crop 0.23 g. of II, m.p. 148–150°, and an additional 0.095 g., m.p. 144–147° was isolated from the mother liquor by further concentration. Recrystallization from methylene chloride–hexane raised the m.p. to 149–150°, $[\alpha]^{25}D$ +94.3° (acetone).

Anal. Calcd. for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.34; H, 9.32.

The sample of II prepared from III had an infrared spectrum identical with that of II isolated from the detosylation procedure.

CHEMICAL RESEARCH DIVISION
SCHERING CORPORATION
BLOOMFIELD, N. J.

A Method for Determining the Solubility Characteristics of Components in Protein Mixtures[1]

BY E. L. HESS AND DORIS S. YASNOFF

RECEIVED SEPTEMBER 19, 1953

A rational approach to the chemical separation of a mixture of proteins requires some knowledge of the effect of *p*H and ionic strength upon the solubility of the components. In many fractionation studies the available quantity of starting material severely limits the investigation of these parameters. This report outlines a method for obtaining such information. As little as 0.1 g. of a crude mixture is sufficient for a fairly complete study of ionic strength and *p*H effects. The simplicity of the method and the economy of material recommends its use, particularly in work with tissue extracts. The earlier work of Kober,[2] Morse,[3] and Wannow[4] indicated the possible use of the method in the fractionation of protein mixtures.

The method consists of diluting a protein mixture to a concentration appropriate to optical density measurements in an ultraviolet spectrophotometer and reading the scale deflection given by the mixture.

A series of readings are made at various *p*H values with the concentration and ionic strength held constant. When *p*H and concentration are held constant the ionic strength may be varied. At those *p*H and ionic strength conditions where precipitation occurs, the optical density resulting from turbidity is superposed upon the intrinsic absorption from the soluble portion of the system. The precipitate, in our experience, remains suspended at the low concentrations employed. The turbidity is an inverse function of the solubility as can be seen in Fig. 1. The solubility curve in Fig. 1 was obtained by centrifuging the turbid solutions and reading the optical density (λ 280 mμ) of the clear supernatants in a Model DU Beckman spectrophotometer. For protein systems, where the solubility relationships are such that precipitation does not occur, the optical density remains constant over the *p*H range normally of interest. Although most of our studies have been carried out at 260 and 280 mμ, it would seem preferable to make the turbidity measurements at 250 mμ. At this lower wave length most proteins are close to the minimum of their characteristic ultraviolet absorption curve. Because the scattered radiation is an inverse function of the fourth power of the wave length the measured turbidity will be greater at the lower wave length. When precipitation does not occur within the *p*H and ionic strength deemed suitable for separation, the effect of salts such as ammonium sulfate or miscible solvents with low dielectric properties, *e.g.*, ethanol, can be explored. The use of thermospacers on the spectrophotometer allowing temperature control of the solution is recommended when organic solvents are employed.

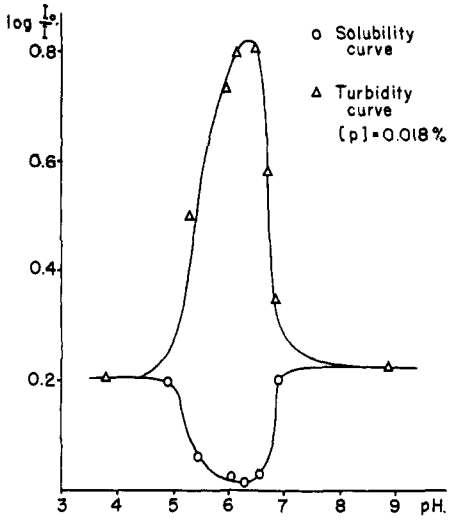


Fig. 1.—Comparison of solubility and turbidity measurements as a function of *p*H: fraction 6.2P, μ = 0.0; λ 280 mμ.

The application of the method is illustrated in Figs. 2 and 3. The turbidity curves of several fractions from bovine palatine tonsils are shown in Fig. 2. The ionic strength effect can be seen in Fig. 3. The electrophoretic patterns of the corresponding fractions are given in Fig. 4. In this instance the turbidity curves are much more revealing than are the electrophoretic patterns. For example, compare 3.0S curve in Fig. 2 and the electrophoresis pattern in Fig. 4 C.

The various fractions listed in Fig. 2 are related as outlined below. Fraction 5.1P, the crude starting material in this report, is the equivalent of Ppt.B in the fractionation scheme of a preceding publication.[5] It is of interest that although 5.1P shows a fairly symmetrical pattern at *p*H 8.6, μ = 0.10, veronal buffer in the Tiselius apparatus this fraction is shown to contain at least four separate components. 5.1P is separated at *p*H 3.0 and μ = 0.10 into a precipitate (3.0P) amounting to 70% of 5.1P. The supernatant (3.0S) constitutes the remaining 30% of 5.1P. 3.0S is separated at *p*H 6.2 and μ = 0.0 into a precipitate (6.2P) amounting to 80% of 3.0S while the supernatant (6.2S) constitutes the remaining 20% of the 3.0S fraction.

