Thermal field-flow fractionation of polymeric and particulate materials: applications and fundamental aspects
Mes, E.P.C.

Citation for published version (APA):
Mes, E. P. C. (2002). Thermal field-flow fractionation of polymeric and particulate materials: applications and fundamental aspects

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General Introduction

Since the first synthesis of a polymer in 1862 an ever-increasing variety of polymeric materials have become available and synthetic polymers play a more and more important role in modern society as raw materials for plastics, fibers, elastomers, and adhesives.\(^1\)

Polymers (Greek: *polys* = many, *meros* = part) consist of repeating segments of small molecules (strictly defined, polymers and macromolecules are not synonyms). Usually, polymeric products consist of polymer(s) mixed with so-called auxiliaries (additives such as: antioxidants, flame retardants, and light stabilizers; and modifiers such as: cross-linkers and fillers).\(^2\) The list of applications of polymers is virtually endless.

For many years the plastics market has been the strongest expanding materials sector and it continues to expand into markets not historically reserved for plastics, \textit{e.g.}, as replacement for wood and steel.\(^3\) Apart from commodity plastics, produced in bulk, there is an increasing demand for polymers with tailor made properties to be used in high-tech applications.\(^4\)

Essential to the successful development and production of polymers is the ability to characterize these materials. This requires a range of sophisticated analysis methods.\(^5\)

If we focus on the raw polymer material - thus excluding auxiliaries - properties of polymers and polymeric particles are determined by their chemical composition, mass/size, and conformation. The strong correlation between molecular mass/size and polymer properties has led to the development of a number of important separation methods to determine the molecular mass/size, or better, the mass- or size-
distribution. Well-known methods are Size-Exclusion Chromatography (SEC),\textsuperscript{6} Hydrodynamic Chromatography (HDC),\textsuperscript{7} and Field-Flow Fractionation (FFF).\textsuperscript{8}

By far the most often used separation method for polymers is Size-Exclusion Chromatography. SEC is carried out in a packed column, through which a liquid is forced to flow. A dissolved polymer sample is injected onto the column. Separation is achieved by the size-dependent distribution of the sample molecules between the stagnant mobile phase inside the pores of the packing material and the moving mobile phase outside the pores. Low-molecular-mass (LMM) polymers are more retained than high-molecular-mass (HMM) polymers. The range of polymer sizes that can be separated is determined by the pore size. SEC is a robust technique that does not require an expert to be run. The technique can be used to determine the molecular mass distribution of polymers with a molecular mass up to $\approx 10$ MDa. For materials with higher molecular mass or particulate material SEC is not suited. Shear degradation of polymer chains can occur and large molecules or particles can even block the column.\textsuperscript{9}

An alternative to SEC is Hydrodynamic Chromatography (HDC), also known as Hydrodynamic Fractionation (HDF). Separation in HDC occurs in narrow open tubes or in packed columns under laminar flow conditions. The separation is based on the steric exclusion of large molecules from the wall (tube wall or particle wall). In an HDC column, the mobile phase flows with a parabolic velocity profile. Therefore, the distance from the solute to the wall determines the longitudinal velocity. The separation is solely based on size. The retention order is the same as for SEC; HMM solutes elute first. HDC is suited to analyse polymeric material\textsuperscript{10} as well as particulate material.\textsuperscript{11} The selectivity is low, but this is partly compensated by a very high efficiency. Still, HDC is seldom used for polymer analysis. More successful is its use for particle analysis.\textsuperscript{12}

A third technique is Field-Flow Fractionation, capable of separating polymers as well as particles. This technique is the main topic of this thesis and will be discussed in more detail in the remainder of this chapter.
Field-Flow Fractionation

FFF is an emerging separation technique complementary to SEC that gained slowly in popularity during the last decade. The FFF principle was suggested by Giddings in 1966. Nowadays, FFF comprises a number of techniques all based on a one-phase system in which separation is achieved by applying an external field perpendicular to a laminar solvent flow.

In practice, a carrier liquid is forced to flow under laminar conditions through a ribbon-like channel without packing material (Figure 1). A sample is injected and is taken along with the liquid stream through the channel.

Figure 1: Principle of FFF. F is a force, D is the diffusion coefficient, w is the channel height, and l is the mean layer thickness equal to the ratio of the diffusion coefficient D and transverse component velocity $u_F$.

