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Chemical characterization of cellulose acetate by non-exclusion liquid chromatography

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ABSTRACT

The physical properties of cellulose acetate polymers are not governed solely by their average chemical properties, but are also dependent upon molecular mass and acetyl distributions. Currently, most analytical techniques that are routinely used to characterize cellulose esters, provide only a mean value of the property that is measured. Non-exclusion liquid chromatography was investigated as a means of analyzing cellulose acetate polymers over a compositional range from 37–43% (w/w) acetyl. Under optimum conditions this technique was highly selective for the chemical composition of these polymers, providing information on both mean acetyl composition and acetyl distribution. The purpose of this paper is to describe chromatographic variables required to successfully characterize cellulose acetate.

INTRODUCTION

Because heterogeneous polymers (or heteropolymers) possess both molecular mass and chemical composition distributions, they are a difficult class of polymers to characterize. To measure any distribution of a polymer, *e.g.*, molecular mass or chemical composition, requires the implementation of some separation method. When measuring a single distribution of a heteropolymer, a separation is required which either is dependent predominantly upon this one distribution variable or provides a means of readily extracting from the raw data information on this variable.

Gel permeation chromatography (GPC) is the most common separation method used to characterize the molecular mass distribution of homopolymers. The application of GPC for heteropolymers has been limited since a polymer's molecular volume is dependent on both molecular mass and chemical composition. Because of this shortcoming, alternative separation techniques, such as non-exclusion liquid chromatography separations, have become increasingly invaluable when characterizing heteropolymers.

To date, several polymers have been analyzed by non-exclusion liquid chromatography. They include poly(styrene methacrylate) [1–3], poly(styrene acrylate) [4,5], poly(styrene acrylonitrile) [6,7], and poly(styrene–vinyl acetate) [8]. Specific separation factors should be known when assessing the usefulness of liquid chromatography for heteropolymer characterization. Polymer separations routinely require mobile phase gradients. Changes in the mobile phase may alter the underlying retention mechanism for a polymer. This is most pronounced when polymer solubility is effected, since it will influence the predominance of either an adsorption or a phase separation mechanism [9]. Mobile phase and stationary phase conditions will also affect the dependence of elution upon either the polymer's molecular mass and/or chemical composition [10–12].

In this work liquid chromatography was used to determine the acetyl composition of cellulose acetate

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polymers. This was performed on a conventional high-performance liquid chromatograph using reversed-phase separation conditions. The eluted polymer was detected using an evaporative light scattering detector. As opposed to previous chromatographic work on cellulose acetate, neither prederivatization or off-line detection was necessary using this unique detector [12–17]. The observed separation of cellulose acetate was strongly dependent upon mass percent acetyl composition. Under reversed-phase HPLC conditions cellulose acetate was retained on the column primarily due to adsorption.

In the context of this paper acetyl distribution or "acetyl spread" is defined as both the distribution of acetyl groups along the cellulose backbone and the distribution due to intermolecular mixing of dissimilar cellulose acetate molecules. Cellulose ester chemists have theorized that many physical properties of cellulose acetate may be attributed to its acetyl distribution. This theory has been difficult to validate since all historical analytical methods have relied on tedious and irreproducible separation techniques. It is agreed that much is to be gained in the development of an accurate and automated means of characterizing the chemical composition of this polymer.

CELLULOSE ACETATE FRACTIONATION

Cellulose esters form a unique class of synthetic polymers. Unlike either polyolefins or polyesters, cellulose esters are synthesized from a natural polymer. Cellulose acetate is synthesized by the complete acetylation of cellulose followed by a controlled back hydrolysis to a desired degree of acetyl substitution. As shown in Fig. 1, acetylation can take place along the cellulose chain at three available hydroxyl groups per glucose monomer. Cellulose acetate can have an acetyl content from >0% to 44.8% (w/w). As the acetyl content of cellulose acetate is increased, it changes from a hydrophilic to a hydrophobic polymer. Completely acetylated cellulose is referred to as cellulose triacetate. Polymer solubility is highly dependent on both the degree of acetyl substitution (DS) and degree of polymerization (DP) [10,18–26]. From a manufacturing standpoint, the extent and consistency of both composition and molecular mass are important

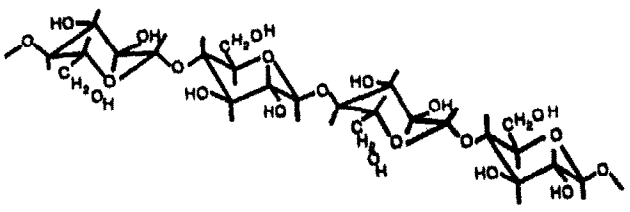

Fig. 1. Structure of α-cellulose.

variables that should be measured when controlling cellulose acetate reactions.

Four primary techniques historically used in the separation of cellulose acetate have been solutional fractionation, precipitative fractionation, thin-layer chromatography, and column chromatography. Solutional fractionation involves taking a solid polymer and extracting the polymer with a successively stronger solvent. The solvent is chosen so that it dissolves a select fraction of the polymer. Precipitative fractionation involves dissolving a polymer in a good solvent, and then decreasing the solubility and precipitating out a desired polymer fraction by either successively adding a controlled volume of poor solvent or changing the temperature. Comparisons between these two bulk separation techniques, used for cellulose ester fractionation, have been previously reported [18,19]. The terms "good" solvent and "poor" solvent will be used throughout this paper to describe the strength of solvent for a polymer in relation to either solubility or chromatographic elution.

