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NOTE

On the Interpretation of Some Field-Flow Fractionation Experiments

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In a recent article (*1*), Kesner et al. reported data on protein separations in an electrical field-flow fractionation (EFFF) column where the channel walls are formed by stretched membranes. In their experiments conducted at a pH of 4.5, they found retention to be generally better than expected. Based on the formation of an uneven ion distribution on either side of the membranes, they have postulated some explanations for this apparent deviation from theory. One consequence of such an ion buildup is to modify the electric field experienced by the proteins during their passage through the column. This could explain some of the differences between theory and experiments since their theoretical calculations assumed the field (obtained by dividing the potential drop between the electrodes by the distance between them) to be unaffected by the presence of the membranes. The consequence of a field modification, *to a first-order approximation*, is the multiplication of the idealized migration velocities for the various species by the same species-independent factor. This factor may, of course, vary with the field strength. We have shown in Ref. *2* that the crucial parameter characterizing FFF is a dimensionless group P defined by

$$P = bv/D \tag{1}$$

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TABLE 1
Literature Values of Electrophoretic Mobilities and Diffusion Coefficients Used in the Calculations

Protein[a]	Electrophoretic mobility $\left(\mu \times 10^5 \frac{cm^2}{V\text{-}sec}\right)$	Diffusivity $\left(D \times 10^5 \frac{cm^2}{sec}\right)$	Conditions
Albumin	1.3	5.9	0.1 *M* solution at pH 4.5; 0 to 4°C
Hemoglobin	4.9	8.2	0.1 *M* solution at pH 4.5; 0 to 4°C
Lysozyme	6.35	10.4	0.1 *M* solution at pH 4.5; 0 to 4°C
γ_1-Globulin	2.4	3.84	0.1 *M* solution at pH 4.5; temperature unspecified
γ_2-Globulin	3.0		

[a] The data for albumin, hemoglobin, and lysozyme are from Kesner's thesis (*3*), and the data for the γ-globulins are reported in Ref. *5*.

Here, b is the channel half depth (in EFFF, b = half the spacing between the membranes), v is the migration velocity for a given species, and D is its diffusivity. P is related to λ from nonequilibrium theory by $P = (2\lambda)^{-1}$. For a given field, one may determine the value of P_{expt} for each species from the data of Kesner et al. (*1*). Also, for each species P_{ideal} may be computed by using literature values of the mobilities and diffusivities (shown in Table 1) and the nominal field strength E (calculated by dividing the potential drop by the distance between the electrodes). If the field modification hypothesis is to have a *predictive* value, the ratio P_{expt}/P_{ideal} should be species-independent for a given nominal field strength even though it may conceivably vary with the value of the nominal field strength. The results of our calculations are plotted in Fig. 1. The data for the four different proteins show some degree of correlation with the nominal field strength even though there appears to be considerable scatter at high values of E. Some of the observed scatter may be caused by the following reasons:

(1) The charge buildup near the membranes may cause electroosmotic flow through the membranes into and out of the channel. Such flow would modify the migration velocities as an additive contribution. Furthermore, Kesner et al. (*1*) found that there appeared to be a net flow of solvent into the channel through the membranes

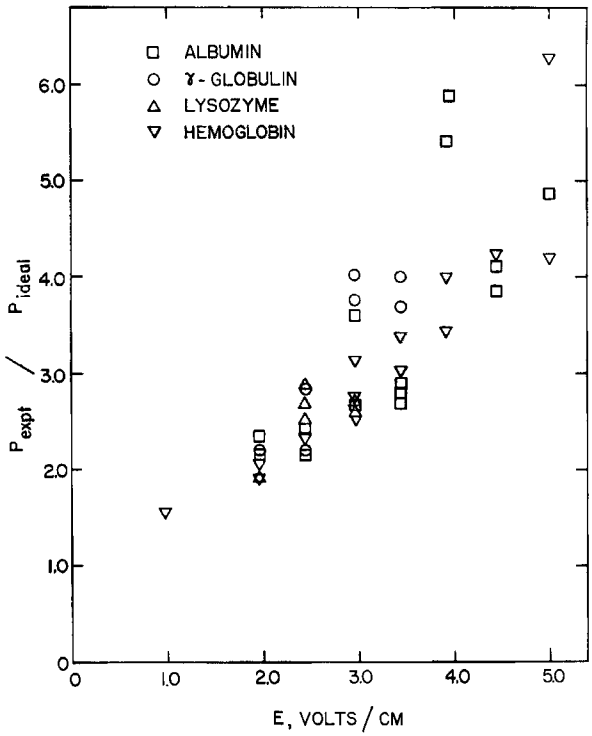

FIG. 1. The data of Kesner et al. for four proteins at pH = 4.5 replotted with P_{expt}/P_{ideal} against the nominal field strength E.

because of unequal electroosmotic flows into and out of the channel—this increase in flow was found to increase at higher field strengths. A proper accounting of this effect would be quite complex.

(2) The literature data are reported under conditions of pH, ionic strength, and temperature different from those of the experiments. Since all these factors influence the properties of interest, some deviations from predictions from literature data may be expected.

(3) There is a possibility of free convection currents, especially at the higher field strengths, due to possibly nonuniform power dissipation.

The utility of a hypothesis lies in its predictive value. Choosing hemo-

globin as a reference species, we computed the field modification factor at each nominal field strength and used this information along with literature data to predict the experimental P values for other species. Excellent agreement with data was obtained for albumin with moderate success in the case of the γ-globulins and lysozyme. It may be mentioned that most of the difficulties associated with Kesner's (*3*) flexible membrane channel have been eliminated by Giddings et al. (*4*) who used porous backing plates for supporting the membranes. These authors employed lysozyme as a reference species to estimate the actual field experienced by the proteins and obtained good agreement with theoretical predictions—a result which shows that field modification problems may be solved, at least in part, by the reference-species technique.

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REFERENCES

1. L. F. Kesner, K. D. Caldwell, M. N. Myers, and J. C. Giddings, *Anal. Chem.*, *48*, 1834 (1976).
2. S. Krishnamurthy and R. S. Subramanian, *Sep. Sci.*, *12*, 347 (1977).
3. L. F. Kesner, "Electrical Field Flow Fraction," Ph.D. Thesis in Chemistry, University of Utah, 1974.
4. J. C. Giddings, G-C. Lin, and M. N. Myers, *Sep. Sci.*, *11*, 553 (1976).
5. F. W. Putnam (ed.), *The Plasma Proteins*, Vol. 1, Academic, New York, 1960, p. 163.

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