiol-ene chips with channel dimensions (width x height) ranging from 50 μm×20 μm (separation chips II, III) to 4 mm×200 μm (microreactor I) were successfully replicated by PDMS molding without PI, and the limit of thiol-ene structure dimensions was mainly dependent on the SU-8 master. Visual
Results and discussion

Inspection by light and scanning electron microscopy showed good channel resolution for each device (Figure 11).

**Figure 11.** Scanning electron microscope images of thiol-ene designs used in this study. A) pillar structures of micropillar-based IMER chip (I), B) Injection cross of a MCE separation chip (II), C) electrospray emitter of a bonded MCE-ESI-MS chip (III), and D) pillar structures and electrospray emitter of microPESI chip (IV). Figure D [237]. Adapted by permission of The Royal Society of Chemistry.

Polymer rigidity was noted to be critical for fabrication of thin structures such as electrospray emitter tips (III, IV) and high aspect ratio micropillars (I). The use of 50 mol-% excess of trithiol in the polymer clearly resulted in softer material than the 1:1 stoichiometric and ally-rich compositions, and the production of thin and sharp structures, such as emitters, was complicated. In addition, the elasticity of trithiol-rich thiol-enes hindered their use for pressure-demanding applications such as microreactor studies (I). The replacement of trithiol- with tetrathiol-functional-thiol monomer enabled fabrication of thin but strong structures, while still maintaining the thiol-excess on the surface (I, III).

Micropillars with height and width of 20 μm (aspect ratio of 1) were implemented on a lidless μPESI chip. The chip design was aimed at simple capillary flow-induced sample handling and use for electrospray ionization (IV). The μPESI chip design was based on previous work of silicon [176, 238, 239] and SU-8 [240] fabricated chips. The low-cost replication used here provided a clear improvement to silicon and SU-8-based chips since no
expensive and complex cleanroom methods were needed to produce good-quality pillar structures (Figure 11D). However, for implementing higher aspect-ratio micropillars on chip, the de-molding from the PDMS replica can be challenging. Previously thiol-ene-based high-aspect-ratio micropillar structures have been made by lithography [26, 241] and by contact liquid photolithographic polymerization (CLiPP) [200]. Although these methods provided pillars with aspect ratios of 4-11:1 (height to width) by well-controlled initiator/inhibitor ratios, for some bioapplications the use of initiator and inhibitor must be avoided. Moreover, when increasing the aspect ratio much over 4 the micropillars were vulnerable to collapse during the development step [26]. The molding process studied here enabled high-aspect-ratio (4:1) micropillar fabrication without deformations or pillar breakage during de-molding thanks to the good mechanical strength of the selected tetrathiol and triallyl-based thiol-ene network. The only critical step of pillar production by replication was noted to be the hydrophobicity difference between thiol-ene prepolymer mixture and PDMS mold. This caused air bubbles trapped inside the PDMS microwells and non-uniform micropillars (Figure 12A). The problem was solved by placing the mold with the thiol-ene mixture under vacuum for 2-5 min (Figure 12B).

5.1.2 Effect of photoinitiator and monomer composition on bulk properties

Thiol-enes mechanical properties (Tg, strength, and stiffness) can be tuned by altering the stoichiometric ratio of monomers in the polymer composition [24, 203] or by selection of the monomer’s structure [242, 243]. In addition, the increase of monomer functionality can enhance the thiol-ene network crosslinking, observed as increased Tg values [244]. A similar trend was noted in this study by changing the trithiol monomer to a tetra-functional monomer (Figure 13). The increase of thiol excess in the bulk resulted in lower Tg values,
probably due to a decreased amount of bulky allyl monomer [244]. Comparison of thiol-enes cured with and without initiator indicated roughly 5-15°C higher Tg values with the presence of PI, likely originating from higher polymer cross-linking. The Tg values were below 50°C, which are much less than reached with, for example, thermoplastics. In addition to Tg, the tensile strength of different native thiol-enes at RT were studied (III). As expected, trithiol-rich thiol-enes had the lowest strength, which increased with increasing allyl ratio. Tetrathiol-rich thiol-ene showed, however, a similar strength to allyl-rich thiol-ene, with a strength of ~50-60 MPa compared with 4 MPa for trithiol-rich thiol-enes. Most rigid thiol-enes were obtained by 1:1 stoichiometric molar amount and 50 mol-% allyl-excess compositions.

**Figure 13.** Tg values of thiol-enes polymerized by tetrathiol- (4T) or trithiol-based (3T) thiol monomer with triallyl monomer. Comparison of native thiol-enes and thiol-enes cured with 0.17% TPO-L photoinitiator (PI). Published at the MNE 2018 conference [245].

**Optical properties**

For optical applications, the material should have good optical clarity in the measuring wavelength and be free from impurities that might cause optical losses. The absorbance spectra were measured for the four native thiol-ene compositions, which showed high optical transparency at the visible range and progressive autofluorescence when going below 330 nm (Figure 14) (unpublished data). The recorded spectra were similar between the different thiol-ene compositions, consistent with the findings of Feidenhans’l et al. [222] and for OSTE+ epoxy-based thiol-enes [195]. The use of initiator in thiol-ene polymerization may cause reduced optical properties by generating colored by-product or higher autofluorescence [195, 220]. For this reason, the effect of addition of initiator was also tested and compared with initiator-free thiol-enes (unpublished data). Polymerization with 0.17% photoinitiator
slightly increased the absorption of the bulk polymers in the UV range for all compositions, to a greater extent with stoichiometric thiol-ene and allyl-rich thiol-ene than with thiol-rich thiol-ene.

For more detailed optical characterization, the LOD and LOQ were determined for native 1:1 stoichiometric and allyl-rich thiol-enes in the near-UV (ex 355 nm) and the visible (ex 488 nm) range (II) by MCE-LIF. As expected, the two thiol-ene compositions had similar detection sensitivity in the visible (LOD=40 nM, both compositions) and UV range (LOD=1.5 and 1.8 μM for stoichiometric and allyl-rich compositions, respectively). Comparison with commercial Borofloat glass showed somewhat similar sensitivity in the visible range (DL=30 nM), but 10 times lower sensitivity in the UV range (0.2 μM), probably due to the partial background fluorescence originating from the thiol-ene bulk material.

