Anal. Chem. **2005,** *77,* 4207−4212

Hollow Fiber Flow Field-Flow Fractionation of Proteins Using a Microbore Channel

Dukjin Kang and Myeong Hee Moon*

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

Protein separation through hollow fiber flow field-flow fractionation (HF FlFFF) at microflow rate regime was successfully achieved by employing a microbore hollow fiber. In most of the flow field-flow fractionation (FlFFF) techniques applied to the separation of proteins, including hollow fiber FlFFF (HF FlFFF), an outflow rate leading to a detector has typically been a few tenths of a milliliter per minute. In this study, it is demonstrated for the first time that 10 μL/min outflow rate in HF FlFFF can be employed for a successful separation of proteins by utilizing a small inner diameter (450 μm) hollow fiber. Initial evaluations of microbore HF FlFFF separation were made to improve separation efficiency by evaluating plate heights, sample recovery, and the limit of detection using protein standards. Microbore HF FlFFF was applied for the separation of low-abundance blood proteins depleted of high-abundance proteins from raw serum using immunoaffinity chromatography.

Hollow fiber flow field-flow fractionation (HF FlFFF), a variant FlFFF technique, uses a hollow fiber membrane as a cylindrical separation channel and is capable of separating macromolecular species, including proteins and nano- to micrometer-sized particles.[1−5] Among the many FlFFF channel types, the hollow fiber FlFFF channel has gained interest due to its simple channel construction and its low cost, both of which lead to the possibility of a disposable channel system. Compared to the classic FlFFF channel, which has a rectangular cross section in which separation is achieved through the application of cross-flow to the direction perpendicular to the migration flow,[6,7] separation in HF FlFFF is carried by controlling radial flow through a porous membrane wall as the driving force of separation. Since the flow entering one end of the hollow fiber is divided into two parts (part of the flow leaves the channel wall through membrane pores and the remainder exits the fiber outlet), flow pattern in the HF FlFFF channel resembles to that in asymmetrical FlFFF (AFlFFF).[8,9]

Since the first report on the use of hollow fiber as an alternative to the FlFFF channel,[10] feasibility studies have been carried out by separating latex beads,[1,11] water-soluble polymers,[2,12] and by investigating the effects of ionic strength of carrier solution and sample overloading on HF FlFFF operation.[13] More recently, the efficiency of particle separation in HF FlFFF has been greatly improved to a level similar to that of a conventional, rectangular FlFFF channel.[3,14] Further efforts were made to apply HF FlFFF to the separation of supramicrometer particles, bacteria, cells, and synthetic organic-soluble polymers.[4,15−17] Additionally, HF FlFFF has been recently examined as a means for a possible development of off-line and on-line protein characterization. This would be effected with a conjunction of mass spectrometry (MS) through matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray ionization ESI-TOF MS, respectively.[5,18] On-line HF FlFFF and ESI-TOF MS analysis has demonstrated the viability of fractionating proteins by HF FlFFF followed by a direct analysis of protein ions in MS. Protein aggregation features have been observed with a simultaneous desalting during HF FlFFF separation.[5] Due to the low channel volume, HF FlFFF both reduced sample dilution and increased MS detectability. However, to reduce the inlet flow rate at the MS source, splitting the HF FlFFF outflow for ESI-MS analysis still needed to be done.

Miniaturization in analytical separation techniques generally provides several advantages such as a decrease in the sample injection amount, an enhancement of separation speed, and the possibility of system integration that leads to a comprehensive analytical device. Attempts to miniaturize FFF channel systems have been made by micromachining in thermal FFF and electrical FFF[19,20] or by reducing channel dimensions in thermal FFF.[22,23] While the performance of separation in earlier research was not

