Countercurrent Fractionation of Cellulose Acetate by Preferential Diffusion into Porous Charcoal

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A multi stage countercurrent apparatus is described with which porous charcoal is used as the stationary phase. The apparatus is used to fractionate secondary cellulose acetates with weight average molecular weights of ca. 35 000 and 60 000. Fifty fractions were isolated which increase in intrinsic viscosity with fraction number. The steady increase of intrinsic viscosity with fraction number appears to preclude effects from variations in acetyl substitution on the fractionation. Recoveries above 90 % representative of the entire sample, are demonstrated.

The effect of molecular weight, shaking time and concentration on the diffusion of cellulose acetate from acetone solutions into charcoal is illustrated by means of single batch experiments. A molecular weight gradient is demonstrated in the capillaries of the charcoal. Preliminary intrinsic viscosity weight distribution diagrams are presented.

A method for determining the intrinsic viscosity of cellulose acetate in acetone by single measurements is given. Graphs of $c[\eta]$ vs. t , where c is concentration, $[\eta]$ the intrinsic viscosity and t the efflux time of the solution in seconds, were obtained on several Ostwald pipettes¹¹ of low kinetic energy error. The graphs are based on Martins¹² equation $\log(\eta_{sp}/c) = \log[\eta] + K[\eta]c$, rearranged to the form $\eta_{sp} = c[\eta] \cdot 10^{Kc[\eta]} = \frac{t-t_0}{t_0}$ where t_0 is efflux time of the solvent. The K value was found to be 0.21 on secondary cellulose acetates ranging in intrinsic viscosity from 0.95 to 2.24, in concentrations up to 0.35 %.

Diffusion methods for the preparative fractionation of high molecular weight compounds have not been successfully used. Suitable membranes have not been found and when columns of porous media are used, selective migration is difficult to realize. As far as is known, capillary systems which take advantage of the variation in diffusion constant with molecular weight have not been employed for preparative polymer fractionation.

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Fig. 1. The uptake versus time of cellulose acetate samples of varying molecular weight by charcoal. Circles: sample 0.95, squares: sample 1.62, triangles: sample 2.24.

When charcoal has been used for the chromatography of cellulose derivatives¹⁻⁴ equilibrium conditions have been sought but were difficult to obtain although slow flow rates were used. Nonquantitative elution has been the main difficulty hindering the use of chromatography as a practical fractionation method for high polymers.

The effect of diffusion upon the chromatography of cellulose derivatives and other polymers with charcoal has been noted by Claesson⁵ who found that above a molecular weight of about 10 000, decreased amounts were taken up by the charcoal with increasing molecular weight, reflecting the decrease in diffusion constant.

Diffusion effects were not evident when starch was used by Brooks and Badger⁶ for separations of cellulose nitrate. The rapid equilibrium observed, the preferential uptake of the larger molecules and the marked dependence of the separation on the degree of nitrate substitution indicate that adsorption was the single affecting factor.

The fractionation of polymethyl-siloxanes by gradient elution from charcoal has recently been reported⁷. An increase in molecular weight with increasing eluent was found.

In the method described in this paper, the preferential diffusion of lower weight molecules into porous charcoal is used to effect cellulose acetate fractionation. Adsorption influences the separation by retaining the more deeply diffused material.

The effect of time and of varying molecular weights on the diffusion into charcoal

Three secondary cellulose acetate samples with intrinsic viscosities of 2.24, 1.62 and 0.95 were used which are identified in the following text by their intrinsic viscosity values. Information about the materials used and further experimental details are found in the Experimental part.

Solutions containing 300 mg of the 2.24, 1.62 and 0.95 samples in 15 ml acetone were shaken for 2, 4, 6 and 10 minutes with six grams of acetone-saturated charcoal.

After decantation the charcoal was first washed for 30 seconds and then for ten minutes successively with 25 ml portions of acetone. The total material recovered in this way was then subtracted from the original sample weight to obtain the uptake per gram charcoal. The results are shown in Fig. 1.

The rapidity of the diffusion into charcoal and the pronounced dependence of the uptake on molecular weight is seen. The decreased rate of uptake after about four minutes followed by a nearly linear uptake by the charcoal during extended shaking periods is generally found in all experiments.

The preferential removal of lower weight molecules by charcoal

Solutions of the three samples of cellulose acetate, each in 25 ml acetone, 750 mg in four cases and 500 mg in another, were shaken for five minutes with ten grams of acetone saturated charcoal. After decanting the original solution the charcoal was shaken with successive 25 ml portions of acetone for five minutes until measurable acetate was no longer found. The charcoal was then shaken further with successive 25 ml volumes of dioxan. The amounts and intrinsic viscosities of the acetate first decanted and of that obtained from each of the successive washings and elutions were then measured.