**Solvent stability**

Depending on the final application of the chip, the material must withstand either aqueous or dilute acidic or basic solutions such as the running electrolyte buffers in separation processes and/or organic solvents often used for functionalization reactions. Solvent stability of the thiol-ene compositions used is presented in Table 7. All of the thiol-ene compositions tolerated well alcohols and dilute acid and base solutions, excluding sodium hydroxide, in short (1 h) and long (4 days) -term exposure. Only trithiol-rich thiol-ene underwent partial cracking in long-term exposure to alcohols and acids. Comparison between the thiol-monomers indicated that tetrathiol-based thiol-ene had better solvent tolerance. In general, the thiol-ene formulations used did not tolerate well the tested organic solvents, and underwent cracking or clear material degradation, consistent with other common polymers.
However, thiol-enes are reported to tolerate many aliphatic and aromatic organic solvents not tested here (such as hexane and benzene) [246, 247] and to a greater extent than other thermoplastic polymers [210, 248].

### Table 7. Solvent compatibility of thiol-ene samples. + indicates good resistance and solvent compatibility, ± signifies limited resistance (cracking), and - signifies poor stability (dissolution/depredation). White: non-polar solvent, light gray: polar aprotic solvents and gray: polar protic solvents. na = not analyzed.

<table>
<thead>
<tr>
<th>Solvent compatibility</th>
<th>1:1 Stoichiometric</th>
<th>Allyl-rich</th>
<th>Trithiol-rich</th>
<th>Tetrathiol-rich</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Toluene</td>
<td>na</td>
<td>na</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>+</td>
<td>na</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>+</td>
<td>na</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Acetone</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>10% formic acid</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>10% acetic acid</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>2 M hydrochloric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10% ammonium hydroxide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 M sodium hydroxide</td>
<td>±</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol, ethanol, propanol</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

### 5.1.3 Effect of photoinitiator on bonding

As noted in the thiol-ene bonding section, thiol-enes allow strong bonding by lamination due to the active surface functional groups. With an initiator OSTE system, only two different surface chemistries are bondable. Although this is useful for multiple applications, for analytical purposes the different top and bottom surfaces may lead to band broadening, and hence, reduced performance [72]. The initiator-free curing used here allowed good bonding between two similar surfaces, and the bonding of the inlet (cover layer) and channels (bottom layer) (I-III) was done by simply laminating the two layers with visual alignment. Only in the case of a sharp electrospray tip (III) the bonding needed to be performed under stereomicroscope to ensure perfect alignment of the emitter area. According to preliminary in-house experiments, bonding of two similar surfaces that are fully crosslinked (either in the presence of PI or after receiving direct UV light) was not feasible.

The micropillar array device was utilized as a part of a flow-through enzyme reactor requiring pressure tolerance. For this reason, the bonding strength of micropillar chips was tested. The bonding strengths were measured for tetra-
thiol-rich thiol-enes and compared with trifunctional-rich thiol-ene (I). As expected, tetrathiol-rich chips can withstand pressures of $2.0 \pm 0.3$ bar ($n=4$ chips) or more (the maximum pressure tolerance of the used fluidic system was 2 bars), whereas the trithiol-based chips can only withstand pressures of $1.5 \pm 0.4$ bar ($n=4$ chips) due to the different rigidity of polymer networks. With tetrathiol-rich polymer, the bonding ruptured from the inlet area instead of from the micropillars, indicating strong adhesion from the relatively small bonding area of pillars to cover layer. Another interesting property of thiol-enes is that they can be reversibly bonded when heated above their glass transition temperature [223, 249]. This was tested by heating ready-made and UV-bonded micropillar chips, followed by delamination and re-bonding with 2 min of UV light. The re-bonded tetrathiol-rich chips withstand the same pressure at $2.1 \pm 0.1$ bar ($n=4$ chips) as on the first bonding. Moreover, lamination of thiol-ene layers could be performed multiple times before finalizing by additional UV. If misalignment occurred, it was possibly to re-do the lamination without damaging the structures, which was especially important for the high precision alignment needed for the ESI emitter tip.

5.2 Thiol-enes’ native surface properties

Many comprehensive studies of mechanical and polymeric properties of thiol-ene based microdevices can be found in the literature [24, 26, 200, 203]. However, the use of thiol-enes as separation devices set partly different physicochemical requirements for the surfaces. The surface chemistry and its overall stability are key parameters for analytical devices, especially for surface-sensitive applications such as microchip electrophoresis. In addition, in this research thiol-ene polymers were used without PI (in their native form), which was expected to emphasize the effect of monomer stoichiometry on the surface properties.

5.2.1 Wettability

Similar WCAs were observed for 1:1 stoichiometric, allyl-rich (II), and thiol-rich (I) thiol-enes. The static WCAs of allyl-rich and stoichiometric 1:1 thiol-enes were typically 70-75º (II). For tetrathiol-rich surfaces, the advancing and receding contact angles were 80º and 15º, respectively (I). The effect of curing time on WCAs of stoichiometric 1:1 and allyl-rich compositions was also studied (Figure 15). For 1:1 thiol-ene, the wetting was dependent on the fabrication curing time. When the curing dose was increased, the WCAs decreased from the initial 90-95º to about 70º. The wettability of allyl-rich surface was not affected by the UV dose.
Results and discussion

5.2.2 Functional groups

The surface chemistry of tetrathiol-rich thiol-enes was studied in more detail for further functionalization with gold nanoparticles. The free surface thiol functional groups can be analyzed by known reduction reaction with Ellman’s reagent, but quantitation of surface allyls is more difficult and was thus done by surface charge determination. Use of 50% molar excess of thiol monomer in the polymer bulk resulted in free thiol groups on the surface, with an average density of 162±16 nmol/device (n=3 titrations). As expected, the thiol density on the surface varied based on the stoichiometric ratio of thiol and allyl monomers, similar to the work of Lafleur et al. [25, 204]. Interestingly, also the stoichiometric and allyl-rich surfaces maintained free thiol groups, with the amount of free thiols on stoichiometric surfaces being 2.2±0.3 nmol/device (n=3) and on allyl-rich surfaces 2.1±0.05 nmol/device (n=2). The effect of UV curing on thiol surface chemistry was studied by curing times of 3-15 min on stoichiometric 1:1 thiol-enes. The observed decrease in residual thiol groups on a stoichiometric 1:1 surface followed the same trend as that noted with surface charge determination. This indicates that higher curing doses can fully polymerize the surface if free thiol and allyl groups are present.

