CHARACTERIZATION OF NITROCELLULOSE AND CERTAIN OTHER HIGH MOLECULAR WEIGHT SUBSTANCES INCLUDING STUDIES OF THE GENERAL APPLICABILITY OF THE LIGHT SCATTERING METHOD

Thesis by

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Abstract

A description is given of various instruments which have been designed and constructed. These include: a capillary viscometer for use with volatile solvents; dynamic and static osmometers; a recording apparatus for precipitation titration experiments; and instruments for light scattering experiments, including a photoelectric photometer, a prism differential refractometer, and an angular scattering camera.

Osmotic pressure and intrinsic viscosity measurements on solutions of nitrocellulose fractions were used to establish a new value of the Staudinger Constant, $9.39 \cdot 10^{-3}$, for nitrocellulose dispersed in acetone at 25° C. The suggestion is made that the value of the ratio of the number average to the weight average molecular weight of a polymer varies with the degree of molecular heterogeneity. The use of this criterion indicates that nitrocellulose manufactured from wood pulp is distinctly more heterogeneous than that from cotton linters.

A technique has been developed for the estimation of the degree of molecular heterogeneity of nitrocellulose by means of precipitation titration. The rate of precipitation in a solution is followed by measuring the decrease in light transmission. The method was found to be useful in the characterization of artificial blends of nitrocellulose.

A detailed description is given of the technique for making light scattering measurements on nitrocellulose solutions. Carbon disulfide is recommended as a light scattering standard. The values determined for the absolute scattering power of carbon disulfide for light of wave length 4358 Å and 5461 Å are, respectively, $15.1 \cdot 10^{-5}$ and $4.8 \cdot 10^{-5}$.

Light scattering measurements on solutions of nitrocellulose fractions indicate that the molecule is relatively stiff, and is nearly fully extended up to a degree of polymerization of about 100. The "effective bond length" is about 10 times the length of the monomer unit. The suggestion is made that the validity of the Staudinger viscosity relation for nitrocellulose is fortuitous and results from the fact that there is little change in the value of $[\eta]/z$ over a range of z which is usually encountered in nitrocellulose samples.

The interaction between nitrocellulose and the components of a binary solvent has been studied by light scattering and the results have been interpreted in terms of the recent Kirkwood-Goldberg theory. The data indicate that there is considerable interaction between nitrocellulose and certain non-solvents; for example, water, ligroin, butyl chloride.

An investigation has been made of the special problems involved in the application of the light scattering method to the study of protein solutions, and a technique has been developed for making such measurements. The method has been used to establish the molecular weight of an antibody from rabbit serum.

A tentative value has been established for the molecular weight of a preparation of Blood Group A-specific substance on the basis of light scattering and osmotic pressure data in conjunction with A. Pardee's viscosity and diffusion data.

A preliminary investigation has been made of the usefulness of the light scattering method for the study of heat denaturation of serum proteins.

Acknowledgment

I wish to acknowledge the kind guidance of Professor R. M. Badger in all aspects of my graduate work.

I am indebted to Professor Dan H. Campbell and Dr. Stanley Swingle for advice in my study of proteins.

The research which is described in the first five sections of Part I was carried out under the direction of Professor Badger as a part of a research project conducted at the California Institute of Technology under contract with the Office of Scientific Research and Development (Contract OEMsr-881), Professor Linus Pauling, Official Investigator.

Preliminary experiments with the light scattering method were carried out by T. S. Gilman under the direction of Professor Badger and Dr. Jurg Waser. Dr. A. L. Wahrhaftig aided in the design of the electronic equipment.

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Part I

A Study of the Molecular Properties of Nitrocellulose

Introduction

Nitrocellulose, or more properly, cellulose nitrate, was apparently prepared as early as 1832 by Braconnot in France, who treated wood, cotton, and paper with concentrated nitric acid.*

Nitrocellulose has long been the technically most important derivative of cellulose because of its use in the explosives, plastics, and lacquer industries, although its use as a plastic has declined in recent years because of the fire hazard. It has also been a popular material for the scientific investigation of high molecular weight substances because it is readily soluble in a variety of solvents. Since cellulose is now believed to be a linear macromolecule built up from monomers of anhydroglucose, nitrocellulose is considered as a chain of glucose units nitrated to varying degrees of completion.

There are features of the chemical constitution of nitrocellulose which are not yet understood; for example, presence of carboxyl groups, but since this investigation has dealt with the physical properties these will not be discussed.

In spite of the many scientific investigations of the molecular properties of nitrocellulose in the past there are features which are not yet understood. The purpose of this investigation was to carry out as systematic a study of nitrocellulose as time would

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* A detailed account of the early history of nitrocellulose and a bibliography of the literature up to 1900 are given by E. C. Worden, Technology of Cellulose Esters, Volume I, E. C. Worden, Millburn, N. J., 1921

permit in order to gain information about the mean molecular weight of nitrocellulose, the molecular heterogeneity, and the interaction with solvents in dilute solution. The work was begun during the war years and most of the information in Sections I through V was obtained during the course of work carried out on a research project at the California Institute of Technology under contract with the Office of Scientific Research and Development (Contract OEMsr-881).*

Section I gives a brief description of the proper procedure for drying nitrocellulose.

Section II contains a description of procedures for measuring viscosity of nitrocellulose solutions.

Section III is devoted to a description of the technique of osmotic pressure measurements, results, and application to the estimation of molecular heterogeneity.

Section IV is a description of the application of osmotic pressure and viscosity measurements to the study of the stability of double-base powders.

Section V describes the development of a precipitation titration technique for the estimation of molecular heterogeneity.

In Section VI, after a brief summary of the pertinent part of the theory of light scattering, a description is given of the

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* This work which has been de-classified is contained in OSRD Final Report No. 5946, PB No. 30759, Linus Pauling, Official Investigator

light scattering instruments and techniques which have been developed during the course of this investigation.

Section VII contains a summary of light scattering data on nitrocellulose and its interpretation in terms of the size and shape of the nitrocellulose molecule.

Section VIII describes a brief investigation of the shape of the nitrocellulose molecule in different solvents.

Section IX is devoted to an investigation of the interaction in dilute solutions between nitrocellulose and mixed solvents and an interpretation of this interaction in terms of the Kirkwood-Goldberg theory.

Section I. Procedures for Drying Nitrocellulose

Commercial nitrocellulose as received from the nitration plant usually contains about thirty per cent of water which has been added to reduce the possibility of detonation during shipment.

All nitrocelluloses which have been studied during the course of this investigation have been dried at room temperature in a vacuum desiccator over Drierite. The desiccation has in all cases been continued until a five-gram sample of nitrocellulose loses less than 0.5 mg. during a two-hour period of evacuation. Most commercial nitrocelluloses will come to constant weight after twenty four hours of vacuum desiccation, although nitrocelluloses of low nitrogen content apparently lose water less readily than do nitrocelluloses of higher nitrogen content. The removal of moisture by this procedure is apparently satisfactory because representative desiccated samples lost no weight when they were subsequently heated for a few hours at 100° C.

This mild method of drying nitrocellulose is necessary to reduce the possibility of change in its molecular weight. If a sample of nitrocellulose is to be used for a nitrogen determination it can be dried to constant weight at 100° C. without detectable denitration, but this procedure will often cause a distinct decrease in the intrinsic viscosity of its solutions. Figure 1 shows the change in intrinsic viscosity with time of heating at 100° C. for two representative commercial nitrocelluloses.

The desiccation procedure is slow, and a sample may be dried more rapidly by heating at 70° C., especially if an oven is available

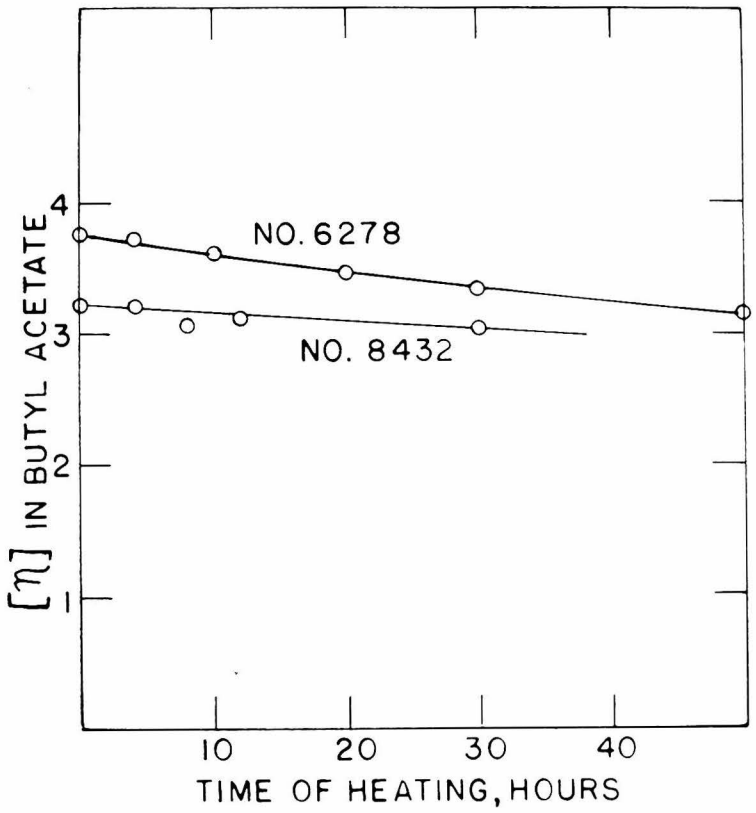

Figure 1. Change of intrinsic viscosity with time of heating at 100° C. Nitrocellulose No. 6278 contains 13.4 per cent nitrogen. Nitrocellulose No. 8432 contains 12.6 per cent nitrogen.

which circulates warm air under forced draft. Selected samples of nitrocellulose have been heated at 70° C. for as long as 100 hours without any detectable change in intrinsic viscosity or nitrogen content.

This latter procedure was not employed in this investigation because of the somewhat elaborate safety precautions which should be observed when nitrocellulose is heated in an oven.

Section II. Viscosity Measurements on Nitrocellulose Solutions

Viscosity measurements are commonly used for the characterization of solutions of high molecular weight substances. Many of the procedures which are used commercially involve measurements on concentrated solutions, because results of great use in the empirical characterization of materials can be obtained from experiments in which the control of conditions need not be inconveniently precise. More valuable results from the scientific standpoint can be obtained from measurements of viscosity on very dilute solutions and the extrapolation of the data to infinite dilution.

In the course of studies of the properties of nitrocellulose, measurements have been made of the viscosity of dilute and concentrated solutions of several nitrocelluloses in various solvents. The use of these measurements for evaluating molecular weights will be described in a subsequent section.

Purification of Solvents

Technical grade butyl acetate was dried over calcium oxide and fractionally distilled under reduced pressure. The middle fraction, which boiled at 53° C. under 55 mm. pressure, was used.

C. P. grade acetone was stored for a day over potassium permanganate and distilled. The distillate was dried over potassium carbonate, decanted into a flask containing anhydrous calcium sulfate, and re-distilled. The resulting material was used for studies of dilute solutions; untreated acetone was used for preparing the concentrated solutions, which were intended to resemble those used for

routine measurements in industrial laboratories.

The alcohol used in preparing the concentrated solutions was commercial 95 per cent ethanol.

Most of the nitrocelluloses used in these studies were representative commercial samples.

Measurement of Intrinsic Viscosity

The intrinsic viscosity, $[\eta]$, of a solute in a particular solvent is defined as follows 1/:

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{\log_e \eta_{rel}}{c} \qquad (1)$$

in which η_{rel} = relative viscosity = η solution / η solvent

η_{sp} = specific viscosity = $\eta_{rel} - 1$, and

c = concentration of solute in grams per 100 ml. of solution

The viscosity of the solutions and the solvent were measured at 25.0° ± 0.1° C. with an Ostwald-Fenske viscometer calibrated according to the procedure recommended by Hatschek 2/. It was found necessary to pass all solutions through Pyrex Fine sintered glass funnels prior to the viscosity determination in order to secure reproducible flow times.

The following routine procedure was adopted for obtaining the necessary data for the extrapolation required by equation (1). A stock solution containing 3 grams per 100 ml. was prepared by

weighing the required amount of dry nitrocellulose. Three solutions were then prepared from the stock solution by successive two-fold dilutions with solvent. The relative viscosity of each of these solutions was then measured.

Measurement of the Viscosity of Concentrated Solutions

The measurement of the viscosity of concentrated solutions of nitrocellulose is described in the following publication.

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A Capillary-Type Viscometer

For Use with Solutions Containing Volatile Solvents, with Application to Measurements of Viscosities of Nitrocelluloses

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A capillary-type viscometer for the study of solutions containing volatile solvents has been designed and tested. Because the solution need not be transferred from the container in which it is prepared, measurements made with this viscometer on concentrated solutions of large molecules, such as those of nitrocelluloses, in volatile solvents are more reproducible than those made with an Ostwald-type capillary viscometer. Because of the small volume of the new viscometer, viscosity determinations may be carried out with much smaller samples than those required by the falling-ball viscometer. The simplicity of this device and its freedom from errors due to concentration changes recommend its application to the study of a variety of substances in solutions containing volatile solvents.

THE standard commercial procedure for measuring the viscosity of nitrocellulose (*1*, *3*) involves the use of a falling-ball viscometer developed by workers at the Hercules Powder Company and described by Speicher (*2*). This procedure requires a nitrocellulose sample of about 20 grams for the preparation of a sufficient volume of 10% solution. The accuracy claimed for the determination is ±3.8%, exclusive of stopwatch errors.

In the course of studies of fractionated nitrocellulose carried out at the California Institute of Technology it became necessary to make accurate measurements of viscosity on small volumes of solution having the same concentration as those used in the standard procedure. The results of measurements made with modified Ostwald capillary-type viscometers varied over a range of several per cent, and the variations appeared to be due to concentration fluctuations arising from volatilization of solvent during transfer of solution to the viscometer. In order to eliminate the necessity for transferring the solution, the authors have developed a modified capillary-type viscometer by means of which it is possible to measure the viscosity of a solution in the container in which it is prepared.

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THE VISCOMETER

A diagram of the viscometer is presented in Figure 1.

The solution to be tested is contained in a glass vial with a threaded mouth, and the viscometer assembly is screwed onto the vial to replace the conventional screw cap. Because several vials can be used interchangeably with the same viscometer, it is essential that all the vials used have nearly the same cross-sectional area, so that the effective head of liquid in the filled viscometer will not be dependent upon the vial to which it is attached. If vials or other containers differing in design and cross-sectional area are used with the same capillary tube, the capillary tube should be calibrated separately for each type of container.

The size of capillary tubing to be used depends upon the range of viscosities to be measured. The times of flow obtained with a given size of capillary tubing should be approximately in the range 100 to 500 seconds; with times shorter than 100 seconds it may be necessary to apply a kinetic energy correction. With viscometers having the dimensions shown in Figure 1, the time of flow for castor oil (viscosity about 8 stokes) is of the order of 200 seconds. The range of viscosities that can be measured with the assortment of capillary tubes used by the authors is 1.8 to 70 stokes. With solutions more viscous than 70 stokes the slowness of drainage from the walls of the bulb may be troublesome.

PROCEDURE

A sample of nitrocellulose (about 1 gram) is weighed into the vial, and enough solvent (usually a mixture of commercial denatured ethyl alcohol and acetone in the ratio 10 to 90 by volume)

is added from a buret to give a solution of the desired concentration (usually 10% of nitrocellulose). The vial is closed with a screw cap lined with tinfoil and is then rotated on a mixing wheel until solution is complete. The screw cap is removed, the viscometer assembly is immediately attached, and the viscometer tube is raised or lowered through the sleeve of rubber tubing until the fiducial mark at the lower end of the tube is level with the meniscus of the liquid in the vial. The assembly is then clamped vertically in a thermostat; it is essential that the mounting clamp be so designed that the vertical mounting of the viscometer is reproducible. After the liquid in the vial has reached thermal equilibrium with the thermostat, it is forced up into the viscometer bulb by connecting the side tube with a source of compressed air. When the bulb has been filled the pressure is released, and the time required for the meniscus to drop from the mark above the bulb to the mark below it is measured with a stopwatch. The viscosity of the solution is calculated from the time of flow in the usual way.

The viscometer is calibrated with liquids of known viscosity. Those used by the authors were glycerol, castor oil, and machine oil (SAE 70).

RESULTS

The new type of viscometer was used in determinations of the viscosities of 10% solutions of several representative commercial nitrocelluloses in 10 to 90 alcohol-acetone. Individual determinations made on a given solution without changing the viscometer setting were usually within 0.1% of each other and showed no systematic trends. Apparently volatilization of the solvent resulting from the "breathing" of the viscometer during the measurement of viscosity has a negligible effect. Determinations of viscosity on seven individual samples of a typical nitrocellulose, Hercules nitrocellulose No. 6278, in 10.0 to 90.0 alcohol-acetone gave an average viscosity of 46.02 stokes with a total spread of 3.3% and a mean deviation of 0.7%; this deviation represents the combined uncertainties of weighing the sample, measuring out the solvent, setting the fiducial mark, and measuring the time of flow. The authors have found that the results are not critically dependent on the volume percentage of denatured alcohol in the solvent; this percentage may be varied between 9.5 and 10.5 without significantly affecting the time of flow.

No large errors are introduced by the uncertainty in adjusting the lower fiducial mark to the meniscus if care is taken. In an experiment in which castor oil was used and in which the fiducial mark was reset before each measurement of time of flow, a total spread of 0.4% in the time of flow was observed in six determinations (averaging 154.3 seconds).

Table I. Viscosities of Representative Nitrocelluloses

Hercules Nitrocellulose No.	Source of Cellulose	Nitrogen, %	Viscosity Hercules Seconds: By new viscometer	Viscosity Hercules Seconds: Reported by manufacturer
3293	Pulp and linters	11.95	0.50	0.5
3713	Linters	10.92	2.05	3.0
6278	Pulp	13.40	10.0	9
5245	Pulp	12.52	14	15, 22
503-3-1	Linters	13.22	15	15
5248	Pulp	13.42	14.2	17, 15

The data obtained with these viscometers can be used to estimate nitrocellulose viscosities in the conventional Hercules smokeless seconds. It has been reported by Speicher (*2*) that one Hercules second is equivalent to 3.77 poises. The density of 10% solutions of nitrocellulose in 10 to 90 alcohol-acetone is 0.837 gram per ml.; hence 1 second is equivalent to 4.50 stokes. The viscosities of several nitrocelluloses, as measured with the new viscometers and calculated in Hercules seconds, are presented in Table I, where they are compared with values reported by the manufacturer.

It may be concluded that the new viscometer may be used satisfactorily for the determination of viscosities of nitrocellulose

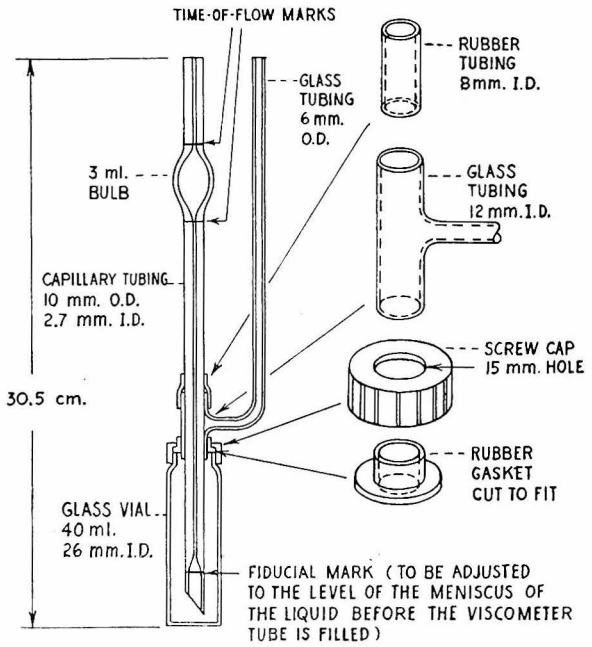

Figure 1. Diagram of Viscometer

samples. Because it is less subject to errors caused by volatilization of solvent than are other commonly used viscometric methods, the new device should likewise be useful for viscosity measurements on many other substances, the viscosities of which in volatile solvents are critically dependent on concentration. Another obvious advantage of its use in research studies, in comparison with the falling-ball method, is the small size of sample required (about 1 gram), although for control use the selection and accurate weighing of a representative sample of this size may be difficult and time-consuming. When enough material is available the use of containers considerably larger than the recommended vial, such as screw-cap bottles, would probably eliminate this disadvantage.

Solutions of some samples of nitrocellulose and other materials may contain large amounts of suspended dirt, lint, and other insoluble solid matter which may lead to results that are not reproducible. Measurements made with large-bore capillaries on solutions of relatively high viscosity, for which the viscometer is most useful, would be much less subject to error from this source than measurements made with finer capillaries on solutions of low viscosity. Of course, much of the advantage of using this viscometer would be lost if it were used with solutions containing enough suspended material to affect the reproducibility of measurement, unless the suspended material could be thrown to the bottom of the vial by centrifugation; filtration and attendant transfers of the solution would in many cases lead to evaporation of solvent.

ACKNOWLEDGMENTS

The authors wish to express their thanks to George J. Doyle and John Hardy, who carried out most of the viscosity determinations.

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(2) Speicher, J. K., *Cellulose*, **1**, 232–4 (1930).
(3) U. S. Army Specification 50-14-3B, p. 10 (Feb. 10, 1943).

Based on work done for the Office of Scientific Research and Development under Contract OEMsr-881 with the California Institute of Technology. Contribution from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, No. 1034.

Section III. Osmotic Pressure

Solutions of high molecular weight substances have long been known to give readily measured osmotic pressures, but early workers were uncertain as to how to interpret the experimental data. After Ostwald 3/ pointed out that data on colligative properties of solutions of high polymers should be extrapolated to zero concentration if values of the molecular weight were to be determined, many workers have successfully employed the method. A. Dobry 4/ presented the first conclusive experimental evidence that the osmotic pressure of a solution of a high molecular weight substance is independent of the solvent employed and is dependent only on the molecular weight of the solute, solute concentration, and temperature.

The following program of osmotic pressure measurements was undertaken primarily to learn whether the degree of molecular heterogeneity of nitrocellulose could be estimated from a comparison of the number-average molecular weight from osmotic pressure with the weight-average molecular weight from viscosity and light scattering.

Since this work was completed a number of articles have been published which describe similar apparatus and techniques for measuring osmotic pressure 5, 6, 7/ . My work with the osmotic pressure method is included, however, in order that this thesis will be a complete record of the research which I have carried out in this laboratory.

Membranes and Solvent

The validity of osmotic pressure data depends on the efficiency of the membrane which is employed. An ideal membrane would have the following characteristics: it would be readily permeable to the solvent but impermeable to the solute molecules; the membrane would not change with time by excessive swelling or dissolving in the solvent, absorbing solute molecules, or becoming impermeable by mechanical plugging; it would have sufficient strength to support itself in an osmometer; and finally, it is desirable that the membrane material be one that is easily obtained or simple to prepare.

Since there are a large number of commercial films now available a semi-quantitative study of the permeability of these films was made. The permeability was tested by the use of the apparatus shown in Figure 2. Each film was clamped between the brass junctions and the apparatus was filled with solvent. The rate of flow of solvent though the membrane was determined by measuring the rate of movement of the solvent through the capillary tube B. Since the pressure on the membrane does not change appreciably during a determination; that is, since the level of the solvent in tube A does not change very much with the movement of the solvent along the capillary tube B, the rate of flow of solvent was taken as being proportional to the permeability of the film.

The permeability of various films to butyl acetate and acetone is presented in Table 1. The choice of solvents was limited to these two because they are the solvents which were used in other

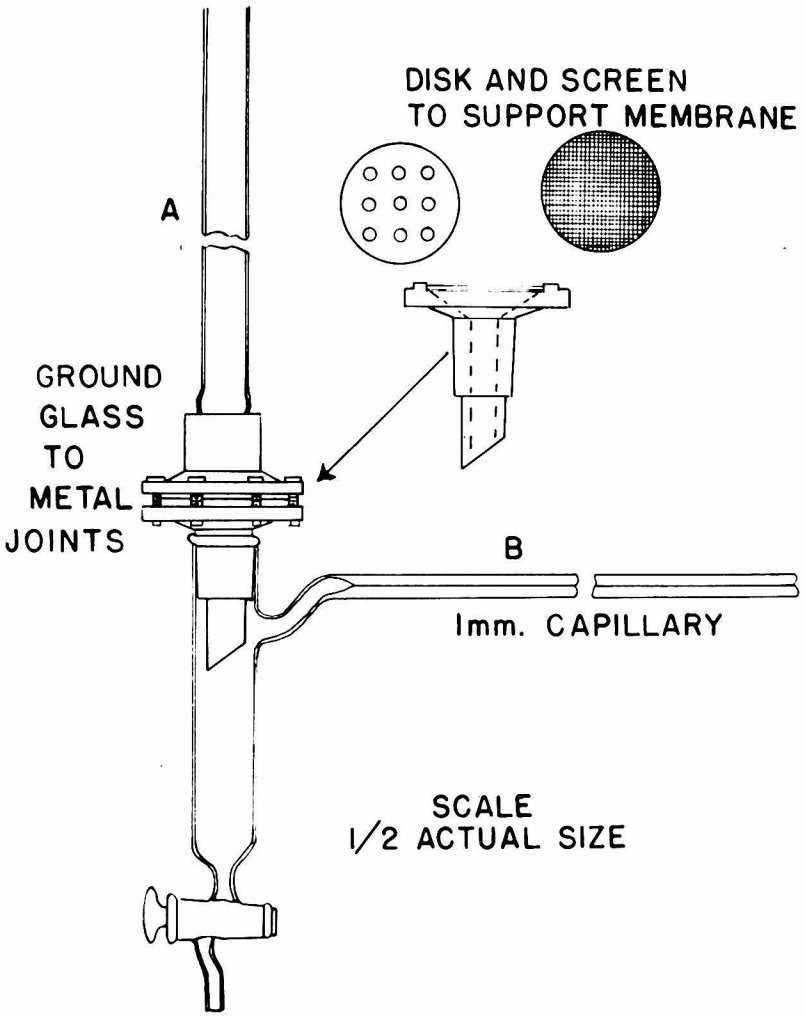

Figure 2. Apparatus to measure permeability of membranes

phases of this investigation. The permeability of each membrane is arbitrarily compared with that of a cellophane membrane; that is, if a film was one-half as permeable as cellophane it is rated as two; if it was one-third as permeable as cellophane it is rated as three, and so on. The reference membrane, the best membrane found, was commercial duPont cellophane (gauge 300, or 0.0008 inches thick) which had been conditioned by soaking for fifteen minutes in hot water, for one hour in 7 N ammonium hydroxide, and then rinsed successively in water, absolute alcohol, and acetone.

Table 1

Relative Permeability of Various Films

Membrane Material	Solvent	Permeability
Conditioned Cellophane Gauge 300	Acetone	1
Pliofilm 120 (P4A)	Butyl Acetate	Impermeable
Pliofilm 140 (NI)	Butyl Acetate	Impermeable
Cellophane No. 300	Butyl Acetate	Impermeable
Victory Film 200 (P9)	Butyl Acetate	Soluble in Solvent
Animal Membrane	Butyl Acetate	Soluble in Solvent
Parafilm	Butyl Acetate	Soluble in Solvent
Cellophane No. 300 Untreated	Acetone	5
Pliofilm 140 (NI)	Acetone	6
Pliofilm 120 (P4A)	Acetone	Impermeable
Rubber film (0.002 inches thick)	Acetone	6
Nylon (0.001 inches thick)	Acetone	Impermeable
Animal Membrane	Acetone	Impermeable
Saran Gauge 125	Acetone	3
Polyvinyl Chloride	Acetone	2

On the basis of these preliminary experiments, commercial cellophane was chosen as a membrane material and acetone as the solvent. Subsequent experiments showed that cellophane which had been soaked in water for twelve hours and then rinsed with acetone served just as well as the especially conditioned cellophane. This treatment gave a membrane satisfactory for use with nitrocellulose which had an average molecular weight greater than 20,000. If the molecular weight was much lower than this, a small fraction of the nitrocellulose often diffused through the membrane.

A peculiar effect was noted with each film tested; that is, each film gradually became less permeable as solvent passed through the membrane, and this rate of decreasing permeability was nearly the same for each membrane examined. After two hours in the apparatus most of the films were completely impermeable to acetone. Since the films did not become impermeable merely by soaking in acetone it seemed as if the flow of solvent through the pores of a membrane changed it in some way. Since the solvent could conceivably contain particles that might plug a membrane, a test was made using redistilled acetone. The results were the same.

Morton 8/ has observed that a viscose film slowly became impermeable if ordinary distilled water was passed through it, but that the same film retained a constant permeability to water that had been redistilled, condensed in a block tin coil, and collected in a stainless steel container.

In order to check the possibility that acetone might dissolve something from the brass parts of the apparatus which might affect

the permeability of films, silver-plated metal parts were substituted in the apparatus. Following this substitution the results were the same as had been observed with the brass apparatus.

This "plugging" of membranes apparently does not affect the osmotic equilibrium in a static osmometer, since only a small amount of solvent flows through the membrane during a determination.

Osmometers

The osmotic pressure of a solution may be determined by two general methods. The first, the so-called static method, involves the measurement of a height of liquid in a capillary tube, caused by the influx of solvent into a solution through a semi-permeable membrane. The second, the dynamic method, involves the determination of the rate at which solvent diffuses through the membrane into the solution, as a function of pressure applied to the solution side of the membrane.

A dynamic osmometer shown in Figure 3 was constructed from a design which is essentially the same as that used by other workers. This instrument has several advantages 9, 10/. The large membrane permits equilibrium to be attained rapidly. The large heat capacity of the stainless steel block reduces errors due to temperature fluctuations. The design is such that a small change in temperature will cause essentially the same change in the height of the fluid in the solution- and the solvent-capillaries.

After preliminary experiments the use of the dynamic osmometer was discontinued. The membrane was found to sag for several hours

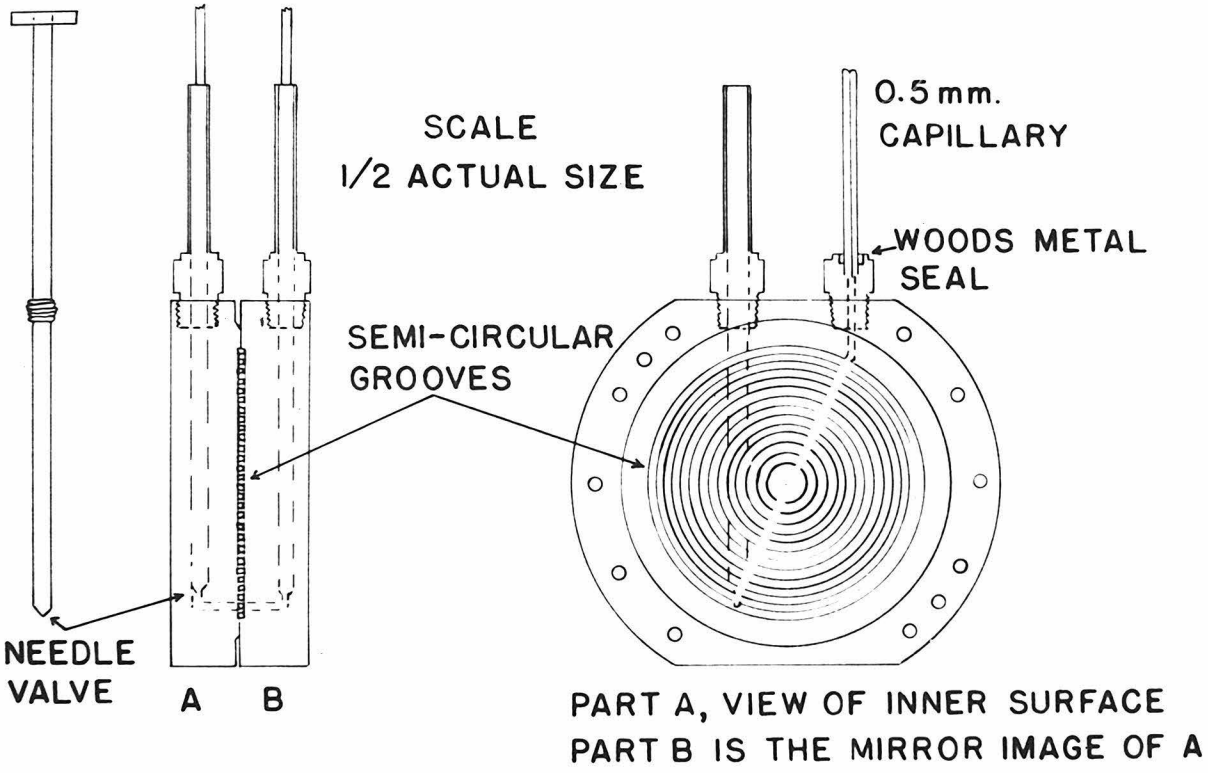

Figure 3. Dynamic Osmometer

as pressure was built up on the solution side, and this spurious capillary rise reduced the usefulness of the osmometer as a rapid instrument. In addition, leakage around the membrane was difficult to prevent because of the inadequate precision with which the inside surfaces of the two parts of the osmometer were finished.

All of the actual osmotic pressure measurements were made with the static osmometer shown in Figure 4. The design follows that described by Wagner 11/. The principal improvement is the short section of capillary tubing which is clipped to the tube of the osmometer to provide a convenient method of correcting for the capillary rise 12/. The assembled osmometer is placed in a glass cylinder with enough solvent to cover the lower end of the short section of capillary tubing. If the two tubes have exactly the same radius, and if the surface tension of the solvent and solution are essentially the same, the difference between the levels of solvent and solution in the respective capillaries is a direct measure of the osmotic pressure of the solution. In practice, however, it was often necessary to apply a small experimentally determined correction to the observed differences in height.

The complete osmotic pressure apparatus consists of five osmometers assembled in a rack which is submerged in a cylindrical glass water bath. The rack can be rotated to bring each osmometer in turn to a point convenient for observation. The water bath was held at a temperature of $25.00^{\circ} \pm 0.005^{\circ}$ C. with the aid of an air bath around the entire assembly. This degree of temperature control was necessary because a change of 0.01° C. was sufficient to cause

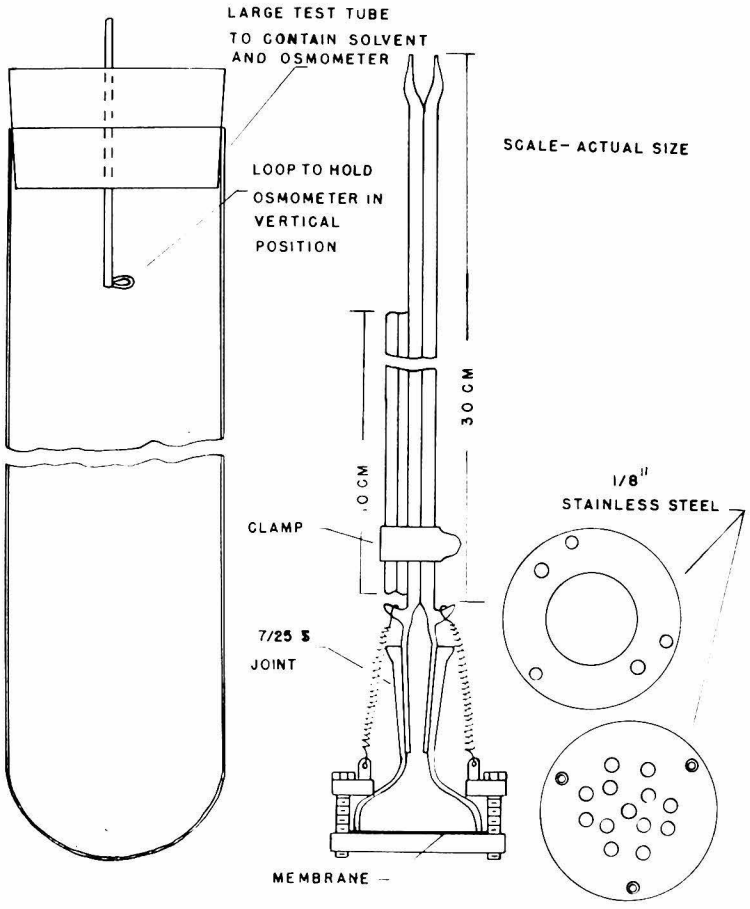

Figure 4. Static Osmometer

a change of 0.2 mm. in the height of the solution in the capillary due to the "thermometer effect" in the osmometer.