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

(1) Jönsson, J. A.; Carlshaf, A. *Anal. Chem.* **1989**, *61*, 11−18.
(2) Wijnhoven, J. E. G. J.; Koorn, J.-P.; Pope, H.; Kok, W. Th. *J. Chromatogr.* **1995**, *699*, 119−129.
(3) Lee, W. J.; Min, B.-R.; Moon, M. H. *Anal. Chem.* **1999**, *71*, 3446−3452.
(4) Bruijinsvort, M. V.; Kok, W. T.; Tijssen, R. *Anal. Chem.* **2001**, *73*, 4736−4742.
(5) Reschiglian, P.; Zattoni, A.; Roda, B.; Cinque, L.; Parisi, D.; Roda, A.; Piaz, F. D.; Moon, M. H.; Min, B. R. *Anal. Chem.* **2005**, *77*, 47−56.
(6) Giddings, J. C. *Anal. Chem.* **1981**, *53*, 1170A−1175A.
(7) Giddings, J. C. *Science* **1993**, *260*, 1456−1465.
(8) Wahlund, K.-G.; Giddings, J. C. *Anal. Chem.* **1987**, *59*, 1332−1339.
(9) Moon, M. H. *Bull. Korean Chem. Soc.* **2001**, *4*, 337−348.
(10) Lee, H.-L.; Reis, J. F. G.; Dohner, J.; Lightfoot, E. N. *AIChE J.* **1974**, *20*, 776−784.
(11) Carlshaf, A.; Jönsson, J. A. *J. Chromatogr.* **1989**, *461*, 89−93.
(12) Wijnhoven, J. E. G. J.; Koorn, J.-P.; Pope, H.; Kok, W. Th. *J. Chromatogr.* **1996**, *732*, 307−315.
(13) Jönsson, J. A.; Carlshaf, A. *J. Microcolumn Sep.* **1991**, *3*, 411−416.
(14) Moon, M. H.; Lee, K. H.; Min, B. R. *J. Microcolumn Sep.* **1999**, *11*, 676−681.
(15) Min, B. R.; Kim, S. J.; Ahn, K.-H.; Moon, M. H. *J. Chromatogr., A* **2002**, *950*, 175−182.
(16) Reschiglian, P.; Roda, B.; Zattoni, A.; Min, B. R.; Moon, M. H. *J. Sep. Sci.* **2002**, *25*, 490−498.
(17) Reschiglian, P.; Zattoni, A.; Roda, B.; Cinque, L.; Mellucci, D.; Min, B. R.; Moon, M. H. *J. Chromatogr., A* **2003**, *985*, 519−529.
(18) Reschiglian, P.; Zattoni, A.; Cinque, L.; Roda, B.; Piaz, F. D.; Roda, A.; Moon, M. H.; Min, B. R. *Anal. Chem.* **2004**, *76*, 2103−2111.

10.1021/ac050301x CCC: $30.25
Published on Web 05/18/2005

superior to that employing a conventional channel, a recent work to miniaturize a frit inlet asymmetrical FlFFF (FI-AFlFFF) channel has demonstrated that an enhancement of the protein separation speed could be attained by scaling down the channel geometry.[23]

For this study, HF FlFFF was performed at a microflow rate regime (down to 10 μL/min outflow rate) using a microbore hollow fiber. The microbore hollow fibers utilized in this study had a geometrical channel volume of only ~40 μL (450 μm × 25 cm). Since the fiber had an inner radius of 225 μm, this dimension was similar to that of the typical channel thickness (254 μm) utilized in a rectangular FlFFF system. However, the perimeter (comparable to the channel breadth of a rectangular channel) of the hollow fiber used in this study was calculated as 14 times narrower than that of a typical channel breadth (2 cm) in an FlFFF channel. This is one of the main advantages of utilizing a cylindrical channel since the channel cross section (largely affected by the breadth of a rectangular channel: 2 cm × 254 μm) can be reduced to a great extent without worrying about the edge effect caused by the frictional drag of flow at the surface of the channel edge. This effect is serious when the channel breadth is not sufficiently larger than the channel thickness. Thus, by simply reducing the radius of hollow fiber, HF FlFFF has a great potential as a microscale separation device in FlFFF. For the minimization of the possible sample broadening that can occur in the connection between hollow fiber and connection tubing, we developed a glue-free connection method with the use of silica capillary tubing for this study.

