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Comparative Evaluation of Ultrafiltration Membranes for Purification of Synthetic Peptides

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Abstract

Published information on the use of ultrafiltration to separate natural and synthetic peptides from each other, and from low-molecular-weight impurities, is reviewed. The suitability of commercial membranes of low nominal molecular weight cut-off (500–8000 daltons) for fractionation of synthetic peptides was evaluated with a model mixture of a hexapeptide (MW 844), insulin (MW 5730), and cytochrome c (MW 12,384) in 5% acetic acid. Diafiltration in a cross-flow thin-channel device allowed graphical determination of the retention coefficient for each solute on each membrane; fouling and cleanability were also assessed. Regenerated cellulose and cellulose acetate membranes did not foul, were chemically resistant, and fractionated efficiently. Other membrane types, including polysulfone and Teflon, fouled and were difficult to clean. Cellulosic membranes can be successfully integrated into the purification of synthetic peptides.

INTRODUCTION

Since the discovery of the asymmetric membrane some 25 years ago (*1*), membrane separation processes, especially ultrafiltration (UF), have emerged as technically and commercially attractive unit operations. In biotechnology, membrane separations are increasingly used in plasma products, protein processing, and fermentation downstream processing (*2–4*). Today, successful operations generally involve rather broad and simple separations, such as cell harvesting, protein desalting, and/or concentration. It is nowadays well-accepted that permeation of molecules

across porous membranes is mainly governed by interactions of the molecules with the polymeric material of the membrane. More specifically, the interrelated aspects of boundary layer formation, interfacial tension, and nonspecific protein adsorption are of particular importance for the permeation behavior of the membrane in the presence of complex biochemical solutions (*5*). As a result, the use of UF as a fractionation process has been restricted, with varying degrees of success, to molecular mixtures with species differing markedly in molecular size by a factor of 10 or more. Albumin (MW 69,000) can be efficiently separated from lysozyme (MW 14,000) on an Amicon PM-30 membrane, provided that conditions are selected to minimize protein-protein interactions (*6*). Bovine serum albumin (BSA) has been successfully separated from alkaline phosphatase (MW 140,000) on an Amicon YM100 regenerated cellulose membrane (*7*). Polyethylene glycol PEG 4,000 (MW 4000) could also be removed from human albumin preparations, in spite of significant irreversible protein adsorption that increases the retention of PEG (*8*). However, BSA could not be cleanly separated from *t*RNA (MW 30,000) (*9*), nor from a lymphocytopoietic protein activity (MW 30,000), although many commercial membranes with nominal molecular weight cut-off (NMWCO) ranging from 10,000 to 60,000 were tested (*2*).

Fractionation depends on the molecular weight difference between the species to be separated, and also membrane charge, hydrophilic properties of the active surface, and overall hydrodynamics. Improvements can be achieved by appropriate selection of operating variables (i.e., ionic strength, pH) or by exploiting other effects such as affinity interactions of enzymes and macromolecules (*10*).

The purification of synthetic peptides, however, is a challenging area for membrane processes, and, like protein UF, has been mostly restricted to solvent exchange and desalting. Peptides are relatively short chains of amino acids (arbitrarily <50 amino acids) that have been isolated from hydrolysates of longer polypeptides or chemically synthesized. Chemical synthesis of various peptides, including antibiotics and hormones, analogs, nucleotides, and various other bioactive molecules is likely to increase in the future due to the availability of modern programmable instruments. Production of 40 to 50 amino acid peptides is feasible today with solid phase technology and fragment condensation. A common problem in these processes is the coproduction of impurities, fragments, or sequence failure molecules in the low molecular weight range (<1500 daltons), along with the desired compound (2500–10,000 daltons). After chemical synthesis, peptides are cleaved from the solid support, lyophilized, and resolubilized, commonly in dilute acetic acid. UF can then

be an efficient purification tool provided that retention of the desired compound is high, adsorption/fouling is low, and the membrane can withstand the low pH.

