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Determination of ester substituents in cellulose esters

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ABSTRACT

A method to determine most ester substituents in cellulose esters has been developed. The cellulose ester is first dissolved in dimethyl sulfoxide. The esters are rapidly hydrolyzed at room temperature by adding a mixture of methanol and sodium hydroxide. Acids in the resulting sample can be determined by gas chromatography, ion chromatography or capillary zone electrophoresis. Capillary zone electrophoresis provides a nearly universal way to determine the hydrolyzed acids and it is the preferred method.

INTRODUCTION

Cellulose esters are an important class of polymers used for the manufacture of fibers, films, plastics, paints and drug delivery systems. The properties of the polymer depend on the degree of substitution of the cellulose hydroxyls as well as the properties of the ester substituents. There are numerous methods to determine the degree of substitution. Most of these methods are specific to one type of ester [1]. For example, phthalate is determined in cellulose acetate phthalate by titration [2]. Acetate in cellulose acetate is determined by a saponification procedure [3]. A method applicable to all, or at least most, cellulose esters and mixed esters would be desirable.

A reagent was recently described that is particularly effective for the hydrolysis of esters for analytical purposes [4]. It consists of a mixture of a polar aprotic solvent, methanol and a strong base, for example sodium hydroxide. It can hydrolyze esters at extraordinary rates. For example, solid polyesters are routinely hydrolyzed in min. A major component of this reagent can be dimethyl sulfoxide (DMSO). DMSO is a solvent for most cellulose esters. A possible analytical scheme employing this reagent would involve dissolving the cellulose ester in DMSO followed by addition of base in methanol. Since the cellulose ester is in solution, the esters would be expected to hydrolyze very rapidly. The hydrolyzed acids could then be analyzed by various chromatographic procedures. This scheme was investigated for the analysis of some commercial cellulose esters. Several approaches for determining the acids in the hydrolysis mixture were investigated. Capillary zone electrophoresis (CZE) was the most versatile approach.

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EXPERIMENTAL

Sample preparation

Approximately 0.1 g of cellulose ester was placed in a 50-ml culture tube. An 8-ml volume (4 ml for GC analysis) of dry DMSO (Burdick & Jackson) were added to the tube. The tube was capped and the contents mixed to dissolve the ester. After the ester dissolved, 2 ml (1 ml for GC analysis) of 5 *M* sodium hydroxide in dry methanol (Burdick & Jackson) was added. During addition, the contents were swirled on a vortex mixer at medium speed. After the addition, the tube was capped and mixed at high speed for 2 min. After mixing, 15 ml of water (20 ml for GC analysis) was added and the tube capped. The contents were swirled at high speed for

2 min to extract the acids. A portion of the contents was filtered through a 0.45-μm Gelman PTFE filter. Final sample dilutions for each method are given below.

For GC analysis, 1 ml of filtrate was transferred to a 10-ml volumetric flask containing 15 μl of phosphoric acid. The flask was diluted to volume with an internal standard solution (300 μg/ml 2-methylbutyric acid in water). For ion chromatographic analysis, 0.1 ml of filtrate was transferred to a 10-ml volumetric flask. The flask was diluted to volume with water. For CZE analysis, 1 ml of filtrate was transferred to a 10-ml volumetric flask. The flask was diluted to volume with water. During standard preparation for CZE, the final dilution for each standard concentration included 1 ml of a sample blank (8 ml DMSO and 2 ml 5 *M* sodium hydroxide in methanol diluted to 25 ml with water).

Data were collected and processed on a PE Nelson Access*Chrom chromatography data system.

Gas chromatography operating conditions

All analyses were performed on a Perkin-Elmer Sigma 3B gas chromatograph. The isothermal separation was performed on a Quadrex Corporation 007-FFAP column, 25 m × 0.53 mm I.D., with a 1.0-μm film thickness. The injection temperature and oven temperature were 122°C. Detection was by flame ionization at 200°C. The on-column injection volume was 1 μl. High-purity helium was used as the carrier gas at a flow of 8 ml/min.

Ion chromatography operating conditions

The separation was performed on a Dionex IonPac AS10 analytical column, 25 cm × 4 mm, with an AG10 guard column in conjunction with an AMMS II micromembrane suppressor. The regenerant was 50 m*M* sulfuric acid at a flow-rate of 5 ml/min. The eluent was 3.5 m*M* sodium borate at a flow of 1 ml/min.