The analytical data, given in Table 1, show the removal of successively lower molecular weight portions of the sample distribution by the repeated washings, evidence of a molecular weight gradient in the capillaries of the charcoal, the smallest molecules having penetrated most deeply. The intrinsic viscosities of the material obtained after prolonged shaking with dioxan evidently represent an average of the cellulose acetate remaining in the charcoal after no more material could be removed by the acetone washings.

That a gradient exists as well in the lower molecular weight material left in the charcoal after the acetone washings, is indicated in one column in Table 1 where the charcoal was shaken with dioxan for successive five minute periods.

The intrinsic viscosity weight average of the recovered fractions is lower than that of the original sample. This appeared to be due to analytical difficulties rather than to the holdup of high molecular weight material.

The effect of prolonged shakings and varying concentrations of cellulose acetate on the uptake

The results are shown in Table 2 which gives analyses of the solutions decanted after shaking solutions of sample 1.62 with the charcoal. Increased uptake with increased concentration is found as predicted by diffusion theory.

The increased uptake with time is reflected by the increasing intrinsic viscosity values.

THE SOLID PHASE COUNTERCURRENT APPARATUS

Although a machine was not developed for its use, Craig and co-workers⁸ suggested that a solid could be used in countercurrent separations. To con-

Table 1. The effect of successive washings of the charcoal with solvent and eluent after shaking cellulose acetate solutions with charcoal for five minutes. a: original sample in 25 ml acetone. Acetone washes five minutes, dioxan washes two hours or more except where superscript b indicates five minutes shaking.

	$[\eta]$	Found mg	$[\eta]$	Found mg	$[\eta]$	Found mg	$[\eta]$	Found mg	$[\eta]$	Found mg
Original sample	0.95	750 ^a	1.62	750 ^a	1.62	750 ^a	2.24	500 ^a	2.24	750 ^a
First decantation	0.96	375	1.61	344	1.64	452	2.33	218	2.27	355
Acetone washes:										
1.	0.98	132	1.52	203	1.65	157	2.30	149	2.44	168
2.	0.94	29	1.54	42	1.65	33	2.12	26	2.42	39
3.	0.76	20	1.31	21	1.48	9		10	2.42	23
Remaining in charcoal		194		140		99		97		165
Dioxane elutions:										
1.	0.48	91	0.86	65	0.69 ^b	43 ^b	1.30	44	1.48	52
2.	0.60	29	0.85	21	0.64 ^b	22 ^b	1.21	17	1.29	23
3.	0.48	10	0.78	11	0.53 ^b	8 ^b	1.20	6	1.63	9
4.					1.09	21				
Remaining in charcoal		64		43		5		30		81
Intrinsic viscosity weight average of recovered fractions										
	0.86	686	1.52	707	1.53	745	2.15	470	2.22	669

struct an apparatus in which a solid is used as the stationary phase, modifications of the Craig⁹ apparatus design were made.

A fifty tube apparatus, blown as a single piece, is seen in Fig. 2. Since decanting rather than separation at the contact level of two liquid phases is involved, and maximum capacity is desirable, the outlet is placed as high as possible on the mixing tube. The transfer reservoirs, best seen in the closeup

Table 2. Effect of concentration and prolonged shaking times on the uptake of cellulose acetate 1.62 and on the intrinsic viscosity of the decanted solutions. Four grams charcoal shaken with each solution. a: 600 mg and b: 135 mg present in 20, 30 and 45 ml acetone, respectively.

% Conc.	Uptake by charcoal mg/g					Intrinsic viscosity				
	Shaking time, minutes					Shaking time, minutes				
	4	15	30	60	600	4	15	30	60	600
3.0 ^a	32.5	36.2	40.0	43.0	48.5	1.71	1.71	1.75	1.75	1.85
2.0 ^a	20.0	24.5	27.3	33.2	35.5	1.67	1.71	1.76	1.79	1.83
1.33 ^a	15.0	16.3	17.3	21.8	25.0	1.67	1.68	1.68	1.77	1.78
0.68 ^b	13.3	10.0	13.3	16.7	18.0	1.85	2.11	1.95	1.86	2.15
0.45 ^b	11.2	12.8	10.0	14.5	16.8	1.82	1.85	1.80	1.99	2.10
0.30 ^b	8.8	11.0	11.3	11.5	17.0	1.83		1.88	1.94	2.05

Fig. 2. The solid phase countercurrent apparatus.

in Fig. 3, have a powder puff shape, which provides good capacity as well as compactness. The volume of the mixing tubes from bottom to outlet, is 50 ml and that of the transfer reservoirs 65 ml.

The reversing motor used for shaking, seen in Fig. 2, has a speed of 4.5 revs. per minute. The extent of tipping is controlled by a rod extending from

Fig. 3. Closeup of apparatus. Solution transfer is from right to left.