Few studies are reported in the literature. We suspect, however, that certain proprietary fractionations are already in operation. ^{131}I-labeled insulin was purified by removal of its low molecular weight contaminant ^{131}I, despite incomplete insulin exclusion by the membrane and significant adsorptive losses. The membrane utilized was an Amicon Diaflo 1,000 NMWCO membrane (*11*). The kinetics of elimination of the peptide hormones pro-insulin (MW 9000), insulin (MW 6000), and C-peptide (MW 3000) were measured on cellulose (Cuprophan) and polyacrylonitrile membranes and allowed the comparison between *in vitro* and *in vivo* hemodialysis on a biological rat model (*12*). Similar work was conducted for the determination of the reflection coefficient and diffusive permeability of vitamin B_{12} (MW 1355), insulin (MW 5200), cytochrome c (MW 13,500), and myoglobin (MW 17,000) in saline and protein solutions using Cuprophan hollow fibers (*13*). These particular membranes are specifically used as artificial kidney devices.

We selected three peptides for a model mixture to evaluate fractionation by membrane technology in the low molecular weight range. Peptide b, with a molecular weight of 844, is typical of the contaminating fragments produced in solid phase peptide synthesis. Insulin and cytochrome c are positively charged at low pH and are representative of synthetic peptides of moderate and high molecular weight. We expected complete retention of cytochrome c by all the membranes tested.

We preferred diafiltration to the concentration mode because complete solvent exchange is theoretically possible, allowing efficient elimination of low molecular weight contaminants. Furthermore, concentration polarization is reduced since no concentration occurs. To minimize the polarization layer (*14*), the shear rate was kept relatively high at 14,400 s^{-1} for all membranes tested. However, a dynamic gel layer on the membrane surface is unlikely to form at low peptide concentrations.

Production and commercialization of asymmetric UF membranes were initially based on cellulose and cellulose derivatives because of their relatively low cost and their good compatibility with biological compounds, especially proteins and peptides. Cellulose is a major membrane polymer used primarily in dialysis and hemodialysis devices. It shows remarkable hydrophilic properties due to its numerous hydroxyl groups. Cellulose is a versatile polymer which can be modified by suitable reagents, leading to cellulose derivatives. Cellulose acetate is also a

common membrane polymer but presents a more hydrophobic surface because of partial acetylation of the available hydroxyl groups.

Cellulose and its derivative tend to undergo hydrolysis at alkaline pH and lack resistance to microbial degradation in general. Thus, the development of UF membranes led to more-resistant polymers such as polysulfone and polyvinyldifluoride (PVDF). Unfortunately, for most synthetic polymers, surface tension and capillarity forces combine to prevent spontaneous wetting of membranes. Polymer modifications to render the polymeric material more hydrophilic have been frequently attempted (*15*, *16*), the sulfonation of polysulfone being a typical success. Synthetic membranes are extensively used nowadays for special purpose applications when protein adsorption is not a significant problem.

We tested various polymeric materials: regenerated cellulose, cellulose acetate, and six other common polymers all listed in Table 1. Polyether sulfone is totally free of aliphatic hydrocarbon groups and exhibits even higher thermal stability than polysulfone. Teflon is naturally hydrophobic, although several fluoropolymers have been successfully modified (i.e., hydrophilic derivatives of PVDF).

Experiments were conducted in the diafiltration mode, allowing the graphical determination of the retention factor k for each species on each individual membrane. The extent of fouling was also measured experimentally in order to assess the degree of peptide adsorption. A standard and simple cleaning procedure was performed to compare the cleanability and chemical resistance of the membranes.

EXPERIMENTAL

Peptides

Peptide b (*p*-Glu-His-Trp-Ser-Tyr-D-LeuOET, MW 844) was kindly donated by K. W. Funk, Abbott Laboratories, North Chicago, Illinois. Insulin (bovine) (MW 5730) and cytochrome c (MW 12,384) were from Sigma Chemical Company.

The peptides were weighed and a measured volume of concentrated glacial acetic acid (99.7% purity) added. Ultrapure water (17 megohm-cm resistivity at 25°C, Zenon Environmental Inc.) was slowly added while sonicating on a Bransonic ultrasonic cleaning bath (Fisher Scientific) until the peptides were dissolved. The final solution was 5% acetic acid, pH 2.25, and contained 500 ppm of each peptide.

