Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Concentration Ultrafiltration and Diafiltration of Albumin with an Electric Field

John M. Radovich[a]; Bahman Behnam[a]
[a] SCHOOL OF CHEMICAL ENGINEERING AND MATERIALS SCIENCE, UNIVERSITY OF OKLAHOMA, NORMAN, OKLAHOMA

To cite this Article Radovich, John M. and Behnam, Bahman(1983) 'Concentration Ultrafiltration and Diafiltration of Albumin with an Electric Field', Separation Science and Technology, 18: 3, 215 — 222
To link to this Article: DOI: 10.1080/01496398308058332
URL: http://dx.doi.org/10.1080/01496398308058332

SEPARATION SCIENCE AND TECHNOLOGY, 18(3), pp. 215–222, 1983

Concentration Ultrafiltration and Diafiltration of Albumin with an Electric Field

JOHN M. RADOVICH and BAHMAN BEHNAM

SCHOOL OF CHEMICAL ENGINEERING AND MATERIALS SCIENCE
UNIVERSITY OF OKLAHOMA
NORMAN, OKLAHOMA 73019

Abstract

The time required to concentrate various starting solutions of bovine serum albumin by ultrafiltration was compared to that by ultrafiltration with an electric field—electroultrafiltration. Diafiltration of bovine serum albumin solutions was also compared to diafiltration with an electric field. In both cases the electric field improved the performance of the ultrafiltration or diafiltration process by increasing the flux through the membrane.

INTRODUCTION

Ultrafiltration is being used extensively for concentrating protein solutions and for microsolute, e.g., salts, removal, or exchange. Over 15 years ago, Blatt et al. (*1*) described a membrane which gave superior flux and protein recovery compared to Visking tubing. Friedli and co-workers (*2, 3*) evaluated hollow-fiber and thin-channel ultrafiltration systems for concentrating albumin solutions. Studies of concentrating albumin (up to 25 wt% protein), γ-globulin, and antihemophilic factor were also completed by Nelsen (*4*). An empirical equation for predicting the membrane flux as a function of fluid velocity and protein concentration was developed by Mitra and Lundblad (*5*) for ultrafiltering human serum albumin and immune serum globulin solutions. Guthohrlein (*6*) compared the efficiency of commercially available ultrafiltration apparatus and found that the choice depended on the feed and process concentration or microsolute removal.

Diafiltration (continuous ultrafiltration with replacement of the ultra-

 0149-6395/83/1803-0215$3.50/0

filtered solution) was initially developed for artificial kidney applications (*7–10*). The use of diafiltration for processing plasma protein solutions in hollow fiber modules was described by Blatt et al. (*11*) and Nelsen (*4*). Mercer (*12*) compared diafiltration and subsequent concentration with other solvent removal systems for plasma protein fractions while Ng et al. (*13*) evaluated the effects of thin-channel ultrafiltration system operating variables on the removal of salt and ethanol from Cohn Fraction V.

A common processing problem in ultrafiltration is the build-up of retained solutes at the membrane surface—concentration polarization. The most widely used ultrafiltration systems for the aforementioned applications minimize concentration polarization by controlling the fluid flow pattern, e.g., thin-channel recirculation and hollow fibers. This increases the rate of diffusive back-transport of retained solute from the membrane into the bulk solution (*14, 15*). We have developed a method which combines electrophoresis and ultrafiltration which eliminates the effects of concentration polarization layer (*16–18*). In electroultrafiltration (EUF), an applied electric field perpendicular to the tangential fluid flow across the membrane acts opposite to the transmembrane pressure drop. The retained proteins are pulled away from the membrane by the electric field. Previous papers (*17, 19*) described the application of EUF to the processing of plasma proteins and other macromolecules at constant concentration. Herein, we will discuss the use of EUF for concentration and diafiltration of bovine serum albumin solutions. In many of the plasma fractionation procedures involving precipitation, the final step for preparation of a therapeutically useful plasma protein solution requires removal of salt and precipitating solvent and the concentration of the plasma protein. The use of EUF could lead to improving the efficiency of these final processing steps. Since the preparation of human serum albumin is the largest and most economically important application of these methods, we chose bovine serum albumin as a model compound because it has properties similar to human albumin and is readily available.