Fig. 4. Intrinsic viscosity versus fraction number. Triangles: five tube run with acetate sample 1.62. Squares: every second fraction from fifty tube run with sample 1.62. Circles: every second fraction from fifty tube run with sample 0.95. The solid symbols indicate the decanted acetone fractions.

the motor shaft to adjustable micro switches. Effective, though gentle shaking is obtained by this arrangement. The shaking time is controlled by a timer wired into the relay. Decanting, transfer and additions of solvent are done by hand.

Five tube countercurrent run

Five grams of charcoal, put into each of the first five tubes of the apparatus, were washed twice with acetone. Fifty ml of acetone containing 2.5 g of the 1.62 cellulose acetate was introduced into the first tube. After four minutes shaking and decantation, 25 ml acetone was added from the first reservoir to the first tube, while the decanted solution automatically transferred to the second tube. Acetone was added in this way four times following each four minute shaking period. At the end of twenty minutes the remainder of the original solution was thus decanted from the fifth mixing tube. Elution was done by shaking the charcoal with 25, 15 and 10 ml volumes successively, of dioxan, for one hour or more. Ten fractions resulted; five decanted to the reservoirs and five obtained by the subsequent elution of the charcoal. For clarity "decanted fractions" will refer to the solutions decanted before the dioxan elution.

Concentrations were determined on all the fractions and intrinsic viscosities on the first elution and on the decanted fractions. 97 % of the acetate was recovered, 20 % by elution from the charcoal.

The degree of separation obtained is shown by the intrinsic viscosity values in Fig. 4. The intrinsic viscosity range, from 0.9 to 1.8, after the short run, illustrates how rapidly the separation is initiated.

Fig. 5. Fraction weight versus tube number. Upper: from fifty tube run with cellulose acetate sample 1.62. Lower: from fifty tube run with sample 0.95. The solid symbols indicate the decanted acetone fractions.

Fifty tube countercurrent run with low molecular weight cellulose acetate

Nine grams of charcoal used per tube was first washed with acetone. Five grams of the 0.95 acetate sample in 70 ml acetone was introduced in two 35 ml portions to the first two tubes as the high viscosity of the full charge when used in the limited volume available in the one tube resulted in poor mixing. The cellulose acetate solution was followed by 25 ml additions of acetone to the first charcoal stage. Five minute shaking periods were used between the successive additions of solvent.

Elution was first done by shaking the charcoal successively with 25 and 15 ml dioxan for two hours or more. 15 % of the original material was in the decanted fractions which, with the eluted material from the two shakings with dioxan, accounted for 66 % of the original sample. The intrinsic viscosities, determined on every second fraction, are shown in Fig. 4.

A third elution was done with 25 ml dioxan. When this material was added to that already obtained, 92 % had been recovered. The viscosities determined on combined, contiguous pairs from this final elution are indicated by crosses on the curve in Fig. 4. They coincide well with the viscosity curve resulting from the previous elutions, showing that after the prolonged shaking with dioxan no molecular weight gradient has been detected. It was also found that the last elution removed nearly like proportions of material regardless of DP, attesting to the effectiveness of the elution.

The weight of each fraction is shown in the lower portion of Fig. 5. It can be seen from the figure that the higher viscosity decanted material

Fig. 6. The intrinsic viscosity weight distribution of the fractions from fifty tube run with acetate sample 0.95.

appears at tube 25, while beyond tube 40, extending to tube 50, acetate is found neither in the charcoal nor in the decanted acetone, illustrating the countercurrent effect.

The intrinsic viscosity weight distribution is given in Fig. 6. It is based on 100 % recovery, assuming that the material eluted was representative of that remaining in the charcoal. To prepare the diagram, the fraction weights were used directly. The intrinsic viscosity values however, were taken from a curve drawn by eye through the viscosity points in Fig. 4. The weight average intrinsic viscosity is the same as that of the original sample, confirming the reliability of the viscosity method used and the effectiveness of the recovery.

Countercurrent run with higher DP cellulose acetate

5 g of the 1.62 acetate in 70 ml acetone were introduced to the first two tubes of the apparatus. Other conditions were the same as those used with the lower DP sample.

25 % was found in the decanted fractions in comparison to 15 % when the lower DP acetate was run under like conditions. When this material was added to that obtained in the elutions with dioxan, 60 % was recovered. No further elution was done. The intrinsic viscosity of each second fraction is shown in Fig. 4 and the amounts recovered, in Fig. 5.

The intrinsic viscosity weight distribution, shown in Fig. 7 is based on 100 % recovery. The intrinsic viscosity weight average, 1.53, is lower than that of the original sample. This may be due to unrepresentative elution or analytical errors.

Fig. 7. The intrinsic viscosity weight distribution of the fractions from fifty tube run with acetate sample 1.62.

