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DETERMINATION OF THE ACIDIC DEGRADATION PRODUCTS, ACETIC, PROPIONIC, BUTYRIC, AND PHTHALIC ACID IN AQUEOUS PSEUDOLATEXES OF CELLULOSIC ESTERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

Roland Bodmeier and Huagang Chen
College of Pharmacy
The University of Texas at Austin, Austin, TX 78712.

ABSTRACT

An isocratic, reversed-phase HPLC method was developed to quantify the organic acids, acetic, propionic, butyric, and phthalic acid, formed as a result of ester hydrolysis, in pseudolatexes of cellulosic esters. Colloidal dispersions of cellulose acetate, cellulose acetate butyrate, and cellulose acetate propionate were prepared by a microfluidization-solvent evaporation method. Dispersions of cellulose acetate phthalate were prepared by redispersion of a spray-dried commercial pseudolatex. The acids were detected at 210 nm, the mobile phase being 0.025 M phosphate buffer : methanol (80:20 v/v%, pH 3.0). The peak height response was linear over the studied concentration range of 2 - 10 mM/L for the aliphatic acids and 20-100 μM/L for phthalic acid. The minimum detectable quantities for acetic, propionic, butyric, and phthalic acid were 0.02 mM/L, 0.05 mM/L, 0.1 mM/L, and 0.0005 mM/L, corresponding to a % change in acetyl, propionyl, butyryl, and phthalyl content of 4.0×10^{-4}, 1.2×10^{-3}, 2.9×10^{-3}, and 2.8×10^{-5} for a 30% w/v pseudolatex. The colloidal polymer particles were separated by ultracentrifugation, filtration, or flocculation with aluminum chloride solution before analysis of the aqueous phase. Similar acid concentrations were obtained for the three separation methods. The recovery from spiked samples was almost complete for acetic, approximately 90% for propionic acid, and less than 80% for butyric acid.

INTRODUCTION

Water-insoluble derivatives of cellulose are popular polymers used in the coating of solid dosage forms to obtain sustained or controlled release drug delivery systems (1, 2). Besides ethyl cellulose, these polymers are based on esters such as cellulose acetate, cellulose acetate butyrate, cellulose acetate propionate and cellulose acetate phthalate. Cellulose acetate is the polymer of choice for the preparation of semipermeable membranes in osmotically driven systems (3, 4). Polymers derivatized with phthalic acid have enteric properties (5).

While coating with organic polymer solutions is still widespread, aqueous colloidal polymer dispersions, called latexes or pseudolatexes, have been developed to overcome the hazards associated with organic solvents (6-8). During the coating and drying process, the colloidal polymer particles are forced together, deform and coalesce into a continuous film.

The cellulosic pseudolatexes are prepared by emulsification of polymer solutions or melts into an external aqueous phase. Since cellulosic esters are prone to hydrolysis in an aqueous environment, the stability of the pseudolatexes has to be monitored. Hydrolysis of the polymer during storage may affect the physical stability of colloidal polymer dispersion and may result in changes in the permeability of the resulting polymeric membranes.

Cellulose acetate has been used in reverse osmosis membranes, and several research groups have studied the hydrolysis and degradation of these membranes (9-12). The degradation resulted in marked changes in water flux and salt retention. The parameters studied to follow polymer degradation were tensile strength, inherent viscosity, molecular weight, and the acetyl content of the membranes.

Standard methods of testing for the cellulose esters include assays of the polymer phase for the hydroxyl, acetyl, propionyl, and butyryl content (13). Except for an UV spectroscopic method for phthalic acid, which formed during storage of pseudolatexes of enteric polymers (5), no studies have been reported that measure the formation of the acidic degradation products in cellulosic pseudolatexes. Various chromatographic techniques including HPLC and GC methods have been reported on the analysis of various organic acids and their derivatives with applications in the food industry (14-17). The objective of this study was to prepare pseudolatexes of the cellulose esters by a microfluidization-solvent evaporation method and to develop a rapid analytical method, which allows the determination of acidic degradation products in the aqueous phase formed during storage.

MATERIALS AND METHODS

Materials

The following chemicals were used as received: cellulose acetate, CA-398-10; cellulose acetate butyrate, CAB-171-15S; cellulose acetate propionate, CAP-482-20 (Eastman Kodak Company, Rochester, NY); cellulose acetate phthalate (Aquateric, FMC Corp., Newark, DE); sodium lauryl sulfate; potassium hydrogen phthalate (99.95 %) (MCB Manufacturing Chemist, Norwood, OH); acetic acid, glacial (99.6 %); methanol; methylene chloride; sodium phosphate, monobasic (Mallinckrodt, Inc., Paris, KY); o-phosphoric acid (85%) (Fisher Scientific, Fair Lawn, NJ); propionic acid (99%) (Aldrich Chemical Company, Inc., Milwaukee, WI); n-butyric acid (99%) (Sigma, St. Louis, MO).

