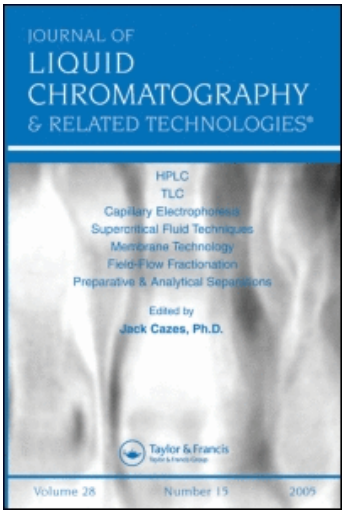

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HYDRODYNAMIC VS. FOCUSING RELAXATION IN ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

Myeong Hee Moon[a]; Inmi Hwang[a]
[a] Department of Chemistry, Pusan National University, Pusan, Korea

Online publication date: 31 December 2001

J. LIQ. CHROM. & REL. TECHNOL., 24(20), 3069–3083 (2001)

HYDRODYNAMIC VS. FOCUSING RELAXATION IN ASYMMETRICAL FLOW FIELD-FLOW FRACTIONATION

Myeong Hee Moon* and Inmi Hwang

Department of Chemistry, Pusan National University,
Pusan 609-735, Korea

ABSTRACT

Two different sample relaxation methods (focusing relaxation and hydrodynamic relaxation) in asymmetrical flow field-flow fractionation (AFlFFF) are examined by using a conventional asymmetrical channel and a frit inlet asymmetrical channel. In this report, sample recovery using a protein standard is studied, comparing focusing relaxation and hydrodynamic relaxation process at various field strengths and outflow rate conditions. It is shown that a conventional AFlFFF channel provides a better resolution than a frit inlet asymmetrical flow field-flow fractionation (FI-AFlFFF) channel, with sample recoveries up to 80% under moderate field strength conditions. Focusing relaxation appears to provide flexibility in selecting high speed run conditions without losing resolution, since sample relaxation is expected to be achieved more completely than hydrodynamic relaxation. The FI-AFlFFF channel utilizing the hydrodynamic relaxation provides a relatively poor resolution and separation

*Corresponding author. E-mail: mhmoon@hyowon.pusan.ac.kr

speed; however, it performs well under higher field strength conditions with higher sample recoveries than the conventional channel and it is remarkably convenient in system operation by using stopless flow sample injection method. The low separation speed can be overcome by a simple application of a field programming technique to FI-AFlFFF.

INTRODUCTION

Flow field-flow fractionation (Flow FFF), as a member of FFF techniques, is a separation method suitable for the characterization of macromolecules and particulate matters.(1-4) Flow FFF separates sample components by employing a pump flow along the flow FFF channel axis. Sample components in flow FFF channel are retained in the channel by a secondary flow called a cross flow, that moves across a thin ribbon like channel driving the sample components toward the one side of channel walls called an accumulation wall. Since macromolecules or particles exhibit diffusions against the external force driven by the movement of cross flow, a sample component will attain an equilibrium where the two counteracting transports balance each other.

Like other FFF techniques, flow FFF requires the pre-establishment of an equilibrium state for a sample component before the separation process begins. This is referred to as the relaxation process, and it is normally obtained by applying the cross flow field simultaneously with the temporary halt of the migration flow (separation flow).(5,6) When the relaxation is achieved, the separation flow is resumed while the cross flow continues. A conventional flow FFF channel utilizes two permeable frits as channel walls and the cross flow passes through them. This is called a symmetrical channel.

Asymmetrical flow FFF, an alternative form of flow FFF, utilizes only one permeable frit at the accumulation wall and the other is replaced with solid wall, normally a glass plate. Since there is no influx of cross flow to an asymmetrical channel, part of the flow entering through the channel inlet acts as a cross flow as it exits through the accumulation wall, and the rest of flow (axial flow) transports the sample along the channel.(7-8) Relaxation in an asymmetrical channel is not achieved in the same manner as in the symmetrical one. It is accomplished by focusing two opposing flow streams (one from the inlet and the other from the channel outlet) for a period of time below an injection point that is slightly apart from the channel inlet, as shown in Figure 1a. When relaxation is achieved, flow coming from the channel outlet is redirected to the channel inlet and the separation begins.

During the focusing process, sample materials are forced to form an initial band which is expected to be narrower than the one achieved by the conventional