EXPERIMENTAL

Materials. The cellulose acetates are Hercules Powder Company products. The samples which have intrinsic viscosities of 2.24, 1.62 and 0.95 — weight average molecular weights of ca. 83 000, 60 000 and 35 000 — are substituted to the extent of 2.45, 2.39 and 2.48, respectively.

The charcoal, a Norit Company product, is a rather soft, activated carbon with a particle diameter of 0.7 mm. Acetone of analytical purity was used throughout. The dioxan used had 99 % purity and was used without further treatment.

Experimental details. To wash the charcoal, 25 ml portions of acetone were added simultaneously from the reservoirs and shaken with the charcoal for fifteen minutes. This was done twice. In addition to removing excessive charcoal fines, the acetone remaining on the charcoal after washing, served to dilute the cellulose acetate solution making it possible to transfer the original charge through the apparatus. Similar diffusion effects were found whether the cellulose acetate solution was shaken with dry charcoal or with acetone-saturated charcoal.

After the run the decanted acetone fractions in the reservoirs were removed with a syringe and centrifuged to remove charcoal fines. To determine concentrations, aliquots were precipitated in water on a steam bath in small, thin drying phials. The phials were hung in a vertical rack in an electrically heated drying column¹⁰ under vacuum for twenty minutes. During the last ten minutes of this period 105–108° C was maintained. Viscosities were determined directly on the decanted acetone fractions after adjustment of concentrations.

For elution, the dioxan was added simultaneously to the charcoal from all the reservoirs and generally shaken two hours or more. Aliquots of the dioxan solutions were precipitated and dried in the same manner as the acetone fractions. The dried samples were dispersed in acetone for the viscosity determination.

Viscosity Measurements. Intrinsic viscosity analyses are laborious and since small quantities were analyzed, measurements at several concentrations would have been very difficult. In consequence means were sought to obtain the value from one efflux time at any single concentration in a low concentration range.

Ostwald pipettes¹¹ recommended for a range between 0.3 and 5.0 centipoises were used at 25° C ± 0.02. 2 ml solution was required. Conventional semi-log plots of spec. viscosity / conc. *vs.* conc. were made on several secondary cellulose acetates and on mixtures of these.

The finding of Martin¹² that the slopes obtained have a linear relation to their limiting viscosity values at zero concentrations was confirmed in concentrations up to 0.35 %

on samples ranging in intrinsic viscosity between 0.95 and 2.24. The low kinetic energy error of the pipettes was found to be a necessary condition in determining the relation.

A K value of 0.21 was obtained by plotting the slopes against the corresponding intrinsic viscosities. This was then used in Martins expression

$$\log \left(\frac{\eta_{sp}}{c} \right) = \log [\eta] + K [\eta] \cdot c$$

rearranged to the form

$$\eta_{sp} = c \cdot [\eta] \cdot 10^{Kc [\eta]} = \frac{t - t_0}{t_0}$$

where t , is efflux time of the solution, t_0 , that of the solvent, c , the concentration and $[\eta]$, the intrinsic viscosity.

By using the equation in this form, graphs of $c \cdot [\eta]$ vs. t were obtained for several pipettes. One efflux time in the appropriate concentration range is thus sufficient. The $c \cdot [\eta]$ value found on the other axis need only be read off and divided by the concentration used.

DISCUSSION

An important aspect of the countercurrent method is the ease with which larger capacities and quantities can be employed. The rapidity of the fractionation is an obvious advantage.

It appears that a single batch separation method could be refined and used to partially characterize the distribution. It is also a convenient means to obtain a preliminary partition of the sample which can then be followed by the countercurrent fractionation of the upper or lower molecular weight segment.

In the countercurrent runs, the smaller molecules, continually pressing into the capillaries of the charcoal at each stage of the moving system, had an opportunity to be adsorbed. The decanted fractions of higher DP, were selectively refused by the charcoal stages, resulting in the progressively increasing intrinsic viscosity of the decanted, as well as the eluted, fractions.

When extended shaking times were used in a run with acetate 1.62, a small percentage of the sample, above viscosity 2.5, failed to diffuse into the charcoal. There was also some evidence that representative elution was more difficult after the extended contact between the charcoal and the cellulose acetate solution.

The steady increase of intrinsic viscosity with fraction number seems to preclude much effect from variations in the degree of substitution on the fractionation. Acetyl analyses are planned on the fractions.

The distributions obtained are asymmetrical with positive skewness conforming with the preponderance of theoretical and experimental evidence^{13,14}. The presence of more than one maximum in the weight distribution diagrams is believed to have resulted from the experimental conditions. Homogeneity measurements of the fractions are planned.

It appears likely that other polymers may be separated with the demonstrated countercurrent method and such experiments are in progress.

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