The production of salted-out films may thus prove to be a rapid and convenient microtechnique for purifying small amounts of various proteins. By the choice of the proper salt and concentration it should be possible to separate proteins from one another and to measure the amounts.

Summary

When a glass or metal plate is dipped into a protein solution, then into a concentrated solution of a salt such as ammonium, sodium or magnesium sulfate, or sodium chloride, a compact film of protein often 200–1000 Å. thick is salted out onto the plate. This can be fixed by 1% tannic acid and can then be washed with water without loss. After it has dried the thickness can be determined optically by interference colors. Insulin films, formed by using 1% sodium chloride solution, need no tannic acid treatment.

Salted-out protein films, in some cases up to 7000 Å. in thickness, are formed on the surface of salt solutions by applying a few drops of the protein solution. These can be deposited as hydrous B-films or as lifted A_L films onto plates or small pieces of metal foil, and they can thus be transferred to clean water surfaces where the amount of the protein can be measured from the area of the monolayer produced, or they can be fixed by tannic acid, washed and dried and the amount of protein per sq. cm. determined optically.

The refractive index of dried salted-out films of crystalline egg albumin was found to be 1.48. This low value and the power of the film to absorb hydrocarbon vapors indicate that the dried film has a skeleton-like structure.

The formation of these films at the surface of the solution or on a plate is aided by the concentration gradient in the salt solution. This carrying effect is analogous to the Ludwig–Soret phenomenon by which a solute originally uniformly distributed through a solution tends to concentrate in a portion of the solution which is cooled.

SCHENECTADY, N. Y. RECEIVED AUGUST 12, 1938

NOTES

Esters of Chlorosulfonic Acid[1]

BY W. W. BINKLEY WITH ED. F. DEGERING

While the lower alkyl esters of chlorosulfonic acid have been prepared, they have not been purified by redistillation and there is some disagreement in the values of the physical constants which have been reported.[2] We have therefore again prepared these substances and have purified them by careful rectification using a modified Podbielniak column and have redetermined with care certain of their physical constants.

The esters were prepared by adding dropwise one-fifth mole of the absolute alcohol to one-fifth mole of sulfuryl chloride contained in a test-tube 20 cm. long immersed in an ice-bath, the sulfuryl chloride being agitated by a brisk current of dry air drawn through it. A rough separation of the alkyl chlorosulfonate from the dialkyl sulfate was first accomplished by distillation under diminished pressure at the lowest possible temperature. The distillate was then rectified under diminished pressure in a jacketed electrically heated column which has a continuous nichrome wire gage No. 18 for packing.[3] An average yield of 50% based on the alcohol was obtained. The characteristic physical constants for these esters and other analyses are collected in Table I.

The chlorine content was estimated by a modified Fajans method[4] after the ester was allowed to decompose in a pressure bottle containing calcium carbonate. Sulfur was determined by the method of Carius.

All the lower normal esters of this series are lachrymators. The tear-producing effect decreases as the length of carbon chain is increased.

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(1) Abstracted from a portion of a thesis submitted by W. W. Binkley in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry, June, 1939.

(2) Bushong, *Am. Chem. J.*, **30**, 212 (1903).

(3) Podbielniak, *Ind. Eng. Chem., Anal. Ed.*, **5**, 119 (1933).

(4) Mellon, "Methods of Quantitative Chemical Analysis," The Macmillan Co., New York, 1937, p. 297.

TABLE I

	Methyl	Ethyl	*n*-Propyl	*n*-Butyl
Sp. gr. at 25°	1.48	1.35	1.28	1.23
Ref. index 20°	1.414	1.416	1.422	1.427
B. p., °C.	48.1 at 29 mm.	42.3 at 10 mm.	53.2 at 10 mm.	69 at 10 mm.
% S, calcd.	24.53	22.18	20.18	18.65
% S, found	24.8 ± 0.2	22.0 ± 0.2	20.4 ± 0.2	18.3 ± 0.2
% Cl, calcd.	27.13	24.54	22.38	20.56
% Cl, found	27.0 ± 0.1	24.5 ± 0.1	22.2 ± 0.1	20.5 ± 0.1

They are colorless liquids, insoluble in water, but quite soluble in the common organic solvents.

