In order to obtain the relation between the molecular weight of the peptides forced into the substrate solution and the pressure exerted on the film, the percentages of the hydrolyzed material remaining in the film were plotted against the pressures exerted. The corresponding molecular weights were then interpolated from Figs. 5 and 6. The logarithms of the molecular weight have been plotted against the pressures exerted and are shown in Fig. 7.

The points in Fig. 7 extrapolate at zero pressure to log molecular weight of about 3. This means that peptides whose molecular weight is about 1,000 or less pass spontaneously into the substrate solution and will not form a film.

The techniques described in this paper are being applied to the study of the molecular weight distribution of peptides resulting from the action of enzymes on proteins. These results will be reported in due time.

It is a pleasure to acknowledge the generous assistance granted this research by Corn Products Refining Company.

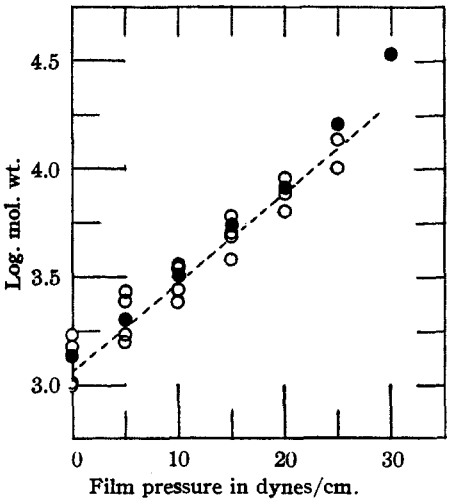

Fig. 7.—Logarithm of molecular weights of displaced peptides plotted against pressure exerted on the film. Open circles are 60° hydrolysates and filled circles two hour hydrolysate at 30° (protein free).

Summary

1. A new method has been described for the determination of the molecular weight distribution of peptides in a partial acid hydrolysate of a protein.

2. An hydrolysate is spread as a monomolecular layer on a 5% ammonium sulfate solution and is compressed to progressively increasing pressures. The logarithm of the molecular weight of the displaced peptides is proportional to the pressure exerted on the film. The molecular weight of the peptides remaining in the film after exposure to a given pressure is determined by the application of the gas laws in two dimensions.

3. The molecular weight distribution of egg albumin hydrolyzed by hydrochloric acid at several degrees of hydrolysis has been reported.

4. It is found that while the molecular weight distribution of peptides departs greatly from that expected for a random hydrolysis, there is no evidence for the accumulation of any considerable amounts of a peptide of a given molecular weight.

CHICAGO 11, ILLINOIS RECEIVED DECEMBER 6, 1947

[CONTRIBUTION FROM THE UNITED SHOE MACHINERY CORPORATION RESEARCH DIVISION]

Partition Chromatography of Amino Acids with Applied Voltage

BY GOTFRED HAUGAARD[1] AND THOMAS D. KRONER

In the one dimensional partition chromatography developed by Consden, Gordon and Martin[2] it is often very difficult to detect the bands of amino acids whose R_F values lie close together. To overcome this difficulty, two dimensional chromatography employing two solvents was developed by these workers.

In our work, we encountered overlapping of R_F values between the basic, acidic and certain neutral amino acids. We have effected a two dimensional chromatography by the passage of current through paper treated with phosphate buffer at *p*H 6.2.[3] The negatively charged acids—aspartic and glutamic—move toward the anode; the basic acids—lysine and arginine—migrate toward the cathode and the neutral amino acids are unaffected by the voltage gradient at the *p*H close to their isoelectric point.

