A chip system for hydrodynamic chromatography
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CHAPTER 1

General Introduction

1. Lab-on-a-Chip, Micro Total Analysis Systems and Analytical Separations

Progress in microfabrication technology in recent years, which originates mainly in the computer silicon chip industry, also allows the miniaturization and novel design of non-electronic devices. As envisioned earlier,\(^1\) in the near future it will be possible to reduce the size of various instruments to micro or nanometer scale with attractive applications spanning from medicine to space travel.\(^2,3\) In a similar context a number of scientists are working towards the miniaturization and integration of procedures common in the chemical laboratory and industry into the so-called Lab-on-a-Chip.\(^4,5\) In general, this would consist of a system of microfluidic channels combined with micromechanical, microelectronic or microoptical transducers and actuators. The basic advantage of such a design is the easy control of temperature and species transport due to the small size and the consequently small time constants. Other important features are portability and low material and energy consumption. For example, microreactors\(^6\) may soon be able to perform many chemical processes including dangerous reactions that would be out of control in macroscale. If many are used in parallel, such microreactors would form the basis of a modern, flexible chemical factory.

Chemical analysis is a field where the reduced scale is of particular importance. The amount of the analyzed sample is usually limited, and specific, expensive or toxic reagents are commonly used. In addition, the speed and the performance of many analytical methods can be significantly increased by miniaturization. The ultimate concept here is the so-called Micro Total Analysis System\(^7,8,9\) (\(\mu\)TAS), introduced by Manz.\(^10\) This is a miniaturized version of the Total Analysis System (TAS),\(^11\) in which all steps of an analytical process
i.e. sample pretreatment, reactions, separations, detection and data processing are integrated into a single piece of equipment (Figure 1).

A μTAS can in principle combine the advantages of the two classical strategies in analytical chemistry. One strategy is the selective detection of a compound in a mixture by a sensor. Simple sensors such as ion-selective electrodes are fast and allow continuous monitoring, but are suitable only for specific cases, and for samples of a simple composition. More complex sensors based on spectroscopic techniques, are more broadly applicable and still fast, but often require expensive, large and delicate equipment. The other strategy, a multistep analysis, is almost universally applicable but slow. In modern automated instrumentation, especially in TAS, the sample is carried through the various steps by a carrier fluid. Although this speeds up the analysis, in macroscopic systems it is still too slow (> 10 min) for e.g. process monitoring or high-throughput screening. Miniaturization and integration can bring the analysis time down to several seconds,\textsuperscript{12,13} which is close to the response time of a sensor.

\textbf{Figure 1:} Miniaturization and integration trend in chemical analysis. An analytical laboratory (1950), Total Analysis System (1980) and μTAS (1990).
The small scale of microdevices is illustrated in Table 1 by typical values for dimensions, volumes, times, and number of molecules in such systems.

**Table 1** Small volumes vs. small resp. large molecules.

<table>
<thead>
<tr>
<th>Volume</th>
<th>1 μL</th>
<th>1 nL</th>
<th>1 pL</th>
<th>1 fL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cube dimensions</td>
<td>1 mm</td>
<td>100 μm</td>
<td>10 μm</td>
<td>1 μm</td>
</tr>
<tr>
<td>Diffusion time a)</td>
<td>8 min / 14 h</td>
<td>5 s / 8 min</td>
<td>50 ms / 5 s</td>
<td>0.5 ms / 50 ms</td>
</tr>
<tr>
<td># of molecules a,b)</td>
<td>$10^{15} / 10^{11}$</td>
<td>$10^{12} / 10^{8}$</td>
<td>$10^9 / 10^5$</td>
<td>$10^6 / 10^2$</td>
</tr>
</tbody>
</table>

a) Small (M = $10^2$ g/mol) / large (M = $10^6$ g/mol) molecule. b) In ~ 1 mg/mL solution.

Despite the earlier optimism, the creation of a Lab-on-a-chip and especially μTAS turns out to be a challenge. Only few systems have been fully integrated so far. A recent example is shown in Figure 2a. Compared to microelectronics, microfluidics has to deal with complex properties of fluids, interfaces and molecules, strong influence of the geometry, surface chemistry, the danger of clogging etc. For these reasons one should try to keep the system as simple as possible. Even then many problems have to be solved. Only few partially integrated microfluidic chips have been commercialized so far, and mainly for the demanding analysis of DNA or proteins (Figure 2b).

**Figure 2:** Examples of recent analytical microsystems. (a) MAFIAS - a true Lab-on-a-chip (μTAS) prototype system for the monitoring of ammonia. [reprinted from reference 14 (p. 665 fig. 1b) with kind permission of Kluwer Academic Publishers] (b) A commercialized microfluidic electrodriven separation chip for proteomics.
Separation methods
In analysis by column separation\textsuperscript{15} ("separation methods"), the analyzed sample is injected as a concentration zone into a column with a continuously flowing carrier liquid. The sample components are transported through the column as concentration zones with different speeds due to differences in some chemical or physical property. The zones thus elute from the column at different times and they can be detected and quantified as peaks in a chromatogram by using a preferably nonspecific detector (Figure 3). In this way very complex mixtures can be analyzed.\textsuperscript{16}

![Figure 3: Retention ($t_r$) and efficiency ($\sigma_r$) for Gaussian chromatographic peaks.](image)

The ultimate parameters in separation methods are the peak resolution $R$, defined as\textsuperscript{15}

$$R = \frac{\Delta t}{4\sigma_t}$$  \hspace{1cm} (1.1)

and the analysis time $t$, equal to the retention time $t_{r,2}$ of the last eluting component.

Ideally, resolution $R \sim 1$ should be obtained (peak overlap < 2\%), and this in the shortest possible time. The separation is always counteracted by the finite peak width, which increases during the process. Because in a properly designed column the relative
displacement increases faster than the peak dispersion, theoretically a separation can always be obtained with a sufficiently long column and time.