To explore the utility of a microbore hollow fiber (490-μm i.d.), flow optimization was performed with an initial evaluation of plate height and sample recovery using protein standards. The microbore HF FlFFF was applied for the separation of the low-abundance proteins (LAP) depleted of high-abundance proteins (HAP) contained in human serum using immunoaffinity chromatography. Serum proteome is of increasing interest in proteomics since it is expected to contain numerous (or even as large as thousands) proteins, and some of them are expected to be indicative tools for disease, or so-called biomarkers.[24,25] While high-abundance serum proteins such as albumin, IgA, IgG, transferrin, haptoglobin, and antitrypsin are known as major constituents (occupying more than 85% in weight of total proteins in serum), other numerous proteins exist at very low abundance (up to 10 orders of magnitude in concentration difference), but these have not been found to be connected to a specific physiological process or disease in terms of their change in concentration. Thus, the separation and isolation of LAP from HAP has been an important subject in the proteomic study of serum. In this study, the elution pattern of HAP and LAP fractions obtained by treating raw serum with immunoaffinity chromatography was examined with microbore HF FlFFF.

(19) Gale, B. K.; Caldwell, K. D.; Frazier, A. B. *IEEE Trans Biomed. Eng.* **1998**, *45*, 1459−1469.
(20) Edwards, T. L.; Gale, B. K.; Frazier, A. B. *Anal. Chem.* **2002**, *74*, 1211−1216.
(21) Janca, J. *J. Liq. Chromatogr., Relat. Technol.* **2002**, *25*, 683−704.
(22) Janca, J. *J. Liq. Chromatogr., Relat. Technol.* **2002**, *25*, 2173−2191.
(23) Kang, D.; Moon, M. H. *Anal. Chem.* **2004**, *76*, 3851−3855.
(24) Poon, T. C.; Johnson, P. J. *Clin. Chim. Acta* **2001**, *313*, 231−239.
(25) Pieper, R.; Su, A.; Gatlin, C. L.; Huang, S. T.; Anderson, N. L.; Steiner, S. *Proteomics* **2003**, *3*, 422−432.

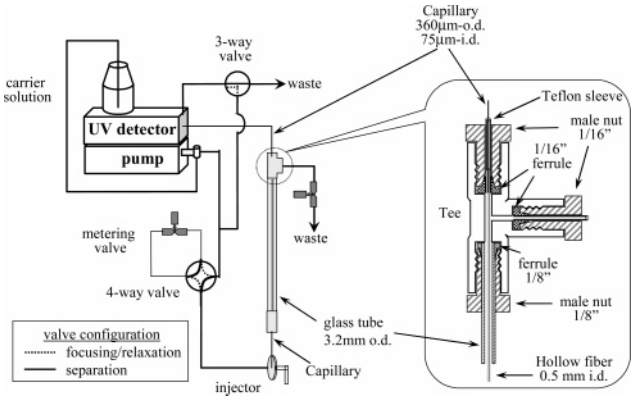

Figure 1. Schematics of the HF FlFFF system with an enlarged view of the glue-free connection between hollow fiber and silica capillary tubing. The same connection method was applied for the union in the initial part of the HF module. During sample focusing/relaxation, sample was injected along the dotted line pathway of both valve configurations. After relaxation, flow direction was set to the inlet of the fiber with a solid line configuration.

EXPERIMENTAL SECTION

HF FlFFF. The HF FlFFF channel was constructed in our laboratory. The HF used in this study was made of polysulfone, having a molecular weight cutoff of 30 000, supplied by the Kolon Central Research Institute (Yongin, Korea). The hollow fiber dimensions used were 25 cm long, 450-μm i.d., and 720-μm o.d., and the geometrical fiber volume was ~40 μL. The HF separation module and the entire HF FlFFF setup are illustrated in Figure 1. In this work, the HF module was made with a minimization of dead volume connection by introducing a glue-free connection method when the HF was immobilized within an exterior housing, such as glass tubing. The fiber was inserted into a piece of glass tube having 3.2-mm o.d. and 1.6-mm i.d., and both ends of the glass tube were connected with silica capillary by means of a union at the one end and a tee at the other, as illustrated in the enlarged diagram of the connection part in Figure 1. The connection between a glass tube and the tee was made simply by using an $^1/_8$-in. ferrule with hand tight male nut ($^1/_8$ in.). Both ends of the hollow fiber inside the glass tubing was connected with silica capillary tubing (360-μm o.d., 100-μm i.d.) in such a way that a few millimeters of capillary tubing end was inserted first into the hollow fiber (500-μm i.d.), which was covered with a piece of Teflon sleeve, and then the entire sleeve was tightened by a $^1/_{16}$-in. ferrule with a hand-tight male nut as shown in the right side of the connection tee at the enlarged diagram of Figure 1. The connection between hollow fiber inlet and capillary tubing from injector was made using a union the same way as described for the tee connection. Since the HF is extended through the tee connector, radial flow can exit the HF module through the tee connector.

