

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/8480848>

Miniaturization of Frit Inlet Asymmetrical Flow Field-Flow Fractionation

ARTICLE *in* ANALYTICAL CHEMISTRY · AUGUST 2004

Impact Factor: 5.64 · DOI: 10.1021/ac0496704 · Source: PubMed

CITATIONS

25

READS

21

2 AUTHORS:



Dukjin Kang

Korea Research Institute of Standards and Sc...

28 PUBLICATIONS 454 CITATIONS

SEE PROFILE



Myeong Hee Moon

Yonsei University

150 PUBLICATIONS 2,417 CITATIONS

SEE PROFILE

Miniaturization of Frit Inlet Asymmetrical Flow Field-Flow Fractionation

Dukjin Kang and Myeong Hee Moon*

Department of Chemistry, Yonsei University, Seoul, 120-749, Korea

A miniaturized frit inlet asymmetrical flow field-flow fractionation (mFI-AFIFFF) channel has been constructed and tested for the separation of proteins. By scaling down the geometrical channel dimension of a conventional FI-AFIFFF system, flow rate ranges that can be manipulated were decreased to 20–30 $\mu\text{L}/\text{min}$, which reduces the injection amount of sample materials. The end effect contribution to plate height was evaluated by varying the inner diameter of the connection tubing between the injector and the channel inlet at various injection flow rates, and the results showed that the use of silica capillary tubing of the shortest possible distance is essential in reducing the initial band broadening prior to the sample injection to the microscale channel. The capability of the $\mu\text{FI-AFIFFF}$ system was demonstrated with the separation of protein standards, polystyrene-sulfonates, and ssDNA strains and for the characterization of replication protein A–ssDNA binding complex regulated by redox status.

Flow field-flow fractionation (FIFFF) is an elution-based technique that is capable of separating and characterizing proteins, DNA, aqueous polymers, cells, and nanometer-sized to micrometer-sized particles.^{1–4} In FIFFF, separation is carried out in a thin channel or in a hollow fiber with the application of cross-flow or radial flow, respectively, in a direction perpendicular to the separation flow. When the applied field plays a role in driving sample components toward the accumulation wall of the FIFFF channel, the force applied to sample components is counterbalanced by the diffusion of sample materials at equilibrium positions that are away from the channel wall. When laminar separation flow is delivered to the sample components that are differentially distributed against the channel wall according to their hydrodynamic sizes, a small component having large diffusion and located further away from the channel wall will be eluted faster than a large one located closer to the wall. Thus, separation is carried out with an increasing order of diameter of sample components.

Since the conventional (or symmetrical) FIFFF channel was first introduced in 1976 by Giddings,¹ the channel system has been diversified into several types such as asymmetrical FIFFF

(AFIFFF),^{5,6} frit inlet and frit outlet FIFFF,⁷ frit inlet asymmetrical FIFFF (FI-AFIFFF),^{8,9} and hollow fiber FIFFF.^{10,11} Typical dimensions of FIFFF channels having a rectangular cross section are 25–40 cm in length, 1–2 cm in breadth, and 100–300 μm in thickness. In the case of a hollow fiber having a circular cross section, fibers of ~ 25 cm long and ~ 1 mm in diameter have been utilized.¹¹ Fractionations by these channels require flow rates of a few milliliters per minute, and the fluid flux leading to the detector is lowered to a few tenths of milliliters per minute.

Miniaturization of separation devices has been of great interest since it brings many advantages such as a decrease in sample injection amount and in consumption of the mobile phase and the possibility of system integration with other analytical techniques. Recently, miniaturization of a field-flow fractionation channel has been tried with electrical FFF¹² and thermal FFF¹³ by utilizing micromachining techniques; however, the performance of microfabricated thermal FFF (TFFF) system was found to be less than that of conventional TFFF. Miniaturization of TFFF was followed by scaling down the conventional channel and it has demonstrated that micro-TFFF can be applicable for the separation of polystyrene and polystyrene latex particles.^{14–16} While FIFFF has shown to be versatile due to its wide applicability toward macromolecules, colloidal particles, cells, proteins, etc., an approach to miniaturization has not been made yet.

