New Approaches to Preparation of Macroporous Monoliths for Use in Liquid Chromatography

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To my parents and my family,

To my teachers
Abstract
High performance liquid chromatography (HPLC) is one of the major techniques in separation sciences. Faster separation and higher efficiency are required to meet ever-growing demands. Despite numerous studies and achievements on improving mass transfer in particulate packings discontinuity seems to be the cornerstone drawback in their development. Macroporous continuous beds or monoliths are therefore a promising alternative to the particle medium. This thesis deals with preparation of new monoliths used as carrier for HPLC. Two different approaches were developed for two polymer systems. One was based on polycondensation of epoxy resins and polyamines which were components of an oil-in-water emulsion. An epoxy resin mixture was dispersed in aqueous polyamine phase with the aid of a surfactant. The other involved a traverse of a ready-made polymer solution around its upper critical solution temperature (UCST). In other words, linear polyamides, non-covalently crosslinked polymers, dissolved in a solvent at temperature higher than their UCST followed by slow cooling to below the critical temperature to precipitate the polymers. Partly re-established hydrogen bonds resulted in the formation of crystallites that interconnected into a network structure. Factors controlling morphology and porosity of final products were investigated. The study also deals with surface modifying for chromatographic applications. Functionalization pathways attempted in the thesis were quaterization of inherent amine of the epoxy-based monoliths and grafting tentacle ion groups via glycidyl methacrylate by atom transfer radical polymerization (ATRP) for ion exchange chromatography (IEC).

Keywords: monolith, polycondensation, dissolution-precipitation, epoxy-amine, polyamide, nylon, emulsion polymerization, characterization, protein separation, liquid chromatography.
This thesis is based on the papers listed below, which are referred to in the text by their corresponding Roman numerals.

I. Epoxy-Based Monoliths. A Novel Hydrophilic Separation Material for Liquid Chromatography of Biomolecules
   Anh Mai Nguyen and Knut Irgum
   Chemistry of Materials, 2006, 18, 6308-6315

II. Sizeable Macroporous Monolithic Polyamide Entities Prepared in Closed Molds by Thermally Mediated Dissolution and Phase Segregation
    Nguyen Anh Mai, Nguyen Thanh Duc and Knut Irgum
    Chemistry of Materials, 2008, 20, 6244-6247

III. Sizeable Macroporous Epoxy-Based Monolithic Supports for Flow-through Systems – Preparation and Characterization
     Nguyen Anh Mai, Dinh Ngoc Phuoc, Quach Minh Cam, Tobias Sparrman and Knut Irgum
     Submitted to Journal of Separation Science *

IV. Thermally Induced Dissolution-precipitation – A Simple Approach for Preparation of Macroporous Monoliths from Linear Aliphatic Polyamides
    Nguyen Anh Mai, Anna Nordborg, Andrei Shchukarev and Knut Irgum
    Submitted to Journal of Separation Science *

V. Functionalization of Epoxy-Based Monoliths for Ion Exchange Chromatography of Proteins
    Dinh Ngoc Phuoc, Nguyen Anh Mai, Quach Minh Cam, Andrei Shchukarev and Knut Irgum
    To be submitted

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerization</td>
</tr>
<tr>
<td>BA</td>
<td>Benzyl alcohol</td>
</tr>
<tr>
<td>BADGE</td>
<td>Bisphenol A diglycidylether</td>
</tr>
<tr>
<td>BDGE</td>
<td>Butadiol diglycidylether</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer-Emmett-Teller</td>
</tr>
<tr>
<td>DAH</td>
<td>Diaminohexane</td>
</tr>
<tr>
<td>DEGDB</td>
<td>Diethyleneglycol dibuthylether</td>
</tr>
<tr>
<td>DEGDE</td>
<td>Diethyleneglycol diethylether</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>EXP-IDA</td>
<td>IDA grafted epoxy-based monolith</td>
</tr>
<tr>
<td>EXP-NR$_4^+$</td>
<td>Quaternary ammonium bearing epoxy-based monolith</td>
</tr>
<tr>
<td>EXP-SO$_3^-$</td>
<td>Sulfonate-bearing epoxy-based-monolith</td>
</tr>
<tr>
<td>GMA</td>
<td>2,3-epoxypropyl methacrylate</td>
</tr>
<tr>
<td>GTGE</td>
<td>Glycerol triglycidylether</td>
</tr>
<tr>
<td>HETP</td>
<td>Height equivalent theoretical plate</td>
</tr>
<tr>
<td>$^2$H NMR</td>
<td>$^2$H Nuclear magnetic resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IDA</td>
<td>Imminodiacetic acid</td>
</tr>
<tr>
<td>IEC</td>
<td>Ion exchange chromatography</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>MIP</td>
<td>Mercury intrusion porosimetry</td>
</tr>
<tr>
<td>PA</td>
<td>Polyamide</td>
</tr>
<tr>
<td>PMDETA</td>
<td>Pentamethyldiethylenetriamine</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEPA</td>
<td>Tetraethylenepentamine</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
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<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
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1. Introduction

A book on separation science would not be completed without a discussion of the various chromatographic techniques. In liquid chromatography separation of a solute mixture is based on differences in relative interaction strength of the compounds with a porous solid bed (stationary phase) and a liquid (mobile phase) percolating through the bed. The two phases are chosen in such a way that the analytes spend significant time in both phases. In a successful chromatography one would expect solutes with different affinities for the stationary phase will emerge successively at the column outlet as narrow discrete bands. Figure 1 shows a schematic diagram of a liquid chromatograph.

![Diagram of a liquid chromatograph](image)

**Figure 1.** Schematic diagram of a liquid chromatograph

In the last few decades we have seen fast progression in the field of liquid chromatography with respect to instrumentation as well as developments of novel stationary phases and interaction modes to meet the stringent demands in quality control, and life sciences. A huge number of applications can be found in academic and industrial environments when browsing literature with the key word “liquid chromatography”.

- 1 -
In my doctoral project I have dedicated myself to the development of new monolithic materials for use as supports in high performance liquid chromatography (HPLC). As opposed to conventional particle-packed column, monolithic columns contain only one piece of material (“monolithus” means a single stone in Latin). The materials are characterized by a solid skeleton surrounded by interconnected voids/pores. The monoliths developed in this thesis must be characterized as rather unconventional polymeric packings for HPLC, considering their unusual methods of preparation. One kind of material developed is based on polycondensation of epoxy resins and polyamines; each component presents in a separate phase of an emulsion. The other is prepared from linear polyamides, i.e., ready-made polymers, using a thermally induced dissolution-precipitation approach. The research focused on investigation of factors effective in controlling the monolithic morphologies and on characterization of the final products. Attempts were also made to apply the materials to protein separation.
2. HPLC stationary phases

2.1 An Overview

2.1.1 What happens when a solute traverses a chromatographic column?

A mixture of solutes to be separated is injected into a HPLC column containing a separation medium, and the compounds get retained at the inlet as a narrow band. By development in elution mode, which is common in analytical and small scale preparative separations, a mobile phase (eluent) containing a compound that will compete with the solutes for active sites on the stationary phase is pumped through the column and consequently, pushes them out of the column according to their affinities with the separation medium. A solute with high affinity requires more volume of the eluent to elute and hence has longer retention time in the column.

Retaining solutes on stationary phase and releasing them to mobile phase stream where they are carried towards column outlet cannot be viewed as a simple two-step process. In reality, the traversal of a solute along a chromatographic column comprises numerous repeated mass transfer steps, which almost reach equilibrium, of the solutes between two phases. The faster the overall rate of mass transfer and the retaining and releasing reactions, the better separation power of the column is.

Solutes get into contact with interaction sites of a separation bed via both convective and diffusive flows. By means of convection solutes are propelled in macropores towards interaction centers on the stationary phase surface facing the mobile phase main stream, which actually percolates through monolithic stationary phases. The separation medium usually also contains a secondary pore system consisting of mesopores and micropores, which hold stagnant mobile phase. Solutes enter these pores, move towards hidden reactive sites and backwards to the flowing stream by diffusion. This intraparticle diffusion is slow process and is one of the main causes of solute band broadening due to the difference in travel time between solute molecules along various diffusive pathways. Diffusion limitation can therefore lead to (partial) overlaps of the solute bands. The diffusion also takes place within stationary and mobile phases.
themselves when the solutes move towards the interphase for mass transfer. As a result dimension becomes one of key parameters to take into account in development of new materials for HPLC.