Reducing the peak dispersion ($\sigma$) will improve the resolution or speed up the analysis. One possibility to reduce the dispersion is miniaturization, providing the detection and other practical problems do not counterbalance the gains.

In (pressure driven) liquid chromatography (LC), where transversal concentration gradients are induced by the nonuniform, in open columns parabolic, flow profile the peak dispersion can be significantly reduced by reducing the (effective) diameter $d$ of the free fluidic path.\textsuperscript{15}

This is accomplished by using smaller beads in a packed column or using a thinner capillary as an open (i.e. non-packed) column. The selectivity in LC is not directly influenced by this change. For fast analysis, when dispersion is dominated by the radial mass transfer, the resolution scales as

$$R_{\text{LC}} \sim \frac{\sqrt{L}}{d}$$

(1.2)

were $L$ is the length of the separation column. High efficiencies can be achieved in LC only with $d < \sim 5 \, \mu m$.

This is different from the situation in electrophoresis, where, because of the plug-like flow profile, maximum efficiency is achieved already with $d$ of several tens of $\mu m$. Further miniaturization only increases detection problems and is not feasible. An exception is perhaps the combination of electrophoresis with entropic effects on large molecules such as DNA in very narrow channels,\textsuperscript{17} or the use of extremely high voltages for ultrafast separations.\textsuperscript{18}

Zones are also broadened outside the separation column, in the injector, detector and connections. For separation systems on a chip\textsuperscript{19} it is therefore important that the fluidic part, starting from the point of the sample zone definition to the point of detection is integrated into a single substrate. This should preferably be replacible and have low fabrication costs, because its lifetime may be limited by gradual contamination when using real life samples. Integration of detector components may increase the sensitivity of the detection techniques used, but is feasible only if the cost and complexity of the device
remain acceptable. Integrated microelectrodes or some passive optical components seem suitable.

In this thesis, a microfluidic pressure driven analytical separation device is presented, which contains a partially integrated injector and, in a newer version, an internal UV detection cell.

2. Size Characterization of Large Analytes. Hydrodynamic Chromatography

Properties of those materials, which contain larger constituents such as macromolecules, micelles or solid particles depend on the size and/or size distribution of these species. For example, the size of latex nanoparticles determines the quality of latex paints or coatings, particles of silica and other minerals influence the properties of soil or natural water. Fluidic lubricants, soft or hard plastics can be made from polymers of the same chemical composition, e.g. polyalkanes, just by varying the degree of polymerization. Therefore, methods for size characterization of large species are important for industrial process monitoring or quality control in many fields.

Although optical techniques such as Light Scattering (LS) in a batch mode or in some cases direct microscope measurements can be used for the analysis, those techniques provide either an average size, or a size distribution in a very small ensemble of particles. Separation methods such as Size Exclusion Chromatography (SEC), Field Flow Fractionation (FFF) or Hydrodynamic Chromatography (HDC) offer a simple way to size characterization including size distribution (Figure 4). These methods are described below.

In the case of biopolymers, such as proteins or DNA, or bioparticles such as viruses, which are typically monodisperse, size separation is mainly used for identification. Although electrodriven methods are superior in this field, SEC, and especially FFF and HDC can be helpful when biological activity needs to be preserved, and thus interactions with a stationary phase or strong electric fields are not desirable.
General Introduction

SEC
In SEC,\textsuperscript{22} formerly called gel permeation chromatography (GPC), columns packed with highly porous beads are used for separation. Larger molecules are more excluded from pores than smaller ones, and thus elute from the column first, followed by ever smaller ones. A lower and an upper limit in size selectivity exist, due to analogous limits in the pore size distribution. Reduction of the particle size (\( \sim d \)) which is in SEC \( \sim 10 \, \mu \text{m} \) would increase the efficiency in the case of the slowly diffusing large molecules. However, extremely porous and thus mechanically unstable packing would be needed in order to maintain the pore size and the selectivity for these molecules. Therefore, further significant improvement in speed of analysis is not likely in SEC. Miniaturization down to a scale of a chip seems not feasible, because of the necessity of the porous structures. However, a reduction in the bore of the packed columns, presently of the order 7 - 4 mm, would lower the consumption of solvents and ease high temperature applications.

FFF
In field-flow fractionation\textsuperscript{15,23} (FFF), "open" (i.e. non-packed) flat channels (typically 2 cm x 100 \( \mu \text{m} \) cross-section) are used as separation columns. A transversal force is applied, perpendicular to the flow direction, which distributes the sample unevenly over the cross-section. This, combined with the different flow velocities over the cross-section in pressure driven flow, results in the separation of the analytes. In flow field-flow fractionation

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**Figure 4:** Size analysis using separation methods.
(FFFF) the transversal force is created using cross-flow. For that purpose, part of the channel walls has to be made as a porous membrane. Recently, suitable membranes resistant to some organic solvents, such as tetrahydrofuran, were introduced and also an axisymmetrical version was demonstrated using hollow porous ceramic fibers (HF5). Other physico-chemical phenomena can also be utilized in FFF to create transversal forces. Thermal gradients (ThFFF), gravitation or centrifugal (sedimentation) forces (SdFFF) and electrostatic forces (ElFFF and DEP-FFF) can be used, however the separation is then not based solely on size, which offers calibration problems, and the methods are more restrictive for the type of solvent or sample.

Because in FFF the species are focussed by the applied transversal force close to the channel wall, reducing the channel thickness does not improve the efficiency. In FFFF, the required complex fluid actuation disfavors an on-chip integration.

**HDC**

In HDC columns narrow fluidic paths are necessarily utilized (Figure 5). Because of a nonuniform flow profile and steric exclusion of analytes from the slowest velocity region near the channel walls, larger analytes are eluted earlier from the column than the smaller ones, similarly as in SEC. Because the separation mechanism is closer to that of FFF sometimes the name "hydrodynamic fractionation" (HDF) is used. Earlier the method was referred to as "separation by flow" and "surface exclusion chromatography." So far HDC has been performed in packed columns (PCHDC) or open-tubular (cylindrical capillary) columns (OTHDC; also CHDF).

