hydrogen bonding, both of which would facilitate packing in the micelle.

The relative viscosities, η, of the solutions of the two compounds discussed here are represented in Fig. 4. Log η is seen to be linear with weight normality, N_w, over most of the range with a sharp change of slope occurring at approximately 0.35 N_w in the case of hexanolamine caprylate. At higher concentrations not shown in the graph, the viscosity falls below the extrapolated curve.

Acknowledgment.—The author wishes to thank Professor J. W. McBain for his interest in this work.

Summary

1. The osmotic behavior, conductivity and relative viscosity of solutions of hexanolamine caprylate and diisopropylamine caprylate have been investigated. The concentration of free cation in solutions of hexanolamine caprylate have also been determined. The results are correlated and discussed.

2. From a comparison of the ratio of free cation to total concentration with the osmotic coefficients and a modified "Arrhenius" conductivity ratio, it is concluded that hexanolamine caprylate associates first to neutral ion pairs followed by secondary association to colloid.

3. The presence of a branched chain cation combined with a straight chain colloidogenic anion causes the osmotic behavior to fall midway between Brady's generalized curves for straight and branched chain compounds, although the cation is of itself non-associating.

4. The logarithms of the relative viscosities of solutions of hexanolamine caprylate and diisopropylamine caprylate are linear with weight normality, and in the case of the former compound exhibit a change of slope at approximately the "critical concentration."

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[CONTRIBUTION FROM BAKER LABORATORY, CORNELL UNIVERSITY]

The Fractionation of Proteins by Electrophoresis-Convection

BY LAWRENCE E. NIELSEN[1] AND JOHN G. KIRKWOOD

Introduction

A new method for the fractionation of proteins in solution was suggested several years ago.[1a] The method depends upon the superposition of differential horizontal transport of the components by electrophoresis upon vertical convective transport of the solution as a whole in a narrow channel connecting two storage reservoirs. The theory of the method was developed under the assumption that the vertical convective transport could be controlled by a horizontal temperature gradient maintained between the channel walls. However, preliminary experiments demonstrated that with solutions not inconveniently dilute, the convection was controlled by horizontal density gradients produced by the electrophoretic transport of the proteins across the channel. This effect dominates that of temperature gradients of convenient magnitude and produces a much greater transport and separation than had been expected. However, when complicated by the effect of electrophoretic transport on density, the differential equations describing the process become rather unmanageable. It therefore seemed most expedient to us to carry out an exploratory experimental investigation to determine in a systematic manner the influence of the various factors affecting transport upon the degree of separation in representative protein mixtures.

The fractionation unit consists schematically of a narrow vertical channel connecting upper and lower reservoirs. In batch operation, the reservoirs and channel are filled with the protein mixture in solution, and a horizontal electric field is applied across the channel walls. Differential transport of all proteins from the top to the bottom reservoir takes place, resulting in a separation. At the end of the operation, the solutions in the two reservoirs are removed and collected for a further stage of fractionation. For a unit of specified geometry, the significant variables affecting transport and separation are (1) dimensions; length, width and wall separation of the channel; (2) temperature and the initial composition of the protein solution; (3) electrophoretic mobilities of the components, determined principally by *p*H; (4) strength of the applied electric field; (5) duration of the operation. The influence of these variables on the transport of several proteins in an experimental fractionation unit and upon the fractionation of representative mixtures of these proteins has been investigated and is the subject of this report.

The results of the exploratory investigation suggest that the method may be of practical value in protein fractionation. It permits operation on a larger scale than in the Tiselius electrophoresis apparatus, and promises to have advantages over solubility methods of fractionation in certain cases.

Experimental

The apparatus used in this investigation is shown in Fig. 1. The channel for the protein solution was formed

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(1a) I. G. Kirkwood, *J. Chem. Phys.*, **9**, 878 (1941).