The choice of *p*H 6.2 and an ionic strength of zero for the separation of 3.0S was made from inspection of the two 3.0S curves shown in Fig. 3. Additional

(1) *This report represents work done under contract with the U. S. Atomic Energy Commission. Project No. 6 to Contract AT (11-1)89 with Northwestern University.*

(2) P. A. Kober, *J. Ind. Eng. Chem.*, **10**, 556 (1918).

(3) J. F. Morse, *Analyst*, **62**, 11 (1937).

(4) H. A. Wannow, *Koll.-Z.*, **87**, 311 (1939).

(5) E. L. Hess, W. Ayala and A. Herranen, THIS JOURNAL, **74**, 5419 (1952).

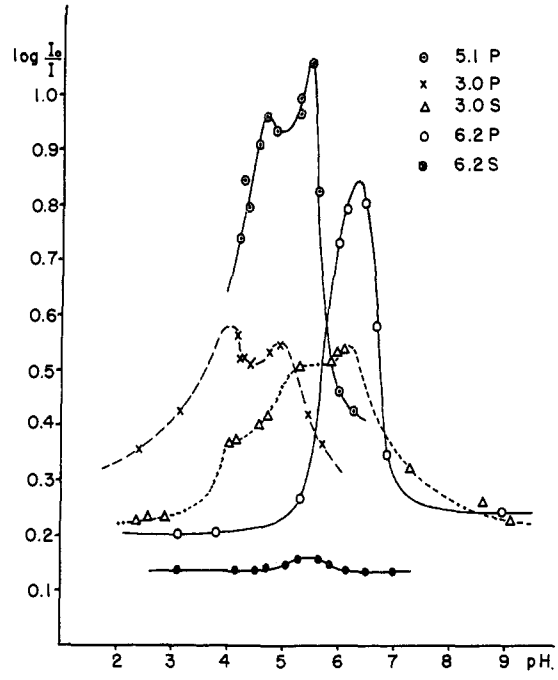


Fig. 2.—Turbidity of various fractions of lymphocyte cytoplasm as a function of *p*H: λ 260 mμ; ionic strength (μ) = 0.00 in all cases.

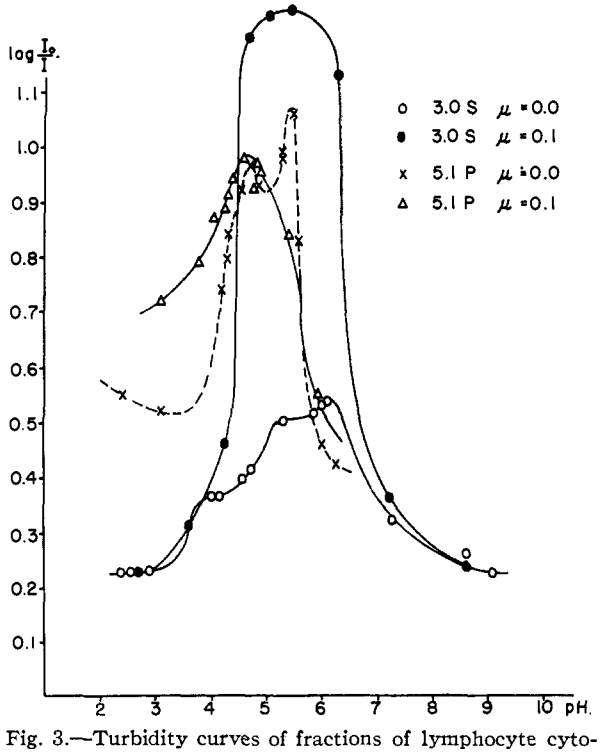


Fig. 3.—Turbidity curves of fractions of lymphocyte cytoplasm as a function of *p*H and ionic strength, λ 260 mμ.

studies, not included in this report, at intermediate ionic strengths added confidence to the choice. The 6.2P curve shown in Fig. 2 as well as electrophoretic patterns verified the prediction.