HDC is faster and more efficient than the previously mentioned methods, because of the faster mass transfer in the narrow fluidic paths and the absence of partitioning as in SEC. However, its size-selectivity is low and therefore the high efficiency must be fully exploited. With packed columns or classical microcapillaries this is complicated by extra-column instrumental problems, nevertheless such devices have recently been developed and commercialized, although only for the analysis of particles. For the analysis of polymers, smaller packing sizes or smaller cylindrical tubes are necessary. This application was demonstrated experimentally, but not yet introduced in practice.
Figure 5: Separation principle and classical instrumentation in HDC.

In hydrodynamic chromatography the conduit thickness has an additional influence on the resolution through the separation selectivity. In the simplest approximation

\[ R_{HDC} \sim \frac{\sqrt{L}}{d^2} \]  

(2.1)

This implies that HDC should substantially gain from reducing d, although the resulting instrumental problems are by no means smaller than in other methods.

Table 2: Comparison of different analytical size-separation methods.\(^{22,23,32,39}\)

<table>
<thead>
<tr>
<th></th>
<th>SEC</th>
<th>Flow FFF</th>
<th>HDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size range (approx.)</td>
<td>1 nm — 1 (\mu)m</td>
<td>10 nm — 100 (\mu)m</td>
<td>10 nm(^a) — 10 (\mu)m(^b)</td>
</tr>
<tr>
<td>Peak efficiency</td>
<td>+/-</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Size selectivity</td>
<td>+</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>Analysis speed</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>Decrease in d</td>
<td>c(^c)</td>
<td>d(^d)</td>
<td>+ c,d(^e)</td>
</tr>
<tr>
<td>State of the art</td>
<td>++</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

\(^a\) Packed column HDC, \(^b\) Open tubular HDC, \(^c\) Packing size \(^d\) Open tube size
3. Research and Development in HDC

Early work

Already more than 100 years ago it was observed that in narrow veins the blood cells and particles move faster than the plasma (Fahreus effect), but no practical application was derived from this observation. In 1962 Pedersen has achieved a separation of proteins on a column with glass beads and related this to the flow profile in the interparticle space. DiMarzio and Guttman (1970) proposed theoretically flow-induced size-separation of flexible polymer molecules in a laminar flow of liquid in an open tube of various cross-sectional shapes and derived basic relations for the elution volumes and zone broadening. In their model only the steric exclusion and the so-called slip velocity is accounted for. Small et al. (1974-1977) have developed packed column HDC into a precise technique for the size characterization of colloids in a size range 10 nm - 1000 nm. Their experiments qualitatively agreed with the DiMarzio and Guttman models. The observed influence of the eluent ionic strength, the particle chemistry and the presence of a surfactant has been explained qualitatively by the presence of colloidal, i.e. electrostatic and Van der Waals forces between the particles and the capillary wall. Small has introduced the name hydrodynamic chromatography for the method. Stoisits et al. (1976) presented a model for packed column HDC based on a parallel array of interconnected capillaries. McHugh et al. (1976) could quantitatively predict retention in packed columns at low ionic strength. In this model the distance of closest approach of the colloids and the surface of the packing was calculated from a balance between electrostatic and van der Waals forces, adding this distance to the radius of the colloid particle to obtain the apparent particle diameter. Brenner and Gaydos (1977) attempted a proper hydrodynamic study of the HDC effect in cylindrical open tubes, arguing that the analysis of DiMarzio and Guttman was oversimplified. The analyte in their model is represented by a hard sphere, and the model accounts for hydrodynamic particle-wall interactions. These interactions limit the selectivity and the efficiency of the method. However, it is difficult to relate their model to practice because in case of permeable polymer coils the hydrodynamic interactions may be less pronounced, and in the case of colloids, the colloidal forces may dominate.
In 1978, HDC in open capillaries was also demonstrated experimentally. Although until today the research has concentrated more on packed column HDC, significant work was also carried out on open-tubular HDC systems (see further). The research in the two techniques is presented separately, noting that many results are important for both cases.

**Packed column HDC (since 1978)**

Priev and Hoysan\textsuperscript{28} and Silebian McHugh\textsuperscript{45} (1978) derived a quantitatively correct model for the retention of colloids in packed column HDC by incorporating colloidal forces in the model of Brenner and Gaydos. The models agreed well with the experiments of Small on polymer latexes.

Silebi\textsuperscript{46} et al. (1979) presented algorithms for the conversion of HDC chromatograms into true particle size distributions, including corrections for size-dependent detector response and axial dispersion. In addition, detection of latexes was studied extensively, notably the turbidimetric detection common in colloidal sciences. This method was found to be more sensitive than refractive index detection and the highest sensitivity was obtained when the incident light was both absorbed and scattered.

Nagy\textsuperscript{47} et al. (1981) tested the validity of the so-called universal calibration in HDC, which allows the size characterization of particles from different materials than particle size standards. The elution of various polymer latexes at low ionic strength was consistent with the model.

McGowan and Langhorst\textsuperscript{48} (1982) developed an integrated computer controlled HDC system for routine size-characterization of colloids. Efficiency and selectivity was improved by using smaller, 15-\(\mu\)m packing particles. Van Gilder and Langhorst\textsuperscript{49} (1985) used this system for monitoring particle growth during latex polymerization. Thorton\textsuperscript{50} et al. (1985) used a comparable system for characterization of latexes of a size 40-1100 nm. The data agreed with measurements by transmission electron microscopy (TEM), photon correlation spectroscopy and sedimentation FFF.

