ELSEVIER

Journal of Chromatography A, 782 (1997) 159–165

JOURNAL OF CHROMATOGRAPHY A

Module performance of anion-exchange porous hollow-fiber membranes for high-speed protein recovery

Noboru Kubota[a], Yoshitaka Konno[a], Kyoichi Saito[a,*], Kazuyuki Sugita[a], Kohei Watanabe[b], Takanobu Sugo[c]

[a]*Department of Specialty Materials, Faculty of Engineering, Chiba University, Inage, Chiba 263, Japan*
[b]*Industrial Membranes Development Department, Asahi Chemical Industry Co., Ltd., Fuji 416, Japan*
[c]*Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, Takasaki 370-12, Japan*

Received 26 July 1996; received in revised form 31 December 1996; accepted 30 May 1997

Abstract

A laboratory-scale module was fabricated by housing eight anion-exchange porous hollow-fiber membranes with an effective length of 8 cm in parallel to each other in a cartridge. The hollow fibers were prepared by radiation-induced graft polymerization of glycidyl methacrylate and subsequent chemical modifications using diethylamine and ethanolamine. The resultant hollow fibers had diethylamino and ethanolamino group densities of 2.1 and 1.2 mmol/g of the dry hollow fiber, respectively, with inner and outer diameters of 2.8 and 4.4 mm, respectively, in a wet state. A low operating pressure of 0.1 MPa in the module provided a flow-rate of 100 ml/min for a bovine serum albumin solution. The dynamic binding amount of bovine serum albumin for the module was 270 mg, irrespective of the flow-rate, for rates ranging from 10 to 100 ml/min. The dynamic binding amount for the module was equivalent to eight times that of a single hollow fiber with an identical effective length, which indicates a linear relationship between the module and the number of single fibers for dynamic binding amount. © 1997 Elsevier Science B.V.

Keywords: Hollow-fibre membranes; Proteins; Module performance; Radiation-induced grafting

1. Introduction

A membrane module is used in membrane chromatography to process proteins on an industrial scale based on adsorptive interactions. Porous structures with flat-sheet and hollow-fiber forms are effective in transporting a target protein to a ligand immobilized on the pore surface by convective flow through the pores, driven by a pressure gradient across the porous membranes [1–6]. Ligands can adsorb the protein with a negligible diffusional mass-transfer resistance.

Previous studies on membrane chromatography using such modules are summarized in Table 1. The modules produced by Millipore [3,7,8], Nygene [9] and Sartorius [10,11] consist of a stack of flat-sheet membranes containing immobilized affinity ligands or ion-exchange groups. Sepracor, on the other hand, has commercialized a hollow-fiber membrane module containing Protein A as an affinity ligand [12,13]. The hollow-fiber membrane modules have the advantage over flat-sheet membrane modules that scale-up of the chromatography can be achieved by simple

*Corresponding author.

PII S0021-9673(97)00687-0

Table 1
Results of previous studies on membrane chromatography using a module

Membrane form	Interaction mode	Ligand	Supplier	Reference
Flat sheet	Affinity	Protein A	Millipore, Nygene	[7,9]
		Protein G	Nygene	[9]
		Cibacron blue F3GA	Sartorius	[10]
		Immobilized metal	Sartorius	[11]
	Ion-exchange	Diethylaminoethyl group	Millipore	[3,7,8]
		Diethylaminoethyl group	–	[22]
		Carboxymethyl group	Millipore	[7,8]
		Quaternary ammonium group	Millipore	[7]
		Sulfopropyl group	Millipore	[7]
Hollow fiber	Affinity	Protein A	Sepracor	[12,13]
		Antibody	Sepracor	[23]
	Ion-exchange	Diethylamino and ethanolamino groups	–	this work

bundling of a number of hollow fibers, which is suitable for the production scale and process requirements.