5.2.3 Surface charge

The surface charge was studied for 1:1 stoichiometric and allyl-rich thiol-enes since they were deemed most suitable for MCE. Thiol-rich thiol-enes were excluded due to relatively strong biomolecule adsorption when used in their native form. The surface charge of native 1:1 stoichiometric and allyl-rich thiol-enes was analyzed by MCE-LIF and EOF determination. Both studied thiol-

Figure 15. Effect of UV curing dose on water contact angles (WCA, degrees) of 1:1 stoichiometric thiol-ene and allyl-rich thiol-ene surfaces.
ene compositions showed high cathodic EOF, indicated by the apparent net mobilities ($\mu_{\text{app}}$) of the four model fluorescein compounds and by the current monitoring method used for EOF determination. The effect of fabrication conditions on surface charge was evaluated with curing doses from 50 to 350 J/cm² (II) (corresponding to curing times of 5-25 min). The surface charge was affected by the varying curing times on both thiol-ene compositions. An increase of curing time produced an increase of $\mu_{\text{app}}$ from 1.3 to $2.4 \times 10^{-4}$ cm² s⁻¹ V⁻¹ in 1:1 thiol-ene channels. In allyl-rich channels, the opposite effect was observed, with $\mu_{\text{app}}$ values slightly decreasing from 2.6 to about $1.8 \times 10^{-4}$ cm² s⁻¹ V⁻¹ (Figure 16). The surface charge reached a stable state at a curing dose of 200 J/cm² (excitation time of 15 min), after which only minor changes of $\mu_{\text{app}}$ values were observed, indicating full polymerization of the surface [190]. The detection difficulties of the most lipophilic compound FDL were related to wettability changes of thiol-ene surface with low curing doses (Figure 15).

The surface charge was also studied over a pH range of 4.5 to 12 and compared with commercial glass microchip (Figure 17). For these studies, the optimal curing doses were chosen for both thiol-ene compositions, being 270 J/cm² for 1:1 thiol-ene and 70 J/cm² for allyl-rich thiol-ene. The EOF was similar between thiol-ene compositions and comparable to that of glass, varying from $2.5$ to $5.8 \times 10^{-4}$ cm² V⁻¹ s⁻¹ (depending on the electrolyte and pH used). Similar EOF mobility and pH dependence for native thiol-rich thiol-ene microchip were described by Mesbah et al. [230]. In summary, native thiol-ene microchips with excess of thiols [230], stoichiometric molar ratio, or excess of allyl functional groups (II) maintain high cathodic EOF, comparable to that of glass and in general higher or equal to other polymer materials (Table 1), making thiol-enes a suitable substrate for electrophoresis chips.
5.2.4 Storage stability

Polymer-based systems may suffer from reduced storage stability, reflected as changes in surface properties. Thiol-enes are reported to suffer reduced shelf-lives in prepolymer mixtures (premature polymerization), which can be overcome by various stabilizers [250]. However, the stabilizers as well as the photoinitiators may cause yellowing or rearrangements in the final crosslinked polymer [190], resulting in decreased performance over time. Thiol-ene surfaces remained stable for extended periods when optimized curing time was used. The surface charge of stoichiometric and allyl-rich thiol-enes (II) remained unchanged over 14 days of storage. Only on the 1:1 stoichiometric surface were small hydrophilicity changes observed during the first days of storing. Native thiol-enes did not face hydrophobic recovery, but instead stayed (relatively) hydrophilic (WCA of 70º) for the time period of the study. The surface stability was also evaluated by extreme long storing test (unpublished data). The thiol-ene samples were stored for 1-2 years (at RT and covered from light) followed by FTIR and absorbance analyses. The qualitative FTIR analysis showed that the surface functional groups (evaluated by thiol peak at 2550 cm⁻¹ and =CH₂ at 3080 cm⁻¹) remained unchanged for all compositions for the study period. Interestingly, the absorbance spectra of old 1:1 stoichiometric and allyl-rich thiol-enes were slightly changed in the near-UV range, whereas absorbance of thiol-rich thiol-enes stayed the same. However, in general the optical properties and surface functional groups evaluated by FTIR of the used native thiol-enes remained unchanged up to 1-2 years, which can be attributed to the use of additive free polymer.


5.3 Thiol-ene microdevices for peptide research

The bulk and surface characterization presented above provide a strong foundation for the development of thiol-ene based microdevices for peptide research. The first step in protein (and peptide) analysis is often sample digestion into smaller units, which are then separated (in microsystems by MCE) and identified commonly by mass spectrometry. Here, thiol-enes suitability for all of the steps were demonstrated for 1) an immobilized enzyme digestion device with the help of an immobilized chymotrypsin microreactor (I), 2) MCE separation of peptides by aqueous and non-aqueous conditions with a view to improving the sometimes limited resolution of peptides (II, III), and 3) identification by MS demonstrated in off-chip ionization mode (microreactors, I) and in two different on-chip ionization modes, including direct infusion chips (uPESI, IV) and integrated MCE-ESI chips (III).

5.3.1 Enzymatic digestion (I)

In this study, chymotrypsin was selected as a model enzyme due to its appropriate simple bradykinin model substrate hydrolysis reaction. Based on the material characterizations, the best thiol-ene composition and thiol-surface chemistry for the microreactor was 50 mol-% excess of tetrathiol based thiol-ene due to its available thiol functional groups and good material rigidity.

