Characterisation of polymers and particles by asymmetrical flow field-flow fractionation
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CHAPTER 1

Introduction

For the analysis of macromolecules and particles, several analytical separation techniques are available to the modern chemist. The classical separation methods of High-Performance Liquid Chromatography (HPLC)\(^1\) and Size Exclusion Chromatography (SEC)\(^2\) are the workhorses of polymer analysis. Other methods, such as Capillary Electrophoresis (CE),\(^3\) Hydrodynamic Chromatography (HDC)\(^4\) and Field Flow Fractionation (FFF)\(^5\) have mainly been developed in the 1980's and 1990's. Of these techniques, FFF is particularly suitable for the separation and characterisation of large polymers (with molar masses in the MDa range) and particles. Many of the drawbacks, connected to packed columns as employed in chromatographic techniques, are avoided.

The general concept of FFF originates from Giddings.\(^6\) To a large extent, FFF has matured to today's level from the ideas and efforts of this master. The basic principle of FFF is depicted in Figure 1, where a channel of trapezoidal shape serves as an example. Polymers or particles are injected in the channel as analytes. While a laminar flow (\(F_{ax}\)) flushes the analytes out of the channel in the axial (length) direction, a second force is exerted in the direction perpendicular to the channel. Essentially for the separation, this cross-field forces the solutes into a layer close to a wall of the channel (the so-called accumulation wall). Depending on the interaction with the cross-field and the molecular diffusion counteracting this force, different solutes occupy on average different velocity lines of \(F_{ax}\). This way, they can be eluted from the channel separated in time and detected.

![Figure 1. Principle of FFF.](image-url)
With FFF techniques the (molecular) size distribution of macromolecules or particles in solution is measured. Alongside the chemical composition, the size distribution plays a significant part in determining the physical properties of the materials manufactured of such compounds. For example, the brittleness, (melt) viscosity, morphology, tensile strength and glass transition temperature are all related to molecular size, while the viscosity of particles in solution, the coarseness and toughness of coatings are related to the particle size distribution. Essentially, the size distribution of synthetic polymers and particles provides a direct link between material properties and synthesis conditions.

There are several sub-techniques of FFF that follow the general principle, but vary in the nature of the cross-field that is applied. Sub-techniques are named after the cross-field; the most common field types that are used are a flow field (flow FFF), a centrifugal force (Sedimentation FFF) and a thermal gradient (Thermal FFF). As these fields act differently on analytes, different selectivities can be obtained. Each of the sub-techniques has its merits for specific application areas. Several overviews of the complete FFF family can be found in the literature.

**ASYMMETRICAL FLOW FFF**

**Theory (normal mode)**

The cross-force in flow FFF is induced by the hydrodynamic drag of the solvent moving through the accumulation wall consisting of a semi-permeable material (usually an ultrafiltration membrane) that allows the solvent to pass through but retains analytes larger than a certain cut-off size inside the channel.

In asymmetrical flow FFF, the technique used throughout this thesis, the axial flow generates the cross-flow as it is pushed through the accumulation wall. Thus, $F_{ax}$ decreases over the length of the channel. Asymmetrical flow FFF channels exist in two geometries: a flat channel, and a tubular channel (Figure 2). In modern literature "asymmetrical flow FFF" typically refers to the flat-channel geometry, a convention that will be used throughout this thesis. Flow FFF in a tubular channel (a hollow fiber membrane) is named hollow-fiber flow FFF (HF5). In this section, the theory of retention in a flat channel is described.

As a result of the dynamic equilibrium between the drag force induced by the cross-flow and in the opposite direction molecular diffusion (described by Fick’s second law), a concentration gradient of the analyte is formed as a function of the distance from the accumulation wall ($z$). This equilibrium is described by the following differential equation:

$$u_{cr} c (z) = -D \frac{dc}{dz} \quad (1)$$

where $u_{cr}$ is the velocity of the cross-flow, $D$ the diffusion coefficient and $c(z)$ the concentration of the analyte at distance $z$. 