Sample materials examined in this study were as follows. Protein standards were carbonic anhydrase (29 kDa), BSA (66 kDa), alcohol dehydrogenase (150 kDa) from yeast, apoferritin (444 kDa), and thyroglobulin (669 kDa) from Sigma (St. Louis, MO). Human serum was obtained from the Yonsei University Medical Center (Seoul, Korea). A separation of LAP and HAP from raw serum was carried out with immunoaffinity chromatography using the multiple affinity removal column (MARC, 4.6 × 50 mm) packed with mixed resins of antibodies having specific affinity to

albumin (64 kDa), IgG (28 kDa for light chain and 49.4 kDa for heavy chain), IgA (61 kDa), α-1-antitrysin (44.9 kDa), transferrin (68.5 kDa), and haptoglobulin (38.2 kDa) from Agilent Technologies (Palo Alto, CA). A depletion of HAP from serum was carried out by immunoaffinity chromatography with a model 1100 HPLC system from Agilent Technologies. Two mobile-phase solutions (A for sample loading, washing, and reequilibration of column, and B for the elution of LAP) were purchased from Agilent Technologies. Approximately 100 μL of diluted (5 times) human serum sample was injected into the multiple affinity column, and the flow-through low-abundance proteins (individual proteins that were not expected to bind with HAP) were eluted at 0.25 mL/min 100% mobile phase A. After the elution of flow-through LAP, the composition of mobile phase B was increased to 100% at 9 min from the start and the flow rate was raised to 1.0 mL/min. After the bound high-abundance proteins were eluted, the mobile-phase composition of A was brought up to 100% for a column regeneration for 7.5 min. The same collection process was automatically repeated 20 times to accumulate low-abundance proteins. The accumulated protein fractions were concentrated about 140 μg/μL for HAP and 20 μg/μL for LAP, both of which were measured by the Bradford method.

For the HF FlFFF separation of proteins, carrier solution was used with 10 mM NH_4HCO_3 solution that was prepared from ultrapure water (>18 MΩ) and filtered with a membrane filter having a pore size of 0.1 μm prior to use. The carrier solution was delivered to the HF module by an SP930D solvent delivery pump from Young-Lin Instrument (Seoul, Korea). The HF FlFFF operation detailed in this study is divided into two different steps: (a) sample injection while focusing flows are introduced and (b) elution. At the focusing/relaxation step, the pump flow is divided into two parts (1:9 ratio): one part enters the fiber inlet at one-tenth of the total flow rate, and the rest (9/10) is made to enter through the fiber outlet by adjusting the metering valve with both the four-way and the three-way valves simultaneously positioned with a dotted line configuration in Figure 1. A sample injection is made when the focusing/relaxation mode is established. After a period of time to allow for the sample to enter the fiber inlet and for relaxation, both valve configurations are switched back to a solid line connection (as they appear in Figure 1) so that the flow is only directed to the inlet of HF and the separation begins. During the separation, the control of both the outflow rate and radial flow rate was effected with a metering valve located at the radial flow outlet. Eluted proteins were monitored by a model UV 730D absorbance detector from Young-Lin at a wavelength of 280 nm.