Before discussing essential parameters of a chromatographic column it is sensible to clarify some technical terms used in the thesis, which could be ambiguous to readers because they may be named differently by others.

- **Macropores/throughpores, mesopores, and micropores.** Pores in separation media are often classified as macropores/throughpores, mesopores, and micropores based on the pore diameter, with definition trigger values of > 50 nm, 2-50 nm, < 2 nm, and respectively, according to IUPAC.

- **External porosity** refers to the void volume created by throughpores or macropores. In packed beds external porosity is voids between particles.

- **Internal porosity** indicates the porosity contributed by mesopores and micropores within solid structures of stationary phases.

- **Skeleton** refers to the solid structure of a monolithic bed.

- **Domain size** is the combined size of skeleton and throughpore which is considered as unit size of a network structure; it is difficult to determine exactly and requires electron spectroscopy for proper assessment.

### 2.1.2 Essential parameters of a chromatographic column

Whatever the objectives of a chromatographic separation, *e.g.*, scale (preparative or analytical) and types of analytes, some common essential parameters of a separation medium including hydraulic resistance, efficiency, retention ability and dynamic capacity have to be considered in the development of new stationary phases. Why are these parameters so important? And how will the morphology and chemistry of a material affect these parameters?

- **Hydraulic resistance** is a measure of how easy a fluid can percolate through a column. It is evident that columns with high permeability can be used at higher flow rates, or longer columns can be employed to afford larger number
of plate counts without collapse due to excessive pressure. Permeability of monolithic and packed columns have been extensively discussed by Guiochon.\(^1\) Beside the fact that permeability of packed columns increases with decreased particle diameter (\(\sim d_p^{-2}\)), packed beds share the same dependency on external porosity, \(\varepsilon_e\), as monoliths (Equations 1 and 2). Packed columns with external porosity ranging from 30-40 % of total column volume obviously have higher hydraulic resistance than the monolithic counterparts where \(\varepsilon_e\) is typically in the range of \(\sim 60-70\) %. In addition, monoliths with large and tube-like throughpores have higher permeability.

\[
\Delta P = \frac{\eta u_F L}{k_{p,F}} \quad (1)
\]

\[
k_{p,F} \sim \frac{\varepsilon_e^3}{(1-\varepsilon_e)^2} \quad (2)
\]

where \(\eta\) is the mobile phase viscosity, \(u_F\) the superficial velocity, \(L\) the column length, and \(k_{p,F}\) the superficial-based permeability.

- **Efficiency** is a measure of the axial dispersion inflicted on a solute band as it travels along a column. The efficiency is hence an indication of the separation power of a chromatographic column. Due to the fact that diffusion coefficients are finite and that longitudinal diffusion is not negligible, solute bands tend to spread as they move towards the column outlet. Based on the plate theory and the kinetic rate theory, van Deemter et al. proposed (with the assumption of linear isotherm) an equation (3) expressing contributions of various limiting processes on the band broadening. The height equivalent to a theoretical plate (HETP), \(h\), is conceptually the distance a solute travels between each interactive instance. It is a function of the linear velocity of mobile phase, the diffusion coefficients of solutes in both the mobile and stationary phases, and the column geometry.\(^2\)

\[
h = A + \frac{B}{u} + (C_m + C_s)u \quad (3)
\]
The A-term corresponds to the convective dispersion by flows through a tortuous separation bed. The B-term indicates the longitudinal molecular diffusion, which is dependent on the nature of analyte in question, on the molecular diffusion rate of the solute in the eluent, and on the type and density of the packing. The B-term is significant in liquid chromatography only for small molecules separated at low eluent flow velocities. The \( C_m \) and \( C_s \)-terms express the contribution from limited mass transfer of solutes in mobile phase and stationary phases, respectively. The C-term is of importance when dealing with high molecular-weight compounds, e.g., polymers and biomacromolecules, which have low diffusion rates. The contribution from the C-terms also increases with the mobile phase velocity. The combined contributions from the A, B and C terms take the form of a van Deemter curve (Figure 2), where a minimum exists at a certain flow rate, specific for each solute and separation condition. Chromatographic separations should thus be operated at a linear eluent velocity in the vicinity of the average HETP minimum for solutes to be separated, in order for the column to perform at peak efficiency.

As mentioned above, an ideal material for HPLC should promote mass transfer by convection through wide macropores and minimize the diffusion pathways by a shallow and non-tortuous mesopore system. Micropores cause very slow diffusion and complex sorption phenomena and should be avoided, at least when molecules small enough to enter these micropores are separated. It is worth noting that materials with narrow pore size distributions also usually offer even flow profiles. The mesopores are necessary to provide sufficient surface area for solute-stationary phase interaction.
Retention ability refers to the capability of a material in retaining compounds via interactions with functional groups on its surface. Separation calls for retention, and in order for a separation to be established the retention must therefore be strong enough for compounds to spend a significant fraction of the time attached to the separation material, but not excessively strong in order for the most retained compounds to be eluted in reasonable time scale using moderate eluents. Retention ability can be adjusted by manipulating the availability, identity, and topology of functional groups, and also by the phase ratio (the volume ratio of eluent to stationary phase). Tuning the surface chemistry as well as the porosity of stationary phases can thus result in high selectivity. A good example of the effect of functionality density on retention behavior of stationary phase is the comparison of separation power of cellulose gels grafted with polyallylamine (PAA) and diethylamine (DEAE) on human serum albumin and bovine serum albumin in anion exchange mode, as demonstrated by Kim. The denser distribution of amino groups of PAA-cellulose is believed to account for its stronger interaction and its higher selectivity (Figure 3).
Separation media should further not have any undesirable interactions, for example interactions of silanol group on silica supports with basic compounds, or between excessively hydrophobic surfaces with proteins. In general, the surface of separation media should be densely covered by designed functional groups. If this is not the case, shielding groups should spread over the remaining areas to prevent compounds from unintentional interactions. End-capping silica stationary phases by reactions with trimethylsilyl reagents targeting silanol group for reverse-phase chromatography and coating polymer surfaces with hydrophilic films\textsuperscript{5,6} when working with proteins are common approaches to solve the problem.

- **Dynamic capacity** expresses how much of a solute that a column can hold before it reaches the saturation level, when the compound is continuously driven through the separation bed. It is an important parameter, especially in preparative chromatography. Ways to enhance the dynamic capacity are via improvement of the mass transfer and enrichment of accessible functional group population. Once again the morphology of supports and their surface chemistry are decisive factors.

In practice, the dynamic capacity is usually expressed as the weight of bound analyte per weight or volume unit of an absorbent. It is measured by continuously feeding a column with a solution of analytes of known concentration until saturation, followed by extensively flushing the column with weak mobile phase to remove any un-bound analyte. The strength of mobile phase is then increased to completely elute the retained compound. Information about the dynamic capacity as well as the recovery percentage can be calculated.
by integration of the obtained chromatogram, which is known as a breakthrough curve.

2.2 Particulate packings

Spherical particles have been, and still are the dominant format of HPLC packings. In the last 50 years we have seen numerous efforts in tailoring the structures of particulate stationary phases coupled with novel approaches for manipulating other parameters of chromatographic systems, *e.g.*, control of temperature to improve the performance of HPLC columns.

Non-porous, sub-2μm particles have been synthesized by several groups aiming at improving mass transfer by totally excluding intraparticle diffusion phenomena.\(^7\)\(^-\)\(^8\) Despite the demonstrated advantages of substantially reduced analysis time, high efficiency, and high sensitivity, short columns packed with sub-2μm particles have not gained much acceptance in routine analysis due to their low sample capacity and excessive backpressures (> 100 MPa). Particles that small further impose new and stringent requirements for HPLC systems, which, *e.g.* on connection tubes and connectors, detector cells that have to be miniaturized to reduce extra-column band-broadening, the use of small columns in order to reduce frictional heating effects, development of frits with special porosity to retain the particles.