Hoagland and Prud'homme\textsuperscript{51,52} et al. (1982-1988) presented HDC experiments on ultrahigh molecular mass species, which were too large for conventional SEC. The latter were xanthan polysaccharides, dextran, hydrolyzed polyacrylamides, TMV viruses and DNA's. Fluorescence detection was used. Dependence of the elution behavior on the flow rate was
observed and was explained by shear induced orientation or elongation of the large species in the flow. HDC was also applied to monitor polymer degradation by sonication. Leitzelement\textsuperscript{53} et al. (1984) applied HDC to analysis of paints and milk, using columns packed with 20 \( \mu \text{m} \) ion exchange resins. Lecourtier\textsuperscript{36} et al. attempted analysis of large (M > \( 10^6 \)) xanthan polymers with HDC using differential refractive index detection. Langhorst\textsuperscript{54} et al. (1986) used low-angle light scattering detection in HDC separation of polyacrylamides.

Secchi\textsuperscript{55} et al. (1987) investigated the recovery of latexes in columns packed with ion-exchange resin. The recovery was found to increase with decreasing solute size, decreasing eluent velocity, increasing surfactant concentration and repeatedly injecting the sample. Kraak\textsuperscript{30} et al. (1989) achieved separation of polymers in the molecular mass range \( 10^4-10^7 \) and a preliminary separation of proteins in columns packed with 2.1 \( \mu \text{m} \) non-porous silica particles.

Stegeman\textsuperscript{32,56} et al. (1990-1994) and Venema\textsuperscript{34,57} et al. (1994-1998) extensively studied the separations of mainly polymers in columns packed with 0.75-2.7 \( \mu \text{m} \) non-porous silica particles. The feasibility of the method was demonstrated by separation of polystyrene standards in the molecular mass range \( 10^4-10^6 \) in THF. Other polymer materials and solvents were also used. Packing problems and extracolumn peak broadening were found to be the limiting factors. Coupling of HDC to ThFFF, and pressure and electrodriven HDC in packed capillaries was also attempted.

Klavons\textsuperscript{58} et al. (1997) applied HDC to analysis of waxy maize starch. Peyrin\textsuperscript{59} et al. (2000) studied HDC effects and separations of DNA molecules.

Williams\textsuperscript{37,60} et al. (2002) presented recently a modern version of a packed column HDC system for routine size analysis of colloids.

**Open-tubular HDC (since 1978)**

Noel and Mullins\textsuperscript{44} et al. (1978) used stainless steel tubes with inner diameters of 250-500 \( \mu \text{m} \) and length of 80-200 m to fractionate various particulate materials with sizes of 0.02-50 \( \mu \text{m} \) in aqueous buffers. Latexes, pollens, bacteria spores and silica particles were used as the samples. An influence of flow velocity and viscosity on the elution behavior was observed and attributed to radial hydrodynamic forces. Brough\textsuperscript{61} et al. (1981) carried out
similar but more extensive studies, and demonstrated the use of the system for the analysis of paint and metal particles in used engine oil. Methanol and THF were used as eluents. De Jaeger\(^6\) et al. (1986) focused on the influence of the eluent composition on the elution of carboxylated polystyrene latexes. Elie and Renaud\(^6\) (1987) used very large tubes of 4-15 mm internal diameter and 12-15 m long to obtain size distribution of paper fibers of lengths up to 4 mm. Shiragami\(^6\) (1990) used stainless steel tubes of 260 \(\mu\)m diameter to analyze contaminants in a bioreactor.

Tijssen and Bos\(^6\) et al. (1983-1995) demonstrated the possibility to analyze solutes of size down to 3 nm in an open-tubular HDC setup, using fused silica capillaries of internal diameter 1.2-2.6 \(\mu\)m and 0.8-3.3 m length with UV detection at 210 nm. Polystyrene standards in the molecular mass range \(10^4-10^6\) g/mol in THF were separated in \(~10\) min with a resolution similar or better to state of the art SEC. Efficiency up to 400,000 theoretical plates was achieved\(^6\). However, detection in these microcapillaries was difficult. The models of Brenner and Gaydos and DiMarzio and Guttman were evaluated, the results being more in favor of the latter model. In the same system influence of temperature on dissociation of micelles was studied\(^6\). In a later publication (1992), the influence of the flow velocity was studied and some new theoretical concepts such as reptational chromatography were introduced\(^6\). Silebi and DosRamos\(^6\) (1989) used 4-60 \(\mu\)m ID fused silica capillaries 1-20 m long for the analysis of polystyrene latexes of size 100-1100 nm. The influence of eluent velocity and surfactant concentration were studied and the separation performance and feasibility of OTHDC for obtaining size distribution was discussed.

Ploehn\(^3\) (1987) and DosRamos and Silebi\(^6\) (1989) presented a detailed and rather complete theoretical analysis of relative retention and later also axial dispersion\(^6\) (1990) of particles in open tubular HDC with cylindrical capillaries. Steric exclusion, hydrodynamic particle-wall interactions, colloidal and inertial (radial hydrodynamic) forces were all included in the model which could accurately describe the influence of ionic strength and flow velocity on the elution behavior of colloids. Miller\(^7\) et al. (1995) applied open-tubular HDC to monitoring of the evolution of the particle size distribution during emulsion polymerization.
Hollingsworth and Silebi\textsuperscript{71} (1998) presented a more detailed analysis of electrostatic and electrokinetic forces in open tubular HDC of colloids.

Willemsen\textsuperscript{72} et al. (2002) used simulations by dissipative particle dynamics (DPD) to study the behavior of a polymer in a square capillary with laminar flow.

**HDC chip proposal**
In 1997 Tijssen\textsuperscript{73} et al. proposed an integrated micromachined HDC system (Figure 6) as a potential solution to both the detection problems in microcapillary OTHDC and the limited efficiency, packing difficulties and shear stress on large polymers in PCHDC. The development of this system, further referred to as HDC Chip, forms the scope of this thesis.