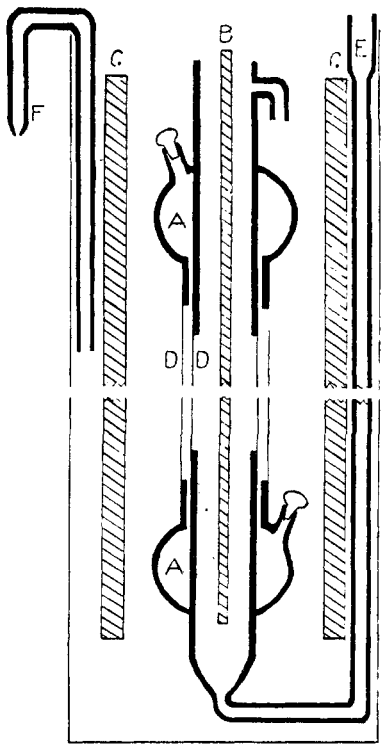

Fig. 1.—Electrophoresis convection apparatus: A, reservoirs; B, inner electrode; C. outer electrodes; D, cellulose membranes; E, inlet for buffer solution; F, outlet for buffer solution.

by the annular space between two Visking Corporation cellulose sausage casings of 19 mm. and 24 mm. diameter and about 165 mm. long. The upper and lower ends of this annular space were connected to glass reservoirs of 20-ml. capacity; the reservoirs had a glass tube extending through them of the same diameter as the inner membrane. The apparatus was mounted on a rigid support. Buffer solution was placed in the inside of the inner cellophane membrane, and the whole apparatus was immersed in buffer solution up to the top of the upper reservoir. A graphite electrode $^1/_8''$ in diameter and 12″ long was placed in the inner cellulose membrane; four other graphite electrodes $^1/_4''$ diameter and 12″ long were connected electrically and were symmetrically placed outside the outer membrane. By making the inner electrode positive, for instance, and the four outer electrodes negative, a fairly homogeneous electric field could be set up across the annular space that contained the protein solution. Electrolysis occurred which tended to change the *p*H of the buffer solutions. To counteract this, buffer solution was circulated by a gravity system through the inner membrane from bottom to top at a rate of about 10 to 20 ml. a minute. The buffer discharged into the solution in which the apparatus was immersed and was eventually discarded by an overflow tube. The *p*H of all the solutions remained constant to within a few hundredths of a *p*H unit with this arrangement. The temperature of the system was regulated in a constant temperature cold bath provided with cooling coils from a refrigeration machine.

The usual buffer was 0.01 *M* borax solution with enough boric acid to give a *p*H of 8.70 and a specific conductance of 1.56×10^{-3} reciprocal ohms at 25°. Another buffer used in some of the runs was 0.01 *M* sodium hydroxide solution with enough succinic acid to give a *p*H of 6.0 and a specific conductance of 9.5×10^{-4} reciprocal ohms at 25°. The *p*H of the solutions was determined by the use of a Beckman glass electrode *p*H meter.

The nominal field strength in the solutions was determined from measurements of the specific conductance of the solutions and from the average current density.

Proteins used in this investigation were purified horse hemoglobin, crystalline bovine serum albumin and 1-amino-8-naphthol-3,6-disulfonic acid azo ovalbumin. The concentrations of the single colored proteins were determined colorimetrically by means of a Fisher electrophotometer and a calibration curve. Kjeldahl determinations were made to check on the concentrations of the proteins. The concentration of bovine serum albumin solutions was determined by coagulating the protein with Exton reagent (5 g. of sulfosalicylic acid, 2 g. of sodium sulfate plus water to give a total of 100 ml.) and measuring the turbidity of the solution after a given time (ten minutes) with the electrophotometer. The concentrations of hemoglobin and the violet azo-protein in mixtures of the two were determined by readings with a green filter and then a blue filter in the electrophotometer and comparing with the results obtained from a series of mixtures of known composition. The concentration of hemoglobin in mixtures of hemoglobin and bovine serum albumin was determined colorimetrically while the serum albumin was determined nephelometrically by using Exton reagent and comparing with solutions of known composition.

The mobility of the horse hemoglobin was -5.0×10^{-5} sq. cm./volt sec. at *p*H 8.70. The mobility of the bovine serum albumin was -6.3×10^{-5} sq. cm./volt sec. at *p*H 8.70 and -2.2×10^{-5} sq. cm./volt sec. at *p*H 6.0. The mobility of the azo-albumin was -7.5×10^{-5} sq. cm./volt sec. at *p*H 8.70.