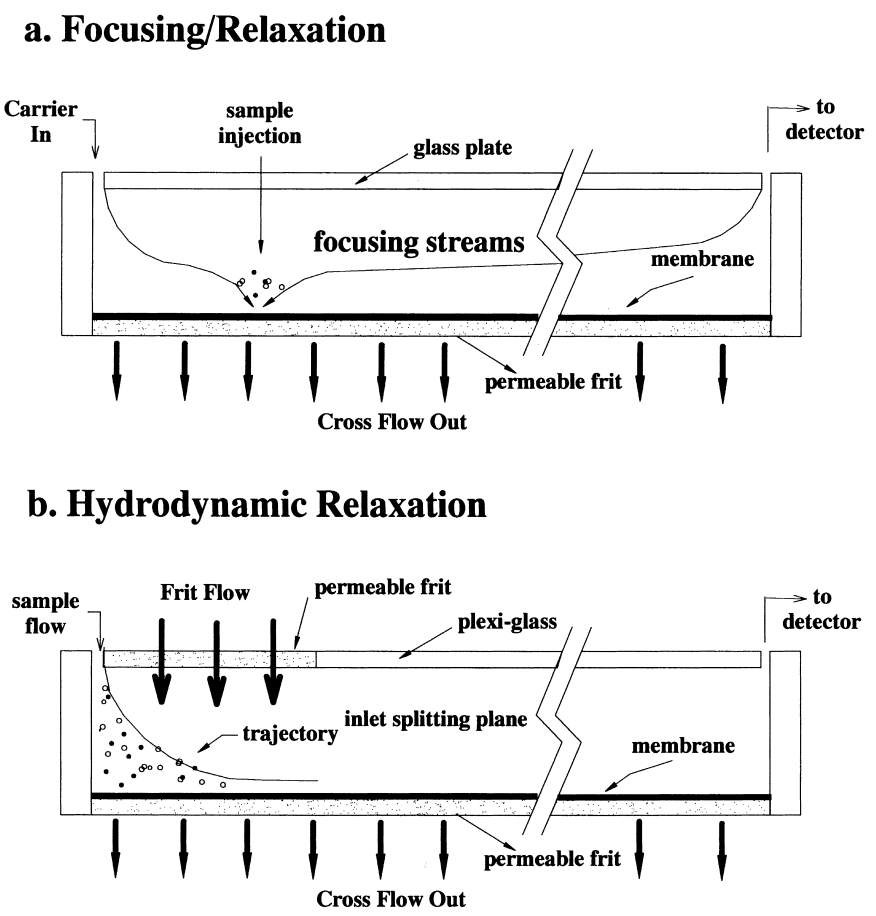

Figure 1. Schematic diagrams showing the side views of a) a conventional asymmetrical flow FFF channel and b) a frit inlet asymmetrical flow FFF channel during relaxation process.

stop flow method of a symmetrical channel, and this helps to reduce the final width of an eluted peak. In addition, the linear flow velocity of down stream decreases further down the channel due to the loss of the cross flow through the accumulation wall; and this effect is expected to reduce the longitudinal width of a migrating sample band because of the zoom effect. In spite of these effects, the asymmetrical flow FFF channel is known to provide an improved separation resolution, and it has been widely used to separate biological macromolecules and water soluble polymers.(8-11) However, both symmetrical and asymmetrical

channels require a temporary halt of migration flow, while a complicated valve is operated both before and after the relaxation process.

Unlike the conventional flow FFF channels, a frit inlet asymmetrical flow FFF (FI-AFlFFF) channel can be operated with stopless flow injection by using a small frit element near the channel inlet of an asymmetrical channel.(12,13) The sample is injected directly into the carrier stream flowing through the channel inlet, while the secondary flow (the frit flow) is entering through the small frit element. Relaxation of sample components in FI-AFlFFF is achieved hydrodynamically by using the compressive force of frit flow that is about 20 times faster than the sample flow.(14,15) The schematics of hydrodynamic relaxation in FI-AFlFFF channel can be seen in Figure 1b. Thus, sample injection and separation processes are smoothly accomplished without interrupting the migration flow, and retention of sample components, after a complete hydrodynamic relaxation in FI-AFlFFF channel, is expected to be similar to that in a conventional asymmetrical channel.

While the FI-AFlFFF system provides an ease of system operation with the removal of valve operations, separation in FI-AFlFFF relies strictly on the successful achievement of the hydrodynamic relaxation that is controlled by the ratio of sample flow rate to frit flow rate. In terms of relaxation efficiency, it is interesting to study the differences between the advantage of each specified technique. In this study, the hydrodynamic relaxation of a frit inlet asymmetrical channel is compared with the focusing relaxation method utilized in a conventional asymmetrical channel. Sample recoveries for both systems are examined using a protein standard by varying the cross flow rates under different migration flow rates. Channel efficiencies, which depend on the ratio of the outflow rate to the sum of outflow and cross flow rate, are investigated by examining the experimental values of plate height and the optimum separation. In order to enhance resolution and separation speed of FI-AFlFFF, a field programming technique has proven useful for separating proteins by circulating the cross flow to the frit flow.

THEORY

Retention in both the frit-inlet and the conventional asymmetrical flow FFF follows the general theory of FFF expressed by retention ratio as(1-3)

$$R = \frac{t^0}{t_r} = 6\lambda\left(\coth\frac{1}{2\lambda} - 2\lambda\right) \quad (1)$$

where t^0 is the passage time for a nonretained material through channel, t_r is the retention time of a species, and λ the retention parameter representing the ratio of equilibrium height of a sample zone to the channel thickness under a given field

strength. The force in flow FFF is generated by the transverse movement of cross flow across the channel, and it is dependent on the cross flow velocity. The retention parameter, λ, is given by(2)