In this study, miniaturization of FIFFF has been attempted first to an FI-AFIFFF system by simply reducing the channel length and breadth. FI-AFIFFF has a channel design modified from AFIFFF by introducing a small inlet frit at the beginning end of the depletion wall so that sample relaxation can be made hydrodynamically by use of the compressing action of the frit flow entering through the small inlet frit. In FI-AFIFFF, sample materials are introduced to the channel via the channel inlet at a low speed while a relatively high speed frit flow is entering

* Corresponding author. Phone: (82) 2 2123 5634. Fax: (82) 2 364 7050. e-mail: mhmoon@yonsei.ac.kr.

- (1) Giddings, J. C.; Yang, F. J.; Myers, M. N. *Science* **1976**, *193*, 1244–1245.
- (2) Giddings, J. C. *Anal. Chem.* **1981**, *53*, 1170A–1175A.
- (3) Giddings, J. C. *Science* **1993**, *260*, 1456–1465.
- (4) Schimpf, M. E.; Caldwell, K. D.; Giddings, J. C. *Field-Flow Fractionation Handbook*; Wiley-Interscience: New York, 2000.

- (5) Wahlund, K.-G.; Giddings, J. C. *Anal. Chem.* **1987**, *59*, 1332–1339.
- (6) Wahlund, K.-G.; Litzén, A. *J. Chromatogr.* **1989**, *461*, 73–87.
- (7) Stevenson, S. G.; Ueno, T.; Preston, K. R. *Anal. Chem.* **1999**, *71*, 8–14.
- (8) Moon, M. H.; Kwon, H. S.; Park, I. *Anal. Chem.* **1997**, *69*, 1436–1440.
- (9) Moon, M. H.; Williams, P. S.; Kwon, H. *Anal. Chem.* **1999**, *71*, 2657–2666.
- (10) Jonsson, J. A.; Carlshaf, A. *Anal. Chem.* **1989**, *61*, 11–18.
- (11) Lee, W. J.; Min, B.-R.; Moon, M. H. *Anal. Chem.* **1999**, *71*, 3446–3452.
- (12) Gale, B. K.; Caldwell, K. D.; Frazier, A. B. *IEEE Trans Biomed. Eng.* **1998**, *45* (12), 1459–1469.
- (13) Edwards, T. L.; Gale, B. K.; Frazier, A. B., *Anal. Chem.* **2002**, *74*, 1211–1216.
- (14) Janca, J. *J. Liq. Chromatogr., Relat. Technol.* **2002**, *25* (5), 683–704.
- (15) Janca, J. *J. Liq. Chromatogr., Relat. Technol.* **2002**, *25* (13–15), 2173–2191.
- (16) Janca, J.; Berneron, J.-F.; Boutin, R. *J. Colloid Interface Sci.* **2003**, *260* (2), 317–323.