The first reported fractionation of cellulose acetate was by Mardles in 1923 [20]. Fractionation was performed by precipitation with acetone and water as the respective "good" and "poor" solvent pair. The fractionation order was strongly dependent upon molecular mass. The effect of temperature on solutional fractionation of cellulose diacetate was studied by Sookne and co-workers [21,22] and by Morey and Tamblyn [23]. As expected, solubility increased for a given solvent fraction as temperature was increased. Fractionation was found to proceed primarily as a function of molecular mass.

Howlett and Urquhart [24] performed an extensive study on solvent–non-solvent pairs in fractionating cellulose diacetate. While performing precipitative fractionation they found that it was desirable

to use a pair of solvents where the change in polymer solubility was small relative to non-solvent addition. Rosenthal and White [10] were the first to use solubility parameter information in choosing "good"/"poor" solvent pairs in the precipitative fractionation of cellulose acetate. They demonstrated that the choice of "poor" solvent can influence the dependence of intrinsic viscosity and weight percent acetyl on fractionation.

Most polymers exhibit a low and high critical solution temperature, the points where precipitation begins. Cowie and co-workers [25,26] determined that the upper critical solution temperature for cellulose acetate increased as a function of acetyl content. Kamide and co-workers [13,27] evaluated the use of thin-layer chromatography for the fractionation of cellulose acetate. Silica gel was used as the stationary phase. A binary mobile phase solvent system of methanol–methylene chloride was used. The migration of cellulose acetate polymers of varying acetyl composition ranging from 39% to 44% acetyl were influenced by changes in the mobile phase volume ratio. Under these conditions polymer migration was found to be independent of molecular mass. A binary solvent system of methylene chloride–butanol was also useful in fractionating by composition. Interesting enough, while both of these binary mobile phases fractionated by composition, the fraction of polymer that migrated (*i.e.*, either high or low acetyl fraction) was different. Apparently this difference resulted from two opposing separation mechanisms; either phase separation or adsorption. This was controlled by the alcohol–methylene chloride volume ratio.

Mark and Saito [12] were the first to use a chromatographic support to separate cellulose acetate. A number of supports, including calcium carbonate, alumina, starch and carbon, were investigated. Polymer adsorption was studied using both acetone and dioxane. In all cases the fraction of adsorbed polymer was less when dioxane was used instead of acetone. Tarakanov and Okunev [14,15] used column chromatography for the fractionation of cellulose triacetate. Using a binary mobile phase gradient of methylene chloride and heptane, the separation was shown to be directly dependent upon polymer viscosity.

Tung [16] used column chromatography to fractionate cellulose esters. Both solvent and thermal gradient conditions were investigated. This work focused on fractionating various cellulose esters based upon molecular mass. Whether the separation mechanism was due either to phase-separation (precipitation) or adsorption determined the significance of the stationary phase in the separation process.

EXPERIMENTAL

Liquid chromatography was performed on a Perkin-Elmer (Norwalk, CT, USA) Model 410 gradient pumping system with a Perkin-Elmer LC-600 autosampler and a 20-μl injection loop. All exploratory separation experiments were done at ambient temperature, nominally 25°C. Statistical and long-term precision studies were performed using a Waters TCM column heater set at 30°C for cellulose diacetates. Data storage and analysis were performed using the PE/Nelson Access-Chrom software, version 1.6, on a 8600 VAX mainframe computer. The statistical plates programme was used to determine peak controid (M1) and peak variance (M2). The Foley–Dorsey plates programme was also used to generate width at one tenth height and number of theoretical plates (N). Column dead volume was estimated by injecting a 500 mg/l solution of acetone, while detecting the eluted peak with a Waters Model 481 variable-wavelength detector set at 300 nm. Gradient delay volume was estimated by tracking the rising baseline of a binary gradient using acetone in the "B" solvent reservoir.

Several HPLC columns were evaluated during this study. These columns are listed in Table I. Spherisorb ODS2, Spherisorb Si, and Spherisorb Al were purchased from Keystone Scientific (Bellefonte, PA, USA). Hamilton PRP-1 column was purchased from Hamilton (Reno, NV, USA) and the Polymer Lab. PLRP-S columns were purchased from Polymer Lab. (Amherst, MA, USA) (all of these columns had a diameter of 4.6 mm). Methanol, *n*-propanol, ethanol, tetrahydrofuran and acetone were purchased from Burdick & Jackson (Muskegon, MI, USA). Distilled/de-ionized water was produced in-house.

A Varex Mark II evaporative light scattering detector was used to detect the cellulose acetate polymer. The detector settings were as follows: attenuation range, 5; evaporator tube temperature, 105°C;

TABLE I

HPLC COLUMNS USED IN COMPOSITIONAL ANALYSIS OF CELLULOSE ACETATE

Column type	Particle size (μm)	Mean pore diameter (nm)	Column length (cm)
Spherisorb Si	5	8	15
Spherisorb Al	5	13	15
Spherisorb ODS2	5	8	15
Hamilton PRP-1	10	10	15
Polymer Lab. PLRP-S	10	10	15
Polymer Lab. PLRP-S	10	10	5
Polymer Lab. PLRP-S	10	30	5
Polymer Lab. PLRP-S	10	100	5

nitrogen pressure, 20 p.s.i. (1 p.s.i. = 6894.76 Pa); time constant, 5 s.