TABLE 1
Specifications and Performance of UF Membranes for the Fractionation of Peptides[a]

Polymer	Membrane	NMWCO (daltons)	Pure water permeability L_p ($L \cdot h^{-1} \cdot m^{-2} \cdot atm^{-1}$)	Experimental filtration rates			Fouling ratio	Cleanability
				Pure water before sample ($L \cdot h^{-1} \cdot m^{-2}$)	With sample ($L \cdot h^{-1} \cdot m^{-2}$)	Pure water after sample ($L \cdot h^{-1} \cdot m^{-2}$)		
Aliphatic alcohol	Zenon CV32TX	1000	30.4	52.1	25.0	32.1	0.62	e,f
Cellulose acetate	DDS CA865PP	RO(100–500)	13.0	20.0	16.5	21.4	1.07	g
	Osmonics SX01	500	7.3	7.1	5.6	6.8	0.96	g
	Amicon YC05	500	15.2	10.9	9.2	14.0	1.28	g
	Millipore PCAC	1000	9.9	13.7	10.7	15.3	1.12	h
	Osmonics SG15	1000	18.7	37.8	38.3	38.6	1.02	g
	DDS CA800PP	8000	30.4	50.9	51.4	61.4	1.21	h
Cellulose (regenerated)	Amicon YM2	1000	8.3	11.6	10.6	12.5	1.08	g,i
	SMI Type C	1000	19.0	33.4	29.2	32.1	0.96	g
	Amicon YM5	5000	26.6	39.1	34.4	35.8	0.92	g
	SMI Type C	5000	37.1	61.1	49.4	56.0	0.92	g

(continued)

TABLE 1 (*continued*)

Polymer	Membrane	NMWCO (daltons)	Pure water permeability L_p ($L \cdot h^{-1} \cdot m^{-2} \cdot atm^{-1}$)	Experimental filtration rates: Pure water before sample ($L \cdot h^{-1} \cdot m^{-2}$)	With sample ($L \cdot h^{-1} \cdot m^{-2}$)	Pure water after sample ($L \cdot h^{-1} \cdot m^{-2}$)	Fouling ratio	Cleanability
Fluoropolymer	DDS FS81PP	6000	419.5	43.5[c]	189.5	16.8[c]	0.39	*j,f*
Polyether sulfone	EPM PES	2000	55.0	100.5	26.5	25.1	0.25	*f*
Polysulfone (sulfonated)	DDS GB90PP	2000	42.9	40.5	16.3	17.6	0.43	*k,g*
	DDS GR81PP	6000	96.1	56.3	37.7	38.6	0.68	*e,f*
Polysulfone (modified)[b]	Bio-Rec. 01K-PSB03	1000	17.4	23.9	17.7	21.1	0.88	*g,k*
	Bio-Rec. 03K-PSB03	3000	55.1	86.4	43.6	43.6	0.51	*k,h*
	Bio-Rec. 05K-PSB03	5000	109.5	139.5	67.2	70.7	0.51	*g*
Teflon	Osmonics AP01	1000	207.4	322.3	107.2	104.9	0.33	*e,f*
	Osmonics AP02	3000	256.1	112.8[d]	119.8	54.3[d]	0.48	*e,f*

[a]Total peptide concentration: 1500 ppm–5% acetic acid, pH 2.25, 23°C, transmembrane pressure 35 psig, fluid velocity = 0.96 $m \cdot s^{-1}$, shear rate 14,400 s^{-1}, effective membrane area = 40 cm^2.

[b]Proprietary chemical modification for low-protein binding properties.

[c]Measurements made with no pressure applied due to the very high flux rate (916 $L \cdot h^{-1} \cdot m^{-2}$).

[d]Measurements made with no pressure applied due to the very high flux rate (498 $L \cdot h^{-1} \cdot m^{-2}$).

[e]Membrane was heavily stained with cytochrome c.

[f]Membrane was difficult to clean and was discarded.

[g]Membrane was easily cleaned.

[h]Upon cleaning, the flux rate increased by twofold.

[i]Membrane was pierced during the fractionation assay.

[j]The fluoropolymer was detached from the membrane support.

[k]Membrane was slightly stained with cytochrome c.

