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Experimental Investigation of Conditions of Transport in the Electrophoresis-convection Apparatus

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A study has been made of the conditions affecting operation of the electrophoresis-convection apparatus for fractionation of proteins. Systems containing a single protein have been investigated for the dependence of the time of transport upon the electric field strength and the mobility, initial concentration, and fraction of protein transported. The experimental results show satisfactory agreement with the theoretical treatment of the rate of transport. The characteristic times of transport correspond to effective field strengths bearing ratios of between unity and one-fourth to the nominal applied field strength.

Introduction

A systematic experimental investigation of the conditions determining the rate of transport of proteins by electrophoresis-convection is reported in the present article. The results demonstrate that the theory of transport may be used to obtain practical estimates of the time required to transport a specified fraction of a given protein from the top to bottom reservoir of the apparatus if a factor of safety between 2 and 4 is applied to the theoretical field strength.

The electrophoresis-convection apparatus as described by Cann, Kirkwood, Brown and Plescia[1a] consists of two reservoirs connected by a narrow, vertical, semi-permeable channel formed between two sheets of Visking Corporation sausage casing. The top of the upper reservoir is open so that the reservoirs and channel may be filled with a solution of the proteins to be fractionated. In operation the apparatus is filled and immersed in a suitable buffer solution between two flat platinum electrodes arranged to provide a homogeneous electric field across the channel upon the passage of a direct current between them. The field effects a horizontal transport of the protein components establishing horizontal density gradients in the channel, which, under the action of gravity, result in differential migration of the proteins into the lower reservoir. In the case of a single protein system the protein is simply concentrated in the bottom reservoir. External circulation of the buffer is maintained to prevent accumulation of electrolysis products.

Kirkwood and co-workers[2] have shown that for a system containing a single mobile protein the time, t, required to transport a fraction $1 - \gamma$ of mobile protein from the top reservoir is, for equal volumes, V, in top and bottom reservoirs

$$t = \theta I(\gamma)$$

where

$$I(\gamma) = (5/3)^{5/4} \int_0^{1-\gamma} \{(1 - y)^{-3/5} - (1 + y)^{-3/5}\}^{5/4}\, dy \qquad (1)$$

and

$$\theta = 2VD/hbl\mu^2E^2,\ h = (4\eta lD)/\alpha\rho g C^0)^{1/4}$$

The integral I, equal to t/θ, is presented as a function of $1 - \gamma$ in Table I. In these expressions, μ is the electrophoretic mobility; D, the diffusion constant; γ, the fraction of protein remaining in the top reservoir; and C^0, the initial concentration in grams per 100 ml. of the mobile component. ρ is the density and η, the viscosity of the solvent. b is the channel width; l, the channel length; g, the acceleration of gravity; E, the electric field strength; and $\alpha\rho$, the density increment produced by 1 g. of protein per 100 ml. of solution. This theory is derived under the conditions

$$\mu Ea/D << l, \qquad h/a << 1 \qquad (2)$$

where a is the channel thickness. The second condition was reasonably well satisfied in all the experiments, h/a ranging in value from 0.08 to 0.25. The first was realized approximately in a few cases. However, since under practical operating conditions $\mu Ea/D$ is greater than unity, many of the runs were made in violation of the first of Eq. (2). The theory for a system of a single protein component applies to two component systems if one component is immobilized by operating at its isoelectric point. This is the usual procedure in practice.

TABLE I

TIME OF TRANSPORT AS A FUNCTION OF FRACTION OF MOBILE COMPONENT TRANSPORTED

t/θ	$1 - \gamma$	t/θ	$1 - \gamma$
0.006	0.10	0.631	0.70
.029	.20	0.972	.80
.074	.30	1.57	.90
.145	.40	2.13	.95
.253	.50	3.21	.99
.407	.60	5.57	1.00

Experimental

Four proteins were used in this study: human carboxyhemoglobin, bovine serum albumin, ovalbumin and pepsin. The crystallized bovine plasma albumin and crystallized porcine pepsin were obtained from Armour and Company. The hemoglobin was prepared by the method of Drabkin,[3] and the ovalbumin by the method of Kekwick and Cannan.[4] The protein solutions were prepared and dialyzed for 24 hours against the appropriate buffer before being placed in the electrophoresis-convection cell. Several runs were made with each protein, varying the electric field strength, mobility, initial protein concentration, and in one case the dimensions of the apparatus, as indicated in Table II. The progress of the transport was followed by withdrawing small samples from the top reservoir during the runs and analyzing them for their protein content.