Mean acetyl composition of cellulose acetate was determined either by saponification followed by an acid titration or by ^{1}H NMR. Inherent viscosity was determined using acetone. These analytical methods are described in detail elsewhere [28,29].

GPC was performed with both a 100 Å and a mixed-bed columns in series. These columns were purchased from Polymer Lab. Tetrahydrofuran was used as the mobile phase when performing GPC on cellulose diacetate polymers (<42.5%Ac). Polymer samples were made up in mobile phase at a concentration of 1000 mg/l. Polystyrene standards were used to calibrate for number average and mass average molecular mass. Analytical information for all cellulose acetate polymers studied during this work are listed in Table II. When performing non-exclusion liquid chromatography, the cellulose diacetate (samples A–K in Table II) polymer solutions were made up to a concentration of approximately 2000 mg/l using acetone. All cellulose acetate samples were supplied by Tennessee Eastman (Kingsport, TN, USA).

RESULTS AND DISCUSSION

Chromatographic conditions used in the analysis of cellulose acetate

Cellulose acetate polymers were separated by reversed-phase liquid chromatography. Chromatograms of a series of cellulose acetate polymers, ranging in acetyl substitution (or mean mass percent acetyl) from 38.3% to 42.6%, are shown in Fig. 2. As the degree of acetyl substitution increased, the samples elution time increased. These polymers were chromatographed on a Hamilton PRP-1 (150 × 4.6 mm, 10 μm) column under a mobile phase condition of acetone–water–methanol (4:3:1) to 100% acetone in 15 min at a flow-rate of 0.8 ml/min. A strong linear correlation existed between mean mass percent acetyl and the polymer elution time. This is shown in Fig. 3. The slope, intercept, and correlation coefficient were determined to be 2.01, 21.0 and 0.993, respectively, over a acetyl range from 37.9% to 43.5%. In generating the retention time data peak centroid, the first statistical moment, was chosen instead of peak maximum. This was used because the eluting polymer peak would rarely conformed to a gaussian profile and because there was no reason to assume *a priori* that the polymer peak would be gaussian.

TABLE II

CELLULOSE ACETATE ANALYTICAL DATA

M_n = Number-average molecular mass; M_w = mass-average molecular mass.

Sample	Acetyl (%, w/w)	Inherent viscosity (dl/g)	M_n	M_w
A	37.9	1.34	64 000	130 000
B	38.3	1.26	52 000	129 000
C	38.8	1.30	53 000	139 000
D	39.3	1.43	58 000	173 000
E	39.9	1.30	69 000	158 000
F	40.3	1.35	51 000	165 000
G	41.0	1.32	78 000	170 000
H	39.8	1.05	52 000	111 000
I	39.9	1.37	72 000	164 000
J	41.5	1.20		
K	42.6	1.09	52 000	106 000

During development of this HPLC method a number of solvent conditions were evaluated. Factors such as detector response, polymer solubility and column back pressure restricted the number of potential mobile phase choices. Since this analysis required a mobile phase gradient, both "good" and "poor" solvents were required [30]. In the past,

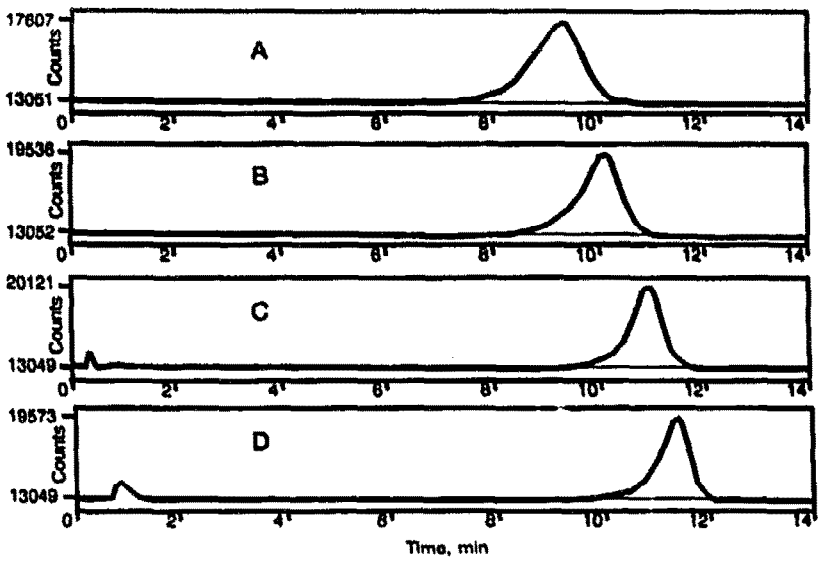

Fig. 2. Superimposed chromatograms of four cellulose acetate samples; A = 37.9%, B = 39.9%, C = 41.5% and D = 42.6% (w/w) acetyl. A 15-min linear gradient from acetone–water–methanol (4:3:1) to 100% acetone at a flow-rate of 0.8 ml/min was used.

"good" solvents that have been used for solubilizing cellulose acetate have included; acetone, tetrahydrofuran, ethyl acetate, dimethyl sulphoxide, and a binary solvent system of methylene chloride–methanol. Dimethyl sulphoxide was eliminated due to its high viscosity, which would have created problems both due to high back pressure and high detector background noise [31]. In this study ethyl acetate and methylene chloride–methanol were rejected due to their immiscibility in water. Acetone and tetrahydrofuran were the only "good" solvents that fulfilled all initial requirements.

