to considerable lengths to suggest alternative explanations of the phenomenon (including the cataphoresis of colloidal metallic aluminum, and the existence of complex ions such as $Al^{+++}Al_2$), which would avoid the hypothesis of a simple aluminum ion of charge lower than + 3. On the other hand, abundant evidence is reported in the literature for the existence of such compounds as AlO[18] and AlF,[19] although at elevated temperatures only.

In the experiments here reported, both the excessive weight of aluminum dissolved from the anode during electrolysis and the liberation of hydrogen in the anolyte appear to leave small room for doubt that a singly or doubly charged aluminum ion does indeed exist, at least momentarily, in acetic acid solutions of acetates. The data do not point unequivocally either toward a unipositive or toward a bipositive ion, but analogy with the known behavior of other members of this family, as well as the electron structure of aluminum, with its single 3 *p* electron, lends strong support to the former hypothesis.[20] If this supposition is correct, it must be concluded that aluminous ion, Al^+, is a considerably stronger reducing agent than gallous ion, Ga^+. For, in the first place, the amount of reducing agent remaining in the solution at the end of the electrolysis was always much smaller in the case of aluminum; and secondly, the amount of hydrogen liberated out of contact with the cathode was usually far less than sufficient to account for the oxidation of Al^+ to Al^{+++}. It seems not unlikely that acetate ion, which is, of course, present in the electrolyte in enormously higher concentration than hydrogen ion, may enter into the reaction also, and that such a reaction may account for the yellow color[21] observed in the electrolyte.

(18) Coheur, *Bull. classe sci. Acad. roy. Belg.*, [5] **28**, 569 (1943).

(19) Klemm and Voss, *Z. anorg. allgem. Chem.*, **251**, 233 (1943).

(20) It may also be pointed out here that in later unpublished work in this laboratory (Mazzitelli, Master's Thesis, University of Kansas, 1949), initial mean valence numbers for aluminum in acetic acid solutions as low as 1.80 were obtained.

Summary

1. Anodic oxidation of thallium in acetic acid solution of an acetate yields thallous ion, Tl^+, exclusively.

2. In the anodic oxidation of gallium, indium, and aluminum in similar electrolytes, the loss of metal from the anode is always considerably greater than that corresponding to Faraday's law, if the product is assumed to be a triply charged cation; and free hydrogen is in every case evolved from the anolyte.

3. The behavior of the solutions resulting from such anodic oxidation of gallium, indium, and aluminum, as well as the coulometric data, may readily be explained in terms of the hypothesis that the primary anode product is in each case a mixture of a singly and a triply charged cation.

4. If this hypothesis is correct, it follows that gallous ion, Ga^+, and especially aluminous ion, Al^+, are to be regarded as very strong reducing agents; whereas indous ion, In^+, is subject not only to oxidation but to rapid disproportionation into indic ion, In^{+++}, and metallic indium.

(21) It was found possible to extract with ether, from an aqueous solution of the electrolyte after electrolysis, a minute amount of a yellow oil, obviously organic in nature.

LAWRENCE, KANSAS RECEIVED AUGUST 13, 1949

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1312]

Partition Systems for the Fractionation of Nitrocellulose with Respect to Molecular Weight[1,2]

BY MARVIN C. BROOKS AND RICHARD M. BADGER

Many studies of the molecular weight distribution of high polymers which would be of considerable interest are at present impracticable because of the limitations and tediousness of conventional fractionation procedures. The recent success of adsorption and partition methods in the separation of closely related chemical species has directed our attention to the possibility of applying such methods to the fractionation of high polymers. In this communication we describe a partition system applicable to the fractionation of nitrocellulose with respect to molecular weight. Studies on adsorption systems are in progress and will be reported separately.

Partition separations depend upon differences in the way the solutes to be separated distribute themselves between immiscible phases. They have the great advantage that they can be brought about by the same convenient type of procedure used in ordinary chromatography, which automatically effects a repeated fractionation.[3] The method at present most commonly employed in fractionating high polymers, namely,

(1) This paper is based on work supported by the Bureau of Ordnance and done under contract with the Office of Naval Research, Contract N6-ori-102, Task Order VI.