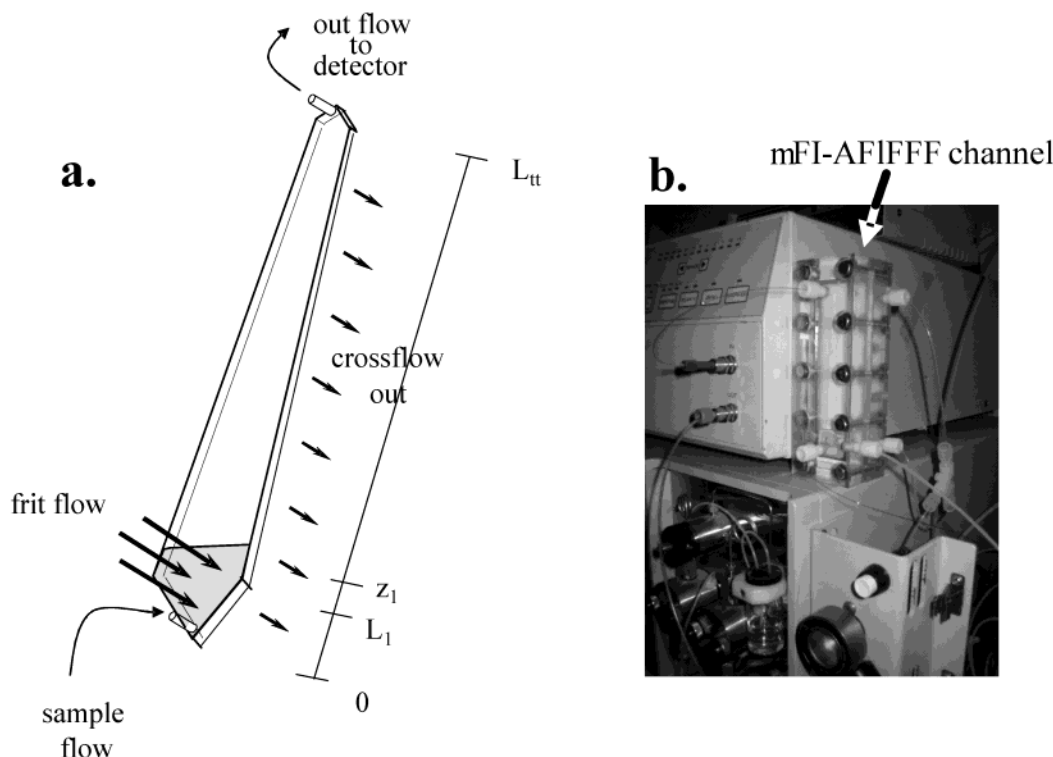


Figure 1. (a) Schematic view of frit inlet asymmetrical flow field-flow fractionation (FI-AFIFFF) channel and (b) photograph of mFI-AFIFFF channel system. The channel was vertically placed with the channel inlet at the bottom for the upstream flow passage. The connection tubings before and after the channel shown in the photograph were fused-silica capillary (75- μm i.d.).

through the inlet frit. Sample components injected to the channel are pushed toward the accumulation wall by frit flow, and they can undergo hydrodynamic relaxation without halting their migration along the channel. The schematic view of the FI-AFIFFF channel is shown in Figure 1a. Therefore, the separation process can be continuously achieved without stopping the flow. When hydrodynamic relaxation procedure is utilized, injected sample components are expected to achieve relaxation while they continuously migrate along the channel.^{17–19} The continuous fluid motion helps reduce the chance of sample adhesion to the channel wall.

Though the separation efficiency of FI-AFIFFF has been reported as somewhat lower than that of a conventional AFIFFF channel due to the unavoidable band spreading during hydrodynamic relaxation compared to focusing/relaxation, it has some advantages of convenience in system operation, in reducing the possibility of sample adhesion toward the channel membrane during the period of static relaxation, and in simplicity of adapting field programming²⁰ for the separation of a broad molecular weight range of macromolecules. In the case of eluting a low-concentration sample such as biological macromolecules, sample loss from a possible contact with the channel wall during the conventional stop-flow procedure may result in a serious loss of information. This can be enlarged when the flow FFF channel is miniaturized and uses a very small amount of sample concentration. However, miniaturization of the FI-AFIFFF channel can reduce the risk of

sample adhesion once the hydrodynamic relaxation procedure is utilized.

The purpose of this study is to examine the possibility of using miniaturized frit inlet (mFI)-AFIFFF for the separation of macromolecules without losing performance of the macroscale channel. By downscaling the channel system, a substantial reduction of channel outflow rate leads to a decrease in the injection amount required. This also leads to the possibility of applying the direct interface of μ FI-AFIFFF to electrospray ionization mass spectrometry (ESI-MS), and thus, it will bring great potential toward biological applications. Evaluation of mFI-AFIFFF is carried out for the separation of polystyrenesulfonate standards, proteins, and protein complex with ssDNA in a microflow regime. The end effect contribution to plate height was evaluated by varying the inner diameter of the connection tubing between the injector and the channel inlet at various injection flow rates.