Methods

Pseudolatex preparation - The pseudolatexes were prepared by a high pressure emulsification - solvent evaporation method. A polymer solution [polymer, 22.5 g, methylene chloride or methylene chloride:methanol (9:1 v/v), 200 ml] was pre-emulsified into an aqueous phase (280 ml) containing sodium lauryl sulfate 0.5 w/v % to form an O/W emulsion. The particle size of the internal phase was further reduced to the colloidal size range with a microfluidizer (operating pressure = 7000 psi, 5 cycles; standard M-110 laboratory model, Microfluidics Corporation, Newton, MA), a high pressure homogenizing device, to form the pseudolatexes. The nanosuspensions were stirred for 48 - 72 h at room temperature and ambient pressure prior to storage. Cellulose acetate phthalate (Aquateric) was redispersed in water prior to analysis to result in a 10% w/v pseudolatex.

Chromatographic conditions - The chromatographic system consisted of an HPLC pump (Beckman 110B Solvent delivery module), a variable wavelength UV absorbance detector (Beckman 163 Variable wavelength detector), a low dead volume sample injector with 20 microliter-loop (Rheodyne Model 7125, Cotati, CA), a column inlet filter (0.5 μm filter element, Rainin Instrument, Woburn, MA), an analytical column (Beckman-Ultrasphere, C-18, 5 μm particle size, 25 cm X 4.6 mm ID), and an integrator (C-R3A Chromatopac, Shimadzu, Kyoto, Japan). The mobile phase consisted of 0.025 M phosphate buffer-methanol (80:20 v/v, pH

3.0). The solvents were mixed, vacuum-filtered through a 0.45 µm nylon 66 filter (Applied Science Lab., Deerfield, IL), and degassed by ultrasonication under reduced pressure prior to use. The flow rate was 1.2 ml/min, resulting in a pressure of 2000-2500 psi. The sensitivity was set at 0.02 AUFS at 210 nm. Quantitation was by peak height measurements and temperature was ambient.

Stock, standard and sample solutions, precision - Stock solutions were prepared by dissolving acetic (6.0 mL), propionic (7.5 mL), butyric acid (10.0 mL), and potassium hydrogen phthalate (20.42 mg) in distilled water to make 100 ml. The standard solutions were obtained by diluting the stock solutions with water to concentrations of 2, 4, 6, 8, and 10 mM/L for the aliphatic acids and to concentrations of 20, 40, 60, 80, 100 µM/L for phthalic acid. Linear regression analysis of peak height versus concentration gave slope, intercept, and coefficient of correlation.

The sample solutions were obtained after separation of the colloidal polymer particles from the aqueous phase by either ultracentrifugation (40,000 rpm, 1hour, Beckman L5-50), filtration through a 0.01 µm filter (Sartorius, Westburg, NY) or flocculation with a 1 %w/v $AlCl_3$ solution (1ml $AlCl_3$ solution was added to 4 ml of pseudolatex). The supernatants were diluted with water, if necessary.

The pH of the standard and sample solutions was adjusted below 3 with phosphoric acid (5% v/v) prior to injection (n = 2).

Within-run precision was determined by calculating the coefficient of variation on 5 replicate analyses of standard solutions containing 1 and 10 mM/L of aliphatic acids and 10 and 100 µM/L of phthalic acid.

Recovery study - The pseudolatexes were analyzed before and after the addition of known amounts of acid to the pseudolatexes (n = 2; 100, 1000 mM per liter latex for the aliphatic acids) to determine the percent recovery [100% * (total acid in pseudolatex - acid in original pseudolatex) / acid added to pseudolatex]. The polymers were separated from the aqueous phase by ultracentrifugation.

Particle size measurement - The average particle size of the pseudolatexes was determined by photon correlation spectroscopy (BI-200SM goniometer, BI-2030 digital correlator, Brookhaven Instruments Corporation, Holtsville, N.Y., Melles Griot 10 mW He-Ne laser).

