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HPLC analysis of aliphatic and aromatic dicarboxylic acid cross-linkers hydrolyzed from carbohydrate polyesters for estimation of the molar degree of substitution

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Abstract

Quantitative analysis of carboxylic acids in hydrophilic matrixes remains a challenge despite the introduction of derivatization reagents for carboxylic acids nearly three decades ago. Analysis of many bifunctional ester forming polymer cross-linkers are additionally complicated by the their amphiphatic character. We report a sensitive and selective HPLC method to quantify organic dicarboxylic acids resulting from the hydrolytic degradation of cross-linked carbohydrate polyesters. An important group of cross-linkers encompassing C_7–C_{10} aromatic and non-chromophoric aliphatic diacid chlorides were investigated. Specific validation is reported for terephthalic acid, diethylmalonic acid and sebacic acid. Linear calibration curves were obtained in the 0.5–15.0 μg/ml range corresponding to a mass fraction of 0.05–1.5% diacid to polyester. The accuracy and precision in this range was 94.4–114.1 and 6.1–24.8% RSD, respectively. This method is suitable for the routine determination of the molar or mass degree of substitution in carbohydrate polyesters.

Keywords: Dicarboxylic acids; Carbohydrate polyesters; Molar degree of substitution; Phenacyl ester derivatives; HPLC

1. Introduction

Carbohydrates are the most abundant natural polymers available on earth. Among them, starch, in all its natural and semi-synthetic forms, is of great interest to food and pharmaceutical scientists because of its inherent advantages regarding safety and biocompatibility. Unmodified starch has limited application because of the undesirable properties of gelation and retrogradation. Cross-linking starch polymer molecules with bifunctional reagents is an important chemical modification used to control and minimize these undesirable properties (Langan, 1986), as well as to be used to create desirable properties such as in the development of biodegradable drug delivery devices. In this context, material development requires a routine method which is efficient and sensitive to quantify organic dicarboxylic acids resulting from modified carbohydrate polymers in order to relate the degree of cross-linking to physical, structural or chemical properties of the polymer.

Since most polyesterification reactions result in only a 0.5–2.0% degree of molar substitution of cross-linkers in the polymer (Jarowenko, 1986), sensitive methods of detection of the diacid cross-linkers are required. In addition, many dicarboxylic acids commonly employed as cross-linkers have limited solubility profiles in aqueous and organically based solvent systems. For instance, terephthalic acid, an important cross-linker in condensation polymerizations, is practically insoluble in any solvent other than aqueous alkali.

Although many methods have been reported for the analysis of monocarboxylic acids (Coenen, Kerkhoff, Heringa, & van der Wal, 1992; Mehta, Oeser, & Carlson, 1998; Toyo'oka, 1995), only a few methods have been reported for the analysis of dicarboxylic acids (Bazirama-kenga, Simard, & Leroux, 1995; Docherty & Ziemann, 2001; Fischer, Chodura, Kotalik, Bieniek, & Kettrup, 1997; Grushka, Durst, & Kikta, 1975) with requisite sensitivities in the upper nanomolar to lower micromolar concentration

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range. Ion chromatographic methods (Baziramakenga et al.; Fischer et al.) utilizing ion-exchange separation and conductance detection report the analysis of $\leq C_6$ aliphatic dicarboxylic acids and a number of aromatic dicarboxylic acids with good sensitivity. The acidic mobile phase in one method (Fischer et al.), however, limits the method to only the most soluble of the aliphatic dicarboxylic acids. No accuracy and precision data are given for this method. The other method (Baziramakenga et al.), requires two separate chemical analyses using different analytical columns for the separation of aromatic and aliphatic dicarboxylic acids. Reported accuracy and precision for this method are good. Both methods require ion suppression to achieve optimal sensitivity. A gas chromatographic method using on-column trimethylsilane derivatization (Docherty & Ziemann), is described for the analysis of C_2–C_{10} dicarboxylic acids with excellent selectivity, but inadequate sensitivity.

Sensitive and selective HPLC methods for analysis of fatty carboxylic acids are reported based on derivatization with fluorescent chromogenic reagents (Naganuma & Kawahara, 1989; Yamaguchi, Iwata, Inoue, Hara, & Nakamura, 1990). These methods, however, require synthesis and purification of the fluorescent reagents, which are not commercially available. Even with the power of tandem mass spectrometry, derivatization is necessary to obtain adequate selectivity in complex matrixes (Kushnir, Komaromy-Hiller, Shushan, Urry, & Roberts, 2001).