EXPERIMENTAL SECTION

The mFI-AFIFFF channel was built in-house in a way similar to that used for the construction of a typical macroscale FI-AFIFFF channel,^{8,9} except for the channel dimensions. The channel length and breadth of the mFI-AFIFFF channel in Figure 1b were reduced to ~ 3 times to those of a macroscale channel. The channel space was made by cutting a 250- μm -thick Mylar spacer in a ribbonlike shape. The tip-to-tip length, L_{tt} , of the mFI-AFIFFF channel was 9.0 cm, while the initial channel breadth of 0.7 cm was decreased to a final 0.3 cm as a trapezoid. The length of the inlet frit measured from the channel inlet to the end of the relaxation segment (see Figure 1a), z_1 , was 1.1 cm. The total channel area was reduced to 4.29 cm^2 from 38.8 cm^2 (for the macroscale channel used in ref 8), and the area of inlet frit was reduced to

(17) Giddings, J. C. *Anal. Chem.* **1985**, 57, 945–947.

(18) Giddings, J. C. *Anal. Chem.* **1990**, 62, 2306–2312.

(19) Moon, M. H.; Myers, M. N.; Giddings, J. C. *J. Chromatogr.* **1990**, 517, 423–433.

(20) Moon, M. H.; Kang, D.; Hwang, I.; Williams, P. S. *J. Chromatogr., A* **2002**, 955 (2), 263–272.

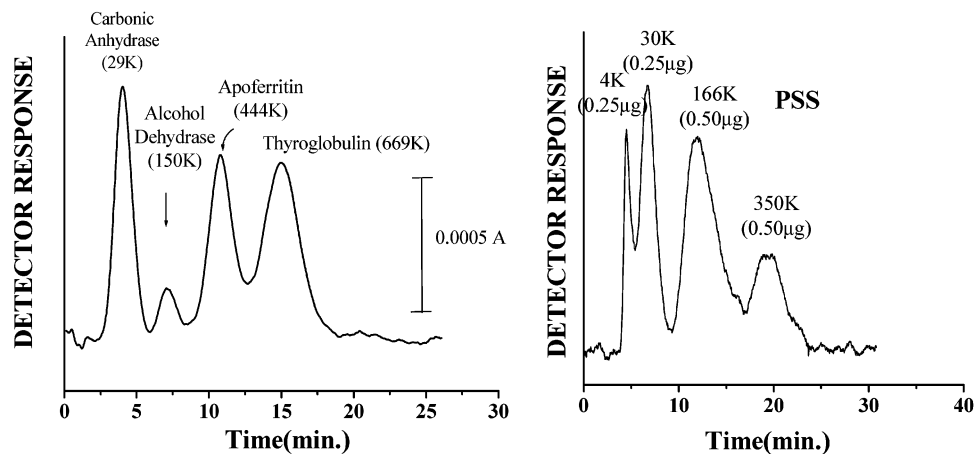


Figure 2. Fractograms representing the separation of (a) protein standards and (b) polystyrenesulfonate standards obtained by mFI-AFIFFF. Flow rates used (a) $\dot{V}_s = \dot{V}_{out} = 30 \mu\text{L}/\text{min}$ and $\dot{V}_f = \dot{V}_c = 0.50 \text{ mL}/\text{min}$ and (b) $\dot{V}_s = \dot{V}_{out} = 20 \mu\text{L}/\text{min}$ and $\dot{V}_f = \dot{V}_c = 0.30 \text{ mL}/\text{min}$.

0.52 cm² from 4.17 cm². Both connections from the injector to the channel inlet and from the channel outlet to the detector were made with a fused-silica capillary (75- μm i.d., 360- μm o.d.) for the minimization of band broadening caused by the end effect. At the accumulation wall, a sheet membrane, PLCCG (MWCO, 10 kDa) from Millipore Corp. (Danvers, MA), was placed above the frit to keep sample materials from penetrating the wall. Carrier solution used for sample delivery and for frit flow was 0.1 M Tris-HCl buffer adjusted at pH 7.4. The solution was prepared from ultrapure water (>18 M Ω) and was filtered through a membrane filter with a pore size of 0.45 μm prior to use. Sample delivery was made at 10–60 $\mu\text{L}/\text{min}$ by using a model 590 HPLC pump from Waters Co. (Milford, MA), and carrier solution through the inlet frit was pumped with a model 920 HPLC pump from Young-Lin Co. (Seoul, Korea). A metering valve, model Whitey SS-22RS2 from Crawford Fitting Co. (Solon, OH), was located after the detector to provide back pressure and to regulate flow rates.