The experimental results are not entirely reproducible from run to run, due probably to the fact that the membranes are not rigid structures and, therefore, can move relative to one another thus changing the width of the channel containing the protein solution. Furthermore, there is some tendency for the membranes to stretch and get out of shape if they are used for a series of runs. It was also found that runs made with different sets of membranes could not be compared without an appreciable margin of error because of slight changes in the length of the channel and in the width of the annular space between the membranes.

The concentration of the hemoglobin could be determined to within about 0.2 mg. per 100 ml. of solution by careful use of the electrophometer. Similarly, the maximum error in determining the concentration of the bovine serum albumin was about 0.4 mg. per 100 ml. of solution and of the violet azo protein was 0.4 mg. per 100 ml. of solution. Therefore, the largest relative errors in determining the ratio of the concentrations in the bottom reservoir to the top reservoir would be in the more dilute solutions and in the runs in which this concentration ratio was large. The determination of the concentrations of the proteins in mixtures was in general slightly less accurate than the above values because any error in the concentration of one protein affected the value obtained for the other protein.

Experimental Results

A series of transport experiments for each of the three proteins, horse hemoglobin, bovine serum albumin and the violet azo-ovalbumin were carried out to obtain preliminary data for the fractionation experiments. The length of the convection column was 165 mm. and the *p*H was 8.70 in each of the first three series. The results are presented in Tables I, II and III. Data for bovine serum albumin at a *p*H of 6 is given in Table IV. The following symbols and units are employed in the tables

E = nominal field strength in volts/cm.
T = temperature in degrees centigrade
t = duration of run in hours

C_0 = initial concentration of protein in milligrams per 100 ml. of buffer solution
C_T = final concentration of the protein in the top reservoir
C_B = final concentration of the protein in the bottom reservoir

TABLE I

TRANSPORT DATA FOR HORSE HEMOGLOBIN

t, hrs.	E, volts/cm.	T, °C.	C_0 mg./100 ml.	C_B/C_T
1	0.318	12.0	20	1.31
1	.322	11.5	20	1.33
2	159	12.0	20	1.28
2	.238	12.5	20	1.58
2	.318	12.0	20	1.70
2	.397	12.0	20	2.25
2	.464	13.0	20	2.40
2	.794	12.0	20	4.87
2	.953	12.0	20	6.35
2	.326	11.5	10	1.67
2	.318	12.0	40	1.95
2	.127	20.5	17	1.30
2	.260	20.0	28	2.11
2	.266	19.0	20	2.24
4	.159	12.0	20	1.78
4	.318	12.0	20	2.86
4	.482	11.5	20	6.02
4	.482	11.5	20	5.51
4	.618	13.0	20	6.70
6	.161	11.5	20	2.01
6	.318	12.0	20	3.56
9	.159	12.0	20	2.35
9	.318	12.0	20	4.47

TABLE II

TRANSPORT DATA FOR BOVINE SERUM ALBUMIN AT A pH OF 8.70

t	E	T, °C.	C_0	C_B/C_T	$C_0 - C_T$
1	0.643	11.5	22.4	2.09	8.8
1.16	.333	10.0	22.5	1.40	3.7
2	.159	12.0	22.4	1.34	3.6
2	.330	10.5	21.3	2.02	7.6
2	.658	10.5	22.4	4.55	13.8
2	.266	19.0	7.0	1.90	2.5
2	.266	19.0	23.5	2.08	9.0
2	.269	18.5	36.0	2.23	15.0
4	.163	11.0	22.0	1.92	6.7
4	.329	10.5	22.4	3.60	13.0
4	.476	11.8	23.5	5.80	17.0
8	.163	11.0	22.4	2.74	10.6
8	.244	11.0	22.5	4.23	13.6

Some experiments were tried with horse hemoglobin at a pH less than 6, but difficulty was encountered with denaturation and collection of the protein along the membranes. In the more alkaline buffer of pH 8.70 it was verified that the proteins did not denature during a run, by subsequently bringing them to their isoelectric points without precipitation.