(2) Presented before the High Polymer Forum at the San Francisco meeting of the American Chemical Society, March 28, 1949.

(3) Martin and Synge, *Biochem. J.*, **35**, 91, 1358 (1941). Partition chromatography has been applied with notable success to the separation of amino acids in protein hydrolysates, Stein and Moore, *J. Biol. Chem.*, **176**, 337 (1948).

fractional precipitation, is in effect a partition method, but has several inherent limitations, and does not offer the particular advantage just mentioned. The presence of polymer is in general necessary for the existence of two phases, one of which is consequently very concentrated and viscous, and no convenient and automatic systemization is evident for repeated fractionation.

Two-phase liquid systems suitable for the fractionation of high polymers have been conspicuous by their absence from the reported literature. Notable exceptions are those employed by Schulz and Nordt in the fractionation of polyethylene oxide[4] and by Lovell and Hilbert in the fractionation of lignin.[5]

A number of attempts have been made by the present authors to find a two-phase liquid system suitable for the fractionation of nitrocellulose by partition. However, the presence of water was always required to obtain a two-phase liquid system at low nitrocellulose concentration, and in none of the cases studied was the maximum concentration of nitrocellulose obtainable in the aqueous phase sufficient to give promise for practical use of the system. Although systems which exist in two liquid phases *per se* seemed to offer no possibility for use, it was conceived that an effective two-phase system might be brought about by addition of a solid which though insoluble would swell by the selective absorption of one component of a two-component, normally miscible, solvent mixture. It was anticipated that such a two-phase system might be conveniently adapted to a partition chromatographic process. This approach proved fruitful.

In a mixture of methyl acetate and water it was found that cellulose triacetate[6] swells but does not dissolve appreciably. The swollen gel is apparently richer in the methyl acetate than is the supernatant phase. Nitrocellulose polymers distribute between the gel and supernatant phases, and the distribution constants, other factors held constant, depend strongly upon the molecular weights of the nitrocelluloses. Further, the ability to control the relative amounts of methyl acetate and water furnishes a powerful means by which the magnitudes of the distribution constants can be modified, though not independently of molecular weight.

In this paper are reported the results of a number of distribution experiments which indicate that fractionation of nitrocellulose by partition between gel and supernatant phases is feasible, though subject to some limitations.

Experimental

Materials.—Cellulose triacetate was obtained from the Hercules Powder Co. as cellulose acetate, type TH-2. Acetate composition for TH-2 is specified as 61.5–62.5% combined acetic acid; calcd. for cellulose triacetate, 62.5% combined acetic acid. The product was obtained in granulated form. In the distribution experiments here reported only that portion (*ca.* 10–15%) which would pass through a 100-mesh screen was used.

Methyl acetate, technical grade (n^{20}D 1.3554), which presumably contained methyl alcohol was purified by extraction and distillation. Four successive extractions with 25% aqueous solution of potassium carbonate were carried out. For each extraction the amount of the carbonate solution was about half that of the methyl acetate phase. The acetate was dried over anhydrous potassium carbonate and distilled, b. p. 55–56° (uncor.), n^{20}D 1.3598, yield 60–70%; lit. values, b. p. 57°, n^{20}D 1.3594. A sensitive test for the purity of methyl acetate with respect to methyl alcohol is the extent of miscibility with water. The purified product had a solubility of 46 g./100 g. of water at 20°; this compares to the lit. value of 33 g./100 g. of water. One drop of methyl alcohol added to a 5-ml. sample of the purified methyl acetate brought about complete miscibility with water. To reproduce results in distribution experiments, it has been found necessary to use freshly distilled methyl acetate.

Acetone, Shell C. P. grade, was used without further purification. Butyl acetate, technical grade, was dried over calcium oxide and fractionally distilled. A middle fraction boiling at 55° at 55 mm. was retained and stored until used over calcium oxide. Absolute ethanol from U. S. Industrial Alcohol was used without further purification.