Enzyme immobilization possibilities

The efficiency of the microreactor is highly dependent on the enzyme immobilization method, and often covalent bonding is preferred. Here, the enzyme immobilization was achieved via gold nanoparticle-mediated linking by the thiol groups on the enzyme (cysteine residues), but also through thiol groups on the thiol-rich chip surface. The strong covalent Au-S coordination bond is known to efficiently link proteins to gold [251, 252] and has been demonstrated in, for example, the PET thermoplastic trypsin microreactor [96] and conventional capillary electrophoresis of trypsin [253] and L-glutamic dehydrogenase [109]. All of these required, however, pretreatments to first introduce the thiol groups to the surface. Off-stoichiometric thiol-rich thiol-enes provided inherently thiol groups on the surface capable of GNP bonding, and no surface pre-treatments were needed, making the system simpler. The micropillars of IMER chip were used as supports for GNP enzymes. Since the micropillars can be fabricated simultaneously with the microchip, no post-processing to implement support structures, such as commonly used monoliths, on-chip were needed.

GNPs of 10 nm were selected for enzyme linking. Dodecanethiol-functionalized GNPs (d-GNP) of 3-6 nm were used as a reference for native GNPs. First, functionalization of GNPs on the thiol-rich chip surface was
analyzed by XPS (Figure 18A), AFM (Figure 18B), and indirectly by water contact angle (WCA) goniometry (see details for WCA from I). To confirm the Au-S binding, thiol-rich thiol-enes were compared with allyl-rich surfaces. Surface analyses indicated GNP and d-GNP linking on thiol-rich surfaces, and, as expected, to a lesser extent on allyl-rich surfaces in the case of GNP. However, d-GNP did attach also on the allyl-rich surface, possibly by both hydrophobic and gold-thiol interactions. CHT immobilization was tested preliminarily by WCA analysis, which showed a clear change in the surface wettability for CHT-functionalized surfaces. Advancing contact angles shifted from the initial values of 80° (native) and 60° (GNP-treated) to clearly below 20°.

**Figure 18.** A) XPS surface elemental analysis of triallyl-rich, tetrathiol-rich, and gold nanoparticle functionalized surfaces and B) AFM images of differently functionalized thiol-ene surfaces: (1) thiol-rich treated with 10 nm GNP particles (2) allyl-rich treated with 10 nm GNP particles, (3) thiol-rich native surface, (4) thiol-rich treated with 3-6 nm d-GNP particles, (5) allyl-rich treated with 3-6 nm d-GNP particles, and (6) thiol-rich treated with toluene solvent. Sq = root mean square, Sdr = surface area ratio of the interfacial (real) surface compared with the area of the projected (flat) x,y plane.

**Immobilized enzyme microreactors**

Chymotrypsin can hydrolyze bradykinin from two potential cleavage sites next to the carboxylic side of phenylalanine Phe [254]. The hydrolysis of Phe8-Arg9 was noted to be more favorable, in accord with the findings of others [255]. The substrate bradykinin Bk1-9 and the hydrolysis product Bk1-8 were identified as double charged ions [M+2H]2+ of 531 and 453 m/z, respectively. Evaluation of the microreactor efficiency was done by comparing the intensity ratio of [M+2H]2+ ions from the substrate and the reaction product. The effect of stability, flow rate, and temperature on enzyme activity was tested, and good performance was observed for CHT-IMER. The reactor stability was evaluated by collecting fractions every 15 min, and stability for at least 105 minutes was observed, with average [M+2H]2+ metabolite ion signal stability of 3.6±0.3×10^7 cps (RSD 19 %, n=6, excluding first void volume fraction) (Figure
Increased CHT activity was observed with reduced flow rates (increased residence time) and increased temperature. Heating the chip to physiological 37°C increased the turnover ratio by 2- to 3-fold, whereas even greater turnover ratio was obtained by decreased flow rates (Figure 19B-C).

**Figure 19.** A) Photograph of the CHT-IMER with the fluidic couplings and the stability of the product ion intensity (m/z 453) over time. Error bars represent the standard deviation of the direct infusion signal intensity in ESI-MS. In all analyses, the substrate solution contained 20 μM bradykinin in 20 mM ammonium acetate (pH 8.2). B) Effect of flow rate on product/substrate conversion rate at RT for two IMERs 1 and 2. C) ESI-MS spectra obtained at a flow rate of 2.5 μL/min.

### 5.3.2 Separation of peptides in aqueous and non-aqueous modes (II, III)

The off-stoichiometric allyl-rich (50 mol-% excess) thiol-ene composition was selected for fabrication of microchip electrophoresis chips for fluorescence and ESI-MS detections due to good mechanical strength and sufficient solvent and surface stability. Separation performance of thiol-ene microchips was studied by analysis of angiotensins and substance P (6-11) peptides in aqueous electrophoresis by MCE-LIF (II) and by aqueous and non-aqueous electrophoresis by MCE-ESI-MS (III). Angiotensins are peptide hormones
having a biologically important task as indicators for activity of angiotensin-converting enzyme (ACE) and blood pressure regulation [256]. Angiotensins have been used in many earlier MCE studies and were therefore good comparison samples for evaluating a new material.

In aqueous conditions by LIF detection, the FITC-labeled peptides (Ang I, II, and Sub P) were baseline resolved in less than 50 s with repeatabilities (n=4) of migration times within 0.7-1.1 % RSD and peak areas within 6.1-16.7 % RSD (FITC as an internal standard). MCE-LIF analysis by allyl-rich chips showed good separation efficiency of $2 \times 10^5$ plates/m (Figure 20A, Table 8), comparable to values of separation of the same peptides with glass (this work II, Figure 20B), Ormocer ceramics [177], SU-8 [56], and traditional CE [257] and better performance than with PDMS chips [258].

![Peptide separation](image)

Figure 20. Peptide separation by MCE-LIF by A) allyl-rich thiol-ene microchip and B) commercial Borofloat glass microchip. In both runs, FITC-labeled peptides were all 2.5 μM in 20 mM sodium borate buffer (pH 10.0), electric field 400 V/cm, effective separation length 4.0 cm, and LIF detection 488 nm. Sub P= substance P(6-11), Ang I and II= angiotensin I and II, FITC= fluorescein isothiocyanate and * indicates FITC-related impurity.