The procedure for making a measurement was as follows: The osmometer was assembled as shown in Figure 4. Since the membrane of conditioned cellophane could not be allowed to become dry, it was placed in the osmometer while water-wet and then was washed free of water with acetone. The membrane would shrink somewhat when washed with acetone and become taut enough to remove wrinkles. The osmometer cell was then filled with the nitrocellulose solution and the capillary tube was put in place in such a manner that no air bubbles were trapped in the cell or the capillary. The height of the solution was adjusted to be about midway of the length of the capillary. The osmometer was then placed in the cylinder which contained enough solvent to cover the lower end of the auxiliary capillary tube. Usually five osmometers were assembled for each determination of the same nitrocellulose. These were then placed in the rack and mounted in the constant temperature bath. After two hours the contents of the osmometers were assumed to have reached temperature equilibrium with the bath, and the difference in the height of the solvent and of the solution in the respective capillary tubes was measure to the nearest 0.1 mm. with a cathetometer. Subsequent observations were made at two-hour intervals during the normal working day for the next twenty-four to thirty-six hours.

Treatment of Osmotic Pressure Data

A graph was made by plotting the observed differences in height of the capillaries against e^{-t} where t is the time in units of ten hours. The difference in height at equilibrium was determined by extrapolation to infinite time. The density of the solution in the osmometer was assumed to be the same as its density before the measurement was made. From the value of the density of the solution and of the difference in height of the solvent and solution in their respective capillaries at equilibrium, the osmotic pressure was calculated.

The results of an osmotic pressure determination can be given a simple interpretation only if the membrane is truly impermeable to solute molecules. If the data could be extrapolated to a definite value for the equilibrium pressure, and if less than 0.5 per cant of the nitrocellulose diffused through the membrane during a determination, the assumption was made that the observed pressure did not differ significantly from the true osmotic pressure.

At sufficient dilution van't Hoff's law in its simple form as a limiting law is applicable to solutions of nitrocellulose 13/. Since osmotic pressure determinations which are made with extremely dilute solutions are not very accurate, molecular weights can be determined more satisfactorily by extrapolating to infinite dilution the results of measurements on solutions of a relatively high concentration.

The usual method of treating osmotic pressure data is to plot

the osmotic pressure divided by the concentration, π/c, against the concentration and extrapolate the curve to a value of π/c at infinite dilution. This type of plot does not differ significantly from a straight line and has a slope which within the limits of experimental error, is independent of the molecular weight of the solute, but is different for different solutes or solvents 14/.

This method is illustrated by Figure 5. The nitrocelluloses are fractions which were supplied us by Dr. J. W. Williams of the University of Wisconsin and which are described in detail in a subsequent section.

The molecular weight of a nitrocellulose is calculated by the expression:

$$\overline{M}_n = RT/(\pi/c)_{c \to 0} \qquad (2)$$

in which

$\overline{M}_n$ is the number average molecular weight

R is the gas constant in 100 ml. atm./mole degree

π is the osmotic pressure in atmospheres

T is the absolute temperature

c is the concentration in grams per 100 ml. of solution, and

$(\pi/c)_{c \to 0}$ is the value of π/c obtained by extrapolation to infinite dilution

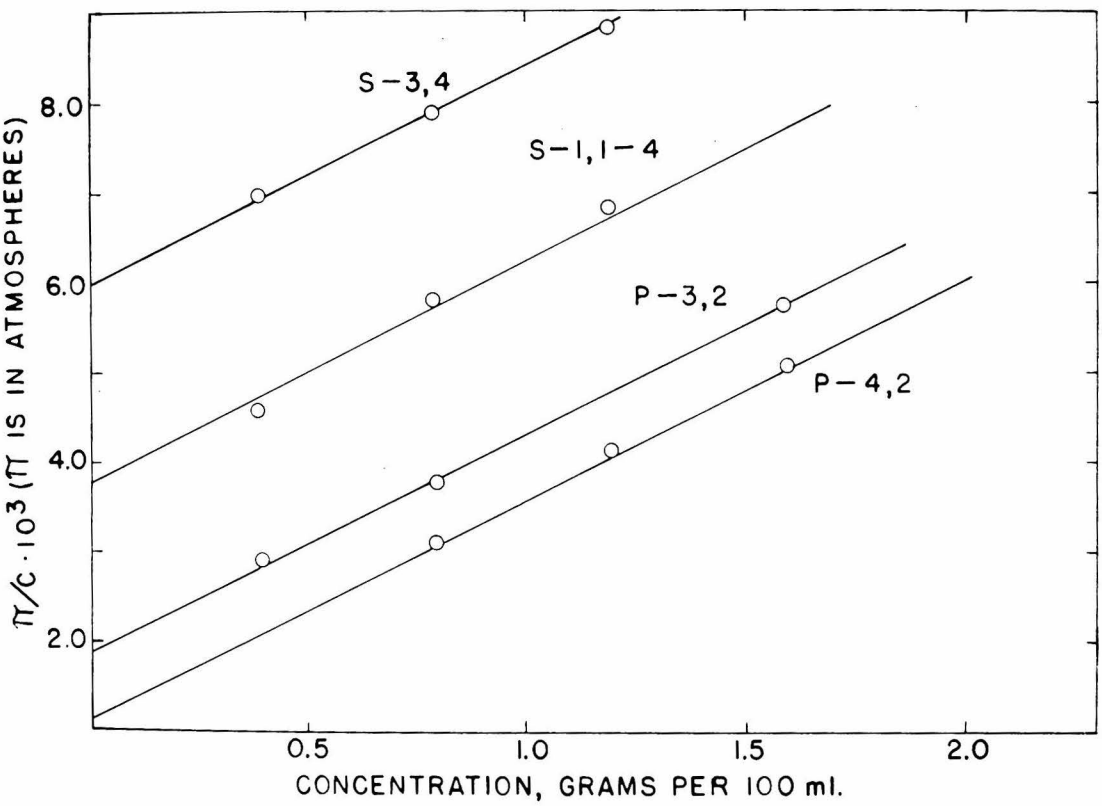

Figure 5. Osmotic pressure data for four nitrocellulose fractions in acetone solution

Application of the Osmotic Pressure Method to the Study of Nitrocellulose

The use of osmotic pressure data to evaluate the Staudinger Constant for nitrocellulose and to estimate the degree of molecular heterogeneity of nitrocellulose is described in the following publication.

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MOLECULAR PROPERTIES OF NITROCELLULOSE. II

STUDIES OF MOLECULAR HETEROGENEITY[1]

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INTRODUCTION

Of the methods currently employed for determining the average molecular weights of high polymers, procedures involving measurements of osmotic pressure and viscosity are probably the most frequently used. These procedures require comparatively simple apparatus and are capable of precision satisfactory for many purposes; however, they lead to different results when used for measurements on mixtures of polymeric species of different molecular weight.

When measurements of osmotic pressure on solutions of a polymeric mixture

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are extrapolated to infinite dilution, the results can be used to calculate the number-average molecular weight defined by the relation

$$\bar{M}_n = \frac{1}{\Sigma f_i/M_i} \tag{1}$$

in which $\bar{M}_n$ is the number-average molecular weight, f_i is the fractional weight of the i^{th} species in the mixture, and M_i is the molecular weight of the i^{th} species. In this average the lower-molecular-weight material has a predominant effect, and traces of material of very high molecular weight have little influence.

When measurements of viscosity are made on similar solutions, the data cannot necessarily be related to the molecular weight of the solute by any simple expression (3); however, for many straight-chain polymers the following empirical equation is found to hold reasonably well:

$$[\eta] = K_s\bar{M}_w/M_0 \tag{2}$$

In this so-called Staudinger expression K_s is a constant dependent upon the polymeric material and the solvent but independent of molecular weight, M_0 is the molecular weight of the monomeric unit in the polymer, $\bar{M}_w$ is the weight-average molecular weight defined by the relationship

$$\bar{M}_w = \Sigma f_i M_i \tag{3}$$

and $[\eta]$ is the "intrinsic viscosity" of the solute defined by the relationship

$$[\eta] = \lim_{c\to 0} \frac{\ln \eta_{rel}}{c} \tag{4}$$

where η_{rel} is the viscosity of the solution relative to that of pure solvent and c is the concentration in grams per 100 ml. The weight-average molecular weight as defined by expression 3 is predominantly influenced by the presence of material of high molecular weight.

The number-average and weight-average molecular weights of a sample are equal only if the sample is molecularly homogeneous; otherwise the ratio of the weight-average to the number-average molecular weight is greater than unity, and its magnitude may be taken as a rough measure of the heterogeneity of the sample. In cases where the molecular-weight distribution function has a single broad maximum of the kind obtained in the polymerization of a monomer or in the degradation of a single high polymer (4), the ratio may be expected to be slightly less than two. Other forms of distribution function yield larger values of the ratio, but it seems probable that values in excess of two will only be encountered in practice when the distribution function has more than one maximum.

We have made measurements of osmotic pressure on acetone solutions of several supposedly homogeneous nitrocellulose fractions of different molecular weights and have used the results to evaluate K_s for nitrocellulose containing about 13.4 per cent of nitrogen. With the aid of data reported by previous workers we have used our results to calculate K_s as a function of nitrogen content

and have studied the molecular heterogeneities of various nitrocelluloses by means of measurements of number-average and weight-average molecular weights on samples prepared from different sources.

It should be emphasized that a polymeric mixture is not completely characterized by determinations of its weight-average and number-average molecular weights, for identical values of these quantities can be obtained with different molecular distribution functions. The object of the determinations described below is to obtain approximate information about molecular heterogeneity by procedures which are much simpler than the extensive fractionations necessary for determining complete molecular distribution functions.

EXPERIMENTAL

Materials

The nitrocellulose fractions used in the evaluation of the Staudinger constant, K_s, were prepared by Dr. J. W. Williams and coworkers at the University of Wisconsin. The fractionation procedure involved dissolving a sample of nitrocellulose in a mixture of 3 parts (by volume) of commercial 95 per cent alcohol and 5 parts of acetone and then precipitating part of the sample by the addition of ligroin under controlled conditions. The fractions from the first precipitations were refractionated by the same procedure. The final fractions are thought to be more nearly homogeneous with regard to nitrogen content and molecular weight than most other nitrocellulose samples that have been available for such studies.

The nitrocellulose samples used in the studies of heterogeneity were taken from standard commercial lots prepared for use in the manufacture of smokeless powder.

The acetone used for solvent in the osmometric and viscometric studies was purified by the procedure described in the first paper of this series (1).

Osmometric measurements

Measurements of osmotic pressure were made with static osmometers modeled after the one described by Wagner (6). The capillary rise of the solution in the osmometer was corrected for with the use of an open section of capillary immersed in the solvent by a procedure similar to that developed independently by Zimm and Myerson (8).

Commercial cellophane was the most satisfactory membrane material investigated. The only pretreatment given to the cellophane was to soak it in distilled water for at least 12 hr. before it was used and to wash out the water with acetone at the time the osmometer was being assembled. Membranes treated in this way were satisfactory for use with nitrocelluloses having average molecular weights greater than 20,000, but a few per cent of samples of lower average molecular weight diffused through the membranes.

The data were treated by plotting the osmotic pressure, π, divided by the concentration, c, against concentration and extrapolating the curve to a value of

π/c at infinite dilution. This type of plot does not differ significantly from a straight line and has a slope which, within the limits of experimental error, is independent of the molecular weight of solute but is different for different solutes or solvents (2). The molecular weight of the sample was calculated by the equation

$$\bar{M}_n = \frac{RT}{\lim_{c \to 0} (\pi/c)} \tag{5}$$

in which T is the absolute temperature, R is the gas constant in 100 ml. atm./mole degree, and the other quantities have been defined previously.

TABLE 1

Staudinger constants for nitrocellulose fractions in acetone

FRACTION NUMBER	NITROGEN	NUMBER-AVERAGE MOLECULAR WEIGHT, $\bar{M}_n$ (OSMOTIC VALUE)	DEGREE OF POLYMERIZATION	INTRINSIC VISCOSITY	STAUDINGER CONSTANT $K_s \times 10^3$
	per cent				
S-3,4	13.36	41,000	144	1.32	9.15
S-1,1–4	13.44	64,700	227	2.24	9.88
P-3,2	13.41	130,800	459	4.37	9.52
P-4,2	13.42	216,400	759	6.83	9.00
Average					9.39

Viscometric measurements

Measurements of viscosity were made with Ostwald-type capillary viscometers, and the intrinsic viscosities of the solutes were calculated by the procedure described in the first paper of this series (1).

RESULTS AND DISCUSSION

Evaluation of Staudinger constant

The results of measurements of osmotic pressure and viscosity on four nitrocellulose fractions are presented in table 1. The data indicate that the Staudinger relation is obeyed within the probable accuracy of the measurements by nitrocelluloses having molecular weights between 40,000 and 200,000.

The value of the constant presented in table 1 is valid only for use with nitrocellulose samples containing about 13.4 per cent of nitrogen, and then only if the solvent is purified acetone and if the viscosities are measured at 25°C. In order to obtain values applicable to all nitrocelluloses, we have used the data of Wannow (7). He nitrated cellulose to various degrees under conditions such that the degrees of polymerization of the products were the same as indicated by osmotic-pressure measurements. By means of measurements of viscosity on these samples he was able to express the fractional change in K_s with change in

nitrogen content, but he was not able to obtain an absolute value for K_s because his samples were not molecularly homogeneous. With the use of the value of K_s for nitrocellulose containing 13.4 per cent of nitrogen obtained from table 1 and with the data of Wannow we have calculated values of K_s for various nitrogen contents from 10.7 to 13.6 per cent and have plotted the results in figure 1.

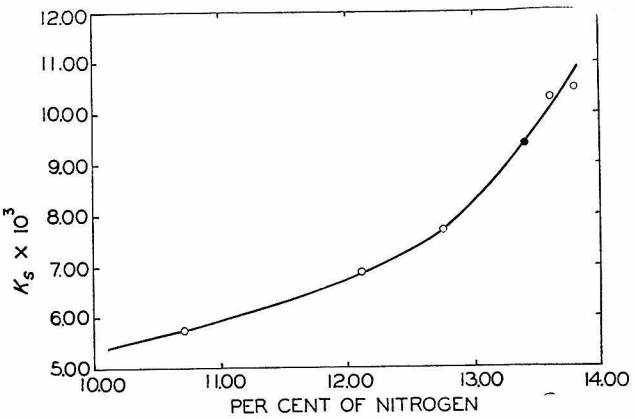

FIG. 1. Dependence of Staudinger constant in acetone on nitrogen content of nitrocellulose. ●, determined in these laboratories; ○, calculated from the data of Wannow with the use of the other point.

TABLE 2

Heterogeneities of commercial nitrocelluloses

NITROCELLULOSE NUMBER	SOURCE	NITROGEN	NUMBER-AVERAGE MOLECULAR WEIGHT, $\bar{M}_n$	INTRINSIC VISCOSITY, $[\eta]$	DEGREE OF POLYMERIZATION	WEIGHT-AVERAGE MOLECULAR WEIGHT, $\bar{M}_w$	$\bar{M}_w/\bar{M}_n$
		per cent					
6278.....	Wood	13.40	43,300	3.07	327	93,100	2.15
8432.....	Wood	12.67	41,600	2.65	351	96,000	2.31
10405.....	Wood	13.26	44,200	3.00	333	94,200	2.13
5167.....	Cotton	13.46	65,900	3.50	365	104,200	1.58
5168.....	Cotton	12.60	53,400	2.85	383	104,200	1.95
10411.....	Cotton	13.23	56,700	3.12	351	98,700	1.74

It should be emphasized that the curve presented in figure 1 is very approximate and can probably be refined by subsequent experiments. The absolute values of all points are based on the assumption that the fractions used in these experiments were molecularly homogeneous, and the relative values of the Staudinger constant taken from Wannow's data are based on the assumption that the distribution of molecular species was the same in each of his samples. This second assumption is not necessarily justified by the fact that the number-average degrees of polymerization of all Wannow's samples were virtually identical.

Molecular heterogeneity of commercial nitrocelluloses

Measurements of osmotic pressure and viscosity were made on dilute acetone solutions of six commercial nitrocelluloses, three of which had been prepared from wood pulp and three from cotton linters. The results of these measurements were used to calculate the weight-average and number-average molecular weights of these samples by the procedures described above, and the data are presented in table 2.

The results demonstrate that the nitrocelluloses prepared from wood are distinctly more heterogeneous than those prepared from cotton. It is interesting to note that the heterogeneities of the homogeneously nitrated samples containing about 12.6 and 13.4 per cent of nitrogen are not significantly different from those of the nitrocelluloses containing about 13.2 per cent of nitrogen, which were blended from materials of the other two types. Since the samples studied in table 2 were all prepared to be of approximately the same viscosity or weight-average molecular weight, the greater heterogeneity of these particular wood nitrocelluloses is probably due to the presence of a considerable fraction of low-molecular-weight material. These observations are consistent with those reported by other workers (5).

SUMMARY

The absolute value of the Staudinger constant for nitrocellulose in acetone has been determined by means of measurements of osmotic pressure and of viscosity and by the use of data previously reported in the literature.

Measurements of number-average and weight-average molecular weights on six commercial nitrocelluloses indicate that materials prepared from wood pulp are distinctly more heterogeneous molecularly than those prepared from cotton linters.

We are indebted to Dr. J. W. Williams for the nitrocellulose fractions which rendered this investigation possible. We are also indebted to Dr. Robert B. Corey for helpful suggestions during the progress of the research.

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Section IV. Application of Osmotic Pressure and Viscosity Measurements to the Study of Smokeless Powder

Considerable time was spent during the war in making osmotic pressure and viscosity measurements on nitrocellulose samples from domestic and foreign double-base powders. Most of this work is still classified, but the following application of these methods has been released in OSRD Report No. 5946, PB No. 30759.

During the course of an investigation of the stability of double-base powders various samples of powder were heated at 65° C. to accelerate the ageing process. Samples of a cordite, JP 76, which were heated for periods longer than 30 or 40 days, were found to contain material which was insoluble in acetone and all common nitrocellulose solvents. One or two drops of dilute ammonium hydroxide, when added to 25 ml. of a suspension of this insoluble material in acetone, would cause immediate solution. Dilute sodium hydroxide had the same effect, while dilute acid apparently had no effect. The nitrogen content of the material was not significantly different from that of the powder which did not insolubilize. An X-ray diffraction photograph of the insoluble material was practically indistinguishable from similar photographs of nitrocellulose of the same nitrogen content.

These preliminary experiments suggested that the material was a nitrocellulose which had become insoluble by some chemical reaction which resulted in the formation of cross-linkages between molecules, rather than by extreme denitration. A series of osmotic pressure and viscosity determinations were then made on

solutions prepared from aged powders in order to verify this hypothesis.

Procedure for Measurement of the Osmotic Pressure of Solutions of Powders

If the membrane which is used in osmotic pressure measurements is permeable to all species of molecules in solution except the nitrocellulose molecules, the presence of substances other than nitrocellulose should have little effect on the osmotic pressure.

The osmotic pressure of a sample of JP 76 was measured and the result compared with that of a similar measurement on a sample of the same powder which had been extracted with ether to remove the nitroglycerin, plasticizer, and stabilizer. The osmotic pressure of the two solutions reached the same steady state value, but the solutions of unextracted powder took about twice as long to reach equilibrium. The slopes of the curves made by plotting π/c against c for the two solutions were the same within experimental error.

The procedure adopted for preparing a powder sample for an osmotic pressure determination involved the extraction of the powder with a suitable solvent, the removal of most of the solvent from the residue by several hours of vacuum desiccation, and the preparation of a solution in chemically pure acetone. After twenty-four hours of mixing, the insoluble material was removed by filtration, and an osmotic pressure determination was made on the solution. The weight of nitrocellulose in solution was calculated from the known percentage of nitrocellulose in the unextracted powder and from the amount of insoluble material as determined in

a separate experiment.

Procedure for Viscosity Measurement of Solutions of Powders

Viscosity measurements were made on solutions of the soluble material from the whole powder. The assumption was made that constituents other than nitrocellulose had but little effect on the viscosity of the solutions and that this effect was substantially constant through any series of heated samples of powder.

For the purpose of comparing the viscosities of solutions prepared from samples of powder degraded to different degrees, a function of the viscosity was calculated which was called the "apparent intrinsic viscosity" and was defined as follows:

$$[\eta]_{app} = \lim_{c \to 0} \frac{\log_e \eta_{rel}}{c} \qquad (3)$$

in which $[\eta]_{app}$ is the apparent intrinsic viscosity,

η_{rel} is the relative viscosity of the solution, and

c is the calculated grams of nitrocellulose per 100 ml. solvent

Results and Discussion

Viscosity and osmotic pressure data on heated samples of JP 76 are tabulated in Table 2 and presented in Figure 6.

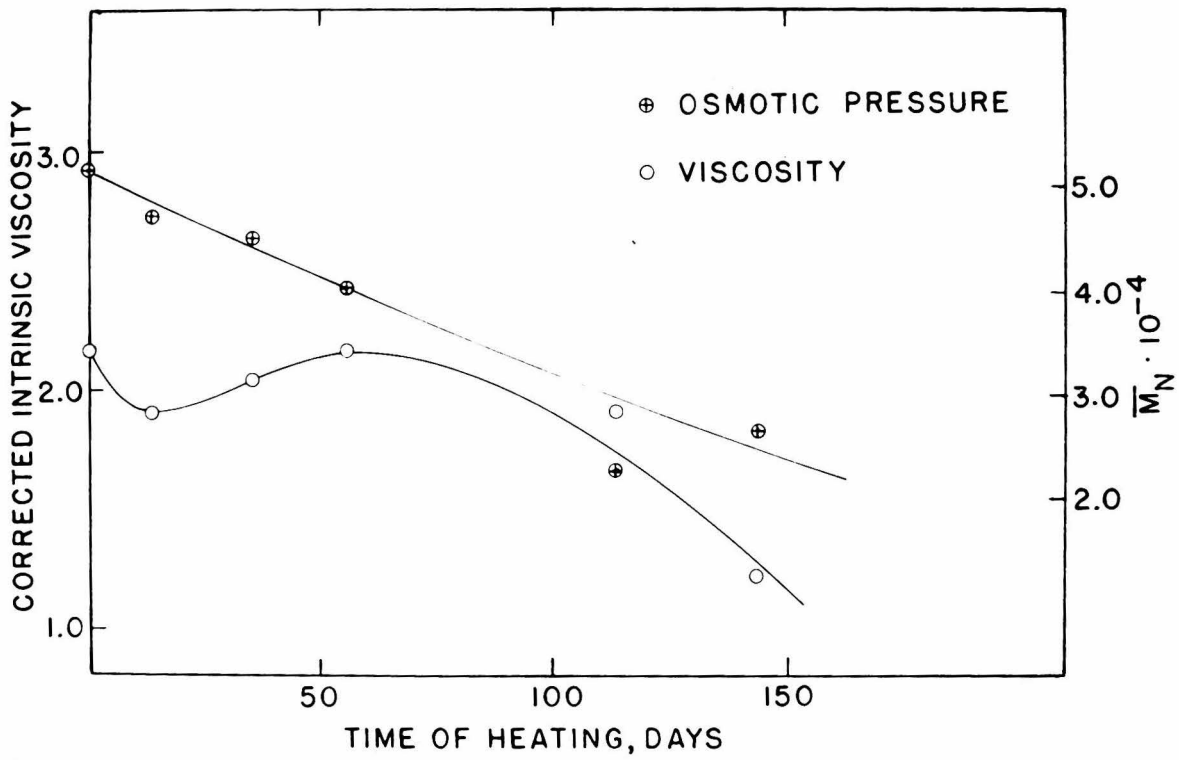

Figure 6. Viscosity and osmotic pressure data for nitrocellulose from heated powders

Table 2

Viscosity and Osmotic Pressure Data for Heated Samples of JP 76

Time of heating at 65.5° C., days	Percent of Nitrogen	Apparent Intrinsic Viscosity in Acetone	Corrected Apparent Intrinsic Viscosity*	Number Average Molecular Weight from Osmotic Pressure
0	12.18	2.17	2.17	53,000
14	12.14	1.90	1.91	48,000
36	12.05	2.02	2.05	46,000
57	11.76	2.10	2.17	41,000
114	11.78	1.85	1.92	22,000
145	11.63	1.16	1.23	26,000

* Corrected to the viscosity the sample would have if the nitrogen content had been 12.18 per cent. The data of Wannow 15/ were used for the calculation of this correction.

The difference in the two curves shown in Figure 6 is quite striking. With the exception of the anomalous point at 114 days the osmotic pressure (number-average) molecular weight decreases continually with time of heating. The viscosity does not show a correspondingly rapid decrease in the early stages, and indeed, passes through a minimum at about 15 days of heating. This minimum is followed by a slight maximum before a final more rapid decrease sets in. The powder begins to contain insoluble material after about 50 days of heating with 0.8 per cent of insoluble material at 57 days and 12.5 per cent at 145 days.

A plausible interpretation can be advanced to account for these observations. It is clear from the osmotic pressure data

that an uninterrupted degradation of the nitrocellulose is occurring. This degradation, however, appears to be opposed by some polymerization reaction which may well involve the type of process referred to as cross-linking. The production of a relatively small fraction of very large molecules in this manner can more than compensate for the effect of the general degradation on the viscosity, which is proportional to the weight-average molecular weight. Eventually the association process culminates in complexes which are so large as to be insoluble. As these complexes are removed from solution, the general degradation is no longer compensated for, and the viscosity of the solution then experiences a rapid drop.

Section V. Characterization of Nitrocellulose by Means of Precipitation Titration

The suggestion was made in Section III that the ratio of the number-average and weight-average molecular weights of a nitrocellulose is of value in estimating the degree of molecular heterogeneity of the material. Such a ratio is valuable in that it gives some information about the distribution of molecular weight in the nitrocellulose but it is limited in that it indicates only the average degree of heterogeneity. It is conceivable that two nitrocelluloses of widely different molecular weight distributions could give the same ratio of weight-average to number-average molecular weight. The ideal approach to the problem of the heterogeneity of nitrocellulose would be to evaluate completely the molecular weight distribution curve. This would involve the separation of each molecular species and the determination of the molecular weight and relative amounts of each.

There are several methods of estimating the degree of molecular heterogeneity other than that described in Section III. The existence of different molecular weight species in a heterogeneous polymer sample can apparently be established on the basis of diffusion experiments 16/. Isolation of fractions of different average molecular weight by a chromatographic adsorption procedure have been reported 17, 18/. Ultracentrifuge experiments on a polymer solution can be interpreted in terms of the width of a molecular weight distribution curve, if certain assumptions are made.

A fractional precipitation or fractional solution procedure can be used to isolate sizable portions of fractions of different molecular weight. The fractional precipitation method, the one most frequently used, involves the separation of small increments of the polymer from solution by means of the addition of a non-solvent and the determination of the average molecular weight and relative amount of each increment. From these data it is possible to plot an integral distribution curve for the polymer. This procedure is quite laborious and in practice the fractions obtained include an appreciable range of molecular weights. It will not be possible to secure even a reasonably sharp fractionation of nitrocellulose by this method unless the material is perfectly homogeneous in regard to nitrogen content.

Considerable time has been spent in an effort to develop a precipitation titration procedure for the estimation of the degree of molecular heterogeneity of nitrocelluloses. If a precipitant is slowly added to a solution of nitrocellulose, the solution will become turbid after a certain amount has been added and the turbidity of the solution will increase until the nitrocellulose is completely precipitated. If the solubility of each molecular weight species is a simple function of its molecular weight 19, 20/ and if the turbidity of a solution is a measure of the amount of nitrocellulose which has precipitated, then the progressive increase in turbidity as precipitant is added is an indication of the number and the amount of the molecular weight species which have become insoluble. If the turbidity of the solution is plotted against the

amount of precipitant added the curve will bear some resemblance to an integral molecular weight distribution curve for the sample.

Description of Apparatus

An apparatus was designed and constructed which records the decrease in light transmission of a solution of nitrocellulose as the nitrocellulose is progressively precipitated. The apparatus was built around a precipitation cell which is shown in Figure 7. The cell is constructed of Pyrex glass; part A is from a section of tubing which was selected to be free of imperfections which might affect the light transmission. The propeller, which is driven by a small motor controlled by a Variac, is pitched so as to throw the liquid down into that part of the cell which will be in the light path.

Transmission measurements were made with a Fisher Electrophotometer and were recorded with a General Electric Photoelectric Recording Potentiometer. The only modification of the Electrophotometer was the installation of a double-pole double-throw switch so that the recorder could be substituted for the galvanometer in the instrument's circuit.

Precipitant was added by a gravity feed from a large reservoir. With a head of about 90 cm. and a reservoir of one liter capacity it was found that reproducible amounts of precipitant could be added to the cell merely by measuring the time of flow with a stopwatch. Sufficiently good temperature control was secured by placing a jacket around the delivery tube from the reservoir and pumping

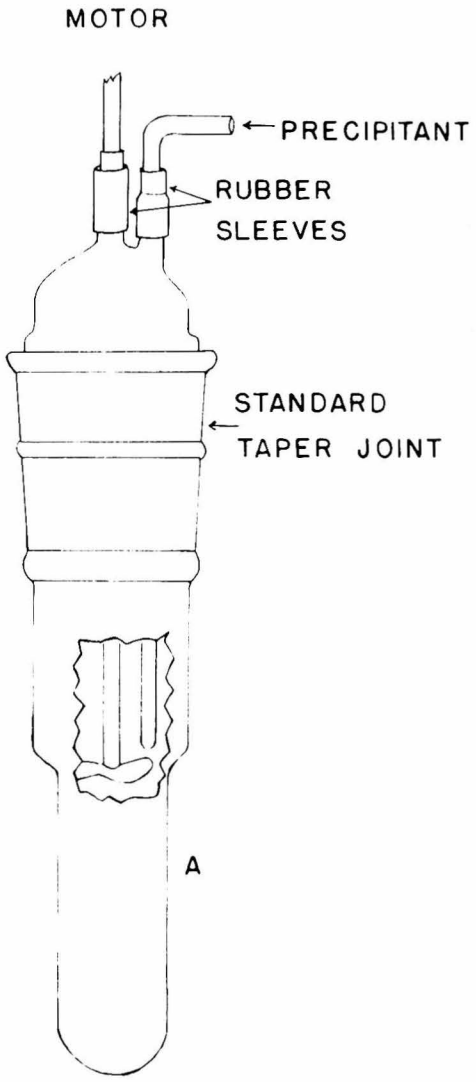

Figure 7. Precipitation cell

water from a constant temperature bath through the jacket.

Experimental Procedure

The nitrocellulose to be studied was dispersed in a mixture of three parts of ethyl alcohol and five parts of acetone and was made up to a concentration of 1/3 mg. per ml. The alcohol was added to the acetone to reduce its solvent power so that a smaller amount of precipitant could be added during the course of a determination. A 30-ml. portion of the solution together with 1 ml. of a 0.05 per cent aqueous solution of Duponol M.E. was introduced into the cell and the cell was placed in the regular cell holder of the Electrophotometer. The stirring motor was connected to the cell and water as a precipitant was added at a constant rate, the change in light transmission being followed by the recorder.

Experimental Precautions

The temperature of the solution had an effect on the rate of precipitation, and the turbidity curves for aliquot portions of nitrocellulose solutions varied rather widely until a reasonable degree of temperature control was maintained. All solutions were brought to 25° C. before they were introduced into the cell. The water which was used as the precipitant was maintained at this temperature by a water jacket on the inlet tube.

The addition of a trace of wetting agent was found to be essential for the reproducibility of the turbidity curves. Duponol M.E. was used, but other wetting agents may be satisfactory.

The addition of Duponol increased the maximum turbidity of the solution by about ten per cent, apparently insured reproducibility of the precipitation, and stabilized the particle size so that the turbidity of a solution after precipitation would not change over a period of several hours.

The addition of one or two drops of dilute hydrochloric acid had a large effect on the shape of the turbidity curves. Apparently a little acid causes the nitrocellulose to precipitate more rapidly, and after precipitation the material coagulated. A trace of calcium chloride also caused the nitrocellulose to precipitate more rapidly than normal, while ammonium hydroxide had little effect.

These experiments emphasize the importance of making sure that no impurities were introduced into the nitrocellulose solutions. Traces of salt and acid ions have a large effect on the formation of the colloid particles and as in all turbidimetric procedures care must be exercised to use well-characterized materials and to reproduce the precipitation procedure in every detail 21/.

Experimental Results

Each determination was made with 30 ml. of solution which contained 10 mg. of nitrocellulose and a trace of Duponal M.E. Turbidity curves were plotted with per cent transmission as ordinate and ml. of water added as the abscissa. Selected curves are shown on Plate 1.

An inspection of these curves reveals the following:

(i) The maximum turbidity which a given concentration of nitrocellulose will give depends on some unrecognized property of the

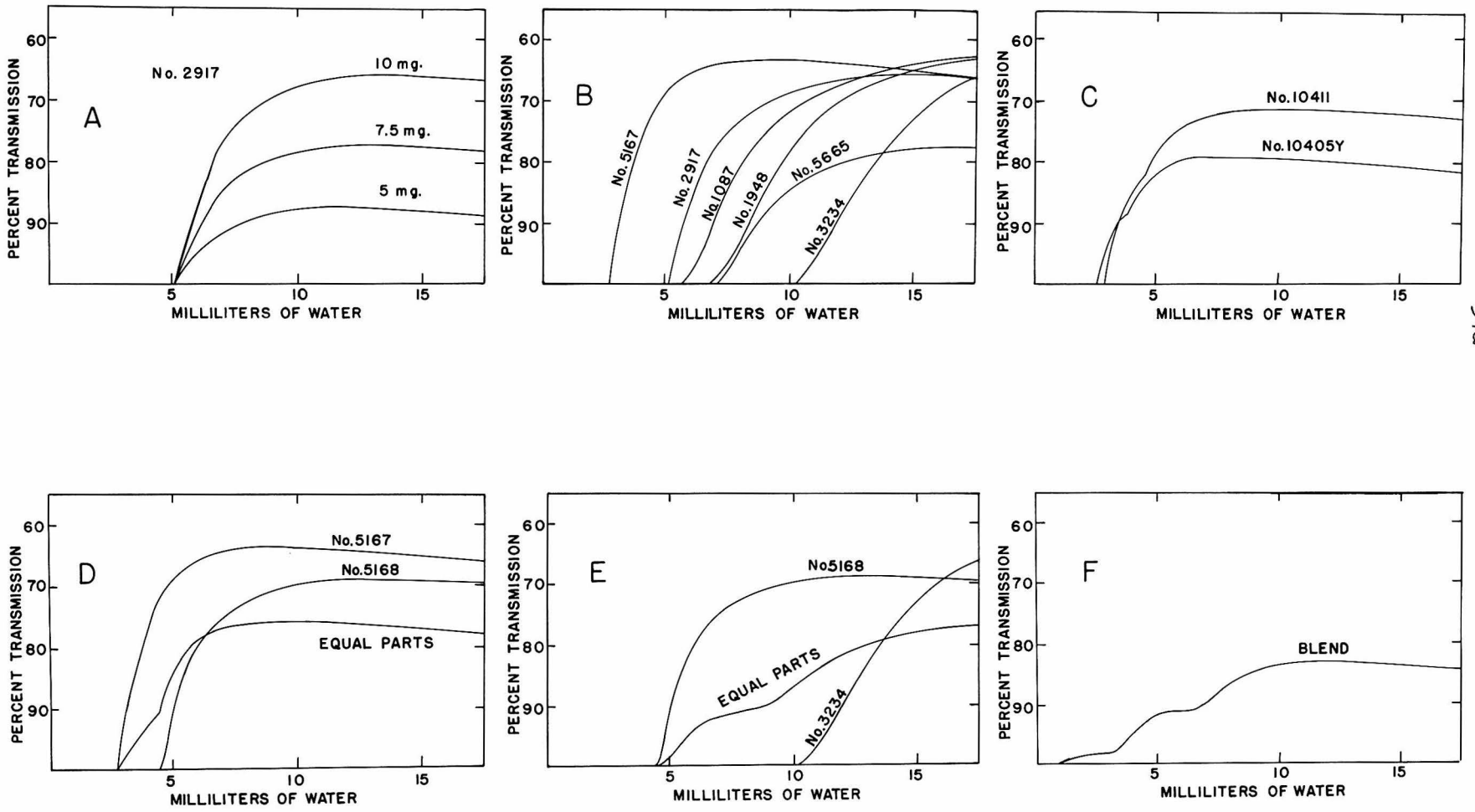

Plate I. Turbidity curves for selected nitrocelluloses

Legend for Plate 1

Figure A. The effect of change of concentration of nitrocellulose on the shape of the precipitation curve. Hercules No. 2917; 11.89 per cent nitrogen; intrinsic viscosity, 2.81

Figure B. Precipitation curves for a variety of unblended nitrocelluloses

Nitrocellulose Hercules No.	Nitrogen Content %	Intrinsic Viscosity	Source
5167	13.46	3.50	cotton
1087	11.99	0.67	wood
1948	11.82	0.64	cotton
5665	11.01	2.60	cotton
3234	10.96	0.55	cotton
2917	11.89	2.81	cotton

Figure C. Precipitation curves for two commercial nitrocelluloses which are blends of materials of different nitrogen contents

Nitrocellulose Hercules No.	Nitrogen Content %	Intrinsic Viscosity	Source
10411	13.23	3.12	cotton
10405	13.26	3.26	wood

Figure D. Precipitation curve for a 50-50 mixture of two nitrocelluloses which differ both in viscosity and nitrogen content

Nitrocellulose Hercules No.	Nitrogen Content %	Intrinsic Viscosity	Source
5167	13.46	3.50	cotton
5168	12.60	2.85	cotton

Legend for Plate 1 (continued)

Figure E. Precipitation curve for a 50-50 mixture of two nitrocelluloses which differ rather widely both in viscosity and nitrogen content

Nitrocellulose Hercules No.	Nitrogen Content %	Intrinsic Viscosity	Source
5168	12.60	2.85	cotton
3234	10.96	0.55	cotton

Figure F. A precipitation curve for a nitrocellulose of undisclosed origin which was found by a gravimetric fractionation procedure to contain a small amount of a very high nitrogen content, high viscosity material, and two larger components, each of lower nitrogen content and viscosity

nitrocellulose or on some impurity in the solvent.