Integrating Equation 1 yields an expression for $c(z)$:

$$c(z) = c(0) \exp\left(-\frac{z u_{cr}}{D}\right)$$  \hspace{1cm} (2)

where $c(0)$ is the concentration at the accumulation wall. The ratio $D/u_{cr}$ defines the characteristic layer thickness $\ell$. In the so-called normal mode, described in this section, the size of the analyte is negligibly small compared to $\ell$ and the height of the channel $w$. For convenience, $\ell$ is usually expressed in its reduced form as the dimensionless retention parameter $\lambda$:

$$\lambda = \frac{\ell}{w}$$  \hspace{1cm} (3)

This important parameter describes the level of retention of the analyte with the height of the channel taken into account.

The velocity of the analyte is the result of the average of the velocity lines of the laminar flow ($F_{L}$) that the concentration profile samples. The velocity in the axial direction at a distance $z$ from the accumulation wall is given by:

$$v(z) = \frac{z}{w} - \left(\frac{z}{w}\right)^2$$  \hspace{1cm} (4)

where $<v>$ is the average velocity over the cross-section of the channel. In Figure 3, two examples of concentration and velocity profiles as a function of $z$ are depicted.

\[\text{Figure 2. Principle of flat-channel asymmetrical flow FFF (A) and hollow-fiber flow FFF (B).}\]
Figure 3. Exemplary concentration (left axis) and velocity profile (right axis). $\ell = 17 \, \mu m$, $w = 254 \, \mu m$ and $<v> = 5 \, c m \, s^{-1}$.

Evaluating the integral of Equations 3 and 4, it can be shown that the retention ratio ($R_f$) of the analyte is:

$$ R_f = \frac{t_0}{t_r} = 6 \lambda \left( \coth(2\lambda^{-1}) - 2\lambda \right) $$

(5)

Here, $R_f$ is defined as the ratio of the void time ($t_0$) and the retention time ($t_r$). The void time (the residence time of an unretained compound) can be calculated from the channel dimensions and flow rates. This requires a precise value of the height of the channel, which in practice is obtained by calibration with a standard with known diffusion coefficient (e.g. the protein ferritin). Alternatively, $t_0$ can be derived from the breakthrough time of a high molar mass probe.

Equation 5 relates the retention time to $\lambda$. At sufficiently high retention ($R_f < 0.48$), Equation 5 is approximated by:

$$ R_f = 6 \lambda $$

(6)

with an error of less than 10 %. At low retention, it is more correct to solve Equation 5 analytically to obtain $\lambda$. From $\lambda$, the characteristic layer thickness $\ell$ and the diffusion coefficient $D$ can be calculated. At sufficiently high retention, there is an alternative, more direct route from retention time to the diffusion coefficient. In asymmetrical flow FFF, the cross-sectional flow velocity in the axial direction ($u_{ax}$) decreases linearly along the length of the channel. The linear velocity at position 1 (counted from the inlet of the channel) is:

$$ u_{ax}(l) = u_{in} - \frac{1}{L} u_{cr} $$

(7)
where \( L \) is the length of the channel. When this is combined with the velocity of a solute \((v_s)\):

\[
v_s = 6 \frac{l}{R} \langle v_{ax} \rangle
\]  

(8)

it can easily be shown that the retention time of a compound is:

\[
t_r = \int_0^L \frac{dz}{v_i} = \frac{w^2}{6D} \ln \left( \frac{F_{in}}{F_{out}} \right)
\]  

(9)

More detailed expressions, taking into account the channel dimensions and focussing point, can be found in the literature.\(^{14}\) Small deviations from the general theory, occurring because in an asymmetrical channel the velocity lines are not parallel to the accumulation wall, have been described elsewhere.\(^{18}\)

From the diffusion coefficient, other important molecular parameters such as the hydrodynamic radius \((r_H)\) or the molar mass \((M)\) can be estimated. Under the assumption that the molecule has a spherical conformation, \(r_H\) is related to \(D\) by:

\[
r_H = \frac{kT}{6\pi \eta D}
\]  

(10)

where \(k\) is the Boltzmann constant, \(T\) the temperature and \(\eta\) the viscosity of the carrier liquid. The molar mass is related to \(D\) by an equation, derived by Flory on the basis of the Mark-Houwink theory:\(^{19}\)

\[
D = AM^{-b}
\]  

(11)

where \(A\) and \(b\) are empirical constants, the latter being a measure of the solvent quality.