Nitrocelluloses were unfractionated commercial polymers: NC No. 17, 10.98% N, $[\eta] = 5.1$[7,8] obtained from cotton linters; NC No. 18, 10.96% N, $[\eta] = 0.71$,[7] obtained from cotton linters; NC No. 19, 10.93% N, $[\eta] = 1.64$[7], obtained from cotton linters; NC No. 41, 11.99% N, $[\eta] = 0.85$[7], obtained from wood pulp.

Determination of Distribution Constants.—Aliquots of standard nitrocellulose solutions were placed in 15-ml. graduated centrifuge tubes and sufficient amounts of the appropriate solvents were added to provide a total of 8 ml. of solvent mixture. One half gram of cellulose triacetate was then added and the tubes were corked and agitated by mounting on a small wheel which revolved in a vertical plane at about 50 r. p. m. After an appropriate length of time, agitation was stopped and the gel and supernatant phases separated by centrifugation. An aliquot of supernatant phase was removed and analyzed for nitrocellulose concentration.

The extent of distribution can be reported as the fraction of nitrocellulose apparently absorbed by the cellulose triacetate or if the relative volumes of the gel and supernatant phases are known, as a distribution constant. When mixtures of methyl acetate and water were used as co-solvents, the degree of swelling of the cellulose triacetate was observed to be practically independent of solvent composition throughout the range of interest, and the effective volume occupied by 1.0 g. of cellulose triacetate was found to be 4.2 ml. This was determined by packing a column with gel with the minimum amount of methyl acetate, above which a nitrocellulose solution was placed. The volume of elute collected before nitrocellulose could be detected was taken equal to the free volume not occupied by gel. Conditions were such that absorption of the nitrocellulose was negligible.

In absorption experiments the total volume of gel and supernatant phases was found to be 8.2 ml. when 8 ml. of the co-solvents methyl acetate and water and 0.5 g. of cellulose triacetate were used. Consequently, within experimental accuracy the ratio of the volumes of supernatant and gel phases was 3.0:1.0. All distribution constants have been calculated on that basis. In all of the

(4) Schulz and Nordt, *J. prakt. Chem.*, **155**, 115 (1940).

(5) Lovell and Hilbert, THIS JOURNAL, **63**, 2070 (1941).

(6) Cellulose acetate treated with butyl alcohol was used by Boscott, *Nature*, **159**, 342 (1947), in a partition chromatographic method of extracting cresol and benzoic acid from saline solution.

(7) All intrinsic viscosities were determined in butyl acetate.

(8) This value was not obtained directly. A Hercules smokeless second value of 20 was determined and the corresponding intrinsic viscosity estimated with the use of the relations given by Doyle, Harbottle, Badger and Noyes, *J. Phys. Colloid Chem.*, **51**, 569 (1947).

sets of runs, controls to give the original concentration of nitrocellulose in solution before cellulose triacetate was added, were included. The concentration in the supernatant phase was determined from an aliquot directly, and the concentration in the gel phase was calculated from the difference of original and final concentrations in the supernatant phase. The distribution constants reported are the ratios of the equilibrium concentration in the supernatant phase to the concentration in the gel phase.

Volumes of the gel of cellulose triacetate in systems other than the methyl acetate–water co-solvent system have not been determined, so distribution results are reported as the per cent. of nitrocellulose apparently absorbed by the cellulose triacetate.

Analytical Determination of Nitrocellulose.—A colorimetric test was used which is a modification of a classical method for the determination of nitrates in water.[9] The method depends upon the reaction of 1-phenol-2,4-disulfonic acid with nitrates to give 2-nitrophenol-4,6-disulfonic acid. Our method of preparation of the reagent is to heat 50 g. of purified phenol in 200 ml. of concentrated sulfuric acid and 60 ml. of oleum (65% free SO_3) for two hours in a boiling water-bath. The product is a light brown liquid which can be stored without deterioration in a glass-stoppered bottle. Aliquots of the nitrocellulose solution to be analyzed are volumetrically transferred to a vial and heated to dryness at about 75–100°. About 1 ml. of the reagent of 1,2,4-phenoldisulfonic acid is added to the dry residue while still warm. Solution is brought about by stirring and if necessary reheating at 75° for about one minute. The acid mixture is diluted and made alkaline with excess potassium hydroxide and then further diluted to standard volume. An intense yellow color appears when the solution is made alkaline; it is presumably due to the ion of 2-nitrophenol-4,6-disulfonic acid. A Fischer electrophotometer was used with a double thickness blue filter for measurement of color intensity. At 50 ml. dilution amounts of nitrocellulose corresponding to $1–5 \times 10^{-5}$ g. nitrate nitrogen could be conveniently measured. The precision has been found to be good to about 1% under optimum conditions of operation.