MATERIALS AND METHODS

Electroultrafiltration

The Plexiglas ultrafiltration cells were similar in construction to the parallel plate cell described previously (*17*). The cells in these studies, however, had circular membranes (15.1 cm^2 area) and a distance of 2.3 cm between the electrodes. Dupont 215PD-62 cellophane separated the circulating buffer in the electrode compartments from the protein solutions.

The buffer and protein solutions were cooled as necessary to remove any heat generated during EUF. Nitrogen was used to pressurize the system for the ultrafiltration driving force. The applied voltage during electroultrafiltration was measured between the electrodes and across the retentate compartment.

Amicon Diaflo ultrafiltration XM-50 membranes were used. The external flow system and reservoirs were used for normal ultrafiltration/concentration operation and switched to the diafiltration mode by adding another reservoir and an Amicon CDS-10 switch in the flow circuit.

Bovine serum albumin (BSA) obtained from Sigma Chemical Co. (St. Louis, Catalogue number A-4503) was dissolved in sodium phosphate buffer (pH 7.4 ionic strength of 0.05 *M*) for all experiments. The protein solution flowed through the cell at 162 mL/min and the transmembrane pressure drop was 5.0 psia.

Protein concentration was determined by ultraviolet light absorption at 280 nm. A standardization curve of absorbance values of bovine serum albumin solutions of known concentration was prepared. A Hitachi Model 19 digital spectrophotometer was used. Changes in salt concentration were detected by measuring the pH and conductivity of the process solution.

RESULTS AND DISCUSSION

Concentration of Bovine Serum Albumin (BSA) Solutions

The changes in concentration with time for an initial 500 mL, 1 wt% solution of BSA are shown in Fig. 1 for ultrafiltration and electroultrafiltration. As the applied electric field E is increased up to about 11 v/cm, the time required to increase the concentration by a given factor decreases drastically (2–4 times less) compared to ultrafiltration (note that the elapsed time axis is logarithmic). This is due to a higher flux for electroultrafiltration during the concentration operation as seen in Fig. 2. At a given applied electric field strength (4 v/cm) the increase in concentration is more rapid for an initial concentration of 1 wt% albumin versus 2 wt% as seen in Fig. 3. This trend is the same as expected for normal ultrafiltration (*20*). We are currently working on a mathematical model to predict this electroultrafiltration behavior during concentration operation.

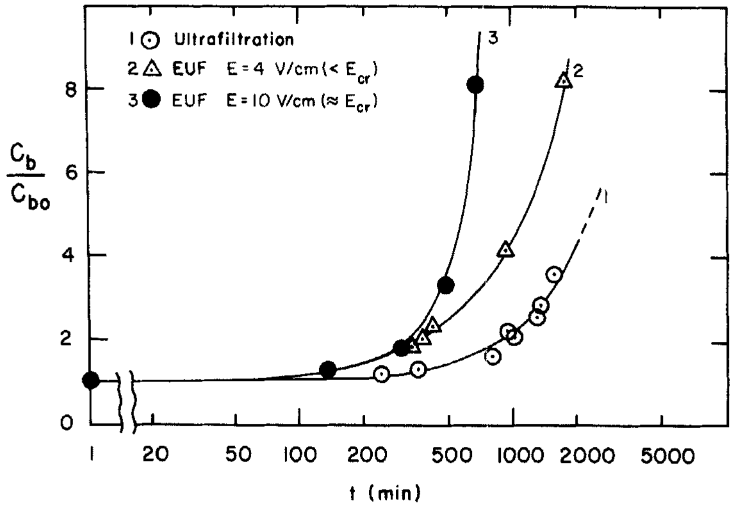

FIG. 1. Enhancement of bulk concentration (C_b) for ultrafiltration and EUF ($C_{bo} = 1\%$ wt BSA).