The mechanism of flow FFF is such that in the normal mode direct information can be obtained about molecular diffusion, which is defined according to Fick's second law. Several features of flow FFF, such as the the open channel structure, soft forces exerted on the sample and relatively low concentrations of sample, make flow FFF an excellent tool to study the diffusional behaviour of macromolecules and particles under unperturbed conditions. This characteristic of flow FFF is exploited throughout this thesis.

**Efficiency**

Following an ancient analytical custom (stemming from the time when distillation was the separation method of choice) the efficiency of a separation is captured in terms of the numbers of plates \((N)\). In case of differential migration methods, such as flow FFF, \(N\) is defined as the broadening of a zone in time:\(^{10}\)

\[
N = \left( \frac{t}{\sigma} \right)^2
\]  

(12)

where \(\sigma\) is the standard deviation of the zone in time units. The efficiency of an analytical column is described by the plate height \(H\):

\[10\]
\[ H = \frac{L}{N} \]  
\[ H = \frac{B}{v} + C(v) + \sum H_i \]  
(13)  
(14)

where \( L \) is the length of the separation channel. The separation system is used most efficiently per length unit at the minimum of \( H \). \( H \) depends on several factors, which in flow FFF are:

\[ H = \frac{B}{v} + C(v) + \sum H_i \]

where the B-term represents the diffusion in the longitudinal direction and the C-term non-equilibrium effects. Other contributions, such as non-idealities of the separation channel, dispersion in the detector and the tubing, are collected in \( \sum H_i \). If experiments are performed carefully, the B-term and the non-idealities are negligible and non-equilibrium effects are the main causes for peak broadening. It can be shown that the non-equilibrium term can be calculated as:

\[ C = \chi \frac{w^2}{D} \]

(15)

where \( \chi \) is a complex function of \( \lambda \) that is approximated by:

\[ \chi = 24 \lambda^3 \]

(16)

at sufficiently high retention. This implies that flow FFF is most efficient for smaller molecules (with large \( D \)) and that in principle the non-equilibrium contribution can be minimized by decreasing \( w \) and \( \lambda \) (i.e., by increasing the cross-flow). However, in the latter case \( \lambda \) is decreased at the expense of analysis time so that a compromise has to be found between analysis time and separation efficiency. Moreover, because at higher retention the sample becomes more concentrated and is situated on average closer to the accumulation wall, concentration effects or interactions with the accumulation wall are more likely to cause deviations from ideal normal mode behaviour.

**Steric Inversion**

For a sample of which the size is substantial compared to \( \ell \), retention is no longer purely governed by diffusion because the movement of the sample is sterically hindered by the accumulation wall. Then, the concentration profile deviates from the exponential shape which is characteristic for the normal mode. In this case, the retention factor has to be corrected with an additional sterical term, and \( R_f \) is approximated by:

\[ R_f = 6 \gamma a + 6 \lambda \]

(17)

which is valid for small \( a \) and \( \lambda \). Here, \( a \) is the reduced particle diameter \( (r_H/w) \) and \( \gamma \) a factor that describes the effect of hydrodynamic lift-forces (absent when \( \gamma \) equals 1) counteracting the cross-flow. These lift-forces are caused by complex hydrodynamic phenomena. Although it is known that \( \gamma \) depends on the flow rate, density of the carrier liquid, temperature, and the size and shape of the particle lift forces, as yet the mechanisms behind the lift-forces cannot be described exactly. Since this renders \( \gamma \)
unpredictable, it prevents the translation of retention time to size by Equation 17 when calibration standards are not available.\textsuperscript{26}

Above a certain size, steric inversion (also called steric foldback) occurs.\textsuperscript{27} The steric inversion radius ($r_i$) is the point where the hydrodynamic radius of a particle (Equation 10) equals $l$. Taking into account lift forces, this gives:

$$r_i = \frac{kT}{6\pi\eta u_{cr}}$$

For particles larger than $r_i$, it is only the size of the molecule that governs retention. This retention mode is named the steric mode. Hydrodynamic lift forces accompany the steric mechanism under many practical conditions; the combination of the steric mode with lift forces is called the steric/hyperlayer mode. In Figure 4A and 4B, the principle of the steric and steric/hyperlayer mode, respectively, is depicted. The lift-forces counteract the cross-flow and cause the samples to focus into small bands (hyperlayers) some distance from the accumulation wall.\textsuperscript{28} In the steric and steric/hyperlayer mode, larger particles sample faster velocity lines than smaller particles. Consequently, they are eluted earlier. This elution order is opposite to that of the normal mode.