Capillary zone electrophoresis operating conditions

Analyses were performed on a Waters Quanta 4000 capillary electrophoresis system. The separation was performed on a 60 cm × 75 μm I.D., fused-silica column with an applied potential of 20 kV negative. The injector to detector distance was 52.7 cm. The electrolyte was 7 m*M* 3-nitrophthalic acid (Aldrich) with 0.5 m*M* myristyltrimethylammonium bromide (Aldrich) as the electroosmotic flow modifier, adjusted to pH 7.0 with sodium hydroxide. Detection was by indirect UV at 254 nm. Sample injection was by hydrostatic flow for 30 s.

RESULTS AND DISCUSSION

Before the utility of the hydrolysis reagent could be explored, a means of determining aliphatic acids in the hydrolysis mixture had to be developed. Chromatography of the hydrolysis solution is not trivial, particularly if a universal procedure is sought. The acids will be present as minor components in the DMSO–methanol mixture. Ion exclusion chromatography with a 300 × 7.8 mm Aminex HPX-87H (Bio-Rad) and dilute sulfuric acid eluent or reversed-phase liquid chromatography with a 150 × 4.7 mm C22 (ES Industries) column and methanol–0.1 *M* pH 2.2 phosphate buffer are preferred ways to determine aliphatic acids in our laboratories. However, DMSO is sufficiently retained in these methods to be a major interference in determining aliphatic acids in the hydrolysis mixture. These methods could be useful for the determination of some acids, but they are not broadly applicable.

Gas chromatography is adequate for the determination of acetic, propionic and butyric acids, but not aliphatic diacids, in the hydrolysis mixture. Methanol is not retained under the conditions used. DMSO elutes between propionic and butyric acid

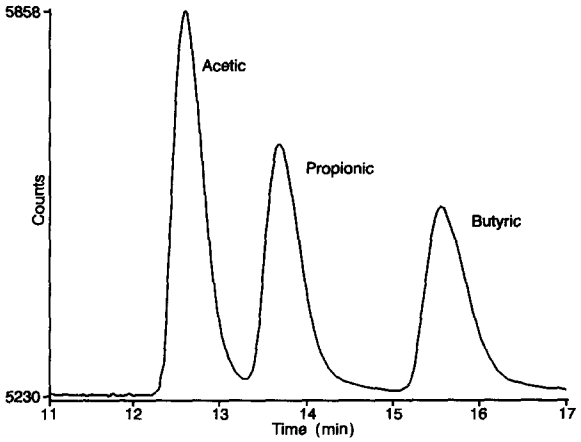

Fig. 1. Ion chromatographic separation of aliphatic acids. See Experimental for conditions; 20 μg/ml of each acid.

and it does not interfere with either component. Calibration of acetic acid is a problem if it is necessary to analyze it at low levels, for example 50 μg/ml. At 50 μg/ml it is necessary to condition the column with a duplicate injection to get reproducible data. Above 100 μg/ml there was no problem reproducing standards or samples. Repeated injection of hydrolysate eventually led to degraded column performance that could be restored by removing a short inlet section. This inconvenience and the inability to determine diacids were the only significant limitations encountered.

Ion chromatography provides a barely adequate separation between acetic and propionic acid (Fig. 1). DMSO and methanol are not retained so neither is an interference. Aliphatic diacids must be eluted with a gradient or determined with another column, for example a Dionex AS5 with NaOH eluent, or an AS10 guard column, which is an inexpensive equivalent to the AS5 analytical column for many applications. The most serious disadvantage of ion chromatography is the small linear range available when a conductivity detector is used. A typical calibration curve for acetic acid is shown in Fig. 2. The severe curvature is a result of the weak dissociation of aliphatic acids [5]. The acids generated by the suppressor column can be ion exchanged back to fully dissociated salts before detection, but the added complexity makes this approach impractical for many situations. While good nonlinear fits are possible, many more standards are necessary to achieve accuracy comparable to linear situations. In spite of these limitations, ion chromatography provides a robust method for the determination of acids in the hydrolysis mixture.

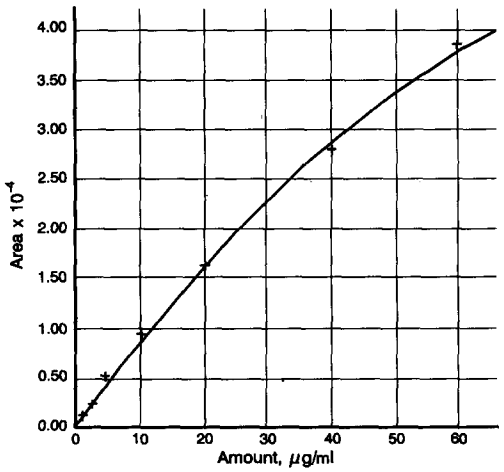

Fig. 2. Ion chromatography calibration curve for acetic acid.