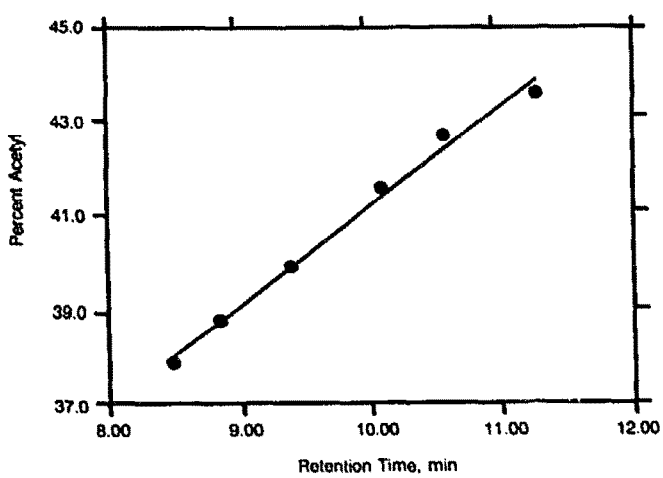

Fig. 3. Graph of retention time in minutes *versus* mass percent acetyl. Linear least squares fit provided a slope of 2.01, an intercept of 21.0 and a correlation coefficient of 0.993.

In choosing the most appropriate "poor" solvent, several conditions were also required. First, the initial mobile phase should not precipitate the polymer at the head of the column. Second, the initial solvent should not allow the polymer to be excluded through the HPLC packing. Third, the initial solvent should not produce an excessively high back pressure on the system. Methanol and water, as well as binary solvents of methanol–water, methanol–acetone, water–acetone and water–tetrahydrofuran failed at least one of these initial three criteria. Ternary solvent mixtures were then investigated as a possible "poor" mobile phase. A number of suitable solvent combinations were ultimately identified. They are listed in Table III.

These five solvent systems were further evaluated. Chromatographic selectivity toward acetyl composition relative to molecular mass was chosen as the final criteria. This was measured using three different cellulose acetate polymers. Both mass percent acetyl (%Ac) and inherent viscosity (IV) are listed in Table I for these three polymers. One sample pair had a comparable acetyl substitution (39.9% *vs.*

TABLE III

EFFECT OF MOBILE PHASE ON CHROMATOGRAPHIC SELECTIVITY OF CELLULOSE ACETATES

%Ac = Acetyl (%, w/w); IV = inherent viscosity.

No.	Mobile phase	Retention time (min)			Δ%Ac I – A	ΔIV I – H	|Δ%Ac/ΔIV|
		Sample I	Sample A	Sample H			
1	Water–methanol–tetrahydrofuran (2:1:1) to 100% tetrahydrofuran	9.67	9.01	9.82	0.66	−0.15	4.4
2	Water–ethanol–tetrahydrofuran (2:1:1) to 100% tetrahydrofuran	8.81	8.46	8.94	0.35	−0.13	2.7
3	Water–isopropanol–tetrahydrofuran (2:1:1) to 100% tetrahydrofuran	8.95	7.08	9.41	1.87	−0.46	4.1
4	Acetone–water–methanol (4:3:1) to 100% acetone	9.40	8.48	9.31	0.92	0.09	10.2
5	Acetone–water–methanol (3:4:1) to 100% acetone	12.22	11.41	12.34	0.93	−0.12	7.8

39.8%Ac) but a different IV (1.37 *vs.* 1.05). The second pair had a comparable IV (1.34 *vs.* 1.37), but a different acetyl substitution (37.9% *vs.* 39.9%Ac). In evaluating different mobile phase conditions a relative selectivity index was devised by determining the absolute ratio of the change in retention due to %Ac at a constant IV, relative to the change in retention time due to the change in IV at a constant %Ac. This has been abbreviated as $|\Delta\%Ac/\Delta IV|$ and is listed in Table III for the five different mobile phase conditions.

Solvent systems 1–3 used tetrahydrofuran as the "good" solvent. The "poor" solvent in these first three mobile phase systems consisted of water–alcohol–tetrahydrofuran (2:1:1, v/v/v) where the alcohol was either methanol, ethanol or isopropanol. No advantage in selectivity was observed in replacing methanol with the other two alcohols. Substituting acetone instead of tetrahydrofuran in solvent systems 4 and 5 produced a significant improvement in compositional selectivity. Weakening the "poor" solvent's composition from 4:3:1 to 3:4:1 acetone–water–methanol did not improve acetyl selectivity. Conversely when the volume fraction of water was decreased further, partial exclusion of the lower acetyl (*i.e.*, <38%Ac) polymeric fraction occurred.

Five HPLC columns were compared using the same three cellulose acetate samples previously discussed. Initially, care was taken to choose stationary phases of narrow pore diameter. This was done

TABLE IV

EFFECT OF COLUMN TYPE ON CHROMATOGRAPHIC SELECTIVITY OF CELLULOSE ACETATES

No.	Column type	Retention time (min)			Δ%Ac I – A	ΔIV I – H	|Δ%Ac/ΔIV|
		Sample I	Sample A	Sample H			
1	Polymer Lab.	11.76	10.93	11.89	0.83	−0.13	6.4
2	Spherisorb ODS2	11.16	10.33	11.29	0.83	−0.13	6.4
3	Hamilton PRP-1	12.22	11.41	12.34	0.93	−0.12	7.8
4	Spherisorb Al	11.98	NR	12.79	–	−0.81	–
5	Spherisorb Si	NR[a]	NR	NR	–	–	–

[a] NR = polymer not retained.