(ii) The point of first precipitation depends on both the nitrogen content and the viscosity of the nitrocellulose, but is more strongly influenced by the nitrogen content.

(iii) The slopes of the curves are more strongly influenced by viscosity than by nitrogen content.

(iv) Blended materials give curves with smaller slopes and lower maximum turbidities than do unblended materials.

(v) Nitrocelluloses from wood pulp give smaller slopes than do those from cotton linters of equal nitrogen content and viscosity.

(vi) Blended nitrocelluloses give curves which are characterized by points of inflection. Some information about the nature of the component nitrocelluloses can be gained from the relative position of the inflection points.

Discussion

An article was published while this investigation was being conducted in which the authors attempted to calculate differential distribution curves from precipitation titration data on cellulose acetate butyrate 22/. The procedure involved the experimental determination, by means of carefully prepared fractions, of the point of first appearance of turbidity as a function both of concentration and of molecular weight of the polymer. A precipitation titration curve on an unfractionated polymer was then regarded as the summation of turbidities produced by the precipitation of each of the molecular species in turn as the concentration of pre-

cipitant was increased. By making several assumptions and utilizing nomograms, a precipitation titration curve could be analyzed and a differential distribution curve constructed for the sample of polymer.

The application of a similar method of calculation to the data for nitrocellulose did not seem to be justified. An important feature of the method is the assumption of a constant relation between turbidity and amount of precipitated polymer. This assumption implies that the particle size of the precipitated polymer is the same in all experiments and does not change during the course of a determination.

The data presented in the figures of Plate 1 indicate that although the turbidity is approximately proportional to the amount of precipitated nitrocellulose for solutions containing different amounts of the same nitrocellulose (Figure A), solutions containing the same amount of different nitrocellulose gave different turbidities when the precipitation was complete. This uncertainty in relation between the turbidity and the amount of precipitated nitrocellulose made it impossible to give an unambiguous interpretation of the precipitation titration curves.

As a strictly empirical method, the precipitation titration procedure was of considerable value in the investigation of nitrocellulose from foreign propellants. As a preliminary experiment, titration curves were made of the unknown nitrocellulose. The shape of this curve suggested whether or not the material was a blend, and if so, the approximate viscosity and nitrogen content

of the components. This information was useful in the selection of a solvent-nonsolvent system for a gravimetric fractional precipitation and as a guide in carrying out this procedure. The possible presence, however, of unknown constituents which might affect the particle size of the precipitated nitrocellulose made it inadvisable to rely on the precipitation titration method.

Summary

Instruments and a technique have been developed for the estimation of the degree of molecular heterogeneity of nitrocellulose by means of a precipitation titration procedure. The method has been found to be useful in indicating the width of a molecular weight distribution, but it does not give unambiguous information as to the shape of the distribution curve.

Section VI. Application of the Light Scattering Method to the Study of Nitrocellulose in Solutions*

Introduction

A theoretical basis for the calculation of the molecular weight of large molecules from the measurement of the amount of light scattered from solution has been established during recent years 23, 24/. The theory is not in its final form at the present time, but is being developed by workers both in this country and abroad 25, 26, 27/.

Experimental investigations have shown that the light scattering method is capable of giving a value for the molecular weight which is in essential agreement with that obtained from other experiments, and in addition may give valuable information about the size and shape of the molecules in solution 28, 29, 30/. The results of these experiments have emphasized, however, that further developments are desirable both in the design of instruments and in the technique of preparing solutions.

After a very brief summary of the pertinent part of the theory of light scattering, the purpose of this section is to describe instruments which have been constructed in this laboratory and techniques which have been developed for making light scattering

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* This section is a somewhat expanded version of a paper entitled "The Determination of the Size and Shape of Nitrocellulose Molecules in Solution by Light Scattering Methods. I. Experimental Procedures", Blaker, R. H., Badger, R. M., and Gilman, T. S., J. Phys. and Coll. Chem., 53, 794 (1949)

measurements on solutions of nitrocellulose and other high molecular weight substances.

Theory

The first theoretical treatment of light scattering was carried out by Lord Rayleigh 31/ some eighty years ago when he derived an expression for the turbidity of a perfect gas as a problem in electromagnetic theory. This treatment became quite complicated when the attempt was made to apply it to the case of real gases and liquids because of intermolecular interactions.

A different and more successful approach due to Smoluchowski 32/ and Einstein 33/ considered turbidity as being due to statistical fluctuations in the refractive index of a medium. In a binary solution of a single solute molecular species these fluctuations may arise from two effects, one caused by density fluctuations and the other by concentration fluctuations thoughout the liquid.

If $Id\Omega dV$ is defined as the amount of light scattered by a volume element dV of solutions into a solid angle $d\Omega$ at 90° to the incident beam, the quantity I can be represented by the expression:

$$I = I_1 + I_2 \qquad (4)$$

in which I_1 represents the scattering due to density fluctuations and I_2 that due to concentration fluctuations. If the solute particles are small compared with the wave length of light, a consideration of the work necessary to produce these fluctuations

leads to the equations

$$i_1 = \frac{I_1}{I_0} = \frac{2\pi^2 n^2}{\lambda^4 N_0}\left\{ RT\kappa\rho\left(\frac{\partial n}{\partial \rho}\right)^2 \right\}\frac{V}{r^2} \qquad (5)$$

and

$$i_2 = \frac{I_2}{I_0} = \frac{2\pi^2 n^2}{\lambda^4 N_0}\left\{ \frac{M_1 c_2 \left(\frac{\partial n}{\partial c_2}\right)^2}{\rho\left(-\frac{\partial \log_e f_1}{\partial c_2}\right)_{P,T}} \right\}\frac{V}{r^2} \qquad (6)$$

in which

I_0 is the intensity of the unpolarized incident light. (Intensity is here defined as the energy carried through 1 $cm.^2$ per second.)

R is the gas constant (in $cm.^3$-atm. per mole degree)

T is the absolute temperature

κ is the isothermal compressibility (in reciprocal atmospheres)

λ is the wave length (in vacuo) of the light used (in cm.)

N_0 is Avogadro's number

ρ is the density of the scattering medium (in grams per cm^3.)

n is the refractive index of the scattering medium

M_1 is the molecular weight of the solvent

c_2 is the weight fraction of the solute (in grams per gram of solution)

f_1 is the fugacity of the solvent

V is the scattering volume (in $cm.^3$)

r is the distance (in cm.) at which the scattering is observed

If the plausible assumption is made that I_1 and I_2 are essentially independent of each other in dilute solution, I_1 may be replaced by I_s where $I_s d\Omega$ represents the light scattered per unit volume of pure solvent. The quantity, I_2, can thus be determined as the difference between the scattering from the solution and that from the solvent.

The value of I_2 bears the following relation to the turbidity of the solution if, as Rayleigh showed was true for solutions of particles small compared to the wave length of light, the angular dependence of scattering obeys a $(1 + \mathrm{Cos}^2\theta)$ relation. This expression is:

$$\tau = \frac{16\pi r^2 I_2}{3 V I_o} \qquad (7)$$

where τ is the turbidity of the solution due to scattering defined by the relation:

$$I_t = I_o e^{-\tau x} \qquad (8)$$

I_t is the intensity of the light which has traversed a layer of scattering medium of thickness x.

The assumptions which were used in the derivation of equations (5) and (6) lead to the conclusion that with unpolarized incident light the scattered light should be linearly polarized in a plane perpendicular to the plane formed by the incident beam and the direction of observation. Actually, a certain amount of depolarization is observed. Cabannes 34/ has shown that, in certain circumstances at least, the expressions for total intensity

scattered at 90° (that is, the sum of the intensity of the perpendicular and parallel components) must be multiplied by the factor known as the Cabannes factor

$$\frac{6 + 6\Delta}{6 - 7\Delta} \qquad (9)$$

in which the depolarization, Δ, is the ratio of the intensity of the parallel and perpendicular components of the light scattered at 90°. (The incident unpolarized beam and the direction of observation determine the plane of reference.) This term corrects for the contribution to the observed turbidity of fluctuations due to random orientation of anisotropic particles. The turbidity, τ, and the 90° scattering, i_2, are not increased equally by depolarization since the angular dependence of scattering does not follow the $(1 + \text{Cos}^2\theta)$ relation if the scattered light is depolarized. The proper correction factor to apply to the turbidity is 35/:

$$\frac{6 + 3\Delta}{6 - 7\Delta} \qquad (10)$$

Debye 24/ proposed that the quantity $\left(-\frac{\partial \log_e f_1}{\partial c_2}\right)_{P,T}$ from equation (6) be related to a colligative property of the solution, in particular, to the osmotic pressure. Since in terms of osmotic pressure

$$\left(-\frac{\partial \log_e f_1}{\partial c_2}\right)_{P,T} = \frac{\bar{V}_1}{RT}\left(\frac{\partial \pi}{\partial c_2}\right)_T \qquad (11)$$

where $\bar{V}_1$ is the partial molal volume of the solvent, π is the osmotic pressure, and since the osmotic pressure of a dilute

solution of a high molecular weight substance can be expressed as 14/:

$$\pi = \frac{RT}{M_2} p c_2 + B p^2 c_2^2 + \cdots \qquad (12)$$

an expression can be given for the turbidity of a solution in terms of the molecular weight of the solute, its refractive index properties, depolarization, and solute concentration. This expression is ordinarily written as:

$$\tau = \left\{ \frac{32\pi^3 n^2 \left(\frac{\partial n}{\partial c_2}\right)^2 c_2}{3\lambda^4 N_0 \left(\frac{1}{M_2} + 2B\frac{c_2}{RT}\right)} \right\} \frac{6+3\Delta}{6-7\Delta} \qquad (13)$$

where M_2 is the weight-average molecular weight of the solute.

If the particles are larger than one-tenth of the wave length of light, the intensity of the 90° scattering may be considerably reduced because of intramolecular interference. This interference also causes the radiation envelope to differ from the simple $(1+\cos^2\theta)$ relationship which holds for small particles. The dissymmetry of the radiation envelope is a function of the size and shape of the dissolved molecules and offers a valuable means for the investigation of the shapes and absolute dimensions of large molecules. Various equations have been proposed which relate the angular distribution of scattered intensity to the size of particle for several molecular models 28, 36/. The application of these models to the angular scattering data for nitrocellulose solutions will be described later in this Section and in Section VII.

The above paragraphs have presented that part of the light scattering theory which is applicable to the experimental data

of this and the subsequent two Sections. More complete reviews of the method have been presented recently by several authors 37, 38, 39/.

Light Scattering Instruments

When the investigations to be described were undertaken, no commercial instruments were available which were regarded as suitable for the experimental determination of light scattering data. It was consequently necessary to design and construct three special instruments described below to measure the 90° scattering of the solutions, the angular distribution of the scattering, and the refractive index increment of the solute.

Differential Refractometer

Two refractive index measurements enter into equation (13), the refractive index of the solution and the refractive index increment of the solute. The first of these presents no special problem. The determination of the refractive index increment of the solute is not practicable with the ordinary Abbe refractometer because of its limited precision. The difference between the refractive index of acetone and a one-per cent solution of nitrocellulose is only about 0.001. A Pulfrich refractometer might be used, but a differential instrument is much to be preferred since it greatly reduces the difficulties of temperature control.

A differential refractometer consequently was built which allows the measurement of the difference between the refractive index of the solution and the solvent with an accuracy of about

$3 \cdot 10^{-6}$. This instrument consists of a double, hollow, 90° prism and a simple optical system which transmits an image of a slit through the prism to a focus on the scale of a microscope micrometer eyepiece. Figure 8 is a drawing of the instrument. The light source is a GE 100-watt AH-4 mercury arc. The filters which give monochromatic light are those which are used in the photometer to be described below. Lens 1 and Lens 2 have focal lengths of 26 and 61 mm. respectively. The eyepiece micrometer is one which was purchased from the American Optical Company.

The prism is a block of stainless steel with two milled apertures which is enclosed in a short length of stainless steel tubing. Pieces of 6-mm. plate glass are cemented to the faces of the prism and onto the ends of the tube with sodium silicate. This cement is not very durable, but no more suitable substance with resistivity to acetone has been found. Figure 9 gives details of the construction of the prism.

If the two cavities of the hollow prism contain liquids of different refractive index, two images of the slit will appear on the scale of the eyepiece and the distance between the images will be proportional to the difference in the refractive index of the two liquids. The enclosing tube is filled with a liquid whose refractive index is not widely different from that of the liquids under investigation. This liquid serves two purposes. It provides two 90° prisms in the light path, one on either side of the prism,

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* This instrument is a modification of the refractometer described by Rau and Roseveare 40/

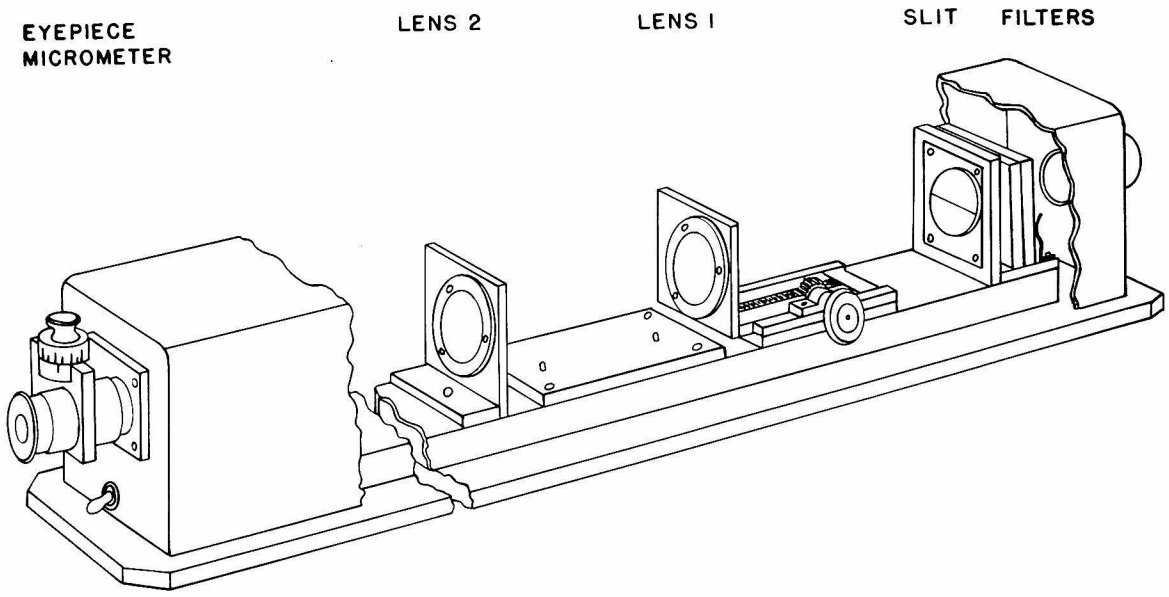

Figure 8. Differential refractometer

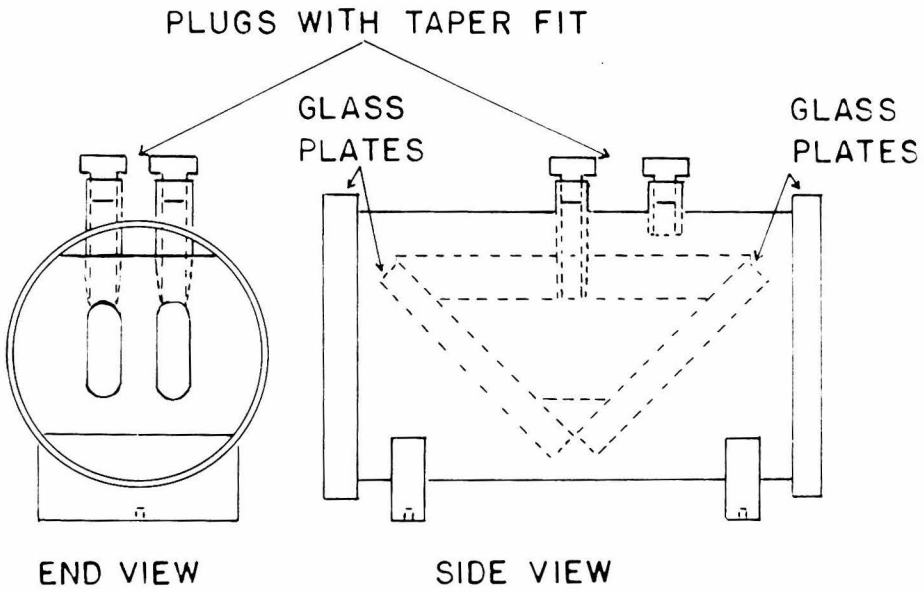

Figure 9. Prism of differential refractometer

which reduce the magnitude of the deflection of the slit image, but do not change the difference in deflection if this is small. The liquid increases the heat capacity of the cell and therefore helps to reduce temperature fluctuations.

The image deflection, X, can be calculated from the equation 41/.

$$X = 2f(n - n_o) \tan(A/2) \qquad (14)$$

where f is the focal length of lens 2

A is the top angle of the prism, and

$n - n_o$ is the difference in refractive index between the two liquids in the cavities of the prism.

Absolute measurements may consequently be made, or the refractometer may be calibrated by measuring the image deflection when one cavity contains pure water and the other contains aqueous solutions of known refractive index. Absolute measurements made on the refractive index increment of sodium chloride and sucrose in aqueous solutions were found to be in agreement with previously reported values 42, 43/.

No special precaution was taken to control the temperature of the prism since the value of $n - n_o$ is insensitive to small temperature changes.

Light Scattering Photometer

A photoelectric photometer which was constructed for measuring the 90° scattering and the depolarization of this scattering is illustrated in Figure 10 and is described below.

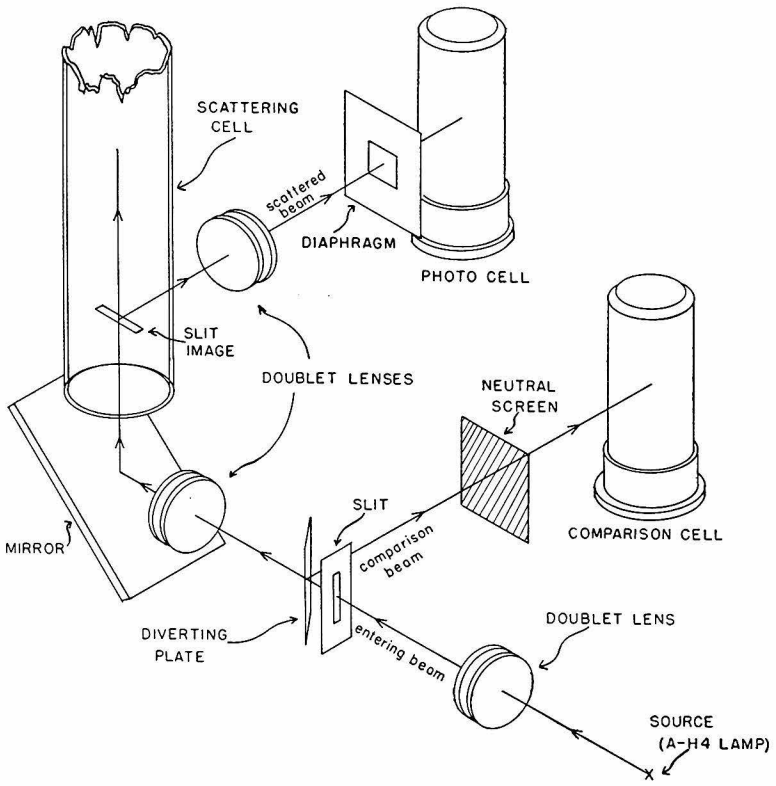

Figure 10. Scheme of the optical path in the photoelectric photometer. (Figure is from an article by Blaker, H. H., Badger, R. M., and Gilman, T. S., J. Phys. and Coll. Chem., 53, 794 (1949).)

The light source, an AH-4 mercury arc operated by a constant voltage transformer, is focused on a vertical slit about 3 x 18 mm., before which are located filters for monochromatizing the beam. For isolating the 4358 Å line, 4 mm. of Corning No. 585 in combination with a thickness of Wratten No. 2A is used. The 5461 Å line is isolated with 4 mm. of Corning No. 5120 and 2 mm. of Corning No. 3486. The red light transmitted by this combination is unimportant because of the insensitivity of the photocell to long wave lengths. At some little distance behind the slit is a rotatable disc with three apertures (not shown in the figure). Two of these apertures are covered with Polaroid sheet (Polaroid J film), thus permitting polarization of the incident beam in either of two mutually perpendicular planes. The beam is then diverted upward by a mirror at 45° and enters the scattering cell. Immediately in front of the mirror a lens pair serves to focus an image of the slit in the scattering solution at a point opposite to the observation window in the side of the cell. The illuminated volume is consequently a thin sheet somewhat over 3 mm. in average thickness.

Opposite the window a third pair of lenses focus the scattered light on a square aperture, 1 cm. on edge, which defines the two additional dimensions of the effective scattering volume. A slide with three openings (not shown in the figure) is located between this square aperture and the photocell. Two of the openings in the slide are covered with Polaroid sheet oriented to pass light polarized in horizontal and vertical directions, respectively.

The intensity of the scattered light is compared with that

of the incident beam by means of a second photocell which receives light diverted by a thin glass plate mounted immediately behind the slit and at 45° to the direction of the entering beam. The two photocurrents are compared by the use of a bridge circuit. Adjustment of one of the potentiometers is made until a null reading is obtained on a galvanometer. Direct calibration has shown that measurements of relative intensity may be made with an error not exceeding one per cent.

The photocells employed are RCA electron multiplier tubes type No. 931-A. The tubes are normally operated at 90 volts per stage by a conventional 1000-volt power supply with electronic voltage regulation. The complete electrical circuit is shown in Figure 11.

Electron multiplier photocells are quite sensitive to the direction of polarization of light incident upon them. This effect may give rise to errors in the polarization measurements and has been compensated for by the introduction of a suitably inclined glass plate which slightly polarizes the light just before it enters the photocell. This plate is orientated in such a manner that the photocell will give a correct indication of the ratio of the intensities of vertically and horizontally polarized light.

The instrument polarizes the incident beam slightly because of the use of a glass plate to reflect light to the comparison photocell and because of the reflection by the mirror. This effect is compensated for by a slight inclination of the glass filters located before the initial slit. The instrument was adjusted

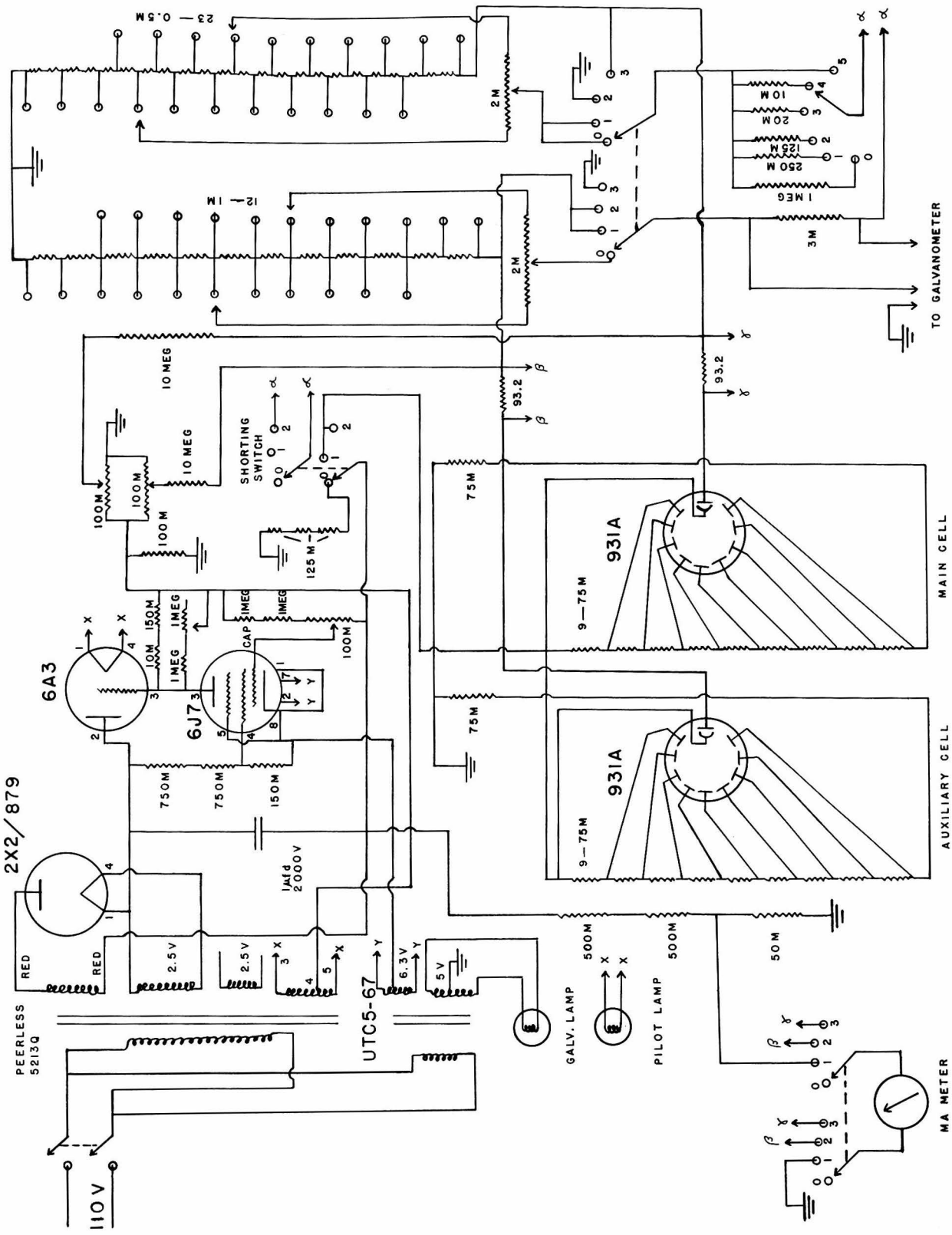

Figure 11. Electrical circuit for photoelectric photometer

until the measured value for the depolarization of the light scattered from carbon disulfide agreed with the value reported by previous investigators.

The scattering cell used in the apparatus described above was constructed from a 20-cm. section of Pyrex tubing, 35 mm. in outside diameter, with a 24/40 standard taper joint on the top, and a disc of polished Pyrex glass cemented to the bottom with an easily fusible glass 44/. The standard taper joint is closed with a short "horn" which serves as a light trap. The outside walls and top of the cell were painted with opaque lacquer except for a window 2 x 3 cm. near the bottom, which is opposite the viewing lens when the cell is located in the photometer.

Absolute Calibration of the Light Scattering Photometer

The selection of a scattering standard was necessary since the photometer is capable of giving only relative values of scattered intensity. Some previous workers have made calibration measurements using a diffusely reflecting screen for comparison. This method has some difficulties and may involve a number of corrections, since scattering by a volume in one case, and by a surface in the other case, is compared. The use of a standard scattering liquid is consequently much more convenient in routine measurements.

Carbon disulfide has been found to be a very convenient standard since its scattering power is comparable with that of the solutions ordinarily studied, and it can be rather easily prepared in a dust-free condition. In a sealed vessel it is stable if not exposed to

violet light. One sample of carbon disulfide used for three years as a standard, chiefly with light of 5461 Å, showed no sensible change in scattering power.

The scattering standard was prepared in the following manner. The top of a scattering cell was drawn down and sealed to a U-tube which in turn was sealed to a bulb with a short side arm. Portions of 40 ml. of reagent grade carbon disulfide and 20 g. of phosphorus pentoxide were then introduced into the bulb through the side arm. The bulb was placed in a liquid air bath, the system evacuated, and the side arm sealed off. The carbon disulfide was distilled from the bulb into the scattering cell by placing the cell in an ice bath and leaving the bulb at room temperature. The distilled carbon disulfide was swirled gently in the cell and then run back into the bulb. This process was repeated a number of times until the scattering power of the carbon disulfide reached a constant minimum value.

The scattering power of carbon disulfide has been previously measured by several investigators but it was thought very desirable to check these determinations. The procedure employed was somewhat similar to that used by Martin 45/ for ethyl ether.

The intensity of the scattered light is so faint in comparison with the incident beam that it was not convenient to compare the two light levels directly; instead the calibration experiment was carried out in two steps. In one experiment the incident beam was compared with the intensity of light scattered by a freshly ground porcelain plate when the angles of incidence and scattering were both 45°. In the second experiment the intensity of the 90° "volume"

scattering of carbon disulfide was compared with the "surface" scattering of the porcelain plate, with angles of incidence and scattering again at 45°. These procedures could be carried out without using neutral filters of excessively high density the calibration of which is difficult.

In both experiments intensity measurements were made with the photometer described above. Cells of the same design as normally used in light scattering measurements were used. The porcelain plate was suspended in one of these cells and immersed in carbon disulfide to insure that light reflected from the plate was subject to the same reflections and refractions at the cell walls as the light scattered by carbon disulfide.

In the first experiment an auxiliary arc was employed which could be moved about the circumference of a circle centered in the scattering cell. The initial beam was so defined by lenses and apertures that in the direct measurement of its intensity all of the light, except for reflection losses, was captured by the viewing lens and was transmitted to the photocell.

In the second experiment the following precautions were taken to simulate the effect of a volume scattering when the scattering by the porcelain plate was measured. The porcelain plate was moved step-wise in a direction parallel to the incident beam, and intensity readings were taken at each step. The effective intensity of scattering by the plate to be compared with the volume scattering of carbon disulfide was then obtained by integration under the plot of intensity versus plate position.

The results of the calibration experiment are presented in Table 3 together with the results of earlier investigators.

Table 3

Scattering Power of Carbon Disulfide

Investigator	Wave Length Å	Temperature °C	Scattering Power i/I_o
Bai 46/	4358	30	$120 \cdot 10^{-6}$
Cabannes* 47/, Bhagavantam 48/	5461	15	$44 \cdot 2 \cdot 10^{-6}$
Martin 45/	4358	20	$138 \cdot 10^{-6}$
Pai**	5461	30	$39 \cdot 6 \cdot 10^{-6}$
This investigation	4358	25	$151 \cdot 10^{-6}$
This investigation	5461	25	$47 \cdot 8 \cdot 10^{-6}$

In view of the difficulty of eliminating systematic errors the final value of the scattering power of carbon disulfide may be somewhat in error. Since the results obtained by four investigators are not widely different, an average value has been taken as more representative of the true value than the value obtained by any one investigator. The values of i/I_o for carbon disulfide which are used in this laboratory are, therefore, $43 \cdot 9 \cdot 10^{-6}$ for a wave length of 5461 Å and $136 \cdot 10^{-6}$ for 4358 Å.

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* Cabannes gives the scattering power of benzene. The scattering power of carbon disulfide is calculated by the use of the relative scattering power of carbon disulfide and benzene given by Bhagavantam.

** Calculated from Bai's value of the scattering at 4358 Å with the aid of the Lorenz-Lorentz formula.

Camera for Measurement of Angular Distribution

The camera designed to measure the angular distribution of intensity of the scattered light is somewhat similar to one previously described by P. P. Debye 43/, but has several improvements. The photographic method of measurement was chosen because it was desired to have satisfactory angular resolving power. This is attended with low intensity levels and makes photoelectric measurements somewhat difficult. Furthermore the intensity of light scattered at various angles can be measured simultaneously and consequently any error which might be introduced because of periodic fluctuations or drift in the sensitivity of a photoelectric instrument can be avoided. The camera designed permits the direct comparison of light scattered at any angle with that scattered at 90°. This feature practically eliminates errors due to lack of uniformity in the photographic film, and makes it unnecessary to correct for effects due to the "spreading" of the scattered beam because of refraction as it passes through the cell window. It reduces errors due to possible incorrect allowance for losses by reflection at the cell window, although these are not entirely eliminated since the polarization of the two beams being compared may not be the same.

The essential features of the camera are shown in Figure 12. The light from a mercury arc is focused on the rectangular aperture 2 x 3 mm. (O). Lens (L) renders the light essentially parallel and the diaphragm (D) limits the beam to a narrow shaft 4 mm. by 18 mm.

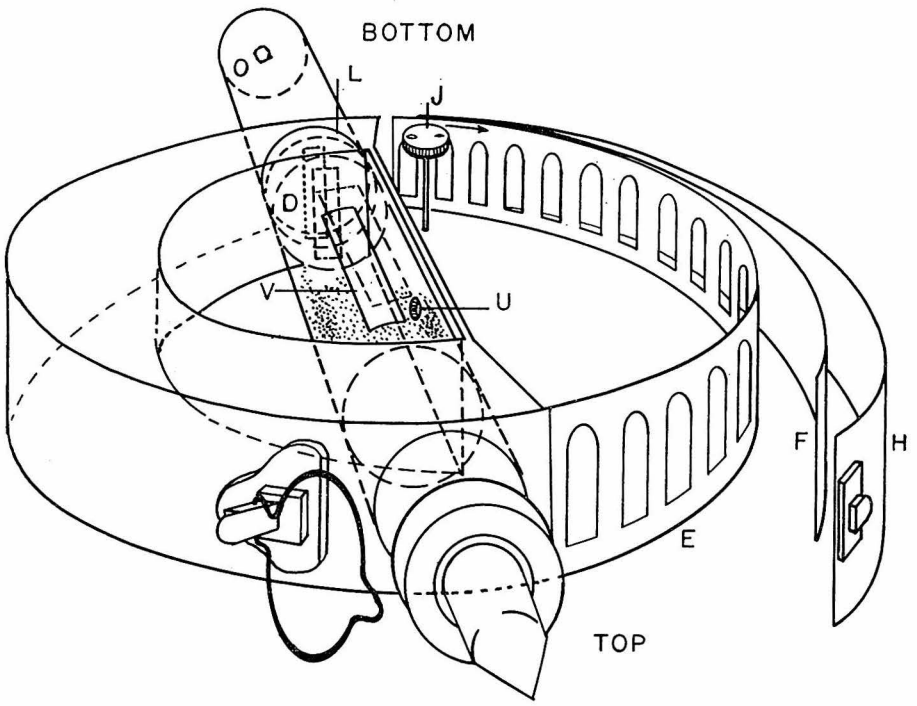

Figure 12. Camera for the determination of the angular distribution of scattering. (Figure is from an article by Blaker, R. H., Badger, R. M., and Gilman, T. S., J. Phys. and Coll. Chem., 53, 794 (1949).)

