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COMMUNICATION

Coupling Electrophoresis with Ultrafiltration for Improved Processing of Plasma Proteins

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Abstract

The feasibility of coupling electrophoresis with ultrafiltration (electroultrafiltration) in order to increase the flux and improve the selectivity of the ultrafiltration process was demonstrated experimentally for protein solutions. An electric field controlled the build-up of retained proteins at the membrane surface. Electroultrafiltration fluxes were 3 to 5 times greater than normal ultrafiltration fluxes. Retention and separation factors were also higher. The effects of important process variables were studied.

INTRODUCTION

Membrane processes such as ultrafiltration are being used on an ever-increasing scale to concentrate and purify biological materials. Ultrafiltration (UF) has become the method of choice for concentrating proteins and removing salt, alcohol, and other low molecular weight solutes (*1–4*), but it has seen only limited use in separating the protein components of blood plasma (*5*, *6*).

The efficiency of UF is limited by concentration polarization, the build-up of retained molecules at the membrane surface. Concentration polarization decreases membrane selectivity and flux, causing UF to compare poorly to other protein separation techniques (*7*). Protein fractionation by UF is possible only at low concentrations even when the molecules differ in molecular weight by an order of magnitude (*8*).

Concentration polarization in protein solutions has been modeled extensively. At steady state, the pressure-driven transport of retained proteins with the solvent toward the membrane is balanced by the reverse diffusive transport away from the high protein concentration at the

membrane into the bulk stream. Under these conditions the volumetric flux through the membrane, J, is independent of the membrane pressure drop. The membrane flux can be increased by adjusting the membrane geometry and fluid flow conditions (*9*).

The most widely used membrane systems for concentrating protein solutions or removing microsolutes are thin-channel, laminar-flow recirculating systems and hollow fiber modules. However, for separating protein mixtures such systems are not practical because the retained protein layer becomes impermeable or partially permeable to solutes that would normally pass through the membrane (*10*).

It would be ideal to prevent the formation of the retained protein layer or to remove it often enough to limit its deleterious effects on flux and selectivity. In electroultrafiltration (EUF), an electric field is used to pull the retained proteins in a direction opposite to the pressure-driven membrane flow. By proper adjustment of pH and field strength, protein layer formation may be minimized. Separation of the proteins is then controlled by the membrane, which discriminates on the basis of size and shape, and the electric field, which controls the number of molecules of a given charge and mobility that reach the membrane. Maintenance of a clean surface by electrophoresis has been demonstrated in plasmapheresis (*11*) and in electrofiltration of clay–water solutions (*12*).

MATERIALS AND METHODS

Electroultrafiltration

A continuous flow, parallel plate, Plexiglas (UV transmitting type) cell was constructed for studying ultrafiltration with and without an electric field (Fig. 1). The electrode compartments were separated from the ultrafiltration compartments by impermeable cellophane membranes to prevent contamination of the protein solutions by electrolysis products and to prevent coating of the electrodes by the migrating proteins. These electrode compartments contained a circulating buffer solution which was compatible with the buffered, protein solution. Heat generated during EUF was removed by cooling the circulating protein and buffer solutions. The ultrafiltrate and retentate were recycled to the feed to maintain constant concentrations. Low Reynolds numbers (<40) guaranteed laminar flow in the retentate compartment.

Membranes

Amicon Diaflo ultrafiltration membranes, XM-50, XM-100A, and

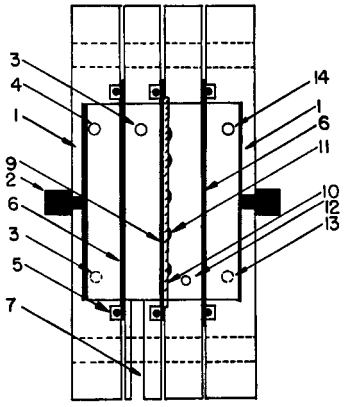

FIG. 1. Cross section of electroultrafiltration cell: (1) electrodes, (2) electrode binding post, (3) high-pressure buffer inlet, (4) high-pressure buffer outlet, (5) O-ring, (6) dialysis membrane, (7) high-pressure protein solution inlet, (8) high-pressure retentate outlet, (9) ultrafiltration membrane, (10) membrane support, (11) membrane support grid, (12) low-pressure ultrafiltrate outlet, (13) low-pressure buffer inlet, (14) low-pressure buffer outlet.