An alternatively route for enhancing the mass transfer without sacrificing capacity are to prepare particles with exceptionally large pores,\(^9\)-\(^11\) called “throughpores”, “gigapores” or “superpores”, through which solutes can traverse with the aid of convective flow while diffusive mass transfer takes place in mesopores or micropores (Figure 4). This is termed perfusion chromatography. The existence of “gigapores” allows part of the mobile phase to flow through the interior of particles, thereby improving the separation kinetics by reducing diffusion path length. Note that the particle sizes of such particles have to be increased to accommodate the very wide pores. Large interparticular voids and low filling volume of the packing, however, somewhat deteriorate their performance.
Throughpore particles are another option for limiting the diffusion pathway. Developed by Kirkland, the controlled porosity supports demonstrated superior performance both in gas and liquid chromatography. Beside efforts made on particulate sorbents chromatographic chemist have been working on high temperature HPLC to accelerate mass transfer by reduction of the mobile phase viscosity. This allows the use of columns packed with very small particles, where the optimal flow rates become higher because of short diffusion parts in the porous network. Low consumptions of solvents and high efficiency are the direct consequences of fast separations with high-temperature HPLC. Unfortunately, the use of high temperature is not preferable in some cases e.g. in separation of proteins which are susceptible to thermal denaturation.

2.3 Monoliths

2.3.1 Monolithic material - from ideas to reality

Despite a number of successes in dealing with mass transfer problems in packed beds, the thrust for novel formats of separation medium that could meet the growing demands for fast and highly efficient chromatography has been firing up scientists in the development of new stationary phases. Let us imagine that if we can extend the structure of “gigaporous” particles to whole column volume, it would be possible to completely remove interparticular voids. This requires that throughpores are enlarged to such an extent that all mobile phase can percolate through the separation bed at moderate back pressures. This would definitely have great impact on mass transfer and improve the column...
efficiency, and was probably the initial thoughts conceived by the pioneers in monolith area. The monolith concept in the field of HPLC could be traced back to the early years of 1950s but it had not gained any popularity until pioneering researches of Hjertén, Tennikova, Svec and Frechet, and Nakanishi and Tanaka.

Monolithic materials can be divided into two categories based on chemical nature, silica and organic polymer-based monoliths ( polymer monoliths for short hereafter). The two types are well distinguished from each other by their typical morphologies and main fields of applications. However, they share same advantages of high permeability and fast mass transfer which allow the use of long columns for high separation power. Consequently, monolithic columns can be used for complex mixtures and when fast analysis is required. The history of monolith evolution and comparisons of their characteristics with traditional particulate packings has been excellently reviewed in a number of publications. The following section will deal with discussions on monoliths derived from polymer and silica.

2.3.2 Polymer monoliths

• Preparation. This section is devoted to the synthesis of macroporous polymer monoliths with permanent porosity that persists even in the dry state. Free radical polymerization of vinyl monomers e.g. styrene-divinylbenzene, methacrylates, or acrylamides, in closed molds have been an almost exclusive method in preparing organic polymer monoliths for HPLC. The ring-opening methathesis reaction is a less common approach for polymerization of strained olefins. Polycondensation of epoxy resins and polyamines[Paper I and III], urea and formaldehyde are another mechanism used for preparing macroporous monoliths. By free radical polymerization a basic mixture is composed of mono- and polyfunctional vinyl monomers (crosslinkers), a solvent(s) which is is capable of dissolving all precursor components but is a poor (non-Θ) solvent (mixture) for the formed polymer, one of several porogen(s), and an initiator providing free radicals upon heating or UV radiation. The mechanism of pore formation and the control of porosity have been explicitly described by Svec and Frechet in a review published few years after their pioneering works. The polymerization process is initiated by
free radials from the decomposed initiator, and since the growing crosslinked polymer cannot dissolve in the non-$\Theta$ solvent it precipitates from the solution as nuclei as the conversion proceeds. The nuclei solvated by remaining monomers continue to grow and associate as clusters, which later contact with their neighbors to form an interconnected matrix of essentially globularly shaped entities. Macropores are formed where the solvent(s) is trapped in voids of the polymerization system, while micropores and mesopores form due to the incorporation of porogen(s) into polymeric skeleton. For HPLC applications porosity and flow characteristics of polymer monoliths have to be controlled carefully by means of three main factors, $^{32,33}$ polymerization temperature, porogen identity, and proportion of crosslinker. Average pore sizes become smaller as the polymerization temperature increases which gives rise to the formation of numerous nuclei. Poorer solvents result in an early onset of phase separation and the solvation of polymer chains by monomers during the early stage of polymerization explain the shift of pore size to higher ranges. In contrast, higher crosslinker level results in a decrease in pore size due to a reduced tendency of coalescence. Figure 5 presents the typical morphology of a polymer monolith.

![Figure 5](image)

**Figure 5.** Monolith prepared from a zwitterionic monomer ($N,N$-dimethyl-$N$-methacryloyloxyethyl-$N$-(3-sulfopropyl) ammonium betaine) and ethylene dimethacrylate as crosslinkers by photopolymerization. Reprinted with permission from ACS$^{34}$

- **Features.** The most attractive characteristics of organic monoliths are the ease of preparation and the availability of wide variety of crosslinkers and (functional) monomers. To be used as HPLC stationary phase monolithic beds can be prepared by post-synthesis modification of core materials [Paper V] or simply by one-step polymerization of monomers carrying functional groups.
Formats of monolithic separation media vary from membranes, disks, columns, capillaries to large bore preparative columns. Heterogeneous morphology of most organic monoliths which is characterized by interconnected globular entities forming dense clusters separated by relatively wide channels is still a challenge to material chemists. Efforts have been made to obtain connected-rod type structures with organic monoliths. Applications. Organic monolithic materials find most applications in the separation of macromolecules whose mass transfer relies on convection more than diffusion. Successful separations of biomolecules in different chromatographic modes such as affinity, hydrophobic interaction, reverse phase, and ion exchange emphasize their usefulness.

Drawbacks. Low surface area, tendency of swelling in organic solvents which leads to the dependency of mesopores and micropores on swelling effect and lower mechanical properties in comparison to silica counterparts are targets for ongoing research.

2.3.3 Silica Monoliths

Preparation. Macroporous silica monoliths prepared by the sol-gel approach from alkoxysilanes were a milestone in the field of monolithic materials. The hydrolysis and polycondensation of an alkoxysilane solution accompanied by phase separation results in the formation of a three-dimensional continuous silica gel skeleton surrounded by the fluid-filled interstices, which become interconnected macropores after the fluid removal. Tetramethoxysilane and poly(ethylene glycol) (PEG) are typical starting material and additive, respectively, for the preparation of silica monoliths. Original micropores on the silica gel network can be enlarged to mesopores by a subsequent treatment with ammonia at elevated temperature. Varying formulation of reactant mixtures and conditions of the post-gelation reaction with ammonia makes it possible to tune the domain size and the total porosity, as well as the mesopore size. silica monoliths are characterized by a bimodal porosity consisting of micrometer-sized macropores and mesopores in the tens of nanometer size range. Narrow pore size distributions imply homogenous pore structures throughout the material entities. A typical
morphology of a state-of-the-art silica monolith is shown in Figure 6. Currently, silica monolithic columns are available as encased “rods” of conventional diameters (Chromolith, Merck) and as narrow-bore columns of tens to few hundreds micrometers in diameter. Nunez and the Kyoto research groups recently summarized the current situation of silica monoliths for HPLC.\textsuperscript{54}

Figure 6. SEM image of typical morphology of silica monolith. Reprinted with permission from Elsevier.\textsuperscript{52}

- **Features.** As near “ideal” stationary phase the most attractive characteristics of silica monoliths are the primary contribution of convection to mass transfer and the lower hydraulic resistance compared to particulate stationary phases at similar column efficiency. The fast mass transfer in silica monoliths by convection is especially of importance in the chromatography of high molecular-weight analytes. As can be seen from their corresponding van Deemter curves, the smaller HETPs and the lower dependency of HETP on mobile phase velocity of silica monoliths in comparison to packed columns allows their use at comparatively high flow rates with minimal efficiency penalty (Figure 7).\textsuperscript{20} The high permeability is attributed to the large throughpores and the exceptionally high external porosity (~ 35 \% for packed columns, compared to 65-70 \% for silica rods and more than 85 \% for capillary columns).\textsuperscript{55} Commercially available monolithic silica columns (Chromolith, Merck) have the same permeability as a packed bed column with 9 \( \mu \)m particles but the efficiency is as high as columns packed with 5 \( \mu \)m particles. Their retentive properties of silica monoliths is satisfactory for most applications in spite of large phase ratios (of mobile phase to stationary phase volume) due to the high specific
surface areas (100-300 m²/g), a level that is comparable to porous silica particles and much higher than polymer monoliths.