“Aqueous” MCE-ESI-MS analysis of peptides Ang I and II was performed in 60:40 % water: MeOH (+ 1% AcA) BGE (Figure 21A). Additional 40% methanol in BGE was needed to obtain good electrospray, and the above-mentioned conditions are referred to as “aqueous conditions” when later compared with purely non-aqueous BGEs. Ang I and II peptides were analyzed in less than 30 s, with migration time repeatabilities of 2.4-2.7% RSD (n=4, Ang III as an internal standard). However, the similarity of pI values of angiotensin I and II (7.7 and 7.54, respectively [137, 259]) caused a challenge for separating the peptides in a short (2 cm) MCE-ESI chip. The plate numbers $5.7-6.2 \times 10^3$ plates/m and the peak widths at half height of 3.3-4.1 s in MS were similar to other ESI-MS-based MCE chips, indicating minimal peak broadening due to sheath flow. Although the peptides were not separated by MCE-ESI-MS, the good migration time repeatability in both LIF and MS aqueous analyses indicates good surface stability arising from good feature resolution of replica molded thiol-ene separation channels.
Results and discussion

To solve the resolution problem of Ang I and II in MCE-ESI-MS, the peptide separation was studied further in non-aqueous electrophoresis (NACE) (Figure 21B, Table 8). Due to changes in the solvent’s properties, such as dielectric constant $\varepsilon$ – viscosity $\eta$ ratio, non-aqueous background electrolytes can provide better separation resolution and selectivity [260]. Thiol-enes have good solvent compatibility against methanol and ethanol, which were selected for BGE solutions for NACE. Another commonly used solvent in NACE is acetonitrile, but it was excluded due to poor compatibility with thiol-enes. A list of NACE solvent properties can be found in the Supplementary material of Publication III.

![Figure 21. Extracted ion chromatograms of angiotensin peptides I and II (each 100 μg/mL) in A) 20 mM ammonium acetate containing 40% methanol and 1% (v/v) acetic acid and B) 20 mM ammonium acetate in ethanol containing 1% (v/v) acetic acid. In both runs, the separation electric field was 250 V/cm, effective separation length 2.0 cm, ESI voltage 3.5 kV, and sheath liquid methanol-water 80:20 containing 1% (v/v) acetic acid.](image)

The apparent net mobilities of the angiotensins decreased from $\sim 4.5 \times 10^{-4}$ cm$^2$ s$^{-1}$ V$^{-1}$ in aqueous and methanol-based electrolytes to $1.2 \times 10^{-4}$ cm$^2$ s$^{-1}$ V$^{-1}$ in ethanol-based electrolytes (Figure 22A) due to the decreased EOF and the change in BGE’s $\varepsilon/\eta$ ratio. This affected the electrophoretic mobilities of the peptides, and better resolving power (resolution= 0.9) was obtained in acidified ethanol than in aqueous or methanol-based electrolytes (Figure 21B). In addition, the peak areas and the theoretical plate numbers increased 2-fold in ethanol-based electrolytes, probably due to improved electrospray and thus increased ionization efficiency (Figure 22B-C). However, peak widths in ethanol-based BGEs were much wider because of a longer migration time. Moreover, the migration time repeatability was poorer in ethanol-based BGEs than in aqueous or methanol BGEs (Figure 22D), and more rigorous optimization of separation conditions would be needed for further quantitative studies.
Results and discussion

Comparison of the aqueous and non-aqueous MCE-ESI–MS analyses by A) apparent mobility, B) plate heights, C) peak area, and D) migration time repeatability. The aqueous electrolyte used was 20 mM ammonium acetate containing 40% methanol with or without 1% (v/v) acetic acid. The non-aqueous electrolytes used contained either 10 mM (methanol) or 20 mM (ethanol) ammonium acetate in pure organic solvent, with or without 1% (v/v) acetic acid. The apparent pH ranges of the BGEs were 4.5–6.4 (with acid) and 7.1–8.1 (without acid), the electric field strength was 250 V/cm, and ESI voltage was 3.5 kV. The sheath liquid was methanol-water 80:20 containing 1% (v/v) acetic acid. The error bars represent the standard deviations of n = 4–5 repeated runs. Modified from the original publication [261] with permission.

Figure 22. Comparison of the aqueous and non-aqueous MCE-ESI–MS analyses by A) apparent mobility, B) plate heights, C) peak area, and D) migration time repeatability. The aqueous electrolyte used was 20 mM ammonium acetate containing 40% methanol with or without 1% (v/v) acetic acid. The non-aqueous electrolytes used contained either 10 mM (methanol) or 20 mM (ethanol) ammonium acetate in pure organic solvent, with or without 1% (v/v) acetic acid. The apparent pH ranges of the BGEs were 4.5–6.4 (with acid) and 7.1–8.1 (without acid), the electric field strength was 250 V/cm, and ESI voltage was 3.5 kV. The sheath liquid was methanol-water 80:20 containing 1% (v/v) acetic acid. The error bars represent the standard deviations of n = 4–5 repeated runs. Modified from the original publication [261] with permission.
### Results and discussion

Table 8. Separation parameters of angiotensins by MCE-LIF and MCE-ESI-MS obtained by allyl-rich thiol-ene microchips. Ang I=angiotensin I, Ang II=angiotensin II, Ang III=angiotensin III, and Sub P=substrate P (6-11) (n=4 each). The LIF detection parameters are for optimized MCE conditions. For MS detection, aqueous and EtOH containing 1% acetic acid represent the conditions with the best performance values.