Samples used for this study were as follows. Polystyrenesulfonate (PSS) standards (the certified weight average molecular masses in Da of 4K, 30K, 166K, and 350K) were obtained from American Polymer Standards Corp. (Mentor, OH). Protein standards were carbonic anhydrase (29 kDa), alcohol dehydrogenase (150 kDa), apoferritin (444 kDa), and thyroglobulin (669 kDa), from Sigma (St. Louis, MO). Two single-stranded DNA oligomers, having bases of 50 (oligo(dT)₅₀) and 100 (oligo(dT)₁₀₀), and a codon element-forward strand DNA (CE-F₂₀) were purchased from Takara Biochemical (Kyoto, Japan). Replication protein A (RPA) was obtained from the laboratory of Prof. Jang-Su Park at Pusan National University, and the RPA–DNA complex was made after the procedure in the literature.²¹ Sample injection was made via a model 7125 loop injector having a 5- μL loop from Rheodyne (Cotati, CA). Eluted materials were monitored by a model 720 UV detector from Young-Lin Co. at a wavelength of 280 nm for proteins and by a Shodex RI-71 differential refractometer from Showa Denko K. K. (Tokyo, Japan) for polystyrenesulfonate standards. Detector signals were recorded by the Autochro-Win software from Young-Lin.

RESULTS AND DISCUSSION

Performance tests of the mFI-AFIFFF system were carried out by separating protein standards, shown in Figure 2a which were obtained at flow rates of $\dot{V}_s = \dot{V}_{out} = 30 \mu\text{L}/\text{min}$ and $\dot{V}_f = \dot{V}_c = 0.50 \text{ mL}/\text{min}$, where \dot{V} is the volumetric flow rate specified with each subscript representing as s is the sample injection flow, out is outflow, f is frit flow, and c is cross-flow. The flow rates used in Figure 2 were about 7–10 times lower than those used for a typical separation of the same standards when used with a conventional FI-AFIFFF channel. Injection amounts were $\sim 0.3 \mu\text{g}$ for each standard, which was ~ 10 times less than those used at a macroscale FI-AFIFFF channel. It is demonstrated that the prototype microchannel shows a successful separation of protein standards at a micro flow rate regime with a reduced amount of sample injection. In addition, the separation was achieved within 20 min, which was faster than a typical run time (30–40 min.) observed at an ordinary FI-AFIFFF channel without losing resolution. Figure 2b shows a separation of PSS standards by a mFI-AFIFFF channel obtained at flow rates of $\dot{V}_s = \dot{V}_{out} = 20 \mu\text{L}/\text{min}$ and $\dot{V}_f = \dot{V}_{out} = 0.30 \text{ mL}/\text{min}$. Detection was made with an RI detector. Similarly to the protein separation shown in Figure 2a, loaded amounts of PSS standards, as marked in the figure, were reduced (reduced more than 10 times) compared to that used for the macroscale channel. This shows the potential of the miniaturized channel in the separation of other water-soluble polymers, which in some cases (very large molar mass polymers) need to be injected at a diluted concentration to minimize the chance of being aggregated with each other.

The main advantage of operating an FI-AFIFFF channel is to bypass the static relaxation procedure, which requires a temporary halt of sample migration along the channel. A possible disadvantage in using hydrodynamic relaxation is that it brings some additional band broadening that occurs due to a residual field driven relaxation and a passage of sample components through the inlet connecting tubing. The latter case of band broadening is the so-called end effect contribution H_{end} to overall plate height and is represented by¹⁸

$$H_{end} = \frac{L}{t_r^2} \frac{\sigma_v^2}{\dot{V}_s^2} = \frac{L}{t_r^2} \sigma_t^2 \quad (2)$$

(21) Park, J.-S.; Wang, M.; Park, S.-J.; Lee, S.-H. *J. Biol. Chem.* **1999**, 274 (41), 29075–29080.