By radiation-induced graft polymerization, we have so far prepared functional porous hollow-fiber membranes for purifying proteins based on pseudo-affinity [14,15], ion-exchange [16–18] and hydrophobic interactions [19], and have evaluated the protein adsorption performance using a single hollow fiber. For example, a bovine serum albumin solution was permeated through the pores of a diethylamino-group-containing hollow-fiber membrane to determine the protein binding rate and capacity, and the percentage of protein eluted [17]. Moreover, by comparison of the protein adsorption performance based on an anion-exchange interaction, we demonstrated that the dynamic binding capacity of a single hollow-fiber membrane was constant, irrespective of flow-rate, while that of a bead-packed column with an identical volume decreased with increasing flow-rate [20]. In order to promote the extensive use of membrane chromatography on an industrial scale, the performance of modules fabricated with functional porous hollow-fiber membranes should be evaluated.

The objectives of this study were three-fold: (1) to fabricate a module with anion-exchange porous membranes with a hollow-fiber form, (2) to determine the dynamic binding amount of the protein and the operating pressure as a function of the flow-rate of the protein solution fed into the module and (3) to compare the performances of the module and a single hollow fiber. Bovine serum albumin was employed as a model protein and a diethylamino group was introduced into the hollow fiber as an anion-exchange group.

2. Experimental

2.1. Materials

A porous polyethylene hollow-fiber membrane was used as a trunk polymer for grafting. This hollow fiber had inner and outer diameters of 1.9 and 3.0 mm, respectively, with a pore diameter of 0.3 μm and a porosity of 70%. Technical grade glycidyl methacrylate (CH_2=$CCH_3COOCH_2CHOCH_2$) was purchased from Tokyo Kasei (Tokyo, Japan) and used without further purification. Bovine serum albumin was obtained from Sigma (A-7030; St. Louis, MO, USA). Tris–HCl buffer solution (0.02 *M*, pH 8.0) was employed. Other reagents were of analytical grade or higher.

2.2. Preparation of anion-exchange porous hollow-fiber membrane

A porous hollow-fiber membrane containing a diethylamino (DEA) group (-$N(C_2H_5)_2$) and an ethanolamino (EA) group (-NHC_2H_4OH) was prepared by a pre-irradiation grafting technique and subsequent chemical modifications as reported by

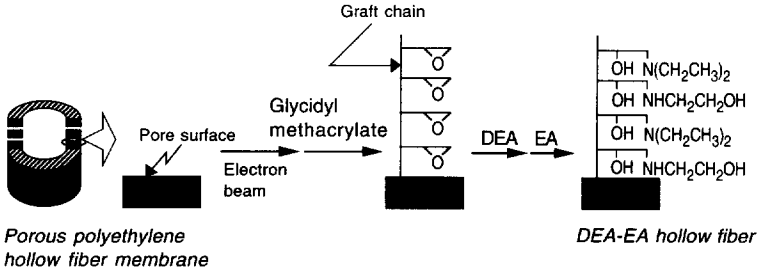
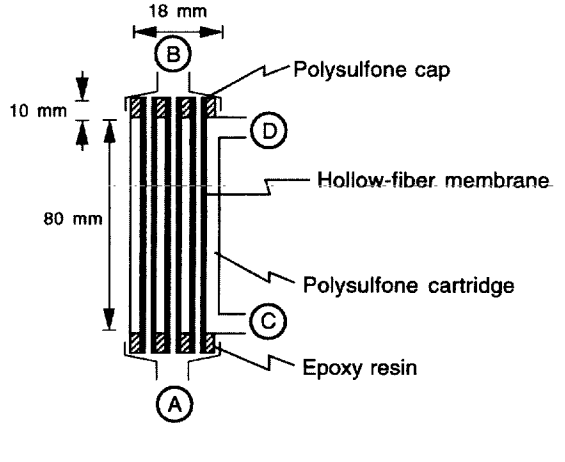


Fig. 1. Scheme of the preparation of a porous hollow-fiber membrane containing diethylamino (DEA) and ethanolamino (EA) groups.