<table>
<thead>
<tr>
<th>Detection and MCE conditions</th>
<th>Analyte</th>
<th>Migration time (s)</th>
<th>Peak width at half-height (w1/2 / s)</th>
<th>Theoretical plates (N/ m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIF/ aqueous</td>
<td>Ang I</td>
<td>29.0 ± 0.5</td>
<td>0.8 ± 0.1</td>
<td>2.1 x 10^5</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>30.9 ± 0.6</td>
<td>0.8 ± 0.1</td>
<td>2.0 x 10^5</td>
</tr>
<tr>
<td></td>
<td>Sub P</td>
<td>27.0 ± 0.5</td>
<td>0.7 ± 0.1</td>
<td>2.0 x 10^5</td>
</tr>
<tr>
<td>MS / aqueous + 1% AcA</td>
<td>Ang I</td>
<td>17.7 ±1.1</td>
<td>3.9 ± 0.3</td>
<td>5.7 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>19.1±1.7</td>
<td>4.1±0.5</td>
<td>6.2 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Ang III</td>
<td>16.7±1.4</td>
<td>3.3 ± 0.4</td>
<td>7.7 x 10^3</td>
</tr>
<tr>
<td>MS/ non-aqueous (EtOH + 1% AcA)</td>
<td>Ang I</td>
<td>70.1±20.0</td>
<td>10.1±2.3</td>
<td>1.1 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Ang II</td>
<td>88.8±23</td>
<td>9.0±1.4</td>
<td>2.2 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Ang III</td>
<td>67.0 ±18</td>
<td>8.1 ±1.5</td>
<td>1.6 x 10^4</td>
</tr>
</tbody>
</table>

### 5.3.3 Mass spectrometry interfacing on-chip (III, IV)

A simple method to integrate microchips with ESI-MS is off-chip capillary. This is, however, impractical for separation purposes due to a decreased separation resolution, and a better approach is on-chip ionization. In this work, on-chip electrospray chip designs were fabricated by the replication method. Two different ESI designs were fabricated from thiol-ene: an open channel format (lidless) micropillar array ESI (μPESI) chip and a closed, bonded electrophoresis separation ESI chip.

Good solvent compatibility is a critical requirement for microchip ESI-MS. Insufficient solvent resistance may cause polymer material (e.g. monomers) [66], developers from the fabrication process [185], or other additives to leak from the bulk material and cause deterioration of MS spectra quality. For this reason, the chemical background of different thiol-ene compositions were tested prior to MS measurements. Monomer leaching from different thiol-ene compositions was evaluated by direct infusion MS studies and drug molecule verapamil (m/z 455.4) (Figure 23). The allyl (m/z 250.1), trithiol (m/z 399.1), and tetrathiol (m/z 489.0) monomers leached out from the chip whenever used in excess ratio in the bulk thiol-ene. The increase of curing time from 10 to 20 min was also tested to reduce monomer leaching. The curing time did not reduce the background interference, but efficient flushing of the chip prior to use was sufficient to eliminate the background.
Results and discussion

**Figure 23.** ESI–MS spectra of different thiol-ene chips (cured for 10 min) fabricated from A) allyl-rich, B) 1:1 stoichiometric, C) trithiol-rich, and D) tetrathiol-rich compositions. The sample solution was 5 μM verapamil in methanol-water 80:20% containing 1% acetic acid, and the ESI voltage applied was 3 kV. Picture modified from the original [262] with permission.

**Micropillar electrospray ionization chip (IV)**

Lidless μPESI-MS chips, fabricated from silicon [176, 238, 239] or SU-8 [240], have been developed to solve the problems of channel clogging faced with common direct infusion nanospray methods. In addition, the lidless micropillar array format induces capillary flow towards the ESI tip without the need for external pumps and provides easy sample handling. However, fabrication of silicon or SU-8 chips requires cleanroom facilities and relatively complicated fabrication. Here, μPESI chips were produced by the thiol-ene replication method, thus at low cost and with a simple protocol.

The μPESI chips fabricated from native allyl-rich thiol-enes provided efficient capillary self-filling, but only with high (>90%) organic content solutions. In various bioapplications, such as metabolite analyses [263], the sample volumes are extremely low (few μLs or less) and even slight sample adsorption will cause sensitivity issues for measurements. For these reasons, the thiol-ene μPESI chips were coated with thiol-PEG by UV grafting. After the PEG functionalization, the capillary flow was clearly improved, allowing use of even 100% aqueous solvents. This same protocol was used for another study [261], where the PEGylation reduced the surface hydrophilicity from an initial value of static WCA of 80º to 50º. Furthermore, the higher wettability of non-functionalized areas of μPESI chips reduced cross-contamination between neighboring tips and decreased sample spreading from the tip. This improved the ESI, leading to remarkable sensitivity and analysis of various biomolecules (see details of MS sensitivity measurements in IV).
Microchip electrophoresis-electrospray ionization chip (III)
The performance of the bonded, thiol-ene ESI emitters was examined by comparing the total ion current (TIC) and the extracted ion current (EIC) of drug molecule verapamil stabilities by direct infusion. The most rigid thiol-ene compositions, allyl-rich (50 mol-%), tetrathiol-rich (50 mol-%), or 1:1 stoichiometric, provided stable electrospray with TIC stabilities of 4.6-7.0% RSD, whereas their EIC stabilities were 6.1-8.9% RSD (n = 3 chips, over 2 min range) (Figure 24). As explained previously, the trithiol-rich composition caused elastic tip structures and poorer TIC and EIC stabilities of 9.6% and 16% RSD, respectively. Thiol-ene emitters, excluding the trithiol-rich composition, could maintain stable ion current stability for as long as 20 min with 10.4% RSD. The chip-to-chip repeatability was 13.3% RSD (n = 4 chips). The obtained stability values were in a similar range to other reported microfabricated emitters, made of glass [31], SU-8 [56], organically modified ceramics [177], or PDMS [184, 264], indicating that the presented replication method is suitable for producing on-chip sharp emitters. The feasibility of the allyl-rich thiol-ene emitter was demonstrated also for direct infusion of a cytochrome c protein, showing good accuracy (12230.5 ± 0.5 Da, 0.004% accuracy) in terms of molecular weight.