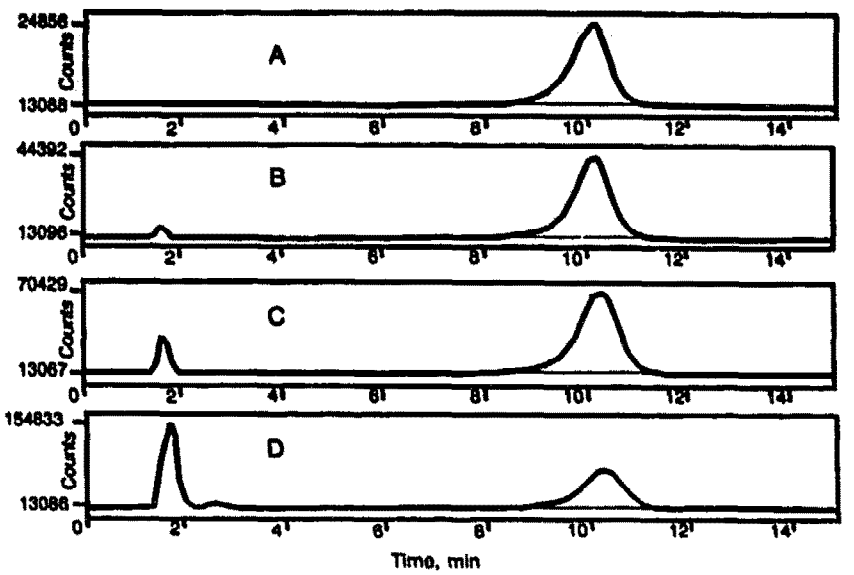

Fig. 4. Effect of injection volume on polymer exclusion. A single polymer (39.9%Ac) was injected using three different injection volumes: A = 20-, B = 50-, C = 100- and D = 200-μl loops. Chromatographic conditions as in Fig. 2.

in order to minimize molecular mass discrimination due to preferential migration of lower-molecular-mass subpopulations into more accessible pores. The column types, particle sizes, and pore sizes for these four columns are listed in Table I. A mobile phase gradient of acetone–water–methanol (3:4:1) to 100% acetone in 15 min at 0.8 ml/min was used for this study. The results from this study are shown in Table IV. No difference in the $|\Delta\%Ac/\Delta IV|$ selectivity between the Polymer Lab. PLRP-S (styrene–divinylbenzene based) and Spherisorb ODS2 (octadecylsilane bonded silica gel) columns was observed. The selectivity index was slightly better using the Hamilton PRP-1 column (styrene–divinylbenzene based). Cellulose acetate was retained longer on the polystyrene–divinylbenzene based supports. The Spherisorb Al (alumina) column provided no retention (NR) for the 37.9%Ac/1.37 IV cellulose acetate polymer. At a constant acetyl substitution, the Spherisorb Al column had a significantly longer retention for the lower IV polymer. Presently no reasons are known to explain this observed selectivity profile. The Spherisorb Si (silica gel) column failed to retain any of the cellulose acetate samples. This result was consistent with earlier work reported by Mark and Saito [12].

The effects of both injection volume and polymer concentration were studied. Previous work had shown that polymer exclusion will occur at high injection volumes [2]. This effect was studied in order to avoid injection discrimination. Fig. 4 illustrates the effect of injection volume on polymer exclusion. At both 100 and 200 μl, a large fraction of the cellulose acetate sample was excluded from the column. When collected this excluded polymer was found to have the same acetyl composition and inherent viscosity of the non-excluded polymer fraction. Lochmüller *et al.* [32] have shown under isocratic conditions that polymer exclusion can be influenced by the strength of the injection solvent. This has been shown to be due to poor equilibrium of the polymer with the mobile phase prior to introduction onto the column. This effect is dependent upon injection volume and solvent strength. In this work initial conditions were weak enough that at an injection volume of 20 μl polymer exclusion was not evident. As a result of this study, injection volumes were kept at 20 μl.

Separation mechanism for a polymer can primarily result from either phase separation or stationary phase adsorption. One way of determining the presence of phase separation (polymer precipitation) is by studying the dependence of polymer concentration on retention [30]. In Fig. 5, a cellulose

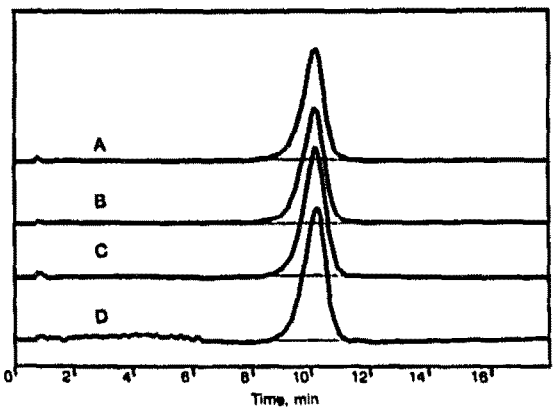

Fig. 5. Effect of cellulose acetate concentration on chromatographic retention. A single polymer (39.9%Ac) was injected at four different concentrations: A = 10 000, B = 8000, C = 3000 and D = 1000 ppm. Chromatographic conditions as in Fig. 2.

acetate polymer at four different concentrations was chromatographed using the standard conditions described earlier. No dependence of polymer concentration on retention was observed, both with regards to peak centroid retention time and peak dispersion, measured as peak variance.