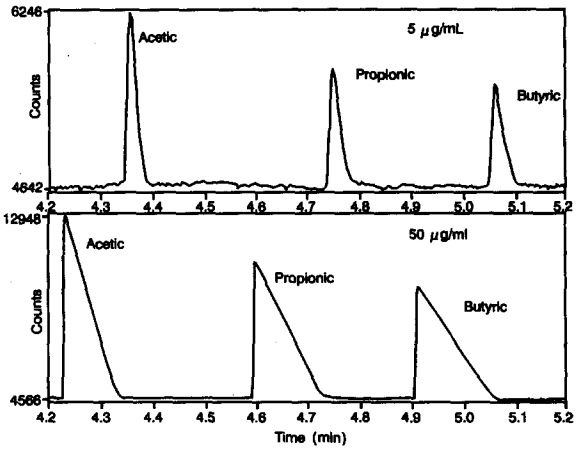

Fig. 3. Comparison of 5 and 50 μg/ml standard by CZE. See Experimental for conditions.

Altria and Simpson [6] proposed using CZE with an alkyl quaternary salt for anion analysis. Hjertén *et al.* [7] described the CZE separation and indirect detection of aliphatic acids and small anions. Several more recent papers [8–10] and a patent [11] describe the use of capillary zone electrophoresis for the determination of aliphatic acid anions. CZE would seem to be an ideal technique to apply to the problem of determining acids in the hydrolysis mixture. CZE provides a class separation of charged anions from neutral species, in this case DMSO and methanol, and within the charged species it offers the possibility of high resolution and fast separations of nearly any acid of interest, including aliphatic diacids and aromatic acids. In addition, consumables cost is insignificant compared to alternative methods.

In our investigation of CZE we encountered a limitation not emphasized in the above references. The conditions previously described result in a dynamic range of less than two decades for the determination of aliphatic acids. This range is too limited for many applications. The bottom of the dynamic range is set by the limit of quantitation; the top of the range is limited by resolution which is deter-

mined by peak shape at high concentration. Unless there is a good match between the mobility of the analyte and the electrolyte, the peaks severly broaden at high concentration. This phenomena was shown by Hjertén [12] and a mathematical description of the origin of peak broadening was provided by Mikkers *et al.* [13] and Poppe [14]. The problem is illustrated in Fig. 3. The criteria to achieve the best dynamic range in indirect detection have been discussed [15–20]. The chromophore for indirect detection should have the largest possible molar absorbtivity to achieve the best limit of quantitation. The mobility of the electrolyte should closely match the mobility of the analytes to preserve peak shape at high concentration. None of the chromophore-electrolytes previously used were optimized for aliphatic acid determination. The mobility of chromate and phthalate is too large; benzoate mobility is too small. Small aliphatic acids either severely tail, or front, with these electrolytes.

Chromophore-electrolytes were investigated that might provide a better mobility match to the aliphatic acids of interest and provide an anion with a large molar absorbtivity. These improvements would provide a more useful dynamic range. We discovered that hydroxy- or nitro-substituted phthalic, isophthalic or terephthalic acids provide a good mobility match for C_2 to C_4 aliphatic acids and these compounds have a large molar absorptivity. The phthalic acid derivatives were readily soluble and stock solutions were more convenient to prepare than the less soluble terephthalic acid derivatives.

With 3-nitrophthalic acid as the chromophore-electrolyte, two decades of useful concentration range could be quantitated with good precision, 1–100 μg/ml, at 254 nm. Calibration curves were slightly curved over this range, but accurate cali-

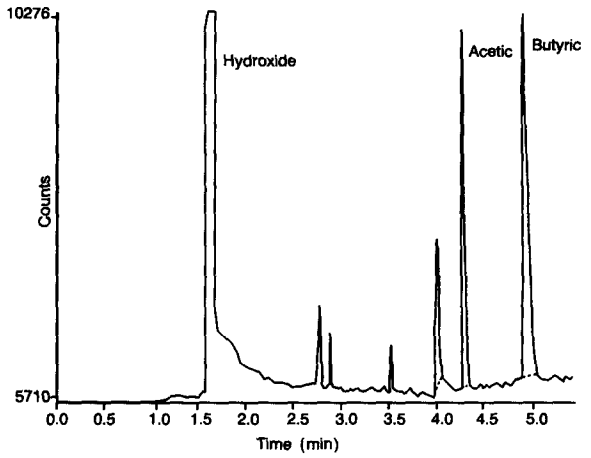

Fig. 5. Electropherogram of a typical hydrolysis solution. See Experimental for conditions.