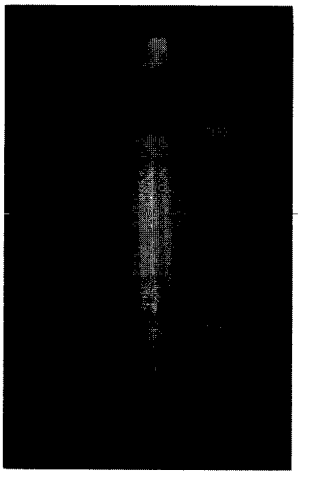
Tsuneda et al. [17] and shown in Fig. 1. Briefly, the porous polyethylene hollow-fiber membrane, irradiated with an electron beam, was immersed in a 10 (v/v)% glycidyl methacrylate–methanol solution for graft polymerization of glycidyl methacrylate onto the pore surface of the porous polyethylene hollow-fiber membrane. The weight of grafted glycidyl methacrylate was set to be twice that of the porous polyethylene hollow-fiber membrane. By immersion of the glycidyl methacrylate-grafted hollow fiber in diethylamine–H_2O (1:1, v/v) for 65 min at 303 K and subsequently in ethanolamine for 24 h at 303 K, a porous hollow-fiber membrane containing DEA and EA groups (DEA–EA hollow fiber) was obtained. The densities of the DEA and EA groups in the DEA–EA hollow fiber were determined by titration. After the hollow-fiber membrane was lyophilized, its cross-section was observed by scanning electron microscopy.

2.3. Fabrication of a hollow-fiber membrane module

A schematic illustration and a photograph of a hollow-fiber membrane module are shown in Fig. 2a and b, respectively. The inner diameter and length of a module cartridge made of polysulfone were 1.8 and 10 cm, respectively. Eight DEA–EA hollow fibers, 10 cm long, were housed in the cartridge in parallel and plugged at both ends using 1 cm thick epoxy resin. Thus, the effective membrane length was 8.0 cm. Four openings, i.e., two on the lumen side (A and B) and two on the shell side (C and D), were



(a) Schematic illustration of the DEA-EA module



(b) Photograph of the DEA-EA module

Fig. 2. Schematic illustration and photograph of the DEA–EA module. The number of DEA–EA hollow fibers in a module is eight.

made. The resultant hollow-fiber membrane module is referred to as a DEA–EA module.

2.4. Adsorption and elution of bovine serum albumin

The adsorption–elution performance of the DEA–EA module was evaluated in a dead-end mode at constant flow-rates ranging from 10 to 100 ml/min. Throughout the procedures, (B) and (C) were closed with silicone caps. The following three solutions were fed into the lumen side of the DEA–EA module at (A), and the permeate at the shell side was sampled from (D): (1) the buffer solution, in order to condition the DEA–EA hollow fiber, (2) a 2 mg/ml bovine serum albumin solution in the buffer to allow the adsorption of bovine serum albumin and (3) the buffer solution, in order to wash the pores of the hollow fiber and the shell side of the module. Then, a buffer solution containing 0.5 *M* NaCl was fed into the module from (D) to (A) to elute the bovine serum albumin adsorbed on the DEA–EA hollow fiber. For comparison, the same solutions were permeated radially outwards through a single DEA–EA hollow fiber with an effective length of 8 cm. All experiments were performed at 298 K.

The effluents from the DEA–EA module and the single DEA–EA hollow fiber were continuously sampled, and the bovine serum albumin concentration was determined by measuring the UV absorbance at 280 nm. The dynamic binding amount, equilibrium binding amount and elution percentage for bovine serum albumin are defined as

$$\text{Dynamic binding amount (mg)} = \int_0^{V_B} (C_0 - C)\,dV \quad (1)$$

$$\text{Equilibrium binding amount (mg)} = \int_0^{V_E} (C_0 - C)\,dV \quad (2)$$

$$\text{Elution percentage (\%)} = 100\,(\text{amount eluted}) / [(\text{amount adsorbed}) - (\text{amount washed})] \quad (3)$$

where C and V are the bovine serum albumin concentration and the effluent volume, respectively. C_0 is the feed concentration of bovine serum albumin (2 mg/ml). V_B and V_E are the effluent volumes at which C is 10 and 100% of C_0, respectively.

3. Results and discussion

3.1. Properties of the DEA–EA membrane

The densities of the DEA and EA groups in the anion-exchange porous hollow-fiber membrane (DEA–EA membrane) were 2.1 and 1.2 mmol/g of dry hollow fiber, respectively. The fibers had inner and outer diameters of 2.8 and 4.4 mm, respectively, in a wet state. Magnified images of the cross-section of a DEA–EA hollow fiber are shown in Fig. 3. Pores with a diameter of less than 1 μm penetrated through the membrane, and the pore network looked like a sponge. Radiation-induced graft polymerization of an epoxy-group-containing vinyl monomer (glycidyl methacrylate) and the subsequent addition of diethylamine and ethanolamine resulted in a uniform distribution of DEA and EA groups throughout the membrane thickness [21].