XM-300, with nominal cutoffs of 50,000, 100,000, and 300,000 molecular weight, respectively, were used. Membranes were used only once.

Protein Solutions and Concentrations

The proteins were dissolved in phosphate or acetate buffer solutions with minimal agitation. The pH range was 4.7 to 8.0. Bovine albumin (BSA), γ-globulin (BγG), and fibrinogen were obtained from Sigma Chemical (St. Louis, catalog numbers A-4503, BG-11, and F-4753). ^{125}I-labeled albumin was purchased from Mallinckrodt Nuclear (St. Louis, catalog number 350). ^{131}I-labeled bovine γ-globulin was prepared from labeled sodium iodide in 0.1 N NaOH obtained from Industrial Nuclear Corp. (St. Louis) (*13*).

Concentration in single-protein solutions was measured by ultraviolet light absorption (280 nm). Binary solutions were prepared with ^{125}I-labeled albumin and ^{131}I-labeled γ-globulin. This permitted concentration measurement by scintillation counting.

RESULTS

Single Proteins: Process Variables

The electric field strength, E, was the most important variable affecting

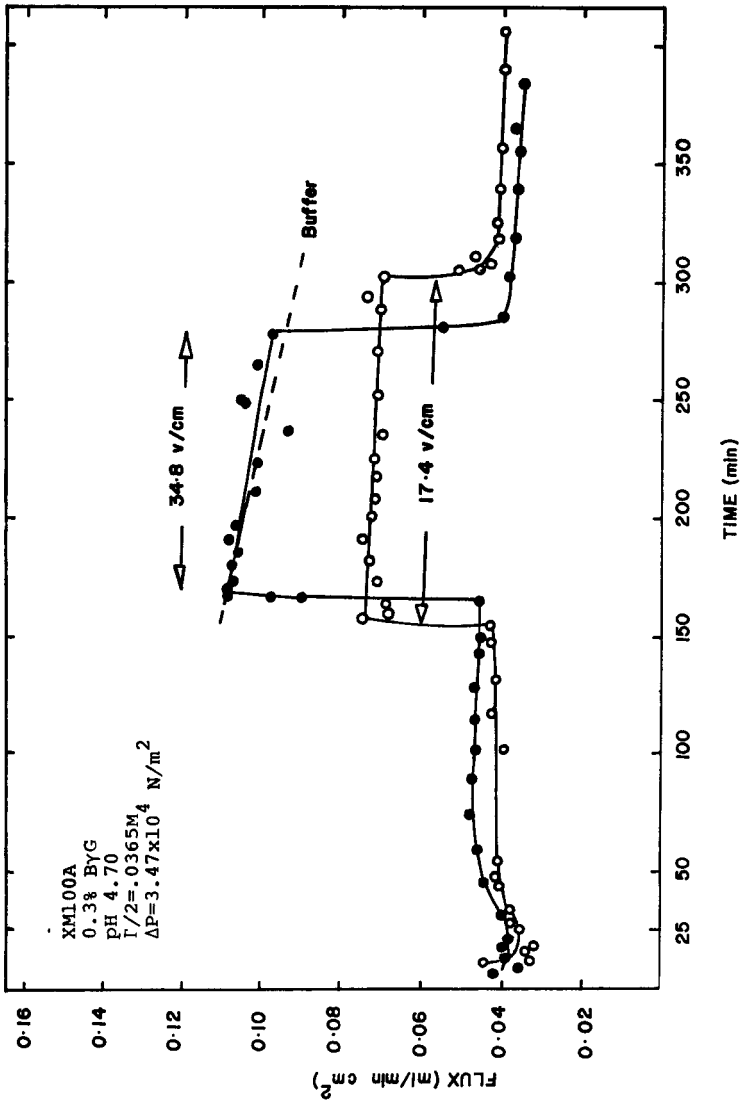

FIG. 2. Effect of electric field strength on flux of 0.3% ByG.