RESULTS AND DISCUSSION

An initial evaluation of microbore HF FlFFF began with an optimization of flow rate condition for the separation of protein standards. Figure 2 shows the two HF FlFFF fractograms for the separation of protein standards obtained with a microbore hollow fiber. The separation of five proteins (carbonic anhydrase (29 kDa), BSA (66 kDa), alcohol dehydrogenase (150 kDa), apoferritin (444 kDa), and thyroglobulin (669 kDa)) in Figure 2a was successfully achieved within 13 min with a nearly baseline resolution. This demonstrates that a microbore HF FlFFF separation of proteins provides excellent results, for it does so without destroying protein conformations, an undesirable effect often

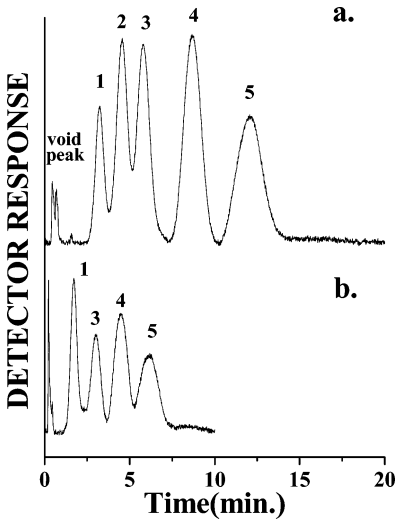

Figure 2. Fractograms of protein standards (1, carbonic anhydrase (29 kDa); 2, BSA (66 kDa); 3, alcohol dehydrogenase (150 kDa); 4, apoferritin (444 kDa); and 5, thyroglobulin (669 kDa)) by microbore HF FlFFF. Flow rates were $\dot{V}_{in}$ = 0.60 mL/min., $\dot{V}_{out}$ = 60 μL/min. for run a. and $\dot{V}_{in}$ = 0.60 mL/min., $\dot{V}_{out}$ = 170 μL/min for run b.

accompanying the denaturation or change in protein structure during separation in a typical HPLC separation. It has recently been demonstrated by on-line analysis of protein using HF FlFFF and ESI-MS that protein conformation was maintained during HF FlFFF.[26] It is noted that the separation shown in Figure 2a details by far the highest level of separation achieved with HF FlFFF for a broad molecular weight range of proteins. The flow rate condition for Figure 2a was $\dot{V}_{in}$ and $\dot{V}_{out}$. A faster separation was obtained when an outflow rate was increased to $\dot{V}_{out}$ while the total flow in, $\dot{V}_{in}$, remained the same. The injection amount for these runs was ~0.5 μg of each standard. In the case of Figure 2a, the radial flow rate ($\dot{V}_{rad}$) used was 0.54 mL/min, which is equivalent to 0.14 cm/s, the average linear velocity of cross-flow at the wall of the hollow fiber membrane obtained by dividing the radial flow rate with the surface area of the inner wall. The average linear velocity of radial flow at the wall used in Figure 2a was equivalent to that of ~7.0 mL/min of cross-flow rate ($\dot{V}_c$) for a rectangular asymmetrical FlFFF (or AFlFFF) channel having a dimension of 2.0 × 25 cm (breadth × length). Compared to a similar separation obtained with an AFlFFF channel at such a high field strength,[9] the speed of separation shown in Figure 2a was enhanced nearly two times, and the resolution was analogous to that obtained with an AFlFFF channel. This demonstrated that microbore HF FlFFF in a microflow rate regime can be successfully applied for the separation and isolation of proteins without a dilution effect at the channel. In addition, separation resolution appears to be improved by reducing the inner fiber diameter compared to the previous work done with larger diameter hollow fibers.[5]

To estimate the technical performance of the microbore HF FlFFF, retention of apoferritin (444 kDa) was measured by varying field strength and outflow rate. The results for this are reported in Figure 3a. The total inlet flow rate, $\dot{V}_{in}$, was fixed at 0.5 mL/min through all the runs in Figure 3a, and $\dot{V}_{out}$ was decreased

(26) Reschiglian, P.; Zattoni, A.; Parisi, D.; Cinque, L.; Roda, B.; Piaz, F. D.; Roda, A.; Moon, M. H.; Min, B.-R. *Anal. Chem.* **2005**, *77*, 47−56.