A few runs with convection columns of different lengths were made in another apparatus. They

TABLE III

TRANSPORT DATA FOR AZO-OVALBUMIN

t	E	T, °C.	C_0	C_B/C_T
2	0.155	13.0	15	1.80
2	.318	12.5	15	3.50
2	.392	12.5	15	5.37
2	.476	12.0	15	8.00
2	.488	11.0	15	5.90
2	.326	11.0	25	5.75
3	.235	12.5	15	3.80
3	.488	11.5	15	10.00
6	.154	13.0	15	3.40
6	.309	13.0	15	6.77

TABLE IV

TRANSPORT DATA FOR BOVINE SERUM ALBUMIN AT A pH OF 6.0

t	E	T, °C.	C_0	C_B/C_T	$C_0 - C_T$
1	0.270	11.0	25.0	1.10	1.4
1	.556	10.0	25.0	1.32	4.0
1	.572	9.0	25.0	1.33	3.4
1	.859	9.0	25.0	1.57	5.6
2	.221	19.0	10.0	1.50	2.2
2	.270	11.0	25.0	1.43	4.5
2	.432	20.0	25.0	2.38	10.0
2	.540	11.0	25.0	1.96	9.0
2	.556	10.0	25.0	1.84	7.7
2	.648	20.0	25.0	4.40	14.2
2	.834	10.0	25.0	2.50	11.0
4	.278	10.5	25.0	1.63	7.9
4	.291	8.5	25.0	1.86	8.8

TABLE V

TRANSPORT WITH TEMPERATURE GRADIENT

Protein	t	E	T_i	T, °C.	C_0	C_B/C_T	P
Azo ovalbumin	3½	0.000	18.0	13.0	15	1.08	
Azo ovalbumin	6	.000	18.5	13.0	15	1.0	
Azo ovalbumin	6¼	.215	17.5	14.0	15	4.7	+
Azo ovalbumin	6¼	.588	16.5	13.5	15	13.4	−
Azo ovalbumin	2	.140	21.5	13.0	15	1.2	+
Azo ovalbumin	2	.142	19.5	13.5	15	1.6	−
Hemoglobin	7	.000	21.0	13.0	40	1.00	+
Hemoglobin	2	.252	23.5	20.0	20	1.40	+
Hemoglobin	2	.252	23.0	20.5	20	1.85	−
Hemoglobin	2	.125	23.5	21.0	20	1.17	−
Hemoglobin	2	.122	24.5	21.5	20	0.965	+
Hemoglobin	2	.238	26.0	23.0	20	1.35	−
Hemoglobin	2	.235	27.0	23.0	20	0.953	+
Hemoglobin	2	.120	26.5	22.0	15	1.0	+
Hemoglobin	2	.120	25.5	22.5	15	1.04	−
Hemoglobin	2	.242	24.5	22.5	15	1.39	−
Hemoglobin	2	.242	25.5	21.0	15	1.0	+
Serum albumin	2	.235	28.0	22.5	21.6	1.01	+
Serum albumin	2	.240	26.0	22.5	22.3	1.42	−

indicate that log C_B/C_T is proportional to the length of the column.

The influence of moderate horizontal temperature gradients on the transport was studied in several runs by placing a small nichrome wire heater in the inner part of the apparatus containing the central electrode. The pH was 8.70 in these runs. The results are presented in Table V, in which T_i denotes the inner temperature, T_0 the outer temperature and P the polarity of the inner electrode. In the absence of the electric