Results and Discussion

Effect of Molecular Weight Variation on the Distribution Constant.—The distribution constants of nitrocelluloses of several different molecular weights have been determined in the system: the gel of cellulose triacetate equilibrated with co-solvents, methyl acetate and water. The polymers used had nitrogen contents substantially equal and were all prepared from cotton linters. Hence, differences shown should be due primarily to molecular weight variation. The data are shown in Fig. 1.

It should be borne in mind that the polymers studied were heterogeneous with respect to molecular weight so the distribution constants which have been measured and reported are average values. It can easily be shown that the particular average which applies to the experimental values is peculiarly weighed according to the weight fractions, x_i^g of the various components of the gel phase, namely

$$\bar{D} = \sum_i D_i x_i^g \qquad (1)$$

It follows that the experimentally determined distribution constant depends on the relative amounts of supernatant and gel phase present. Consequently, all of the distribution measurements, the results of which are here reported, were made on systems in which the ratio of supernatant to gel phase was the same, namely, 3:1.

(9) Snell and Snell, "Colorimetric Methods of Analysis," Vol. I, D. Van Nostrand Co., N. Y., 1936, p. 629. References are given and also examples of the application of the method.

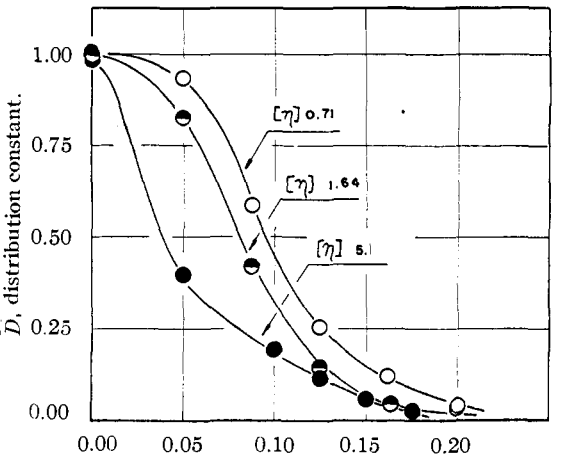

Fig. 1.—Distribution constants of nitrocelluloses of several different molecular weights in the two phase system: the gel of cellulose triacetate equilibrated with co-solvents methyl acetate and water: ○, NC 18, 10.96% N; ◒, NC 19, 10.93% N; ●, NC 17, 10.98% N.

From Fig. 1, we conclude that no one mixture of water and methyl acetate yields a system with which quantitative separations of nitrocelluloses of widely different molecular weight could be brought about conveniently. Rather, it is indicated that the practical procedure is to perform first a distribution in a system containing a relatively large amount of water in the solvent mixture, thereby removing in the supernatant phase primarily low molecular weight polymer. The volume fraction of the water may be progressively decreased in further distributions and fractions of successively higher molecular weight obtained until, finally, substantially all of the nitrocellulose has been recovered from the gel phase. In partition chromatography, analogously, one might use first a developer containing a high volume fraction of water and thereafter decrease the fraction of water gradually as the fractions of nitrocellulose of successively higher molecular weight are eluted.

Effect of Variation of the Nitrate Content of Nitrocellulose on the Distribution Constant.—Nitrocelluloses of significantly different degrees of nitration but in other respects quite similar have been studied to compare distribution constants. The results are shown in Fig. 2. It is evident that the effect of nitrate content is such as to make impossible molecular weight fractionations by partition methods of polymers which are not homogeneous with respect to degree of nitration.