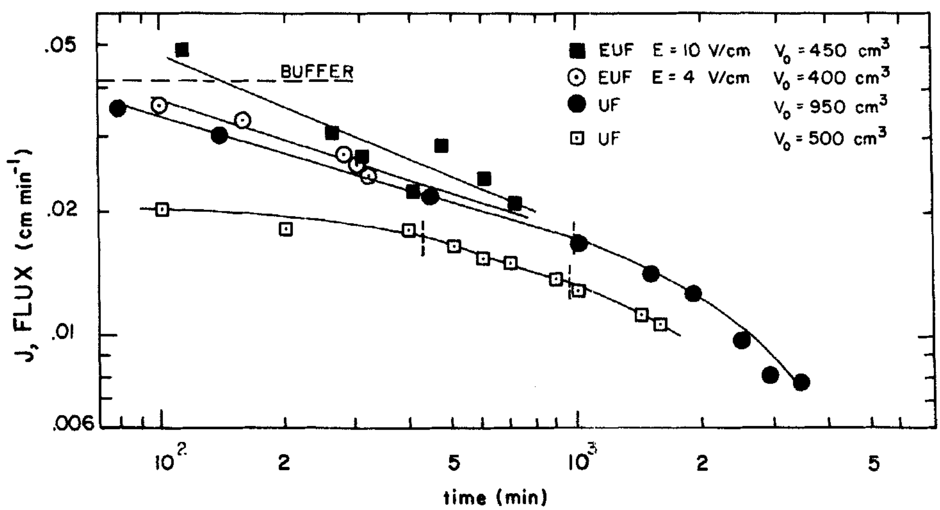

FIG. 2. Flux vs time for concentrating albumin solutions by ultrafiltration (UF) and electro-ultrafiltration (EUF).

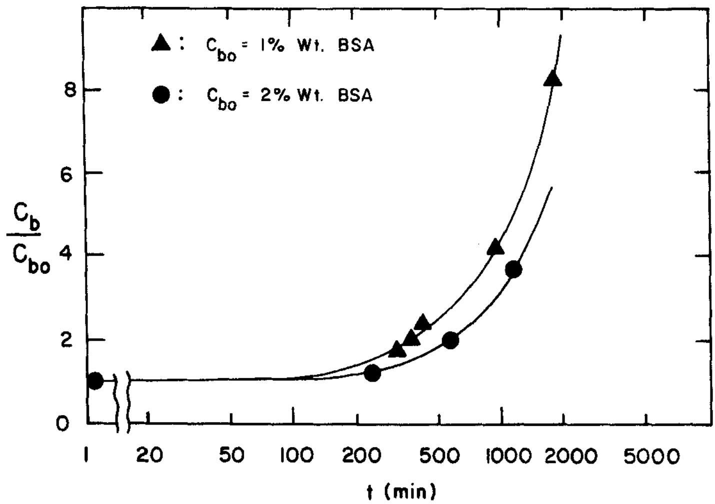

FIG. 3. Enhancement of bulk albumin concentration (C_b) for EUF at different initial concentrations (C_{bo}).

Diafiltration of BSA Solutions

Both wash-in and wash-out procedures were done simultaneously for the diafiltration and electodiafiltration process. The starting solution contained 1 wt% BSA plus 0.16 *M* NaCl in a 0.5 *M* sodium phosphate buffer at pH 6.25. The reservoir contained pH 8.10 sodium phosphate buffer. As the diafiltration or electrodiafiltration proceeded, the NaCl was washed out of the protein solution while the pH increased from 6.25 to 8.10 as the new buffer was washed-in.