3.2. Binding amount and elution percentage of bovine serum albumin

Typical breakthrough and elution curves for a module consisting of eight DEA–EA hollow fibers (DEA–EA module) are shown in Fig. 4. The equilibrium binding amount of the DEA–EA module was calculated using Eq. (2) as being 380 mg of bovine serum albumin. This is equal to eight times the equilibrium binding amount of a single DEA–EA hollow fiber with an identical effective length (8 cm), and is converted to 67 mg of bovine serum albumin/ml of the membrane volume in a wet state. The membrane volume for the single hollow fiber is defined as

$$\text{membrane volume [ml]} = (\pi/4)(d_o^2 - d_i^2)L \quad (4)$$

where d_o, d_i and L are the outer and inner diameter and the length of the hollow fiber, respectively.

The dynamic binding amount of the DEA–EA module, which is defined by Eq. (1), is shown in Fig. 5 as a function of the flow-rate of the bovine serum

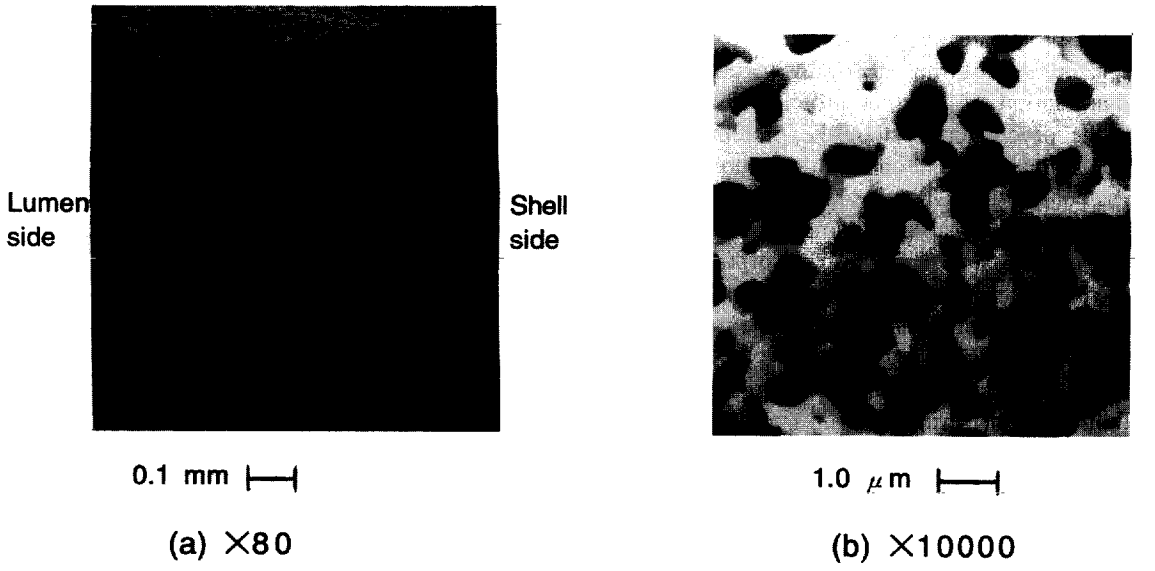


Fig. 3. Scanning electron micrographs of the cross-section of a DEA–EA hollow fiber.

albumin solution. The dynamic binding amount of the module was constant at 270 mg, irrespective of the flow-rate, for rates ranging from 10 to 100 ml/min, which was equivalent to 47 mg of bovine serum albumin/ml of the membrane volume in a wet state. This is because the convective flow of the protein solution through the pores of the hollow fiber minimized the diffusion path between proteins and the anion-exchange groups on the graft chains extending from the pore surface towards the pore interior.