$$\lambda = \frac{DV^0}{w^2 \dot{V}_c} \tag{2}$$

where D is the diffusion coefficient of a sample component, w the channel thickness, V^0 the channel void volume, and $\dot{V}_c$ the volumetric flow rate of cross flow. By substituting Eq. (2) into Eq. (1), one can calculate the diffusion coefficient or hydrodynamic radius of a species from an experimental retention time. When retention is sufficiently long ($R \cong 6\lambda$), retention time in flow FFF is calculated as

$$t_r \cong \frac{6D}{w^2}\frac{\dot{V}_c}{\dot{V}} \tag{3}$$

where $\dot{V}$ is the volumetric channel flow rate replaced with V^0 / t^0. The prediction of retention time in flow FFF is based on Eq. (3) once void time, t^0, is properly calculated. For a symmetrical channel, it is relatively straightforward, since mean flow velocity along the channel remains constant (thus, $\dot{V}$ is constant). However, for asymmetrical channels, longitudinal velocity varies along the channel and they are calculated differently. When a conventional asymmetrical flow FFF channel is used, sample migration starts at the focusing point and, thus, the void time, t^0_{AS}, is expressed as(7)

$$t^0_{AS} = \frac{V^0}{\dot{V}_c}\ln\left(\frac{\dot{V}_{in}'}{\dot{V}_{out}}\right) \tag{4}$$

where $\dot{V}_{out}$ is the volumetric flow rate measured at the channel outlet (or outflow) and $\dot{V}_{in}'$ is the carrier flow rate at the focusing point. $\dot{V}_{in}'$ is found by subtracting the fraction of cross flow rate that exits the channel up to the focusing point from the inlet flow rate, $\dot{V}_{in}$, as in the following:

$$\dot{V}_{in}' = \dot{V}_{in} - \dot{V}_c \frac{A_{fp}}{A_c} \tag{5}$$

in which A_{fp} and A_c are the geometrical areas of the accumulation wall from the channel inlet up to the focusing point and the entire accumulation wall, respectively. For a frit inlet asymmetrical flow FFF channel, void time calculation is more complicated since the frit flow mixes with sample flow. The calculated void time for FI-AFlFFF, t^0_{FI}, is given as(14)

$$t^0_{FI} = \frac{V^0 A_f / A_c}{\dot{V}_f - \dot{V}_c A_f / A_c}\ln\left(\frac{\dot{V}_s + \dot{V}_f - \dot{V}_c A_f / A_c}{\dot{V}_s}\right) + \frac{V^0}{\dot{V}_c}\ln\left(\frac{\dot{V}_s + \dot{V}_f - \dot{V}_c A_f / A_c}{\dot{V}_{out}}\right) \tag{6}$$

where A_f is the geometrical area of the inlet frit element and $\dot{V}_s$ and $\dot{V}_f$ are the volumetric flow rate of sample injection and frit flow, respectively. In order to use Eq. (6) to predict retention time, the hydrodynamic relaxation of sample materials must be completed within a very short period of time, right after injection.

According to Eq. (3), retention time in flow FFF is based on $\dot{V}_c / \dot{V}$. In asymmetrical flow FFF, it is rather expressed as $\dot{V}_c / \dot{V}_{eff}$ where $\dot{V}_{eff}$ is the effective channel flow rate and is the equivalent of $\dot{V}$ in a symmetrical channel. Therefore, the effective channel flow rate for a conventional asymmetrical channel is $\dot{V}_{eff,AS}$, and for a frit inlet asymmetrical channel is $\dot{V}_{eff,FI}$, and can be calculated by using Eqs. (3) and (6) respectively, as

$$\dot{V}_{eff,AS} = \dot{V}_c \left[\ln\left(\frac{\dot{V}_{in} - \dot{V}_c (A_f' / A_c)}{\dot{V}_{out}} \right) \right]^{-1} \tag{7}$$

$$\dot{V}_{eff,FI} = \dot{V}_c \left[\frac{\dot{V}_c A_f / A_c}{\dot{V}_f - \dot{V}_c A_f / A_c} \ln\left(\frac{\dot{V}_s + \dot{V}_f - \dot{V}_c A_f / A_c}{\dot{V}_s} \right) + \ln\left(\frac{\dot{V}_s + \dot{V}_f - \dot{V}_c A_f / A_c}{\dot{V}_{out}} \right) \right]^{-1} \tag{8}$$

As expressed in Eqs. (7,8), effective channel flow rate in both asymmetrical channels increases as cross flow rate increases. Thus, in asymmetrical channel systems, a selection of $\dot{V}_c / \dot{V}_{eff}$ needs to be carefully considered for an efficient separation.

EXPERIMENTAL

A frit inlet asymmetrical flow field-flow fractionation channel and a conventional asymmetrical channel were built as described earlier. For the conventional asymmetrical channel, a glass plate is utilized for the accumulation wall with an injection hole drilled 2.5 cm from the inlet, as shown in the assembly I in Figure 2. The depletion wall of the frit inlet asymmetrical channel is made with a Plexiglass block with a small inlet frit implanted at the injection end, as shown in the assembly II of Figure 2. Both systems utilize the same accumulation wall block, as depicted in the right hand side of Figure 2. The membrane layered over the porous frit wall, mounted on the accumulation wall block, is PLGC with MW cut off of 10,000 Kda, a regenerated cellulose from Millipore Corp (Bedford, MA, USA). Each block is clamped together with spacer and the accumulation wall block to form the channel. Both channels utilize the same width spacer, a thickness of 254 μm. The channel is shaped trapezoidal, having an inlet breadth of 2.0 cm and an outlet breadth of 1.0cm with a tip-to-tip length of 27.2 cm. When the frit inlet asymmetrical channel is assembled, the inlet frit extends 2.0 cm from the channel inlet.