![Figure 6: The layout of the proposed HDC chip.](image)

**4. Microtechnology for the HDC Chip**

In this section is a brief overview is presented of the technology used in the fabrication of the HDC chip and its connections. Details on the procedures can be found elsewhere.\textsuperscript{74} The choice of materials for the HDC chip follows from the practical requirement of the compatibility with various, mainly organic solvents and the basic requirement of a precise, stiff geometry for the HDC channel. Silicon,\textsuperscript{19} due to its monocrystallinity, allows etching of structures with an almost atomic resolution.\textsuperscript{75,76} It is, as well as the other used inorganic materials (glass, fused silica and stainless steel), inert to almost all chemicals.
The fabrication of a silicon-glass chip is shown here, however similar processing technology was used for the recently developed chips from fused silica (cf. Chapter 6).

The chip fabrication process (Figure 7-10) starts with the creation of the etching masks, using electron beam lithography. Three masks are needed: one for definition of the shallow channels, one for patterning of the deeper structures and one for the through-holes. The silicon wafers are thermally oxidized to allow the easier etching by HF. A photoresist layer is applied by spin-coating and the mask pattern of the channels is transferred into this layer using photolithography (Figure 7).

![Figure 7: Fabrication - step 1: Thin layer technology and patterning by photolithography.](image)

Subsequently, the shallow channels are defined into the silicon oxide layer with HF (Figure 8). The etching stops at the silicon wafer. For the creation of the deeper slits, Reactive Ion Etching,\(^\text{77}\) (RIE) is used in order to achieve both a sufficient etch depth and preserve the required narrow width (before this etching step, another transfer of a mask is performed, analogous to Figure 7).

Finally, a Pyrex glass wafer is processed which is used as the cover wafer to close the channels from the top. Fluid access holes are created in this wafer by powderblasting,\(^\text{78}\) the wafer is polished and fusion-bonded\(^\text{79}\) to the bottom silicon wafer (Figure 9).
Figure 8: Fabrication - step 2: Etching of the channels.

Figure 9: Fabrication - step 3: Processing and bonding the cover wafer.
Fluidic connection of the chip is realized with a stainless steel clamp (Figure 10) to which stainless steel connection tubing is soldered using a silver based alloy. The clamp-chip interface is sealed by chemically resistant Kalrez™ O-rings. A photograph of the prototype HDC-chip system is shown in Figure 11.

Figure 10: The chip is clamped to external tubing.

Figure 11: The test setup for separation experiments on the HDC chip prototype with fluorescence imaging microscopy (FIM) detection.
5. Perspectives of Detection Techniques for the HDC Chip

The crucial problem in microfluidic chip systems in general still seems to be the detection. Recent reviews of the development in this field and in the broader context of μTAS are available.\textsuperscript{80,7,9} In most of the miniaturized detection techniques, low detection volumes\textsuperscript{81} limit the sensitivity. Fluorescence (mainly laser induced, LIF) detection, being the most successful detection technique on chips so far, can only be used for fluorescently labeled analytes. Hence, it is the method of choice for biomacromolecules in aqueous buffers. Miniaturized electrochemical detectors offer a good perspective for many microsystems. However, neither electrochemical nor fluorescence detectors are directly applicable to synthetic (uncharged) polymers in mainly organic solvents, which represent an important field for the HDC chip.

More universal detection principles are UV light absorbance and especially differential refractive index (RI) measurement. For large analytes, for example in SEC, also other universal detection techniques are often applied. Those are viscosity detection (for solutions of flexible polymer molecules) and light scattering (LS). The techniques are sensitive both to concentration and mass and are mostly used in combination with a concentration detector such as UV or RI.

UV-VIS absorption detection and related techniques

In liquid chromatography (LC) the most used detection principle is UV light absorption. In classical HDC, it is applied almost exclusively, mainly because the detection volumes of other standard detectors using refractive index, viscosity or light scattering are too large with respect to the high peak efficiency obtained in the method. The integration of UV detection on a chip is more difficult than in the case of fluorescence, however, promising results have recently been reported.\textsuperscript{82,83,84,85} In the present work, a UV cell is implemented in a fused silica HDC chip.

In a solution of large particles, the intensity of the transmitted light is reduced both by absorption and scattering. This so-called turbidimetric detection is often used for colloids. An interesting version of light absorption measurement is the so-called thermal lens technique,\textsuperscript{86,87} in which a strong focused laser beam is passed through a specimen. The
more the light is absorbed by the analytes the more it increases the sample local temperature and changes its refractive index. This is sensed by a second weaker beam with a different wavelength.

**RI detection**

Two classes of RI detection schemes can be distinguished. In the classical RI detectors for normal scale LC, the measurement is based on the *refraction* of an external light beam. This generally insensitive method was also demonstrated in a microsystem where a laser beam was employed.\(^8^8\) External beam RI measurement in microsystems can also be realized by continuously probing the angle of total internal reflection\(^8^9\) or by using backscatter interferometry.\(^9^0\)

The second class of RI detection methods has been realized in various microsystems and uses integrated optical techniques. It is based on *evanescent field* effects known from wave optics. The discrete propagation modes of light in a waveguide are influenced by the refractive index of the closest surrounding environment e.g. the sample.\(^9^1,9^2\) This is because the electromagnetic light wave travels partly on the outer side of the waveguide as an evanescent field, with depths typically \(\sim 100\) nm. The sensitivity of this method is \(\sim 10^{-5}\) RI units and is largely independent of the cell volume. This makes it a good candidate for the miniaturized cells. Higher sensitivity can be achieved with integrated optical ring resonators.\(^9^3\) Using a spiral waveguide, RI can be measured using so-called whispering gallery modes.\(^9^4\) The most sensitive integrated optical RI detection technique can be achieved in an interferometric setup.\(^9^5\) Sensitivity values of better than \(10^{-8}\) RI units have been reported. As this device employs an active, modulated Mach-Zehnder interferometer, it is also the technologically most challenging technique.