Perpendicular to the flow a force F is applied, acting on the solutes to be separated, that makes the injected components move towards a channel wall with a velocity $u_F$. This is counteracted by the Brownian motion (D), which results in an equilibrium distribution of the components to be separated close to one wall (the accumulation
The separation in the axial direction is, just as in the case of HDC, the result of the velocity gradient of the carrier liquid near the wall. However, the elution order is the reverse of HDC, i.e., small molecules/particles elute before large. Due to the open geometry of the channel, FFF can be used for dissolved polymers as well as for colloids and particles. The separation efficiency is low compared to SEC and HDC but the selectivity is relatively high.\textsuperscript{15}

\textbf{Separation mechanism}

\textit{Normal mode}

The net flux of material in the transversal direction in an FFF channel is given by:\textsuperscript{14}

\[ J_x = u F c(x) - D \frac{\partial c}{\partial x} \tag{1} \]

Here, \( c \) is concentration and \( x \) is the position relative to the accumulation wall. The first term of the right-hand side of Eq. [1] is the result of the field-force in the direction of the accumulation wall and the second that of the opposing Brownian motion. A dynamic steady state is reached when the cross-force and the counteracting diffusive force are balanced, so that the net flux is zero and a concentration profile according to

\[ c(x) = c_0 e^{-\frac{x u F}{D}} = c_0 e^{-\frac{x}{f}} \tag{2} \]

is formed. Here, \( c_0 \) is the concentration at the accumulation wall.

Under laminar flow conditions the velocity profile in a rectangular FFF channel takes the form

\[ v(x) = \delta(v) \left( \frac{x}{w} - \left( \frac{x}{w} \right)^2 \right) \tag{3} \]

where \( w \) is the channel height and \( \langle v \rangle \) denotes the average cross-sectional liquid velocity.\textsuperscript{16} Superposition of the above concentration profile \( c(x) \) across the velocity profile \( v(x) \) allows the calculation of the retention of a component:
Genera l Introductio n

\[ t_R = \frac{\langle v \rangle}{V}, t_0 = \frac{t_0}{6\lambda \left( \coth \left( \frac{1}{2\lambda} \right) - 2\lambda \right)} \]  

[4]

Here, \( V \) is the mean velocity of an injected component. The dimensionless retention parameter \( \lambda \) is given by \( D/(u_F w) \) or \( l/w \).

For small values of \( \lambda \), Eq. [4] can be simplified to:

\[ t_R \approx \frac{t_0}{6(\lambda - 2\lambda^2)} \]  

[5]

or

\[ t_R \approx \frac{t_0}{6\lambda} \]  

[6]

The approximate Eq. [5] can be used at relatively low retention. Retention ratios \( (t_R/t_0) \) of 1.5 or higher give an error of less than 1 %. Eq. [6], however, is only useful at high retention. Retention ratios > 7.5 give errors less than 5 %.

Steric mode

In the normal mode of FFF it is assumed that the sample moves to the accumulation wall and forms a zone with an exponential concentration distribution. This relatively simple model holds well for polymers and small particles. However, due to their finite size, particles can approach the wall no closer than their radius \( (r) \). This leads to a sterically corrected FFF retention equation:

\[ t_R \approx \frac{t_0}{6 \left( \lambda + \frac{r}{w} \right)} \]  

[7]

Eq. [7] is an approximate equation, valid only at high retention. The exact equation can be found in reference 18. Figure 2 illustrates the influence of this so-called steric effect. Plotted are the predicted retention ratio \( t_R/t_0 \) against particle diameter \( d \) for FFF with different field strengths. Plots a, b, and c indicate the retention ratios in the nor-
mal mode at increasing field strengths. Plots d, e, and f give the values in the steric mode at increasing field strengths. The maximum $t_R/t_0$ values found in the steric mode are the so-called steric inversion points that depend on the particle size and the field strength. The steric inversion point marks the point at which steric effects start to dominate and a foldback in elution order occurs, with still larger particles eluting earlier. Often, the steric inversion diameter is around 1 μm.\(^{19}\) However, as can be seen from Figure 2, this is dependent on the experimental conditions.

Moreover, the figure shows that as the particle size approaches the channel size and we enter the realm of hydrodynamic chromatography, the field strength becomes of less influence and the retention is more and more controlled by the ratio of the particle diameter and the channel height.