**Figure 7.** Van Deemter curves for C18 silica packed and silica monolithic columns with insulin as analyte. (●), (▲) silica rods (1.7 μm throughpore, 1 μm skeleton, 7 mm diameter × 8.3 cm length) with 14 and 25 nm mesopore, respectively. (○), (Δ), (□) 5 μm-particle packed columns (4.6 mm i.d × 15 cm) with mesopores of 12, 30, and 30 nm from different brand names and HPLC conditions. Reprinted with the permission from ACS.

- **Applications.** The advantages of low backpressure and high efficiency make silica-based monoliths very useful for high throughput analysis and for complex mixtures. Silica monolithic columns integrated into multidimensional LC coupled with mass spectrometry detectors become an extremely powerful analytical technique for studies in the field of life sciences.

- **Disadvantages.** Despite the advantageous features there are a few research groups involved in the silica monolith area albeit a large number of studies on their applications. Difficulty in preparation of the materials with high reproducibility could account for this situation. Moreover, silica monoliths still suffer from the same problem as silica particles regarding limited pH operation range.
3. Characterization of Porous Material for HPLC

Chemical, mechanical, and morphological features are equally vital characteristics of a material used as separation medium. As a result, these parameters have to be “monitored” in the course of developing new materials.

At the very beginning of the synthesis macro-morphology of core materials is one of targets to be controlled. Scanning electron microscopy (SEM), nitrogen gas adsorption, and mercury intrusion porosimetry (MIP),60 are common techniques to assess porosity, homogeneity, and surface area of a material. There is no single method covering the whole range from micropores to macropores. While gas adsorption is mostly used to characterize micropores and mesopores, MIP is mainly employed for mesoporous and macroporous materials. Note that clean samples in dried state are required for these two techniques. Pore size distributions can be different from those in the wet/solvated state due to possible shrinkage upon drying. 2H NMR cryoporosimetry is an option since the measurement is carried out on samples saturated with solvents (D2O-enriched water). The success of modification step of core materials can be judged by means of X-ray photoelectron spectrometry (XPS), elemental analysis, and titration. In the following parts techniques involved in the thesis will be discussed successively.

3.1 Techniques for investigation of morphology

- Visualization of surface by scanning electron microscopy (SEM).

SEM seems to be a primary approach to study macro-structures of materials. It uses electrons to form an image of a surface. A beam of electrons generated from a heated metallic filament in vacuum is directed through electromagnetic lenses towards a sample. The focused beam hits the sample surface and releases secondary electrons. When the beam scans over a surface area of the specimen a detector collects the secondary electrons, converts them to signal and produces a corresponding image. Samples to be viewed by SEM microscope must be electrical conductive. Sputter coating is employed to cover non-conductive specimens with a nanometer overlayer of gold or platinum. Topological structures are visualized on fresh fractures of samples made in frozen state by
liquid nitrogen. High resolution, high magnification (up to 200,000×), and wide range of sample types make SEM a heavily used technique in many research areas.

Imaging monolithic materials with SEM allows a general overview of the size and homogeneity of the macrostructure (through pore and skeleton) but SEM cannot be relied on to provide complete answers to interconnectivity, tortuosity of pore networks, etc.

- **Gas adsorption for determination of surface area.** The measurement principle is based on the adsorption theory of Brunauer, Emmet and Teller; the method is therefore named after the inventors as BET. An inert gas, nitrogen in most of the cases, is physically adsorbed on a solid surface at the cryogenic temperature of liquid nitrogen. The amount of adsorbate \( V_a \) increases as the pressure \( P \) rises and the relationship can be expressed by Equation 4

\[
\frac{P}{V_a(P_0 - P)} = \frac{1}{V_mC} + \frac{C-1}{V_mC} \left[ \frac{P}{P_0} \right]
\]  

(4)

where \( C \) is a constant, \( P_0 \) the saturation pressure of \( \text{N}_2 \) at liquid nitrogen temperature, and \( V_m \) the quantity of the gas adsorbed when entire surface is covered by monomolecular layer.

\( V_m \) and \( C \) can be determined from the slope and intercept of the plot of \( P/[V_a(P_0-P)] \) vs. \( P/P_0 \). In practice, the linear relationship is usually found in the \( P/P_0 \) range of 0.05 - 0.3 for most solid materials using \( \text{N}_2 \) as adsorbate. Opposed to multi-point BET method, where the adsorbed amounts of gas are measured at different pressures, single-point BET provides a simpler but less accurate approach to obtain \( V_m \) assuming that \( C >> 1 \), then Equation 4 becomes Equation 5

\[
\frac{P}{V_a(P_0 - P)} = \frac{1}{V_mC} \left[ \frac{P}{P_0} \right]
\]  

(5)

The specific surface area (m²/g) is then calculated from \( V_m \) by Equation 6 assuming close packing at the surface.
\[ s = \frac{V_m \sigma N_A}{m V_o} \]  

where \( \sigma \) is the area of surface occupied by an adsorbate molecule (16.2\( \times 10^{-20} \) m\(^2\) for N\(_2\)), \( N_A \) the Avogadro constant, and \( m \) is the mass of the adsorbing sample (g).

For accurate results samples with clean surfaces must prior to BET measurements be completely dried by vacuum or under nitrogen flow at elevated temperature to remove adsorbed humidity.

- **Gas adsorption for assessment of micropores and mesopores.**

The shape of isotherms which are plots of \( V_a \) against relative pressure \( P/P_o \) reveals information about porous characteristics of a sample (Figure 8). A sharp increase of \( V_a \) at very low \( P/P_o \) (Type 1) is an indication of micropores. Note that as pressure increases nitrogen gas adsorbs onto the surface of pores of smaller size before onto bigger ones. Type 2a characterizes non-porous solids where adsorption and desorption branches overlap. Type 2b with a hysteresis loop indicates mesopores and macropores where condensation and evaporation process are different in curvature.

The calculation of pore size distribution is based on the assumption that the amount of adsorbed gas lost in a stepwise decrease of pressure presents the pore volume emptied at this step. The procedure starts from the state that the material is completely filled by the adsorbate (usually taken at \( P/P_o = 0.995 \)) until all pores are empty. The method was developed by Barrett, Joyner and Halenda and is hence named as the BJH method. It should be noted that the calculation is made with an assumption of straight cylindrical shape of pores. The pore size range covered by this technique is typically 0.55 - 360 nm.
Figure 8. Some typical adsorption/desorption isotherm types. Curve 1, 2a and 2b present microporous, non-porous and mesoporous/macroporous material, respectively. Reproduced with permission from Micromeritics Instrument Corporation.  

- Mercury intrusion porosimetry (MIP) for assessment of mesopores and macropores. Mercury is used as fluid intruding into pores of a material to gain information about their porous characteristics. The most distinguished features of mercury (apart from being the only metal liquid at room temperature) is its high surface tension and non-wetting behavior on the (non-metallic) surfaces it is applied (Figure 9). As a result, it cannot penetrate into pores until the applied pressure reaches a certain level. The smaller pore opening the higher pressure is required to force mercury to intrude into the pores. The pore diameter is inversely proportional to the applied pressure, as shown in the Washburn equation (7). Once again pores are assumed to be cylindrical for simplicity of the calculation although it is not always the case.
\[ D = \frac{-4\gamma \cos \theta}{P} \]  

(7)

where \( D \) is the pore diameter, \( \gamma \) the surface tension of mercury (485 dyne/cm), \( \theta \) the contact angle (130° is the most accepted), and \( P \) the applied pressure.

**Figure 9.** Mercury in contact with porous solid. Reprinted with permission from Micromeritics Instrument Corporation.

By varying the applied pressure from \( 3.4 \times 10^{-3} - 414 \) MPa one can force mercury to enter pores with diameter ranging from 360 μm - 3 nm. The steeper rise in
cumulative intrusion as the pressure increases, the narrower the pore size distribution is. An example showing the bimodal pore size distribution of silica-based monoliths is illustrated in Figure 10. Disadvantages of MIP are the use of toxic mercury and a risk of compression of materials due to the high pressure. Furthermore, large pores with small orifices (ink-bottle shaped pores) are thus filled at high pressures, and detected as smaller pores than they actually are.