Less obvious, was the choice *p*H 3.0 and $\mu = 0.1$ for the separation of 5.1P into 3.0P and 3.0S. This step was developed before we understood the

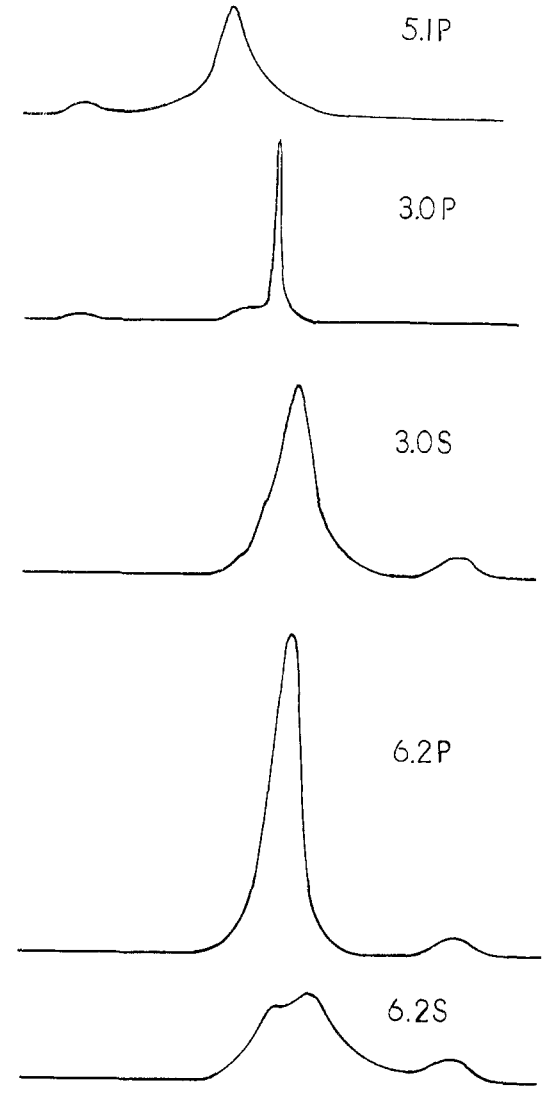


Fig. 4.—Electrophoretic patterns (descending) of various fractions of lymphocyte cytoplasm after 120 minutes under the potential gradient specified.

fraction 5.1P, fraction 3.0P: veronal buffer, $\mu = 0.10$, *p*H 8.6, $E = 6.5$ volt cm.$^{-1}$

fraction 3.0S, fraction 6.2P, fraction 6.2S: sodium phosphate, $\mu = 0.10$, *p*H 2.9, $E = 5.5$ volt cm.$^{-1}$

usefulness of the turbidity curves. In retrospect, however, the selection of *p*H 3.0 and $\mu = 0.1$ can be rationalized with the turbidity behavior. In the *p*H region 4–6 precipitation of all components in 5.1P is virtually complete throughout the ionic strength range 0.0–0.1. This was verified by centrifuging the turbid solutions and reading the optical density of the supernatants. At *p*H 3, however, there is an ionic strength effect as can be seen in Fig. 3.

Material insoluble at *p*H 5 becomes increasingly soluble as the *p*H is lowered. The solubility of this material is greater at $\mu = 0.0$ than at $\mu = 0.1$. Fractionation followed by analysis of the subfractions indicated that the best separation of components occurred at *p*H 3.0 and $\mu = 0.1$.

The relationship between the number of maxima in the turbidity curves and the number of demonstrable components in the system is not direct. Turbidity does not develop if all components in the system are soluble under the conditions of the experiment and no interaction to produce insoluble products occurs. Where turbidity does occur, if the turbidity is simply insolubility without interaction, one peak apparently develops for each insoluble component. The *p*H at which the maximum occurs is presumably related to the isoelectric point of the component. When an interaction between two or more components does occur, the result may be the formation of an insoluble complex. The maximum with respect to *p*H is then indicative of the *p*H of interaction. Conversely, an interaction to alter the solubility of an otherwise insoluble component likewise may occur. As can be seen in Fig. 2 component 6.2P by itself or mixed with 6.2S gives a maximum at *p*H 6.2 in the turbidity curve. The maximum at *p*H 6.2 is not present in starting fraction 5.1P, suggesting that one or both of the two components in 3.0P interacts with 6.2P and that the reaction product is soluble at *p*H 6.2.

We have found turbidity analyses helpful in suggesting *p*H and ionic strength conditions at which separations may be expected to occur. Our experience with the method is too limited, however, to generalize the relationship. The purpose of this communication is merely to direct attention to the method and to indicate that a relationship exists.

It seems likely that turbidity analysis can be adapted to the study of a variety of protein interactions. Under proper conditions the method is useful as an additional, and possibly fairly sensitive, criterion of purity. A simple turbidity curve or failure to obtain turbidity provides no information about purity; a complex turbidity curve, however, almost certainly indicates heterogeneity.