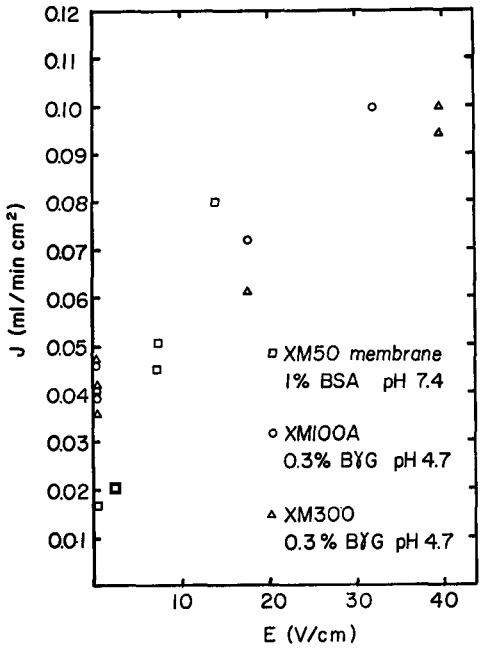

FIG. 3. Effect of electric field strength on flux of protein solutions.

the flux as illustrated in Fig. 2. The dependence of flux, J, on electric field strength is shown in Fig. 3. At steady state, the volumetric flux was proportional to the applied electric field strength. During EUF of protein solutions, transmembrane pressure drop, pH, and ionic strength ($\Gamma/2$) were of secondary importance [see Radovich (*13*) for details]. The flux was no longer independent of ΔP as in normal ultrafiltration. Changing pH and $\Gamma/2$ affected flux because they affected the protein's electrophoretic mobility.

Single Proteins: Retention

Retention was calculated as

$$R = 1 - \frac{\text{concentration in ultrafiltrate}}{\text{concentration in retentate}}$$

The retention of charged protein always increased when the electric field was applied to move it away from the membrane. At pH 7.4 and $E = 8.5$ V/cm, BSA retention increased 9 to 14% for XM-50 membranes. At pH = 4.7 and $E = 17.4$ and 39.2 V/cm, BγG retention increased 3 and 6% for XM-100A and XM-300 membranes, respectively.

Separation of BSA and BγG Mixtures

A solution of 1.0 wt-% BSA and 0.3 wt-% BγG was ultrafiltered with and without the electric field. For ideal BSA–BγG separation, the build-up of a layer of retained BγG must be prevented while the BSA is allowed to pass through the membrane. Tests were done at pH 4.7 (BγG has a net positive charge and BSA is at its isoelectric point) and at pH 8 (BSA and BγG both have a net negative charge). The flux for any electric field strength at pH 8 is greater than at pH 4.7 because both molecules were transported away from the membrane. However, the goals in fractionation are high flux plus good separation.

The separation factor, α, is defined as $(1 - R_{BSA})/(1 - R_{B\gamma G})$. The measured separation factors and the calculated retention coefficients are listed in Table 1. At pH 8 the retention for both proteins is high and increases when the electric field is on. For the XM-300 membrane at pH 4.7 the field holds back BγG while uncharged BSA is forced through the membrane. This leads to a 2- to 6-fold increase in α.

DISCUSSION

In EUF an electrophoretic force is used to counter the pressure-driven convection of retained proteins, preventing their build-up on the membrane surface. The field increased the flux for single protein solutions

TABLE 1

Protein Retention and Separation Factors (α) in Mixtures of BSA and BγG

Membrane	pH	E (V/cm)	R_{BSA}	$R_{B\gamma G}$	α
XM-300	4.72	0	0.370	0.792	3.03
		39.15	0.487	0.975	20.52
XM-300	4.70	0	0.569	0.900	4.31
		39.15	0.543	0.946	8.46
XM-300	4.70	0	0.641	0.927	4.92
		39.45	0.59	0.976	17.08
XM-300	8.18	0	0.923	0.887	0.68
		30.45	0.984	0.972	0.57
XM-100A	8.00	0	0.964	0.937	0.57
		30.45	0.987	0.987	1.0
XM-100A	8.08	25.9	0.976	0.972	0.86
		0	0.970	0.963	0.81
Amicon XM-100A[a]			0.45	0.90	5.50[b]
Amicon XM-300[a]			0.10	0.65	2.57[b]

[a]Catalog values for ultrafiltering *single* protein solutions.

[b]*Calculated* from single-solute retention values.

(albumin, γ-globulin, fibrinogen) and increased flux and selectivity for separating a binary solution (albumin + γ-globulin).

In general, the effect of the electric field on proteins will depend on their electrophoretic mobility and the pH of the solution. The differences in the amphoteric nature of proteins will permit the control of concentration polarization and improve separation. However, it may not be possible or economical for EUF to separate homogeneous protein fractions from plasma in a series of single steps. The practical uses of EUF may be to increase the efficiency of microsolute removal and protein-solution concentration, and perhaps to provide subfractionation of crude blood plasma fractions obtained by conventional techniques.

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