UF Equipment

The experimental set-up used for all the experiments is shown in Fig. 1. The system consisted of a standard TCF10 thin channel unit equipped with a peristaltic pump (Amicon Corp.). Reservoir volume was automatically maintained at a constant level by use of a concentration/dialysis selector (Model CDS10, Amicon Corp.), connected to a nitrogen gas supply for pressurization and to a dialysate tank (Model RC800, Amicon Corp.). The selector allowed instant manual switching of the unit from concentration to diafiltration or vice versa, and permitted sequential sampling of the peptide mixture. The cross-flow rate was 230 mL/min and the transmembrane pressure was 35 psig for all experiments. Channel geometry was rectangular (0.4 × 10 mm). Fluid velocity was calculated at 0.96 $m \cdot s^{-1}$, shear rate at 14,400 s^{-1}. No temperature control was used. Ambient and operating temperatures were stable at 23 ± 1°C. The system's reservoir was covered with a foil sheet to protect the cytochrome c against photooxidation. The volume in the TCF10 cell was 100 mL. As the solution passed through the membrane, it was replaced with 5% acetic acid from the pressurized reservoir. A 0.5-mL sample from

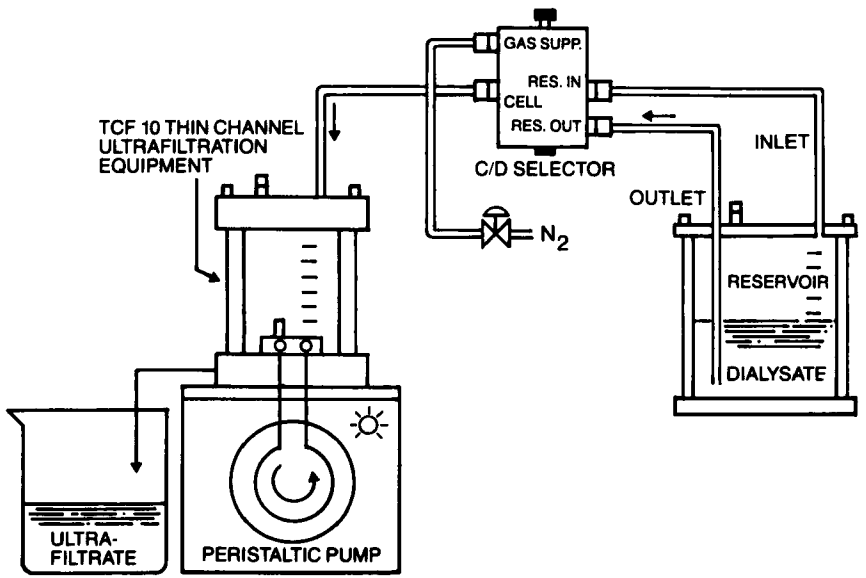

FIG. 1. Ultrafiltration set-up for the fractionation of peptides in the diafiltration mode.

the TCF10 cell was removed after each diafiltration volume (total diafiltration volumes = 4). Samples were stored at −20°C for later analysis by high performance liquid chromatography (HPLC).

The retention factor k was determined graphically from the slope of a semilog plot of % solute remaining versus the number of volumes exchanged:

$$\ln (C/C_0) = -k_s(V/V_0) \tag{1}$$

with

$$k_s = \text{sieving coefficient} = 1 - k$$

or

$$k = \frac{\ln (C/C_0) + (V/V_0)}{(V/V_0)} \tag{2}$$

Membranes

Twenty commercial membranes, with NMWCO between 500 and 8000 daltons, and representing eight polymer materials, were tested. All membranes used were 90 mm diameter disks (effective membrane area = 40 cm^2), either purchased directly from the manufacturers or cut from a flat sheet. Table 1 summarizes membrane characteristics, manufacturers, and pure water permeabilities L_p as measured on the TCF10 system. Aliphatic alcohol membranes were from Zenon Environmental Inc., Burlington, Ontario, Canada. Cellulose acetate membranes came from DDS (Nakskov, Denmark), Osmonics Inc. (Minnetonka, Minnesota), Amicon Corp. (Danvers, Massachusetts), and Millipore Corp. (Bedford, Massachusetts). Regenerated cellulose disks were from Amicon and Spectrum Medical Industries (Los Angeles, California). The polyether sulfone membrane was kindly provided by Dr. C. Bouchard (Department of Chemical Engineering, Ecole Polytechnique de Montréal, Canada), and modified polysulfone flat sheets (proprietary modification) came from Bio-Recovery Inc., Northvale, New Jersey.