TABLE VI

SEPARATION OF PROTEIN MIXTURES

Protein	E	C_{0H}	C_{0A}	$\frac{C_{BH}}{C_{TH}}$	$\frac{C_{BA}}{C_{TA}}$	$\frac{C_{BA}/C_{TA}}{C_{BH}/C_{TH}}$
Hemoglobin	0.353	19.1	0	2.22		
Serum albumin	.357	0.00	20		3.59	
Hemoglobin and serum albumin	.357	11.0	5	2.18	3.3	1.5
Hemoglobin and serum albumin	.353	22.3	5	2.21	3.6	1.6
Hemoglobin and serum albumin	.357	14.3	15	2.21	3.20	1.45
Hemoglobin and serum albumin	.357	14.3	15	2.05	2.78	1.36
Hemoglobin and serum albumin	.357	15.8	15	2.15	3.30	1.53
Hemoglobin and serum albumin	.357	12.5	15	2.05	3.33	1.62
Hemoglobin and serum albumin	.357	11.0	15	2.30	3.50	1.52
Hemoglobin and serum albumin	.357	21.6	15	2.20	3.78	1.72
Hemoglobin and serum albumin	.353	21.5	15	2.28	4.10	1.80
Hemoglobin and serum albumin	.353	16.7	20	2.22	3.71	1.67
Hemoglobin and serum albumin	.357	17.6	20	2.19	3.90	1.78
Hemoglobin and serum albumin	.353	11.1	25	2.15	3.42	1.59
Hemoglobin and serum albumin	.353	7.4	25	2.12	2.80	1.32
Hemoglobin and serum albumin	.353	12.4	25	2.23	3.30	1.48
Hemoglobin and serum albumin	.353	7.5	25	2.0	2.81	1.40
Hemoglobin and serum albumin	.357	11.0	25	2.32	3.57	1.54
Hemoglobin and serum albumin	.350	21.5	25	2.40	3.22	1.34
Hemoglobin and serum albumin	.353	30.0	25	2.10	2.86	1.36
Hemoglobin and serum albumin	.357	33.4	25	2.44	3.43	1.41
Hemoglobin and serum albumin	.353	51.0	25	2.42	4.22	1.75
Hemoglobin and serum albumin	.175	16.5	20	1.55	1.97	1.27
Hemoglobin and serum albumin	.179	16.5	20	1.61	2.06	1.28
Hemoglobin and serum albumin	.500	15.8	20	3.11	6.63	2.13
Hemoglobin and serum albumin	.536	16.5	20	3.45	8.22	2.37
Hemoglobin and serum albumin	.706	16.5	20	2.66 (1 hr.)	4.88 (1 hr.)	1.83 (1 hr.)
Hemoglobin and azo ovalbumin	.350	11.8	15	2.17	4.04	1.86
Hemoglobin and azo ovalbumin	.350	18.0	15	2.12	3.04	1.43
Hemoglobin and azo ovalbumin	.342	7.1	20	2.32	3.86	1.66
Hemoglobin and azo ovalbumin	.350	18.7	20	2.30	4.03	1.75
Hemoglobin and azo ovalbumin	.350	26.5	25	2.64	4.40	1.67

field, little or no transport was observed. Thermal diffusion therefore appears to play no role for small temperature gradients in question. In every case the temperature gradient appears to diminish the transport produced by a given electric field and time of run. The effect is very marked when the effect of the temperature gradient on the horizontal density distribution in the channel opposes that due to the protein concentration by electrophoresis.

Fractionation experiments were carried out on mixtures of horse hemoglobin and azo-ovalbumin and of hemoglobin and bovine serum albumin. A series of representative runs is presented in Table VI. In all of these experiments, the length of the convection column was 165 mm., the pH equal to 8.70 and the temperature 8.5 ± 0.7°. The duration of the run was in each case two hours. C_{0H} and C_{0A} denote the initial concentrations of the hemoglobin and albumin, respectively. The separation factor f is defined by the relation

$$f = \frac{C_{BA}C_{TH}}{C_{TA}C_{BH}} \qquad (1)$$

where C_{TA}, C_{BA}, C_{TH} and C_{BH} are the final concentrations in top and bottom reservoirs of the albumin and hemoglobin, respectively. The experimental error in the separation factor is estimated to be roughly 10%.

Discussion

An analysis of the experimental results presented in Tables I–VI leads to the following conclusions. Within the range of conditions covered by the experiments, log C_B/C_A appears to be proportional to the applied field, the duration of the run, and the length of the convection column, for each of the individual proteins studied. The experiments show that log (C_B/C_A) is greater for the protein with greater electrophoretic mobility, other conditions being the same. For a given protein, the transport is greater the higher the temperature for runs of the same duration at the same field strength. These conclusions may be summarized in the following formula

$$\log \frac{C_B}{C_A} = \beta(\mu) lEt \qquad (2)$$

where l is column length. The coefficient β is an increasing function of the electrophoretic mobility μ of the protein, an increasing function of tempera-

ture, and depends upon the geometry of the apparatus. The effect of the wall separation of the convection column has not been investigated. Equation (2) must be regarded as only approximately verified by the experimental data, but it represents their trend quite satisfactorily.