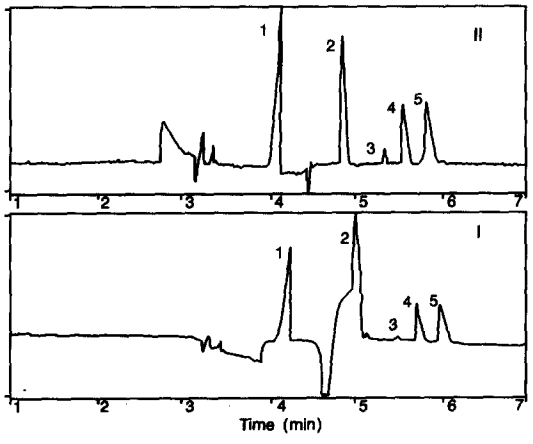

Fig. 4. Comparison of standards prepared from aliphatic acids (I) and aliphatic acids neutralized with sodium hydroxide (II). Peaks: 1 = formic; 2 = acetic; 3 = propionic; 4 = isobutyric; 5 = isovaleric acid. See Experimental for conditions.

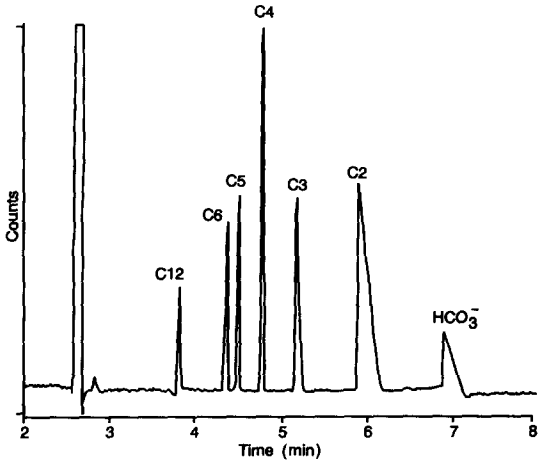

Fig. 6. Separation of C_2–C_{12} aliphatic acids. Conditions: 75 cm × 50 μm column, 30 kV, detection at 220 nm, 0.007 m*M* benzoate at pH 6. Separation performed on an ABI 270A-HT instrument.

brations could be made with five points. It is necessary to use acid salts for calibration. When the standard is acidic, a negative dip occurs which can interfere with acids of interest (see Fig. 4). The migration time of the dip is the same as for the electrolyte, so a plug of electrolyte is the likely cause. We do not know what causes this plug to form when an acidic standard is analyzed.

A typical electropherogram for a hydrolysis solution from a mixed acetate–butyrate ester is shown in Fig. 5. The mobility match of 3-nitrophthalate is not perfect for C_2 to C_4 aliphatic acids; they front at high concentrations. A slightly less mobile salt could extend the dynamic range even further, which could be an advantage for some applications. Without the constraint of detection at 254 nm, other chromophore-electrolyte possibilities are available. Electrolyte optimization for anion analysis will be the subject of a subsequent publication.

Recent work suggests the osmotic flow modifier may be unnecessary for separating many aliphatic and aromatic acids. Fig. 6 shows the separation of C_2–C_{12} aliphatic acids on a 50-μm column at high pH. Under these conditions the electroosmotic flow is fast enough to counter the high mobility of these ions. An investigation into the utility of this approach is in progress.

Validation of hydrolysis procedure

To be successful, the hydrolysis reagent must quantitatively hydrolyze the ester, the resulting acid salt must quantitatively extract from the cellulose, these acid salts must not be lost by decomposition or volatilization, and the reaction must not form interfering acids or other products. The hydrolysis procedure described meets these essential criteria.