Reported reverse-phase HPLC methods (Capristo et al., 1999; Grushka et al., 1975) with potentially adequate sensitivity are based on the derivatization reaction of carboxylic acids with *p*-bromophenacyl bromide (Durst, Milano, Kikta, Connelly, & Grushka, 1975) or phenacyl bromide (Borch, 1975) both of which are commercially available. The utility of this reaction was demonstrated for non-chromophoric C_4–C_6 dicarboxylic acid esters of phenacyl bromide, but not *p*-bromophenacyl bromide (Grushka et al.). These authors found the *p*-bromophenacyl derivatives of some diacids, especially fumaric and succinic, to be insoluble in almost all solvents. Phenacyl bromide derivatives showed improved solubility characteristics, but were still compromised. Chromatographic resolution of the dicarboxylic acid derivatives was poor and exhibited peak distortion on a nonyl bonded stationary phase column. Another study (Capristo et al.) showed much improved specificity and peak shape of detected dicarboxylic acids resulting from the in vivo deesterification of triglyceride esters of sebacic and dodecanedioic acids on an octadecyl bonded stationary phase. Analytical accuracy and precision was only provided with respect to the triglyceride esters for this method.

The purpose of this study was to develop a sensitive and selective HPLC assay for routine analysis of organic dicarboxylic acids resulting from the hydrolytic degradation of cross-linked polyester carbohydrates in order to determine the molar or mass degree of substitution from various polymeric materials. Particular emphasis was placed on assay methodology for an important category of dicarboxylic acid chloride cross-linkers encompassing C_7–C_{10} aliphatic and aromatic diacid chlorides. Validated analytical accuracy and precision is reported specifically for terephthalic acid, diethylmalonic acid and sebacic acid, although the method is suitable for a variety of $\geq C_6$ diacids.

2. Experimental

2.1. Preparation of reagents and sources

Acetonitrile was used as a reverse phase HPLC solvent (Optima grade, Fisher Scientific/Acros Organics, Pittsburgh, PA). *N*,*N*-dimethylformamide (Fisher), glacial acetic acid (Fisher), ammonium hydroxide (Fisher) and potassium hydroxide (Fisher) were reagent grade. Terephthalic acid (Acros), phenacyl bromide (2-bromoacetophenone, Acros), sebacic acid (Sigma-Aldrich/Fluka, St Louis, MO), diethylmalonic acid (Aldrich), suberic acid (Aldrich), azelaic acid (Fluka), and triethylamine (Sigma) were of purity >98%. Water for the mobile phase was double glass distilled. For the polymer condensation reactions, terephthaloyl chloride >99% (Aldrich), sebacoyl chloride 92% (Acros), and diethylmalonoyl dichloride 98% (Aldrich) were used as reagents.

Analytical derivatization standard solutions were prepared as follows. A 200 mM (40 mg/ml) phenacyl bromide solution was made by dissolving 400 mg phenacyl bromide in 10 ml of acetonitrile. A 60 mM triethylamine solution was prepared by adding 832 μl of triethylamine to a 100 ml volumetric flask and diluting to volume with acetonitrile.

2.2. Synthesis of carbohydrate polyesters

Polyesterification of a variety of starches and modified starches with different mass percentages of amylose and amylopectin were investigated. These included maltodextrin from corn starch (Maltrin M40, Grain Processing Corporation, Mucatine, IA), maltodextrin from waxy maize starch (C*Pharm 01980, Cerestar USA, Inc., Hammond, IN), corn starch (Sigma), high-amylose corn starch (C*Amylogel 03003, Cerestar USA, Inc.), waxy maize starch (PFP 2850, Cargill Foods, Minneapolis, MN), hydroxypropylated starch modified from tapioca starch (C*Aratex 7570, Cerestar USA, Inc.), and hydroxyethyl starch modified from corn starch (Sigma). Polyesterification of the starches was accomplished using an emulsion polymerization technique similar to that reported by Lévy and Andry (1992). Briefly, a specified carbohydrate was dispersed in aqueous 200 mM borate buffer, pH 10. Carbohydrate concentrations ranged from 3 to 20% (w/v) for starches and was 50% for maltodextrins. The dispersion was heated to approximately 100 °C to produce a solubilized colloidal solution, and then allowed to cool to about room temperature. The aqueous colloid was emulsified with