The evaporative light scattering detector's response is non-linear and known to be dependent on a number of variables [31]. In chromatographing cellulose acetate, it was important to know if the detector's response was dependent upon the degree of acetyl substitution. A series of cellulose acetate samples of differing acetyl substitution and similar molecular mass were chromatographed using the conditions listed for Fig. 2. Normalized area counts were compared within this series and are listed in Table V. Detector response (normalized area counts) was observed to be slightly dependent on %Ac over this acetyl series. Approximately a 30% increase in area was observed in going from a cellulose acetate polymer of 38% to 41.5%Ac. In conjunction a 30% decrease in peak width was also observed over this same range. Since the response of the evaporative light scattering detector is non-linear, it is believed that the increase in area counts is mostly due to the decrease in peak width than due to the polymer's compositional changes.

The comparison of these detector responses would be more accurate if they were run under identical isocratic conditions. This study was designed to measure the potential response differences under the working range of the mobile phase. This experiment was set-up using conditions which the polymers would typically elute under. Over the 2–3-min window by which most of these polymers eluted solvent composition did not significantly change, for instance water composition decreased from 28 to 20%, methanol decreased from 9 to 7% and acetone increased from 63 to 73%.

Polymer peak dispersion

An important aspect of the liquid chromatography method is the ability to determine acetyl spread by measuring peak dispersion. Due to the novelty of using chromatographic peak dispersion to measure compositional distribution in polymers, it was necessary to evaluate several dispersion descriptors in

TABLE V

RETENTION TIME, NORMALIZED PEAK AREA, AND PEAK DESCRIPTORS OF VARIOUS CELLULOSE ACETATE POLYMERS

The values in parentheses are the respective standard deviations of 9 replicates.

Sample	Acetyl (%, w w)	Retention time (min)	Normalized area counts	Plate efficiency (N)	1 10 Peak width (s)	1 2 Peak width (s)
A	37.9	8.30 (0.00)	93 077 (3200)	555 (18)	96.9 (1.7)	47.1 (0.5)
C	38.8	8.80 (0.01)	105 650 (7600)	583 (14)	103.1 (1.1)	47.6 (0.4)
D	39.3	9.10 (0.01)	117 148 (2500)	729 (13)	95.7 (0.7)	44.2 (0.6)
E	39.9	9.32 (0.02)	121 024 (1400)	757 (10)	97.5 (1.1)	43.3 (0.4)
F	40.3	9.53 (0.01)	127 474 (2500)	963 (14)	89.2 (0.6)	39.4 (0.5)
K	41.5	10.17 (0.01)	123 521 (1000)	1279 (30)	84.4 (0.8)	35.1 (0.7)

order to arrive at the most accurate and precise one. Three original peak dispersion descriptors are listed in Table V. They are (1) plate efficiency, (2) width at 1/10 height and (3) width at 1/2 height. Both plate efficiency and width at 1/10 height were determined using the Foley–Dorsey calculations in the PE Nelson Access-Chrom software, while the width at 1/2 height was calculated by hand. Plate efficiency was found to be less precise than the others. In addition the Foley–Dorsey calculations for plate efficiency assume a modified gaussian peak which the polymer peaks usually did not resemble [33]. These two factors led us to immediately abandon its consideration as a descriptor. The coefficients of variance for both the width at 1/2 height and the width at 1/10 height were between 1 and 2%. Since the width at 1/10 height was more sensitive to peak asymmetry arising from skewed peaks, it was the preferred choice.

Statistical moments were also evaluated as peak descriptors. Fundamentally the second statistical moment or peak variance (M2) would be a sound descriptor of peak dispersion. Skew (M3) and excess (M4) would in addition be valuable descriptors of non-gaussian behavior, which is believed to result from over or under hydrolyzed sub-populations of cellulose esters. In comparing several cellulose acetate polymers the square root of peak variance (σ), was found to be slightly less precise than the width at 1/10 height: 3% vs. 1%. Skew and excess exhibited even greater variability. Since statistical moments are more influenced by the selection of peak start and stop points they are generally excepted to be less precise [34].

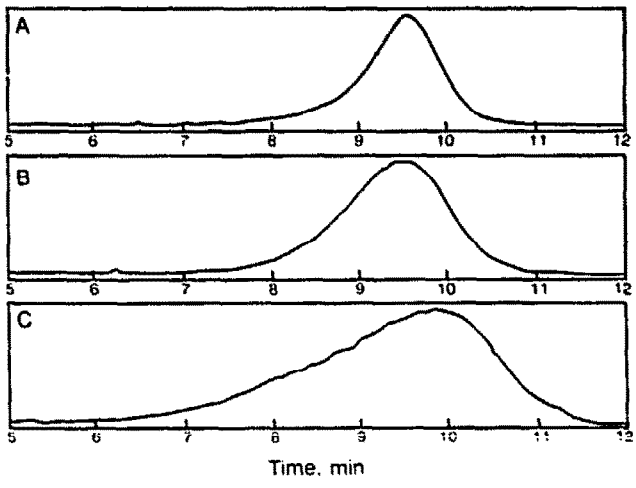

Fig. 6. Comparison of three cellulose acetate polymers, having a mean mass percent acetyl of 39.9% and a comparable molecular mass. Chromatographic conditions as in Fig. 2.