![Graph showing particle diameter vs. $t_R/t_0$.](image)

**Figure 2:** Retention ratio as function of the particle size without (plot a, b, and c) and with (plot d, e, and f) correction for steric effects for Thermal FFF under different field strengths. Assumed conditions: (a and d): $\Delta T = 30$ K, (b and e): 45 K, (c and f): 60 K; $D_T = 3.8 \times 10^{-12}$ m²/sK; $w$: 125 μm; $\eta = 1$ cP (assumed constant); $T_{\text{cold}} = 298$ K. Calculations are based on the exact equation given in reference 18.
Hyperlayer mode
Although the model discussed for steric FFF proved to be satisfactory to explain most experimental observations, it was discovered that, especially in the case of supramicron particles (d > 1 \( \mu \m\)), other factors also influenced retention. Well-known are the hydrodynamic lift forces that push the particles away from the wall and increase with the carrier flow rate and particle size. The combined mechanism of the field-dependent driving forces and the flow-dependent lift forces give rise to the formation of small sample layers or sample zones at certain positions from the wall. If the elevation exceeds one particle radius, the technique is called lift-hyperlayer FFF. The theoretical description of lift forces is a very complicated problem that has only been partly solved. Nevertheless, lift-hyperlayer FFF has proven to be very valuable to separate particles in a size range of 0.5 – 50 \( \mu \m\).

Other hyperlayer-FFF modes have been developed but these seldom-used techniques fall beyond the scope of this chapter and will not be discussed.

Band broadening
Dispersion of a solute during migration through an FFF channel is characterized by the variance \( \sigma^2 \) of the sample zone relative to the distance \( L \) travelled, which is expressed as the plate height \( H = \sigma^2/L \). The plate height in FFF can be divided into a number of independent contributions attributed to: the longitudinal diffusion (\( H_0 \)), the non-uniformity of the flow profile (or non-equilibrium) (\( H_a \)), the relaxation process (\( H_r \)), and several instrumental contributions (\( \Sigma H_i \)). The major contribution to plate height in FFF, under normal conditions, is the non-equilibrium (\( H_a \)) term given by

\[
H_a = \frac{\chi w^2 \langle \nu \rangle}{D}
\]

where \( \chi \) is a complicated function of \( \lambda \) that at high retention can be simplified to \( 24\lambda^3 \). It follows from Eq. [8] that the highest efficiency is achieved at high retention. Still, the plate number of FFF is low, usually in the range of a few hundred.

In practical cases, the major contribution to the observed peak widths is the sample’s polydispersity. The polydispersity \( \mu \) is not considered to be a plate height contribu-
tion, as this peak broadening is actually a reflection of the selectivity of the method.\textsuperscript{25} The peak variance resulting from polydispersity is given by

$$\sigma^2 = L^2 S_m^2 \left( \zeta(\mu) - 1 \right)$$

[9]

where $S_m$ is the mass selectivity, equal to $|\partial \ln(t_c/t_0)/\partial \ln(M)|$, and $\zeta(\mu)$ is a function of the polydispersity $\mu$.\textsuperscript{26}

The limiting values of mass selectivity $S_m$ are for Flow FFF as well as for Thermal FFF in the range of 0.5 – 0.6. This compares favourably with SEC, where the limiting mass selectivity is considerably lower, in the vicinity of 0.1 – 0.2. However, the efficiency is typically much higher in a SEC column.

Band broadening effects will lead to inaccuracies in determining the polydispersity of narrowly distributed polymers. Nevertheless, it has been shown that the accuracy and precision of polydispersity determinations of narrow polymer standards is higher with FFF than with SEC.\textsuperscript{26}
FFF sub-techniques

The type of cross-force determines the type of FFF. In principle, every force that shows interaction with the solute can be used as a cross-force for an FFF experiment. Since the invention of FFF in 1966 by Giddings, a number of sub-types have been studied, and a few have been implemented. Table I lists the most important FFF techniques in use today.

Table I: FFF sub-techniques.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Size range (nm)</th>
<th>Carrier*</th>
<th>Applications#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation FFF</td>
<td>$10^1 - 10^5$</td>
<td>Aq.</td>
<td>Env./Bio., and Ind. particles and colloids</td>
</tr>
<tr>
<td>Flow FFF</td>
<td>$10^0 - 10^5$</td>
<td>Aq./Org.</td>
<td>Env./Bio., and Ind. particles, colloids and polymers</td>
</tr>
<tr>
<td>Thermal FFF</td>
<td>$10^1 - 10^5$</td>
<td>Org./Aq.</td>
<td>Ind. polymers, colloids, and particles</td>
</tr>
<tr>
<td>Electrical FFF</td>
<td>$10^1 - 10^5$</td>
<td>Aq.</td>
<td>Ind. and Bio. particles and colloids</td>
</tr>
</tbody>
</table>

* Aq. = aqueous, Org. = organic.