This beam then passes up through a cylindrical scattering cell. Scattered light passes through aperture (U) and is recorded on a strip of film (F) held in place by film holder (H). The sliding shutter (E) is located just in front of the film and has 16 equally spaced apertures, each 7 mm. by 36 mm. A step wedge of nine steps is held tightly in place against the inside surface of the shutter. The position of this shutter can be shifted the distance of a width of one aperture by a rack and pinion at (J). This device allows a record to be made of the scattering at 16 angles. The position of the shutter is then shifted to mask the exposed areas on the film and to allow a second exposure to be made. In the second exposure the 90° scattering is recorded on the film adjacent to each of the exposures which record the angular scattering. For this operation the first lamp is turned off and the cell is illuminated through the side slit (V), 3 mm. by 50 mm., with a parallel beam of light from a second mercury arc. The film now receives light which is scattered at 90° to the incident beam and which passes through the cell window at the same angles as the angularly scattered light.

The complete angular scattering apparatus is shown in Figure 13. Light from an AH-4 mercury arc in the small lamp housing is made monochromatic by passage through a set of Corning filters in a holder on the housing. The light is focused on the aperture (designated as (O) in Figure 12) of the camera after being reflected by a mirror set at 45° to the beam. The light is polarized with the electric vector perpendicular to the plane including the incident and scattered

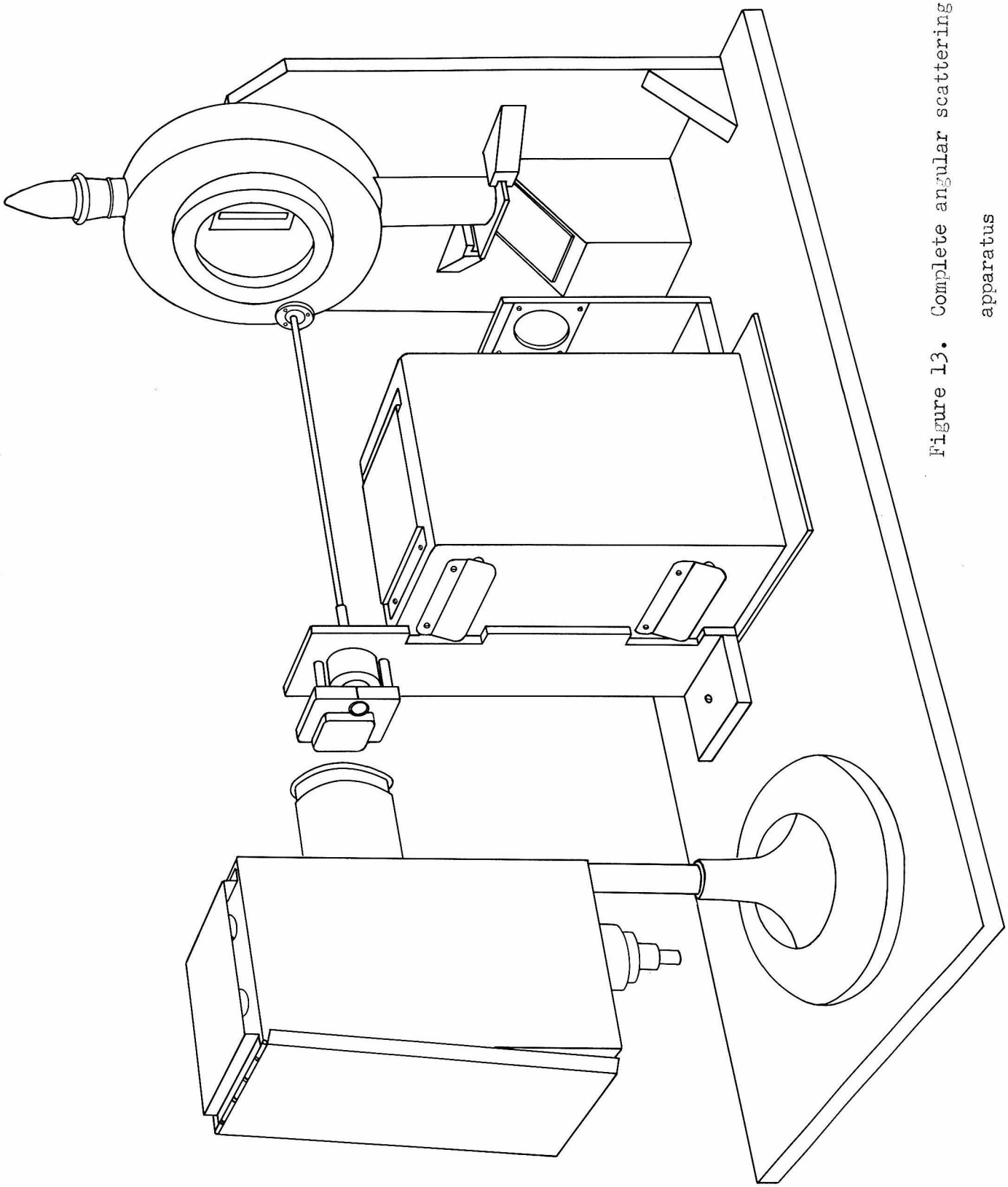

Figure 13. Complete angular scattering apparatus

beam by a section of Polaroid film located just before the aperature of the camera. Light from an AH-4 arc in the large lamp housing illuminates the camera for the 90° exposure. The filters for this light are located in the camera body just before the side slit (V) of Figure 12.

In practice, a solution is carefully introduced into the scattering cell. A 2-inch by 10-inch strip of film* is placed in the film holder, and the small lamp is turned on. An exposure of about three hours is used for solutions of normal scattering. This lamp is then turned off and the shutter is moved so as to cover the exposed area of the film and to uncover the unexposed part of the film. The second lamp is then turned on for a three-hour interval.

The developed film has 32 exposed areas, each of which has received light through the nine steps of the step wedge. Density readings are made with an Eastman Densitometer (Model D). A comparison is made between the density of an area which received angular scattered light and the two areas on either side which received the 90° scattered light. An interpolation is made to determine which steps of the step wedge allowed equal intensity of the angular scattered light and the 90° scattered light to fall on the film. Since the transmission of the various steps of the step wedge is known, the ratio of the intensity of the angular scattered light to that of the 90° scattered light can be calculated for the angles corresponding to the sixteen apertures in the shutter. The apertures cover the range of 22° to 161° in air.

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* Eastman cut film, Panchromatic Super XX

The symmetry of construction of the camera was tested by measuring the angular scattering of carbon disulfide. With vertically polarized incident light there should be no angular dependence of scattering. Slight changes were made in the original design until the observed scattering curve of carbon disulfide showed no angular dependence between angles of 60° and 120° as measured in the liquid.

Preparation of Solutions for Light Scattering Measurements

Great care must be used in the preparation of solutions for light scattering measurements if the results are to have significance. Low molecular weight materials, such as impurities in the solvent, may cause slight error because of their influence on the refractive index increment of the solute. A more frequent and serious source of error is the presence of particles which are large in comparison with the solute molecules. Since the molecular weight which is calculated from light scattering measurements is the weight average molecular weight a relatively small amount of a high molecular weight impurity can completely invalidate a molecular weight determination. The presence of a trace of a high molecular weight impurity may also affect the angular distribution of scattered light so much that the estimation of molecular size and shape becomes impossible.

It has been found impossible to prepare satisfactory solutions for light scattering studies by any filtration procedure in cases where the solvent is even moderately polar. Carbon disulfide may

be reasonably well freed of motes by filtration, but solutions in acetone or particularly in water appear always to be contaminated by particles detached from the filter. This contamination may be constant and may lead to erroneous conclusions. This is especially true when the dissymmetry of the scattered light is being measured.

A procedure for preparing solutions for light scattering measurements has been worked out which has given satisfactory results for nitrocellulose in acetone solution and for several proteins in aqueous solutions.

The scattering cell is carefully cleaned by washing with soap and warm water. It is then rinsed successively with water which contains a little Aerosol O.T., with distilled water, and with chemically pure acetone. Since it is virtually impossible to remove all dust from the rinsing liquids the scattering cell will still contain a trace of material which will show up as dancing motes in the Tyndall beam. Most of this material is removed by boiling out the cell with acetone vapor. A water jacket is slipped on the scattering cell; it is inverted and placed on a still. There the cell acts as a condenser where the remainder of the dust is washed out by the condensing acetone.

The solution is made up with chemically pure solvent. If the solution is obviously turbid it is forced through a Seitz filter disc (Hercules Filter Corp., No. 3). Filtration is more effective with organic solvents than with aqueous solutions, but is not completely satisfactory for either.

The solution is then centrifuged in a field which will throw

down all particles of a molecular weight from ten to a hundred times greater than the molecular weight of the material which is being studied. Most of the solutions are centrifuged in a field of 32,000 g for 20 minutes in a centrifuge which was designed and built in this laboratory*.

While solutions which are prepared in this manner can conceivably contain some impurity of an appreciably higher molecular weight than the solute, experience has shown that its presence can usually be disregarded. This has been found to be true for nitrocellulose and certain proteins.

The amount of material which is centrifuged out is usually very small, often so small that no change can be detected in the concentration of the solution.

The turbidity of the solution and the dissymmetry of scattering are usually markedly decreased by this treatment. Figure 14 shows the effect of preparation on the angular distribution of the light scattered from a nitrocellulose solution. The molecular weight which was calculated from the turbidity of the filtered solution of this nitrocellulose was 373,000, while that from the centrifuged solution was 185,000. The value from viscosity and osmotic pressure measurements was 163,000.

The calculation of a value for the molecular weight requires that the turbidity of a solution be measured at several concentrations and that a value of concentration divided by turbidity at zero

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* This centrifuge, constructed under the direction of Dr. Stanley Swingle, is capable of running at 20,000 rpm (50,000 g) for 30 minutes without undue heating.

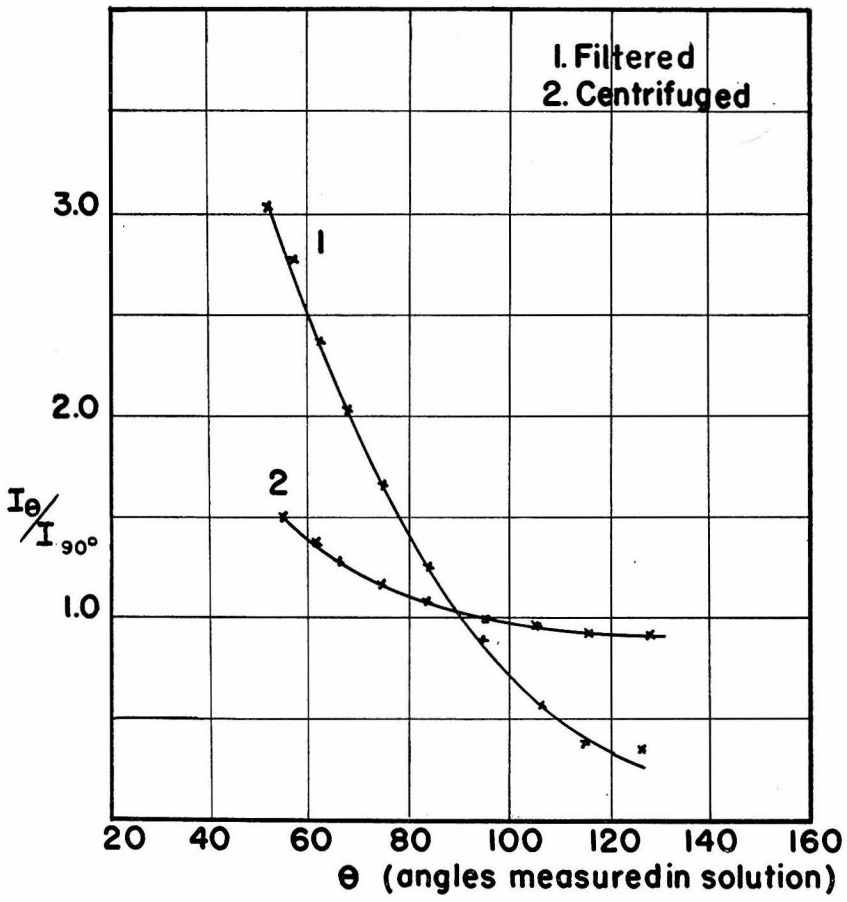

Figure 14. The angular scattering of a nitrocellulose solution after filtration and after centrifugation. (Figure is from an article by Blaker, R. H., Badger, R. M., and Gilman, T. S., J. Phys. and Coll. Chem., 53, 794 (1949).)

concentration be determined by extrapolation. The following procedure has been adopted for making these measurements.

A portion of solvent and solution are centrifuged. A clean scattering cell is weighed on a Chainomatic balance. The centrifuged solvent is introduced, the cell weighed, and the scattering measured. A small portion of solution is carefully added to the cell; the contents are thoroughly mixed by gentle but extensive swirling; the cell is weighed; and the scattering is measured. The process is continued until sufficient data is obtained for making the extrapolation to infinite dilution. This procedure has been found to give better results than the reverse process whereby a solution is progressively diluted by the addition of solvent. The effect of contamination is most serious in the dilute solutions, and if these are measured first the danger of contamination is decreased.

Treatment of Light Scattering Data

If the scattering particles are small compared with the wave length of light the angular distribution of scattered intensity obeys Rayleigh's relation $(1+\mathrm{Cos}^2\theta)$, and therefore equation (13) is directly applicable to the data. In general the direct determination of the turbidity, τ, is not practicable because of its small value and because of the complication often presented by true absorption. In practice the value of the turbidity is calculated by the use of equation (7) from the experimentally determined value of the intensity of the 90° scattering.

If a plot of c/τ against c is made and the value of c/τ at infinite dilution is determined by extrapolation, the molecular weight is given by the expression

$$M_2 = \frac{3\lambda^4 N_0}{32\pi^3 n^2 \left(\frac{\partial n}{\partial c_2}\right)^2 \left(\frac{c_2}{\tau}\right)_{c_2 \to 0}} \qquad (15)$$

which follows from equation (13).

The working equation, however, is

$$M_2 = \frac{\lambda^4 N_0}{2\pi^2 n^2 \left(\frac{\partial n}{\partial c_2}\right)^2 \left(\frac{c_2}{i/i_{cs_2}}\right)_{c_2 \to 0} \left(\frac{I_0}{i_{cs_2}}\right)_{90°}} \qquad (16)$$

where i/i_{cs_2} is the ratio of the intensity of the 90° scattering of the solution to that of carbon disulfide. This value has to be multiplied by the proper Cabannes factor. By use of a value of $\left(\frac{i_{cs_2}}{I_0}\right)_{90°} = 4{\cdot}4{\cdot}10^{-5}$ and a wave length of 5461 Å this expression reduces to

$$M_2 = \frac{12.0}{n^2 \left(\frac{\partial n}{\partial c_2}\right)^2 \left(\frac{c}{i/i_{cs_2}}\right)_{c_2 \to 0}} \qquad (17)$$

In general the molecular weights calculated by the above equation will be low because most solutions of high molecular weight materials exhibit a definite angular dissymmetry. For particles larger than about a tenth of the wave length of light the 90° scattering is reduced because of intermolecular interference, and the scattering envelope around the illuminated particle becomes quite asymmetric. The general treatment of such scattering is quite difficult and has been handled rigorously only for homogeneous spherical particles 49, 50/.

By the use of various approximations among which are, that

there is little difference between the refractive index of the solvent and the solute, and that the solute particles are of a size comparable to the wave length of light, Debye 28/ and Kuhn 36/ have derived expressions for the angular scattering of suspensions of spheres, rods, and randomly coiled chains when such suspensions are irradiated with vertically polarized light.

Plots of the angular distributions given by these models are shown in Figures 15 to 17. S/λ for each model is the ratio of a characteristic dimension of the particle to the wave length of light: D for diameter of the sphere, L for length of the rod, and R for the distance between the ends of a randomly coiled chain.

Although in principle direct comparison of the experimental angular scattering curves with the theoretical curves would establish the shape of the scattering particle, such a comparison is of little value because the shapes of the theoretical curves differ but little from one another for the values of S/λ usually encountered. This approach would require greater experimental accuracy than the light scattering method is now capable of giving.

In practice the angular scattering curves have been merely characterized by a dissymmetry coefficient, q, which has been defined in this investigation as the ratio of the scattered intensity at two angles, namely at 60° and 120° measured in solution. The value of q is sufficient characterization since the curves are monotonic. The value of q could be determined merely from two intensity measurements, but it is more reliably determined from a smoothed plot of data taken over a range of angles.

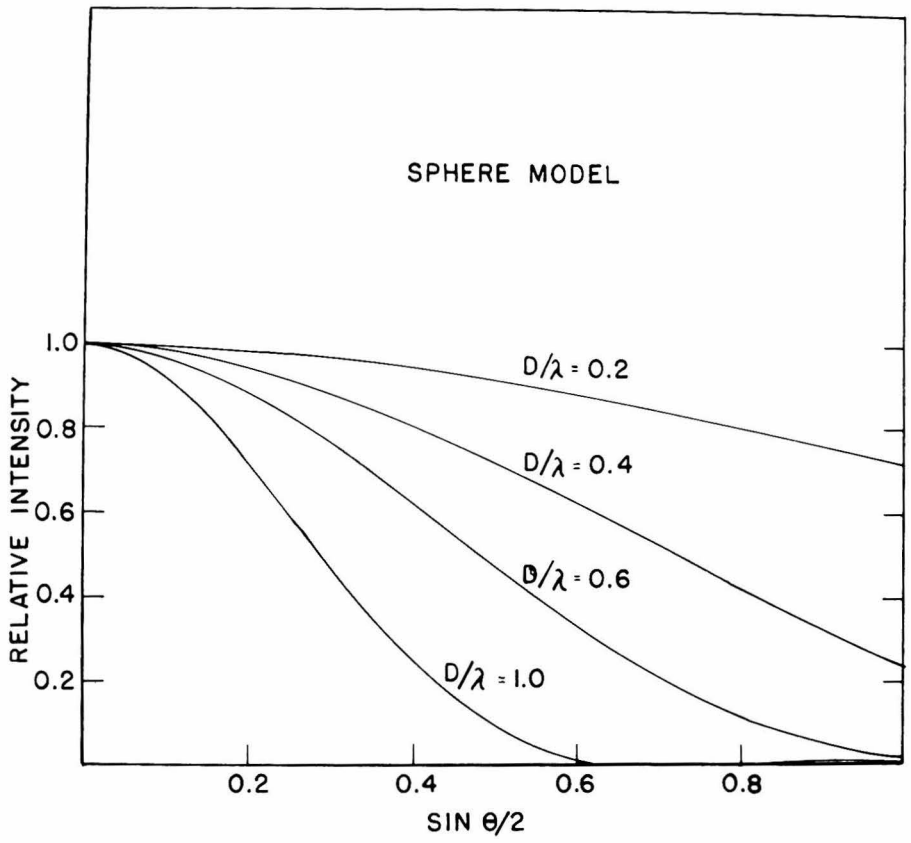

Figure 15. Relative intensity of scattering as a function of angle for the sphere model

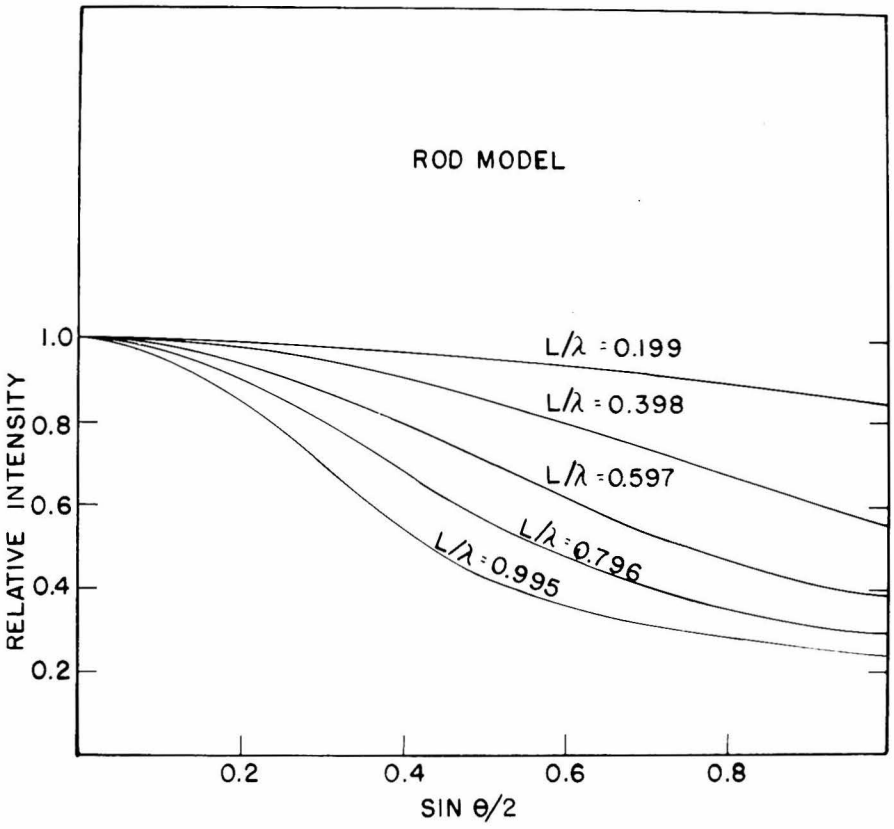

Figure 16. Relative intensity of scattering as a function of angle for the rod model

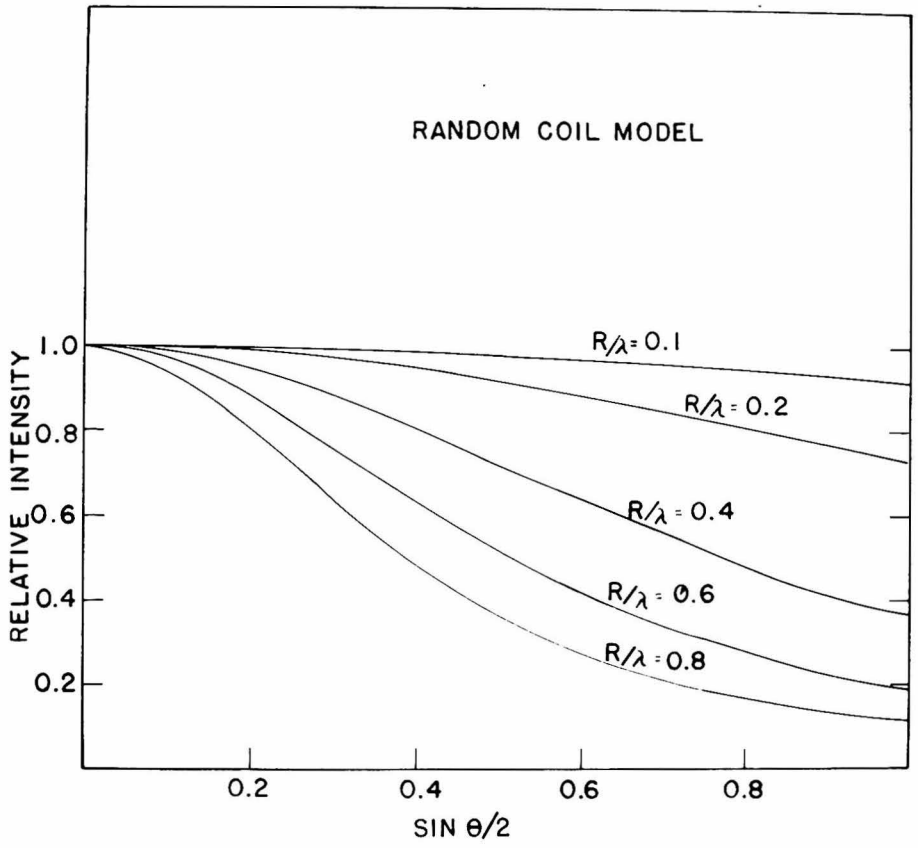

Figure 17. Relative intensity of scattering as a function of angle for the random coil model

For a given value of q, dimensions of the scattering particle equivalent to each of the three models can be evaluated from plots of the theoretical angular intensity for the various models. The relation between q and these characteristic dimensions is shown in Figure 18.

The correction factor which must be applied to the value of the molecular weight determined from the 90° scattering is merely the ratio of the intensities of the scattering at 0° and 90°, respectively. This factor for different values of S/λ, again calculated from the theoretical scattering curves for the three models, is shown in Figure 19.

Summary

A description is given of three instruments which were designed for use in a light scattering investigation of nitrocellulose. These are: a differential refractometer, a photoelectric photometer for measuring 90° scattering, and a camera which measures the angular distribution of scattered light.

The use of carbon disulfide is recommended as a light scattering standard, and a new determination of the absolute scattering power of carbon disulfide has been made.

A detailed procedure is described for the preparation of solutions for light scattering measurements.

A brief summary of the pertinent part of the light scattering theory is presented and a description is given of the methods of handling light scattering data.

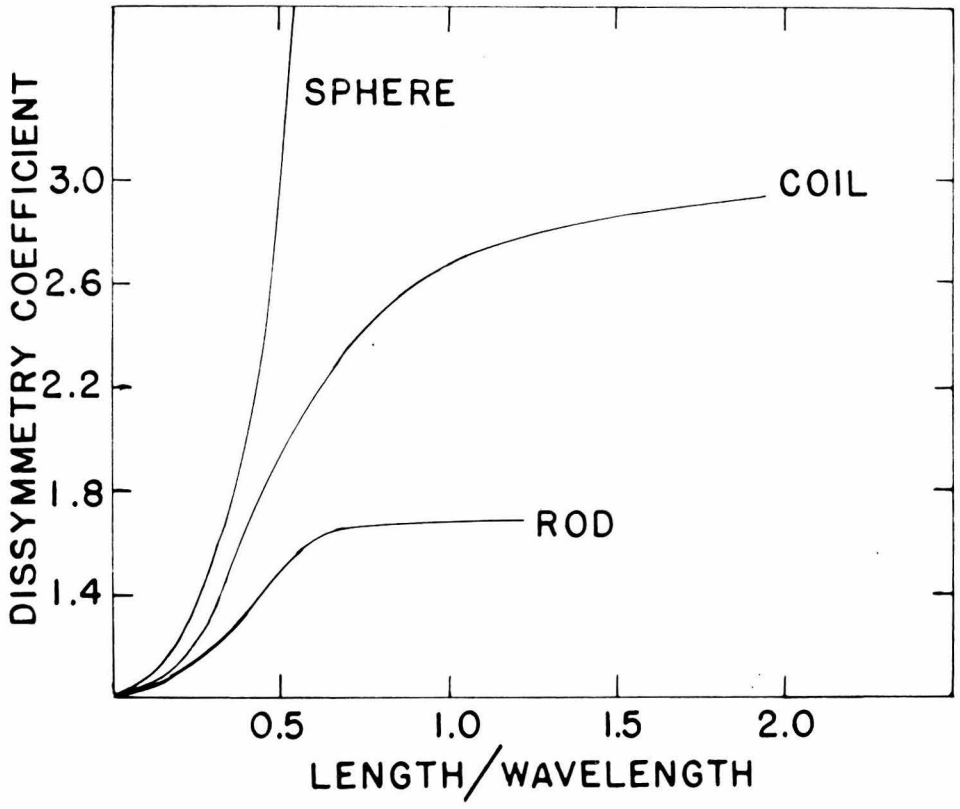

Figure 18. Dissymmetry coefficient as a function of the ratio of the characteristic dimension to wave length of light for three models

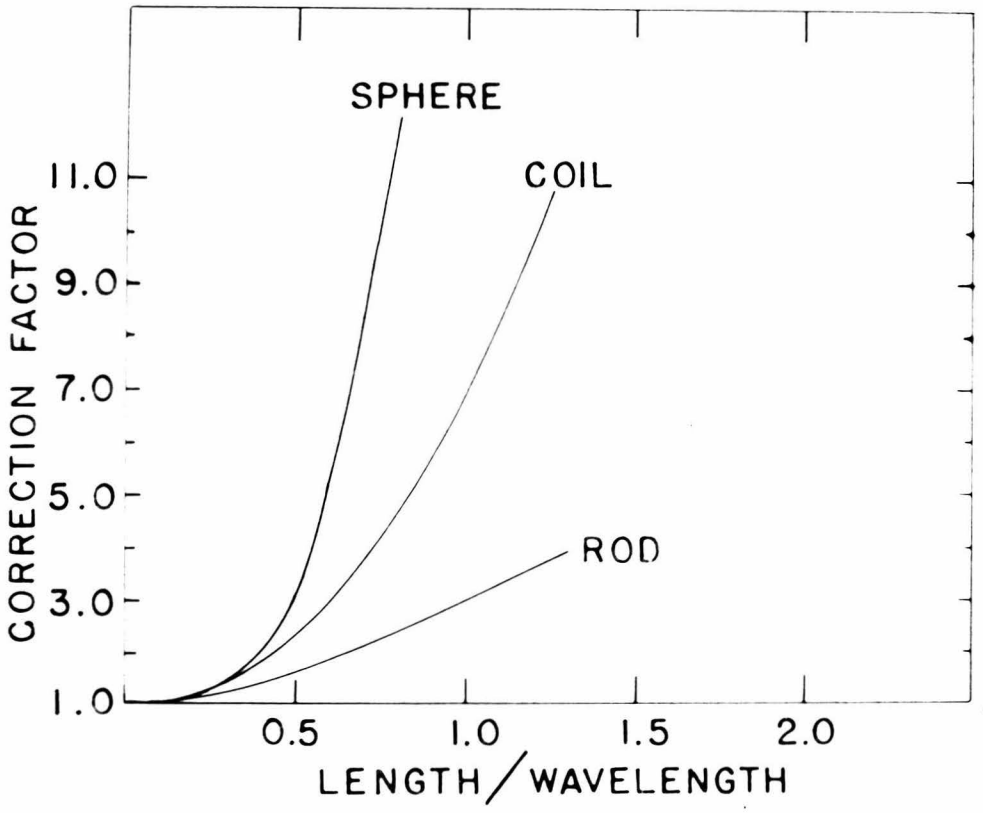

Figure 19. Correction factor as a function of the ratio of the characteristic dimension to wave length of light for three models

Section VII. The Determination of the Properties of Nitrocellulose Molecules in Solution by Light Scattering Methods*

Techniques and instruments developed in this laboratory for the determination of the size and shape of high polymer molecules by the light scattering method were described in the previous section. This section will describe the application of these methods to a study of a series of nitrocellulose fractions which has resulted in rather definite conclusions regarding the character of the nitrocellulose molecule in solution. The results obtained from the light scattering measurements are correlated with viscosity and diffusion data by the use of recently developed theories.

Experimental

The nitrocellulose specimens employed in this investigation include several unfractionated commercial materials and seven fractions. Fractions S-4,3; S-3,4; S-1,1-4; P-3,2; and P-4,2 all of approximately 13.4 per cent nitrogen content, were provided by the courtesy of Professor J. W. Williams of the University of Wisconsin.** Fractions designated as A and B were kindly supplied by Dr. R. L. Mitchell of Rayonnier, Inc. The solvent used in all

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* "The Determination of the Size and Shape of Nitrocellulose Molecules in Solution by Light Scattering Methods II. Experimental Results and Interpretation" by Badger, R. M., and Blaker, R. H., J. Phys. and Coll. Chem. (in press)

** The details of the fractionation procedure are described in OSRD Report No. 4123, PB No. 18861, "The Characterization and Solubility of Fractionated Wood Pulp and Cotton Linters Nitrocelluloses" by J. W. Williams et al.

experiments was chemically pure acetone.

Viscosity Measurements

The viscosity measurements were all made at 25° C. with an Ostwald capillary viscometer according to procedures described in Section II.

Refractive Index Increment

With each of the specimens examined the difference between the refractive index of solution and pure solvent was determined at three concentrations below one per cent, with the use of the differential refractometer described in Section VI. Measurements were made at 25° C. with the 5461 Å and the 4358 Å mercury lines. In all cases the refractive index was found to increase linearly with concentration, within experimental error. The refractive index increment, dn/dc, was obtained from a plot of n versus c. No dependence of this quantity on molecular weight was observed but a marked dependence on nitrogen content is shown in Table 4 and Figure 20.

The trend of dn/dc with nitrogen content is similar to that reported by Jullander 51/, but the absolute values obtained by that investigator appear to be slightly low.

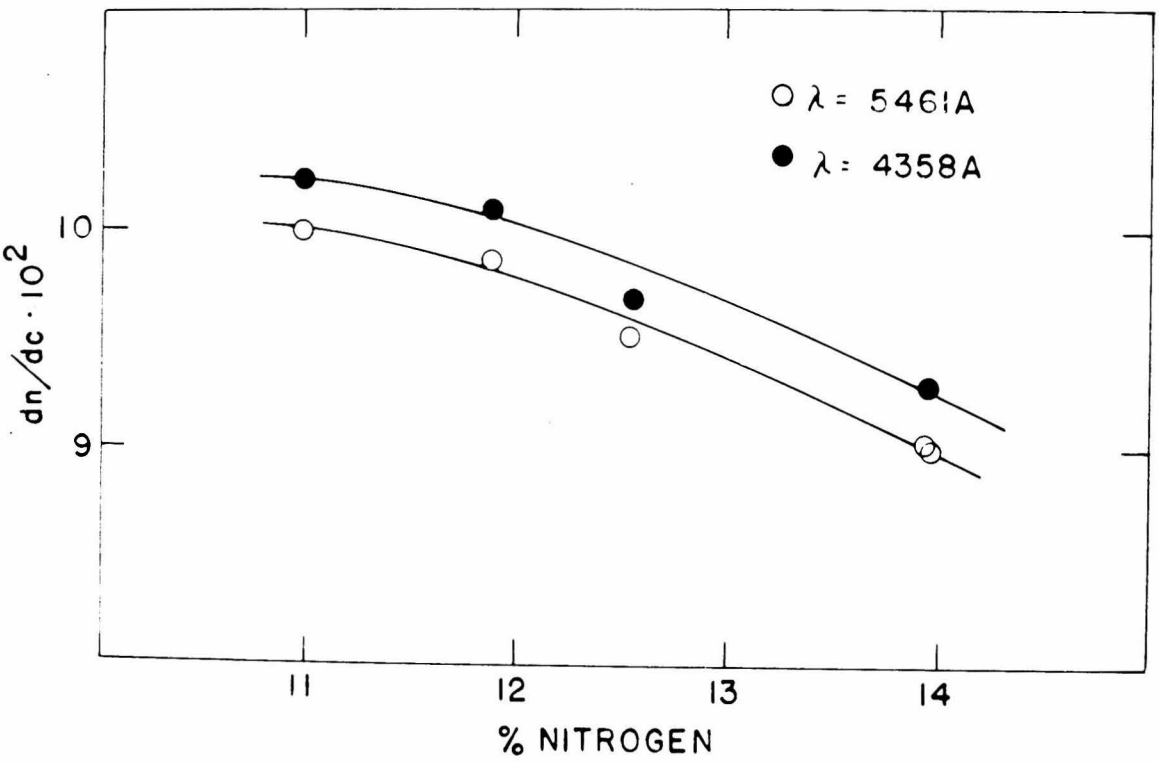

Figure 20. Variation of refractive index increment of nitrocellulose with nitrogen content

Table 4

Refractive index increment of nitrocelluloses of several nitrogen contents in acetone. The concentration c is expressed as weight fraction.