The dependence of $R_f$ on the particle radius $r$ for three different cross-flow velocities, calculated from the simplified Equation 17, is shown in Figure 5. Here, it can be seen that the retention factor decreases with particle radius in the normal mode until the steric inversion point is reached. Above the steric inversion radius, the retention factor rapidly increases with the particle radius. This enables rapid and efficient analyses. However, for polydisperse samples in a size range that overlaps $r_i$, small particles, fractionated in the normal mode, are co-eluted with larger particles in the steric mode.\textsuperscript{29} Although to a certain extent the steric inversion point can be shifted by varying experimental conditions, this complication may hamper the analysis of such samples.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Principle of the steric (A) and steric / hyperlayer mechanism (B).}
\end{figure}
Figure 5. Theoretical retention curve as a function of particle radius $r$. $w = 254 \mu m$, $A = 38.6 \text{cm}^2$. a: $F_\alpha = 0.2 \text{ ml min}^{-1}$; b: $F_\alpha = 1 \text{ ml min}^{-1}$; c: $F_\alpha = 5 \text{ ml min}^{-1}$.

Applications

Since there are, except for the pore size of the membrane and channel dimensions, no physical limits to the size range that can be handled, flow FFF is one of the most universal of all separation techniques. Flow FFF covers a size range of a few nm's up to to $\approx 100 \mu m$ and can provide size separation of any type of soluble macromolecules or suspended particles in this size range. It is a prerequisite that the solute does not interact with the membrane and that the membrane resists the carrier liquid that is used. Since few membranes resist organic solvents, mostly aqueous carrier liquids have so far been used in flow FFF.

Although flow FFF covers a very wide size range, at the lower mass end (up to several MDa's) SEC is generally the method of choice, as it provides a resolution and performance superior to that of flow FFF. Some comparisons between SEC and flow FFF and Thermal FFF (a closely related sister-technique of flow FFF) can be found in recent literature.

For large macromolecules, and certainly for aggregates or particles, secondary interactions with the packing material may cause decreasing recoveries and distorted peak shapes; both anomalies seriously hamper the accurate determination of the molar mass distribution. For such applications, flow FFF has the advantage that a much smaller surface (i.e., the membrane area) is available for interactions. Therefore, the focus of flow FFF is nowadays shifting more towards larger macromolecular systems. This trend is visible in a small selection of recent publications in popular research areas of flow FFF (Table 1). Numerous other examples can be found in the text.
Table 1. Selection of recent articles on flow FFF.

<table>
<thead>
<tr>
<th>Area</th>
<th>subject</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical</td>
<td>liposomes, vesicles</td>
<td>34, 35</td>
</tr>
<tr>
<td></td>
<td>gene-carrier complexes</td>
<td>36</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>starches, gum arabic, κ-carrageenan</td>
<td>37-39</td>
</tr>
<tr>
<td>Particles</td>
<td>(core-shell) latices</td>
<td>40, 41</td>
</tr>
<tr>
<td></td>
<td>submicron particles</td>
<td>42</td>
</tr>
<tr>
<td>Environmental</td>
<td>natural colloids</td>
<td>43-46</td>
</tr>
<tr>
<td>Syntheticspolymers</td>
<td>polyacrylamides, aggregated co-polymer</td>
<td>17, 48</td>
</tr>
</tbody>
</table>

As mentioned, one of the limitations of flow FFF is the vulnerability of the membrane towards organic solvents. Since a wide range of hydrophobic synthetic polymers only dissolve in solvents of organic nature, this limits the variety of polymers that can be analysed with this technique. Although with several types of membranes separations in organic solvents have been reported\textsuperscript{49-53}, none of these membranes provides the long-term resistance required for a robust system. In Chapter 3, the efforts made to expand the application range of flow FFF by making use of a polycrionitrile membrane are presented.