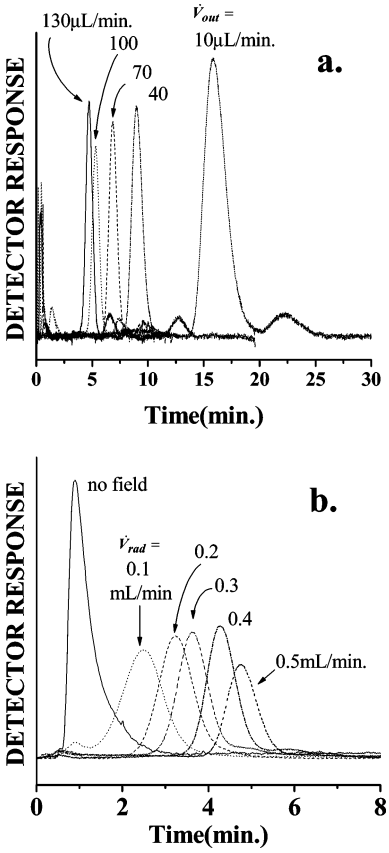

Figure 3. (a) Effect of field strength and outflow rate on the retention of apoferritin in microbore HF FlFFF at a fixed rate of total flow in as $\dot{V}_{in}$ = 0.5 mL/min and (b) fractograms of BSA for sample recovery measurement at a fixed outflow rate of $\dot{V}_{out}$ = 70 μL/min.

from 130 to 10 μL/min. As the outflow rate decreased, radial flow rate correspondingly increased from 0.37 to 0.49 mL/min. For the entire run conditions, dimers of apoferritin appeared as clearly separated from the monomer peak. Experimental plate height values were calculated for each monomer peak and are listed in Table 1a. When the ratio of radial flow rate to outflow rate, $\dot{V}_{rad}/\dot{V}_{out}$, was ~4, the experimental plate height profile showed a minimum and the corresponding number of plates was calculated as 109, a figure somewhat smaller than the reported value of 180 for ferritin (440 kDa) used with a thicker fiber (1-mm i.d.) in the literature.[4] However, the demonstration of protein separation at an outflow rate of 10 μL/min shows that microbore HF FlFFF has a great potential as a means for dealing with small amount of proteins and biological materials, and especially for applying to a direct ESI-MS analysis without splitting channel outflow.

An important consideration in developing a microflow separation was the recovery of sample materials during an HF FlFFF run. Since the HF FlFFF relies on the focusing/relaxation method as does a conventional AFlFFF system, sample recovery after a run depends on the strength of the radial flow applied for separation. This effect was examined by measuring the peak area of BSA obtained at various field strengths (radial flow rate) at a fixed outflow rate of 70 μL/min in Figure 3b. As the radial flow rate increased, the retention of BSA appeared to increase to some extent but not linearly. The nonlinear increase in retention is due to the fact that the effective migration rate increases with the increase in HF FlFFF radial flow rate. The recovery percentage

Table 1. (a) Experimental Plate Height Values Measured with Apoferritin Obtained by Varying Outflow Rates and (b) Recovery Values of BSA Calculated from the Peak Area at Increasing Field Strength by HF FlFFF

(a) Plate Height Values[a]

outflow rate (μL/min)	plate height (cm)
10	0.36 ± 0.02
40	0.30 ± 0.01
70	0.24 ± 0.02
100	0.23 ± 0.01
130	0.29 ± 0.01

(b) Recovery Percentage of BSA[b]

radial flow rate (mL/min)	recovery (%)
0	100
0.1	77.8 ± 0.5
0.2	76.3 ± 0.4
0.3	70.2 ± 3.1
0.4	69.6 ± 0.6
0.5	53.7 ± 7.7

[a] The total flow rate at the fiber inlet was fixed at 0.5 mL/min. [b] All runs were made with a fixed outflow rate of 70 μL/min.