The large increase in absorbability with nitrogen

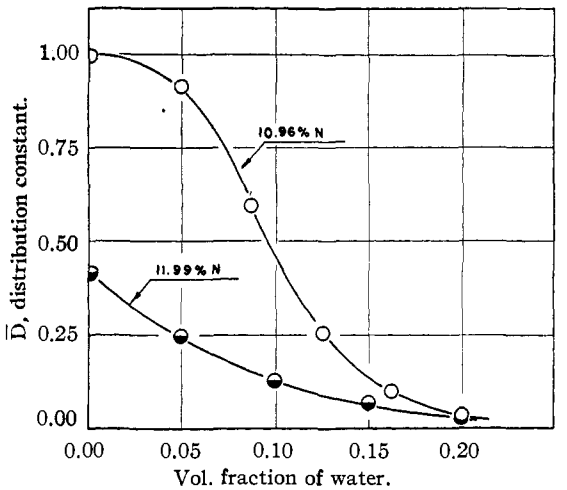

Fig. 2.—Distribution constants of nitrocelluloses of two different nitrogen contents in the two phase system: the gel of cellulose triacetate equilibrated with co-solvents methyl acetate and water: ○, NC 18, $[\eta] = 0.71$; ◒, NC 41, $[\eta] = 0.85$.

content shown in Fig. 2 suggests that nitrocelluloses of nitrogen content 12% or greater may well be so strongly absorbed from methyl acetate–water as to render fractionation impracticable with this system. As is shown below, the substitution of acetone for water causes the distribution to favor the supernatant phase to such an extent that 12–13% N nitrocellulose is readily extractable from the gel phase, but the dependence of the distribution constant on molecular weight has not yet been investigated in acetone–methyl acetate systems.

Nitrocelluloses having nitrogen contents of 13% or greater are not readily soluble in methyl acetate. Consequently, a system in which a solvent other than methyl acetate is the principal co-solvent should be sought if it is desired to fractionate very highly nitrated celluloses.

Co-solvents with Methyl Acetate Other than Water.—Several co-solvents have been tried with methyl acetate and the distributions of nitrocellulose between gel and supernatant phase which have been obtained show the systems to have promise for application to partition separations. A summary of the results using one nitrocellulose with the three different methyl acetate co-solvents: butyl acetate, ethanol, and water, is given in Fig. 3. The data have been reported as per cent. of the nitrocellulose apparently absorbed by the cellulose triacetate, since for the ethanol–methyl acetate and butyl acetate–methyl acetate systems the degree of swelling of the cellulose triacetate gel decreased significantly as the fraction of co-solvent was increased, and reliable estimates of the gel volume could not be made easily. A distribution constant of 1.00 corresponds to 0% adsorption.

Distribution experiments have also been made

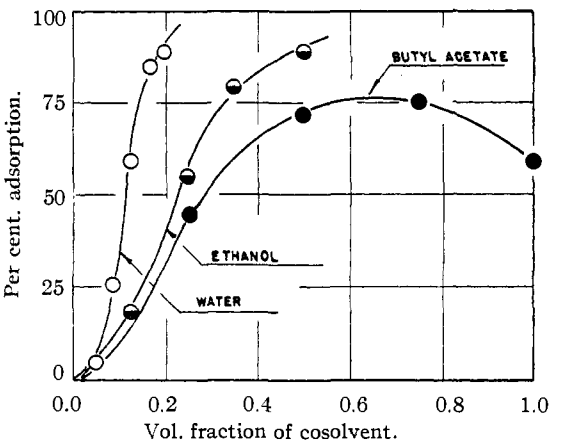

Fig. 3.—The apparent adsorptions of nitrocellulose, NC 19, by cellulose triacetate from various solvent mixtures of methyl acetate and co-solvent. In each run 0.50 g. of cellulose triacetate was equilibrated with 8 ml. of solvent mixture; co-solvents are indicated.

using benzene as a co-solvent. Analysis of nitrocellulose in the supernatant phase was complicated by the interference of benzene in the colorimetric test; hence, accurate quantitative measurements could not be made. Qualitatively, benzene has an effect equal to that of water in causing the nitrocellulose to concentrate in the gel phase.