Although some of the methods based on evanescent light roughly compare in size, type of materials and sensitivity with the requirements for the HDC chip, implementing of those techniques would likely be an extensive task in the current stage of developments.

**LS detection**

A solution of larger species scatters incident light. The intensity of the scattered light varies with the angle of the measurement with respect to the incident beam, increases with the
species size and strongly increases with the decreasing wavelength of the light. However, the intensity of the effect is in general low and thus laser light is mostly used for the illumination. In practice, the intensity can be measured at several angles\(^9^6\) (MALLS) or at one, mostly right angle (RALLS) or a very small acute angle where the scattered light has the highest intensity (LALLS). From the LS techniques, RALLS seems the least demanding one for instrumentation and may be thus interesting for the HDC chip. However, also here an extensive development would be necessary.

**Viscosity detection. A microviscodetector**

Polymer in a solution\(^9^7\) increases its viscosity, \(\eta\), according to

\[
\eta = \eta_0 (1 + \eta_p) = \eta_0 (1 + c[\eta])
\]  \hspace{1cm} (5.1)

where \(\eta_0\) is the viscosity of the pure solvent, \(c\) the local polymer concentration, \(\eta_p\) is the so-called specific viscosity and \([\eta]\) the so-called intrinsic viscosity of the polymer in the solvent. \([\eta]\) is related to the molecular mass \(M\) through the Mark-Houwink relationship

\[
[\eta] = kM^a
\]  \hspace{1cm} (5.2)

where \(k\) and \(a\) are empirically determined constants, known for many polymer-solvent pairs.\(^9^8\)

Differential viscometry in a capillary-bridge configuration\(^9^9\) (Figure 12 left) is commonly used as a detection method in combination with analytical polymer separations. From the measured pressure drops the specific viscosity can be obtained as

\[
\eta_p = \frac{4\Delta P}{P_1 - 2\Delta P}
\]  \hspace{1cm} (5.3)

Using (5.1) and (5.2) the molecular mass can then be determined, if \(c\) is measured independently. Alternatively, when the separation method is calibrated for size for a certain
Genera ll Introductio n

Type of species, and the same relations are applied, the viscometer can be used as indirect concentration detector.

The smallest commercially available viscometer has, however, a cell volume of several \( \mu \text{L} \)'s, which is much too large for the HDC chip, but also for semi-miniaturized methods e.g. capillary size exclusion chromatography. Miniaturization of such a viscometer to a chip-scale was attempted in the context\(^\text{100}\) of the present HDC chip project. Small pressure drops of \( \sim 1 \text{ Pa} \) have to be measured by the differential pressure sensor and only very small hydraulic compliance (membrane deflection) is allowed because of the very small detection volumes. Therefore, a unique pressure sensor had to be developed (Figure 12 right). Subsequently, this sensor was integrated into a prototype of a chip microviscometer (Figure 13, Figure 14).

![Figure 12: Scheme of a capillary bridge viscometer (left). The ultrasensitive pressure sensor (right).](image)

![Figure 13: Layout of the microviscometer.](image)
Figure 14: Fabrication results. SEM image of the split region (left), stand alone microviscometer prototype (right).

Preliminary experiments show that the device is able to detect an injected plug of liquid of different viscosity than the carrier fluid (Figure 15).

Figure 15: Response of the bridge pressure sensor and the differential sensor on an injected plug of ethanol in water in the prototype of the microviscometer.

At this stage the viscometer is not suitable yet for integration into the HDC chip, because of its insufficient sensitivity, by a factor of ~40. Although increase in the sensitivity should be achieved via further optimization, the application to the HDC chip will likely remain difficult because of the extremely small flow rates involved. Possible application of the microviscometer for semi-miniaturized separation methods however looks promising.
Imaging methods for inspection of flows in microsystems

In the presented work, imaging by fluorescence microscopy (FIM), apart from its use as a simple detector in the test separations, is utilized for a simple inspection of the species transport in the system. This can, in principle, also be used to obtain information about the flow profile in the systems, through monitoring the concentration profiles of species of known diffusion coefficient at different times. However, this so-called scalar imaging velocimetry (SIV), would require a very sensitive camera with a high acquisition rate if applied to 1 μm depth.

6. Transport Equations and Computational Fluid Dynamics

In the field of separation techniques, the study of transport phenomena is important in the design and optimization of the methods. In a macroscale chromatographic system, standard components are used, such as a packed column or a standard detection cell and cylindrical connection tubing. Those can be described individually by well established models. Contrary, in integrated microfluidic devices design-unique features have often to be created. Here a detailed description of flows and species transport is important in order to fully exploit the advantages of miniaturization.

In general, the motion of fluids is described by the Navier-Stokes equations which, for a pressure-driven flow of an incompressible Newtonian fluid can be written as

\[ \nabla \cdot \mathbf{u} = 0 \]  \hspace{1cm} (6.1)

\[ \frac{\partial \mathbf{u}}{\partial t} = \frac{\eta}{\rho} \nabla^2 \mathbf{u} - (\mathbf{u} \cdot \nabla) \mathbf{u} - \frac{1}{\rho} \nabla p \]  \hspace{1cm} (6.2)

where \( \mathbf{u} \) is the velocity profile vector, \( p \) the pressure profile, \( \eta \) the dynamic viscosity and \( \rho \) the density of the fluid. The equations represent mass continuity (6.1), and momentum conservation (6.2). The properties of the flow equations depend on the Reynolds number \( Re \), which represents the ratio of viscous and convective transport of momentum.
\[ Re = \frac{\rho ud}{\eta} \]  \hspace{1cm} (6.3)

In this definition, \( u \) is a characteristic velocity and \( d \) the characteristic dimension. For \( Re < 10^3 \) flows are laminar, furthermore, for \( Re < 1 \) the second term in RHS in (6.2) is negligible and the equations become linear (so-called creeping or Stokes flow) and therefore much simpler. In microdevices \( Re << 1 \) is typical for liquids.