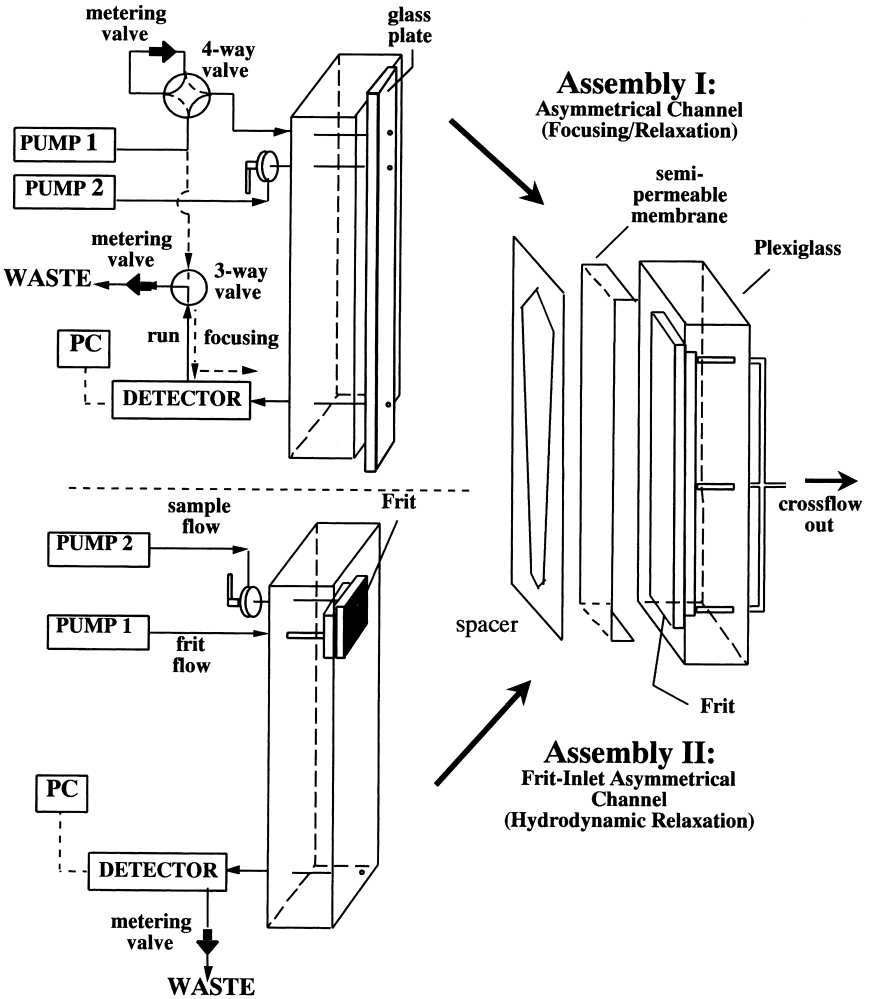

Figure 2. System assemblies of the two asymmetrical channels: the assembly I for focusing relaxation process and the assembly II for hydrodynamic relaxation.

The carrier solution used for the separation of protein standards is 0.1M Tris-HCl buffer solution (pH=7.8) containing 0.02% NaN_3 prepared with deionized water (>18MΩ) and filtered with 0.45 μm pore sized membrane filter prior to the use. Protein standards are carbonic anhydrase (29 Kda), alcohol dhydrogenase (150 Kda), apoferritin (444 Kda), and thyroglobulin (669 Kda) from Sigma (St. Louis, MO, USA).

The carrier liquid and sample injections were delivered by two HPLC pumps: Pump 1 is a model 305 HPLC pump from Gilson (Villiers-le-Bel, France) and pump 2 is a model Vintage 2000LC pump from OromTech (Seoul, Korea). For the focusing relaxation process after sample injection in the asymmetrical channel (the assembly I), both 4-way and 3-way valves were used to control the directions of the carrier flow as shown in Figure 2. In order to pump carrier liquid from the channel outlet toward the focusing point, carrier flow from pump 1 was divided into two parts for both the channel inlet and channel outlet by using a model Whitey SS-22RS2 metering valve from Crawford Fitting Co. (Solon, OH, USA). The amount of time used for focusing relaxation varied from 24 ~ 120 seconds depending on the total flow rates of the cross out flow that are used for the entire run. When the focusing relaxation was achieved, the backward flow was reversed to channel inlet with the bypass of the metering valve located at the 4-way valve and the separation began. To control the outflow and the cross flow during runs, another metering valve was located after the detector. For assembly II, a flow stopping process was not used, since sample relaxation is hydrodynamically obtained by using frit flow.