- **\(^{2}H\) NMR cryoporometry.**\(^{52-63}\) The principle of this technique is based on line broadening of water in the frozen state and probes liquid water that melts at temperatures substantially below zero when confined in narrow pores as tiny crystals. Melting point depression \(\Delta T_m\) for crystals in pores of diameter \(d\) (it is also taken as the size of the crystals) can be predicted by Gibbs-Thomson equation (8)

\[
\Delta T_m = T_m - T_m(d) = \frac{4\sigma_{sl}T_m}{d \Delta H_f \rho_s}
\]  

(8)

where \(\sigma_{sl}\) is the surface energy of liquid-solid interface, \(T_m\) the bulk melting point, \(T_m(d)\) the melting point of crystals of size \(d\), \(\Delta H_f\) the enthalpy of fusion of the bulk (per g material), and \(\rho_s\) the density of the solid.

For a particular liquid Equation 8 can be simplified as

\[
T_m - T_m(d) = k / d
\]

(9)

Due to the supercooling phenomenon the melting temperatures of tiny crystals are determined by raising the temperature after all the liquid has been frozen. The distribution of melting temperature reflects the pore size distribution. While differential scanning calorimetry records melting temperatures to trace pore size, the magnitude of the \(^{2}H\) signal at liquid state is measured in \(^{2}H\) NMR cryoporosimetry to give information about volumes of pores of certain sizes. Pores can be evaluated by \(^{2}H\) NMR cryoporosimetry in the size range 20-350 Å which corresponds to 30-1 °C below the normal melting point of bulk ice D\(_2\)O.\(^{64}\)

In Paper III the skeleton of epoxy monoliths was found nonporous by BET and it was proven to turn into a gel form when solvated with D\(_2\)O with \(^{2}H\) NMR cryoporosimetry. Figure 11 shows that 95 % water resided in macropores and the
rest is best considered as water in the hydrogel state rather than in mesopores of broad size distribution.

![Figure 11. Corrected integrals and line width for epoxy monolith subjected to 2H NMR cryoporosimetry without any drying step between the preparation and pore determination [Paper III]](image)

### 3.2 Techniques for investigation of chemical composition

- **X-ray Photoelectron Spectroscopy.** XPS is preferred as the first investigation means for the surface of monoliths since it is sensitive (semi-quantitative), fast and informative. An XPS spectrum provides information about the elemental composition and the chemical and electronic state of the detected elements. A monoenergetic X-ray radiated to a solid sample under ultra high vacuum conditions cause emission of photoelectrons with their kinetic energies, $KE$, given by

\[
KE = h\nu - BE - \Phi_s \tag{10}
\]

where $h\nu$ is the energy of the photon, $BE$ the binding energy, and $\Phi_s$ the spectrometer work function.

Identification and quantification in XPS are based on the facts that each element has a unique set of binding energies and the number of emitted photoelectrons is proportional to the concentration of elements. Only photoelectrons that originate within tens of Å from the surface can escape without loss of energy due to interactions with matter, therefore, XPS is an analytical technique for surface chemistry but not for bulk. Variations in
elemental binding energies (chemical shifts) can be used to identify the chemical state of elements. Detection limits are in most cases around parts per thousand; however, this can be improved by extending the collection time of photoelectrons.

Identification of functional groups can solely be relied on XPS if difference in binding energies is large enough (>±0.3 eV) e.g. quaternary amine can easily spotted from primary, secondary, and tertiary amines (Figure 12) [Paper V] but the last three amines cannot be differentiated. This is also the case of oxygen of hydroxyl, linear and cyclic ether (epoxy ring). In such a case, simple titration set-ups using specific reactions for each or a group of functional groups is a specific approach though it is time consuming due to two-phase reactions.

![Figure 12. Survey XPS spectrum of a modified epoxy-based monolith bearing quaternary ammonium (left) and an expansion of the N 1s band (right) [Paper V]](image)

- **Elemental analysis by combustion.** The elemental compositions of bulk materials can be analyzed by burning samples in an excess of oxygen. Combustion products e.g. CO₂, NO₂, SO₂ from C, N, and S, respectively are detected and quantified. Bear in mind that the combustion converts elements of different chemical states to only one product per element hence no information but the presence of the elements can be gained from this technique. Elemental analysis can be used to identify grafted functionality containing elements which are not present in starting materials. For example, the magnitudes of C and S
signals are evidences of grafting efficiency of C18 chains on silica or sulfonic groups on divinylbenzene particles, respectively.

4. New approaches for preparation of macroporous organic monoliths

A general objective of my doctoral project is to develop new materials targeting on applications in biomolecule chromatography. Epoxy and polyamide-based materials were studied with great interest since we successfully developed simple routes for their preparation, and possibilities of surface modification on these materials have been well documented. Additionally, their good mechanical properties, chemical resistance, and hydrophilicity are also attractive features. In the following parts the novel preparation approaches and basic characteristics of the monoliths will be summarized.

4.1 Epoxy-based monoliths prepared by emulsion polymerization

4.1.1 Polycondensation of epoxy resins and polyamines

In a curing process of epoxy resins by polyamines amine hydrogen, a nucleophile, attacks oxirane carbon leading to opening the epoxy ring and formation of hydroxyl group. Figure 13 illustrates the SN₂ mechanism of epoxy-amine reaction where step (1) is rate determining.

![Figure 13. Mechanism of epoxy-amine reaction. Reproduced with permission of ACS](image)

Higher reactivity of primary amines relative to secondary amines can be accounted for by steric factors and solvent effects. Aliphatic amines are able to cure at temperatures markedly lower than aromatic amines. Increase in functionality of curing amines augments the crosslinking and hence gives rise to high glass transition temperature ($T_g$) and low tensile strength of the polymeric products. The polymerization can continue after all amines are used
up if temperature is raised high enough for the reaction of hydroxyl groups and remaining oxirane to take place, which is referred to as homopolymerization. It is well known that compounds with hydroxyl groups, e.g., water, alcohols, acids, accelerate the reaction by hydrogen bond effects, whereas hydrogen-bond acceptors, e.g., ethers, esters, and ketones show retarding effects. Bear in mind that curing by amines generates hydroxyl groups from the epoxy oxygen therefore the polymerization is self-accelerated with proceeding conversion.

Figure 14 shows the schematic functionality of an epoxy-amine polymer, on which abundant hydroxyl groups play a key role in hydrophilicity and functionalization of the material.

---

Figure 14. Schematic functionality of crosslinked polymer prepared by polycondensation of epoxy resin and diamine.

We were apparently not the only ones working on epoxy-amine systems for the preparation of porous monoliths for HPLC. While the final hand was put on Paper I, Tanaka’s group reported success in the synthesis of epoxy-based polymers by phase separation, which has subsequently proven to yield excellent performance in separation of small molecules by HPLC. Temperature, monomer weight ratio, and molecular size of polyethyleneglycol have been found to be determining factors controlling the monolith porosity and morphology.
Papers I and III present our approach to prepare porous epoxy-based monoliths by emulsion polymerization.

4.1.2 Synthesis of epoxy-based polymer by emulsion polymerization

In our method the two main components were added in the two phases of an oil-in-water emulsion stabilized by a block copolymer surfactant. Epoxy resins were in the dispersed phase while the continuous aqueous phase contained the polyamines. Aliphatic amines were preferred over aromatic amines as curing reagents because the later require higher temperatures that are destructive to emulsion stability. Two different ethers were added into the aqueous and oil phases as retarders to compensate the catalytic effect of water, so that we were able control the polymerization by external thermal regulators. After initial interfacial polymerization, the “frozen” emulsions were subjected to temperature programs which were carefully optimized in order to form strong bicontinuous rod-like monolithic networks. Experiences showed that the emulsions had to undergo an incubation step at low temperature, typically at 24 °C for 2 h, when an interfacial polymerization took place to fixate the structure of the low temperature dispersion. The curing was then continued with a stepwise increase in temperature until complete conversion.