**Instrumentation**

Nowadays, the equipment for asymmetrical flow FFF is at a matured stage and can be acquired from several specialized manufacturers.\textsuperscript{54-56} The basic design of flat-channel flow FFF is shown in Figure 6. A supporting block contains a frit and drainage for the cross-flow. The frit consists of a microporous ceramic or metal material. A membrane, serving as accumulation wall, covers the frit. Flow FFF can also be practiced without membrane,\textsuperscript{57} but since this system is only suitable for the analysis of particles larger than the pore size of the frit (1 μm or greater), this seriously reduces the application range. The channel is cut out of a spacer, which is clamped between the membrane and a covering plate. In order to maintain a reasonable axial flow velocity at the outlet of the channel, channels are cut into a trapezoidal shape.\textsuperscript{58}

The channel is installed in a flow scheme that enables injection, relaxation, fractionation and detection. The general scheme is shown in Figure 7. Samples are injected using standard HPLC injection valves. After injection, the sample is forced in a layer close to the accumulation wall before it is subjected to fractionation. During this process, called (stop-flow) relaxation or focussing, samples are focussed by two opposing flows, entering through the front and back end of the channel, respectively. At the point where the net velocity is zero, a sample zone with a width of a few millimetres is formed. Normally, the sample is introduced through the front of the channel. Large volumes, containing low concentrations of solutes, can also be injected through the back end of the channel.\textsuperscript{59}
Figure 6. Construction of an asymmetrical flow FFF channel. Legend: supporting block (a), frit (b), membrane (c), spacer (d) and cover plate (e).

Figure 7. Scheme of the flow-FFF set-up. Arrows show the direction of the flow during relaxation (r) and fractionation (f). NV: Needle valve; I: Injection valve; R: cross-flow regulator.

After relaxation of the sample, valve V switches the system to the fractionation mode and the inlet flow is set at the required value. There are several ways to control the cross-flow. It can directly be regulated by a syringe pump that sucks the cross-flow through the membrane. This method is used in Chapter 3. It has the advantage that gradients can be applied, but since the syringe pump generates an underpressure in the module that contains the membrane, air bubbles are liable to form, which can make the operation of such a system a tedious task. Alternatively, the cross-flow can be regulated by needle valves (as used in Chapter 3 and 6) that control the pressure difference between outlet- and cross-flow. This is common practice in asymmetrical flow FFF. With needle valves, the system is more robust than with a syringe pump.
and the costs are lower. However, cross-flow gradients cannot easily be applied. A third possibility is the use of an automated cross-flow regulator, such as supplied with the commercial Consenxus apparatus.

Several improvements on the standard flat channel are being investigated. One improvement involves the use of frits in the upper wall to improve relaxation and detection. The other improvement, the use of a hollow-fiber membrane as a separation channel (addressed in the next section) allows for a more elegant set-up.

Frits can be inserted in the covering plate of the channel at the inlet or outlet. In frit-inlet flow FFF, an auxiliary stream is provided through the frit (Figure 8A). This stream forces the sample directly towards the accumulation wall, bypassing the need for a relaxation period prior to fractionation. Compared to stop-flow relaxation, this so-called hydrodynamic relaxation saves time, reduces possible interactions of the sample with the membrane surface and simplifies the automation of the system.60-62 The potential value of frit inlet flow FFF has been exemplified by the analysis of (lipo)proteins.63,64

![Figure 8. Principle of frit-inlet flow FFF (A) and frit-outlet flow FFF (B).](image-url)
With a frit-outlet (see Figure 8B), the outlet flow is split and the layer close to the upper wall that does not contain a significant amount of analyte is led away. Thus, higher concentrations of analyte are fed to the detector. Under optimal conditions, a ten-fold increase in concentration, with negligible loss of sample, can be achieved.\textsuperscript{65} This increase in concentration can enable detection of species that are untraceable in a conventional channel.\textsuperscript{66,67} Although frits in the upper wall offer significant advantages, the system may tend to become complex in set-up, handling and optimization of conditions.

**Multi-angle light scattering detection**

The use of a multi-angle light scattering (MALS) detector is described in several chapters. When combined with a separation method, MALS is one of today's most powerful on-line detection methods for macromolecules and particles, unveiling a wealth of information that is not available when either the separation or the detection technique is used separately.