Env. = environmental, Bio. = biological, Ind. = industrial.

**Sedimentation FFF (SdFFF)**

Sedimentation FFF is mostly used for the analysis of colloids and particles in the range of approximately 50 nm to 100 μm. Retention is achieved by rotating the channel in a centrifuge. The imposed centrifugal force separates the particles based on their effective mass ($m_{eff}$) according to:

$$
\lambda = \frac{kT}{m_{eff}Gw} = \frac{6kT}{\pi d^3|\Delta \rho|Gw}
$$

[10]
where \( k \) is Boltzmann’s constant, \( d \) is the particle diameter, \( T \) is the absolute temperature, \( \Delta \rho \) is the difference between the density of the particle and of the carrier liquid, and \( G \) is the field strength. SdFFF is technically a very complicated technique, as it requires a special centrifugal apparatus with rotating seals. Nevertheless, it was the first FFF technique to be commercialized (1986).

SdFFF is a highly size-selective technique (\( t_R \sim d^3 \)), and allows excellent separation of particles in a wide size range. It can be used in both the normal mode and the steric mode. The limiting factor for SdFFF is the maximum rotation speed, determining the minimum particle size that can be separated. Furthermore, as can be seen from Eq. [10], at least a small difference between the density of the carrier and of the particle is required.

The technique has proven to be a very valuable tool for analyzing industrial, environmental, and biological particulate material. Well-known applications are bacteria, viruses, human cells and animal cells, soil colloids, and industrial particles.

Recent developments include the coupling of SdFFF to Inductively Coupled Plasma Mass Spectrometry (ICPMS) and to Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). Furthermore, new instrumental developments have resulted in a decrease of the minimum attainable particle size down to 20 nm.

**Flow FFF (FIFFF)**

Flow FFF is usually regarded as the most universal FFF technique. The cross-force is generated by a cross-flow that permeates through one or both of the channel walls. The accumulation wall is overlaid with a semi-permeable membrane to prevent the passage of sample material. This membrane determines the actual minimum molecular-mass cut-off.

The retention parameter \( \lambda \) can be related to experimental parameters according to:

\[
\lambda = \frac{DV^0}{V_c w^2} \tag{11}
\]

where \( V^0 \) is the channel void volume and \( V_c \) is the cross-flow rate. Retention is solely dependent on the diffusion coefficient of the solute and the generated field. Elution volumes can therefore be directly related to sample diffusivities without the need for calibration. FIFFF is mostly used for aqueous samples. The technique is applicable in the range of 2 nm – 50 \( \mu \)m and can be used for particles as well as for polymers.
Its size selectivity is relatively high ($t_r \sim d$), though smaller than SdFFF. FIFFF is used in the normal and steric mode.

After its introduction in 1976, several types of FIFFF have been developed, *i.e.*, Symmetrical FIFFF, Asymmetrical FIFFF, and Hollow Fibre FIFFF. Symmetrical and Asymmetrical FIFFF instruments consist of a flat rectangular channel. In Symmetrical FIFFF both walls are permeable, in Asymmetrical FIFFF only one wall (the accumulation wall) is permeable. In Hollow Fibre FIFFF the separation takes place in a cylindrical fibre instead of in a rectangular channel. The first two techniques are commercially available.

Interesting FIFFF applications for a great variety of samples have been developed. Well-known applications are the separation of (aqueous) polymers, latex particles, biological samples such as proteins and DNA, and environmental samples such as natural colloids.

A relatively large number of studies on the coupling of FIFFF to Multi-Angle Light Scattering detection have been done. Other successful recent couplings are FIFFF with Laser-Induced Breakdown detection for the analysis of nanocolloids and FIFFF with ICPMS for the determination of the size and trace element distribution of colloidal material in natural water.

Until recently, FIFFF could only be used for aqueous applications, mainly due to incompatibility of the membrane material with organic solvents. The development of new membranes paves the way for the use of FIFFF with organic solvents. A commercial Asymmetrical FIFFF instrument that is applicable with organic solvents is now available. Applications are still limited to a few organic solvents (e.g., THF), but new membranes are currently under development. Experiments with rubber analysis in THF showed excellent stability of the membrane.