The fraction of protein remaining in the top reservoir, γ, was corrected for osmosis by taking for γ the ratio of the concentration in the top reservoir to the initial concentration multiplied by the ratio of the volume of the protein

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TABLE II

CHARACTERIZATION DATA FOR ELECTROPHORESIS-CONVECTION APPARATUS

Run	$\mu \times 10^5$	$D \times 10^7$	C^0, g./100 ml.	E, v./cm.	a, cm.	$\frac{\mu Ea}{D}$	θ, hr.	θ^*/θ	E^*/E
				Carboxyhemoglobin in phosphate buffer					
1	+2.09	4.56	2.10	0.090	0.14	0.56	1.9×10^3	3.9	0.51
2	0.83	4.71	2.82	.21	.15	.57	1.8×10^3	0.6	1.3
3	2.02	4.41	0.45	.12	.14	.78	6.4×10^2	17	0.24
4	0.83	4.71	2.79	.30	.15	.81	8.8×10^2	0.8	1.1
5	2.13	4.64	2.35	.12	.15	.84	8.1×10^2	0.9	1.1
6	2.19	4.71	2.82	.12	.15	.86	7.9×10^2	1.2	0.91
7	2.13	4.64	2.97	.24	.15	1.7	2.1×10^2	1.2	.91
8	2.02	4.41	2.81	.23	.16	1.7	2.9×10^2	1.5	.82
9	2.13	4.64	2.88	1.50	.14	9.8	6.8	5.1	.44
				Serum albumin in phosphate buffer					
10	−3.28	3.34	0.60	0.054	0.10	0.53	1.1×10^3	3.3	0.55
11	3.28	3.34	2.41	.054	.11	0.55	1.5×10^3	4.6	.47
12	4.46	3.70	2.31	.060	.19	1.4	6.6×10^2	6.6	.39
13	3.66	3.77	2.21	.085	.18	1.5	5.2×10^2	5.1	.44
14	4.33	3.60	0.58	.12	.19	2.7	1.1×10^2	11	.30
15	4.68	3.77	2.66	.12	.20	2.9	1.6×10^2	8.0	.35
16	4.33	3.60	2.21	.23	.14	3.8	5.4×10	7.6	.36
17	4.68	3.77	2.44	.24	.20	5.9	4.2×10	5.3	.43
18	4.68	3.77	2.14	1.50	.17	32	0.92	10	.32
19	3.98	3.34	2.57	2.83	.17	56	0.32	11	.30
20	3.98	3.34	0.53	2.83	.17	56	0.22	13	.28
				Ovalbumin in acetate buffer					
21	−3.28	4.56	0.52	0.070	0.12	0.62	8.6×10^2	2.1	0.69
22	3.28	4.56	2.52	.070	.18	0.90	9.9×10^2	2.8	.60
23	3.08	4.56	2.96	.19	.14	1.7	1.6×10^2	2.0	.71
24	3.08	4.56	3.22	1.17	.12	9.8	4.7	4.1	.49
				Pepsin in acetate buffer					
25	−3.94	5.36	1.51	0.097	0.16	1.1	3.4×10^2	1.9	0.73
26	3.94	5.36	1.51	.097	.20	1.4	2.2×10^2	1.7	.77
27	3.94	5.36	1.65	.19	.14	1.9	1.1×10^2	2.9	.59

solution to the initial volume assuming a constant rate of osmosis. The amount of osmosis was found by measuring the volume of solution before and after the run together with that of the samples removed for analysis.

The electrophoretic mobilities, μ, used in the calculations were computed from the descending boundary patterns obtained with a Tiselius–Longsworth[5,6] electrophoresis apparatus. The concentration of hemoglobin was determined colorimetrically, that of the other proteins, by the method of Koch and McMeekin.[7] Measurements of the resistance between the electrodes with the electrophoresis-convection cell filled first with buffer and then with air showed that about 95% of the electric current is carried across the channel and the rest through the external buffer. Hence, the effective field strength, E, was taken as 95% of that calculated from the measured total current, the specific resistance of the buffer, and the area of the convection channel.

The channel thickness, a, is an average value computed from the channel area and the volume of solution in the channel under its operating hydrostatic head. The channel length, l, and width, b, were 24.1 and 5.1 cm., respectively, except in run 26 where b was 1.75 cm. The volumes, V, of solution in the top reservoir were between 44 and 60 ml. except in experiment 26 in which V was 10 ml. In all the experiments the ratio of the volume of the solution in the top reservoir to that in the bottom was between 0.88 and 1.2, which is sufficiently close to 1.0 to permit application of the simplified theory of Eq. (1).

The other data used in the calculations were obtained from the literature. The mobilities and diffusion constants were corrected to the temperatures and viscosities of the experiments. All the experiments were carried out in a refrigerated room at 4° to avoid denaturation of the proteins and temperature fluctuations.

The experiments with hemoglobin are probably slightly less reliable than those with the other proteins since an atmosphere of CO was not maintained during the runs, the temperature control was poor, and some denaturation was observed in the channel at the end of each run. For the most part these difficulties were not encountered with the other proteins.