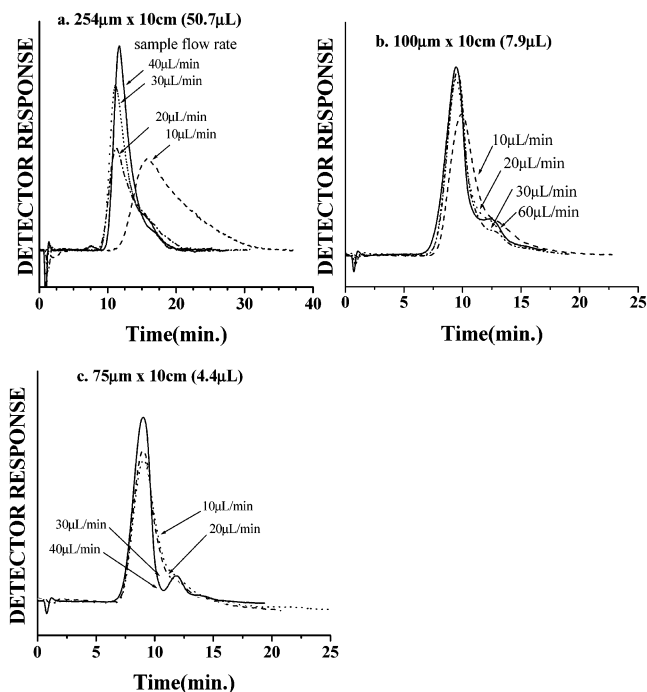


Figure 3. Elution profiles of apoferritin obtained at different injection flow rates (marked in the figure) by varying inner diameter of connection tubing between the injector and the channel inlet. Tubings are (a) 254- μm Teflon, (b) 100- μm silica capillary, and (c) 100- μm silica capillary. The length of connection tubings was fixed as 10 cm. Volumes of each tubing used were marked inside. All runs have an identical flow rate condition of $\dot{V}_{\text{out}} = 40 \mu\text{L}/\text{min}$ and $\dot{V}_i = 0.50 \text{ mL}/\text{min}$.

where L is the channel length, t_r the retention time of the sample component, σ_v^2 the volume-based variance of the incoming band, and σ_t^2 the time-based variance of the sample band during injection. The broadening source of the end effect in a mFI-AFIFFF system can be serious since a successful hydrodynamic relaxation requires a small ratio of \dot{V}_s/\dot{V}_i , which normally runs at a reduced flow rate of sample injection. The optimum ratio \dot{V}_s/\dot{V}_i found from earlier experiments was ~ 0.05 .^{8,9} However, the end effect contribution to plate height increases significantly when the flow rate of the sample inlet substream decreases, as shown in eq 1. This can be reduced to some degree if a narrow-bore or capillary tubing is utilized for the connection between the injector and the $\mu\text{FI-AFIFFF}$. To evaluate the effect of inlet tubing volumes on additional band spreading, experiments were set up to measure plate height values by varying the inner diameter of the connection tubing from the injector. For the minimization of any possible spreading of the sample band, an injection loop having a volume of $10 \mu\text{L}$ was used with a PEEK tubing (length, 20 cm; i.d., $127 \mu\text{m}$), and the distance for the connection tubing between injector and the channel inlet was fixed at 10 cm. The channel was plumbed right above the injector as shown in Figure 1b. The capillary tubing was inserted directly on channel by using a Microtight tubing sleeve. Figure 3 is the elution profile of apoferritin obtained by varying the inner diameter of the inlet connection tubing: 254- μm -i.d. Teflon tubing and 100- and 75- μm -i.d. fused-silica capillary tubing. The sample injection amount was fixed as $4.0 \mu\text{g}$, and the run condition was fixed at $\dot{V}_{\text{out}} = 40 \mu\text{L}/\text{min}$ and $\dot{V}_i = 0.50 \text{ mL}/\text{min}$. Figure 3a shows the change in the elution profile of apoferritin when 10 cm of 254- μm -i.d. Teflon tubing was used for the connection to the channel inlet from the injector. When the