**Thermal FFF (ThFFF)**

Thermal FFF was the very first FFF technique to be studied. The cross force in ThFFF is generated by heating one of the walls and cooling of the accumulation wall. The retention depends on the temperature difference between the walls and on the so-called Soret coefficient, *i.e.*, the ratio of the thermal diffusion coefficient and the normal diffusion coefficient ($D_T/D$) of the solute, according to:

$$
\lambda = \frac{D}{D_T \frac{\partial T}{\partial x}} \approx \frac{D}{D_T \Delta T}
$$

[12]
where $\partial T/\partial x$ is the temperature gradient across the channel thickness and $\Delta T$ is the temperature difference applied between both walls. The Soret coefficient depends on the molecular mass as well as on the chemical composition of the solute. The thermal diffusion mechanism, which is the driving force of ThFFF, is not well understood and requires an empirical approach. This has limited the applicability of ThFFF. Nevertheless, ThFFF has been used for a wide variety of samples. Up to now, ThFFF has been the only FFF method suitable for the analysis of synthetic polymers in organic solvents (lipophilic polymers). It appears, however, that it will lose its unique position due to new developments in FIFFF, as described earlier. It remains, however, the only FFF technique that is able to detect chemical composition differences of lipophilic polymers (Although, in principle EIFFF could also be used, its applicability in organic solvents has been very limited). This feature has successfully been used to detect compositional differences in copolymers. In addition, ThFFF has been used for the analysis of microgels, and aqueous and non-aqueous particles. Furthermore, biopolymers such as starch and cellulose have occasionally been analyzed. In general, ThFFF is not suited to analyse hydrophilic polymers due to the often negligibly low thermal diffusion in aqueous media.

During the past five years the study of ThFFF has mainly been focussed on the development of new applications and the understanding of the underlying principles. There have been no major instrumental developments. A relatively large number of experimental studies of the relation between the thermal diffusion and polymer behaviour in ThFFF have been carried out as well as several theoretical thermal diffusion studies. Despite these studies the knowledge about thermal diffusion remains rudimentary. Successful couplings between SEC and ThFFF and between ThFFF and HDC have been developed. Both methods allow the direct calculation of the thermal diffusion coefficients and were used to detect chemical composition changes in heterogeneous copolymers.

One of the most interesting developments in ThFFF is its increased use for particle analysis. A study on core-shells by Rathatanatanawongs et al. has shown that ThFFF can detect chemical composition differences in the particle shell, and Jeon et al. experimentally confirmed the hypothesis that thermal diffusion is mainly determined by the outer layer of a particle. Recently, Wahlund and Myers showed that ThFFF can be used to separate supramicron particles in the range of 2 – 20 µm within two minutes.
Another interesting area for ThFFF is the development of high-temperature ThFFF for the analysis of polyolefins. Cold wall temperatures exceeding 400 K have been reached. Technically this is hard to accomplish and puts very high demands on the instrument.

Coupling of ThFFF to Light Scattering detection has resulted in a very powerful technique for the determination of ultra-high-molecular-mass materials and microgels. Surprisingly enough, no studies on the use of ThFFF-MALS for particle analysis have been done yet. ThFFF will be discussed more in detail further on in this chapter.

_Electrical FFF (EIFFF)_

EIFFF is a relatively new development that has received much attention during recent years. Although the feasibility of EIFFF was already demonstrated in 1972, interest faded quickly due to instrumental problems. However, since 1993 new technical developments have helped to overcome these instrumental problems. A new channel design that utilizes two graphite plates that served both as channel walls and electrodes renewed interest in EIFFF.

Retention in EIFFF is dependent on the diffusion coefficient $D$ and the electrophoretic mobility $\mu_{em}$ and is given by:

$$\lambda = \frac{D}{E \mu_{em} w} = \frac{D}{\mu_{em} \Delta V}$$

[13]

where $E$ is the electric field and $\Delta V$ is the voltage drop across the channel. EIFFF can be used in the normal and steric mode. In common electrolytes, steric inversion already occurs at particle diameters of about 300 nm. It appears to depend on the particle diameter and the Debye double layer thickness. Applications of EIFFF have been limited so far. Caldwell lists a number of applications including the separation of paints and pigments, viruses, and yeast cells. Palker et al. used EIFFF to separate colloidal sized polymers. Recent studies focus mainly on the development of the technique using model compounds. Another area of interest is the development of miniaturized EIFFF (μ-EIFFF) with an integrated detector.