a continuum phase in a 1:5 solvent ratio, respectively, consisting of a mixture of cyclohexane and chloroform 68:32 (v/v) containing an appropriate surfactant in a homogenizer. After homogenization, an aliquot of cylcohexane/chloroform containing the diacid chloride is added to the emulsion to give a final concentration of 60 mM in the continuum phase. The reaction was carried out for 3 h at ambient temperature with stirring. Esterified carbohydrate was precipitated by adding the reacted emulsion to excess ethanol while stirring. The precipitated residue was collected either by gravitational settling or centrifugation, decanting, and washed three additional times with ethanol. The product was dried in a vacuum oven at 20–25 °C at 20 psi for subsequent analysis of cross-linking efficiency and as material to be used to determine the suitability of this assay.

2.3. Calibration standards, sample preparation, and polyester hydrolysis

Depending on the application, either terephthalic acid, diethylmalonic acid, or sebacic acid was chosen as the internal standard. The internal standard primary working solution was prepared by dissolving 40 mg of the appropriate acid in 100 ml of 5 M ammonium hydroxide to make a final concentration 400 μg/ml. The other two acids were regarded as analytes. Working solutions were prepared by dissolving 40 mg analyte with an appropriate volumes of 5 M ammonium hydroxide to give two working solutions with final concentrations of 40 and 400 μg/ml. Calibration standards were prepared by adding 500 μl of the 40 μg/ml analyte solution, and 100, 500, 1000 and 1500 μl of the 400 μg/ml analyte solution, respectively, to 4 ml screw-top glass vials containing 40 mg of non-esterified carbohydrate. A 1000 μl aliquot of the internal standard solution was added, and aliquots of 5 M ammonium hydroxide were added to each vial to give a total volume of 4.0 ml for each sample. For carbohydrate polyesters, 40 mg of the polymer, 1000 μl of the internal standard solution, and 3.0 ml of 5 M ammonium hydroxide were added to the vial.

The vials were tightly capped and hydrolyzed at 130 °C in a dry bath for 60 min. After hydrolysis, the sample was allowed to cool to ambient temperature, and 1.0-ml aliquots of hydrolysate were transferred to 15-ml centrifuge tubes. A 9.0 ml aliquot of a 60 mM triethylamine in acetonitrile solution was then added to each tube in order to precipitate any remaining insoluble carbohydrate. The tubes were vortexed and centrifuged at 2500 rpm for 30 min separating any insoluble material from the dissolved diacids. Exactly 1.0 ml of the clear supernatant was retained for subsequent analytical derivatization and transferred to a reaction vial. The theoretical final concentrations of the calibration standards are 0.5, 1.0, 5.0, 10, and 15 μg/ml corresponding to 0.5, 1.0, 5.0, 10, and 15 μg/mg carbohydrate, or 0.05–1.5% (w/w).

2.4. Analytical derivatization

The 1-ml aliquot of retained supernatant from each sample is evaporated until completely dry in a vacuum manifold. It is important that sample is dry and any excess ammonium hydroxide is removed by evaporation of the ammonia. To each sample is added 300 μl dimethylformamide, 100 μl acetonitrile, 300 μl 60 mM triethylamine in acetonitrile, and 300 μl phenacyl bromide solution. The reaction vials are capped and heated at 60 °C for 60 min in a dry bath. Following the reaction, the samples are removed from the heating block and allowed to cool to ambient temperature before subsequent processing.

2.5. Separation of soluble polysaccharides

It is necessary to reduce the soluble polysaccharide background of the sample resulting from the hydrolytic decomposition of polyesters and oxidative decomposition of the carbohydrate backbone. Although residual polysaccharides do not interfere with the derivatization, they can effect the chromatography. This separation was achieved by solid phase extraction using PrepSep (Fisher) C_{18} SPE cartridges (500 mg sorbent/6 ml). The SPE cartridge is conditioned by passing 4 ml of distilled water through the cartridge. The derivatized sample ($\sim$1 ml) is diluted with 2 ml of distilled water and drawn through the sorbent with a vacuum manifold, washed with 4 ml water, and eluted with 1.0 ml of acetonitrile into autosampler vials.