In practice there is no clear preference between width at 1/10 height and second statistical moment. Both have been valuable in helping to characterize the chemical composition of various cellulose acetates. In Fig. 6 there is a comparison of three cellulose acetates. All three polymers have comparable molecular mass and a nominal mass percent acetyl of 39.3%; however, both peak variance (A = 910 s^2, B = 1400 s^2 and C = 4300 s^2) and width at 1/10 height (A = 122 s, B = 159 s and C = 261 s) varied significantly. From these results it was not surprising to learn that sample C exhibited significant haze when dissolved in acetone while samples B and A did not.

There are a number of factors that could potentially contribute to peak dispersion. These contributions include gradient profile, normal column variance, acetyl distribution and molecular mass. The gradient profile was an important factor when understanding external dispersion effects. All polymer samples should see the same gradient change over time. The system dead volume and gradient delay time were calculated in order to better understand this method's working range. Even though the gradient started at injection, the gradient did not reach the head of the column until 2.4 min and the detector for 4.1 min. Since the lower acetyl polymers did not elute until after 7 min, all polymers experienced a constant gradient change.

External and internal column variance will contribute to the total peak dispersion. Since external variance would be constant for all polymer samples, it was simply ignored. It should be noted that without compensating for this contribution, acetyl spread values constitute a relative measurement.

While over the range of 37 to 43% acetyl polymer mean retention time behaved linearly, further proof was required to show that peak dispersion did not change relative to mean acetyl. To study this effect a 38.8% cellulose acetate polymer was fractionated in 0.3-min slices and re-chromatographed. The chromatograms of the original polymer along with six successive fractions (peaks 6–10) are shown in Fig. 7. It is evident from this figure that the original polymer separation could be reconstructed from the individual fractions and that peak dispersion was negligible. In reviewing the results of this experiment in Table VI, 50–60% of the polymer was re-chro-

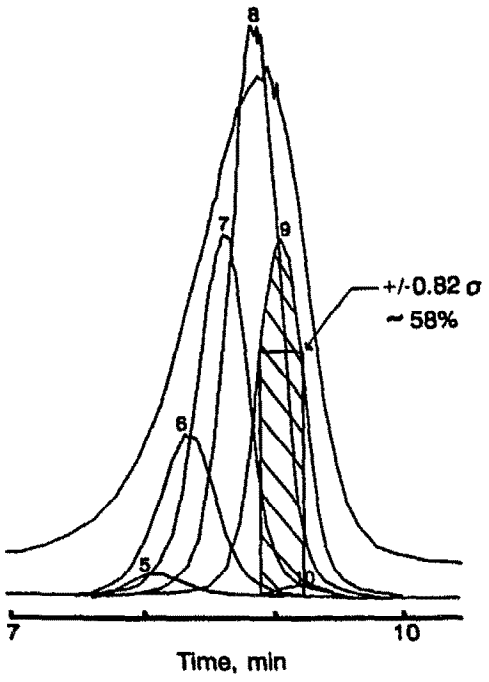

Fig. 7. Superimposed chromatograms of a single polymer (38.8%Ac) before and after fractionation. Chromatographic conditions as in Fig. 2.

matographed within the fractionated time period. When closely examining the width at 1/10 height and the area percent within the original fraction period, there was a slight dependence between the peak dispersion (measured as both width at 1/10 height and peak variance) and retention time (%Ac). The peak width of fraction 5 may be called into question because of the low signal-to-noise ratio for this fraction. This may have made it difficult to accurately measure peak dispersion for this fraction. By ignoring this fraction one could contend that no bias exists, while including this fraction one could postulate that an approximate 15% chromatographic bias exists over a range from 37 to 40% acetyl.

TABLE VI

PEAK DESCRIPTORS OF FRACTIONATED CELLULOSE ACETATE

Fraction No.	Peak centroid (min)	Peak variance (s^2)	Width at 1/10 height (s)	Area percent within 0.3 min fraction window (%)
5	8.10	356	72	46
6	8.35	235	63	50
7	8.62	186	56	54
8	8.86	166	53	57
9	9.07	159	52	59
10	9.25	163	55	55
Original	8.74	903	121	–

To validate the usefulness of this liquid chromatography method, it was important that the effect of cellulose acetate molecular mass on elution time be minimal or altogether absent. A careful study was undertaken to verify this condition. Again, the 38.8% cellulose acetate sample was fractionated, however, this time by GPC. A 20 × 300 mm I.D. mixed-bed Polymer Lab. GPC column was used. This polymer was fractionated using 0.3-min time slices. Six fractions were collected and reanalyzed by GPC to determine M_w, M_n and polydispersity. These data are listed in Table VII. M_n covered a range from 30 000 to 200 000 u and the polydispersity was generally between 1.2–1.3. Mean retention times varied approximately 6 s over this molecular mass range, while peak dispersion increased about 30–40%. As shown in Table VII, several fractions over the molecular mass range were analyzed by ^{1}H NMR for mean mass percent acetyl. This analysis confirmed that the mean acetyl composition of this cellulose ester did not vary over the molecular mass range. It was concluded from these results that the change in peak dispersion is a reflection of increased "acetyl spread" at lower molecular mass.

Effect of stationary phase pore diameter

A pore diameter study was conducted using a series of Polymer Lab. PLRP-S columns having pore diameters of 10, 30 and 100 nm. These stationary phases were packed into 50 mm × 4.6 mm columns. The 10 nm pore diameter stationary phase was also evaluated in a 150 mm long column format. Peak variance and retention time were measured for these supports. A cellulose acetate polymer series varying in acetyl composition from 38.3–40.3% acetyl was studied. The results for the chemical composition series is listed in Table VIII. The column temperature for the chemical composition series was 30°C. Butyl octyl phthalate was used as a low-molecular-mass control to determine total system variance under an ideal case.