_Other FFF techniques_

Apart from the sub-techniques mentioned above, a number of other FFF techniques have been studied. We suffice with a brief summary of the most important sub-techniques: Dielectrophoretic FFF, mainly used for cell separations, appears to be a
highly promising technique for biological samples.\textsuperscript{112,113} Split Flow-Thin Cell Fractionation (SPLITT) is an FFF spin-off showing a rapidly growing number of applications for (supramicron) colloids and particles. It is a continuous technique that can be used to prepare relatively large quantities of fractionated material.\textsuperscript{114-116} Magnetic FFF (MgFFF) has been pioneered by Vickrey and Garcia-Ramirez.\textsuperscript{117} The technique has been applied to the separation of nickel complexes of bovine serum albumin\textsuperscript{118} and egg albumin and EDTA.\textsuperscript{119}

**Programmed field operation**

The open geometry of an FFF channel allows the analysis of samples ranging from a few nanometers up to 100 microns. In order to deal with broadly distributed samples, programmed cross-fields are required. (Strictly speaking, programmed FFF also comprises flow programming and eluent composition programming. However, these techniques are seldom used and will not be discussed here.\textsuperscript{40}) Programming in FFF has already been studied since 1974.\textsuperscript{120} Initially, linear and parabolic field decay programs were developed.\textsuperscript{121,122} However, these were quickly superseded by the exponential field decay program and the power programmed field decay program that showed much better results.\textsuperscript{123-125} The exponential decay function is given by

\begin{equation}
S(t) = S_0 e^{-\left(\frac{t-t_i}{\tau}\right)}
\end{equation}

where \(S(t)\) is the field strength at time \(t\), \(S_0\) is the initial field strength, \(t_i\) is an initial time period during which the field strength is held constant, \(\tau\) is the decay constant and \(t \geq t_i\).

The power programmed field decay function is given by

\begin{equation}
S(t) = S_0 \left(\frac{t_i-t_s}{t-t_s}\right)^p
\end{equation}

where \(t_s\) and \(p\) are variable parameters (\(t \geq t_i > t_s\) and \(p > 0\)). When field programming is employed during an FFF experiment, Eq. [4] is not applicable anymore and more complicated models are required.
Thermal FFF

As said before, the driving force of ThFFF is thermal diffusion. Although the discovery of the thermal diffusion effect dates back to 1856, it remains a poorly understood phenomenon up to the present day.\textsuperscript{126} Thermal diffusion can be described by Onsager's irreversible thermodynamics theory.\textsuperscript{127} Unfortunately, this theory cannot be used to actually predict thermal diffusion coefficients. Thermal diffusion in liquids is difficult to determine experimentally. The effect is very weak and often overshadowed by the normal diffusion. For polymers, D is small enough to allow thermal-diffusion-driven separations such as ThFFF.

ThFFF is not the first polymer separation technique based on thermal diffusion. Several researchers already studied the possibility to separate polymers by thermal diffusion in the 1950's and 1960's with thermogravitational columns or static cells.\textsuperscript{126} During the first ten years after the invention of ThFFF, several types of channels were constructed, tested, and improved.\textsuperscript{128-131} However, since the late 1970's the basic design of the ThFFF channel has remained virtually unchanged.

In order to separate polymers in a reasonable time by ThFFF, very strong temperature gradients are required. Present-day ThFFF channels are able to generate temperature gradients of typically 0.4 – 0.8 K/µm. Such extreme experimental conditions put high demands on the construction of the channel and the materials used.

The design of the ThFFF instrument is illustrated in Figure 3. The channel is constructed of two highly-polished chromium- or nickel-plated copper bars with a sheet of Mylar clamped in between. A ribbon-shaped channel with approximate dimensions of 45 cm × 2 cm × 0.0125 cm is cut from the Mylar spacer. Liquid, entering and leaving through two holes that have been drilled in the copper bars, is pumped through the channel. Heating the top bar with heater rods and cooling the lower bar with water creates the temperature gradient. The temperature is monitored by thermistors placed in the top and lower bars. A computer is used to control the temperature gradient by cycling the heater rods on and off.
**Figure 3:** Basic design of a ThFFF channel.