One comparison of electrodiafiltration (at 16.9 v/cm) and diafiltration is shown in Fig. 4. These experimental curves show that the electric field decreases the time (by 13 to 21%) needed to wash out the sodium chloride (as measured by the changes in the conductance of the solution). The difference in time can be determined by comparing the elapsed time (x value) to reach a given % of original value (y axis). The amount of time saved is also shown in Fig. 5. The accuracy of the results in Fig. 4 depend on the measurement of total solution flux and the measurement of the conductance. The flux measurements, which are average values for the time intervals, are accurate to within ±1%. The fluxes are compared in Fig. 5, which shows about a 50% increase in flux during electrodiafiltration versus diafiltration. The conductivity–salt concentration relationships are probably accurate to within ±5%. The increase in salt transport through the membrane (as

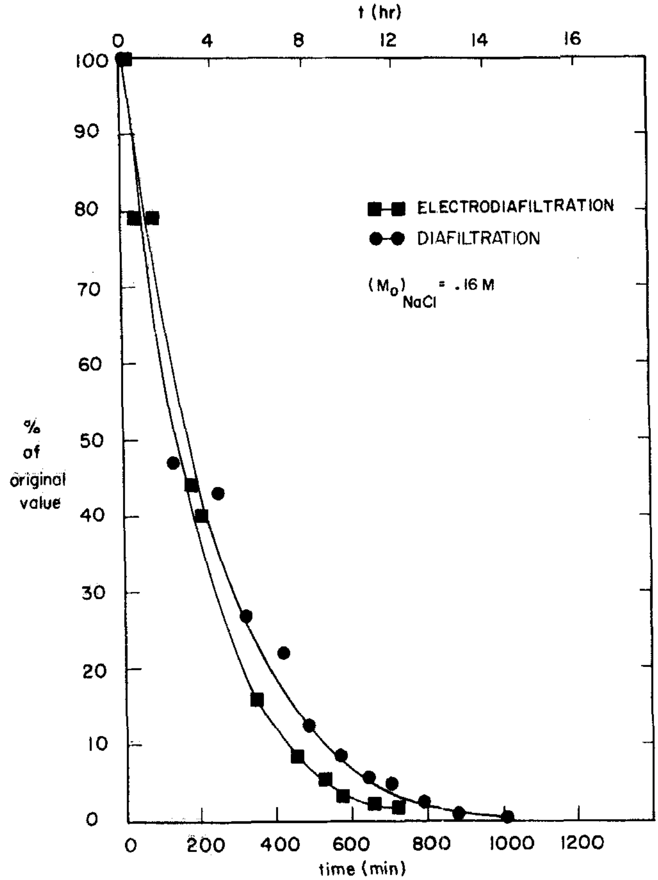

FIG. 4. Change in amount of salt in feed solution during electrodiafiltration and diafiltration.

indicated by a more rapid decrease in the solution conductivity) for electrodiafiltration is due to increased convective or bulk transport with the higher solution flux and possibly electrokinetic transport due to electro-osmosis or ionic migration in the electric field. We do not know the relative magnitudes of these transport terms for the salt (NaCl) in solution. We do know that the *total* solution flux increased. Figure 5 also shows the current and voltage drop in the retentate compartment during electrodiafiltration. The total voltage across the cell was held constant, but the change in conductance of the solution in the retentate (feed) compartment caused a decrease in current and consequently an increase in compartment voltage drop as the cumulative volume of ultrafiltrate increased.

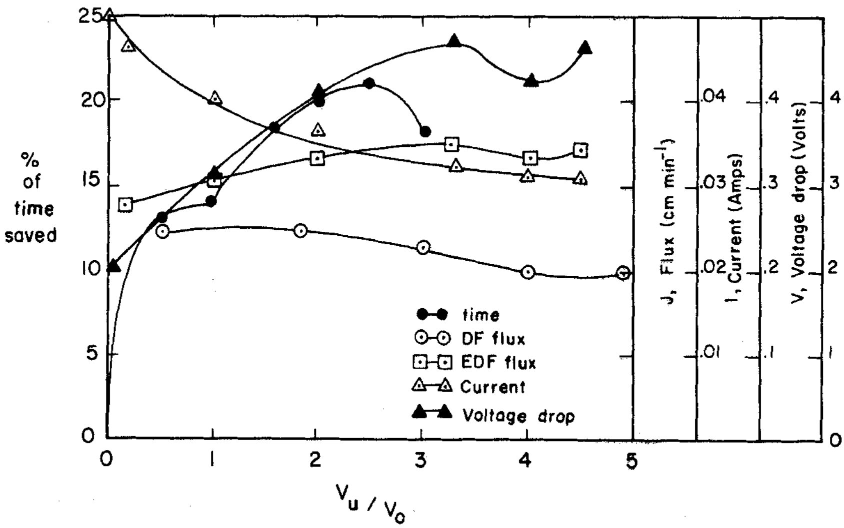

FIG. 5. Process variable comparisons during electrodiafiltration (EDF) and diafiltration (DF) as a function of cumulative ultrafiltrate volume (V_u) and initial solution volume ($V_0$3).