For runs of sufficient duration, the linear dependence of log C_B/C_A on time must finally fail, since a stationary state must eventually be approached in the apparatus. From the data we conclude that the durations of the experiments were short relative to the time required to approach closely the stationary state, and that this state evidently corresponds to a rather high concentration of the proteins in the bottom reservoir.

In the representative fractionations of Table VI, it will be observed that the transport of each component of the mixture is nearly that which would occur if it were transported alone under the same conditions. It also appears that the separation factor does not depend to a marked degree on the initial composition of the protein solutions. To the extent that these approximations are valid, we may estimate the separation factor by the equation

$$\log f = \Delta\beta lEt \qquad (3)$$

where $\Delta\beta$ is the difference of the transport coefficients β of equation (2) for the individual proteins of the mixture. We surmise that $\Delta\beta$ is approximately proportional to $\Delta\mu$ the difference of the electrophoretic mobilities of the proteins, but the data are not sufficient to permit a definite conclusion on this point.

The representative separation factor 1.5 obtained in the series of exploratory runs indicates that the method has practical possibilities. By suitable modifications in design, it is hoped that batch operation in a single unit may be replaced by continuous operation in a series of fractionating units of the type described.

Acknowledgment.—The writers wish to express their thanks to Professor E. J. Cohn of the Harvard Medical School for a supply of the bovine serum albumin,[2] and to Dr. C. L. Hoagland of the Rockefeller Institute for Medical Research for a supply of the azo-ovalbumin. They also wish to thank Dr. T. Shedlovsky and Dr. D. A. McInnes of the Rockefeller Institute and Professor J. B. Sumner of Cornell University for helpful suggestions.

Summary

An electrophoresis convection apparatus has been described for the fractionation of protein mixtures in which an electric field is used to transport the proteins in a horizontal direction while the density gradient thus developed brings about convection currents in a vertical direction.

Factors affecting the concentrating of proteins by this method are discussed, and pertinent data are presented.

The partial separation of horse hemoglobin and bovine serum albumin has been accomplished. Horse hemoglobin and an azo-ovalbumin have also been partially separated.

(2) The protein was prepared in the course of work carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

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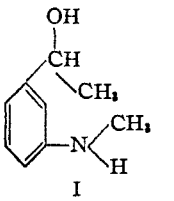
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Some Derivatives of *m*-Ethylaniline. A Novel Disproportionation of an Aminophenylmethylcarbinol[1]

BY C. S. MARVEL AND C. G. OVERBERGER

The desire to obtain styrene derivatives containing basic groups led us to attempt the dehydration of *m*-N-methylaminophenylmethylcarbinol (I) over activated alumina at 450–500°. To our surprise the only product isolated in these experiments was *m*-N-methylaminoethylbenzene (II). That this was obtained in yields of 40–50% suggests that a disproportionation reaction occurred and half of the starting material was reduced whereas the other half was oxidized and lost probably as tar during the reaction.

The following evidence indicates that an ethylbenzene rather than a styrene derivative was obtained. The analytical data on II and its picrate agree with the proposed structure; the base (II) did not take up hydrogen over an Adams platinum oxide catalyst[2] and the picrate obtained from a sample before this treatment was identical with one prepared after the treatment; the carbinol (I) was reduced readily by hydrogen over a copper



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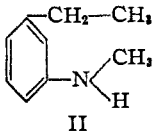

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(1) The work described in this manuscript was carried out under the sponsorship of the Office of Rubber Reserve, Reconstruction Finance Corporation, in connection with the Government Synthetic Rubber Program.

(2) Adams, Voorhees and Shriner, "Organic Syntheses," 2nd ed., Coll. Vol. I, John Wiley and Sons, Inc., New York, 1941, p. 463.