In Table VIII no significant changes in retention time or peak variance for any of the cellulose diacetate polymers were observed for the pore diameters studied. Even though the total surface

TABLE VII

ANALYTICAL RESULTS FROM CELLULOSE ACETATE POLYMER FRACTIONATED BY GEL PERMEATION CHROMATOGRAPHY

Fraction No.	GPC data			HPLC acetyl data			Acetyl (%, w/w) by ^{1}H NMR
	M_n (u)	M_w (u)	Polydispersity	Retention time (min)	Width at 1/2 height (min)	Width at 1/10 height (min)	
4	$1.81 \cdot 10^5$	$2.21 \cdot 10^5$	1.23	8.74	0.751	1.79	–
6	$1.10 \cdot 10^5$	$1.32 \cdot 10^5$	1.20	8.71	0.699	1.44	38.6
8	$7.46 \cdot 10^4$	$9.44 \cdot 10^4$	1.27	8.72	0.813	1.69	–
10	$4.97 \cdot 10^4$	$6.71 \cdot 10^4$	1.35	8.69	1.05	2.02	38.8
12	$3.34 \cdot 10^4$	$4.28 \cdot 10^4$	1.28	8.63	1.22	2.20	–
14	$3.48 \cdot 10^4$	$8.96 \cdot 10^4$	1.35	8.73	1.09	2.37	38.8

area decreased 65% from 410 to 270 m^2/g, no difference in effective surface area was observed for this series of polymers. As expected butyl octyl phthalate, which would be able to penetrate smaller pore diameters and thus accessible to more surface area, showed a greater dependence between retention time and pore diameter.

It has been well reported in the literature that in precipitation liquid chromatography peak efficiency of macromolecules is not dependent upon column length [35]. This theory was tested by comparing the 10 nm pore diameter stationary phase packed in both 5 and 15 cm long columns. The results for these two columns are shown in Table VIII. In all cases the retention time difference between the 15- and 5-cm columns was 1.5 min which at a flow-rate of 0.8 ml/min would correspond to an observed volume for these polymers of 1.2 ml for the last 10 cm of column. The dead volume for the 15-cm column was previously determined to be 1.36 ml for cellulose diacetate using a mobile phase of 100% acetone. Over a length of 10 cm this dead volume would relate to 0.91 ml. Since the dead volume is significantly lower than the observed volume, this would suggest that the polymer is retained even beyond 5 cm.

In Table VIII a significant difference in peak variance was also revealed between the 15 and 5 cm long columns. Column efficiency (N) is both directly dependent upon column length and inversely dependent upon peak variance. In comparing the inverse peak variance for butyl octyl phthalate for the two column lengths, the 15-cm column is three times as efficient as the 5-cm column. This nicely follows fundamental chromatographic theory for a small molecule. For the cellulose acetate polymers the inverse variance difference for the 15-cm column was 1.3–1.7 times as efficient as the 5-cm column. Clearly this increase in efficiency supports an ad-

TABLE VIII

STUDY OF PORE DIAMETER FOR CELLULOSE ACETATE

Sample	Acetyl (%, w/w)	Retention time (min)				Peak variance (s^2)			
		10 nm, 15 cm	10 nm, 5 cm	30 nm, 5 cm	100 nm, 5 cm	10 nm, 15 cm	10 nm, 5 cm	30 nm, 5 cm	100 nm, 5 cm
B	38.3	9.53	7.97	7.82	7.86	980	1360	1310	1380
C	38.8	9.75	8.22	8.06	8.09	890	1200	1220	1270
D	39.3	10.06	8.55	8.41	8.43	790	1140	1120	1220
E	39.9	10.27	8.77	8.61	8.64	820	1200	1130	1140
F	40.3	10.52	9.00	8.85	8.89	670	1140	1090	1070
Butyl octyl phthalate	–	15.83	11.75	11.43	9.30	30	110	110	250

sorption rather than a precipitation retention mechanism. While there is some advantage to a longer column in chromatographing cellulose acetate polymers, the advantage is less than what is observed for smaller solutes.

CONCLUSIONS

A new analytical method for determining the composition of cellulose acetate polymers, that differ in degree of acetate substitution, has been developed. This method is performed on a conventional HPLC instrument under reversed-phase conditions. Chromatographic peak shape and retention time of cellulose acetate samples can be used to distinguish samples of various acetyl substitution and the extent of acetyl spread.

Chromatographic conditions were optimized to selectively separate cellulose acetate as a function of acetyl content. Polymer retention was independent of molecular mass over an M_n range from 33 000 to 180 000. Cellulose acetate was found to elute under adsorption rather than precipitation chromatography conditions. Polymer exclusion was avoided by the judicious choice of the initial mobile phase conditions and injection volume.

Peak width descriptors were found to be useful as a means of quantifying acetyl spread. Acetyl spread is a relative measure of compositional variability since it is inclusive of all contributors to peak variance. Changes in pore diameter over a range from 100 to 1000 Å had little effect upon polymer peak variance. A 15% bias in peak width was observed over a compositional range from 37–40% acetyl. This measurement provided information about cellulose acetate substitution which is more detailed than any other contemporary analysis.

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