Experimental

The papers used in the chromatograms were prepared as follows. Whatman no. 1 paper was dipped in *M*/15 phosphate buffer at *p*H 6.2 and the excess fluid was removed by pressing with a photographic roller over a glass plate. The paper strips (570 × 120 mm.) were air dried before use. We have employed aluminum, nickel and platinum as electrodes and have found little difference between them. The nickel ribbon (6.35 × 0.025 mm.) is woven into slits cut into the edges of the paper and the electrodes extend not more than one half the length of the paper. The electrodes may also be attached to the paper by stapling. The mixture of amino acids consisted of two dicarboxylic acids—aspartic and glutamic; two basic acids—lysine and arginine; and six neutral amino acids—serine, glycine, alanine, valine, leucine and proline. The concentration of the individual amino acids in the

(1) Present address: National Dairy Corporation, Oakdale, Long Island, New York.

(2) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

(3) R. R. Goodall and A. A. Levi, *Nature*, **158**, 675 (1946).

mixture was 0.1 mg. of NH_2-N/ml. and the mixture was applied from a micropipet at the center of the strip. The chromatograms were developed overnight (sixteen to eighteen hours) in a constant temperature room (23°) and phenol was the developing solvent. The potential used in most of the experiments was 100–105 v.

An example of the separation obtained with platinum electrodes and a potential of 105 v. is shown in Fig. 1. The separation of the bands is clear and sharp. It is obvious that little or no differentiation would have been possible without the applied voltage. The identity of the basic and acidic amino acids is substantiated by both the characteristic R_F value and the direction of migration in the electric field.

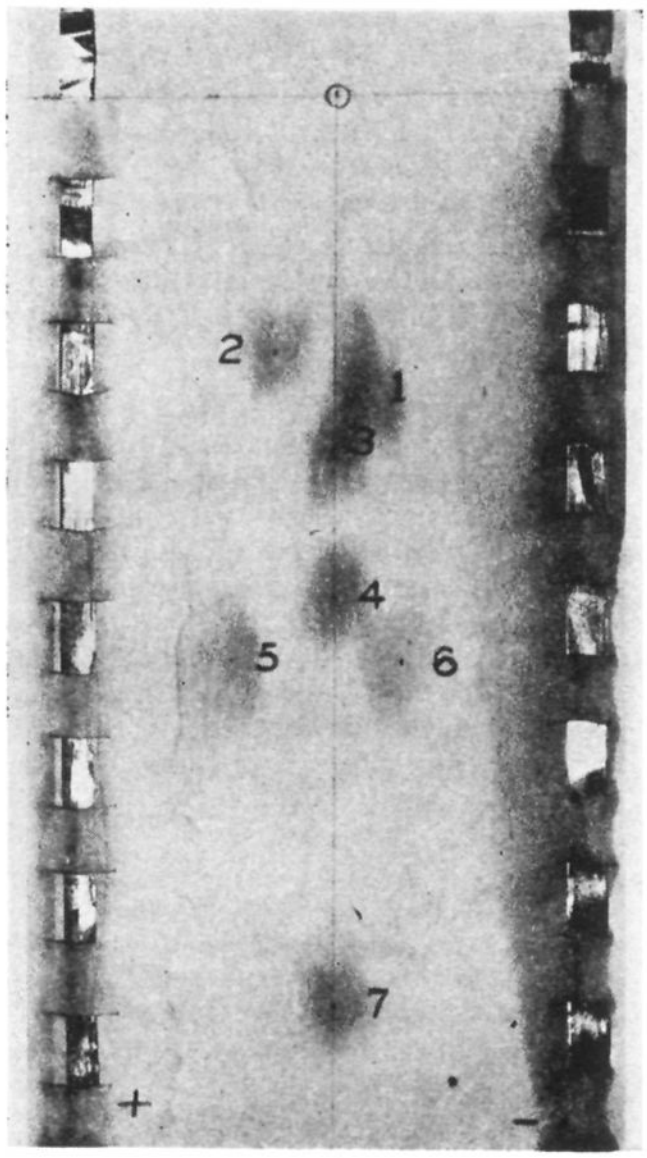

Fig. 1.—Partition chromatography of amino acids with applied voltage: 1, lysine; 2, aspartic acid; 3, serine; 4, glycine; 5, glutamic acid; 6, arginine; 7, alanine. Phenol is the developing solvent. Valine, leucine and proline with greater R_F values than alanine not shown.