The dynamic binding amount value of the DEA–EA module was also equivalent to eight times that of a single DEA–EA hollow fiber with an identical effective length. This linear relationship between the module and the number of single hollow fibers is

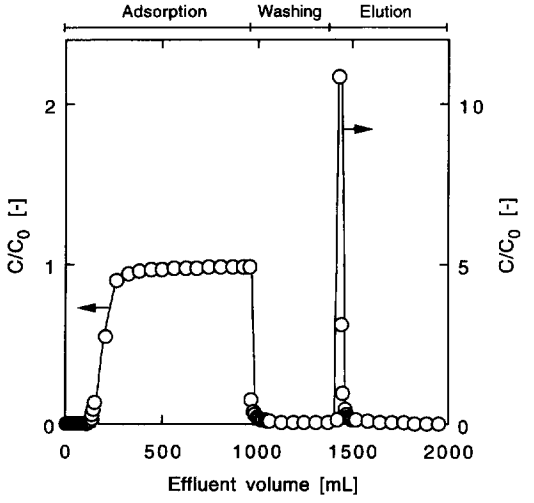


Fig. 4. Typical breakthrough and elution curves for a DEA–EA module obtained at a flow-rate of 20 ml/min.

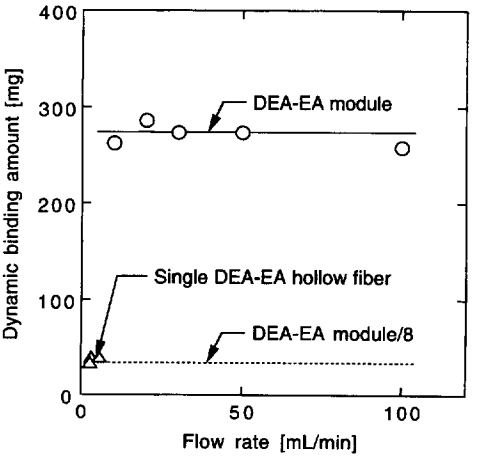


Fig. 5. Dynamic binding amount of the DEA–EA module and that of a single DEA–EA hollow fiber as a function of flow-rate.

favorable for scaling up the membrane chromatography for protein recovery.

Repeated use of the DEA–EA module for adsorption–elution of a target protein is required to lower the production cost of the protein. The dynamic binding amount and elution percentage remained constant at 270 mg and 100%, respectively, after five cycles. This binding amount was equivalent to eight times that of monolayer adsorption because of the multilayer binding of bovine serum albumin onto the graft chains extending from the pore surface towards the pore interior [17]. Moreover, this quantitative elution demonstrates that non-selective adsorption of bovine serum albumin onto the DEA–EA hollow fibers as well as the polysulfone case of the DEA–EA module was negligibly small. The hydrophilic graft chains containing the DEA and EA groups satisfactorily mask the pore surface of the hydrophobic polyethylene hollow fiber used as a trunk polymer, resulting in a reduction in the non-selective adsorption of bovine serum albumin onto the DEA–EA hollow fiber.

3.3. Flow-rate through the module

A linear relationship between the operating pressure and the flow-rate of the bovine serum albumin solution through the DEA–EA module was observed, as shown in Fig. 6. The flow-rate for the module containing eight DEA–EA hollow fibers was equal to eight times that of a single hollow fiber. This result indicates an advantage of membrane chromatography over conventional bead-packed column chromatography: scale-up of the former does not require a higher pressure pump, whereas scale-up of the latter does, unless a thin column with a large diameter, i.e., the equivalent of a functional porous membrane, is used.

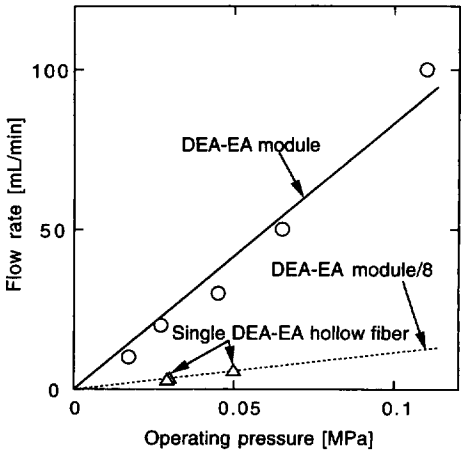


Fig. 6. Flow-rate of the DEA–EA module and of a single DEA–EA hollow fiber as a function of operating pressure.