Previous work has shown the reagent attacks ester bonds at extraordinary rates. When the methanol–NaOH solution is added to the cellulose ester solution, the cellulose formed after hydrolysis does not instantly precipitate. Undoubtably, this delay helps to achieve complete hydrolysis and extraction of the acid salts. Experiments were performed to measure the extent of hydrolysis as a function of hydrolysis temperature and time. A cellulose butyrate ester was hydrolyzed at temperatures between 20 and 120°C and times from 1 to 60 min. The amount of acid recovered was essentially the same for all conditions. At higher temperatures acidic components formed that could be an interference with some acids. Since the recovery was the same at room temperature there was no reason to use higher temperature for hydrolysis and the potential problem was avoided. Extraction times up to 24 h were investigated. There was no significant difference in the amount of acids recovered by increasing the extraction time. The insensitivity of recovery to hydrolysis and extraction conditions suggested these steps were quantitative. To prove this point cellulose recovered after hydrolysis was washed and hydrolyzed with dilute sulfuric acid to yield a completely soluble product. No aliphatic acids were detected in this hydrolyzate which proves they were quantitatively hydrolyzed and extracted by the original hydrolysis procedure.

Four commercial and one experimental cellulose ester were used to evaluate the proposed hydrolysis and analysis procedures. Five replicate samples of each ester were hydrolyzed and analyzed by each of the chromatography techniques described in the Experimental section, as well as traditional methods. These data are shown in Table I. In general there is good agreement between the various approaches for determining the ester substituents.

The precision of the gas and ion chromatography methods was typical for these techniques, about 2% relative standard deviation (R.S.D.). We were able to analyze standards with CZE with a precision of 1–2% R.S.D. Overall method precision, hydrolysis plus acid determination, was typically about 2% R.S.D. for the major component acids (10–50%). For the minor component acids (1–5%), R.S.D.s were in the range of 2–13%. The poorer precision results were always associated with the determination of low concentrations of acetic acid and may reflect inadequate precautions to prevent contamination by acetate. During the course of this work it was discovered that glassware used for processing samples was a significant source of acetate contamination. To reliably determine lower levels of acetate it was necessary to rinse glassware in deionized water just before use. Acetic acid is used as a solvent in our laboratory and we speculate that it adsorbs from the air onto the relatively basic surface of some glassware, particularly autosampler vials.

The main advantage of this sample preparation procedure is speed and convenience. Traditional methods involve refluxing the sample to achieve hy-

TABLE I

WEIGHT % SUBSTITUENTS IN VARIOUS CELLULOSE ESTERS

Cellulose ester	Method	Substituent (%, w/w)			
		Acetyl	Propionyl	Butryl	Succinyl
CAB-171-15S	GC[a]	28.7		18.3	
	NMR[b]	28.1		19.1	
	GC[c]	31.3		19.6	
	IC[d]	30.0		19.3	
	CZE[e]	29.4		19.1	
CAB-381-20	GC[a]	13.6		36.8	
	NMR[b]	12.9		38.2	
	GC[c]	16.4		38.3	
	IC[d]	15.1		37.5	
	CZE[e]	13.8		37.5	
CAP-482-0.5	GC[a]	1.3	44.6		
	NMR[a]	0.7	45.8		
	GC[c]		42.0		
	IC[d]	1.3	43.0		
	CZE[e]	1.6	45.8		
CAB-500-5	GC[a]	3.3		50.9	
	NMR[b]	2.8		53.4	
	GC[c]	3.7		51.6	
	IC[d]	3.7		52.5	
	CZE[e]	4.2		49.5	
CABSU 160	GC[a]	1.9		40.8	10.5[f]
	IC[d]				10.1
	CZE[e]	1.9		40.7	9.8

[a] Internal method. Sample is hydrolyzed in pyridine–methanol–KOH and hydrolyzed acids determined by GC.
[b] Internal method. Substituents determined by integration of proton NMR spectrum.
[c] Hydrolysis as described in Experimental section. Acid determination by GC.
[d] Hydrolysis as described in Experimental section. Acid determination by ion chromatography.
[e] Hydrolysis as described in Experimental section. Acid determination by CZE.
[f] Determined by titration with base.

drolysis. Reflux times are typically 30 min or more and sample sizes are large. The proposed hydrolysis is nearly instantaneous and it can be done in a test tube. We have performed the analysis on as little as one milligram of material.

CONCLUSIONS

Cellulose esters can be rapidly hydrolyzed at room temperature in a mixture of DMSO, methanol and sodium hydroxide. Acids in the resulting sample can be determined by GC, ion chromatography, or CZE. CZE provides a nearly universal way to determine the hydrolyzed acids and it is the preferred method.

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