Table 1. Experimental Plate Height Data Measured for Apoferritin Obtained at Different Connection Tubing Diameters between the Injector and the Channel Inlet by Varying Injection Flow Rate^a

\dot{V}_s ($\mu\text{L}/\text{min}$)	H_{exp} (cm)		
	254- μm i.d.	100- μm i.d.	75- μm i.d.
10.0	2.26 ± 0.04	0.42 ± 0.02	0.37 ± 0.01
20.0	0.63 ± 0.09	0.27 ± 0.01	0.27 ± 0.07
30.0	0.43 ± 0.06	0.20 ± 0.02	0.22 ± 0.02
40.0	0.32 ± 0.01	0.21 ± 0.01	0.21 ± 0.02
50.0		0.22 ± 0.01	
60.0		0.24 ± 0.01	

^a All runs were measured under the following conditions: $\dot{V}_{\text{out}} = 40 \mu\text{L}/\text{min}$ and $\dot{V}_i = 0.50 \text{ mL}/\text{min}$.

injection flow rate, \dot{V}_s , was $10 \mu\text{L}/\text{min}$, retention of apoferritin appeared to be seriously broad due to the time delay during the sample transportation, since the volume of the connection tubing to channel inlet from the injector was $50.7 \mu\text{L}$. The tubing dimension used in Figure 3a was that typically used for the macroscale FI-AFIFFF channel. When the injection flow rate increased, the apparent peak became sharper and shifted toward the shorter retention time. This demonstrates that the end effect contribution to plate height decreases when injection flow rate increases, as expected from eq 1. The plate height values measured for this set of experiments are listed in Table 1, and the data were compensated by subtracting the injection delay time from total retention time for each case. It appears that plate height value decreases as the injection flow rate increases. When the connection tubing was replaced by a 100- μm -i.d. fused-silica capillary ($7.8 \mu\text{L}$) in Figure 3b, the elution pattern of apoferritin at the lowest injection rate was not as serious as was observed in Figure 3a. In addition, it appeared with the shoulder peak, which was presumed to be from dimers of apoferritin, and this became clearer when the injection flow rate increased. Experimental plate height values were listed to be minimum in Table 1 when injection flow rates were around 30–40 $\mu\text{L}/\text{min}$. Further decrease of the tubing inner diameter to 75- μm -i.d. fused-silica capillary shows a better resolution in Figure 3c, with the minimum plate height (in Table 1) at the similar injection rate range used for Figure 3b. However, at the 75- μm -i.d. tube, retention time at the lowest injection rate was not seriously different from each other, except for the resolution of dimers due to the short transient time in the connection tubing. As plotted in Figure 3, experimental plate height seems to increase dramatically as the channel inlet tubing diameter increases at the lowest injection rate. The substantial increase in the plate height according to the increase in the tubing volume suggested that the initial band spreading during the sample transportation through the channel inlet tubing influenced the increase of total band broadening during hydrodynamic relaxation, and the use of a narrow capillary tubing was effective in reducing the end effect contribution of plate height.

The mFI-AFIFFF channel has been applied to the separation of a DNA binding protein complex, which is normally identified with the electrophoresis method. Figure 4a shows the elution profiles of single-strand DNA (ssDNA) oligomers: CE-F₂₉, oligo-(dT)₅₀, and oligo-(dT)₁₀₀ obtained at $\dot{V}_s/\dot{V}_{\text{out}}$ (in $\mu\text{L}/\text{min}$) and $\dot{V}_i/\dot{V}_c = 0.40/0.38$ (in mL/min) using mFI-AFIFFF. It is demonstrated