Sample injections were made at a flow rate of 0.2 mL/min by using pump 2 for both channels via a model 7125 loop injector from Rheodyne (Cotati, CA, USA). For assembly II, sample materials were injected directly into the flowing carrier stream. When recovery measurements were performed, injections using a fixed volume (5.0 μL) loop, which is the equivalent of about 32 μg for carbonic anhydrase was used. For the muticomponent separations, injection amounts were approximately 4, 14, 25, and 28 μg for carbonic anhydrase, alcohol dehydrogenase, apoferritin, and thyroglobulin, respectively. Eluted proteins were monitored with a model M720 UV detector from Young-Lin Co. (Seoul, Korea) at a wavelength of 280 nm. The detector signals were recorded with the Chromastar II, a data acquisition and pump control software from OromTech.

RESULTS AND DISCUSSION

The measurements of peak recovery of carbonic anhydrase were compared in asymmetrical flow FFF using either the focusing or hydrodynamic relaxation techniques shown in Figure 3. Figure 2 shows how the flow FFF systems were assembled, the channel assembly I for focusing relaxation, and the assembly II for hydrodynamic relaxation. By varying field strengths and outflow rates, the peak area of a detector signal was compared to a reference value, which was the area of a passage signal without a cross flow (field strength) applied at a given outflow rate. Figure 3a shows the recovery data obtained during focusing relaxation using the channel assembly I at three different outflow rates (0.2, 0.5, and 1.0 mL/min.) with the variation of cross flow rate. During focusing relaxation,

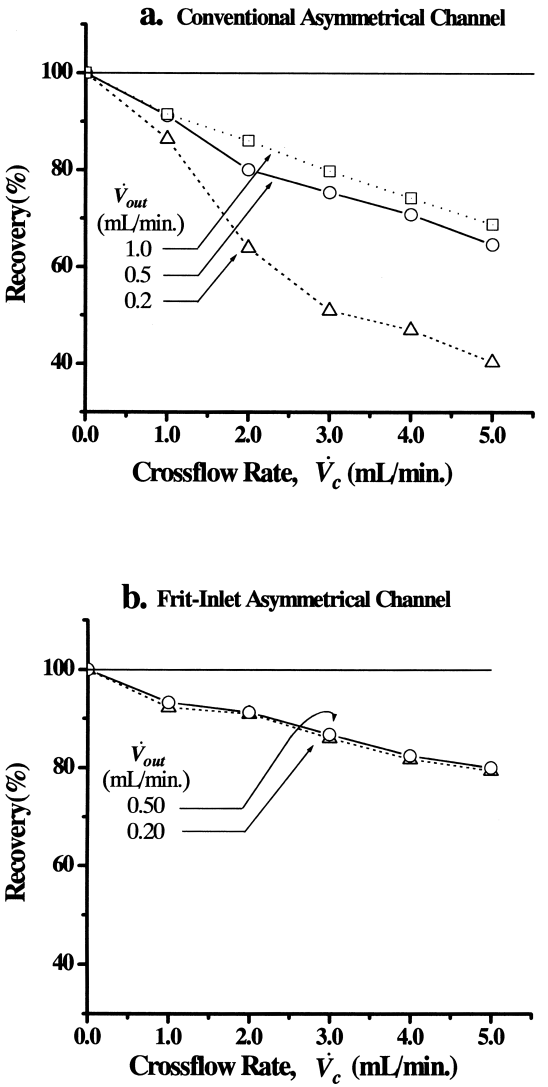

Figure 3. Effect of crossflow rate on the sample recovery obtained at different outflow rates for a) conventional asymmetrical channel and for b) frit inlet asymmetrical channel. For focusing relaxation, sample injection is made at $\dot{V}_{inj}$=0.2mL/min and the injection flow is blocked after focusing relaxation. For hydrodynamic relaxation, sample flow is maintained through a run and sample material is injected at $\dot{V}_s$=0.2mL/min.

the total focusing flow rates (one from channel inlet and the other from channel outlet) for each run is adjusted to be the same as the cross flow rate of each run. When the cross flow rate increases, recovery of carbonic anhydrase appears to gradually decrease. When the cross flow rate increases, recovery of carbonic anhydrase decreases to approximately 68% at an outflow rate of 1.0 mL/min., which are marked as open squares in Figure 3. When outflow rate decreases, recovery values at $\dot{V}_{out}$=0.5mL/min. are not significantly changed but they decrease seriously at $\dot{V}_{out}$=0.2mL/min. At a fixed cross flow rate of 3.0 mL/min., the recovery decreases to approximately 51% from 80% when the outflow rate changes to 0.2 from 1.0 mL/min. In this case, channel inlet flow rate, $\dot{V}_{in}$, decreases only about 20% (from 4.0 to 3.2 mL/min), but the effective channel flow rate, $\dot{V}_{eff}$, in Eq. (6) reduces by half (from 2.16 to 1.08 mL/min for $\dot{V}_{out}$ of 1.0 and 0.2 mL/min, respectively). Since the decrease in sample recovery could originate during the focusing relaxation or during elution, it is difficult to distinguish which factor dominantly causes the observed phenomena. The difference in the recovery values of the two outflow rate conditions becomes larger as the cross flow rate increases. This supports the idea that the loss in peak recoveries may come during elution.