Acetone, as a co-solvent with methyl acetate, has an effect opposite to those of the other co-solvents studied. Acetone causes the nitrocellulose to favor the supernatant phase to an extent greater than that when pure methyl acetate is the sole solvent. In Table I are given the distribution constants determined for different nitrocelluloses using several different volume fractions of acetone. The results are reported as distribution constants since the degree of swelling of cellulose triacetate has been observed to be approximately the same in methyl acetate–acetone mixtures as it is in pure methyl acetate.

TABLE I

DISTRIBUTION CONSTANTS

Description of nitrocellulose	Vol. fraction of acetone	Distribution constant
NC No. 19	0.125	2.23
$[\eta] = 1.64$	.0625	1.18
10.93% N	.00	1.00
NC No. 41	.15	1.44
$[\eta] = 0.85$	.10	0.74
11.99% N	.05	.63
	.00	.44

The Effect of Nitrocellulose Concentration on the Distribution Constant.—A sharp separation by partition chromatographic methods can be accomplished only if the distribution constants of the components to be separated are substantially independent of concentration throughout the ranges of concentration which exist during a column operation. Hence, it has

been of interest to determine the distribution constant as a function of concentration. The results indicate that approximately 10 mg. of nitrocellulose may be equilibrated with 1 g. of cellulose triacetate (dry weight) gel before a saturation effect becomes evident. In Table II are given the data determined in experiments in which 0.50 g. of cellulose triacetate was allowed to swell in 8.0 ml. of solvent: 0.9 ml. water, 7.1 ml. methyl acetate.

TABLE II

EFFECT OF NITROCELLULOSE CONCENTRATION ON THE DISTRIBUTION CONSTANT[a]

Mg. NC no. 19 per g. cellulose triacetate	Distribution constant
1.88	0.15
3.76	.16
5.64	.15
7.52	.16
10.28	.16
15.04	.21
19.05	.24
38.1	.42
76.2	.61

The distribution constants reported in Fig. 1 and Fig. 2 and Table I were determined from experiments in which less than the indicated saturation amount of nitrocellulose was used.

Rate of Equilibration of Distribution.—The rate of equilibration of nitrocellulose between gel and supernatant phases is limited by the rate of diffusion of nitrocellulose in the gel particles. The time of equilibration can be reduced by reduction of the size of the gel particles or an increase in the swelling factor of the gel (*e. g.*, to cut down the viscosity of the gel phase). The experiments, the results of which are reported in this paper, were all carried out using cellulose triacetate which had been passed through a 100-mesh screen. The screening was carried out with the object of securing uniformly small particles. However, periods of one day and in some cases, *viz.*, when very high viscosity nitrocellulose was used, longer periods were required to obtain substantially complete equilibration.

A slow rate of equilibration, as represented by these times, would constitute a serious limitation of the application of these distribution systems to a partition chromatographic operation. Fractions tend to become diffusely distributed in column operations under non-equilibrium conditions. Fortunately, a precipitation method has been found by which much smaller cellulose triacetate gel particles can be produced. The time for essentially complete equilibration has been reduced to a period of about two hours. It is intended that quantitative rate equilibration data will be reported in a subsequent paper when the method of preparation of cellulose triacetate gel suitable for column partition chromatographic operation has been standardized.

Summary

Several two-phase systems in which cellulose triacetate gel is equilibrated with mixtures of methyl acetate and co-solvent have been investigated with respect to the distribution of nitrocellulose between the phases. The distribution constants of nitrocellulose were found to be dependent both on the molecular weight and on the degree of nitration. The distribution constants were found to be independent of nitrocellulose concentration in the range 0–10 mg. of nitrocellulose per g. of cellulose triacetate. Rate of equilibration of nitrocellulose between gel and supernatant phases is apparently limited by diffusion within the gel particles; the rate was increased by reducing the size of the gel particles.

A system in which methyl acetate and water are used as co-solvents shows promise for application in a partition chromatographic fractionation of nitrocellulose.

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