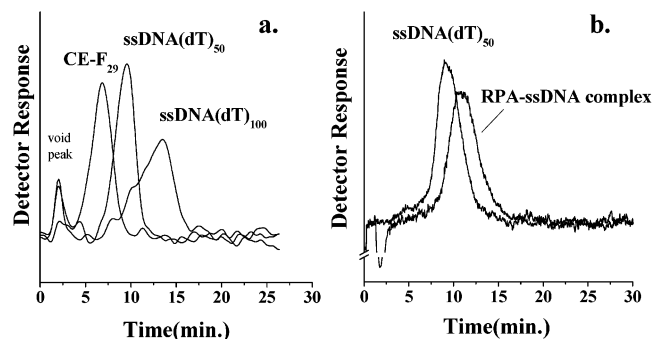


Figure 4. mFI-AFIFFF fractograms of (a) CE-F and ssDNA oligomers (\dot{V}_s/\dot{V}_{out} in $\mu\text{L}/\text{min}$ and $\dot{V}_i/\dot{V}_c = 0.40/0.38$ in mL/min) and (b) RPA–ssDNA binding complex ($\dot{V}_s/\dot{V}_{out} = 30/40$ in $\mu\text{L}/\text{min}$ and $\dot{V}_i/\dot{V}_c = 0.50/0.49$). Injection amounts are 50.4 ng (CE-F_{29b}), 12 ng each for (oligo(dT)₅₀ and oligo(dT)₁₀₀), 0.45 μg (ssDNA), and 0.60 μg (RPA).

that retention of each ssDNA in mFI-AFIFFF increases with increasing numbers of base in nucleotides. When RPA (115 kDa), which is known as a ssDNA binding protein having multiple functions in DNA replication, repair, and genetic recombination, is treated with ssDNA in the presence of a reducing agent such as DTT, a stable RPA–DNA complex is readily formed and its activity is of interest since it is controlled by redox regulation.^{21–24} The mFI-AFIFFF separation showed a clear difference in the elution times according to the increase of hydrodynamic radius upon the formation of RPA–DNA complex as shown in Figure 4b. The separation given in Figure 4b shows a potential in the application of mFI-AFIFFF toward the fast characterization of such

complex formation and the study of binding activity with regard to the redox status.

CONCLUSION

A miniaturization of flow field-flow fractionation has been successfully accomplished for an FI-AFIFFF channel system and tested for the separation of proteins. By reducing the channel dimensions and utilizing silica capillary tubing connections, separation in a mFI-AFIFFF system was achieved with a micro flow rate regime for both sample injection and detection and was performed faster than in a macroscale channel. Investigation of the end effect contribution to experimental plate height shows that an initial band broadening prior to the sample injection to the channel is critical in the mFI-AFIFFF system, and it suggests using narrow-bore capillary tubing for the connection between the injector and the channel inlet (in the current experiment, pressure was maintained below 120 psi). The separation of the ssDNA–protein complex shows a potential applicability of the mFI-AFIFFF system for the fast identification of DNA–protein binding activity. An advantageous feature of utilizing mFI-AFIFFF is a potential toward the direct interface to ESI-MS or ESI-MSMS for the top-down proteomic analysis of intact proteins. Since the outflow rate leading to the detector in mFI-AFIFFF is $\sim 30 \mu\text{L}/\text{min}$, it is thought to be suited for direct electrospray without a significant flow splitting. Further optimization of the prototype mFI-AFIFFF channel is needed to improve the efficiency of separation and to minimize the detection limit.

ACKNOWLEDGMENT

This work was supported by Yonsei University.

- (22) Eckerich, C.; Fackelmayer, F. O.; Knippers, R. *Biochim. Biophys. Acta* **2001**, 1538, 67–75.
- (23) Smith, J.; Zou, H.; Rothstein, R. *Biochimie* **2002**, 82, 71–78.
- (24) Jeong, H. S.; Kim, D.-H.; Kim, A.; Jeong, U.-C.; Kang, H. S.; Kim, Y.-J.; Kang, S.-W.; Park, J.-S. *Bull. Kor. Chem. Soc.* **2000**, 21 (12), 1171–1172.

Received for review March 2, 2004. Accepted April 15, 2004.

AC0496704