Nitrocellulose Designation	%N	$dn/dc \cdot 10^3$ $\lambda = 5461$ Å	$\lambda = 4358$ Å
Hercules 609-68-2	10.98	99.8	102.2
Hercules 2917	11.89	98.5	101.0
Hercules 5250	12.55	95.0	96.8
Hercules 2465	13.94	90.3	93.0
Rayonnier A	13.96	90.0	

Light Scattering and Depolarization Measurements

The general procedure employed in the light scattering measurements has been described in Section VI. As was there discussed, no filtration procedure has been found at all adequate for preparing solutions in polar solvents, and the acetone solutions were consequently centrifuged for 20 minutes in a field of 32,000 g. which was found adequate to remove dust particles. All measurements here reported were made at approximately 25° C. with the 5461 Å mercury line. The measurement of the 90° scattering was made with unpolarized incident light and the absolute intensity of scattering was determined by comparison with a carbon disulfide standard. In Figure 21 representative plots are shown of c/i versus c, where c is the concentration in weight fraction and i is the intensity of scattering at 90°

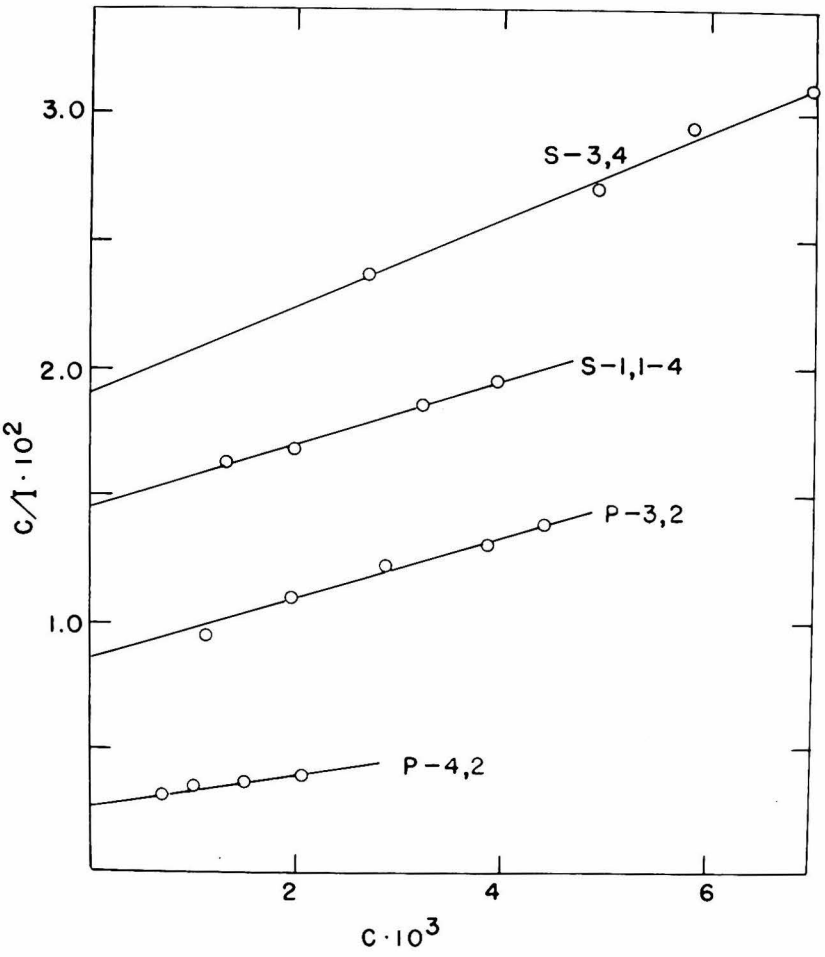

Figure 21. A plot of c/I versus c for four nitrocellulose fractions. c is concentration expressed as weight fraction. I is the ratio of the 90° scattering of the solution to that of carbon disulfide.

relative to that of carbon disulfide.

In connection with the 90° scattering measurements the depolarization of scattered light was determined with the incident light unpolarized. No dependence on concentration or on molecular weight was observed. The average depolarization for four nitrocellulose fractions of 13.4 per cent nitrogen was found to be 0.029.

In studies of the angular distribution of scattering the incident light was polarized with the plane of vibration perpendicular to the plane including incident and scattered beams, since this simplifies somewhat the interpretation of the results. Measurements of the intensity of scattering relative to the intensity at 90° were made at a series of angles between 53° and 124° in solution. Plots showing the results obtained in six fractions are shown in Figure 22. The curves for fractions P-4,2 and for Rayonnier B are practically identical and coincide in the plot.

In the concentration range 0.2-1.0 per cent no dependence of angular distribution on concentration was found, which is in agreement with the observations of Stein and Doty on cellulose acetate 30/.

Results of Measurements

Although in principle direct information regarding molecular shapes should be obtainable from the shapes of the angular distribution curves, this is in practice not yet possible. The curves calculated for different molecular models differ little in shape in the angular range of practical measurement, and very high accuracy of measurement would be necessary to determine uniquely the molecular model which is applicable. Consequently the scattering curves

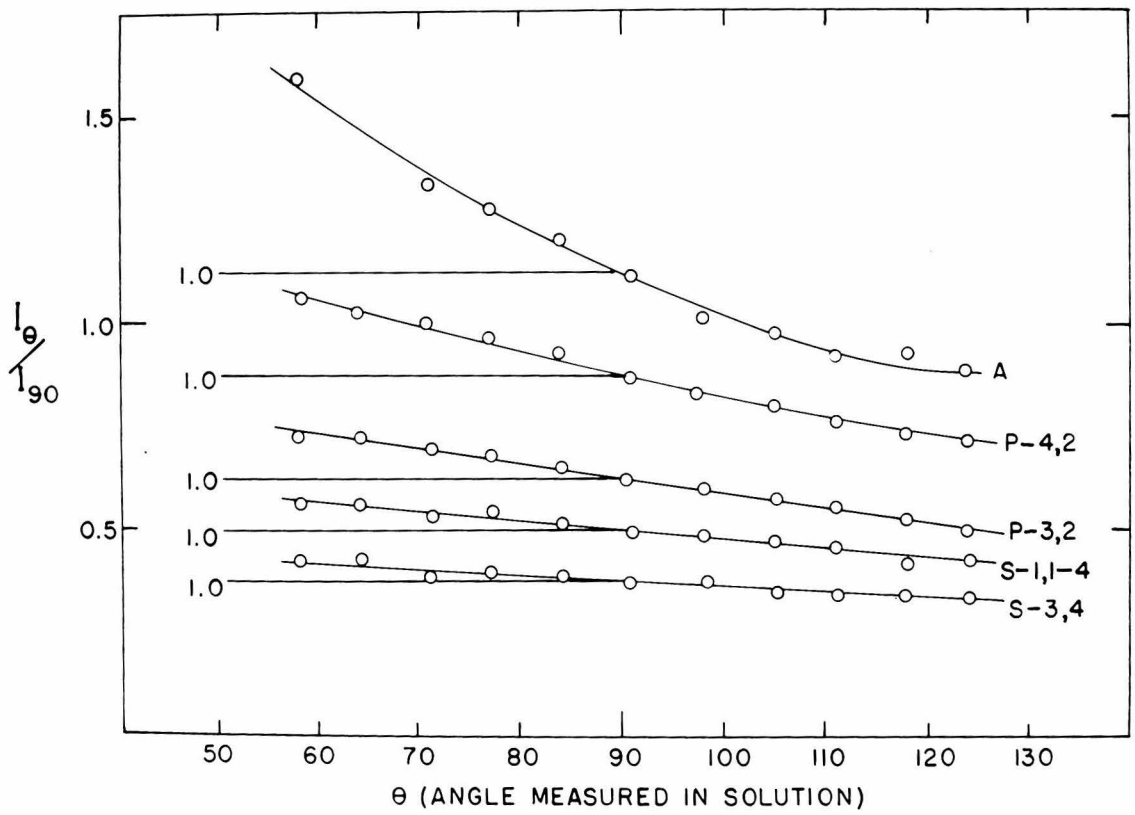

Figure 22. The angular distribution of scattering for five nitrocellulose fractions

have been characterized merely by a dissymmetry coefficient q, which is the ratio of scattered intensity at two angles, namely at 60° and 120°, measured in the solution. This quantity can, however, be obtained more reliably from the smoothed plot of data taken over a range of angles than from two measurements alone.

With the use of this dissymmetry coefficient a characteristic dimension has been calculated for three different molecular models, the sphere, the rod, and the random coil, by the use of methods which have previously been described in the literature and have recently been reviewed 38/. The dimension calculated in the respective cases is the sphere diameter D, the rod length L, and the root mean square distance between ends of the coil, $\sqrt{\langle R^2 \rangle_{av}}$, which we shall designate simply by R. These quantities are given in Table 6.

It should be mentioned that the behavior of the Rayonnier fraction B seemed to be somewhat anomalous in regard to the low asymmetry of scattering as compared with the high molecular weight and high viscosity . The quantities calculated from the asymmetry are consequently of somewhat doubtful significance.

In the determination of molecular weights from the light scattering data not only has the Cabannes factor been applied in all cases, but for all except the fraction of lowest molecular weight for which the asymmetry of scattering was negligible, equation (13) has been corrected by multiplication of its right hand member by a factor calculated from the dissymmetry of scattering. This factor is the ratio of the intensities of scattering at 0° and 90°, respectively. Since the scattering at 0° is not observed it must be

calculated from the observed dissymmetry of scattering, by employing one of the probable molecular models. Molecular weights calculated on the basis of three models are given in Table 6. They differ significantly only for the higher molecular weights.

At very high molecular weights the spherical model may have some validity, but the two models of particular interest are, of course, the random coil and the rod. As will be shown below, when the degree of polymerization, z, is much less than 100 the rod model is presumably the more nearly applicable. At higher molecular weights the cellulose molecule presumably assumes the characteristics of a random coil. It should be pointed out, however, that the particular random coil model on which the light scattering equations, and the viscosity and diffusion equations later to be discussed, are based does not adequately represent the cellulose molecule. Consequently it is not certain to what extent these equations are applicable to cellulose and the present considerations must be ragarded as tentative.

A portion of the molecular model upon which existing statistical considerations of the random coil molecule have been based is shown in (A) of Figure 23. The internal configuration of such a chain is specified by one set of coordinates, ϕ_l, the angles between successive bond pairs. This model is presumably adequate for representing the behavior of polyethylene and other similar substances. Now in cellulose the two bonds connecting the two glucoside oxygens to a given glucose ring may be approximately parallel, but their projections are certainly far from coincident. Consequently if

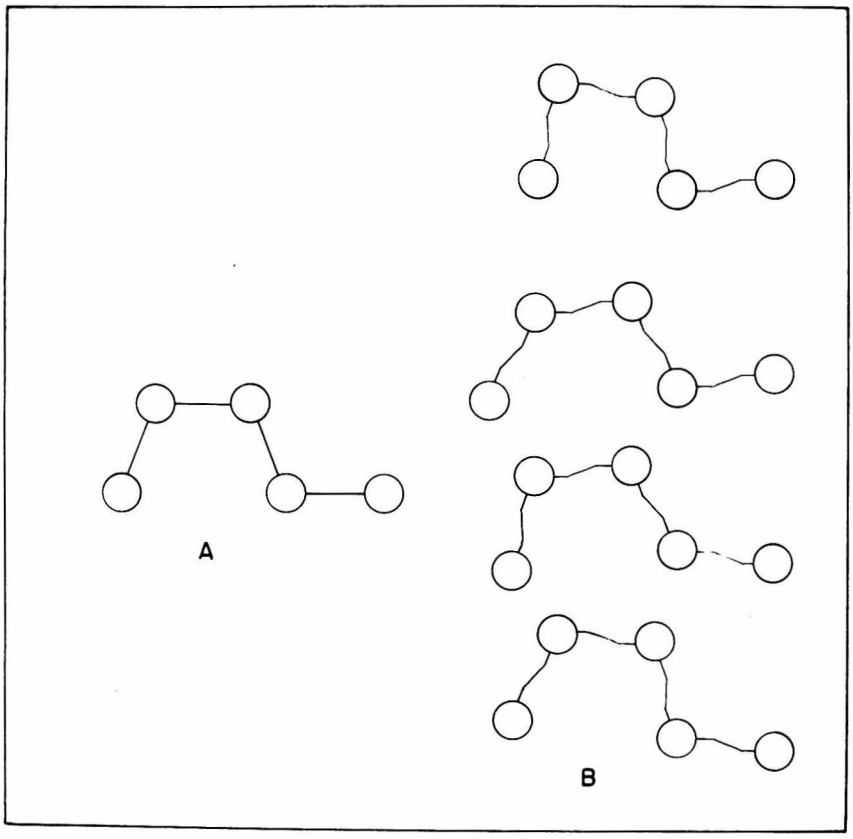

Figure 23. Portions of two models for the random coil molecule

one represents the cellulose molecule by a simplified model consisting of a string of oxygen atoms connected by bonds representing the glucose rings, these bonds must each have an "offset", as shown in (B) of Figure 23. To describe the configuration of the chain a new set of coordinates is required which relate to the rotation of the "kinked bonds" about axes parallel to their extensions. The importance of the new degrees of freedom is shown in the figure, which presents four of the sixteen possible planar configurations corresponding to one planar configuration of the polyethylene model.

A theoretical treatment of the cellulose model must certainly be made before one can be quite certain that conclusions based on statistical considerations of the simpler polyethylene model are valid in the case of cellulose and its derivatives. If these conclusions are valid we should expect that R, the root mean square distance between ends of the molecule, would increase with the square root of z, the degree of polymerization, provided that z is not too small. Actually the calculated $R/z^{\frac{1}{2}}$ is found to decrease slightly with z, as may be seen in Table 7 and Figure 24. An even stronger trend was observed for cellulose acetate by Stein and Doty 30/. Whether this trend is due to the reasons just mentioned or results from experimental error remains to be determined.

The results of the light scattering and viscosity measurements are presented in Table 5 and quantities derived from them in Tables 6 and 7. For correlation with these data diffusion constants are much to be desired, but unfortunately accurate diffusion measurements on nitrocellulose fractions do not appear to have been made. We consequently include approximate diffusion constants determined in this

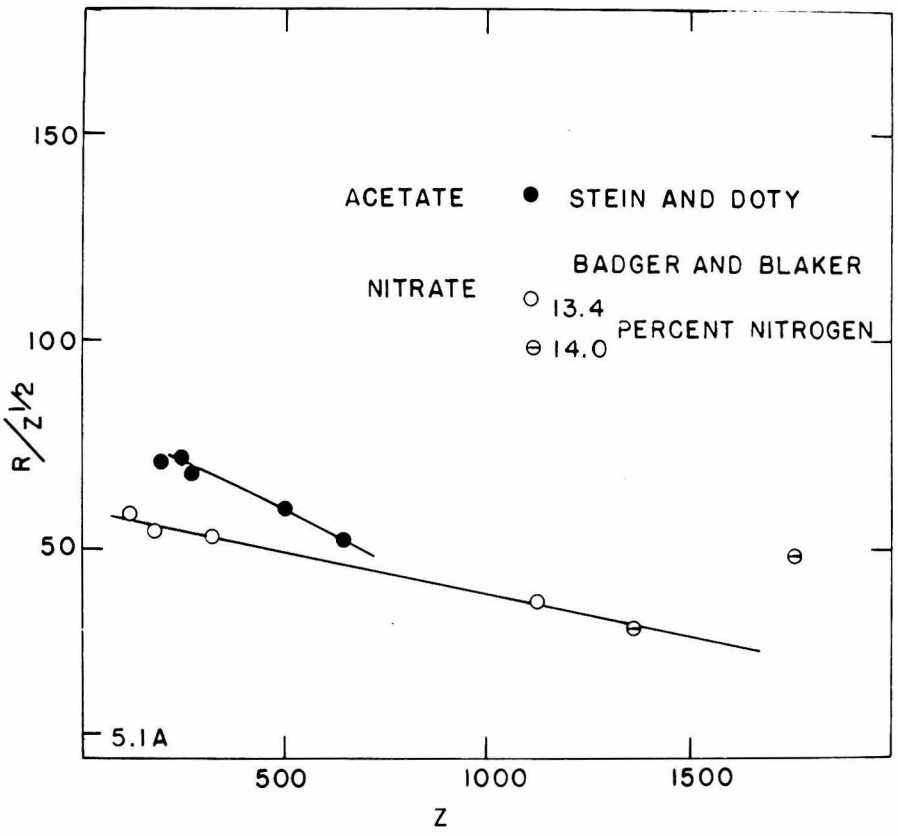

Figure 24. Variation of $R/Z^{\frac{1}{2}}$ with Z for nitrocellulose and cellulose acetate

laboratory by a rapid method 52/ which may be sufficiently accurate to be of use in the following discussion. These constants show precisely the same trend with molecular weight as was found by Jullander for unfractionated material 51/, but are about 25 per cent smaller. This difference is not unreasonable considering that the two sets of measurements involve fractionated and unfractionated materials, respectively.

Table 5

Data on Light Scattering, Viscosity and

Diffusion of Nitrocellulose Fractions in Acetone Solution

Sample No.	Nitrogen Content %	Intrinsic Viscosity $[\eta]$	$\bar{M}_w$ (random coil)	Asymmetry of Scatter ($q = I_{60°}/I_{120°}$)	Diffusion Constant ($cm^2 sec^{-1} \times 10^7$) (D)
S-4,3	13.18	0.30	9,400	(~1)	24
S-3,4	13.36	1.30	35,000	1.10	10.6
S-1,1-4	13.44	2.22	50,000	1.13	6.9
P-3,2	13.41	2.98	93,000	1.22	4.5
P-4,2	13.42	6.86	319,000	1.38	
Ray B	13.96	14.90	400,000	(1.31)	
Ray A	13.96	21.00	518,000	1.86	

Table 6

Molecular Weights and Dimensions for Three Different Molecular Models

Light Scattering Molecular Weights ($\bar{M}_w$)				Molecular Dimension (A)			
Sample	Sphere	Rod	Coil	D (sphere)	L (rod)	R (coil)	Extended Length*
S-4,3	9,400	9,400	9,400				170
S-3,4	35,000	35,000	35,000	605	805	645	630
S-1,1-4	49,000	49,000	50,000	685	890	725	900
P-3,2	87,000	89,000	93,000	800	1210	960	1670
P-4,2	298,000	312,000	319,000	1080	1690	1250	5700
Ray B	356,000	370,000	400,000	(965)	(1450)	(1120)	6550
Ray A	394,000		518,000	1470		2008	9200

* Calculated on basis of the molecular weight for the random coil model

Table 7

"Staudinger Constant" and "Effective Bond Length" for the Random Coil Molecule

Sample	z (D.P.)	$z^{\frac{1}{2}}$	$[\eta]/z \times 10^3$	$R/z^{\frac{1}{2}}$ (A)
S-4,3	33	5.8	9.1	
S-3,4	123	11.1	10.5	58.0
S-1,1-4	176	13.3	12.6	54.5
P-3,2	327	18.1	9.1	53.0
P-4,2	1120	33.5	6.0	37.2
Ray B	1360	36.9	11.9	(30.4)
Ray A	1760	41.9	10.8	49.2

lation between diffusion and viscosity data, but since this relation is rather more explicit in the Kirkwood-Risemann theory, and is based on a more definite molecular model, our discussion will be largely restricted to that case. Both theories represent adequately the molecular weight dependence of viscosity in polystyrene, etc., but the more severe test of correlating viscosity and diffusion data has not yet been made. (Hereafter, the Kirkwood-Risemann theory will be referred to as the K-R theory.)

In attempting to apply either theory to cellulose and its derivatives one meets with serious difficulty. If the parameters are so chosen as to fit the decrease in $[\eta]/z$ which sets in with $z \sim 400$, the region in which it is relatively constant is not well represented, as will be shown below. The reason for the failure of the theory in the low molecular weight range is obvious if one considers the data in Table 7. The statistical methods employed are valid only when z is not too small, and the more restricted the rotation of groups in the molecular chain the larger the value of z at which the theory becomes applicable. The lower limit of applicability may be roughly estimated as follows. The statistical treatment yields the result that for z sufficiently large, $R = bz^{\frac{1}{2}}$, where R is the root mean square distance between ends of the polymer chain, and the proportionality constant b is an "effective" bond length. The more restricted the rotation the greater will be the ratio of b to b_0, the actual length of monomer unit. But since it is physically impossible that $R > bz$ we may expect that the statistical method will be applicable only when $b_0 z > bz^{\frac{1}{2}}$, or when $z > (b/b_0)^2$. Now all measurements agree in indicating that the

molecules of cellulose and derivatives are rather stiff, and at moderate molecular weights are nearly fully extended. In the case of nitrocellulose our light scattering data yield the value $b \sim 54$ Å, as shown in Table 7. From X-ray data on cellulose we may take $b_0 \sim 5.1$ Å. Consequently we may expect the K-R theory to fail when $z < 100$, and to predict too high values of $[\eta]/z$ for smaller values of z. This indeed appears to be the case.

There are few viscosity and molecular weight data for $z < 100$, but examination of all reliable data on fractionated cellulose acetate or nitrate with which we are familiar suggests a rather surprising fact, namely that $[\eta]/z$ appears to have a broad maximum at $z \sim 120$. No one set of data by itself at all adequately supports this conclusion. In some cases the measurements do not extend to sufficiently low molecular weights for the assumed decrease of $[\eta]/z$ to be observed. In other cases the data have not been interpreted in the manner now regarded as most acceptable. It is, however, rather impressive that seven sets of data, involving molecular weight determinations by four methods, all more or less strongly support the conclusion. These data are quoted in Table 8, and a few sets are represented in Figure 25.

The region $z < 80$ would repay investigation, but this will be rather difficult since the range of special interest is precisely that in which molecular weight determination becomes very difficult. One can, however, make a rough estimate of the behavior to be expected in this region.

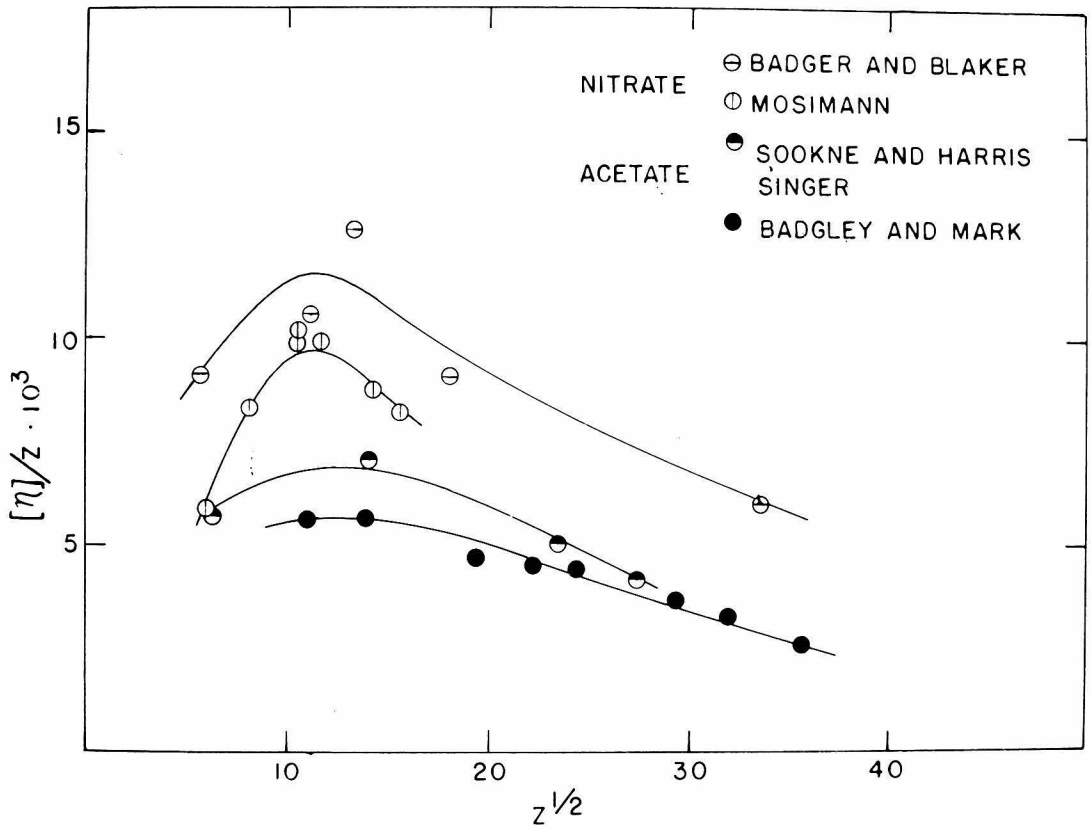

Figure 25. Variation of $[\eta]/Z$ with $Z^{\frac{1}{2}}$ for nitrocellulose and cellulose acetate

Table 8

Cellulose Derivative	Molecular Weight Determination	Investigators	References
Nitrate	light scattering	Blaker	
Nitrate	equilibrium sedimentation	Mosimann	58/
Nitrate	osmotic pressure	Husemann and Schulz*	59/
Nitrate	osmotic pressure	Blaker et al.	60/
Acetate	osmotic pressure	Badgely and Mark	53/
Acetate	osmotic pressure	Sookne and Harris	61/
Acetate	sedimentation rate	Singer, Sookne, and Harris	62, 61/

* These authors used a thermodynamically unacceptable function to extrapolate the reduced osmotic pressure to zero concentration and the reported molecular weights are consequently somewhat in error. If the data are recalculated according to accepted practice the values of $[\eta]/z$ show a trend in reasonable agreement with other data.

When z is small the length of the stiff cellulose molecule will increase with a power of z which is at first nearly unity, but gradually decreases with increasing z. In this region the Simha 63/ treatment of the elongated prolate ellipsoid should be reasonably applicable and $[\eta]/z$ may be expected to increase with a power of z at first somewhat less than unity, and slowly diminishing. As the slight flexibility of the molecule accumulates, the random coil model will eventually become applicable and $[\eta]/z$ will decrease as is predicted by the newer viscosity theories, and as is observed for $z > 120$.

The approximate validity of the simple Staudinger relation over

the range of molecular weights which is of most practical interest is consequently seen to be rather accidental and results from the occurrence within that range of a flat maximum of $[\eta]/z$ in the transition region where the cellulose molecule loses the character of a rigid rod and gradually assumes that of a random coil. This maximum may have interesting consequences when the Staudinger equation is used for estimating weight average molecular weights of heterogeneous materials.

The viscosity and diffusion data on cellulose and derivatives are inadequate for making a really satisfactory test of the K-R theory in the region in which it may be expected to be valid. We nevertheless present our own data together with those of other investigators on cellulose acetate, for comparison. In Figure 26 the diffusion data are plotted. According to the K-R theory the plot of Dz versus $z^{\frac{1}{2}}$ should be a straight line. Although the data on nitrocellulose are admittedly not precise they show the same trend as data on unfractionated material obtained by Jullander 51/ and we consequently believe that the slope of the line drawn through the points can not be greatly in error.

The K-R viscosity and diffusion equations involve two molecular parameters: b, an effective bond length, and f the frictional constant of the monomer unit. Values of these constants obtained from the slopes and intercepts of the plots of Figure 26 are given in the first row of Table 9. In the case of the nitrate the effective bond length, b, lies within the limits determined from our light scattering measurements. In the case of the acetate the agree-

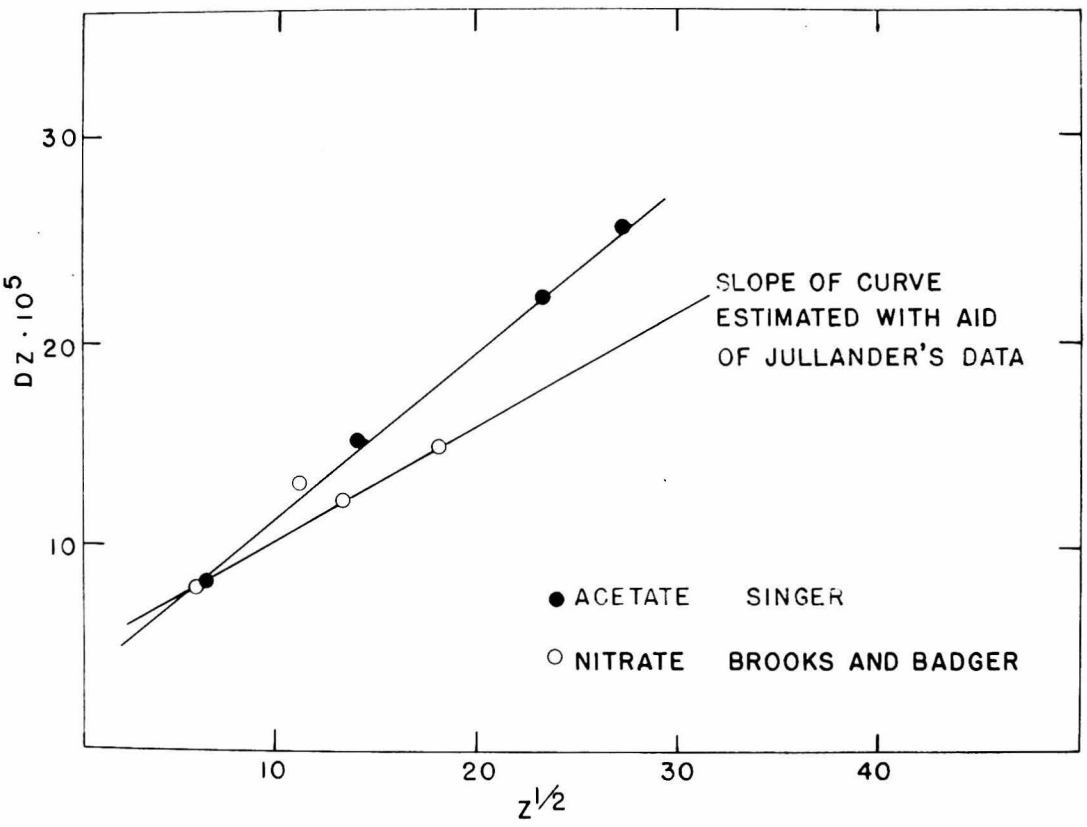

Figure 26. Variation of DZ with $Z^{\frac{1}{2}}$ for nitrocellulose and cellulose acetate

ment is less good. It seems possible to us that Stein and Doty 30/ have somewhat overestimated the asymmetry of scattering due to the presence of contamination. Certainly to judge from the viscosities one should expect b for the acetate to be smaller than for the nitrate if ζ, the frictional constant of the monomer unit is of the same order of magnitude.

It is obviously not possible to obtain a unique set of parameters from the viscosity data alone since the K-R theory approaches validity only at the upper end of the molecular weight range investigated. According to this theory plots of $[\eta]/z$ versus $z^{\frac{1}{2}}$ for all linear polymers should be brought into coincidence by appropriate changes in the scales of abscissae and ordinates. The scale factor of abscissae depends upon λ_o which is proportional to the ratio ζ/b. In Figure 27 curves are drawn for two values of λ_o. The solid curve in each case corresponds to the value of λ_o indicated by the diffusion data, and the shape of the curve appears to fit the viscosity data reasonably well in the upper range of molecular weights. However, in each case the value of K_0, (the limit of $[\eta]/z$ as $z \rightarrow 0$), which must be chosen to make the curves pass through the experimental points, is only about half that predicted from the diffusion data. This discrepancy is not too serious since according to the theory K_0 is proportional to ζb^2. If the discrepancy is proportionally distributed over both ζ and b it consequently amounts to an error of only about 25 per cent in each parameter.

Table 9

Molecular Parameters of the Kirkwood-Risemann Viscosity and Diffusion Equation Determined in Various Ways

Method	Cellulose Nitrate (13.4 per cent nitrogen)				Cellulose Acetate Diffusion Data: Singer 62/ Viscosity Data: Badgely and Mark 53/ Light Scattering Data: Stein and Doty 30/			
	b(Å)	$\zeta \times 10^9$ (g/sec)	$\lambda_0 \times 10^2$	$K_0 \times 10^3$	b(Å)	$\zeta \times 10^9$ (g/sec)	$\lambda_0 \times 10^2$	$K_0 \times 10^3$
Diffusion	46	0.8	4.3	34	32	1.11	8.0	23
Viscosity	—	—	4.0	15	—	—	(4.0)	(8.8)
	—	—	(8.0)	(20)	—	—	8.0	12
Light Scattering	57-32				(73-54)			

b is the effective bond length; ζ the frictional constant of the monomer unit; K_0 the limit of $[\eta]/z$ as $z \to 0$; and λ_0 in the K-R theory is defined as $\zeta/(\sqrt{6\pi^3}\,\eta_0 b)$, where η_0 is the coefficient of viscosity of the solvent.

A question of some interest is whether the large dependence of the intrinsic viscosity of cellulose derivatives upon the degree of substitution, is to be attributed to differences in the frictional constants of the monomer units, or to differences in the "stiffness" of the molecules. It is evident that the effect of these two factors will depend considerably upon the molecular weight range under consideration. In the range of usual interest the K-R theory is approaching validity and predicts that the intrinsic viscosity will increase with somewhat less than the first power of $\mathcal{J}$, and with somewhat more than the second power of b. The high viscosity of the high nitrogen Rayonnier fraction A, in comparison with nitrocellulose of 13.4 per cent nitrogen, consequently is qualitatively accounted for by the more extended, stiffer molecule indicated by the asymmetry of scattering. In the case of the cellulose nitrate and acetate, for which data are shown in Figure 27 and Table 9, the diffusion data indicate that a more extended molecule is responsible for the greater viscosity of the nitrate, although the larger value of b is partially compensated for by a smaller $\mathcal{J}$.

As was previously mentioned, the question must remain open for the present as to the extent to which the K-R theory based on the polyethylene model is quantitatively applicable to cellulose and its derivatives.

Summary

A series of nitrocellulose fractions covering the molecular weight range 9,400-518,000 has been investigated by light scattering

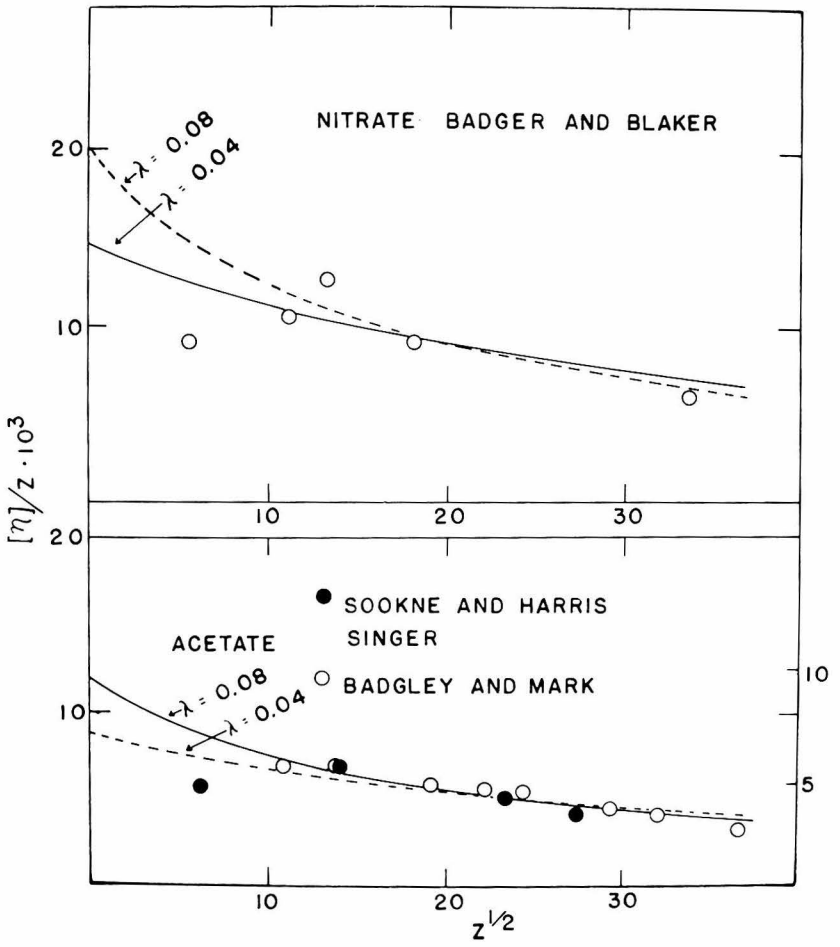

Figure 27. A comparison of experimental values of $[\eta]/Z$ with those predicted by the K-R theory. The scale of the right ordinate is for the data of Badgley and Mark.

methods. The nitrocellulose molecule has been shown to be relatively stiff, and to be nearly fully extended up to a degree of polymerization $z \sim 100$. The "effective bond length" of the random coil observed at high molecular weights is roughly 50 Å, about 10 times the length of the monomer unit. This conclusion is supported by diffusion data interpreted in the light of the recent Kirkwood-Risemann theory of viscosity and diffusion. This theory is, however, shown to be valid for cellulose derivatives only for degrees of polymerization in excess of 100. It is suggested that the validity of the simple Staudinger viscosity relation in the molecular weight range of usual interest is somewhat accidental and results from the occurrence of a broad, flat maximum of $[\eta]/z$ in this region. This maximum presumably corresponds to a transition in which the cellulose molecule loses the properties of a rod and assumes those of a random coil.

Section VIII. Shape of the Nitrocellulose Molecule in Different Solvents

Many of the earlier studies of the molecular properties of nitrocellulose in solution were designed to prove or disprove Staudinger's hypothesis that there is a simple relation between the molecular weight of a polymer and the intrinsic viscosity of its solution. Considerable data was presented to show that the intrinsic viscosity of solutions of nitrocellulose was independent of the solvent 64/. Later experimental investigations have shown that the intrinsic viscosity is really dependent on the solvent, and recent articles have given theoretical justification for the dependence, 65, 56, 57/.