4. Conclusions

A porous anion-exchange hollow-fiber membrane containing diethylamino (DEA) and ethanolamino (EA) groups (DEA–EA hollow fiber) was prepared by radiation-induced graft polymerization of an epoxy-group-containing vinyl monomer and subsequent chemical modifications. A membrane module consisting of eight DEA–EA hollow fibers, 8 cm long, was fabricated, into which bovine serum albumin solution (pH 8) was fed at various flow-rates ranging from 10 to 100 ml/min. The dynamic binding amount and flow-rate of the module were compared to those for a single hollow fiber. It was confirmed that the adsorption kinetics of the module were favorable, i.e., the dynamic binding amount was constant at a high level, irrespective of the flow-rate, and that the dynamic binding amount increased linearly with the number of fibers in the module. Also, the module exhibited a stable performance over five adsorption and elution cycles.

5. List of symbols

C	protein concentration in the effluent (mg/ml)
C_0	protein concentration in the feed (mg/ml)
DEA	diethylamino
d_i	inner diameter of the hollow fiber (cm)
d_o	outer diameter of the hollow fiber (cm)
EA	ethanolamino
L	length of the hollow fiber (cm)
V	effluent volume (ml)
V_B	effluent volume when C reaches 10% of C_0 (ml)
V_E	effluent volume when C reaches C_0 (ml)

References

[1] S. Brandt, R.A. Goffe, S.B. Kessler, J.L. O'Connor, S.E. Zale, Bio/Technology 6 (1988) 779–782.

[2] E. Klein, Affinity Membranes, Their Chemistry and Performance in Adsorptive Separation Process, Wiley, New York, 1991.

[3] D.K. Roper, E.N. Lightfoot, J. Chromatogr. A 702 (1995) 3–26.

[4] J. Thommes, M.-R. Kula, Biotechnol. Prog. 11 (1995) 357–367.

[5] T.B. Tennikova, M. Bleha, F. Svec, T.V. Almazova, B.G. Belenkii, J. Chromatogr. 555 (1991) 97–107.

[6] T.B. Tennikova, F. Svec, J. Chromatogr. 646 (1993) 279–288.

[7] Memsep catalog, Millipore, Bedfold, MA, 1993.

[8] J.A. Gerstner, R. Hamilton, S.M. Cramer, J. Chromatogr. 596 (1992) 173–180.

[9] MASS catalog, Nygene, New York, 1990.

[10] B. Champluvier, M.-R. Kula, Biotechnol. Bioeng. 40 (1992) 33–40.

[11] O.-W. Reif, V. Nier, U. Bahr, R. Freitag, J. Chromatogr. A 664 (1994) 13–25.

[12] Affinity-15 system catalog, Sepracor, Marlborough, MA, 1989.

[13] C. Charcosset, Z. Su, S. Karoor, G. Daun, C.K. Colton, Biotechnol. Bioeng. 48 (1995) 415–427.

[14] H. Iwata, K. Saito, S. Furusaki, T. Sugo, J. Okamoto, Biotechnol. Prog. 7 (1991) 412–418.

[15] M. Kim, K. Saito, S. Furusaki, T. Sato, T. Sugo, I. Ishigaki, J. Chromatogr. 585 (1991) 45–51.

[16] S. Tsuneda, H. Shinano, K. Saito, S. Furusaki, T. Sugo, Biotechnol. Prog. 10 (1994) 76–81.

[17] S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, J. Chromatogr. A 689 (1995) 211–218.

[18] S. Tsuneda, K. Saito, T. Sugo, K. Makuuti, Radiat. Phys. Chem. 46 (1995) 239–245.

[19] N. Kubota, M. Kounosu, K. Saito, K. Sugita, K. Watanabe, T. Sugo, J. Chromatogr. A 718 (1995) 27–34.

[20] N. Kubota, S. Miura, K. Saito, K. Sugita, K. Watanabe, T. Sugo, J. Membr. Sci. 117 (1996) 135–142.

[21] S. Tsuneda, K. Saito, S. Furusaki, T. Sugo, I. Ishigaki, J. Membr. Sci. 71 (1992) 1–12.

[22] D. Josic, J. Reusch, K. Loster, O. Baum, W. Reutter, J. Chromatogr. 590 (1992) 59–76.

[23] M. Nachman, A.R.M. Azad, P. Bailon, J. Chromatogr. 597 (1992) 155–166.