Before each experiment, membranes were soaked in three changes of ultrapure water for 1 h. Fouling ratio was used as a convenient indication of membrane fouling. It is defined as the ratio of pure water flux after sample and a single rinse to the pure water flux before sample

(determined with an unused membrane). Absence of fouling is indicated by a ratio of 1.

The cleanability of the used filters was assessed by soaking them in an enzymatic detergent solution (0.5% P3-ultrasil 53, Henkel) for 15 h at 20°C, and then rinsing them repeatedly with ultrapure water.

HPLC Analysis

Chromatography was performed on a Waters Associates HPLC system consisting of two M510 solvent delivery pumps, a M712 intelligent sample processor with a refrigeration unit maintained at 4°C, a temperature control module, an M490 programmable multiwave-length detector, and an M840 data and chromatography control station (Waters Associates, Bedford, Massachusetts).

The analytical column was a reverse-phase, 300 Å Vydac TP C_{18} (10 μm, 25 cm × 4.6 mm i.d.), from The Separations Group, Hesperia, California.

Acetonitrile (Caledon) and trifluoroacetic acid (TFA) (Pierce Chemical Co.) were HPLC grade. Ultrapure water was used in all the experiments.

The flow rate was 1.5 mL/min (900–1000 psi) and the column temperature was 30°C. The primary solvent (A) was 0.1% TFA/H_2O and the secondary solvent (B) 0.1% TFA/acetonitrile. The solvents were filtered on a 0.45-μm Ultipor N_{66}^{TM} nylon filter (Pall Trinity Micro Corp.) prior to use. The aqueous solvent was also degassed and sonicated in an ultrasonic cleaning bath. Samples of 15 μL were injected and eluted with a linear gradient from 25 to 45% B over 45 min. The absorbance of the effluent at 215 nm was monitored with a sensitivity of 0.8 AUFS. Because the cytochrome c peak was short and broad, automatic integration was not always satisfactory. The chromatograms were reprocessed and manually integrated using the M840 data station to correct any discrepancies.

RESULTS AND DISCUSSION

Individual membranes may vary in their characterization within a batch, and slight batch-to-batch variations are common for polymeric membranes. All experiments were duplicated, using a new membrane each time, and surprisingly satisfactory reproducibility was noticed for each membrane, provided that ultrapure water was the only agent utilized.

Pure water permeability L_p was determined individually for each membrane after 30 min of continuous flushing (Table 1). Flux rate is linearly dependent on the applied pressure across the membrane up to a pressure of 40 psig (maximum pressure allowed on the device). There was no clear correlation between NMWCO and L_p values, since all permeability values were in the same range. However, major discrepancies were observed with the Teflon and fluoropolymer membranes. The FS81PP membrane from DDS had an L_p of 419 $L \cdot h^{-1} \cdot m^{-2} \cdot atm^{-1}$, eight times the flux expected for cellulosic membranes of similar NMWCO. Retention factors will have to be determined for the Teflon and fluoropolymer membranes to verify whether this high flux induces significant losses of the various peptides studied.

Membrane Fouling

Fouling ratios are also indicated in Table 1. Although an incomplete estimate of fouling, this ratio is a practical way to evaluate the extent of peptide adsorption. There is a clear interest in such an estimate. Indeed, the loss of solute due to adsorption could be negligible in the case of high solute concentrations, but remains important when the solute is an expensive compound at low concentration.

Cellulosic membranes show excellent behavior with a ratio close to 1 in all cases, signifying virtual absence of fouling. A ratio over 1.0 could be caused by some degradation of the cellulose acetate material (operating pH 2.25). The same disk should be utilized repeatedly to confirm any variation in the sieving characteristics of the membrane. Noncellulosic materials show consistency in their fouling ratios, most of them being in the 40–60% range. Adsorption of proteins to hydrophobic hemofiltration membranes has been reported to alter their solute rejection spectrum. While no alteration was noticed for cellulosic filters following exposure to plasma proteins, a significant decrease in the apparent NMWCO was evident in the case of polysulfone, polyacrylonitrile, and polyamide membranes (*17*). Full exploitation of the membrane surface sieving properties is thus only possible with highly hydrophilic polymers.