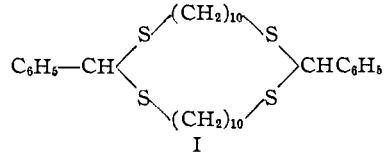
THE RHEUMATIC FEVER RESEARCH INSTITUTE
NORTHWESTERN UNIVERSITY MEDICAL SCHOOL
CHICAGO, ILLINOIS

A Twenty-six-membered Cyclic Dimercaptal[1]

BY C. S. MARVEL, E. A. SIENICKI, M. PASSER AND CHARLES N. ROBINSON

RECEIVED OCTOBER 22, 1953

In a study of the acid-catalyzed reaction of decamethylenedithiol and benzaldehyde in dioxane solutions at 30°, a crystalline solid separated from solution in every experiment to the extent of about 50% of the weight yield. When this solid was isolated by filtration and the solvent evaporated, the expected polymercaptal[2] was obtained. The solid material after repeated recrystallizations from benzene and chloroform melted sharply. Analyses and molecular weight determinations indicated that it was the 26-membered cyclic dimercaptal (I) similar in structure to the 16- and 18-membered



I

ring cyclic mercaptals which were reported by Autenrieth and Beutell from *p*-xylyl dimercaptan and aryl aldehydes and from *m*-xylyl dimercaptan and acetone.[3]

Further evidence for the cyclic structure was furnished from the infrared absorption of the mercaptal since this showed no hydroxyl absorption bands, no carbonyl bands and no mercaptan bands. The X-ray pattern indicated definite crystallinity.[4]

Oxidation of the cyclic mercaptal with monoperphthalic acid in ether solution converted it in good yield to the tetrasulfone which melted at 195–196°.

The ease of formation and good yield of this ring containing 26 members is somewhat unexpected. Other examples of this type of cyclic molecule are being sought.

Experimental

2,15-Diphenyl-1,3,14,16-tetrathiacyclohexacosane.—A solution of 12.9 g. (0.0626 mole) of freshly distilled decamethylenedithiol in 125 ml. of dioxane[5] was added to a solution of 6.6 g. (0.0623 mole) of purified[6] benzaldehyde in 125 ml. of dioxane. A solution of 25 ml. of dioxane saturated with dry hydrogen chloride at room temperature was then added to the mixture. The system was flushed with nitrogen, stoppered, and stirred for four days at room temperature (30–35°). The solid material was removed by filtration and dried to give 9 g. (50%) of white material which melted at 125–128°. After recrystallization from benzene, it weighed 5.7 g. and melted at 133–134°.

Repeated recrystallization from benzene, chloroform (three times) and then benzene again gave a sample of m.p. 135.5–135.8°.

Anal.[7] Calcd. for $C_{34}H_{52}S_4$: C, 69.33; H, 8.90; S, 21.77. Found: C, 70.01; H, 9.07; S, 21.20.

The molecular weight determination on this material was carried out by the method of Menzies and Wright.[8] The ebullioscopic solvent (28 ml.) was thiophene-free reagent-grade benzene which was redistilled and fractionally crys-

TABLE I

EBULLIOSCOPIC MOLECULAR WEIGHT DETERMINATIONS OF BENZALDEHYDE–DECAMETHYLENEDITHIOL CONDENSATION PRODUCT

Expt. no.	*w*, g.	Δ,[a] mm.	Δ*p*,[b] mm.	Δ*t*,[c] °C.	*M*
0	0	8.3(Δ_0)	0	0	...
1	0.0737	11.1	2.8	0.01414	600
2	.1258	13.1	4.8	.02424	596
3	.1884	15.9	7.6	.03838	566
4	.2464	18.1	9.8	.04949	574
				Average	584

[a] The observed differential reading on the Menzies–Wright water thermometer; the first of these is the Δ_0 value. [b] $\Delta p = \Delta - \Delta_0$. [c] $\Delta t = 0.00505\Delta p$.

(1) The work discussed herein was performed as a part of the research project sponsored by the Reconstruction Finance Corporation, Office of Synthetic Rubber, in connection with the Government Synthetic Rubber Program.

(2) C. S. Marvel, Elizabeth H. H. Shen and R. R. Chambers, THIS JOURNAL, **72**, 2106 (1950).

(3) W. Autenrieth and F. Beutell, *Ber.*, **42**, 4346, 4357 (1909).

(4) We are indebted to Dr. R. L. Bohon of the Anderson Physical Laboratory, Champaign, Ill., for the infrared data and to Mr. W. E. Thatcher of this Laboratory for the X-ray examination.

(5) Recently purified by the method of L. F. Fieser, "Experiments in Organic Chemistry," Part II, Second Edition, D. C. Heath and Co., Boston, Mass., 1941, p. 369.

(6) By washing with aqueous sodium carbonate, drying, and twice redistilling under nitrogen.

(7) Microanalyses by C. W. Beazley, Micro-Tech Laboratories, Skokie, Ill.

(8) A. W. C. Menzies and S. L. Wright, Jr., THIS JOURNAL, **43**, 2315 (1921).