In Paper I a screening study on diluent, ether retarder, epoxy and amine components, and curing conditions was performed to find the primary procedure for the formation of bicontinuous matrix. In Paper III, factors related to emulsion components and curing conditions were tuned to improve the material morphology and enhance the reproducibility of the preparation. Figure 15 shows a comparison between morphologies of epoxy materials after the first screening study (Paper I) and the later tuning work (Paper III). A significant increase in specific surface area of the materials from 1.8 m²/g (Figure 15a) to 4.5 m²/g (Figure 15b) could be explained by scaling down the domain size. However, both preparations had non-porous skeletons indicated by low surface areas, a common feature of organic monoliths.
4.1.3 Porosity and chemical characteristics of the epoxy monoliths

Various techniques, namely \( N_2 \) adsorption, MIP, SEM, \(^2\text{H}\) NMR cryoporosimetry, and potentiometric titration were employed to investigate the morphology and chemical features of the monolithic products. The materials exhibited macroporosity with average throughpore diameter of 1.7\( \mu \text{m} \) within a narrow distribution (Figure 16) and therefore high fluid permeability could be realized (Figure 17). Titration revealed an unexpectedly high content of titrable amines (~2.5 mmol/g material) (Paper III), which seemed to contradict the non-porous nature of the skeleton. In an attempt to verify whether there was error in the titration, \(^2\text{H}\) NMR cryoporosimetric was used to determine the size distribution of pores where water resided, after equilibrating with deuterium-enriched water a monolith that had never been dried. From the NMR results it was found that most of the water (~95%) resided in macropores, and the rest (~5%) was present in a state that was best characterized as a hydrogel. The assumption of hydrogel seemed to be rational considering the tendency of swelling of the material in polar solvents such as water. This could be a result of the sub-stoichiometric ratio of polyamines to epoxy resins. Transport of proton in hydrogels can take place by proton hopping according to the Grotthuss mechanism,\(^79\) therefore most of (if not all) the amine inside the skeleton was assessable in the acid/base titration.
Regardless of the interfacial nature of the polymerization, where the two main components were in two phases, elemental analysis and XPS showed similar elemental compositions between the bulk and the surface. The density of hydroxyl groups calculated from the emulsion composition was of ~4.7 mmol/g, which would facilitate functionalization and provide high hydrophilicity.

4.2 Polyamide-Based Monoliths Prepared by Thermal Induced Dissolution/ Precipitation

4.2.1 Polyamide (PA)

Linear polyamides, also named asnylons, are one of the most important classes of thermoplastic polymers. Polycondensation of diamine and diacid (Figure 18, Eq.1) and ring opening polymerization of lactams are common procedures in industry to produce polyamides (Figure 18, Eq. 2).\(^8\)\(^0\) PA\(_{mn}\) is a generally accepted nomenclature for polyamides, where m and n are number of carbons donated from diamine and diacid to the polymer chain, respectively, while PA\(_{m}\) refers to homopolymeric polyamides with m is number of carbons in lactam monomers. The regularity of amide groups on the backbone provides possibilities for formation of multiple hydrogen bonds between polymer chains (Figure 19) giving rise to semi-crystalline properties that impart good mechanical strength. In homologous series of PA\(_{m}\) and PA\(_{mn}\) the increase in density of amide groups innylons of lower numbers leads to increases in melting and glass transition temperatures, density, and mechanical properties.\(^8\)\(^1\) The availability of a wide variety of diamines, dicarboxylic acids, and lactams makes it possible to prepare a large number of polyamide materials spanning over a wide range of hydrophobicity-hydrophilicity, e.g., PA46, PA6, PA66, PA69, PA612, PA11, PA12, etc.

Porous thin PA membranes have been manufactured for a long time, targeting a wide range of applications in the fields of microfiltration\(^8\)\(^2\)-\(^8\)\(^4\) reverse osmosis\(^5\),\(^9\)\(^8\) bioseparations\(^5\),\(^7\)\(^0\)-\(^8\)\(^6\) and bioreactors\(^9\)\(^0\)-\(^9\)\(^2\).
Figure 16. Pore size distribution of an epoxy monolith determined by MIP [Paper III]

Figure 17. Back pressures of an epoxy-based monolithic column (3.8 mm diameter × 50 mm) vs. water flow rate. [Paper III]
Figure 18. Synthesis routes of linear polyamides

Figure 19. Hydrogen bonding in PA6
4.2.2 Dissolution-precipitation approach for preparation of porous monoliths from thermoplastic polymer

- **Dissolution of a non-covalently crosslinked polymer** is a slow process, starts with the adsorption of solvent molecules to the polymer matrix resulting in a swollen gel. The process will continue until the polymer chains are eventually pulled apart from each other and stay in the solution as random coils solvated by solvent molecules. It is noteworthy that when substances with a high degree of crystallinity or multiple hydrogen bonding are involved, where polymer-polymer interactions are strong enough, the process may stop at the first stage. Solvents are frequently ranked as good, poor, extremely poor, or non-solvent dependent on how easily they can dissolve a polymer. From a thermodynamic point of view a dissolution process is spontaneous if the Gibbs free energy is negative.

\[
\Delta G = \Delta H - T\Delta S
\]  

(11)

\(\Delta S\) of polymers is significantly smaller than that of small molecules; therefore, \(\Delta H\) must be substantially decreased to reach a negative \(\Delta G\). The enthalpy change of a binary system is related to concentration and energy parameters by expression (12) where \((\delta_S - \delta_P)\) should be as small as possible.\(^{93}\)

\[
\Delta H = V_{mix} (\delta_S - \delta_P)^2 \phi_S \phi_P
\]  

(12)

where \(V_{mix}\) is the total volume of the mixture (solution), \(\delta_S\) and \(\delta_P\) the cohesive energy densities (solubility parameters) of solvent and polymer, respectively, and \(\phi_S\) and \(\phi_P\) the volume fractions of solvent and polymer, respectively.

In the dissolution-precipitation approach for production of porous polymer membranes,\(^{94}\) polymers are first dissolved in a good solvent or solvent mixture. Afterwards, a gradual removal of the good solvent is performed by exchange with a non-solvent, selective evaporation of good, volatile solvent of a solvent pair, or lowering the temperature to such an extent that phase separation occurs.\(^{95}\) At some point before precipitation, an equilibrium is reached (\(\Delta G = 0\)). This point, where the polymer-solvent and polymer-polymer interactions are
balanced, is known as the \( \theta \) state. It is a function of temperature, the polymer-solvent system, and molecular weight of the polymer. It may be inferred that by lowering the temperature or solvent quality, separation of the polymer in decreasing molecular weight fractions will be obtained. Any polymer could reach its \( \theta \) state by either choosing an appropriate solvent, \textit{i.e.}, a \( \theta \) solvent, at constant temperature, or by adjusting temperature above \( \theta \) temperature in a given solvent.

- **Isothermal immersion-precipitation.** Porous polymer membranes have in general been produced by dissolving the polymer in a good solvent followed by a solvent/non-solvent exchange when cast solution on a flat surface is exposed to a non-solvent bath.\textsuperscript{94-96,99} The technique is therefore referred as immersion precipitation in which phase separation is governed by the change in solvent quality at a given temperature, typically ambient temperature. Formic acid/water is the most studied solvent/non-solvent system for polyamides.\textsuperscript{100-102} It is well known that if the rate of solvent/non-solvent exchange is sufficiently slow, crystallization will dominate the phase separation.\textsuperscript{97,101} As a consequence, formation of a polymeric “skin” at the interface is minimized and more homogenous morphology can be realized throughout the membranes. Mass transfer in immersion precipitation method limits applications to thin entities such as membranes. Monolithic materials in separation sciences are usually sizable and therefore phase segregation originated from solvent/non-solvent exchange is not feasible. Temperature is thus an obvious alternative to the mass transfer.