When hydrodynamic relaxation is employed with a FI-AFlFFF channel (the assembly II), recovery decreases less steeply than are observed in the focusing relaxation procedure, as can be seen in Figure 3b. When $\dot{V}_{out}$ is 0.5 mL/min., peak recovery appears as about 80% compared to about 65% for assembly I at a cross flow rate of 5.0 mL/min. Further decrease in the outflow rate to 0.2 mL/min. for channel II does not show any significant change in recovery. In experiments with assembly II, all sample injections are made at $\dot{V}_s$=0.2mL/min, since a slow flow injection is generally required for obtaining good hydrodynamic relaxation. Since relaxation in a conventional asymmetrical channel is expected to be completely obtained during the focusing relaxation process, a relatively fast outflow can be managed for a high speed separation.

As shown in Figure 3, recoveries in a conventional asymmetrical channel can be maintained above 80% when a large ratio of outflow rate to cross flow rate is used. However in a frit-inlet asymmetrical channel in order to achieve hydrodynamic relaxation, sample flow rates must be minimized within a ratio of sample flow to frit flow rate of about 0.05. When separating low retaining materials (small MW), a low outflow rate is preferred. A focusing relaxation system is likely to be flexible in selecting a high speed separation conditions, but a hydrodynamic relaxation provides for a better sample recovery at a low outflow rate. The recovery data are dependent on the type of membranes used in flow FFF. Though the type of membrane used for Figure 3 is regenerated cellulose, other forms of cellulose membrane yielded higher values during experiments. When the same experiments are repeated with a differently made membrane, recovery values are increased to 92~99% for FI-AFlFFF and 55~95% for a conventional

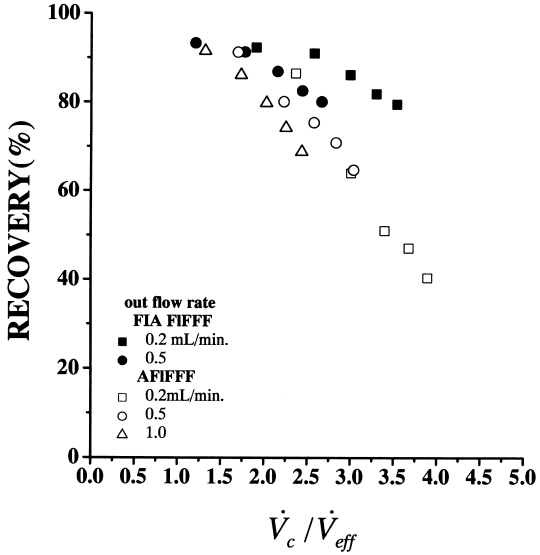

Figure 4. Recovery points plotted against $\dot{V}_c / \dot{V}_{eff}$ for the data points shown in Figure 3.

asymmetrical channel at a fixed outflow rate of 0.2mL/min. with a range of cross flow rate from 5.0 to 1.0 mL/min.

Since the retention in flow FFF is dependent on the ratio of channel flow rate to crossflow rate ($\dot{V}_c / \dot{V}$), it is useful to compare the difference in recovery data between the two different relaxation procedures according to experimental $\dot{V}_c / \dot{V}_{eff}$ using Eq. (6-7). The results are plotted in Figure 4. The filled symbols represent the data obtained by hydrodynamic relaxation and the open symbols are those obtained by focusing relaxation. As shown Figure 4, hydrodynamic relaxation in a frit inlet asymmetrical channel shows recoveries above 80 ~93% within values of about 3.5~1.1 for $\dot{V}_c / \dot{V}_{eff}$. Equivalent recoveries are observed for the focusing relaxation method in an asymmetrical channel when the ratio is below 2.2. Regarding sample recovery, it is suggested that the frit inlet asymmetrical channel provides a higher recovery at a large value of $\dot{V}_c / \dot{V}_{eff}$ but an asymmetrical flow FFF channel requires that a relatively low value of $\dot{V}_c / \dot{V}_{eff}$ be maintained.

Separation efficiency is related to the relationship of outflow to crossflow rate and is examined for both channel systems. Figure 5 represents the experimental plate height data, which were obtained for apoferritin (443Kda) by varying the ratio of $\dot{V}_{out}$ to total out going flow rates, $\dot{V}_{out} + \dot{V}_c$ for each channel assembly. As shown in Figure 5, a conventional asymmetrical channel performs well in the broad ranges of $\dot{V}_{out} / (\dot{V}_{out} + \dot{V}_c)$ with minimum plate height values. A similar level of efficiency is obtained for a frit inlet asymmetrical channel but only when