Results

In all of the experiments the fraction of protein transported from the top reservoir of the apparatus was adequately represented as a function of time by the theoretical relations, Eq. (1), with a suitably assigned empirical characteristic time, θ^*, differing from the theoretical time θ. The experimental results together with the operating conditions are presented in Table II by means of the ratios θ^*/θ. The ratios E^*/E of effective field strength to actual field strength

$$E^*/E = (\theta/\theta^*)^{1/2} \qquad (3)$$

are also presented in the table. The effective field strength E^* is thus defined as that field which would be calculated from the experimental rate of transport by means of the theoretical expression for the characteristic time. It will be observed that the ratio E^*/E ranges from 1.3 to 0.24 in extreme cases. In most of the experiments its

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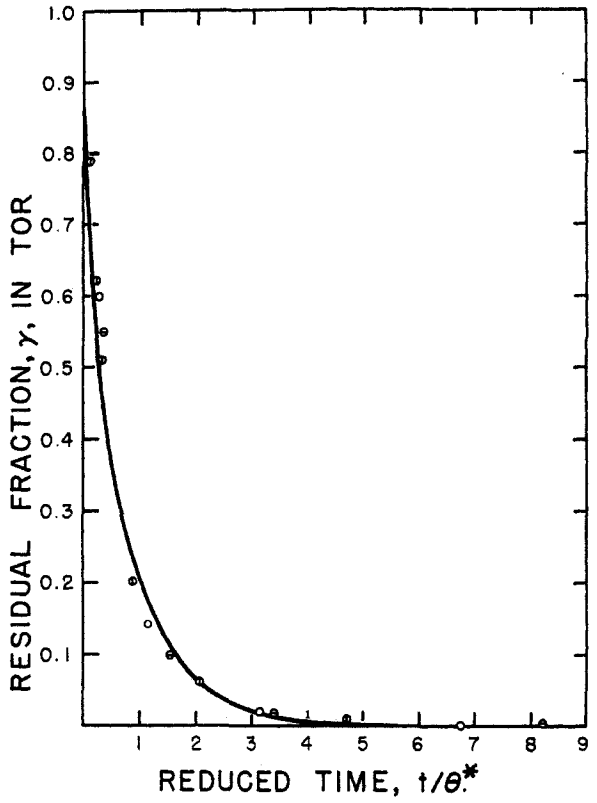

Fig. 1.—Transport of serum albumin by electrophoresis-convection: ⊙, run 18; O, run 19; ⊖, run 20.

value is near 0.5. The ratio depends upon the specific protein under investigation as well as upon electrophoretic mobility and diffusion constant. It decreases in general with increasing values of $\mu Ea/D$, which were carried far beyond the range in which the theory is expected to be quantitatively valid. The curve of Fig. 1 compares the theoretical relation for the fraction of protein, γ, residual in the top reservoir as a function of t/θ^* with the results of three serum albumin runs under practical conditions.

A number of simplifying assumptions underlying the derivation of Eq. (1) are not exactly realized in practice and would be expected to contribute to quantitative disagreement with experiment even when the parameter $\mu Ea/D$ is small relative to unity. Perfect mixing in the reservoirs is assumed but undoubtedly not completely attained. Imperfect mixing would retard transport. The actual electric field in the channel is certainly lower than the measured nominal field in the external buffer due to chemical potential gradients of the ion constituents in the channel and due to field jumps at the membranes where the protein ion constituent disappears. Also retarding the transport is the flow of solvent across the channel opposing the motion of the protein. This flow arises from an osmotic influx of solvent at the membrane at which the protein molecules accumulate and an efflux at the opposite depleted side.

Examination of the data of Table III shows that while the characteristic times of transport in the exploratory experiments vary by a factor of 1000, the ratio E^*/E varies only by a factor of about 5. The practical utility of the theory for the prediction of times of transport and for the planning of separations by electrophoresis-convection is thus clearly demonstrated. A single experiment allows the determination of the ratio E^*/E under the given conditions, and this ratio may be employed to predict transport as a function of time. In the absence of a pilot experiment, assignment of a value of 0.25 to the ratio E^*/E permits the determination of an upper bound to the time required for the transport of a given fraction of protein from the top reservoir of the apparatus.

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A Technique for the Study of Solid-Gas Surface Reactions; The Decomposition of Nitrous Oxide on Iron Oxide–Zinc Oxide Catalysts[1,2,3]

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A method for the measurement of surface area, magnetic susceptibility and catalytic efficiency for solid catalysts is described. The decomposition of N_2O at 500° and 1 atm. pressure over α-Fe_2O_3 and several ZnO–Fe_2O_3 catalysts has been studied by this method. The pure α-Fe_2O_3 is the most efficient of the catalysts studied. The efficiency for equal molar mixtures of ZnO and α-Fe_2O_3 pretreated at a series of temperatures shows a regular decrease in activity with pretreatment temperature. There is no evidence of a catalytically active phase intermediate between the mixture and zinc ferrite. The surface area also decreases regularly with pretreatment temperature, and the loss of catalytic activity may be due to this effect, though other possibilities are discussed. No correlation is observed between magnetic susceptibility and catalytic activity.

A combined interest in surface chemistry and magnetochemistry led to the investigation described in this paper. Studies of the influence of magnetism on catalytic processes occurring at a solid–gas interface have been investigated many times and are reviewed by Selwood.[5] Irregularities and strains in the crystal lattice of solid materials may often impart heterogeneous adsorptive properties to the surface.[6] In addition to the effects of magnetism and surface condition, the absolute magni-

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