CONCLUSIONS

The improvement in the concentration process and diafiltration when an electric field is applied across the ultrafiltration cell appears to be due to the higher fluxes. Mathematical modeling of the electroultrafiltration concentrating process is continuing at this time. Additional work is required to determine the relative contributions of electrokinetic and convective transport of salt in the electrodiafiltration process.

REFERENCES

1. W. F. Blatt, M. P. Feinberg, H. B. Hopfenberg, and C. A. Saravis, *Science, 150,* 224 (1965).
2. H. Friedli, E. Fournier, T. Volk, and P. Kistler, *Vox Sang., 31,* 283 (1976).
3. Friedli, H., E. Fournier, T. Volk, and P. Kistler, *Ibid., 33,* 93 (1977).
4. L. Nelsen, in *Proceedings of the International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation* (H. E. Sandberg, ed.), DHEW Publication No. (NIH) 78-1422, 1977, pp. 133–145.
5. G. Mitra and J. L. Lundblad, *Sep. Sci. Technol., 13*(1), 89 (1978).
6. G. Guthohrlein, in *Proceedings of the International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation* (H. E. Sandberg, ed.), DHEW Publication No. 78-1422, 1977, pp. 146–150.

7. C. K. Colton, L. W. Henderson, C. A. Ford, and M. J. Lysoght, *J. Lab. Clin. Med., 85.* 355 (1965).
8. L. W. Henderson, A. Beserab, A. Michaels, and L. W. Bluemle, *Trans. Am. Soc. Artif. Intern. Organs, 13,* 216 (1967).
9. H. J. Bixler, L. M. Nelsen and L. W. Bleumle, *Ibid., 14,* 99 (1968).
10. E. Quillhorst, E. Fernandez, and F. Scheler, *Proc. Eur. Dial. Transplant Assoc., 9,* 584 (1972).
11. W. F. Blatt, L. Nelsen, E. M. Zipilivan, and M. C. Porter, *Sep. Sci., 7*(3), 281 (1972).
12. J. E. Mercer, in *Proceedings of the International Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation* (H. E. Sandberg, ed.), DHEW Publication No. (NIH) 78-1422, 1977, pp. 160–174.
13. P. K. Ng, G. Mitra, and J. L. Lundblad, *J. Pharm. Sci., 67*(3), 271 (1972).
14. W. F. Blatt, A. Dravid, A. S. Michaels, and L. Nelsen, in *Membrane Science and Technology* (J. E. Flinn, ed.), Plenum, New York, 1970, pp. 47–97.
15. W. F. Blatt, in *Membrane Separation Processes* (P. Meares, ed.), Elsevier, New York, 1976, pp. 81–120.
16. J. M. Radovich, "Electrophoretic Control of Concentration Polarization in the Ultrafiltration of Plasma Proteins," DSc Dissertation, Washington University, 1976.
17. J. M. Radovich, N. S. Mason, and R. E. Sparks, *Sep. Sci. Technol., 15*(8), 1491 (1980).
18. B. Behnam, "Combination of Electrophoresis and Ultrafiltration for Concentration and Purification of Plasma Proteins," MS Thesis, University of Oklahoma, 1980.
19. J. M. Radovich and R. E. Sparks, in *Ultrafiltration Membranes and Applications* (A. R. Cooper, ed.), Plenum, New York, 1980, pp. 249–268.
20. Amicon Corp., *Technical Data Publication No. 429B,* 1976.

Received by editor December 4, 1981
Revised March 15, 1982