A sample is injected into the flow stream. Upon entering the channel the solutes are driven towards the cold wall. In some cases, when the thermal diffusion is low, it is required to temporarily stop the flow in the channel to allow the solutes to migrate to the wall and reach their steady state concentration profiles. The required stop-flow time $\tau$ can easily be estimated by $^{132}$

$$\tau = \frac{w^2}{D_T \Delta T} \tag{16}$$

If, however, a short analysis time is more important than an optimum separation, the stop flow time can often be omitted without significant deterioration of the peak shape.

Figure 4 shows the experimental set-up. A standard HPLC pump is used to deliver a flow of typically 0.1 – 0.5 mL/min. Often a pulse damper and a restrictor are inserted between the pump and the injection valve to suppress pump pulsation. Furthermore, a
back-pressure regulator is generally placed downstream the channel to pressurize the channel a few bars above ambient pressure. In this way, the channel can be heated above the normal boiling point of many organic solvents. The switching valve is used to temporarily bypass the channel during the optional relaxation period.

![Diagram of experimental arrangement](image)

**Figure 4**: Schematic of the ThFFF experimental arrangement.

The sample injection volume ranges from 10 µL to 200 µL. Often used detection methods are Ultraviolet Absorption (UV),\(^\text{133}\) Refractive Index (RI),\(^\text{134}\) Evaporative Light Scattering (ELS),\(^\text{135}\) Viscometry,\(^\text{136}\) and Light Scattering (LS).\(^\text{137}\) Especially the latter technique is gaining in popularity because no calibration of the ThFFF system is required.
Deviation from the FFF retention model

The retention mechanism of ThFFF differs somewhat from the basic retention model, already described earlier in this chapter. This is due to the strong temperature gradient present in a ThFFF channel. The standard FFF retention model assumes an exponential concentration distribution of the solute (Eq. [2]) and a parabolic flow profile (Eq. [3]). These assumptions are, however, only valid if the viscosity and thermal conductivity are constant throughout the channel. In ThFFF, however, both vary with temperature, which leads to deviations in the concentration distribution and the flow profile. Accounting for these effects results in a very complicated retention equation that requires numerical integration to calculate Soret coefficients from retention time.\textsuperscript{138} It has been shown that neglecting this temperature effect can lead to gross errors.\textsuperscript{139,140}

Secondary effects

Secondary effects that cause deviations from the separation models described above can occur for all FFF techniques. An overview of so-called secondary effects has been given by Giddings\textsuperscript{141} and the influence of the individual effects has been computed by Martin.\textsuperscript{142} The most important secondary effect for ThFFF is overloading of the channel. This often-underestimated problem can complicate the analysis of very high-molecular-mass polymeric samples. From the few studies that have been performed on overloading, it became clear that heavy peak distortion and retention shifts can easily occur, especially for high-molecular-mass polymers.\textsuperscript{143,144} This is clearly shown in Figure 5, where the fractograms of polystyrene 700 kDa, injected at different concentrations at two temperature gradients, are depicted.

The peak shifts and distortions are mainly due to the viscosity gradient close to the accumulation wall.\textsuperscript{145,146} The increase in concentration at the accumulation wall compared to the injection concentration commonly falls in the range of 10 – 100 times, and the semi-dilute regime can easily be reached, resulting in entanglement of polymer chains.\textsuperscript{143} However, even at concentrations well below the so-called critical concentration \(c^*\), peak shifts can occur due to increased viscosity. Therefore, especially when physicochemical parameters are calculated from retention data, unknown samples should be injected at a minimum of two concentrations to detect possible overloading.
Figure 5: Influence of sample concentration on retention time and peak shape of PS 700 kDa in THF. (A) $\Delta T = 40$ K; (B) $\Delta T = 60$ K. Injection concentration: $a = 0.05$ mg/mL, $b = 0.1$ mg/mL, $c = 0.25$ mg/mL, $d = 0.5$ mg/mL, $e = 0.75$ mg/mL, $f = 1.0$ mg/mL. Injection volume = 50 $\mu$L; flow: 0.4 mL/min; $w = 125$ $\mu$m; Detection: ELS.
Scope of this thesis

Its ability to separate (ultra)-high-molecular-mass material, including polymers, microgels, and particles and its unique ability to resolve polymeric- or particulate materials according to their chemical composition makes ThFFF a valuable tool. However, our limited understanding of thermal diffusion hinders the implementation of ThFFF.