In Table I, is shown statistical analysis of R_F values of 26–32 determinations on the ten amino acids. The error on the average values is not tabulated. The error in any of the determined R_F values is less than 0.01. The error in a single determination is found in the table and varies between the limits of ±0.02–0.04.

The R_F values found by us, except in the case of glutamic acid, are for the most part less than those reported by Consden, *et al.* The presence of the salts and the fixed *p*H may account for the lower R_F values.

Discussion

The optimum conditions for this method are (1) a voltage of 100; (2) a run of sixteen to eighteen hours; (3) paper buffered with $M/15$ phosphate at *p*H 6.2; (4) controlled temperature. Chromatograms have been carried out at 50 and 70 v.; the migration rates of the charged acids were a little lower but not as much as expected. The explanation of this phenomenon was found experimentally by measuring the potential gradients across the paper. The largest gradients were close to the electrodes and the potential differences at the interior of the paper were not proportional to the applied voltage. Chromatograms developed for eight hours at 100 v. failed to effect separation of the charged amino acids from the neutral acids. Tailing and spreading of the bands occurred on papers prepared with buffer at one-quarter and one-eighth strength. The optimum has been found to be 6.2.

In several runs, it was noticed that although separation had occurred some of the charged amino acids failed to give the typical color with ninhydrin. In these instances, it was shown by testing the paper with *p*H indicators that a high *p*H and a low *p*H zone extended, respectively, from the cathode and anode toward the midline. This phenomenon was associated with a final current greater than 0.3 milliampere. We believe that excessive electrolysis of the buffer resulted in this marked change in the *p*H of the paper. Careful preparation of the paper is necessary to avoid too great salt concentration.

TABLE I

R_F VALUES OF AMINO ACIDS IN PHENOL[a] ON WHATMAN NO. 1 PAPER BUFFERED WITH $M/15$ PHOSPHATE AT *p*H 6.2, AT 23°

Acid	R_F values	Error on a single measurement[b]
Serine	0.21	±0.02
Glycine	.28	± .04
Alanine	.49	± .04
Valine	.74	± .02
Leucine	.82	± .02
Proline	.86	± .02
Aspartic acid	.16	± .02
Glutamic acid	.32	± .03
Lysine	.17	± .02
Arginine	.32	± .03

[a] Hydrogen cyanide added to the tray. [b] $\sqrt{\Sigma\delta^2/(n-1)}$.

Under the conditions of this study, histidine with an isoelectric point of *p*H 7.6 would not be expected to migrate. This has been found to be true experimentally.

We have observed that tailing of certain acids such as lysine, arginine, histidine, aspartic and glutamic acid has occurred on chromatograms developed on plain paper. Dipping the Whatman no. 1 paper in phosphate buffer at *p*H 6.2 has eliminated this phenomenon, except in the instance of histidine.

Control of temperature should be maintained to prevent (1) distillation of solvent from the paper to the walls of the chamber, (2) disruption of the single phase solvent system; also because temperature has an effect on the constancy of the R_F values.

The authors express thanks to Dr. John T. Edsall for the interest he has taken in this work.

Summary

1. A study of partition chromatography with applied voltage has been presented.
2. R_F values for ten amino acids obtained under the experimental conditions are given.
3. Paper buffered with phosphate at *p*H 6.2 has been used.