Figure 24. EIC of verapamil 455.4 ± 0.5 amu obtained by direct infusion from thiol-ene chips (cured for 10 min) fabricated from A) allyl-rich B) 1:1 stoichiometric, C) trithiol-rich, and D) tetrathiol-rich compositions. The sample solution was 5 μM verapamil in methanol-water 80:20% containing 1% acetic acid with ESI voltage of 3 kV. Picture modified from the original [262] with permission.
5.4 Summary and critical review of the work

The key uniqueness of this thesis work arises from the possibility to photopolymerize thiol-enes without the initiators. This resulted in substantial differences compared with other parallelly developed and reported thiol-ene based devices which were fabricated using photoinitiator. The key findings of the work were as follows and are reviewed more thoroughly below in the same order:

- Bulk properties were somewhat unaffected whether or not thiol-ene polymerization was conducted with the initiator or in the absence of it
- Photoinitiator-free curing was shown to be crucial for bonding of two thiol-enes with similar surface chemistry
- In the absence of the photoinitiator, not only the monomer ratio, but also the curing dose affected the surface properties
- Thiol-gold chemistry enabled straightforward enzyme immobilization on thiol-rich surfaces, whereas PEG-coating of allyl-rich surfaces enabled elimination of nonspecific surface interactions
- Native stoichiometric and allyl-rich thiol-enes supported EOF, which permitted their use also in MCE applications
- By optimizing the monomer composition, thiol-ene based on-chip emitters providing stable electrospray could be fabricated and integrated with the MCE separation unit

As already well-studied by others [24, 200, 203], the mechanical bulk properties of thiol-enes can be easily tuned by the selection of monomers’ functionality and stoichiometry. Also here, mechanically different polymer networks were produced. The most critical parameter with a view to the possibility to fabricate thiol-ene based analytical devices by replica-molding was the polymer rigidity, which could be tuned by adjusting the monomer ratio. Allyl-rich thiol-enes were most suitable for MCE and ESI-MS chips (II, III, IV). For biocoupling, a thiol-rich surface was preferred due to inherent surface groups feasible for the thiol-gold bonding. By using a tetrafunctional thiol monomer instead of a trifunctional thiol monomer, sufficiently rigid polymer network, incorporating free thiol functional groups, was achieved, which facilitated replication of the high-aspect ratio micropillar arrays (I). Micropillars with aspect ratio as high as four were successfully produced by replication without bending or deformations. Earlier, similar thiol-ene micropillar arrays have only been achieved via lithographic patterning in the presence of the photoinitiator [26, 241].

Thiol-enes cured in the presence of the photoinitiator showed only slightly higher glass transition temperatures (50°C) than those cured without the photoinitiator (40°C). In general, the Tg values were much more lower
compared to many other thermoplastics [37], which may limit the use of thiol-enes in high-temperature applications such as polymerase chain reaction. However, Tg values as high as 115°C have been reported for certain commercial thiol-ene formulations, such as the dual-cure OSTE+[207]. Also the optical properties were somewhat similar between different thiol-ene compositions with and without the photoinitiator. The optical transparency of thiol-enes was shown to be comparable to low fluorescent Borofloat glass in the visible range and slightly less in the UV range. For applications requiring wavelengths below 330 nm, other materials, such as PDMS [155] or COC [156], still remain superior to thiol-enes.

Curing of thiol-enes without the photoinitiator was shown to have the most substantial impact on bonding two alike thiol-ene layers (I-III), which is a clear benefit over thiol-enes cured in the presence of the photoinitiator as well as most other microfabrication materials (apart from PDMS). In the absence of the photoinitiator, the changes in curing time also affected the surface properties (charge and wetting), especially those of stoichiometric thiol-enes. By increasing the curing dose, the surfaces reverted toward a more hydrophilic state, which typically decreased the nonspecific surface interactions. In general, native thiol-enes’ wettability (evaluated by water contact angles) was in the range of 70º-80º. At lowest curing doses, small changes in the surface properties were shown to occur during the first days of storage suggesting progressive curing of the polymer even after UV exposure. The off-stoichiometric allyl-rich surfaces were more stable against changes in the UV curing dose. For example, the allyl-rich surfaces remained stable over the 2-week study period, and even 1 year after fabrication, similar surface properties were observed by FTIR.

The thiol-rich surfaces were mainly used for facilitating biocoupling reactions with particular emphasis on gold nanoparticle mediated enzyme immobilization. The developed IMER performed in a robust manner at RT and at physiological temperature with a stable enzyme activity even in continuous use. Also, the proposed immobilization strategy can be easily extended to any proteolytic enzymes, such as trypsin, due to feasible thiol-gold chemistry. Until the present work, most IMERs implemented with the help of thiol-enes have exploited functionalized allyl-[226] or thiol-rich [228] surfaces for chemical coupling reactions. Besides, allyl-rich surfaces were functionalized with thiol-linked PEG for bioapplications demanding high surface inertness. Simple grafting was achieved via click reaction, and due to enhanced surface wettability as the result of PEG modification, even 100% aqueous solutions could be analyzed by the μPESI chip.

Thiol-enes, especially the allyl-rich composition, were found to be suitable substrate material for MCE (II) due to stable, inherent cathodic EOF varying from 2.5 to 5.8×10⁻⁴ cm²V⁻¹s⁻¹. Thiol-rich surfaces as such were less suitable for MCE due to the strong nonspecific interactions between the analytes tested and the thiol-rich surface. MCE-LIF analysis of the angiotensin...
peptides showed high separation efficiency (II) with comparable performance to both previously reported cleanroom fabricated devices [177] and conventional CE [257]. The separation efficiency of angiotensin peptides was also compared between aqueous and non-aqueous electrophoresis (III). The use of non-aqueous conditions was shown to improve the ionization efficiency and, under certain conditions, the resolving power compared with aqueous background electrolytes. However, the peaks were relatively wide compared with aqueous conditions, and more comprehensive optimization of separation conditions is needed to reach optimum performance in non-aqueous mode.