- **Thermally induced dissolution-precipitation.** Our approach exploits the fact that a non-crosslinked polymer will precipitate from its solution by cooling the solution below the \( \theta \) temperature. Note that the recommended term by IUPAC for this temperature is upper critical solution temperature (UCST) which is “the critical temperature above which a mixture is miscible”.\textsuperscript{103} UCST is dependent on pressure and molar-mass distribution of constituent polymers. Although this approach was developed for the preparation of microporous membranes already a few decades ago it has not gained the same popularity as immersion precipitation and has not been employed for preparation of porous entities of large dimensions.
4.2.3 Sizable polyamide monoliths prepared by thermally induced dissolution-precipitation

Selected as starting materials in this project were a number of linear polyamides with properties ranging from hydrophilic to hydrophobic, namely PA46, PA6, PA66, PA69 and PA610. Benzyl alcohol was the solvent chosen among other known non-acidic solvents for nylons, e.g., dimethyl sulfoxide, dichloromethane, trichloromethane, 2,2,2-trifluoroethanol, 1,1,1,3,3,3-hexafluoro-2-propanol, phenols, and cresols since it shows UCST behavior to polyamides at a practical temperature (~135 °C). Consequently, it does not require supercooling to precipitate the polymers. Additional selection criteria in favor of benzyl alcohol were low cost and low toxicity.

Scouting experiments were performed on Abulon Top fishing line, made from polyamide of unknown composition, [Paper II] and later extend to the PAs mentioned above [Paper IV]. The dissolution was carried out for different periods of time followed by a cooling step to room temperature. The resulting gels were recovered from glass molds and the solvent was removed by Soxhlet extraction with methanol. Shrinkage was minimal for the gels recovered from molds and in wet state after Soxhlet extraction, but noticeable after drying when the fragile gels changed into more rigid monolithic entities.

The time required to dissolve the polyamides decreased in the following order PA6 > PA610 > PA69 > PA6/66. Among the PAs, PA46 and PA66 could not be brought into solution even after extended heating at 155-165 °C for 7 days. The ease of swelling and finally dissolving the polymers in benzyl alcohol was undoubtedly a result of regularity and density of interchain hydrogen bonds and their molecular weights. The opaqueness of the polymers which could not be dissolved (PA46 and PA66) is a sign of high degree of crystallinity. We further encountered problems attempting making porous monoliths from PA69, since the precipitated monoliths shrank too much to maintain their porosity on drying. Reasons why this polyamide formed compact entities is not clear.

The study revealed that the period of time at the elevated temperature had profound impacts on shrinkage, morphology, and porosity of the monoliths. Figure 20 shows the diverse morphologies of the PA6/66, PA6 and PA610 monoliths.
The PA6/66 monoliths were characterized by coral-like connected clusters and wide, irregular channels, while bicontinuous rod-like networks dominated the macromorphological features of the PA6 monoliths. PA610 also formed macroporous monolith when dissolved for the shorter time, however, it was prone to disintegrate when heated for 48 h.

There was an upward trend in the size of macropores as the dissolution proceeded. Mean macropore sizes of PA6/66 and PA6 monoliths determined by MIP are tabulated in Table 1, indicating an increasing trend as the thermal treatment was extended. Larger (1.8 µm diameter) and narrower size distribution of macropores was obtained with the longer dissolution process (Figure 21). It should be noted that the larger macropores the lesser tendency of shrinkage after drying was seen. Another important parameter when appraising materials for HPLC applications is the specific surface area. The PA6 monoliths with rod-like skeletons had significantly higher specific surface area (14-57 m²/g) than PA6/66 which was composed of coral-like clusters (>1 m²/g) (Table 1). The most interesting features of mesoporous properties was those of PA6 with a family of pores in the 100-150 Å range at surface areas as large as 50 m²/g considering that there was no mesoporogen used in the preparation.

<table>
<thead>
<tr>
<th>Sample*</th>
<th>Specific surface area (m²/g)</th>
<th>Mesopore diameter (Å)</th>
<th>Throughpore diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N6-4h</td>
<td>57.3</td>
<td>106</td>
<td>0.47</td>
</tr>
<tr>
<td>N6-48h</td>
<td>13.9</td>
<td>158</td>
<td>1.8</td>
</tr>
<tr>
<td>N6/66-4h</td>
<td>0.8</td>
<td>150</td>
<td>16.6</td>
</tr>
<tr>
<td>N6/66-48h</td>
<td>1.0</td>
<td>176</td>
<td>45.3</td>
</tr>
</tbody>
</table>

(*) The sample were named PAm(n)-4h and PAm(n)-48h corresponding to the dissolution duration of 4 and 48 h
Figure 20. SEM images at two magnifications of PA6/66, PA610 and PA6 monoliths (~12% PA loading) prepared by dissolving in benzyl alcohol for 4 h and 48 h (time is indicated next to the PA name) [Paper IV]
The impact strength of PA6/66, PA610 and PA6 monoliths was deteriorated when the heating period was prolonging. PA610 represented the worst case among the three, and a likely cause could be polymer degradation during the heat treatment. Traces of oxygen and humidity in the dissolution environment were expected to accelerate such degradation. Degradation was confirmed by a remarkable decline in viscosity, a significant increase in titrable amine groups, and the appearance of low molecular weight polymers in MALDI-MS spectrum [Paper IV]. A possible degradation pathway of PA during the dissolution in benzy alcohol at elevated temperature (Figure 22) could be a result of the
oxidation of benzyl alcohol to benzaldehyde (1), the hydrolysis of polymers by water (2), and the formation of imine in the reaction between amine endgroup and benzaldehyde (3). However, identification of degraded products requires more studies.

\[
\begin{align*}
\text{C}_6\text{H}_5\text{-CH}_2\text{-OH} & \xrightarrow{\text{O}_2} \text{C}_6\text{H}_5\text{-CHO} \quad (1) \\
\text{---NH-CO---} & \xrightarrow{\text{H}_2\text{O}} \text{---NH} \_2 + \text{HOOC ---} \quad (2) \\
\text{C}_6\text{H}_5\text{-CHO} + \text{H}_2\text{N---} & \xrightarrow{} \text{C}_6\text{H}_5\text{-CH} = \text{N ---} \quad (3)
\end{align*}
\]

**Figure 22.** Degradation mechanism of polyamides in benzyl alcohol at elevated temperature

5. **Functionalization of the epoxy-based monolith and applications in separation of proteins**

5.1 **Atom transfer radical polymerization (ATRP)**

Modification of the support surface is a key step in the preparation of stationary phases. A flexible brush-like functionality is often preferred over surface-tethered groups, especially for the chromatography of biomolecules since it provides higher capacity and prevents retained molecules from contortion, which could lead to irreversible unfolding. Two ways to incorporate functional tentacles onto a support surface are “grafting from” and “grafting to” approaches. While the later method is accomplished by coating the surface with pre-made short-chain polymers, in the former polymeric brushes of functional groups are “grown” from initiation sites on the surface by subsequent adding of monomer. “Grafting to” usually results in lower coverage than “grafting from” due to steric crowding of initiation sites by already-attached polymer chains. ATRP is by far the most popular among available approaches for growing polymer brushes via surface-initiated polymerization. Its compatibility with a wide variety of monomers, living and controlling nature, as well as
tolerant conditions have made it more and more preferable since the first studies in 1995.\textsuperscript{117, 118}

Initiation of a surface by incorporation of halogen atoms is the first step of an ATRP process. Pm-X bonds (X = Cl, Br; P refers to the polymer surface) of the initiator will homolytically cleave to generate surface radical $P_m^\cdot$ from which polymerization occurs. Surface-initiated ATRP has surface-confined characteristic and hence prevents the grafted surface from adsorption of polymer chains formed in the solution, which will ease the removal step of unbound materials. One of the distinctive mechanistic features of ATRP is that a dynamic activation-deactivation equilibrium is established between dormant species $P_m^\cdot$X and growing radical $P_m^\cdot$ by exchange of the halogen atom with a transition metal complex $M^zL_n$ in solution to form its higher oxidation state $XM^{z+1}L_n$ (Figure 23). Low concentrations of growing radicals are maintained because the equilibrium shifts backwards to the formation of dormant species. As a consequence, termination by radical-radical coupling is suppressed. An equilibrium constant $K \approx 10^{-8\pm2}$ would be required for successful ATRP of (meth)acrylates, styrene, and acrylonitrile. Note that $K$ is equal to the ratio of activation to deactivation rate constants ($k_{act}/k_{deact}$). In ATRP the propagation process is significantly slower than deactivation and hence in each activation step only one or less than one monomer molecule is inserted into the growing chain. By this fashion well-controlled polymerization can be realized. The living characteristic of ATRP relies on the fact that the dormant R-X will be able to react with other monomers afterwards. This feature offers possibilities to prepare polymers with complex topologies and compositions. There has been a number of publications worth reading devoted to the mechanism of ATRP.\textsuperscript{119-121} A schematic mechanism of ATRP is presented in Figure 23, where $k_{act}$, $k_{deact}$, $k_p$ and $k_t$ are the rate constants for activation, deactivation, propagation, and termination, respectively.
The most commonly used catalysts are complexes of Cu(I) with 2,2'-bipyridine or multidentate amines. Cu(II) complexes are either spontaneously formed in the solution or added at very beginning of the polymerization. Though oxygen should be absent in free radical polymerization the condition is not so stringent with ATRP if appropriate reducing agents are added to the polymerization medium. The rate of ATRP an reaction could be defined as the following equation:

\[ R_p = k_p \left[ M \right] \left[ P_m - X \right] \frac{k_{act}}{k_{deact}} \frac{\left[ M^2L_n \right]}{X^{n+1}M^{n+1}L_n} \] (13)