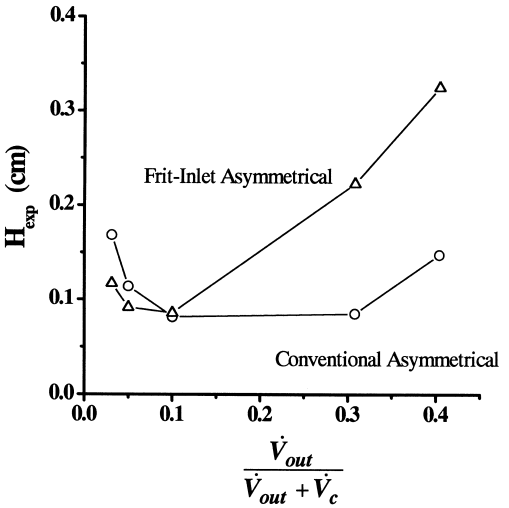

Figure 5. Experimental plate heights of apoferritin measured at different $\dot{V}_{out}$ / ($\dot{V}_{out}$ + $\dot{V}_c$) for both system assemblies. For focusing relaxation, $\dot{V}_{inj}$=0.2mL/min and focusing period = 24s. For all runs, $\dot{V}_{out}$ + $\dot{V}_c$=5.2mL/min.

the ratio is kept around 0.1. The difference shows that focusing relaxation is more efficient in high speed operations than hydrodynamic relaxation. This is reinforced by comparing resolution of the separation of a few proteins when $\dot{V}_{out}$ / $\dot{V}_c$=0.2/5.0 and 1.0/5.0 in mL/min. as shown in Figure 6. When the outflow rate is increased to 1.0 mL/min., as shown in Figure 6b, from 0.2 mL/min., as in Figure 6a, separation resolution is not seriously hampered with focusing relaxation. However, when hydrodynamic relaxation with a frit inlet asymmetrical channel is used, resolution is deteriorated in run b. The peak broadening is caused by the incomplete relaxation as the outflow rate increases.

For fractograms obtained with the hydrodynamic relaxation process, a baseline shift at the beginning of each run disappears as well as a void peak. The baseline shift is presumed to be the result of the pressure change during the valve conversion after focusing process. The void peak becomes larger at a high outflow rate condition in the upper fractogram of Figure 6b. However, the void peaks are negligible at the lower fractograms obtained by hydrodynamic relaxation with the complete removal of the baseline shift. For all of the runs shown in Figure 6, the same amount of each sample component was injected and, thus, the relative peak areas of the two relaxation methods can be directly compared. It shows that peak recovery of each peak is higher when the hydrodynamic relaxation is used with a frit inlet asymmetrical flow FFF.

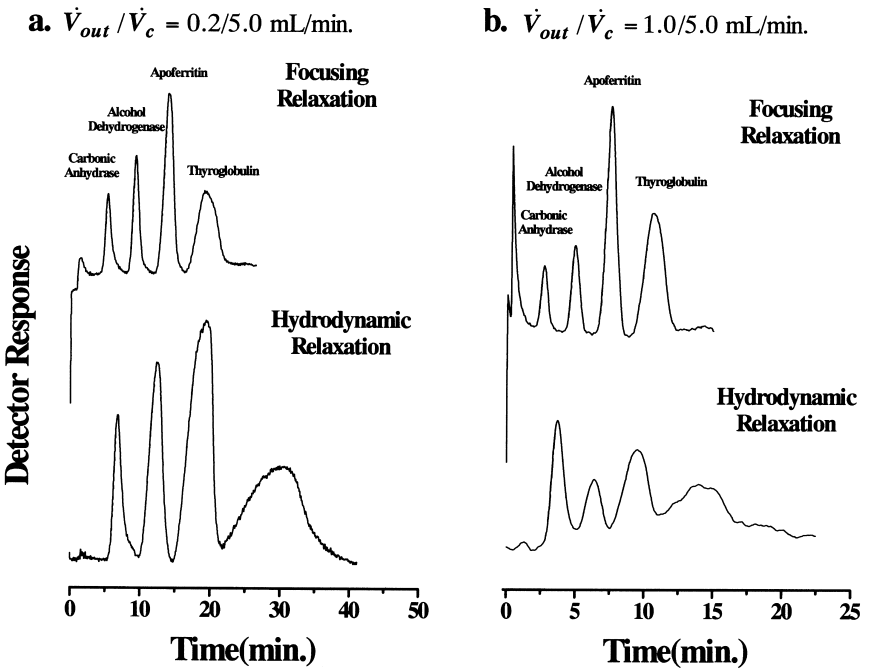

Figure 6. Elution profiles of protein separation obtained at two different $\dot{V}_{out}$ / $\dot{V}_c$ conditions for both channel systems. For focusing relaxation, $\dot{V}_{inj}$=0.2mL/min and focusing period = 24s for the run condition a and 12 s for the run b. For hydrodynamic relaxation, $\dot{V}_s$=0.2mL/min. for both run conditions.

Hydrodynamic relaxation resulted in a broad peak when the thyroglobulin was separated, as shown in Figure 6. It has been demonstrated in other works that higher cross flow rates reduce broad peaks because cross flow rate in both asymmetrical channels eventually increases migration velocity, as well as, field strength, as shown in Eqs (6-7). In this work, however, a higher crossflow rate was not employed since the conventional asymmetrical channel performs well with moderate cross flow rates. The low resolution in FI-AFlFFF is caused by low effective migration flow and this can be improved when the field strength is decreased during the run. A few studies have shown that it is possible to employ field programming in flow FFF, but it has not been widely utilized as other FFF techniques, such as sedimentation FFF and thermal FFF. Field programming in flow FFF requires a programmed decrease of the crossflow rate. Since there are two different flows exiting the channel as outflow and crossflow, it is not easy to simultaneously regulate those flow rates.