Permeate Flux

The filtration rates were measured throughout the diafiltration process at the standard operating conditions indicated in Table 1, always with

freshly prepared feed solutions. The flux remained remarkably stable for all tested disks throughout the four volume diafiltration, except once again for fluoropolymer–Teflon membranes, where up to a 50% drop in flux was observed. The measured pure water flux rates and filtration rates with sample are summarized in Table 1. In parallel to the L_p values, the flux rates were all in the 10–100 $L \cdot h^{-1} \cdot m^{-2}$ range, except for the fluoropolymer and Teflon membranes. Concentration polarization was quite low on cellulosic membranes, as indicated by the flux rates with sample compared to the water flux rates before sample. In the case of noncellulosic membranes, the decrease in flux rate is due to irreversible adsorption rather than polarization, since pure water flux rates after sample are essentially similar to those measured in the presence of sample. Above a certain threshold, flux rates are not the most important factor in the selection of a membrane, due to the high added-value of peptidic compounds. These flux rates did not seem to be related to the hydrophilicity of the membranes.

Cleanability

Membranes were cleaned by overnight incubation in an enzymatic detergent, then rinsed three times with ultrapure water and visually examined to detect any damage or staining. Comments and conclusions are indicated in Table 1 for each particular membrane.

Cellulosic membranes were all easy to clean, and no staining occurred. Flux rates were systematically restored to their original value, except for the DDS CA800PP membrane and the Millipore PCAC 1,000 where they were doubled. A similar effect was noticed for the modified polysulfone Bio-Recovery 3,000 membrane. Most probably, wetting effects were responsible for such an increase in the permeate flux, as already reported (*18*). The Amicon YM2 membrane was pierced several times, although no similar damage was detected with the YM5 membrane from the same manufacturer nor with the other cellulosic membranes. Additional testing is being conducted to confirm the fragility of this membrane at these operating conditions.

For all the other polymeric materials, membranes were either stained with cytochrome c and/or difficult to clean. Using the TCF10 device along with the cleaning solution did not improve the flux. The fluoropolymer active layer detached from its mechanical support. The three Bio-Recovery modified polysulfone membranes behaved surprisingly differently from each other. Though slight staining was noticed on 1000

and 3000 NMWCO membranes, the 5000 NMWCO disk could be easily restored to its original state. Further experiments are underway to elucidate these differences. 5% acetic acid may cause irreversible enlargement of the pores, and more work will be conducted to evaluate the evolution of the flux rates and retention coefficients from run to run.

Separation of Peptide b, Insulin, and Cytochrome c

The retention factor k was graphically determined for each peptide on each individual membrane (Table 2). Separation profiles for the most

TABLE 2
Comparison of Retention Coefficients k for Peptide b (MW 844), Insulin (MW 5730), and Cytochrome c (MW 12,384) Obtained with UF Membranes. k Values Were Determined Graphically from the Slope of a Semilog Plot According to Eq. (2)

Polymer	Membrane	NMWCO[a] (daltons)	k[b]		
			Peptide b	Insulin	Cytochrome c
Aliphatic alcohol	Zenon CV32TX	1000	41.5	86.6	100
Cellulose acetate	DDS CA865PP	RO(100–500)	42.4	91.6	100
	Osmonics SX01	500	62.6	96.6	100
	Amicon YC05	500	100.0	100.0	100
	Millipore PCAC	1000	95.4	97.4	100
	Osmonics SG15	1000	0	83.0	100
	DDS CA800PP	8000	0	11.4	>98[c]
Cellulose (regenerated)	Amicon YM2	1000	57.0	92.2	100
	SMI Type C	1000	62.6	94.8	100
	Amicon YM5	5000	8.0	91.5	100
	SMI Type C	5000	0	94.1	100
Fluoropolymer	DDS FS81PP	6000	0	74.5	>98[c]
Polyether sulfone	EPM PES	2000	65.4	93.9	100
Polysulfone (sulfonated)	DDS GB90PP	2000	42.7	94.0	100
	DDS GR81PP	6000	55.9	97.4	100
Polysulfone (modified)	Bio-Rec. 01K-PSB03	1000	52.0	96.7	100
	Bio-Rec. 03K-PSB03	3000	42.0	96.5	100
	Bio-Rec. 05K-PSB03	5000	23.2	87.6	100
Teflon	Osmonics AP01	1000	17.8	65.0	>98[c]
	Osmonics AP02	3000	0	56.5	>98[c]

[a]As given by the membrane manufacturers.
[b]Operating conditions same as in Table 1.
[c]Permeate was colored, although HPLC could not detect a concentration drop.

successful cellulosic membranes are shown in Fig. 2. The dashed line in each panel represents the theoretical elimination curve for species or solvent not retained by the membrane ($k = 0$). In all cases except the DDS CA800PP (8000 NMWCO), Teflon, and fluoropolymer membranes which leaked traces of cytochrome c, retention of cytochrome c was virtually complete.