The main goals of the work described in this thesis are to better understand the underlying mechanism of ThFFF and to use this knowledge to develop new applications for ThFFF.

In order to calculate $D_T$ values from ThFFF retention data, external diffusion measurements are required. In Chapter 2, a comparison is made of four methods used to determine polymer diffusion coefficients.

Chapter 3 describes the use of ThFFF to detect compositional heterogeneity in random copolymers.

The influence of the composition of the carrier liquid on the retention and thermal diffusion of sub-micron particles is studied in Chapter 4.

In Chapter 5, ThFFF-MALS is applied to the analysis of sub-micron latex particles and core-shells.

In the final Chapter 6 an effort is made to predict polymer thermal diffusion coefficients from their polymer-solvent interaction parameters.

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.

Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>SI units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c$</td>
<td>concentration</td>
<td>kg m$^{-3}$</td>
</tr>
<tr>
<td>$c_0$</td>
<td>concentration at accumulation wall</td>
<td>kg m$^{-3}$</td>
</tr>
<tr>
<td>$d$</td>
<td>diameter</td>
<td>m</td>
</tr>
<tr>
<td>$D$</td>
<td>diffusion coefficient</td>
<td>m$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$D_T$</td>
<td>thermal diffusion coefficient</td>
<td>m$^2$ s$^{-1}$ K$^{-1}$</td>
</tr>
</tbody>
</table>
$E$ electric field $V \text{ m}^{-1}$
$F$ force $N$
$G$ centrifugal field $\text{m} \text{s}^{-2}$
$H$ plate height $\text{m}$
$H_D$ longitudinal contribution to plate height $\text{m}$
$H_n$ non-equilibrium contribution to plate height $\text{m}$
$H_r$ relaxation contribution to plate height $\text{m}$
$\Sigma H_i$ instrumental contributions to plate height $\text{m}$
$J_x$ flux of material $\text{kg m}^2 \text{s}^{-1}$
$k$ Boltzmann's constant $\text{J K}^{-1}$
$l$ mean layer thickness $\text{m}$
$L$ channel length $\text{m}$
$m_{\text{eff}}$ effective mass $\text{kg}$
$M$ molecular mass $\text{kg \text{mol}^{-1}}$
$p$ power program parameter
$r$ radius $\text{m}$
$S$ field strength (in arbitrary dimensions)
$S_m$ mass selectivity
$S_0$ initial field strength (in arbitrary dimensions)
$t$ time $\text{s}$
$t_1$ time period of constant field $\text{s}$
$t_s$ power program parameter $\text{s}$
$t_R$ retention time $\text{s}$
$t_0$ void time $\text{s}$
$T$ temperature $\text{K}$
$T_c$ cold wall temperature $\text{K}$
$\Delta T$ temperature difference $\text{K}$
$u_F$ transverse component velocity $\text{m s}^{-1}$
$v$ velocity $\text{m s}^{-1}$
$\langle v \rangle$ average cross-sectional liquid velocity $\text{m.s}^{-1}$
$V$ mean velocity of a component $\text{m.s}^{-1}$
$V^0$ channel void volume $\text{m}^3$
$V_c$ cross-flow rate $\text{m}^3 \text{s}^{-1}$
$\Delta V$ voltage drop across the channel $\text{V}$
$w$ distance between the channel walls $\text{m}$
$x$ distance from the accumulation wall $\text{m}$
function of polydispersity

dimensionless retention parameter

polydispersity

electrophoretic mobility \[ m^2 V^{-1} s^{-1} \]
difference between particle- and carrier density \[ kg m^{-3} \]
peak dispersion \[ m \]
stop-flow time \[ s \]
exponential decay constant \[ s \]
non-equilibrium dispersion parameter

References

14. J.C. Giddings, Unified Separation Science, Wiley-Interscience, New York,
1991
40. J.C. Giddings, in Field Flow Fractionation Handbook, M.E. Schimpf, K.D. Caldwell, and J.C. Giddings (Eds.), Wiley & Sons, New York, 2000, Ch. 1
Genera l Introduction

84. C. van Batten, M. Hoyos, and M. Martin, *Chromatographia*, 45, 121, (1997)
100. K.-G. Wahlund and M. Myers, Presented at the 23rd Int. Symposium on Chromatography, London, 2000