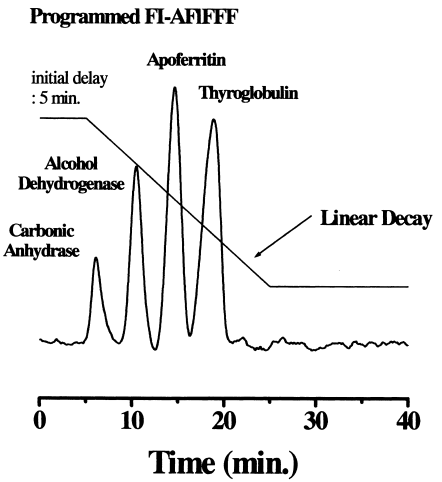

Figure 7. Programmed separation of four protein standards by hydrodynamic relaxation using FI-AFlFFF. $\dot{V}_s = \dot{V}_{out}$=0.2mL/min. and $\dot{V}_f = \dot{V}_c$:5.0→0.2mL/min. with initial delay period = 5min. and linear decay period = 20min.

When using a conventional asymmetrical channel, programming the crossflow is not a simple matter, since part of the incoming flow exits through the channel wall as crossflow. However, for the frit inlet asymmetrical flow FFF system, programming techniques can easily be used by circulating the crossflow into the frit flow and then, by decreasing the flow rates with a linear or power mode. As shown in Figure 7, field programming is used with FI-AFlFFF under the run conditions as used in Figure 6a. Initial field strength is maintained at $\dot{V}_c$=5.0 mL/min. for 5 min. and it is decreased to 0.2 mL/min. for the period of 20 min. During the programmed run, $\dot{V}_f$ was kept the same as $\dot{V}_c$ by flow circulation with $\dot{V}_s$ / $\dot{V}_{out}$.(16) By using field programming in FI-AFlFFF, the separation time is reduced by nearly a half of the constant run and the separation profile is greatly improved.

In this study, both relaxation processes commonly used in the two asymmetrical channel systems were examined. In the case of separation resolution, focusing relaxation provided a more complete relaxation of sample components, which enabled a high speed separation to be performed without introducing serious band broadening. However, hydrodynamic relaxation shows a higher sample recovery, as well as, having a more convenient system to operate, since there is no need of flow converting processes for relaxation. A minor drawback to hydrodynamic relaxation, is maintaining a low outflow rate condition in order to keep an initial sample band during hydrodynamic relaxation from broadening, but it

induces an improvement in detection limits, which leads to a reduction of sample loads. And, the low separation speed with resolution, can be overcome by employing the programmed field operation, which is readily applied for FI-AFlFFF system.

ACKNOWLEDGMENT

This work was supported by the Korea Research Foundation Grant (KRF 00-015-DP0238).

REFERENCES

1. *Field-Flow Fractionation Handbook*; Schimpf, M., Caldwell, K.D., Giddings, J.C., Eds.; Wiley-Interscience, John Wiley & Sons: N.Y., 2000.
2. Giddings, J.C. Science **1993**, *260*, 1456-1465.
3. Yang, F.J.F.; Myers, M.N.; Giddings, J.C. Anal. Chem. **1974**, *46*, 1924-1930.
4. Wahlund, K.-G.; Winegarner, H.S.; Caldwell, K.D.; Giddings, J.C. Anal. Chem. **1986**, *58*, 573-578.
5. Hovingh, M.E.; Thompson, G.E.; Giddings, J.C. Anal. Chem. **1970**, *42*, 195-203.
6. Giddings, J.C.; Caldwell, K.D.; Moellmer, J.F.; Dickinson, T.H.; Myers, M.N.; Martin, M. Anal. Chem. **1979**, *51*, 30-33.
7. Wahlund, K.-G.; Giddings, J.C. Anal. Chem. **1987**, *59*, 1332-1339.
8. Wahlund, K.-G.; Litzén, A. J. Chromatogr. **1989**, *461*, 73-87.
9. Kirkland, J.J.; Dilks, Jr., C.H.; Rementer, S.W.; Yau, W.W. J. Chromatogr. **1992**, *593***,** 339-355.
10. Wittgren, B.; Wahlund, K.-G. J. Chromatogr. A. **1997**, *760*, 205-218.
11. Nilsson, M.; Wahlund, K.-G. Bilow, L. Biotechnol. Tech. **1998**, *12***,** 477-480.
12. Moon, M.H.; Kwon, H.S.; Park, I. Anal. Chem. **1997**, *69***,** 1436-1440.
13. Moon, M.H.; Kwon, H.S.; Park, I. J. Liq. Chrom. & Rel. Technol. **1997**, *20***,** 2803-2814.
14. Moon, M.H.; Williams, P.S.; Kwon, H. Anal. Chem. **1999**, *71*, 2657-2666.
15. Moon, M.H. Bull. Korean Chem. Soc. **2001**, *22***,** 337-348.
16. Moon, M.H.; Williams, P.S.; Kang, D.; Hwang, I. *in preparation*.

Received July 15, 2001 Manuscript 5615
Accepted August 19, 2001