The transport of non-reacting species in the flow field \( u \) is governed by the convection-diffusion equation\(^{102}\)

\[ \frac{\partial c}{\partial t} = D \nabla^2 c - u \cdot \nabla c \]  \hspace{1cm} (6.4)

where \( c \) is the species concentration profile, \( t \) the process time and \( D \) the diffusion coefficient. For this equation, a characteristic dimensionless parameter is the Peclet number \( Pe \),

\[ Pe = \frac{ud}{D} \]  \hspace{1cm} (6.5)

which represents the ratio of the convective and diffusive transport of species. Different characteristic dimensions can be chosen as \( d \). These can be transversal or parallel to the direction of the flow and thus both transversal (radial) and longitudinal \( Pe \) numbers can be defined. For \( Pe >> 1 \) the transport of species is dominated by convection, for \( Pe << 1 \) the transport is dominated by diffusion.

**CFD strategy**

Analytical solution of the transport equations (6.1, 6.2 and 6.4) is possible only for very simple cases.\(^{102}\) However, state-of-the-art Computational Fluid Dynamics\(^{103}\) (CFD) method provides accurate numerical solutions in virtually any geometry, if sufficient computer power is available. Both 2D and 3D problems can be studied, however, 3D problems are
much more demanding in computational resources and time. In the following sections a brief introduction to the CFD concepts relevant to the presented work is given. The information given here was adapted from various sources.\textsuperscript{103,104,105,106}

In CFD (Figure 16) a virtual geometric domain is created, which represents the real geometry of the problem. This domain is divided into very small parts. Those form a mesh (grid) on which the transport equations are numerically approximated and solved. When both the flow and the species transport vary in time, the equations have to be solved simultaneously. When the flow is stationary the flow equations are solved first and with the obtained flow profile the species transport is simulated. This so-called \textit{decoupling} requires much less computation power and can also be used as an approximation for problems where flow varies in a step-like manner.

\begin{center}
\textbf{Figure 16:} The process of the CFD modeling (decoupled).
\end{center}
The computation procedure is rather complex even in 2D models and thus not own but commercially available software is mostly used. Only recommendations rather than solid rules for the simulations can be found in literature, partly because the development in the CFD field is still going on, and partly because of the nature of the fluidic problems. Practice, and a background in numerical mathematics and flow physics minimize the chance of unrealistic solutions. Specifically, the creation of a computation mesh in complex geometries can be a lengthy task and requires experience and often a trial-and-error procedure, even with advanced meshing software.

**Finite volume approach**

In this thesis a commercially available CFD package is applied,\textsuperscript{106} which uses a finite-volume (control-volume) approach to solve the transport equations. In this approach the studied geometry is divided into a large number of small elements, called control volumes (Figure 17) or computation cells, which have simple geometrical shapes.

![Figure 17: Control volumes](image)

The equations (6.1), (6.2) and (6.4) are considered in their integral form for the control volume. For example, the integral form of equation (6.4) can be written as

\[ \int_{\Omega} \frac{\partial c}{\partial t} \, d\Omega = \int_{S} D \nabla c \cdot dS - \int_{S} c \mathbf{u} \cdot dS \]  \hspace{1cm} (6.6)

where $\Omega$ is the cell volume and $S$ its surface. The integral equations are approximated using numerical expressions for the derivatives and the surface and volume integrals. Time is
divided into small, discrete time steps. In these expressions, the concentration in the cell at a specific time is related to the concentrations in the neighboring cells and the closest subsequent times, resulting in a linear algebraic form of the equations. For example eq. (6.6) becomes

$$a_p c_p^0 = \sum_{i} \sum_{j} a_{ij} c_j$$

(coefficients $a$ are functions of the cell size and geometry, the flow field and the diffusion coefficient, subscript $p$ refers to the considered cell and $n$ denotes the neighboring cells. The superscript 0 denotes the current time. When eq. (6.7) is written for every cell, a set of linear equations is obtained which is then solved, and the concentration in every cell is obtained. The same procedure is performed for the following time steps.

In the flow simulations, analogous procedures are applied. Because pressure is implicitly specified with the set of the flow equations (eqn's. 6.1, 6.2), an iterative procedure is necessary, in which the pressure and velocity field are adapted till a consistent solution is obtained. A pressure-correction algorithm SIMPLE (Semi-Implicit Method for Pressure-Linked Equations) is often used.

The finite volume approach is conservative and can be applied to a fluidic problem of an arbitrary geometry. This is an advantage compared to the older and now less used finite difference approach where a grid of points and differential form of transport equations are used. In a finite element approach, so-called weight functions are implemented. The method is otherwise similar to the finite volume approach.

**Numerical schemes in space**

In the numerical approximation process, fluxes on the interfaces have to be calculated from values of quantities which are stored at the cell centers. In an *upwind* scheme (UDS) values from points located upstream are used for this. The UDS scheme is numerically stable, however, its first order version leads to numerical diffusion and should not be used. In a *central* difference (also linear-piecewise) scheme (CDS) values on both sides of the interface are used. CDS is second order and thus more accurate, although less stable and can produce oscillatory solutions. Higher order upwind and *hybrid* schemes e.g. QUICK
(Quadratic Upwind Interpolation for Convective Kinematics) are often used\textsuperscript{103}, and are generally more accurate and stable, however may produce a physical overshoot in the calculation e.g. a small negative concentration on a too coarse grid.

**Numerical schemes in time**

Similarly, the variation in time can be approximated *explicitly* or *implicitly*. In the explicit formulation only the values from the previous time step are included, while in the implicit formulation also the values from not yet calculated time steps occur. Implicit schemes enforce numerical stability and physically correct solutions can be obtained with much longer time steps then with explicit schemes. This scheme is generally preferred, although it requires more computational power. Also, higher order schemes, relating more than two subsequent time steps in the calculation (Runge-Kutta) or hybrid schemes (e.g. Crank-Nicolson), are possible.