values of BSA were obtained by comparing the peak area relative to that of BSA obtained without applying radial flow and are listed in Table 1b. It was shown that the recovery percentage value dropped to 77.8% at 0.1 mL/min radial flow, and it decreased gradually as radial flow rate increased. However, it was noted that a relatively large loss of sample was observed when applying the radial flow only at 0.1 mL/min. It is likely that the sample loss can be induced during the focusing/relaxation process, in which the sample band was focused at ~2.5 cm from the inlet by flow from both the fiber inlet and outlet. Sample adsorption to the fiber during elution cannot be excluded as a possible cause of this. The recovery value for an AFlFFF channel at a relatively high field strength ($\dot{V}_c$ < 5.0 mL/min,) has been noted as being ~80% when using a rather high $\dot{V}_{out}$ (> 0.5 mL/min) in an earlier work.[27] The study showed that the recovery value went below 60% at a further decreased $\dot{V}_{out}$ (= 0.2 mL/min), while those of a FI AFlFFF channel system utilizing hydrodynamic relaxation were above 85% at the same experimental flow rate conditions. However, a certain loss of sample during a HF FlFFF run is inevitable since the sample recovery also depends on the type of hollow fiber material (polysulfone in this work) due to the sample−wall interaction, so it is desirable to examine recovery using different types of membrane material. However, the current work was focused on evaluating the possibility of using microbore hollow fiber for FlFFF at a reduced flow rate. In relation to the sample recovery, a lower limit of detectable amount in the microbore HF FlFFF system was tested by decreasing the injection amount of BSA in Figure 4 obtained under the same conditions described in Figure 2a. It appeared that 30 ng (0.45 pmol) of BSA was clearly resolved by the microbore hollow fiber FlFFF. This was obtained by injecting diluted BSA solution with an injection loop volume fixed at 10 μL. The limit of detection (LOD) for HF FlFFF was reported as

(27) Moon, M. H.; Hwang, I. *J. Liq. Chromatogr., Relat. Technol.* **2001**, *24*, 3069−3083.

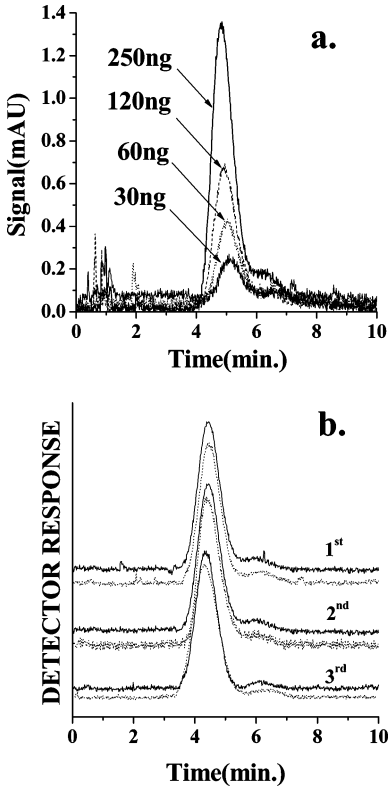

Figure 4. (a) Effect of sample amount of BSA in microbore HF FlFFF obtained at the same flow rate condition used in Figure 2a. Injection amount of BSA for each run was marked inside. (b) The interfiber reproducibility of microbore HF FlFFF of BSA obtained at $\dot{V}_{in}$ = 0.5 mL/min. and $\dot{V}_{out}$ = 50 μL/min. Three fibers were tested individually with repeated runs.

7.4 ng when a peak area measurement of 155-nm polystyrene (PS) latex beads was carried out.[16] Since LOD with PS latex is based on a turbidity measurement, which is different from UV absorption in the case of proteins, it is not directly comparable to the latter. However, the detection of such a low amount of protein by HF FlFFF as detailed in Figure 4a has been effected by using hollow fiber with a reduced diameter, as has been suggested in the literature.[2] Another concern in using microbore hollow fiber as an FlFFF channel has been the reproducibility existing between fibers. The interfiber reproducibility was tested by observing the change in the retention time of BSA (~125 ng) after simply replacing the hollow fibers, as is shown in Figure 4b. The run conditions were $\dot{V}_{in}$ mL/min and $\dot{V}_{out}$ μL/min. As shown with the repeated runs obtained using the same and different fibers, retention time appeared to be reproducible. The RSD value of retention time at three different modules was 1.8%. Improvement in reproducibility may have in part resulted from the complete removal or at least minimization of dead volume between the fiber and tubing connection by using the glue-free connection method. Since glue was applied at both ends of the fiber exterior to immobilize hollow fiber within the housing (usually wide-bore tube) at our earlier setup for HF modules, incorporation of dead spaces at both ends of the fiber was inevitable.