In the MALS detector, the scattering of light of a laser beam inside a detection cell is detected at multiple angles within a single plane. The excess scattering of light (compared to the background scattering of the solvent) is related to the molar mass and the root-mean-square (r.m.s.) radius of the analyte (also, less correctly, called radius of gyration, $r_g$). This entity, the statistical mean of the mass-weighed distance of monomer units to the centre of gravity of a molecule, is a measure of the size of the molecule.

Flow FFF fractionates the sample and each narrow slice of this fractogram is evaluated on-line with the MALS detector. In combination with a concentration detector, MALS enables the direct measurement of the molar mass in these slices. In this mode, a molar mass distribution can be constructed directly from the elution profile supplied by flow FFF. The need for calibration of flow FFF is avoided, which is particularly useful when suitable standards are not available. Additional information may be obtained from the ratio of the r.m.s. (provided by MALS) and the hydrodynamic radius (calculated from the retention time in flow FFF) which reflects the shape and conformation of the analyte in question.\textsuperscript{68}

Many of the practical and fundamental aspects of the coupling of MALS to a separation method have been addressed.\textsuperscript{69-72} The first coupling of MALS to flow FFF has been realised by the group of Kulicke.\textsuperscript{73,74} Wittgren \textit{et al.} have further explored the possibilities that the flow FFF-MALS tandem offers.\textsuperscript{39,75,76} Other applications of flow FFF-MALS include, for example, the analysis of submicron particles,\textsuperscript{42} polystyrenesulfonates,\textsuperscript{77} surfactant vesicles\textsuperscript{35} and biodegradable amphiphilic co-polymers.\textsuperscript{78} Recently, in an interesting article Frose showed how the form factors of co-eluting particles can be deconvoluted with Legendre polynomials. This may be useful when a polydisperse sample is eluted in the normal and steric mode simultaneously.\textsuperscript{79}
HOLLOW-FIBER FLOW FIELD-FLOW FRACTIONATION

In flow FFF, the intrinsic simplicity of hollow-fibre membranes comes fully to its right. While the dynamics and principle of HF5 and asymmetrical flow FFF are basically similar, the main advantage of hollow-fiber membranes compared to flat-channels lies in the more elegant set-up, enabling a faster replacement of the membrane. In addition, far less material is used in the construction of the channel, so that with HF5 disposable columns (comparable to common practice in HPLC, CE and SEC) come within sight.

A short history of HF5

Although HF5 was the first form of flow FFF, flat membranes soon outperformed hollow fibers and forced HF5 into obscurity. Carlshaf and Jönsson revisited HF5 in 1988 and, by successful fractionations of latex standards, showed the potential of the technique. They also studied the effect of the sample load and ionic strength on the overloading of latex beads. However, it was soon recognised that due to variations in the porosities of the membranes, retention times and separation efficiencies can vary significantly between different fibers.

Wijnhoven et al. further investigated the effects of ionic strength and sample load on the retention of polyelectrolytes and the effects of polydispersity and flow rates on the peak broadening of polyelectrolytes. Lee et al. showed a very impressive fractionation of latex particles (see Figure 9). For this type of fractionation their HF5 system performed almost as good as the more-established asymmetrical flat-channel flow FFF. They also exploited the elegance and relatively small dimensions of the HF5 instrument to control the temperature of the system and studied the effect of the temperature on the steric inversion point of latices.

Contemplating this comprehensive list of literature on HF5, it is evident that the practical experience is only limited. The bottleneck in the development of the technique is the varying and inhomogeneous porosity of the fibers. Without doubt, one of the most important reasons for the relatively slow progress of HF5 is that flow FFF poses very high demands on the quality of the hollow-fibre membrane and, especially, on the fibre-to-fibre repeatability. Channels with a flat geometry, although less elegant, provide superior performance. Part of the work in this thesis was aimed to revitalise HF5 and further expand its possibilities.
Figure 9. Hollow-fiber flow FFF fractogram of polystyrene latex beads (reprinted with permission from reference 91).