The intent of this brief investigation was to correlate the dimensions of the nitrocellulose molecule in different solvents as determined by light scattering methods with the intrinsic viscosity in these solvents. It was soon found, however, that those solvents in which nitrocellulose gave a very high viscosity had a refractive index so near to that of nitrocellulose that light scattering measurements did not yield significant information.

Experimental

The intrinsic viscosity of a commercial nitrocellulose, Hercules No. 8432 (cotton linters, 12.6 % nitrogen) was measured in seven solvents, and the results are tabulated in Table 10. The technique employed in making the measurements has been described in Section II.

Table 10

Intrinsic Viscosity of Nitrocellulose in Different Solvents

Solvent	Intrinsic Viscosity at 25° C.
Acetone	2.50
Ethyl Acetate	2.90
Diethylketone	2.95
Nitropropane	3.14
Diisopropylketone	3.30
Nonanone	3.70
Nitrobenzene	4.60

Angular scattering measurements on Nitrocellulose No. 8432 by the technique described in Section VI gave a value for q, the dissymmetry coefficient, of 1.22 in acetone and 1.28 in nonanone. Since this difference is only slightly greater than the experimental error it did not seem profitable to measure dissymmetry coefficients for solutions in the other solvents.

Discussion

The theory of Kirkwood and Risemann 57/ leads to the conclusion that for a polymer molecule which can be approximated by the random coil model the molecular weight is proportional to the quantity $\frac{R^3}{[\eta]}$ where R is, again, the root mean square distance between the ends of the molecule. The value of $\frac{R^3}{[\eta]}$ should therefore be a constant independent of the solvent.

If nitrocellulose is assumed to be a random coil, a value of R corresponding to the observed value of q can be taken from Figure 18 of Section VI. A value of q of 1.22 gives R = 960 Å, and a value of q of 1.28 gives R = 1040 Å.

Since $\frac{(960)^3}{2.50}$ is equal to $\frac{(1040)^3}{3.70}$ within the uncertainty of the respective values of R it follows that the observed difference in intrinsic viscosity of nitrocellulose in different solvents may be attributed, at least to a large degree, to the difference in the degree of extension of the molecule in different solvents.

This result is in agreement with the result of a light scattering investigation of polystyrene in various solvents 66/. In general the observed change of intrinsic viscosity is not as large for nitrocellulose as for various synthetic polymers because the nitrocellulose molecule has been found to resist extensive coiling in solution as shown in Section VII.

Section IX. Properties of Nitrocellulose in Binary Solvents

Although the progressive addition of a non-solvent to a nitrocellulose solution will eventually precipitate the nitrocellulose and is the basis of the common fractionation procedure, it has been recognized that the presence of small amounts of certain non-solvents will enhance the solvent power of even the best nitrocellulose solvents. Kraus 67/ reported that nitrocellulose would go into solution faster in acetone containing a little water than in pure acetone; also that the rate of solution was greater in a mixture of hexane and ethyl lactate than in ethyl lactate alone. Wilson and Miles 68/ concluded that nitrocellulose was solvated to a greater extent in a water-acetone mixture than in pure acetone, and Nakashima and Saito 69/ found that more heat was liberated when nitrocellulose was dispersed in a mixture of benzene and acetone than in pure acetone. Similar data have been reported for other polymer-solvent systems.

Dobry 70/ has reported that the osmotic pressure of a nitrocellulose solution is the same in a binary solvent as in a single solvent, and Gee 71/ has obtained comparable results for rubber in mixed solvents.

Staudinger and Sorkin 72/ found that the intrinsic viscosity of a nitrocellulose solution in a water-acetone mixture did not differ from that in acetone until almost enough water was added to cause precipitation. Weissburg and Simha 73/ have reported the same result for cellulose acetate in mixtures of acetone and methanol.

Ewart, Roe, Debye, and McCartney 74/ found that the turbidity

of a polymer solution was markedly influenced by the addition of a second fluid 75/ and have developed a theory which explains the change in turbidity as being due to changes in the refractive index of the solute particles because of preferential absorption of one of the solvent components. Kirkwood and Goldberg 26/ and Stockmeyer 76/ have recently developed theories of the light scattering of multicomponent systems which explain the increase in turbidity of a polymer solution in the presence of the second solvent component as being due to thermodynamic interactions between the macromolecular solute and the components of the solvent.

This phase of the investigation of nitrocellulose was undertaken to learn whether light scattering experiments would be of value in understanding the behavior of nitrocellulose in mixed solvents. The general treatment of the light scattering of multicomponent systems has been made available to me in advance of its publication through the courtesy of Professor Kirkwood and this theory has guided the light scattering experiments which will be described.

Light Scattering Theory for Multicomponent Systems

Einstein 33/ has derived a general expression for the turbidity due to scattering, τ_0, of any fluid in terms of the wave length, λ, of the incident light and $\langle \Delta\varepsilon^2 \rangle_{av}$, the dielectric constant fluctuations in volume, V.

$$\tau_0 = \frac{8\pi^3}{3\lambda^4} V \langle \Delta\varepsilon^2 \rangle_{av} \qquad (18)$$

Kirkwood and Goldberg have been able to evaluate the contribution of fluctuations in composition and density to the term $\langle \Delta \varepsilon^2 \rangle_{av}$ of the Einstein equation by the use of the grand canonical ensemble of Gibbs. Their equation twelve is a complete expression for the turbidity of a solution containing ν components.

$$\tau_o = \frac{8\pi^3}{3\lambda^4}\left\{ \frac{kT}{\beta}\left(\frac{\partial \varepsilon}{\partial \rho}\right)^2_{T,C} + \frac{1}{N_0 \rho_0} \sum_{\substack{i,k \\ =1}}^{\nu} c_i c_k \frac{|\beta|_{ik}}{|\beta|}\left(\frac{\partial \varepsilon}{\partial c_i}\right)_{T,P,C_j}\left(\frac{\partial \varepsilon}{\partial c_k}\right)_{T,P,C_j} \right\} \quad (19)$$

in which

β is the isothermal compressibility,

ρ_0 is the mass of the major solvent per unit volume,

N_0 is Avogadro's number,

$|\beta|$ is the determinant of the thermodynamic coefficients; for example,

$$\beta_{ik} = \frac{c_i c_k}{M_i RT}\left(\frac{\partial \mu_i}{\partial c_k}\right)_{T,P,C_j}$$

$|\beta|_{ik}$ is the appropriate cofactor of the determinant

When equation (19) is constrained to a system of three components, a high molecular weight solute and two low molecular weight solvent components, it takes the form:

$$\tau = \frac{H_2 M_2 R T}{\left(\frac{\partial \mu_2}{\partial c_2}\right)} \left\{ \frac{1 - 2\alpha w_1 + \alpha^2 w_1^2}{1 - \left(\frac{M_2}{M_1}\right) w_1 w_2} \right\}$$

$$H_2 = \frac{32 \pi^3 n^2}{3 N_0 \beta_0 \lambda^4} \left(\frac{\partial n}{\partial c_2}\right)^2$$

$$\varepsilon = n^2$$

$$w_1 = \left(\frac{\partial \mu_1}{\partial c_2}\right) \Big/ \left(\frac{\partial \mu_1}{\partial c_1}\right) \qquad (20)$$

$$w_2 = \left(\frac{\partial \mu_1}{\partial c_2}\right) \Big/ \left(\frac{\partial \mu_2}{\partial c_2}\right)$$

$$\alpha = \left(\frac{\partial n}{\partial c_1}\right) \Big/ \left(\frac{\partial n}{\partial c_2}\right)$$

where τ is the turbidity of the solution less that of the solvent,

n is the refractive index of the solution,

μ is the chemical potential, and

c is expressed as grams per gram of the major solvent component.

Since $$\mu_i = RT \log_e \gamma_i c_i + \mu_i^\circ(T,P) \qquad (21)$$

where γ_i is the activity coefficient of component i, equation (20) can be put in a more convenient form by expressing $\log_e \gamma_i$ in a power series

$$\log_e \gamma_i = \sum_{K=1}^{2} A_{iK} c_K + \sum_{j,K=1}^{2} B_{ijK} c_j c_K + \cdots \qquad (22)$$

and α as

$$\alpha = \alpha_0 + \alpha_1 c_1 + \alpha_2 c_2 \qquad (23)$$

When these expressions are substituted into equation (20) and all terms involving the cube or higher powers of the concentration are dropped, the following working equation is obtained:

$$H_2 \frac{c_2}{c} = \frac{1}{M_2}\left\{1 + G_{10} c_1 + G_{01} c_2 + G_{20} c_1^2 + G_{11} c_1 c_2 + G_{02} c_2^2 + \cdots\right.$$

where

$$G_{10} = 2\alpha_0 A_{12} \; ; \quad G_{01} = A_{22} \; ; \quad G_{02} = 2B_{222} \qquad (24)$$

$$G_{20} = 4\alpha_0 B_{112} - 2\alpha_0 A_{11} A_{12} + 2\alpha_1 A_{12} + 3\alpha_0^2 A_{12}^2$$

$$G_{11} = 2\left(1 + 2\frac{M_1}{M_2}\alpha_0\right) B_{212} - \frac{M_2}{M_1} A_{12}^2 + 2\alpha_0 A_{12} A_{22} + 2\alpha_2 A_{12}$$

$$\frac{A_{21}}{M_2} = \frac{A_{12}}{M_1} \qquad \frac{B_{211}}{M_2} = \frac{B_{112}}{M_1}$$

where the subscript 2 refers to the high molecular weight species and 1 to the second solvent component. The A's and B's are constants which are defined by equation (22).

The magnitude of the interaction between a polymer and the solvent components may be expressed by the change in the activity coefficient of the polymer in the presence of component ond of the solvent. The activity coefficient of the polymer, γ_2, may be defined by the following explicit expansion of equation (22):

$$\log_e \gamma_2 = A_{21} c_1 + A_{22} c_2 + B_{211} c_1^2 + 2B_{212} c_1 c_2 + B_{222} c_2^2 + \cdots \qquad (25)$$

All of the interaction constants but one, B_{112}, in this equation can be calculated from the values of the G's determined from light scattering data through equation (24). The expression for B_{112} involves the value of A_{11} which is defined by equation (22) in the absence of the high molecular weight species:

$$\log_e \gamma_1 = A_{11} c_1 + B_{111} c_1^2 + \cdots \qquad (26)$$

The value of A_{11} can in principle be determined from partial vapor pressure data on the system composed of the two solvent components.

Light Scattering Measurements

A commercial cotton linters nitrocellulose, Hercules No. 8432 (12.6 per cent nitrogen) was chosen for this investigation. It would have been preferable to use a well characterized nitrocellulose fraction, but a sufficient quantity of any was not available.

The following solvent systems were studied: acetone-water, acetone-ligroin*, acetone-normal butyl acetate, acetone-secondary

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* The boiling point range of the ligroin was 60-70° C.

butyl chloride, and acetone-diethyl phthalate. Acetone was the major solvent component in all the mixtures. These systems were chosen because in each case there was some characteristic property of nitrocellulose which might be explained if the interaction between nitrocellulose and the binary solvent was understood. For acetone-water the unexplained observation was that nitrocellulose will go into solution more rapidly in the mixture than in pure acetone. The acetone-ligroin system was studied because, although ligroin is an efficient precipitant for nitrocellulose, absorption experiments 77/ have indicated that there is considerable affinity of nitrocellulose for ligroin. Diethyl phthalate-acetone was chosen because diethyl phthalate is a widely used plasticizer for nitrocellulose and consequently there must be some interaction between it and nitrocellulose. Mixtures of butyl acetate and butyl chloride with acetone were studied, because these components have essentially the same refractive index, but butyl acetate is a good nitrocellulose solvent and butyl chloride is a non-solvent. Any difference in the turbidities of these two systems might therefore reflect the reason for the difference in solvent power. The solvents, with the exception of ligroin, were C.P. quality. The ligroin (Skellysolve) was used without any further purification.

Measurements of 90° scattering were made at approximately 25° C., with the use of the instruments and technique described in Section VI. Extreme care had to be exercised in the measurements of the refractive index increments, since the refractive index of both the solution and solvent could be changed by the evaporation at different

rates of the two solvent components. This effect which could produce considerable error, especially in the acetone-diethyl phthalate mixture, was not eliminated entirely but was reduced by the rapid transfer of the solutions from the mixing vial to the refractometer. The same effect was operative during the preparation of solutions for the turbidity measurements but was reduced by carrying out the centrifugation in a sealed rotor in a refrigerated centrifuge.

The light scattering results were plotted in the usual way, and are shown in Figures 28 to 32. The proportion of acetone and the second solvent are expressed in volume per cent. Since the value of H_2 in these plots has somewhat different units, following those used in the Kirkwood-Goldberg treatment, from that of H for the usual two-component system, H_2 will be re-defined explicitly.

$$H_2 = \frac{32\pi^3 n^2}{3N_0\rho_0\lambda^4}\left(\frac{\partial n}{\partial C_2}\right)^2 \qquad (27)$$

where n is the refractive index of the solution (acetone, nitrocellulose and the second solvent component),

λ is the wave length of the incident light,

N_0 is Avogadro's number,

ρ_0 is grams of acetone per ml. of solution (solution as defined above),

C_2 is grams of nitrocellulose per gram of acetone, and, for for future reference,

C_1 is grams of second solvent component per gram of acetone.

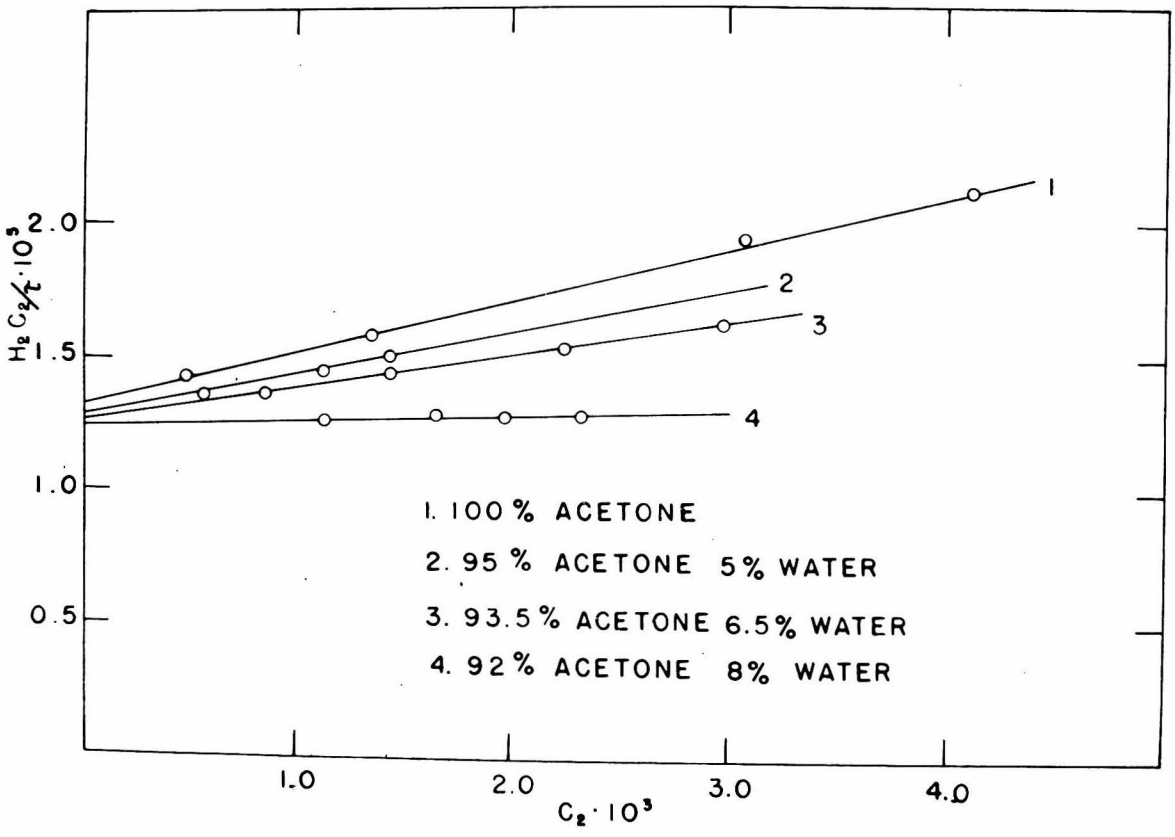

Figure 28. Turbidity data for nitrocellulose in acetone-water mixtures

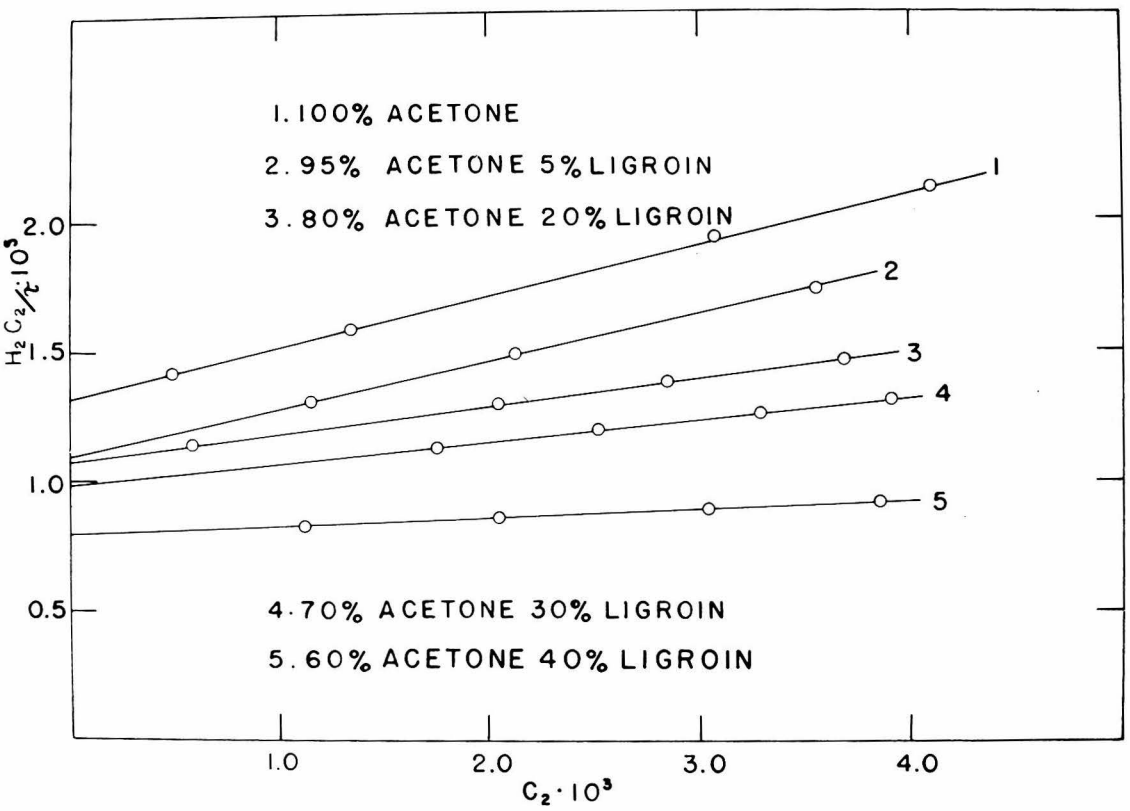

Figure 29. Turbidity of nitrocellulose in acetone-ligroin mixtures

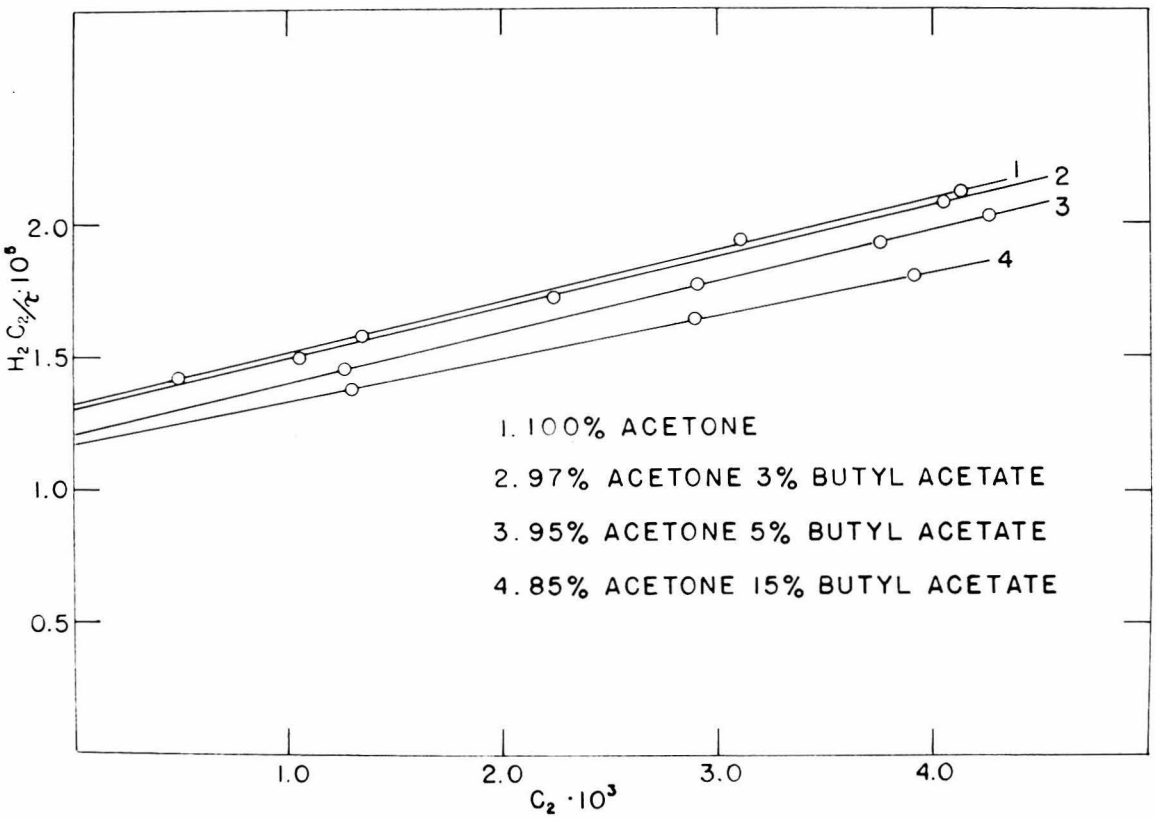

Figure 30. Turbidity of nitrocellulose in acetone-butyl acetate mixtures

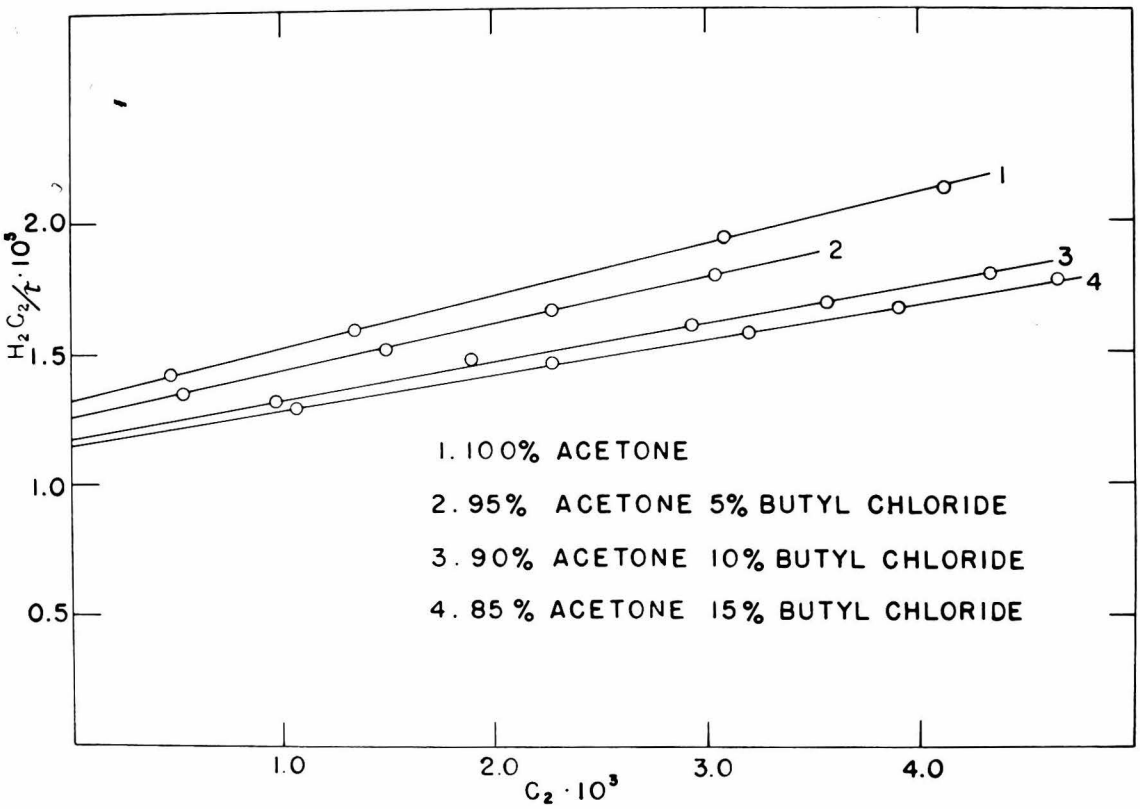

Figure 31. Turbidity of nitrocellulose in acetone-butyl chloride mixtures

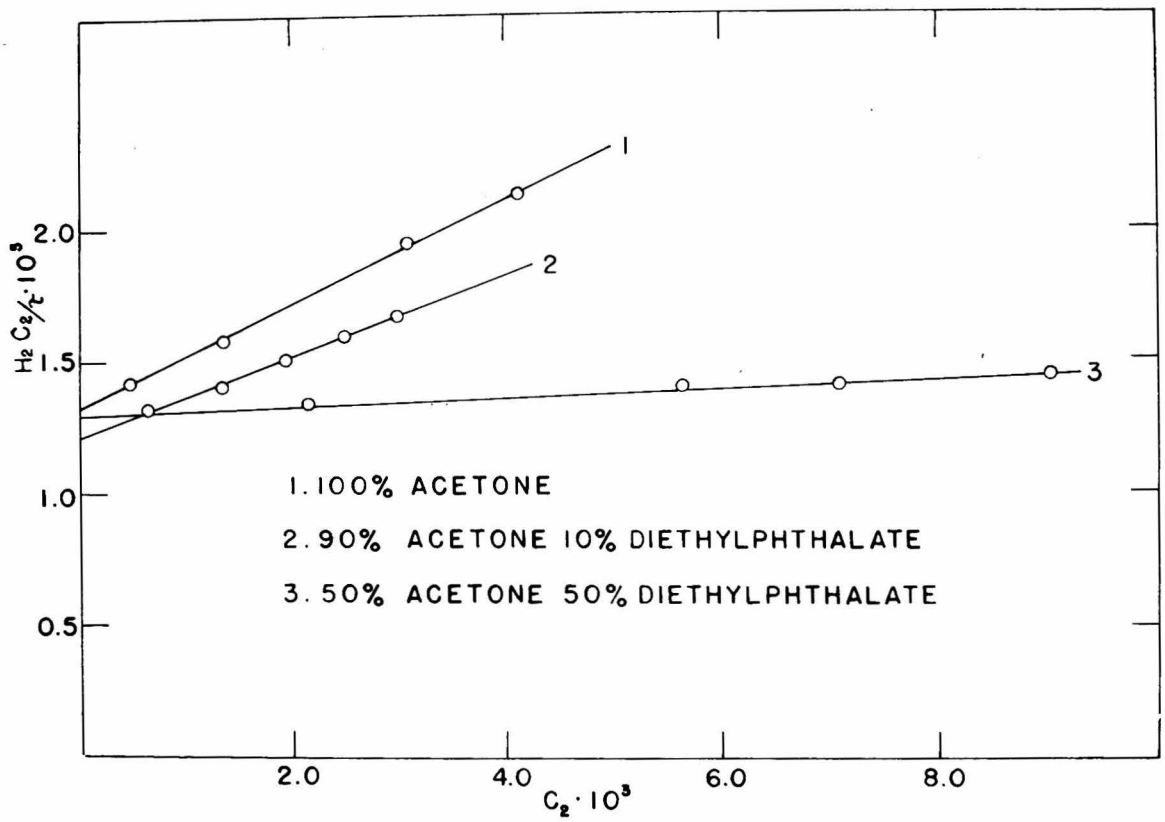

Figure 32. Turbidity of nitrocellulose in acetone-diethyl phthalate mixtures

The turbidity was calculated from the 90° scattering alone since although this nitrocellulose, Hercules No. 8432, exhibits an appreciable asymmetry of scattering (q = 1.22 in acetone) the dissymmetry is not changed appreciably by the addition of the second solvent component. Strictly speaking, the theory of Kirkwood and Goldberg is limited to solutions which exhibit no dissymmetry of scattering.

Vapor Pressure Measurements

As mentioned above, one of the interaction constants of equation (25) can not be evaluated from light scattering data alone, but can be calculated if in addition to light scattering data the partial vapor pressures of the solvent components are known. Vapor pressure data on the system water-acetone at 25° C. was found in the literature 78, 68/, but that of the other systems had to be measured.

A modification of the dynamic method 79, 80, 81/, was used in making the vapor pressure measurements. The procedure is briefly this: Nitrogen (Linde Dry Nitrogen, 99.9%) was bled from a cylinder through a reduction valve at 3 mm. pressure into a series of three saturators containing 50 ml. of the particular solvent mixture, through two absorbers in series (Schwartz drying tubes) submerged in an ether-dry ice mixture, on through a second series of three saturators filled with acetone, and through two absorbers. The saturators were submerged in a constant temperature bath at 25° C. The design of the saturators follows closely that of Bichowsky and Storch 82/, except that the connections were made with 10/30 standard

taper joints. The connections to the absorbers were made with 12/4 spherical joints so that the absorbers could be removed readily for weighing. Corning-Dow stopcock grease was used on all the glass joints. The connecting tubes between the saturators and between the saturators and absorbers were painted black and illuminated with a 250-watt infrared lamp at a distance of about three feet to prevent solvent vapor from condensing in the connecting tubes. The ether-dry ice mixture was used to trap the solvent vapor since Washburn and Handorf 81/ reported that this mixture was nearly as efficient as liquid air for condensing ethyl alcohol-cyclohexane vapor. Open-end manometers were placed in the system before the first series of saturators and after the first series of absorbers to measure the pressure in the system. The flow of nitrogen was adjusted so that after a steady state was reached the pressure was 3 mm. on the first series of saturators and 1.5 mm. on the second. The absorbers were weighed on a chainomatic balance before and after a run to determine the amount of distillate collected. The distillate composition was determined from its refractive index by use of a calibration curve. The refractive index of the solvent mixture in the third of the first series of saturators was measured after each determination to detect any change in solvent composition during the course of the run. There was no appreciable change for the mixtures which were studied. An electric fan was used to pass air over the absorbers before each weighing so that the surface of the absorbers would be rapidly equilibrated with the laboratory air after being removed from the ether-dry ice bath. Barometric readings were taken before, during, and

after each run; since there was little change during the course of the determination an average value of the pressure was used. The solvents were the same as those used in the light scattering experiments with the exception that normal hexane was substituted for 60-70° b.p. ligroin.

Trial runs were made with acetone in both sets of saturators and minor changes were made until the weights of distillate collected in the two absorbers agreed to within one per cent.

The method of calculation, briefly, is as follows: Since the vapor pressure of pure acetone is known 68/ and since the number of moles of acetone which were condensed in the second series of absorbers is known, the number of moles of nitrogen which were passed through the system can be calculated. The vapor pressure of the solvent mixture is given by the total pressure multiplied by the ratio of number of solvent moles to total moles; the former can be calculated from the weight and composition of the mixed solvent distillate. The partial vapor pressure of each solvent species is given by the product of the vapor pressure of the mixture and the mole fraction of that solvent species. The results, for the four systems, are tabulated in Table 11.

Table 11

Partial Vapor Pressures at 25° C.

Acetone-Hexane

Concentration, Hexane		Partial Vapor Pressure
Mole Fraction	Weight Fraction, C_1*	of Hexane, mm.
0.0275	0.0418	12.6
0.0536	0.0836	43.1
0.0784	0.1254	53.6
0.1015	0.1672	68.0
0.1240	0.2090	76.5
1.00		153.5

Acetone-Butyl Acetate

Concentration, Butyl Acetate		Partial Vapor Pressure
Mole Fraction	Weight Fraction, C_1*	of Acetone, mm.**
0.00	0.00	229.2
0.0271	0.0556	223.8
0.0528	0.1112	221.8
0.0772	0.1668	219.5
0.1002	0.2224	216.0

Table 11 (continued)

Partial Vapor Pressures at 25° C.

Acetone-Butyl Chloride

Concentration, Butyl Chloride		Partial Vapor Pressure
Mole Fraction	Weight Fraction, C_1*	of Butyl Chloride, mm.
0.0334	0.0553	8.7
0.0646	0.1101	16.7
0.0940	0.1652	23.3
0.1215	0.2201	30.2
0.1480	0.2752	34.9
1.00		156.0

Acetone-Diethyl phthalate

Concentration, Diethyl phthalate		Partial Vapor Pressure
Mole Fraction	Weight Fraction, C_1*	of Acetone, mm.**
0.00	0.00	229.2
0.0182	0.0708	227.0
0.0358	0.1416	223.5
0.0527	0.2124	217.0
0.0692	0.2832	214.0
0.0850	0.3540	210.2

* Grams per gram of acetone

** Butyl acetate and diethyl phthalate were for practical purposes non-volatile at this temperature and these concentrations

Treatment of Data

The light scattering data presented in Figures 28 to 32 can be fitted by an empirical equation of the form

$$H_2 \frac{c_2}{\tau} = K_0 + K_1 c_1 + K_2 c_2 + K_3 c_1 c_2 + K_4 c_1^2 + K_5 c_2^2 + \cdots \qquad (28)$$

and the values of the K's can be determined from the experimental data. K_0 is the value of the $H_2 \frac{c_2}{\tau}$ intercept at $c_1 = 0$ and is the reciprocal of the weight average molecular weight of the nitrocellulose. K_2 is the slope of the light scattering curve when $c_1 = 0$. K_1 and K_4 can be evaluated from the best empirical equation of the curve obtained by plotting the $H_2 \frac{c_2}{\tau}$ intercept against c_1. K_3 is determined from the initial value of the slope of the curve obtained by plotting the slopes of the light scattering curves against c_1.

The values of the G's in equation (24) can readily be calculated from the K's determined above and in turn all the interaction constants of equation (25) except B_{112} can be calculated from the G's.

The expression for B_{112} involves the term, A_{11}, which is defined by equation (26) and which can be calculated from partial vapor pressure measurements on the solvent systems in the absence of nitrocellulose.

If the assumption is made that the partial vapor pressure of the solvent component one is a measure of its activity the value of A_{11} may be obtained with the aid of the relation

$$A_{11} c_1 + B_{111} c_1^2 = \log_e \frac{P_1}{c_1} - \lim_{c_1 \to 0} \left(\log_e \frac{P_1}{c_1} \right) \qquad (29)$$

which follows from equations (21) and (26).

A plot of $\log_e P_1/c_1$ versus c_1 for the hexane-acetone, water-acetone 78/, and butyl chloride-acetone systems is shown in Figure 33. Some indication of the probable error in the measurements is given by the diameter of the experimental points but even so it is not possible to make an unambiguous extrapolation of the data for the hexane-acetone system. A straight line extrapolation was made although it is quite possible that more accurate data would establish a curvature at low values of c_1.

The value of A_{11} for the above systems was determined from the initial slope of a curve obtained by plotting the quantity on the right side of equation (29) against c_1.

Values of A_{11} for the butyl acetate-acetone and the diethyl phthalate-acetone systems cannot be determined in the above manner since the component one was not volatile in either system. The activities of the butyl acetate and diethyl phthalate can in principle be calculated from the activity of the acetone by the aid of the Gibbs-Duhem equation and a graphical integration device described by Lewis and Randall 83/. Once the activities of the component one are known the value of A_{11} can be determined as above with the aid of equation (29).