RESULTS AND DISCUSSION

The pseudolatexes of the cellulosic esters were prepared by a microfluidization - solvent evaporation method (18). The polymers were dissolved in methylene

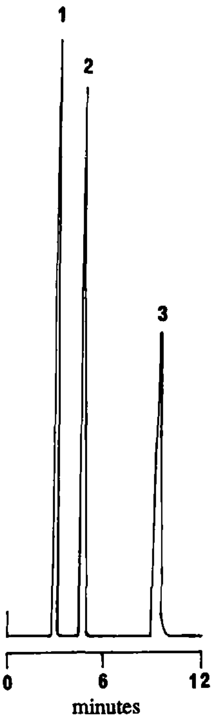

FIGURE 1
Chromatogram of aqueous standard solutions containing (1) acetic, (2) propionic , and (3) butyric acid(mM/L).

chloride (cellulose acetate propionate and cellulose acetate butyrate) or methylene chloride: methanol (9:1 v/v%) (cellulose acetate) and emulsified into an aqueous phase containing sodium lauryl sulfate as surfactant, followed by microfluidization in order to obtain colloidal polymer particles. The particle size of the polymer particles reached a minimum after 5 cycles at an operating pressure of 7000 psi and was in the range of 250 -300 μm. Cellulose acetate did not dissolve in methylene chloride and, in order to solubilize the polymer, methanol, which was also a nonsolvent, had to be added. A cellulose acetate phthalate was prepared by dispersion of a commercially available redispersible powder (Aquateric) in water.

The degradation of cellulosic esters in an aqueous environment is well known. The polymer degradation involves the hydrolysis of the ester groups and, possibly, the breakdown of the polymer backbone as was measured with cellulose triacetate by gel-permeation chromatography (10). Several studies have reported the

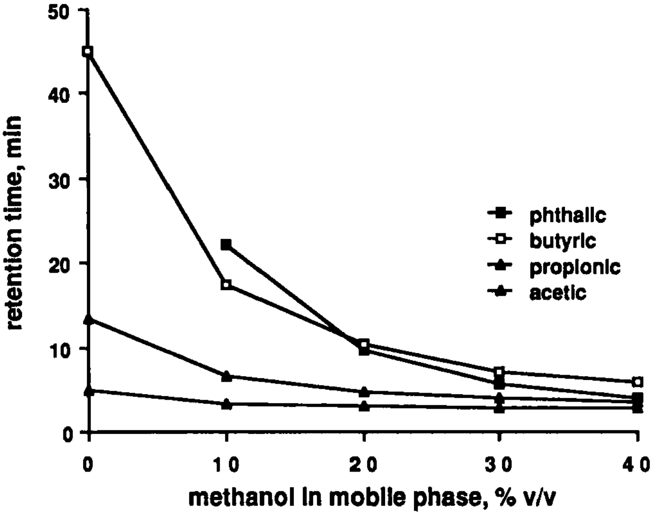

FIGURE 2
Effect of the amount of methanol in the mobile phase (0.025 M phosphate buffer : methanol, pH 3) on the retention time of acetic, propionic, butyric, and phthalic acids.

hydrolysis of cellulose acetate under various pH and temperature conditions (9, 11, 12). The hydrolysis, which will remove acetyl groups from the polymer, is generally followed by analysis of the remaining functional groups on the polymer.

In this study, an HPLC method was developed to determine the acidic degradation products, acetic, propionic, butyric, and phthalic acid in the aqueous phase of the respective pseudolatexes. A chromatogram of the three aliphatic acids with a mobile phase of 0.025 M phosphate buffer : methanol (80 : 20 v/v %, pH 3.0) is shown in Figure 1. The peaks were well separated. The retention times for acetic, propionic, and butyric acid increased with increasing molecular weight of the acids and were 2.6, 4.4, and 9.9 minutes, respectively.

The effect of the amount of methanol in the mobile phase (0.025 M phosphate buffer : methanol, pH 3) on the retention time of the acids is shown in Figure 2. As expected, increasing the amount of methanol decreased the retention time, the effect being more pronounced with the higher molecular weight acids. The retention time of phthalic acid in pure phosphate buffer was larger than 60 minutes. The peak heights increased and the peaks became sharper with increasing methanol concentration. A methanol concentration of 20 % v/v was selected because of only