BEVERLY, MASSACHUSETTS RECEIVED MARCH 16, 1948

[CONTRIBUTION FROM THE KEDZIE CHEMICAL LABORATORY, MICHIGAN STATE COLLEGE]

The Electrical Conductance of Strontium Chloride and Strontium Bromide in Ethanol–Water Mixtures[1]

BY RICHARD LOUIS BATEMAN AND DWIGHT T. EWING

The earliest investigations of electrical conductance in mixed solvents were those of Lenz,[2] and Stephan.[3] This type of work was extended by other workers and particularly by Jones and coworkers.[4]

In general, these investigations showed that the conductance of electrolytes in mixed solvents decreased as the solvent viscosity and degree of solvation became greater but increased as the dielectric constant of the solvent and temperature became greater. A large part of these early investigations concerned the uni-univalent electrolytes with less attention given to those of higher valence types. No recorded data are given for the conductance behavior of strontium chloride and strontium bromide in ethanol–water mixtures.

The purpose of the present investigation was to determine the influence of concentration, solvent composition and temperature on the conductance behavior of strontium chloride in ethanol–water solutions and to note the influence of viscosity and dielectric constant of the solvent on the conductance of these solutions. For purposes of comparison, a limited amount of work was also done on the conductance of strontium bromide in ethanol–water solutions.

Experimental

Purification of Materials.—J. T. Baker C. P. $SrCl_2 \cdot 6H_2O$ was recrystallized from conductance water once above and once below the transition temperature (61.34°) by the method of Richards and Yngve[5] and oven-dried to constant weight.

J. T. Baker C. P. KCl designated "special crystals" (low in Ca, Mg and NH_4OH ppt.) were twice recrystallized from conductance water. The salt was then partially fused in a platinum crucible and transferred to a closed bottle while still hot.

Ethyl alcohol was purified by distilling 95% alcohol from concentrated sulfuric acid (20 ml. of acid per liter of alcohol) to remove amines. The distillate was then treated with alcoholic lead acetate[6] (3 g. of lead acetate in 5 ml. of water and then 5 g. of KOH in 25 ml. of alcohol) and distilled. Absolute alcohol was then prepared by treating each liter of distillate with fresh calcium oxide (200 g. per liter of alcohol), refluxing and distilling. In these distillations, the first and last portions of distillate were discarded. The water content was determined by density measurement and reference to standard density tables.[7] The alcohol thus obtained was 99.9% absolute and had a specific conductance of 2.0×10^{-7} mho at 25°.

Conductance water was prepared by distilling water containing a little potassium permanganate through a block tin condenser. About 50% of the distillate was allowed to condense and only the middle fraction was retained. At 25° the specific conductance of this water was $1.00\text{–}1.04 \times 10^{-6}$ mho.

Apparatus.—A Leeds and Northrup Kohlrausch slide wire bridge with extension coils, tunable head phones, Curtis coil resistance boxes, adjustable air condensers and Leeds and Northup audiooscillator were used. All parts of the bridge assembly were protected by properly grounded shields. A thermostat bath filled with water was kept constant to within 0.01° during the series of measurements. Temperature measurements were made on a thermometer (No. 23044) that had been certified by the Bureau of Standards (certificate No. 49571). Temperature fluctuations were followed by a Beckmann thermometer. The conductance cells were of the Jones and Bollinger[8] type with the filling tubes widely separated. The electrodes were not platinized.[9] The primary standard solution for cell constants was the 0.01 demal KCl solution of Jones and Bradshaw.[10] Cells having low constants were calibrated using a more dilute solution which had been compared to the standard 0.01 demal KCl in another cell.

Procedure.—The ethanol–water solvents were prepared by the weight method. The electrolytic solutions were prepared in volumetric flasks after attaining thermal equilibrium in the thermostat bath. The conductance cells were rinsed several times with the appropriate solution, brought to thermal equilibrium and the conductance determined. All final readings were taken near the center of the bridge with the air condensers adjusted for the most satisfactory null-point. The cells were selected so that the resistance was ordinarily above 1000 ohms. Duplicate determinations were made from two independently prepared solutions. The conductance of the solvent was determined immediately before the preparation of the elec-

(1) This paper represents part of a thesis submitted by Richard Louis Bateman to the Graduate Faculty of Michigan State College in partial fulfillment of the Ph.D. degree, June, 1944.

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