In this thesis, for the first time, on-chip electrospray emitters were prepared from thiol-enes by replica molding (III, IV). The monomer ratio had a huge impact on the quality of the MS spectra, since emitter fabricated out of the off-stoichiometric compositions were shown to give rise to substantial amount of leaching monomers similar to e.g. PMMA [185] and PDMS [167] substrates. However, the uncured monomers could be flushed away in a straightforward manner by simply rinsing the channel with buffer prior to use. In terms of feature resolution, both lidless μPESI chips and MCE-ESI chips were equally well fabricated, although the lack of bonding increased the simplicity of fabrication of the μPESI devices. Correspondingly, the most time-consuming step of the MCE-ESI chip fabrication was the bonding, which required manual alignment of two layers of sharp emitter tips under stereomicroscope. In a later work [232], even 3-dimensional tapered emitter tips have been replicated from stoichiometric thiol-enes using PDMS molds prepared with help of 3-dimensional micromilled masters. These emitters provided similar ESI performance to the ones presented here (TIC stabilities <10% RSD). In all, the thiol-ene replica molding methods developed in this thesis work lay the basis for versatile use of thiol-ene photopolymerization in customization of bench-top test devices. The developed methods may not fulfill the requirements of parallel mass manufacturing, but thiol-ene photopolymerization rather appears as a strong competitor to PDMS soft lithography, which has dominated the rapid chip prototyping efforts for decades.
6 CONCLUSION

Thiol-ene thermosets have been proposed as new, alternative polymer platforms for microfluidic devices due to their low cost chip production and active surface chemistry arising from off-stoichiometry. Thiol-enes have already found wide use in multiple research areas of LOC technology, but they have not been thoroughly investigated as analytical devices.

Already shown by others, thiol-enes can be processed by simple and lower cost fabrication techniques, such as fast replica molding and large volume imprinting and hot embossing. However, most of these works rely on photoinitiator based systems, and the knowledge of fabrication of thiol-enes in the absence of photoinitiator is partly lacking from the literature or have not been systematically studied. In addition, especially for analytical applications the overall stability of the device and its surfaces are in a key role, and thus it is desirable to avoid the use of any additional material components, such as additives and initiators, which are known to reduce the chip performance by time. For this reason, fundamental understanding of the properties of native thiol-enes is important.

In this thesis work, different kind of chip designs were replicated from native thiol-enes. Although thiol-ene polymer conversion kinetics is slower in the absence of initiator, the step-growth polymerization characteristics of thiol-enes provide fast polymer network formation even by UV light, and high structure fidelity microstructures can be obtained even without initiator. For analytical devices, the rigidity of the chip was important and similar to other thiol-ene thermoset it was easily tunable based on the monomer and stoichiometry selection. Thus, the replication of rigid and high resolution thiol-ene structures is not restricted by the use of an inititor, which is important for applications where polymer additives cannot be used. Often the initiator and its decomposition residuals posses also toxicity issues, which restrain their use in, for example, sensitive cell-based systems and medical applications, but also for the device itself followed by final disposal. In addition, as the bulk properties were relatively similar between native and initiator cured systems the same benefit of mechanical tuning of thiol-enes can be obtained even without initiator. Moreover, replica molding facilitates the use of microchip-based techniques in any routine laboratory because of the simple instrumentation, which reduces need of cleanroom based (hazardous) chemicals and facilities.

Two key advantages of thiol-enes over most other polymers are their tunable (inert/reactive) surface chemistry and the possibility for adhesive bonding similar to PDMS. According to the results obtained in this thesis work, both of these properties are strongly interlinked with the crosslinking degree, which can be best tuned in the absence of the photoinitiator. Since the
Conclusion

polymerization is slower in the absence of the initiator, the curing time can be used to control the crosslinking degree in order to achieve full curing in the bulk, yet free functional groups on the surface. This allows adhesive bonding of even two alike thiol-ene layers and was useful especially for stoichiometric thiols-enes, normally forming inert surfaces when cured in the presence of the photoinitiator. The number of surface functional groups was shown to be affected by the curing dose, and when designing, for example, surface-sensitive electrophoresis chips the curing conditions needs to be well controlled to achieve best stability and reproducibility.

Native stoichiometric and allyl-rich thiols-enes maintained strong EOF and were potential substrates for electrokinetic separations. The chips provided good storage stability by time, which can be also attributed to the use of additive-free polymer. Thiols-enes’ good solvent compatibility make them appropriate also for non-aqueous purposes. Native thiols-enes had slightly hydrophilic nature, which was in the same range of common thermoplastics. However, the other benefits of thiols-enes (surface chemistry and bonding) make them superior over PDMS and thermoplastics. Moreover, if further surface modifications would be needed, the active thiol and allyl functional groups can be used via click chemistry reactions, as shown in this thesis work.

In protein and peptide analysis MS is often the desired detection method. For most stable and robust system on-chip electrospray ionization is preferred. This is however difficult to achieve with out-of-cleanroom techniques and most current-day ESI-integrated devices still rely on glass or silicon substrates and thus cleanrooms. Here, thiols-enes’ mechanical tuning was utilized to select the most rigid composition, which enabled out-of-cleanroom replication of thin on-chip emitters. This allowed fabrication of MS compatible devices in low cost, which could eventually promote the use of microchip techniques in routine MS based bioanalyses. The fabrication of the developed on-chip emitters was however strongly dependent on manual postprocessing, i.e., manual accuracy of the bonding two emitter layers. To obtain higher chip production efficiency, further design optimization should be made to fully reach the benefit of non-cleanroom fabrication of on-chip emitters.

All results presented in this thesis evidence the good feasibility of thiols-enes for analytical purposes. Thiols-enes can combine many of the properties of other materials, such as rigidity of thermoplastics, simple bonding of PDMS and controlled surface chemistry of glass. Here, the use of thiols-enes was demonstrated in isolated analytical units belonging to the chemical analysis sequence. Obviously, thiols-enes would also allow for building up fully integrated systems, which appears as a meaningful aim for future research.
7 REFERENCES


References


References

References


[84] C. Yu, M.H. Davey, F. Svec, J.M. Fréchet, Monolithic porous polymer for on-chip solid-phase extraction and preconcentration prepared by photoinitiated in
References

References


References

[165] Y. Tachibana, K. Otsuka, S. Terabe, A. Arai, K. Suzuki, S. Nakamura, Effects of the length and modification of the separation channel on microchip
References


References


References

transthyretin: a step toward an integrated microfluidic system for the follow-up of familial transthyretin amyloidosis, Analyst 143 (2018) 1077-1086.


References


References