In the CFD work described in this thesis, flow is simulated as steady. Second order upwind or QUICK schemes and SIMPLE pressure-velocity correction were used. The convection-diffusion of species has to be considered as unsteady in all cases. Second order implicit unsteady formulation is adopted for this. The data are calculated and stored with double precision (numbers of precision type 'double'), which is recommend for problems with large aspect ratio domains.

**Boundary conditions**

Boundary conditions have to be given for (6.1), (6.2) and (6.4), e.g. by specifying directly the value of the dependent variable (Dirichlet formulation) on the boundaries. In the case of the flow simulation, a uniform velocity is specified (which is alternative to the specification of the pressure) on the inlets. In the case of species simulation, the mass fraction at the inlets is specified, which together with the already specified velocity represents the connective influx of species through the boundary. The mass fraction at the boundary can be specified as a constant or as a time-dependent function.

In the presented simulations, the so-called 'interpreted user-defined-function'\textsuperscript{106} in time was used on the inlet in order to introduce a broad (Gaussian) peak into the domain. Diffusive
fluxes at the boundary have to be set to zero in order to control the amount of the introduced species. An alternative way to introduce species is defining the concentration distribution inside the domain as an initial condition (Cauchy formulation). This 'patching' can be done using the so-called 'custom-defined-function.' A rectangular concentration profile can be introduced using 'adaptation regions.'

Accuracy
The CFD simulation results differ from reality by three types of errors, which are described below.

*Model errors* can occur because of unrealistic assumptions about the nature of the flow e.g. improper use of symmetry, oversimplification of boundary conditions, oversimplification of the geometry or when approximating non-stationary flows as stationary, for example in decoupling. Model errors can be discovered by using a more detailed model or by an experiment. In laminar pressure driven flows model errors are less critical than in turbulent or electrodriven flows.

*(Spatial) discretisation* and *time-stepping errors* result from the necessary discrete representation of space and time in the numerical solution procedure and decreases when finer grids and smaller time steps are used. These errors can be studied by considering the so-called *truncation* error, which is the imbalance between the differential and the discretisation equations. This error can be illustrated by using Taylor series expansions for the variables in the discretisation equations. The discussed discretisation schemes follow from truncating these series to only the lowest order terms. The order $n$ of the first neglected term is the order of the error and the so-called order of the scheme. The error is proportional to the $n^{th}$ power of the grid or time spacing. Generally, every simulation should be checked for grid and time-stepping dependence by refining or coarsening the spatial and temporal discretisation and comparing values of representative quantities in the obtained solutions. In some cases, when the order of the scheme is known, the error and thus the true solution can be determined even with coarse discretisation (Richardson extrapolation). In first order spatial-discretisation schemes the truncation error contributes directly to the diffusive fluxes in (6.2) and (6.4) and causes the so-called *numerical diffusion*. The error can be large when too coarse grids or large aspect-ratio-cells are used in regions with steep
gradients and when the flow is oblique to the grid. A remedy is a refinement and the use of higher order schemes.

The number of cells is limited by the computer memory and acceptable computing time. On current PC's, \(10^6\) cells is approximately the maximum. Nonuniform grids must often be used, especially in large aspect ratio domains, in order to spare cells in less critical regions. The size of the time step should be comparable to the smallest time constant in the problem. This is usually the time of transport of species over one control volume, either by convection or diffusion. For fast diffusing species, more resolution in time and thus more time steps are needed.

*Convergence error (stopping error)* is introduced when the calculation is stopped before the iterative solving procedure for eq. (6.7) reaches the machine accuracy. In practice the so-called sum of residuals is often monitored and the iterations are performed until the sum drops below a certain level or when a chosen maximum number of iterations is reached. This convergence criteria can differ for different problems. Therefore, also monitoring of a change in a representative quantity (key-parameter) such as pressure drop, average fluxes or concentrations at a specific plane is recommended for each iteration. In the time-resolved calculation, each time step has to be converged sufficiently before the next time step is calculated. Here the judgement of the convergence is critical because the time needed to calculate one step multiplies by the large number of time steps that usually have to be calculated.

In the simulations presented in this thesis, double precision was used and the solution of the (stationary) flow was iterated until the sum of residuals dropped by 8-10 orders of magnitude and this required some 200-500 iterations. In the species-transport simulation (non-stationary), 10-15 iterations were performed for each time step, and the sum of residuals dropped by 5-6 orders of magnitude. A typical simulation on a Pentium 500 MHz, 750 MB RAM, with Fluent 5 running under Windows NT for a computation domain of 200 000 cells took about 2-4 hours for the calculation of the flow and 1-2 days for every 1000 time steps of 1 ms in the concentration profile simulation.
7. Scope of This Thesis

The present work was performed within the frame of the project 'Hydrodynamic Chromatography in Integrated Micromachined Separation Systems' (STW-NWO funded project number AAC4556) which was carried out in cooperation between the University of Amsterdam and the University of Twente.

This thesis focuses on the chromatographic and fluidic aspects of the development of this HDC chip system. Microtechnology aspects of the design and details on the development of the microviscometer can be found in the thesis of Marko Blom.\textsuperscript{74}

In the following chapters theoretical and experimental results on the design of the system are presented. In Chapter 2, theoretical aspects of miniaturization of pressure driven liquid chromatography and specifics of hydrodynamic chromatography are discussed in detail. In Chapter 3, the first demonstration of hydrodynamic chromatography in flat microchannels is presented using the first chip prototype. Chapter 4 describes in detail the specific injection system that was used in this device, designed using CFD simulations. In Chapter 5, a CFD design of an optimized transition structure for the flat channel and a detection cell is presented. In Chapter 6, experiments on improved prototypes of the HDC chip are shown, notably a fused silica HDC chip with an on-chip UV detection.

The chapters in this thesis have been written as articles for publication in international scientific journals and can be read independently. Therefore some overlap may occur.

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CHAPTER 1