The value of A_{11} for these systems can also be obtained from the expression *

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* This expression was suggested by Professor R. M. Badger

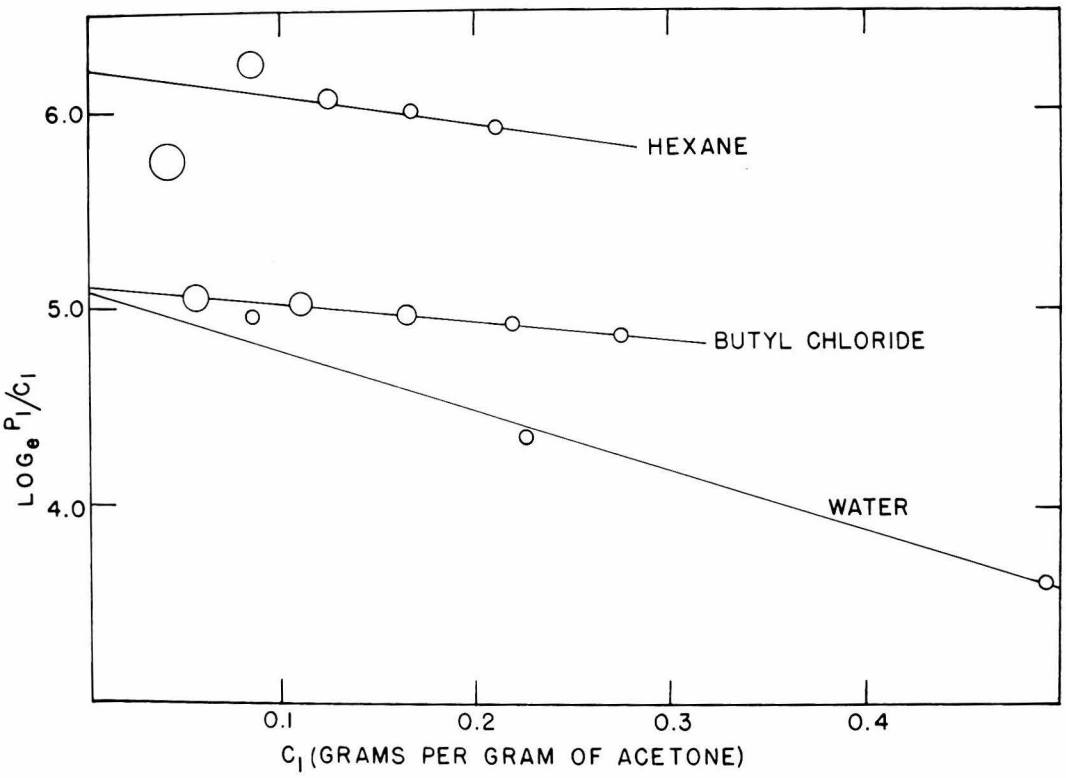

Figure 33. A plot of $\log_e P_1/c_1$ versus c_1 for three solvent mixtures

$$A_{11} c_1 + 2 B_{111} c_1^2 = - \frac{N_0 c_1}{N_1} \frac{d \log_e P_0}{d c_1} - I \qquad (30)$$

which follows from the Gibbs-Duhem equation and the definition of A_{11}.

Each of these methods of evaluating A_{11} give a value not significantly different from zero for both butyl acetate and diethyl phthalate.

A complete tabulation of the data on nitrocellulose in binary solvents is presented in Table 12.

Table 12

A Tabulation of Data for Nitrocellulose in Binary Solvents

Constant	Water-Acetone	Ligroin-Acetone	Butyl acetate-Acetone	Butyl chloride-Acetone	Diethyl phthalate-Acetone
Experimental Constants from Light Scattering Measurements					
$K_0 \cdot 10^5$	1.3	1.3	1.3	1.3	1.3
$K_1 \cdot 10^5$	-0.7	-2.2	-2.2	-1.0	-0.8
$K_2 \cdot 10^3$	1.9	1.9	1.9	1.9	1.9
$K_3 \cdot 10^3$	-4.6	-4.5	-0.7	-4.8	-4.4
$K_4 \cdot 10^5$	0	2.6	7.0	0	0.6
K_5	0	0	0	0	0
Experimental Constants from Refractive Index Measurements					
α_0	0.82	0.15	0.26	0.26	1.03
α_1	-2.8	0.14	0.30	0.30	0.58
α_2	0	0	0	0	0
Experimental Constants from Partial Vapor Pressure Measurements					
A_{11}	-3	-1(hexane)	0	− 0.6	0

Table 12 (continued)

A Tabulation of Data for Nitrocellulose in Binary Solvents

Constant	Water-Acetone	Ligroin-Acetone	Butyl acetate-Acetone	Butyl chloride-Acetone	Diethyl phthalate-Acetone
			Derived Constants		
G_{10}	-0.5	-1.7	-1.7	-0.8	-0.6
G_{01}	150	150	150	150	150
G_{11}	-350	-340	-50	-360	-330
G_{20}	0	2.0	5.3	0	0.4
G_{02}	0	0	0	0	0
A_{12}	-0.3	$-5._7$	$-3._2$	$-1._4$	-0.3
B_{112}	-0.1_3	$5._9$	$4._8$	$0._8$	0.1_2
$A_{22} \cdot 10^{-2}$	1.5	1.5	1.5	1.5	1.5
$A_{21} \cdot 10^{-3}$	$-1._4$	$-5._0$	$-2._0$	$-1._1$	$-0._1$
B_{222}	0	0	0	0	0
$B_{212} \cdot 10^{-4}$	0.0_1	$1._4$	$0._3$	0.0_7	-0.0_1
$B_{211} \cdot 10^{-3}$	-0.6	$5._1$	$3._1$	$0._7$	0.0_4

Discussion

The results of the light scattering experiments on solutions of nitrocellulose in binary solvents can be interpreted from either of two viewpoints: that of Ewart, Roe, Debye, and McCartney, or that of Kirkwood and Goldberg. The data will be discussed first in the language of the former authors.

The change in turbidity of a polymer solution upon the addition of a second solvent component may be attributed to two effects: first, a change in the shape of the swollen polymer molecule in solution; and second, a change in the effective refractive index of the polymer molecule due to selective absorption of solvent. The first effect will be operative at finite polymer concentration and in general will change the slope of the light scattering curve but will not alter the value of the $H_2 \frac{c_2}{\tau}$ intercept at infinite dilution. The second effect will either increase or decrease the value of the intercept depending on the relative refractive indices of the solvent components and the magnitude of the change will depend on the amount of the selective absorption.

Since the addition of each of the second solvent components to the nitrocellulose solution in acetone resulted in a decrease in the $H_2 \frac{c_2}{\tau}$ intercept, and since in all cases acetone had the lowest refractive index of the solvent components the light scattering data presented in Figures 28 to 32 can be explained only if the second solvent components, namely, water, ligroin, butyl acetate, butyl chloride, and diethyl phthalate were preferentially absorbed from solution. This result was not unexpected for butyl acetate

and diethyl phthalate, which are nitrocellulose solvents, but seemed anomalous in the cases of water, ligroin, and butyl chloride. The attraction of these latter fluids to nitrocellulose might be explained in that the nitrocellulose molecule contains both polar and non-polar groups, and that each of these groups may attract molecules which are not solvents for the entire nitrocellulose molecule.

The more detailed theory of Kirkwood and Goldberg does not rely on any particular mechanism, but views the change in turbidity as being due to thermodynamic interaction between the polymer and the components of the solvent. The degree of interaction can conveniently be expressed by the change in the activity coefficient of the polymer upon the addition of the second solvent component.

The activity coefficient of nitrocellulose in the five solvent mixtures can in general be calculated by the use of equation (25) and the values of the interaction constants presented in Table 12.

$$\log_e \gamma_2 = A_{21} c_1 + A_{22} c_2 + B_{211} c_1^2 + 2 B_{212} c_1 c_2 + B_{222} c_2^2 + \ldots \quad (25)$$

Care has to be exercised in the interpretation of these activity coefficients because of the errors in the magnitude of the constants and because of uncertainty in the range of validity of equation (25).

An inspection of the derivation of this equation indicates that it is valid only for small values of c_1 and c_2; so it may be reasonable to assume that it is useful for concentrations as great as 0.001.

The magnitude of the error in the interaction constants is difficult to estimate. Values of K_0 and K_2 can be determined with a probable error of about 5 per cent since these constants refer

to nitrocellulose dispersed in pure acetone. Values of K_1, K_3, and K_4 are determined for a solution in a binary solvent, and the possibility exists that considerable error might be introduced because of changes in refractive index of the solution due to evaporation of the solvent. Although care was used to reduce evaporation the precision of determining the latter constants was less than for K_0 and K_2 since it was sometimes difficult to reproduce the light scattering curves, especially those for the butyl acetate-acetone system.

The percentage error in the A constants is essentially the same as that in K_1, but this error is magnified in the B constants because the value of A_{12} is used several times in the calculation of B_{112} and B_{212}. For these reasons the values of the interaction constants in Table 12 are given with not more than one significant figure.

If the use of equation (25) is limited to a concentration range of $c_1 = c_2 \leq 0.001$ errors in the B constants are not significant because the value of $\log_e \gamma_2$ will then be controlled by the values of A_{21} and A_{22}. Since the value of A_{21} is negative and much larger than A_{22} the presence of a very small amount of component one will cause a large decrease in the value of the activity coefficient of nitrocellulose. This large change in the activity coefficient is difficult to understand as is the fact that butyl acetate apparently decreases the activity coefficient more than does water or butyl chloride, but less than ligroin.

Unfortunately there are no other experimental methods for determining the activity coefficient of nitrocellulose of this

molecular weight with sufficient precision to compare with the values calculated from light scattering measurements. There is also little to be gained by trying to correlate them with the results of kinetic experiments, such as the rate of solution. The magnitudes of the activity coefficients calculated from light scattering will have to stand alone for the present.

Although the activity coefficients cannot be directly correlated with earlier observations of the interaction between nitrocellulose and the components of a mixed solvent (see introduction), light scattering experiments appear to be a convenient method of evaluating such interaction. This method, therefore, has great potential use in the investigation of polymer-solvent systems; in particular, the interaction between polymer and the "non-solvent" type plasticizers.

It is evident from the magnitude of the error which has been assigned to the interaction constants that the technique of making the light scattering and refractive index measurements should be refined. Such refinement will consist largely of developing procedures for carrying out the measurements with a minimum of change in the refractive index of the solution due to evaporation of solvent.

Summary

A brief summary is given of the recent Kirkwood-Goldberg theory of the light scattering of multicomponent systems.

The required light scattering, refractive index, and partial vapor pressure data have been obtained for the application of the theory to the problem of evaluating the nitrocellulose-solvent

interaction in five binary solvents.

A complete tabulation is given of values of the interaction constants for nitrocellulose in five binary solvents, and a discussion is given of their probable accuracy and significance.

Part II

Light Scattering Studies of Protein Solutions

Introduction

The observation that protein solutions are often quite turbid was one of the first reasons advanced for the belief that proteins were high molecular weight substances. Most of the early experimental work was on the light scattering properties of gelatin 84,85, 86, 87/, which was, perhaps, an unfortunate choice because the turbidity of solutions of this material is markedly influenced by small changes in pH, ionic strength, temperature, and trace amounts of inorganic ions in a way which is not yet completely understood. Later work was done on casein solution 88/ the turbidity of which is influenced in much the same manner as that of gelatin, and on ovalbumin which is now recognized as having been a poor choice because of the ease with which it undergoes surface denaturation 89, 90/.

The first light scattering investigation of proteins which were more typical than gelatin or casein was that of Putseys and Brosteaux 91/ on ovalbumin, amandin, excelsin, and a hemocyanin (of Helix pomatia) which had been, at that time, well characterized in Svedberg's laboratory. These investigators concluded that when extreme care was taken to remove denatured material and "dust" from the solutions, the relative turbidities were proportional to the molecular weights from ultracentrifuge data. They found that small variations in pH and ionic strength of the solution had little effect on the observed turbidity.

With the revival of interest in light scattering in 1944 and its subsequent application to the study of high polymer solutions, several

laboratories became interested in the use of the method for the study of protein solutions. Relatively little of this work has as yet appeared in the literature.

The light scattering experiments on protein solutions which are described in Part II were undertaken after some experience with the method had been gained during the study of nitrocellulose solutions.

Section I describes the application of the light scattering method to the study of aqueous solutions of protein materials and presents values of the molecular weight for several serum proteins.

Section II is a study of the size and shape of molecules of Group A-Specific Substance from hog gastric mucin. Data from viscosity, osmotic pressure, diffusion, and light scattering experiments are presented.

Section III is a brief light scattering investigation of the thermal aggregation of serum proteins.

Section I. Light Scattering Studies on Selected Serum Proteins

The first and perhaps the most important problem was to learn how to prepare protein solutions for light scattering measurements. All exploratory experiments were made with solutions of human serum albumin and human serum globulin.* These proteins were chosen because they have been well characterized by other methods, because they were readily available, and because they are relatively insensitive to surface denaturation.

Preparation of Solutions

Considerable difficulty was experienced in preparing a protein solution which was free of obvious dust. Although solutions could easily be prepared which seemed perfectly clear in ordinary laboratory lighting, these same solutions were seen to contain scattering particles when placed in the light scattering apparatus and observed at small angles to the incident beam. The customary filtration through an asbestos pad in the Seitz apparatus was not satisfactory and at times seemed to increase the amount of suspended material. Apparently there was some denaturation** of the protein as it was forced through the pad, or perhaps there was some slight disintegration of the pad. Filter paper did not clear the solution

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* I am indebted to Professor E. J. Cohn of the Harvard Medical School for the preparation of these proteins. The albumin was described as Fraction V Run 35 and the globulin as Fraction II Run 64. The fractionation procedure by which these proteins were prepared has been described 92/

** The term denaturation is used here merely in the sense that a very small fraction of the protein becomes insoluble and is observed in the Tyndall beam as small irregularly shaped particles and fibers

sufficiently, even the hardest which was available (Schleicher and Schull, No. 576)*.

The best filtering medium found was a Pyrex Fine sintered glass funnel which had been blocked with finely powdered silica, but even this did not give optically clean solutions.

Centrifugation at 32,000 g for twenty minutes, the procedure for nitrocellulose solutions described in Part I, Section VI, was finally adopted as a routine procedure. If extreme care was exercised in transferring the centrifuged solution to a clean scattering cell the solution remained essentially free of obvious contamination.

Protein concentration was calculated from the value of total nitrogen determined colorimetrically by the use of a modified Nessler's reagent. In the few instances in which a nitrogen-containing buffer was present a colorimetric method involving the Folin-Ciocalteu reagent was employed 93/.

Depolarization of Scattered Light

Measurements of the depolarization of the scattered light were made in all experiments in order to evaluate the Cabannes factor. The value of the depolarization for unpolarized incident light was 0.032 ± 0.02 for the serum proteins which have been studied and is apparently independent of concentration in the range of 1 to 10 mg. per ml. The value of the depolarization for horizontally

*One filter paper gave promising results but unfortunately it was one small sheet from a sample booklet distributed by a German firm before the first world war.

polarized incident light is evidently influenced by the presence of large particles in solution since the value increased after the solutions were centrifuged. For example, a filtered but uncentrifuged human serum globulin solution gave a value of 0.55 for the depolarization for horizontally polarized light; after the solution was centrifuged for 20 minutes at 32,000 g the value was 0.76. All serum proteins which were studied gave values for the depolarization for incident light horizontally polarized which seemed to approach 0.90 after extended periods of centrifugation; therefore, the magnitude of this value was used as an indication of the effectiveness of a given centrifugation.

Effect of pH on Turbidity

Since early work on gelatin and casein solutions had shown the marked effect of change of pH on their turbidity, the variation of the turbidity of human serum albumin solution with pH was measured. The turbidity of an unbuffered 0.15 N sodium chloride solution containing 0.25 per cent of human serum albumin was observed as the pH was changed by the addition of dilute HCl and NaOH. The solution was centrifuged after each change of pH. The results, shown in Figure 34 indicate that the turbidity of the solution increases rapidly near the isoelectric point of the protein, but that there is little change in turbidity when the pH is changed from 9 to 6. Below pH 4, however, the scattering increases enormously, probably because the protein becomes denatured. Subsequent light scattering experiments on serum proteins were usually made with a pH of the

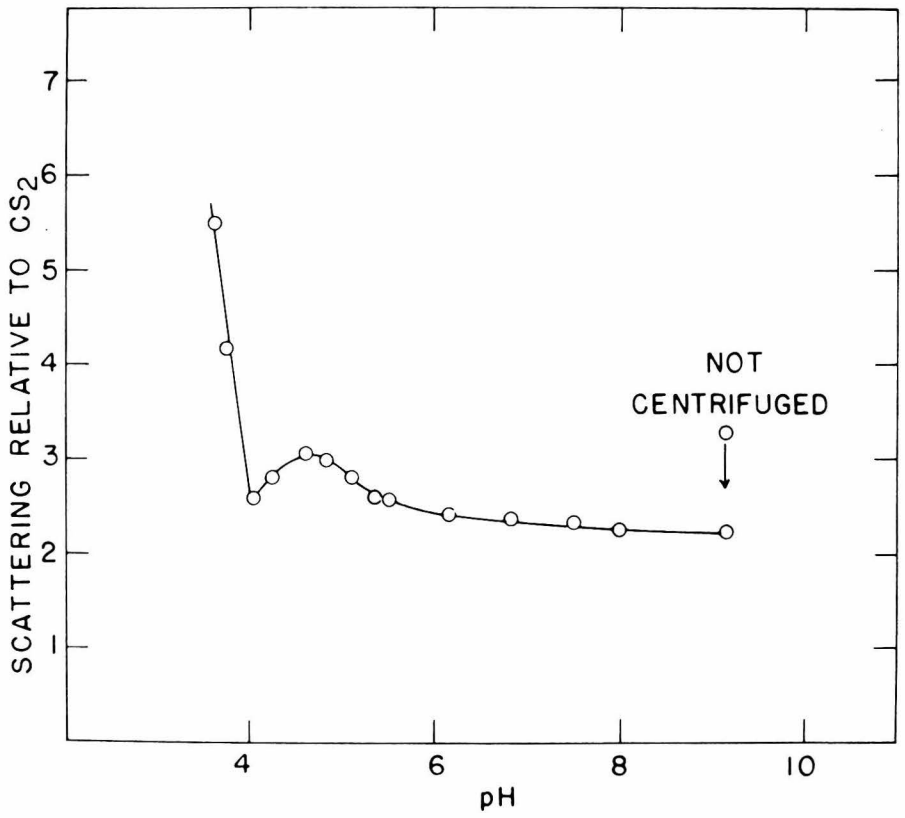


Figure 34. Effect of change of pH on the relative scattering power of a human serum albumin solution

solution between 7 and 9 so as to minimize the effect of pH on the turbidity.

Similar experiments, made to learn if the depolarization of the scattered light was affected by the pH of the solution, were inconclusive because of the relatively large experimental error.

Refractive Index Increment

The differential refractometer was used to determine the refractive index increment of the proteins. Before these measurements were made the solutions were carefully dialyzed to insure that the value of $n - n_o$ as determined by the instrument was really due to protein and not to unequilibrated salt. As a routine procedure 10 ml. of each protein solution was dialyzed with stirring against 2 l. of solvent for 24 hours and then for another 24-hour period against another 2 l. of solvent. In the few instances in which static dialysis was employed two weeks were allowed for equilibration. All dialysis experiments were carried on in the cold (2° to 6° C.)

The change of refractive index increment of human serum albumin with pH was hardly more than the experimental error; however, recent work 94/ indicates that the increment of bovine serum albumin changes about 1 per cent between pH 5 and 8.3 at the same wave length employed in this investigation.

Effect of Ionic Strength on Turbidity

A few short experiments were performed to learn the magnitude

of the effect of ionic strength on the turbidity of protein solutions. The turbidity of human serum albumin solutions in 0.05 N, 0.10 N, and 0.15 N sodium chloride were found to be the same within experimental error; hence it was apparent that small changes in ionic strength would not be important for this protein.

The effect of ionic strength on the turbidity of human serum globulin was then studied briefly. A solution of the protein was dialyzed agsinst periodically renewed distilled water until the euglobulin precipitated. This fraction was then dissolved in 0.1 N sodium chloride and the turbidity was measured. The solution was again dialyzed against distilled water for about 75% of the time required for the protein to precipitate during the first dialysis. After the solution had been centrifuged in the usual manner the turbidity was found to have increased by a factor of about four.

This brief experiment suggests that as salt is removed from a globulin solution the molecules begin to aggregate, but that it is not an "all or none" reaction. The observed increase in turbidity is not due to a few very large particles (which would have been centrifuged out of solution), but to a larger number of small particles. Dissymmetry measurements might give an indication of the shape of the molecular aggregates but these measurements were not made.

Molecular Weight of Serum Proteins from Light Scattering

The procedure for determining the molecular weight of proteins is summarized in the following article on the rabbit antibody against p-azophenylarsonic acid.

[Reprinted from the Journal of the American Chemical Society, 70, 2496 (1948).]

[Contribution from the Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, No. 1178]

The Purification and Properties of Antibody against *p*-Azophenylarsonic Acid and Molecular Weight Studies from Light Scattering Data

By Dan H. Campbell, Robert H. Blaker and Arthur B. Pardee[1a]

It is becoming increasingly evident that many fundamental problems dealing with the structure and behavior of antibody molecules must be studied with purified antibody preparations in solution of known composition rather than in complex solutions such as serum. Methods which are devised for the isolation and purification of antibodies on a practical scale are hence of considerable interest and importance. The following report describes a method for the isolation and purification of antibody against *p*-azophenylarsonic acid in which the antibody is removed from the antiserum by specific precipitation with a polyhaptenic dye and recovered from a solution of the dissociated antigen–antibody complex.

The recovery of antibodies from specific antigen–antibody complexes has been accomplished by a variety of methods.[1b] Perhaps the best known is the one described by Heidelberger and Kendall[2] and Heidelberger and Kabat,[3] in which 15% sodium chloride solutions were used to produce a shift in the antigen–antibody ratio of specific precipitates of SSS or of intact Pneumococcus and antipneumococcus serums favoring the liberation of antibody. Liu and Wu[4] were able to obtain as good if not better yields of antibody preparations by acid dissociation of similar antigen–antibody complexes at about *p*H 4.0 with subsequent isolation of antibody by salt precipitation or removal of antigen by centrifugation if bacterial cells were used. Recently, a report has been made by Haurowitz, *et al.*,[5] which describes the isolation and purification of antibody against *p*-aminobenzylamine, anthranilic, arsanilic, and sulfanilic acids by the use of methods somewhat similar to those used by us in the present investigation. The principal difference was their use of an acid-insoluble conjugated protein for a precipitating antigen. Our own investigations of a number of antigen–antibody systems have indicated that, in general, acid dissociation is the method of choice, at least for the systems involving ovalbumin, polysaccharide, and arsanilic acid antigens. The last of these is a particularly good system since simple polyhaptenic dye antigens can be used for specific precipitating agents. The physical properties of such antigens are so different from those of the antibody proteins that the dissociated complexes can usually be separated into the antigen and antibody components without difficulty. Certain dye antigens have the added advantage that they have a low solubility under acid conditions and hence upon dissociation of the antigen–antibody precipitate the antibody dissolves and the antigen remains behind as an insoluble acid.

Purification of Antibody.—Several methods were studied for the dissociation of antibody from antigen–antibody complexes and its subsequent recovery from the dissociated mixture. For example, treatment of precipitates by alkali at *p*H 9.0–10.0 resulted in considerable dissociation, as evidenced by solution of the precipitates, but the yields of antigen-free protein were low because of the high solubility of the antigen and its tendency to remain attached to the protein. Furthermore, some denaturation of antibody protein always occurred and the purity of antibody as based on the ratio of specifically precipitable protein to total protein usually gave values of only 10 to 20%. Another method which was used with some success was dissociation of dye–antigen complexes with a simple hapten such as arsanilic acid and subsequent dialysis against the hapten until the solution was free of the dye antigen. This

(1a) Present address, McArdle Laboratory, University of Wisconsin, Madison Wisconsin.

(1b) Dan H. Campbell and Frank Lanni, "The Amino Acids and Proteins," edited by D. M. Greenberg, Chapt. XII, "Immunology of Proteins," Thomas Publishing Co., in press.

(2) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **64**, 161 (1936).

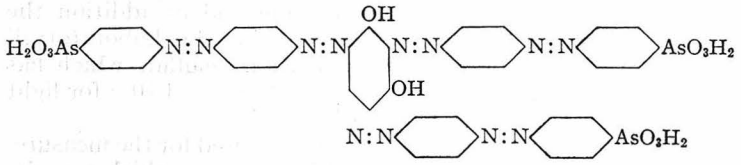
(3) M. Heidelberger and E. A. Kabat, *ibid.*, **67**, 181 (1938).

(4) S. C. Liu and H. Wu, *Proc. Soc. Expil. Biol. Med.*, **41**, 144 (1939).

(5) F. Haurowitz, Sh Tekman, Miervet Bilen and Paula Schwerin, *Biochem J.*, **41**, 305 (1947).

method was limited to precipitating antigens of sufficiently small molecular size to permit diffusion through the dialysis membrane. Such antigens are rather inefficient precipitating agents, and considerable time was required to dialyze away first the dye antigen and then the arsanilic acid. The method of choice in most instances and the one used for the antibody preparation in the present study was (1) the use of a good precipitating dye antigen, (2) dissociation of the antigen–antibody complex with arsanilic acid and then acidification to about *p*H 3.5, and (3) precipitation of the dissociated antibody with salt. The purified antibody used in study of physical properties was a pool obtained by mixing several purified preparations, but all were made by essentially the same method.

Serums from a number of rabbits which had been immunized over a period of many months with sheep serum-*p*-azophenylarsonic acid were pooled and a preliminary precipitation titration was made in order to determine the antigen–antibody ratio for optimum precipitation as well as to obtain an approximate idea of the antibody concentration. The antigen used for all preparations was a trisubstituted resorcinol dye having the following structure



The antigen solutions was adjusted to *p*H 8.0 and dilutions were made which varied by a factor of 2 from 1:1000 to 1:256,000, and these were added in 0.5 ml. volumes to tubes containing 0.5 ml. of a 1:4 or 1:5 dilution of the pooled antiserum at *p*H 8.0. The mixtures were allowed to react for about two hours at room temperature and forty-eight hours at 4°. The precipitates were then washed with 1.0% sodium chloride solution and analyzed for protein by the Folin–Ciocalteu method as modified by Pressman.[6] Most of the pooled sera gave maximum precipitation in slight antigen excess with antigen dilutions around 1:40,000 under the above conditions, and antibody protein values of from about 6 mg./ml. of serum to as high as 15 mg./ml. For precipitation of antibody from an 850-ml. batch of pooled serum which showed a preliminary titration maximum for antibody precipitation of 1:10,000 (1:40,000/1:4) was adjusted to *p*H 8.0 with 0.5 *M* sodium hydroxide, diluted with one volume of saline, and mixed with an equal volume of 1:20,000 antigen solution. After several hours at room temperature and about seventy-two hours at 4° about half of the supernatant was siphoned off and the remainder centrifuged and the precipitate washed free of soluble dye and protein with 1.0% sodium chloride at room temperature. The insoluble antigen–antibody complex was usually dissociated first with sodium arsanilate and was then acidified. Although acid dissociation alone was fairly successful it was found that hapten dissociation facilitated separation and gave higher yields. Thus the precipitate was first suspended in 25 to 50 ml. of 10% sodium arsanilate at *p*H 8.0–8.5 and the mixture carefully stirred until no further solution was evident. This required from two to four hours and usually resulted in a solution with only faint turbidity. When precipitates were allowed to develop over a period of longer than seventy-two hours the dissociation with haptens required much longer time and in a few extreme instances were not complete at twelve hours. The antigen–antibody–hapten solution was then quickly adjusted to *p*H 3.2 and again carefully stirred for about one hour at room temperature. At this *p*H most of the dye antigen became insoluble and the antibody protein remained in solution. A small amount of antibody usually remained with the insoluble dye and arsanilic acid but practically all was recovered in one washing with saline and was added to the original acid extract. The small amount of antibody which remained with the insoluble dye was easily recovered by washing. The antibody was precipitated by addition of a saturated sodium chloride solution in a final concentration of 4.0 *M*. Traces of dye which remained soluble in the acid solution were removed by the careful fractional precipitation with salt solution. The dye being relatively insoluble precipitated with much less salt than was required for antibody globulin. In such instances, 10–20% of the antibody would precipitate with the dye but could be recovered by further fractional precipitation with salt at *p*H 3.2. The final salt precipitated antibody was then re-

TABLE I

DATA ON THE PURIFICATION OF ANTIBODY FROM RABBIT ANTI-SHEEP SERUM-*p*-AZOPHENYLARSONIC ACID

Volume of pooled serum, ml.	Type of antigen	Maximum[c] antibody pptd., mg./ml.	Total protein recovered,[d] mg.	Yield, %	Purity[e]
25	R^1_3[a]	14.81	368	97	98
850	R^1_3	6.27	4487	84	87
230	R^1_3	14.81	3390	99	96
400	R^1_3	9.05	3158	87	93
750	XXX[b]	8.68	4100	63	71
100	XXX	8.68	685	79	83

[a] The trisubstituted resorcinol dye described in text. [b] A chromotropic acid derivative containing two azophenyl-azo-arsonic acid groups. These antibody preparations were not used in the present study. [c] Subsequent experiments with the purified antibody indicated that less precipitate was obtained in the presence of serum proteins, hence these values may represent only relative amounts of antibody. [d] Protein based on microkjeldahl analysis. [e] Purity = specific precipitable protein/total protein in solution.

(6) David Pressman, *Ind. Eng. Chem., Anal. Ed.*, **15**, 357 (1943).

suspended in 0.9% saline and dialyzed against saline until the *p*H became practically neutral.

Representative values for several batches of pooled serum are given in Table I. It will be seen that the preparations obtained by use of the trisubstituted dye antigen were better than those obtained by use of a chromotropic acid derivative. This was due largely to the fact that the latter antigen showed an appreciable solubility at *p*H 3.5 and hence tended to complex with the soluble protein. Serums with lower titers always gave smaller yields.

Electrophoretic Pattern.—Electrophoretic studies of the purified preparations in the Tiselius apparatus indicated a very high degree of homogeneity. The experiments were made with approximately 1.0% protein solutions in 0.15 *M* sodium chloride plus 0.04 *M* phosphate buffer at *p*H 7.2. The current used was approximately 15 ma. and the pattern allowed to develop for two to three hours. The electrophoretic mobility was very similar to that of the gamma globulin fraction of serum.

Molecular Weight Determination. (a) From Osmotic Pressure.—The molecular weight determinations by osmotic pressure were made with simple osmometers of the static rise type with a Visking cellophane bag used for the membrane. The protein concentration was 2.0% in 0.15 *M* sodium chloride and 0.04 *M* phosphate buffer at *p*H 7.3. The values obtained varied from 136,000 to 144,000, as compared to the currently accepted values of 158,000. The slightly lower values were probably a reflection of the *p*H at which the determinations were made.

(b) From Light Scattering Data.—Measurements of the turbidity, refractive index, and depolarization of a protein solution can, under certain conditions, be used to calculate the molecular weight of the dissolved protein. The theoretical bases of these calculations are due principally to Rayleigh,[7] Von Smoluchowski,[8] Einstein,[9] Raman,[10] and Debye.[11]

If the dissolved particles are small compared with the wave length of light the following equation gives a relation between the turbidity of the solution, its concentration, refractive index, depolarization, and the molecular weight of the solute.

$$h = \left\{ \frac{\frac{32\pi^3 n^2}{3\lambda^4 N_0}\left(\frac{\partial n}{\partial c}\right)^2 c}{\left(1/M + \frac{2Bc}{RT}\right)} \right\} \left(\frac{6+3\rho}{6-7\rho}\right) \qquad (1)$$

where

h is the extinction coefficient due to scattering
n is the refractive index of the solution
c is the concentration
$\partial n/\partial c$ is the refractive index increment of the solute
λ is the wave length of the incident light
N_0 is Avogadro's number
M is the molecular weight of the solute
B is a constant which describes the deviation of the system from van't Hoff's law
R is the gas constant
T is the absolute temperature
ρ is the depolarization of the scattered light.

In practice it is difficult to measure h accurately so instead the amount of light which is scattered at an angle of 90° to the incident beam is measured. For solutions of particles which are small compared with the wave length of light the angular distribution of intensity of scattered light obeys a $(1 + \cos^2\theta)$ relation where θ is the angle between the direction of the incident beam and the scattered beam. The relation between h, I_0, the intensity of the original beam and i, the intensity of the light scattered at 90° to the incident beam, is

$$h = \frac{16\pi}{3} i/I_0 \qquad (2)$$

The direct measurement of the quantity, i/I_0, is a time consuming task so that routine measurements in this Laboratory are made by comparing the light scattered from a solution with that scattered from a sealed tube of purified carbon disulfide. Various investigators have reported values of i/I_0 for carbon disulfide and in addition the value has been redetermined in this Laboratory.[12] The value of i/I_0 for carbon disulfide which has been used in this investigation is $4.4 \cdot 10^{-5}$ for light of the wave length of 5461 Å.

The instrument which was used for the measurement of the scattered light is one which was designed and built in this Laboratory. A slightly convergent beam of monochromatic light from a mercury arc (GE-AH-4) is passed up through the bottom of a cylindrical glass cell. The light which is scattered in directions near 90° to the incident beam is focused on a 931-A electron multiplier phototube. A small fraction of the incident beam is reflected to another phototube and the outputs of the two tubes are balanced against one another by means of a potentiometer arrangement. A constant voltage transformer reduces fluctuations in the mercury arc and in the supply of a voltage regulator and rectifier which provides a source of high potential for the plates of the phototubes.

A diaphragm arrangement is installed in the path of the scattered beam which permits sections of polaroid film with known orientations to be switched in and out of the light path. This device gives a convenient way of measuring the depolarization of the scattered light.

The refractive index increment is measured with a differential refractometer similar in design to one which has been described in the literature.[13,14]

(7) Lord Rayleigh, *Phil Mag.*, **12**, 81 (1881).
(8) M. Von Smoluchowski, *Ann. Physik*, **25**, 205 (1908).
(9) A. Einstein, *ibid.*, **33**, 1275 (1910).
(10) C. V. Raman, *Indian J. Phys.*, **2**, 1 (1927).
(11) P. Debye, *J. Applied Phys.*, **15**, 338 (1944).

(12) A more complete description of the light scattering apparatus and technique which have been developed in this Laboratory will soon be published.
(13) D. Rau and W. Roseveare, *Ind. Eng. Chem., Anal. Ed.*, **8**, 72 (1936).
(14) P. Debye, *J. Applied Phys.*, **17**, 392 (1946).

Four solutions of the protein were made with a dilute salt solution (0.15 *M* sodium chloride) and were dialyzed against the same solution for two weeks at 4°. The *p*H of the protein solution at the end of the dialysis was 7.5. The solutions were then centrifuged for twenty minutes in a field 32,000 times that of gravity to remove any suspended dust, placed in a scattering cell, and the intensity of the scattered light compared with that scattered from carbon disulfide for a wave length of 5461 Å. Depolarization measurements were made. The refractive index increment was computed from the difference between the refractive indices of the solution and the solvent. Two of the solutions were slightly colored. Optical density measurements were made on these solutions with a spectrophotometer at the wave length used so that the magnitude of the scattering could be corrected for the true absorption. Concentrations were determined as described in the previous section.

The molecular weight of the dissolved protein is given by

$$M = \frac{\lambda^4 N_0}{2\pi^2 n^2 \left(\frac{\partial n}{\partial c}\right)^2 (c/i/ics_2)_{c\to 0}(I_0/ics_2)} \qquad (3)$$

which follows from (1) and (2)

$c/i/ics_2$ is the concentration of the solution divided by the ratio of the intensity of the light scattered from the solution to that scattered from carbon disulfide. This quantity is corrected for the depolarization of the scattered light and is extrapolated to zero concentration.

A plot of $c/i/ics_2$ *vs.* c is given in Fig. 1. The refractive index increment of this protein is 0.171. The depolarization of the solution is 0.032 and apparently is independent of concentration.

The value of the molecular weight which is calculated from light scattering measurements, 158,000 ± 10,000 compares favorably with previously published data from sedimentation and osmotic pressure studies.

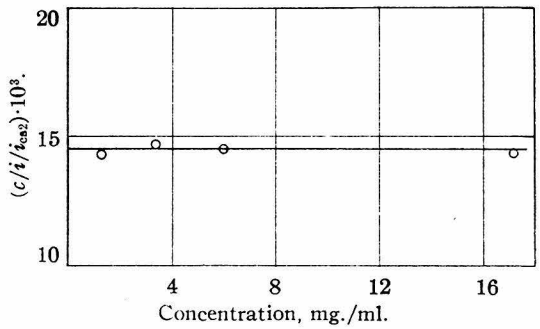


Fig. 1.—Light scattering data for purified rabbit antibody.