**Theory (normal mode)**

The retention of solutes can be described in a way identical to asymmetrical flow FFF (see above), with the one difference that, due to the hollow-fiber geometry, the flow profile in the axial direction is described by:

\[ v(z) = 4 < v > \left\{ \frac{Z}{R} - \left( \frac{Z}{R} \right)^2 \right\} \]  

(19)

where \( R \) is the radius of the lumen of the fiber. Following the treatment for asymmetrical flow FFF (Equation 7 to 9), it can be shown that:

\[ t_r = \frac{R^2}{8D} \ln \left( \frac{F_{in} - \zeta F_{cr}}{F_{in} - F_{cr}} \right) \]  

(20)

where \( R \) is the inner radius of the fiber. \( \zeta \) is the distance of the focusing point from the inlet relative to the length of the fiber.

The number of plates (\( N \)) per unit time are given by the following simple Equation:

\[ N = \frac{u_{cr}^2}{t_r} \left( \frac{4D}{r} \right) \]  

(21)
More details about the theory of HF5 can be found in Chapters 3 and 4. In Chapter 3, the general theory applied to obtain the maximum separation efficiency and to predict the optimal dimensions of a hollow-fiber membrane for flow FFF. In Chapter 4, the influence of the sample load on the retention of polyelectrolytes is described and used to analyse the diffusional behaviour of such charged polymers.

**Instrumentation**

The basic principle and operation procedure of HF5 are similar to that of asymmetrical flow FFF: the same auxiliary equipment and detectors can be used. A fiber is placed in the flow scheme described previously (Figure 7). The distinction with flat channels is that hollow-fibers require a different (and far more simple) module to contain the membrane. The module must enable drainage of the cross-flow and allow for tubing to be connected to the fiber. Several designs have been presented in the literature.\textsuperscript{84,90,91} In the latter two references, epoxy glue is used to fix PEEK tubing to the end of the fiber or glue the fiber into a teflon tube. These procedures are not to be recommended as they are relatively time-consuming and it cannot be guaranteed that the surface of the membrane remains unmodified at the point where glue is applied. Moreover, such systems are not resistant to organic solvents, for which suitable adhesives are not available. An alternative method, making use of standard HPLC materials, is presented in Chapter 4. This way, the trained analyst can replace a membrane within 5 minutes and resistance to most common organic solvents is guaranteed.

**SCOPE OF THIS THESIS**

Although at first sight the subjects of the individual chapters may seem diverse, they share the common objective of this thesis, to present novel ways to characterise polymers and particles with flow FFF. The work is presented in two parts: part I (Chapters 2, 3 and 4) covers hollow-fiber flow FFF and part II (Chapters 5 and 6) applications of the more conventional asymmetrical flow FFF, combined with MALS detection.

Chapter 2 is an introduction to HF5. The technique is placed between other applications of hollow-fiber membranes in analytical chemistry and background is provided of the preparation of hollow-fiber membranes. This may give a deeper insight into the possibilities of modern membrane technology and be a source of inspiration for the improvement and implementation of hollow-fiber membranes in analytical chemistry.

In Chapter 3, the possibilities of flow FFF are extended by a hollow-fiber polycrylonitril membrane that enables the use of a much wider variety of solvents than previously possible. Based on the general theory of HF5, suggestions are made for the ideal dimensions of a hollow-fiber for flow FFF.
In Chapter 4 a ceramic hollow fiber membrane is used as a tool to study the diffusional behaviour of a model polyelectrolyte, sulfonated polystyrene. From the dependence of the retention on the sample load and the ionic strength of the carrier liquid, the diffusional behaviour of the polyelectrolyte in solution is elucidated.

In Chapter 5, flow FFF with MALS detection is applied for the characterisation of hydroxylated and carboxylated core-shell particles. The information on the hydrodynamic and r.m.s. radii is combined to determine the amount of swelling of the particles with varying pH and ionic strength of the carrier liquid.

Chapter 6 describes a study of the retention behaviour of amylopectin, a large polysaccharide, with flow FFF-MALS. The knowledge on the r.m.s. and hydrodynamic radii under different flow conditions is combined to give insight into the retention mechanism of these extremely large macromolecules in flow FFF.

In particular in the last three chapters the intrinsic advantage of flow FFF as a relatively soft method to study the diffusion of macromolecules (and thereby their size) comes to light. The dependence of retention under varying experimental conditions (sample load, ionic strength, flow rates) is shown to be a valuable tool for the examination of the behaviour of macromolecules and particles in solution.
REFERENCES