The polymerization rate can be controlled by the concentration ratio of metal complexes as well as ligand type. Copper complexed with low cost multidentate amines, e.g., tetramethylethlenediamine, 1,1,4,7,7-pentamethyldiethylenetriamine and 1,1,4,7,10,10-hexamethylethylenetetramine provide higher polymerization rates for styrene and methyl acrylate than Cu-bipyridine complex. The effect could be a result of lower redox potentials of the multidentate amines. While fast polymerization could be an advantage in offline modification of particulate materials for HPLC, it is a trouble in inline modification of monolithic substrates where reactant medium is pumped through the materials. Too fast reaction could lead to thicker skeletons, smaller throughpores, and a grafting density gradient with the heaviest density at the inlet and the lowest at the outlet due to quick consumption of monomer at the entrance part of the column. As a result, the modified columns could have excessively high back pressure [Paper V]. It was reported that a way to achieve homogeneous poly(2-hydroxyethyl methacrylate) grafting on a monolithic
entity is to flush it a with an ATRP reaction mixture, then let it stay still and react slowly with the initiated-surface. In addition, very fast grow of brushes could lead to chain termination during initial stages and hence lower grafting density, as well as high polydispersity of brush length. Besides, solubility of copper complexes in monomer solutions and proportionation side reaction in aqueous phase have to be taken into considerations when selecting a catalyst and solvent system.

ATRP has been used extensively in surface modification of silica and polymer particles for HILIC, ion exchange, and affinity chromatography. For monolithic materials most of the works have been done either on membranes or lumps, where monomers and catalysts are brought into contact with the surface via short diffusion pathways, but there are not many studies on sizable entities.

In Paper V tentacle GMA, as active intermediate, was grafted on to the epoxy-based monolith surface which had been activated by anchoring tertiary bromide from bromoisobutiryl bromide (BIBB). GMA was successful incorporated onto the surface using CuBr/CuBr2/PMDETA as catalyst system, however, optimal conditions with respect to homogeneity and controlled thickness of the coating has not been reached yet. The polymerization rate was controlled by the ratio of Cu(I)/Cu(II) added, which varied from 10:1 to 1:1. The reaction rates were too fast to control with copper ratios of 10:1, 5:1 but too slow with the 1:1 ratio. It should be kept in mind that an excess amount of PMDETA (i.e., a concentration of PMDETA larger than the total concentration of Cu(I) and Cu(II)) could initiate opening of the epoxy ring of GMA. Therefore, ATRP composition must be optimized to achieve moderate reaction rate to strike a compromise between homogeneity of the coating and preservation of the epoxy ring.

5.2 Ion exchange chromatography of proteins with modified epoxy-based monoliths

The epoxy-based monoliths prepared by emulsion polymerization with the distinctive features lend themselves to being used for macromolecule separation. The large throughpores promote mass transfer of large molecules by convection. Its hydrophilicity could lessen the risk of protein unfolding due to
adsorption on highly hydrophobic surface. Therefore, the first attempt to find applications for this material was made in protein separation. It should be kept in mind that the surfaces of epoxy-based monoliths are rich in tertiary amines and hydroxyl groups. Unmodified materials showed ability in retaining negatively-charged proteins, e.g., BSA at pH ~7 but the retention was weak. Stronger interaction was expected with strong anion exchange groups\textsuperscript{131} and therefore converting the amine functionalities from tertiary to quaternary was obviously worth trying. The conversion was made by reaction with iodomethane in the presence of a base catalyst. Another approach was incorporation of reactive groups \textit{via} the hydroxyls. Coating the support surface with various polymer brushes carrying functional groups, e.g., SO\textsubscript{3}– and N(COO–)\textsubscript{2} were realized \textit{via} ATRP grafting of GMA. The modification scheme is presented in Figure 24.

Verifying the existence of functional groups on the modified materials was based on XPS analysis and the actual chromatographic retention behavior of the sorbents towards model proteins. The retention orders of the test proteins in three different columns carrying strong anion exchange (NR\textsubscript{4}+), strong cation exchange (SO\textsubscript{3}–) or (metal-chelating) weak cation exchange (N(COO–)\textsubscript{2}) groups were in agreement with literature.\textsuperscript{46, 132-134} The epoxy monolith modified by ATRP \textit{via} GMA showed non-specific interaction with proteins; the chromatograms of protein mixture incubated on EXP-SO\textsubscript{3} column were almost identical to those obtained by immediate elution (Figure 25).

Both classes of functionalized materials, \textit{i.e.}, tethered site and tentacle functionality had moderate capacity and separation power. Although this was not surprising for the fixed-site type because of the low surface area of core material, it was somewhat disappointing with the functional brush type. More work has to be done to optimize the modification procedure using the ATRP \textit{via} GMA so that we can obtain high density of functional groups without lost of permeability and reactive epoxy group.
Figure 24: Modification scheme of epoxy-based monolith for IEC of proteins
Figure 25. Chromatograms of protein mixture [1) myoglobin, 2) α-chymotripsinogen, 3) cytochrom C and 4) lysozyme] after 0, 70 and 150 min incubation on EXP-SO3 column (4.2 mm i.d. × 40 mm). Myoglobin was unretained and used as an indicator of complete loading and used to stop the elution. Eluent A: 20 mM phosphate buffer, pH 7, eluent B: 0.5 M NaCl in 20 mM phosphate buffer, pH 7, linear gradient 0-100 % B in 10 min. [Paper V]
6. Concluding remarks and future aspects

Two novel synthesis approaches, namely interfacial polymerization of an emulsion containing the two monomeric components of an epoxy/amine system in separate phases, and thermally induced dissolution-precipitation, have been developed in this thesis for the preparation of sizable macroporous polymer monoliths targeted for separation science.

The epoxy-based monoliths were prepared by polycondensation of emulsions composed of epoxy resins finely dispersed in aqueous polyamine solutions with the aid of a block copolymer surfactant [Papers I and III]. An incubation step at low temperature followed by a step-wise increase in temperature allowed the formation of a bicontinuous structure characterized by micrometer-sized skeleton and interconnected throughpores with a diameter range of 1.0-2.4 μm [Paper III]. The dimensions of epoxy monoliths can easily be scaled up without observables changes in the structure. Distinctive features of the epoxy monoliths promise applications in macromolecule separation. ATRP grafting is a promising method to functionalize the material. However more work has to be done for optimization of the procedure. Extension to other functional monomers is worth to try as one-step modification.

The second approach was based on phase segregation of polymer from a solution at temperature below UCST [Papers II and IV]. Linear polyamides, non-covalently crosslinked polymers, were brought into solution in benzyl alcohol at elevated temperature and thereafter precipitated when the solutions were slowly cooled to room temperature. The duration of the dissolution and the cooling rate were key factors for controlling the porous characteristics of the monoliths [Paper IV]. We envisage that thermally induced dissolution-precipitation strategy will open up a new possibility to prepare monolithic porous material from other thermoplastic polymers.

The last part of this study was devoted to applications of the monoliths in liquid chromatography [Paper V]. The epoxy monoliths were functionalized ion exchange chromatography of proteins. Quaternization of the amines that are part of the polymer backbone and incorporation of tentacle sulfonic or quaternary ammonium groups via GMA grown off the surface by ATRP were the two modification pathways attempted in the study.
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8. References