There is evidence, however, that the turbidity, depolarization, and refractive index of a protein solution change somewhat with *p*H and perhaps with salt content.[15,16] Not enough work has yet been done to understand how these changes should be taken into account when a value of the molecular weight is to be calculated.

We wish to express our thanks to Professor R. M. Badger and Dr. Stanley Swingle for their suggestions and assistance.

This work was supported in part by a Grant from the Rockefeller Foundation.

Summary

Methods are described for the isolation and purification of rabbit antibody against *p*-azophenylarsonic acid. The purified preparations were electrophoretically homogeneous and similar to gamma globulin.

Molecular weight studies from osmotic pressure and light scattering data gave values of approximately 140,000 and 158,000, respectively.

(15) Unpublished work on solutions of human serum albumin, human serum globulin, and blood group A-Specific substance.

(16) S. Armstrong and others, THIS JOURNAL, **69**, 1747 (1947).

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Additional molecular weight data from light scattering on serum proteins is presented in Table 13. The solutions, with the exception of the bovine serum globulin, were unbuffered and were adjusted to an ionic strength of 0.15 with sodium chloride.

Table 13

Molecular Weight of Serum Proteins

Protein	Remarks	dn/dc	pH	Molecular Weight	Accepted Molecular Weight***
Human Serum Albumin		0.209	6.8	72,000	70,000
Bovine Serum Globulin*		0.193**	8.4	170,000	174,000
Human Serum Globulin	filtered; not centrifuged	0.191	7.2	442,000	176,000
	centrifuged at 32,000 g for 20 minutes			285,000	

* Armour and Company, Fraction II of bovine plasma

** Solution in borate buffer

*** Values given by Cohn and Edsall 95/

No preparative treatment for the human serum globulin solution was found which would reduce its turbidity to that expected from the accepted molecular weight. This sample of protein may have undergone some change during the fractionation procedure or during subsequent handling and storage which was responsible for the high turbidity of its solutions.

Summary

The application of the light scattering method to the study of protein solutions is described. The effect of changes of pH and ionic strength on the turbidity of protein solutions was studied briefly. The light scattering method was used to determine the molecular weight of various serum proteins.

Section II. Studies of the Size and Shape of Molecules of Group A-Specific Substance from Hog Gastric Mucin

In 1936 Landsteiner and Chase 96/ isolated a substance from hog gastric mucin which was effective in inhibiting the isoagglutination of human type-A erythrocytes by type-B serum. Since that time substances of similar properties have been isolated from other sources 97/ but the hog stomach remains the most convenient starting material.

Group A-specific substance (which will be referred to as A-subatance) has been found to consist of polysaccharide material with about 25 per cent of amino acids. It has numerous reactive groups and a quite low isoelectric point. The material is generally considered to be a high molecular weight substance, although most of the experimental work has been with related protein materials. Exact comparisons can not be made between published data because different preparations from similar starting material may be degraded more or less, or changed in size and shape in the process of isolation.

Landsteiner and Harte 98/ have given viscosity, osmotic pressure, and ultracentrifuge data on degraded and "un-degraded" preparations of A-substance from hog stomach. At 22.5° C., 1 per cent solutions had relative viscosities of 1.2 and 3.7, and sedimentation constants of 3.6 and 6.9 Svedbergs, respectively. These data indicate a minimum molecular weight of 40,000 for the degraded material. The authors state that the ultracentrifuge patterns indicated homogeneous preparations. Osmotic pressure measurements gave a molecular weight of

70,000 for the degraded preparation and a variable value of from 120,000 to 200,000 for the un-degraded material. Occasional passage of biologically active material through the cellophane membrane was noted.

Meyer and Palmer 99/ concluded on the basis of viscosity and appearance of the precipitate that their preparations from hog gastric mucin consisted of thread-like molecules of different lengths.

Blix and Snellman 100/ found that hyaluronic acid, a somewhat distantly related compound, consists of molecules from 4,000 to 10,000 Å long. Assuming a diameter of 10 Å they calculated the molecular weight to be in the range of 200,000 to 400,000. Their information was obtained by measurements of the double refraction of flow.

Extensive electrophoretic measurements have been made in this laboratory by D. Brown and E. Bennett on preparations of A-substance similar to those studied in this investigation. They found that the mobility was very low and that the preparations seemed to be electrophoretically homogeneous.

The following investigation of the size and shape of A-substance was carried on between December, 1946 and March, 1947 with the collaboration of A. Pardee 101/.

The A-substance used in this investigation was prepared by G. Holzman, under the direction of Professor Carl Niemann. The essentially identical substances, R18-F10 and R18-F11, were isolated in 5.9 per cent yield from Wilson gastric mucin by three precipitations with 50-60 per cent ethanol and two electrodialyses.

R18-F11 was adjusted to pH 3 and ionic strength 0.0011 with hydrochloric acid. The 15 per cent which precipitated was given the number R19-F2a; the 85 per cent in solution was listed as R19-F1a. Repeated treatment of these fractions showed that the solubilities were actually different. All of the above samples had the same A-activity, about five times that of the original mucin. R18-F10 and R18-F11 had similar absorption spectra while R19-F2a showed diminished absorption in the ultraviolet as compared with R19-F1a. R18-F2 was a crude preparation obtained in an early stage of the purification of R18-F10. A detailed description of the preparative procedure has been submitted for publication 102/.

Light Scattering Measurements

The technique of making light scattering measurements was the same as that described in Part II, Section I. Fraction R18-F2, the fraction which had undergone a minimum of preparative work, gave a very turbid solution when dissolved in a 0.15 N sodium chloride solution but gave a clear solution when dispersed in formamide. Measurement of the refractive index increment indicated that the probable reason for the clear solution was that A-substance has nearly the same refractive index as formamide. The molecular weight which was calculated for this fraction in formamide was $4 \cdot 10^6$ but this value has little significance because of the large experimental error involved in the determination of the refractive index increment.

The material which had undergone more extensive fractionation gave reasonable turbidities in saline. The molecular weights were calculated to be $1.7 \cdot 10^6$, $1.1 \cdot 10^6$, and $0.9 \cdot 10^6$, respectively,

for Fractions R18-F10 in 0.15 N sodium chloride solution and Fractions R19-F2a and R19-F1a in phosphate buffer of pH 7.2 with sufficient sodium chloride to give an ionic strength of 0.15. The refractive index increment was 0.14 and was not detectably different in the two solvents. The light scattering curves are shown in Figure 35.

Osmotic Pressure Measurements

The osmotic pressure was measured in a simple osmometer which was essentially a bag of Visking tubing attached to a 1-mm. capillary tube. The static osmometer described in Part I, Section III was not suitable because the salt solution attacked the stainless steel parts. Four measurements at 25° C. gave an average value of 220,000 ± 20,000 for the molecular weight of Fraction R18-F10. The solvent was 0.02 M phosphate buffer at pH 7.1 plus sufficient sodium chloride to give an ionic strength of 0.15.

Diffusion Measurements

Diffusion measurements were made by A. Pardee in a Neurath type cell 103/ by use of the thermostat and schlieren optical system of an electrophoresis apparatus. Pictures of R18-F10 were taken at two concentrations over a three-day period. The differential diffusion curves at the higher concentration were symmetrical but those at the lower were slightly skew. The diffusion coefficient was calculated by two methods. The first, which depends on the height and area of the curves 104/, gave $0.60 \pm 0.06 \cdot 10^{-7}$ cm.^{2} sec. as an

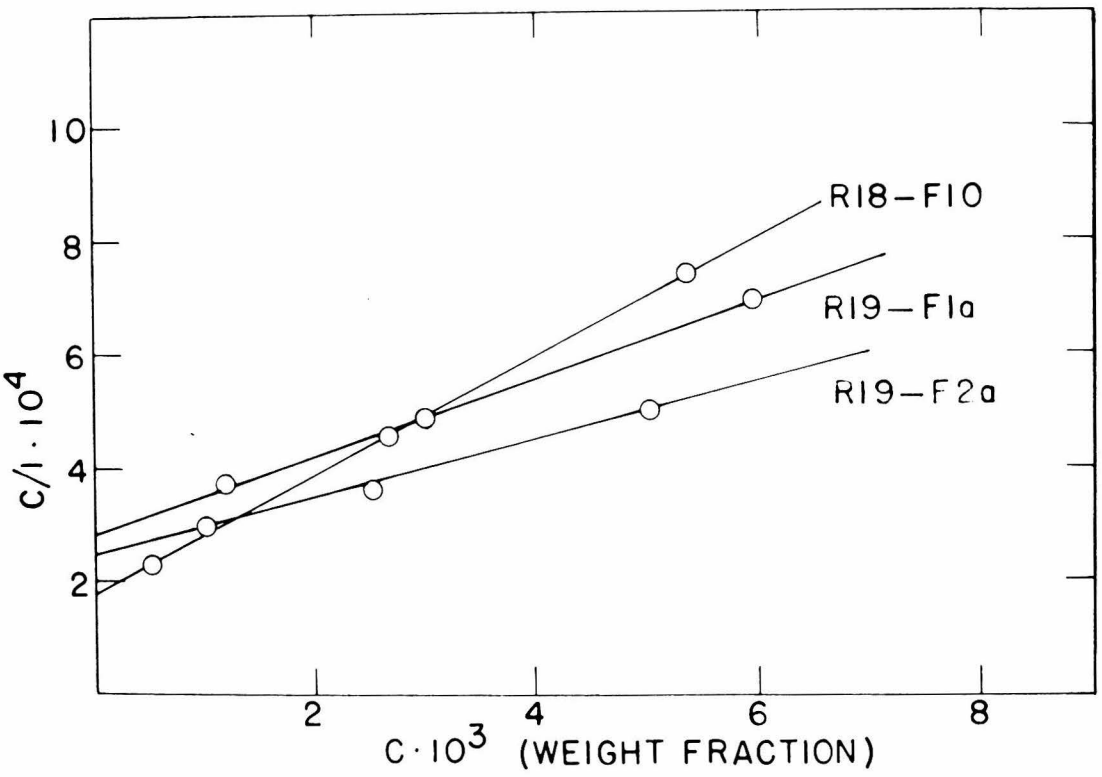

Figure 35. Light scattering data for three preparations of A-substance

average for five pictures, and the second method, which depends on the width at several heights of the curves, gave a value of $0.55 \pm 0.04 \cdot 10^{-7}$ cm.^{2} sec. The two concentrations gave results which agreed within the experimental error.

Partial Specific Volume

The partial specific volume of Fraction R18-F10 in 0.15 N sodium chloride solution is 0.73. This value was determined by A. Pardee.

Viscosity*

An Ostwald-Fenske viscometer was used to measure the viscosity of samples of R19-F1a and R19-F2a at 25° C., at several concentrations, and at different applied pressures. The solvent was 0.02 M phosphate buffer at pH 7.2 which was adjusted to an ionic strength of 0.15 with sodium chloride. The viscosity increment, ν, was calculated from the times of flow of solvent and solution, t_o and t, respectively, and the volume fraction of the solute, ϕ, by the expression**

$$\nu = \left(\frac{t}{t_o} - 1\right) \Big/ \phi \qquad (31)$$

The velocity gradient, $\bar{\beta}$, which is a mean value for liquid flowing through a capillary, was calculated by the use of an expression due to Kroepelin 105/

---

* These measurements were made by A. Pardee

** The viscosity increment when extrapolated to zero concentration is closely related to the intrinsic viscosity defined in Part I, Section II; that is, $\frac{[\eta]}{\nu} = \frac{\bar{\nu}}{100}$, where $\bar{\nu}$ is the partial specific volume of the solute

$$\bar{\beta} = \frac{8V}{3\pi r^3 t} \qquad (32)$$

where V is the volume of liquid flowing through a capillary of radius r in time t.

Values of the viscosity increment at zero concentration and at zero velocity gradient were obtained by a two-stage extrapolation. The extrapolation to zero velocity gradient was guided by a theoretical curve of Kuhn and Kuhn for the viscosity of a suspension of ellipsoids of revolution as a function of velocity gradient 106/. The viscosity data are tabulated in Table 14.

Table 14

Viscosity of A-Substance

$\Phi \cdot 10^3$	ν $(\bar{\beta} = 10200)$	ν $(\bar{\beta} = 5400)$	ν $(\bar{\beta} = 1000)$	ν $(\bar{\beta} = 0.0)$
R19-F1a				
3.91	150	207	302	
1.96	126	168	256	
0.78		144	240	
0.00*	110	135	225	230*
R19-F20				
3.31	188	254	400	
1.65	170	219	330	
0.66	167	217	294	
0.00*	153	200	265	275*

* Extrapolated

Discussion

A considerable structural viscosity exhibited by solutions of A-substance is an indication that the molecules are large and are quite asymmetrical. Such solutions in general exhibit two limiting values of the viscosity increment. At very low velocity gradients the molecules are distributed at random, the resistance to flow is high, and the viscosity increment, ν_p, is high. With very high velocity gradients the molecules are orientated predominantly parallel to the stream lines of the liquid, their influence on the viscosity is a minimum, and the viscosity increment tends towards a lower limiting value, ν_0.

Assuming that the molecules of A-substance are asymmetrical, there are two models: the random coil, and the elongated ellipsoid, which might be applicable. A statistically coiled molecule should have a fairly constant viscosity increment until a high velocity gradient is reached, especially if it is not easily deformed. On the other hand, a rod-like molecule would orientate itself, and the viscosity increment should decrease considerably at high velocity gradients. The variation of ν with velocity gradient of A-substance agrees fairly well with theoretical curves for the rod model presented by Kuhn and Kuhn 107, 108/.

Hydrodynamic treatments have now been made of both ν_p and ν_0 for suspensions of both elongated and flattened ellipsoids 63, 109/. Mehl, Oncley, and Simha 110/ have presented extensive tables based on Simha's calculations 63/ of the relation between the viscosity increment, ν_0, and the axial ratio of suspensions of elongated

ellipsoids. If these calculations are assumed to be applicable to solutions of A-substance with ν_o = 230 and 275, respectively, for fractions R19-F1a and R19-F2a, the A-substance molecule has an axial ratio of about 60 to 1.

A value for the molecular weight of A-substance can be calculated from the viscosity and diffusion data if a variety of assumptions are made.

Einstein 111/ generalized the earlier reasoning of Nernst, Sutherland, and Stokes and showed that the diffusion constant of a solute is a function of the dimensions of the molecules and the viscosity of the liquid. Perrin 112/ carried this approach further and found that the diffusion constant, D, of an ellipsoid of revolution is given by $D = D_o f_o/f$, where D_o is the diffusion constant of a sphere of the same mass and volume as the ellipsoid and f_o/f is the ratio of frictional coefficients of the equivalent sphere and the ellipsoid. Values of f_o/f for a suspension of elongated ellipsoids can be calculated in terms of the axial ratios following a treatment by Perrin 112/, and a tabulation of such calculations has been given by Svedberg and Pedersen 113/. D_o can be calculated by the use of Einstein's relation if the molecular weight, partial specific volume of the solute, and the viscosity of the solvent are known.

The molecular weight which is calculated for A-substance from the following expressions is $5.0 \cdot 10^6$.

$$D = D_o \frac{f_o}{f} \quad \text{where} \quad D_o = \frac{KT}{f_o} = \frac{KT}{6\pi\eta\left(\frac{3\bar{v}M}{4\pi N_o}\right)^{1/3}} \qquad (33)$$

with $D = 0.58 \cdot 10^{-7}$ cm.^{2} sec., $f/f_o = 3.2$ (for an axial ratio of 60 to 1), $\bar{v} = 0.73$, $t = 25^\circ$ C., and $\eta = 0.01$ poise.

The values of the molecular weight from light scattering are not inconsistent with this value. The light scattering measurements were made before the angular scattering equipment was constructed; hence the reported values of the order of one million were calculated from the 90° scattering alone. The correction factor which is calculated from the angular scattering (see Part I, Section VI, Figure 19) could easily be 3 or 4 for a rod-like molecule of reasonable length and could bring the light scattering molecular weight in essential agreement with that from viscosity and diffusion. The different slopes of the light scattering curves for the different preparations and the appreciably lower intercept for Fraction R18-F10 may be due to the fact that the solutions were at different pH and ionic strength. The effects of pH and ionic strength on the light scattering of protein solutions are not yet understood although such data might be interpreted in terms of a treatment similar to the recent Kirkwood-Goldberg theory of light scattering in multicomponent systems (see Part I, Section IX).

The molecular weight from osmotic pressure data, 220,000 for Fraction R18-F10, is not in agreement with the above results unless the difference is ascribed to molecular heterogeneity of the preparations. It is conceivable that the presence of a few per cent of a

rather low molecular weight material could give such a value for the number-average molecular weight. The observation of Landsteiner and Harte 98/ mentioned earlier that biologically active material diffused through the membrane during their osmotic pressure measurements is consistent with this hypothesis. The fractions are not extremely heterogeneous, however, since the two methods of calculating the diffusion constant, one of which is sensitive to heterogeneity, gave essentially the same result.

The only earlier work which is at all suitable for comparison is that of Landsteiner and Harte 98/. By use of their value of the sedimentation constant for "un-degraded" A-substance, $S_{20} = 6.9 \cdot 10^{-13}$ sec., and values for the diffusion constant and partial specific volume, respectively, of $0.58 \cdot 10^{-7}$ cm.^{2} sec. and 0.73, the molecular weight is calculated to be $1.1 \cdot 10^6$.

Summary

Light scattering, osmotic pressure, diffusion, and viscosity measurements have been made on preparations of A-substance. The values of the molecular weight which is calculated from diffusion and viscosity is $5 \cdot 10^6$. Light scattering data indicate only that the molecular weight is greater than $1 \cdot 10^6$. The much lower value of the molecular weight calculated from osmotic pressure, $0.2 \cdot 10^6$, might be ascribed to molecular heterogeneity of the preparation.

Section III. Thermal Aggregation of Serum Proteins

Aqueous solutions of proteins (gelatin is an exception) become turbid when heated to temperatures of the order of 60° C., and in general, proteins become completely insoluble after extended periods of heating at this or somewhat higher temperatures. This process of coagulation of proteins in solution is usually taken as evidence of denaturation since the amount of denaturation determined by this criterion usually agrees with that determined by other methods: for example, change in amount of detectable sulfhydryl groups.

Hardy 114/, many years ago, proposed that heat denaturation of proteins really involves two stages, the first of which is an actual change in the physical properties of the protein, and a second, which is the coagulation of the already altered protein. The mere coagulation of a protein is not sufficient evidence of denaturation, for there are numerous examples of aggregation of native protein: for example, precipitation of serum proteins by the addition of salt, or the coagulation of euglobulin during dialysis against distilled water.

Cooper and Neurath 115/ recently investigated solutions of horse serum albumin which had been heated for various lengths of time at 70° C. and concluded on the basis of viscosity, diffusion, and electrophoretic measurements that there was actually molecular aggregation at certain values of pH and ionic strength even though there was no obvious coagulation. At pH 7.6 the heated solutions exhibited a higher viscosity; the electrophoretic pattern and mobility were different from that of native protein; and finally, the

heated protein was found to be more susceptible to tryptic digestion. Bier and Nord 116/ found that the turbidity of solutions of ovalbumin at pH 4.20 was changed markedly by heating at 40° C., although there was no detectable increase in free sulfhydryl groups and no change in the ability of the protein to crystallize.

The measurement of the turbidity of protein solutions was widely used during the war as an empirical means of evaluating the thermal stability of plasma preparations derived from blood collected by the American Red Cross 117/, but little effort was made to analyze the data.

Experimental

A brief light scattering investigation of the thermal aggregation of human serum albumin and bovine serum globulin was made to learn whether useful information about protein denaturation could be gained in this way. The light scattering apparatus has been described in Part I, Section VI, and the proteins were those described in Part II, Section I. The proteins were dispersed in borate buffer of pH 8 or in barbital buffer of pH 8.6, each with sufficient sodium chloride to give an ionic strength of 0.15. The solutions were centrifuged, transferred to a dust-free scattering cell, and the cell was immersed in a constant temperature water bath. After the required period of heating the cell was removed, brought rapidly to room temperature, and the 90° scattering was determined. The scattering was measured at only one concentration since the data in Part II, Section I indicate that the light scattering curves

for serum proteins at this pH and ionic strength have nearly zero slope. The dissymmetry measurements were made in the usual manner.

Results and Discussion

The turbidity of solutions of humam serum albumin changed very slowly with time of heating at 57.8° C. A solution of 3 mg. per ml. in barbital buffer of pH 8.6 gave a five-fold increase in turbidity after 13 days of heating, which corresponds to a final molecular weight of about 350,000. Solutions which were heated longer than 13 days and then stored for some hours in the cold showed some coagulation.

The study of human serum albumin was not pursued further because of the greater interest in the heat denaturation of γ globulin in connection with the proposed mechanism for the manufacture of antibodies in vitro 118/.

Solutions of bovine serum γ globulin were heated at various temperatures and at various concentrations of protein in borate buffer of pH 8.4, $\mu = 0.15$. The results are plotted in Figures 36 and 37, where I is the ratio of the intensity of the 90° scattering to that of carbon disulfide, and c is the protein concentration expressed as weight fraction. The value of I/c is proportional to the weight average molecular weight of the scattering if, as mentioned above, the slope of the light scattering curve is neglibible and if the scattered light exhibits no angular asymmetry.

Angular scattering measurements were made on a bovine γ globulin solution in the above buffer. The dissymmetry coefficient, for

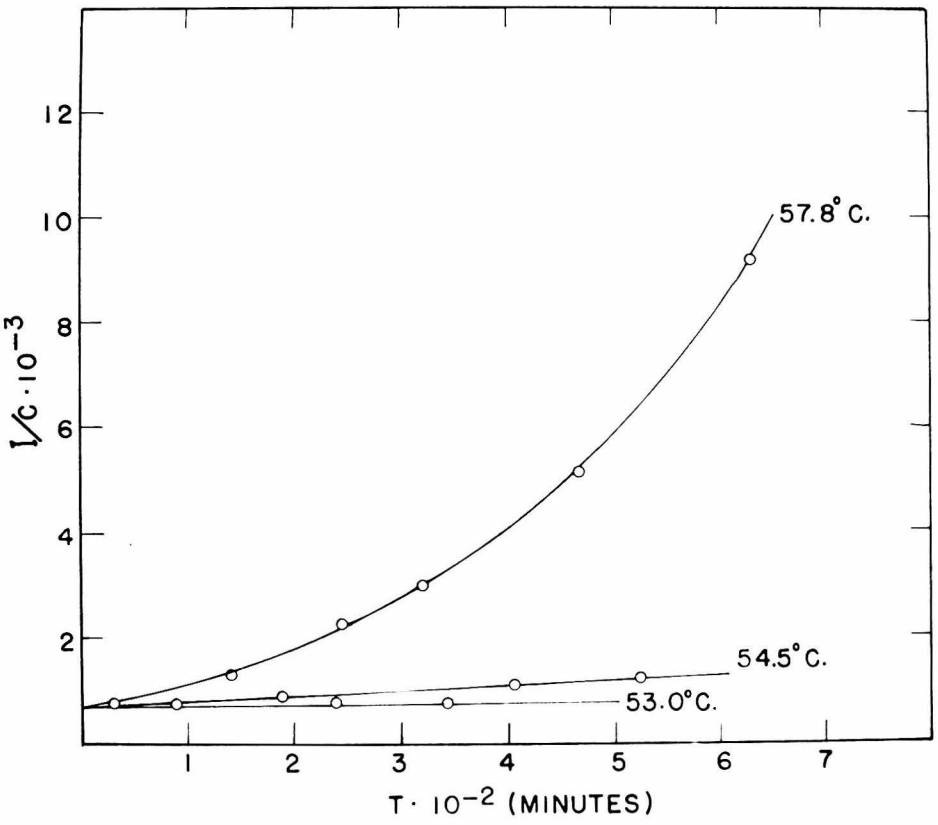

Figure 36. Effect of temperature on scattering power of heated solutions of bovine serum globulin. Initial protein concentration was 0.3 mg. per ml.

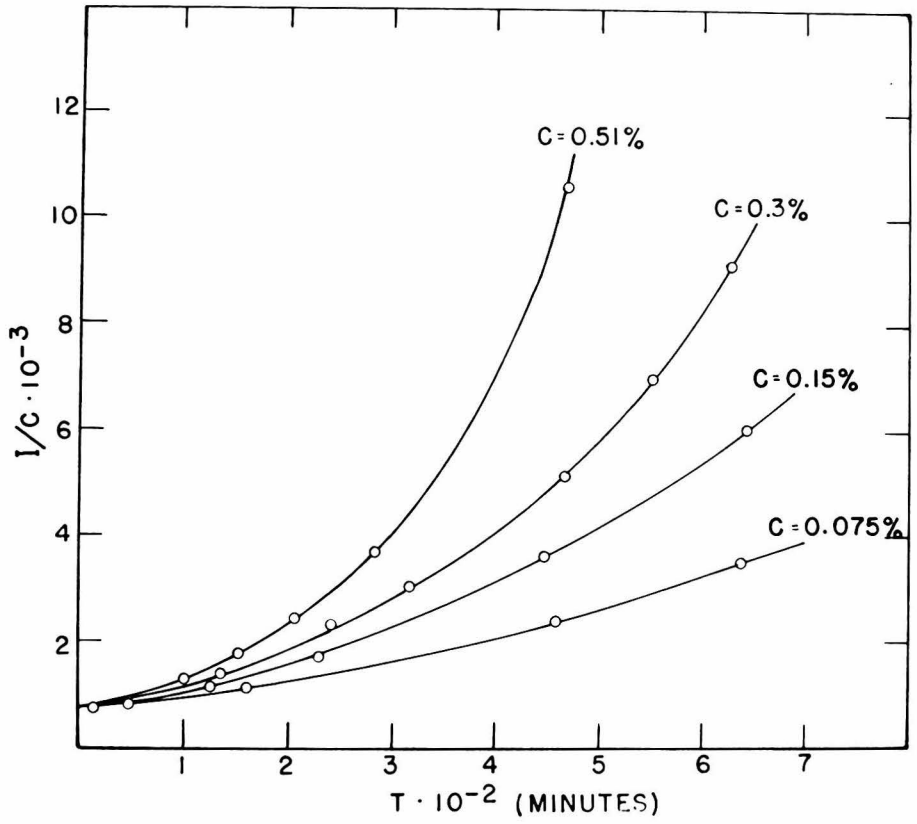

Figure 37. Effect of initial protein concentration on the scattering power of heated solutions of bovine serum globulin. The solutions were heated at 57.8° C.

a solution which contained 3 mg. of protein per ml. of solution, was zero within the experimental error. This result was expected, since the maximum dimension of the globulin molecule is considerably less than the wave length of light. This same solution was then heated for 20 minutes and 76 minutes, respectively, at 57.8° C., but there was no detectable change in the angular scattering.

If a solution of this protein at the above concentration is heated at 57.8° C. for extended periods of time the turbidity of the solution continues to increase for a time, as shown in Figure 37, but reaches a maximum value after about 100 hours. With continued heating there is little change in the turbidity of the solution until the protein begins to precipitate. The appearance of a precipitate was noticed after 380 hours of heating at this temperature.

A differential centrifugation experiment was performed on a solution containing 3 mg. of protein per ml. of solution which had been heated for 120 hours at 57.8° C. in order to learn something about the distribution of particle weights in the solution. It was found that the concentration of protein remaining in solution decreased regularly with increasing time of centrifugation; so it is evident that there was a distribution of particle weights among the aggregates in solution. About half of the protein initially in solution was found to have been centrifuged out of solution after 540 minutes. Since this time of centrifugation at 32,000 g is sufficient to remove from solution all particles having a molecular weight greater than about $2 \cdot 10^7$ it is clear that a considerable percentage of quite large aggregates are formed in the heated solution.

This data may be interpreted as indicating that there are but few large particles in solution before 100 minutes of heating, but after 100 hours of heating quite a large proportion of the total protein is in the form of large aggregates.

The data presented in Figures 36 and 37 indicate that the rate of change of turbidity of a heated γ globulin solution at constant ionic strength and pH is dependent on the initial concentration of protein, the temperature at which the solution is heated, and the time of heating. If these curves represented a change in the amount of denatured protein with time of heating it would be easy to establish the order of the denaturation reaction and to determine the heat of the reaction. The rate of change of turbidity of such solutions is, however, a measure of the rate of aggregation of protein which has presumably undergone thermal denaturation and is therefore no simple measure of the rate of the denaturation.

A possible method of analysis of the turbidity curves would be to consider that heat causes the native protein to change in some manner by a first order reaction and that the altered protein then aggregates according to a second order reaction. The primary reaction of protein denaturation is usually considered to be of first order, and Smoluchowski 119/ has proposed that the coagulation of colloid particles obeys a second order reaction.

The rate of change of "denatured" protein molecules would then be given by the expression

$$\frac{dN}{dt} = -KN^2 + \alpha N_0 e^{-\alpha t} \qquad (34)$$

where N is the number of "denatured" protein molecules,

K is the Smoluchowski rate constant for coagulating systems,

N_0 is the initial number of protein molecules, and

α is a first order rate constant for thermal denaturation.

The turbidity of a polydispersed system can be expressed as

$$\tau = A \sum N_j V_j^2 \qquad (35)$$

where A is a constant for a given system, and N_j is the number of particles per unit volume of the jth kind each having a volume V_j. If the scattering particles are simple aggregates of primary particles, then the volume of the j-mer is j times the volume of the primary particle, V_0, equation (35) can be written as

$$\tau = A V_0^2 \sum j^2 N_j \qquad (36)$$

In principle the turbidity of a heated protein solution can be expressed as a function of time by means of equation (36), with N_j determined by the solution of equation (34).

Summary

The change in the light scattering of solutions of bovine globulin with time of heating has been measured at constant ionic strength and pH but with different initial concentrations of protein and different temperatures of heating.

An incomplete analysis of the data suggests that although the primary reaction of protein denaturation is involved in the change of turbidity of the heated solutions, the most important factor is the aggregation of the denatured protein molecules.

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Propositions

1. Comparison of values of the "apparent molecular weight"* of proteins is at best unsatisfactory and at worst may lead to incorrect interpretation of data. For example, the conclusions of Fraenkel-Conrat and Mecham 1/ concerning the state of aggregation of ovalbumin in an aqueous solution containing urea and iodoacetamide could be questioned.

Although there is no evidence for the aggregation of serum albumin in an isoionic condition in the absence of electrolytes the osmotic pressure of an electrodialyzed isoelectric bovine serum albumin solution has given variable results for the molecular weight 2/. It should be relatively simple to determine the molecular weight of isoionic salt-free serum albumin by light scattering.

* The value of the molecular weight which can be calculated from the magnitude of the osmotic pressure of a solution containing a high molecular weight solute by the use of the van't Hoff equation.

1/ Fraenkel-Conrat, H., and Mecham, D. K., J. Biol. Chem., 177, 477 (1949).

2/ Scatchard, G., Batchelder, A., and Brown, A., J. Am. Chem. Soc., 68, 2320 (1946).

2. It is the practice of certain moonshiners in the Allegheny mountains when preparing spirits for the trade to steep plugs of chewing tobacco in raw distillate for 48 hours. Although this procedure admittedly gives the product a desirable color and eliminates the "slop" taste and odor it probably also reduces the fusel oil content. This hypothesis results from observation of physiological reaction and not from chemical analysis.

3. A recent study of the liquid phase adsorption of acetone by nitrocellulose from an acetone-ligroin mixture was based on the assumption that there is no interaction between nitrocellulose and ligroin 1/. This assumption is contrary to conclusions reached in this thesis 2/.

An independent method of evaluating the magnitude of possible adsorption of ligroin by nitrocellulose from acetone-ligroin mixtures would be the "equilibrium dialysis" procedure 3/. It might be found necessary to use one of the more sophisticated interferometers to evaluate small changes in solvent composition.

1/ Campbell, H., and Johnson, P., J. Polymer Sci., 4, 247 (1949).

2/ This thesis, Part I, Section IX.

3/ Klotz, I. M., Arch. Biochem., 9, 109 (1946).

4. Putman's remarks on the mode of denaturation of proteins by detergents do not include the consideration that surface active agents may be attracted to the protein-solvent interface. Although many factors are involved in protein denaturation this effect should be considered.

The experiments of the Stanford group 2/, Klotz 3/, and others on the adsorption of small molecules by proteins can be interpreted as evidence of decided differences in the configuration of certain protein molecules.

1/ Putman, F. W., "The interaction of proteins and synthetic detergents" in Advances in Protein Chemistry, Vol. IV, edited by Anson, M. L., and Edsall, J. T., Academic Press, New York, 1948.

2/ Papers by Boyer, Ballou, Luck, and others, principally in J. Biol. Chem., 1944 to 1948.

3/ Klotz, et al, J. Am. Chem. Soc., 68, 1486 (1946) and other papers

5. It is often desirable to measure the light scattering of colored solutions. If a solution is colored the optical dielectric constant is no longer given by the square of the ordinary refractive index but is given by the square of a complex refractive index. If the absorption is due to a low molecular solvent component it can be shown that the usual light scattering equation can be used to calculate a molecular weight for a high molecular weight solute. If, on the other hand, the absorption is due to the high molecular weight solute the usual light scattering equation is no longer valid 1/.

1/ Kenyon, A., and LaMer, V., J. Coll. Sci., 4, 163 (1949).

6. The Bureau of Ordnance specifications for the analysis of nitroglycerin solutions should be changed. It would be desirable to carry out the reduction of nitroglycerin to ammonia by means of Devarda's alloy in alkaline solution with subsequent distillation and titration of ammonia with standard acid. The present specifications call for the reduction of nitroglycerin by ferrous ion in acid solution and subsequent back titration of ferric ion with standard titanous chloride solution.

7. The validity of the Staudinger viscosity relation for nitrocellulose solutions is fortuitous and results from the occurrence of a broad flat maximum in values of $[\eta]/z$ for values of z usually encountered in nitrocellulose samples 1/.

1/ This thesis, Part I, Section VIII.

8. The general equation given by Kirkwood and Goldberg 1/ for the light scattering of multicomponent systems, when constrained to a two-component system, gives a simple expression for the light scattering of a mixture of two low molecular weight fluids.

A light scattering method might be developed which would enable the calculation of liquid-liquid interaction with as much accuracy as does the determination of partial vapor pressures.

1/ Kirkwood, J. G., and Goldberg, R., J. Chem. Phys. (in press).

9. Although it is inevitable that technical and scientific workers will give different definitions of what constitutes a good solvent for a high polymer, there now exists a variety of definitions of "good solvents" in the high polymer literature. A statement which is less ambiguous than most is that of two solvents the better solvent is the one in which the activity coefficient of the polymer is least.

It is difficult to predict the ability of solvents to dissolve nitrocellulose. I have found empirically that good nitrocellulose solvents give values of the quantity $\frac{\mu^2}{\epsilon} D$ which are little different from one another. μ is the dipole moment, ϵ is the dielectric constant, and D is the relative internal pressure as determined from the heat of vaporization 1/. Ostwald has proposed the use of the quantity $\frac{\mu^2}{\epsilon}$ in this connection 2/.

1/ Hildebrand, J. H., Solubility, Chemical Catalogue Co., New York, 1924.

2/ Ostwald, Wo., and Ortloff, H., Kolloid-Z., 59, 25 (1932).

10. The classical experiments of Perrin 1/ on suspensions of gamboge particles led to values of Avogadro's number which were about 10 per cent higher than the value accepted at present. The mean square displacement method probably gave high values of Avogadro's number because the resistance factor for such particles is not adequately represented by $6\pi\eta r$ 2, 3/.

The high values of Avogadro's number which were determined by the gravity sedimentation equilibrium method may be attributed to the deviation of the system from perfect solution behavior. The following equation should be a better representation of sedimentation equilibrium of colloid particles than the one usually given.

$$\ln \frac{N_2}{N_1} = \frac{mg}{KT}(h_2 - h_1) - \frac{2B}{KT}(N_2 - N_1)$$

where N is the number of particles of mass m per unit volume,
h is the height above a reference point,
g is the acceleration due to gravity,
K is the Boltzmann constant,
T is the absolute temperature, and
B is a constant for a given system

1/ Perrin, J., Atoms, translated by D. L. Hammick, Constable and Company, London, 1920.

2/ Millikan, R. A., The Electron, Univ. of Chicago Press, Chicago, 1925.

3/ Svedberg, T., Colloid Chemistry, The Chemical Catalogue Company, New York, 1928.