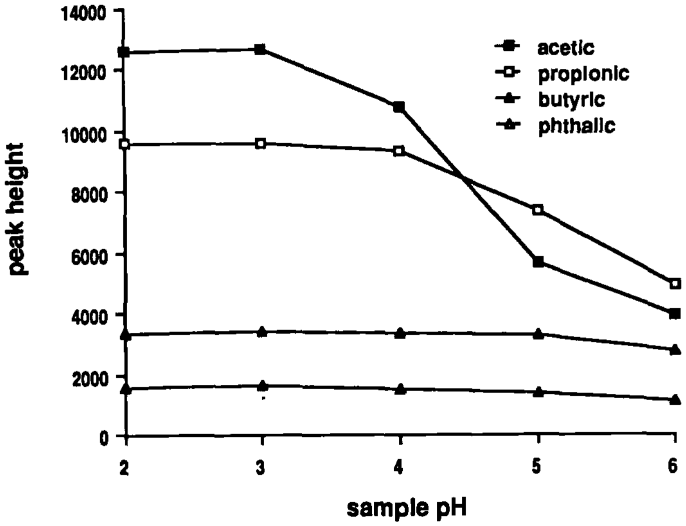

FIGURE 3
Effect of sample pH on the peak height of acetic (3.33 mM/L), propionic (3.33 mM/L), butyric (3.33 mM/L), and phthalic acid (10μM/L).

minor reductions in retention times and increased column pressures at higher methanol concentrations.

The samples had to be acidified with phosphoric acid to a pH < 3 in order to accurately quantitate the amount of acetic and propionic acid, as indicated by the effect of the sample pH on peak height (Figure 3) (15). Solutions of acetic (3.33 mM/L), propionic (3.33 mM/L), butyric acid (3.33 mM/L), and phthalic acid (10 μM/L) were adjusted to pH values between 2 and 6 with phosphoric acid or 0.1 M NaOH. Only the unionized form will be retained on the stationary phase. In addition to the sample pH, the mobile phase was buffered to pH 3, which suppressed the ionization of the acids. The pKa values of acetic acid, propionic acid, butyric acid, and o-phthalic acid are 4.76, 4.87, 4.82 and 2.95 and 5.41, respectively (19). More than 98% of the aliphatic acids was in the unionized form at a pH of three.

Linear responses between peak height and acid concentrations, as indicated by the correlation coefficients > 0.99, were obtained over the studied concentration range of 2 - 10 mM/L for the aliphatic acids and 10 - 100 μM/L for phthalic acid.

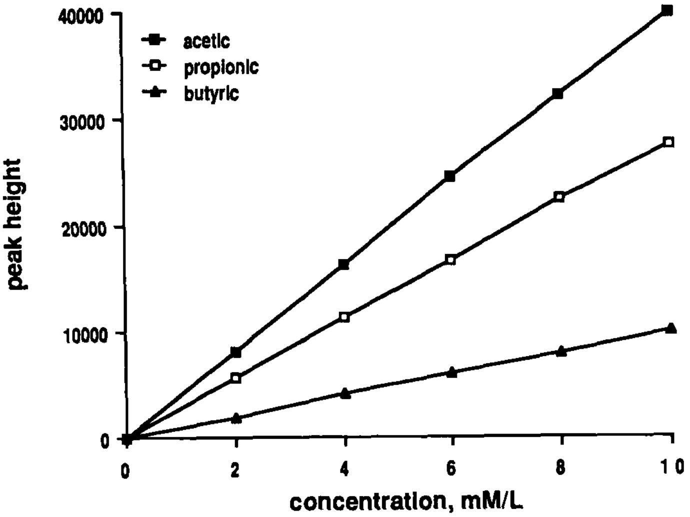

FIGURE 4
Calibration curves for aqueous standard solutions containing acetic, propionic, and butyric acid (2-10 mM/L).

The calibration curves for the aliphatic acids are shown in Figure 4. Within-run precision was determined on 5 replicate analyses of standard solutions containing 1 and 10 mM/L of aliphatic acids and 10 and 100 µM/L of phthalic acid. The coefficients of variation were 3.98 and 0.45 % for acetic acid, 3.83 and 0.77 % for propionic acid, 2.67 and 0.21 % for butyric acid, and 1.36 and 0.81 % for phthalic acid.

The minimum detectable quantity, defined as five times the base-line noise, for acetic, propionic, butyric, and phthalic acid were 0.02 mM/L, 0.05 mM/L, 0.1 mM/L, and 0.0005 mM/L, representing 24, 74, 176 and 1.7 ng in the injection, respectively. For a 30% w/v polymer pseudolatex, these quantities correspond to a % change in acetyl, propionyl, butyryl, and phthalyl content of 4.0 x 10^{-4}, 1.2 x 10^{-3}, 2.9 x 10^{-3}, and 2.8 x 10^{-5}.