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Nitrogen-Terminated Conjugated Systems and Maleic Anhydride

BY FELIX BERGMANN

The ability of 9-vinylphenanthrenes to condense with maleic anhydride[1] induced us to investigate whether conjugated systems consisting of a cyclic C–N double bond and an exocyclic ethylenic linkage would add maleic anhydride. 2-Styrylquinoline gave a well-defined reaction product, which, however, besides the components, contained one molecule of water, and therefore was styrylquinolinium maleate. This became obvious in its reaction with diazomethane, yielding dimethylpyrazoline-4,5-dicarboxylate.[2] Analogous observations have been made recently by La Parola[3] in the case of antipyrine and pyramidone. For comparison purposes, we studied the interaction between maleic anhydride and cinnamylidene-aniline, which contains the nitrogen-terminated conjugated system entirely in an open chain. Here, too, the reaction involves one molecule of water (from the air), but the maleate, presumably formed as above, is unstable and decomposes spontaneously into cinnamic aldehyde and maleanilic acid, HOOCCH=CHCONNH-C_6H_5.

Experimental

2-Styrylquinoline[4] (1.2 g.) and **maleic anhydride** (0.5 g.) were heated on the water-bath or in xylene solution for some minutes. The mixture turned yellow at once and deposited on cooling yellow crystals, which were recrystallized from xylene or butyl acetate. 2-Styrylquinolinium maleate formed long, silky needles, m. p. 165–167°. *Anal.* Calcd. for $C_{21}H_{17}O_4N$: C, 72.6; H, 4.9. Found: C, 72.8; H, 5.3. The salt was kept in contact with an excess of ethereal diazomethane solution; on evaporation a crystalline mass was obtained which was triturated with light petroleum (b. p. 80–100°) and recrystallized from the same solvent. Dimethyl pyrazoline-4,5-dicarboxylate was obtained in the form of leaflets, m. p. 103–105°. *Anal.* Calcd. for $C_7H_{10}O_4N_2$: C, 45.2; H, 5.4; N, 15.1. Found: C, 44.8; H, 5.5; N, 15.6.

Cinnamylidene-aniline[5] (1 g.) and **maleic anhydride** (0.5 g.) were heated together on the water-bath; the mixture turned brown, then red and an intense odor of cinnamic aldehyde developed. After fifteen minutes, benzene was added, whereupon maleanilic acid crystallized, yield 300 mg.; from butyl acetate as rhombohedra, m. p. 210°.[6] *Anal.* Calcd. for $C_{10}H_9O_3N$: C, 62.8; H, 4.7; N, 7.3. Found: C, 62.5; H, 4.5; N, 7.7. Diazomethane gave as above the expected methyl ester-anilide of pyrazoline-4,5-dicarboxylic acid, from xylene or butyl acetate; crystals, m. p. 175° (dec.). *Anal.* Calcd. for $C_{12}H_{13}O_3N_3$: N, 17.0. Found: N, 17.4.

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[1] E. Bergmann and F. Bergmann, THIS JOURNAL, **59**, 1443 (1937).

[2] Von Pechmann, *Ber.*, **27**, 1888 (1894); v. Pechmann and Burkard, *ibid.*, **33**, 3590 (1900).

[3] La Parola, *Gazz. chim. ital.*, **67**, 645 (1937).

[4] Skraup and Boehm, *Ber.*, **59**, 1013 (1927).

[5] Doebner and v. Miller, *ibid.*, **16**, 1665 (1883).

[6] The highest m. p. recorded before is 198° [Auwers and Schleicher, *Ann.*, **309**, 347 (1899)]. A preparation made according to Anschütz [*Ber.*, **20**, 3214 (1897)] showed m. p. 210°, after recrystallization from butyl acetate or butyl alcohol.

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The Calculation of the Dissociation Constant of Weak Acids in H_2O–D_2O Mixtures

BY FRANK BRESCIA

From the exchange equilibria

$$\begin{aligned} 2DCl + QH_2 &= 2HCl + QD_2 & K_1 &= 14.64 & (1)\\ QH_2 + D_2O &= QD_2 + H_2O & K_2 &= 0.96 & (2)\\ H_2O + D_2O &= 2HDO & K_3 &= 3.27 & (3) \end{aligned}$$

Korman and La Mer[1] have calculated the equilibrium constant for the process, $D^+ + H_2O = H^+ + HDO$, to be $K_4 = 7.1$. Their derived equation for the calculation of dissociation constants of weak acids in H_2O–D_2O mixtures

$$K_M = K_D \left[1 + 7.1 \frac{C_{H_2O}}{C_{HDO}} \middle/ 1 + 7.1 \left(\frac{K_D}{K_H}\right) \frac{C_{H_2O}}{C_{HDO}}\right] \quad (5)$$

contains, therefore, the factor 7.1. K_M is the dissociation constant of the acid in the mixtures of H_2O–D_2O, K_D is the dissociation constant of

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[1] Korman and La Mer, THIS JOURNAL, **58**, 1396 (1936).