We were pleased to note that the fractionation of the model peptides was efficiently performed in several cases (principally cellulosic membranes) and that good agreement was found between experimentally determined and rated MWCO. The plots were all linear, probably due to the absence of larger proteins like BSA and relative purity of the solutions.

Peptide b and insulin could be fractionated from each other on the Osmonics SG15 membrane (NMWCO = 1000), the Amicon YM5 (NMWCO = 5000), the SMI type C 5,000 (NMWCO = 5000), and the Bio-Recovery 5,000 (NMWCO = 5000) with satisfactory yields.

Only the membrane listed at 8000 NMWCO (DDS CA800PP) separated insulin from cytochrome c with almost complete exlusion of cytochrome c (98–100%) and 11.4% retention of insulin. Other commercial cellulosic membranes of similar NMWCO could probably also perform this fractionation.

CONCLUSIONS

Membranes were generally able to withstand the low operating pH (2.25), although this value is close to the lower pH limit recommended by the manufacturers in the case of cellulosic membranes. The cellulosic membranes proved to be successful and reliable. Modified polysulfone membranes were satisfactory in terms of separation efficiency but were fouled as a result of significant peptide adsorption. Aliphatic alcohol, polyether sulfone, fluoropolymer, and Teflon membranes were all heavily fouled and stained and could not be cleaned with an enzymatic detergent. Future work includes fractionation of peptide hormones more closely related in size, and establishment of the operating lifetime of the cellulosic membranes in 5% acetic acid.

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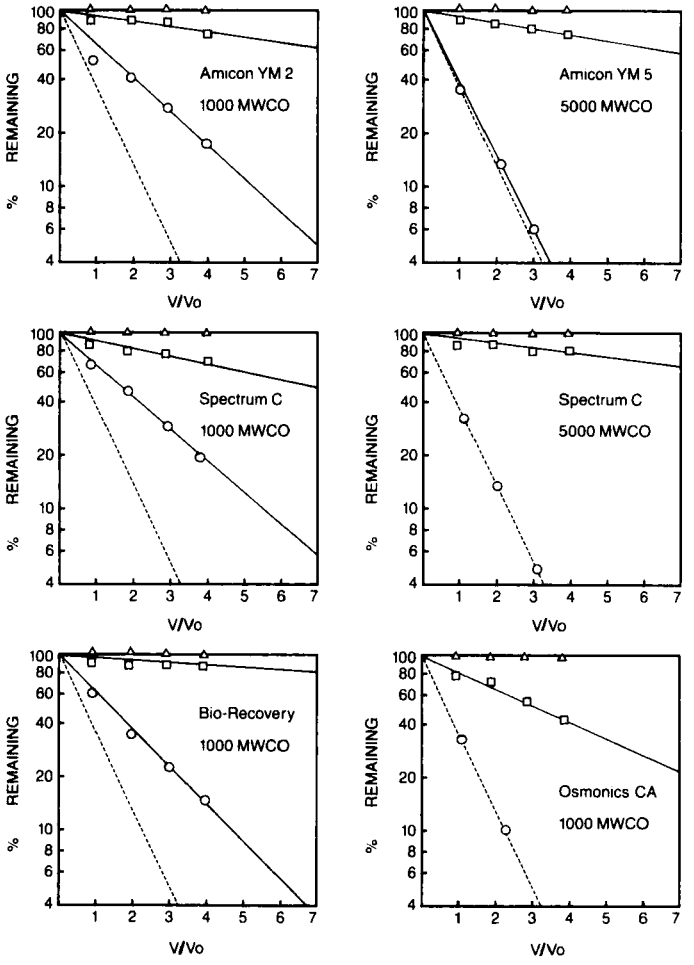

FIG. 2. Separation profiles of peptide b (○), insulin (□), and cytochrome c (△) by cellulosic membranes in the diafiltration mode.

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