Microbore HF FlFFF of Human Serum Proteins. The microbore HF FlFFF system was applied for the separation of serum protein fractions, which were purified using immunoaffinity chromatography. Figure 5 shows the fractograms of raw serum,

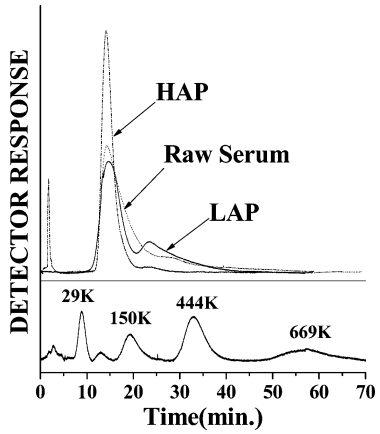

Figure 5. Fractograms of raw human blood serum, LAP, and HAP by microbore HF FlFFF obtained at $\dot{V}_{out}$ = 10 μL/min, $\dot{V}_{rad}$ = 0.49 mL/min along with the fractogram of standard protein separation (shown in Figure 2) for estimating approximate molecular weights. HAP and LAP were purified from the immunoaffinity chromatography explained in the test.

HAP fraction, and LAP fraction, as well as four protein standards obtained at $\dot{V}_{in}$ = 10 μL/min, $\dot{V}_{rad}$ = 0.49 mL/min with the microbore HF FlFFF system. The elution pattern of HAP in Figure 5 showed a single sharp peak ranging between molecular mass of 30−150 kDa. However, the LAP fraction showed a bimodal distribution in which a major part of the LAP fraction corresponded to relatively small molecular weight proteins (<150 kDa), and a small amount of high molecular weight fraction was included. There is a possibility of losing proteins smaller than 30 kDa, the molecular weight cutoff of the fiber used, during HF FlFFF. This can be overcome if a microbore HF membrane of a smaller MW cutoff is utilized. Unfortunately, such materials were not available from the manufacturer. Moreover, the possibility of coeluting HAP with the LAP fraction if there is an overloading during affinity chromatography purification should not be overlooked. The possibility of overloading can be checked by identifying proteins with the use of a nanoflow LC/MS/MS method, but it is beyond the scope of this study. In Figure 5, it is noted that the mobile-phase solution (whose compositions are unclear) used for affinity chromatography was removed in-line when they were resolved in microbore HF FlFFF. It is significant that the mobile-phase solution used for affinity chromatography should be removed before other proteomics analytical procedures such as proteolytic digestion and LC/MS analysis. The microbore HF FlFFF separation of serum proteins in Figure 5 has a potential as a means for fractionating proteins of interest belonging to a certain molecular weight range and for reducing sample complexity for proteomics analysis.

CONCLUSIONS

This work suggests the microbore hollow fiber as a microflow separation device in FlFFF has great possibilities. Our evaluation of microbore HF FlFFF performance showed that separation was comparable to the level obtainable by typical rectangular FlFFF channel with an enhancement of both separation speed and detection limit. In addition, the plumbing of microbore hollow fiber was integrated into a glue-free connection minimizing the dead volume at the interface to lead a possible band broadening.

Utilizing hollow fiber of a reduced inner diameter still has the advantages of HF FlFFF since it is inexpensive to assemble and can be developed into a disposable channel useful when considering run-to-run carryover. Because of the reduced sample dilution and channel outflow, microbore HF FlFFF is obviously very promising when coupled with direct ESI-MS, for this circumvents a splitting of the channel outflow for the analysis of intact low-abundant proteins. A combination of research on microbore HF FlFFF and ESI-MS is continuing with an effort to detect large molecular weight protein molecules with which successful electrospray ionization is difficult to achieve.

ACKNOWLEDGMENT

This study was supported by Korea Research Foundation Grant (KRF-2004-015-C00348).

Received for review February 17, 2005. Accepted April 20, 2005.

AC050301X