In order to determine the acidic degradation products by an HPLC method, the colloidal polymer particles had to be separated from the aqueous phase prior to injection. The pseudolatexes were stabilized with sodium lauryl sulfate, an anionic surfactant. This surfactant has proven its stabilizing effect in a commercially available ethyl cellulose pseudolatex, Aquacoat (1). In this study, the colloidal

TABLE 1

Determination of Acetic and Butyric Acid after Separation of Colloidal Cellulose Acetate Butyrate Particles from the Aqueous Phase by Ultracentrifugation, Filtration, and Flocculation.

Method of separation	Acetic acid (mM/L)	Butyric acid (mM/L)
ultracentrifugation	49.86 ± 0.22	35.96 ± 0.12
filtration	51.01 ± 0.27	34.49 ± 0.37
flocculation	49.16 ± 0.45	35.63 ± 0.20

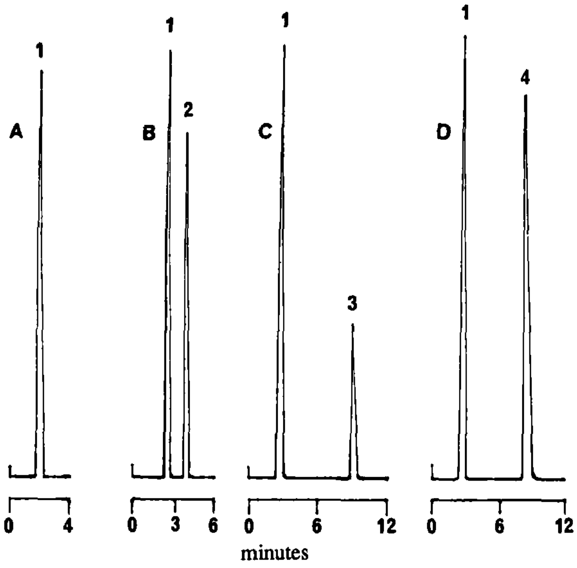

FIGURE 5
Chromatograms of aqueous solutions containing acetic (1), propionic (2), butyric (3), and phthalic acid (4) obtained after ultracentrifugation of the cellulosic pseudolatexes, cellulose acetate (A), cellulose acetate propionate (B), cellulose acetate butyrate (C), and cellulose acetate phthalate (D).

TABLE 2

Recovery of Acetic, Propionic, and Butyric Acid from Cellulose Acetate, Cellulose Acetate Propionate, and Cellulose Acetate Butyrate Pseudolatexes.

Pseudolatex	Acid,	Conc. (mM/L)	% Recovery
cellulose acetate	acetic	100	99.32 ± 1.21
		1000	94.81 ± 3.25
cellulose acetate propionate	acetic	100	96.83 ± 2.17
		1000	96.20 ± 1.15
	propionic	100	89.34 ± 2.49
		1000	90.41 ± 0.84
cellulose acetate butyrate	acetic	100	99.85 ± 3.30
		1000	100.01 ± 0.50
	butyric	100	74.90 ± 2.42
		1000	79.11 ± 0.30

polymer particles were separated from the aqueous phase by ultracentrifugation at 40,000 rpm for 1 hour, filtration through a 0.01 µm filter, or flocculation with $AlCl_3$ solution followed by centrifugation. The polymer phase could be separated from the aqueous phase completely by all methods. As shown in Table 1 with the cellulose acetate butyrate pseudolatex, the amounts of acids determined in the aqueous phase were very similar for the three methods. Chromatograms of the acids after ultracentrifugation of the cellulosic pseudolatexes are shown in Figure 5. The retention times were the same as for the standard solutions. No interference from surfactants or other impurities in the polymers were observed.

To determine the accuracy of the method, known amounts of acid were added to the pseudolatexes. After ultracentrifugation, the recovery in the supernatant was almost complete for acetic acid, however, approximately 90 % of the propionic acid and less than 80 % of the butyric acid added could be recovered (Table 2). The recovery decreased with increasing chainlength of the acids because of a more favorable acid partitioning into the polymer phase. For practical applications of this assay (e.g. stability studies), it is therefore important to determine the recovery or partition coefficient over the relevant acid concentration for the investigated pseudolatex. Similarly, if the acetyl, propionyl, or butyryl content is determined in the separated and dried polymer phase following the ASTM guidelines (13), the free acid present in the polymer phase has to be considered in the calculations. Otherwise, the degree of hydrolysis would be underestimated.

In conclusion, an analytical method was developed for the determination of acidic hydrolysis products of cellulosic esters formulated into pseudolatexes. The method is simple, rapid, and has good sensitivity, precision, and accuracy. It could be used for routine quality control of the pseudolatexes or free powders or for stability evaluation.

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