2.6. Samples for derivatization reaction kinetics

Standard solutions of diacids used in the evaluation of the derivatization reaction kinetics were prepared by dissolving the diacids in an appropriate volume of 60 mM triethylamine in acetonitrile. An aliquot of 1.0 ml was evaporated until dry, and derivatized according to the above procedure. The samples were reacted at 60 °C for varying periods of time. At the completion of each reaction period, sample vials were placed in a dry ice/acetone slurry in order to quench the reaction. Samples were retained in this bath until injected on-column for chromatographic analysis without further cleanup.

2.7. Chromatographic conditions and instrumentation

The chromatographic system consisted of ThermoSeparation (San Jose, CA) Model P2000 pumps used in the isocratic mode, Model AS3000 autoinjector, and Model UV1000 variable-wavelength UV detector. Data collection and processing used Chromquest v3.0 software and a PC computer. The stationary phase was a 25 cm$\times$4.6 mm i.d., 5 μm, 150 A pore size, C_8 bonded phase on silica chromatographic column (Beta Basic 8, ThermoHypersil-Keystone, Bellefonte, PA) and a 10 mm$\times$4.6 mm i.d., Beta Basic 8 precolumn. The mobile phase consisted of 60%

acetonitrile, 40% 100 mM acetate buffer at pH 4.5 delivered at a flow rate of 1.0 ml/min. The column temperature was ambient. The detection wavelength for all derivatized analytes was 242 nm. The injection volume was 15 μl.

3. Results and discussion

3.1. Phenacyl ester formation

The reaction of carboxylic acids with phenacyl bromide typically require the production of a 'naked' carboxylate anion utilizing either triethylamine (Borch, 1975) or crown ethers (Durst et al., 1975) as catalysts. The proper choice of base used to hydrolyze carbohydrate polyesters is essential. The high concentration of base required for the hydrolysis precludes the use of alkali metal bases, which have the potential to overwhelm the catalysts competitively with metal cations, as well as provide a large excess of hydroxide ions, which are good nucleophiles for the production of phenacyl alcohols. Both of these reactions hinder the desired formation of phenacyl esters of the analytes. The use of ammonium hydroxide for hydrolysis was found to overcome these deficiencies since excess reagent can be evaporated leaving a stoichiometric 1:1 ammonium salt with carboxylate anion. The ammonium cation is displaced with excess triethylamine as the catalyst in the reaction solvent.

Another problem is reaction solvent choice. Although the aprotic solvent acetonitrile is generally reported as the preferred reaction solvent for fatty acid derivatization, reaction products from some diacids are insoluble (Grushka et al., 1975). The phenacyl ester derivatives of the diacids investigated is this study were generally found to be solubility limited in neat acetonitrile. Co-solvency with 30% dimethylformamide improved solubility of diacids and products in the reaction medium.

Terephthalic, diethylmalonic and sebacic phenacyl ester formation was facile under the prescribed conditions.

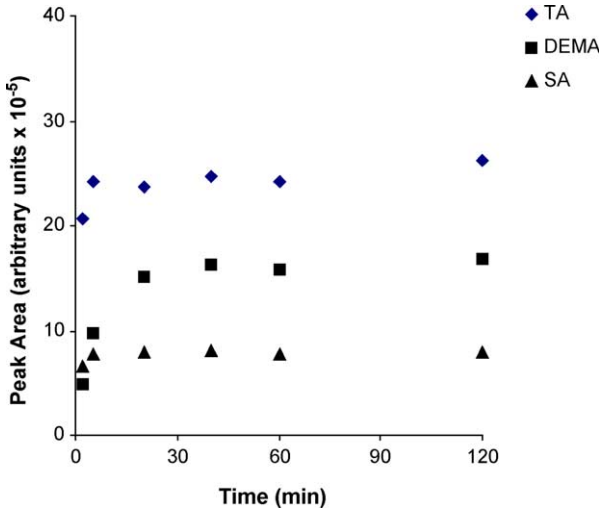

Fig. 1. Phenacyl ester formation kinetics for terephthalic acid (TA), diethylmalonic acid (DEMA), and sebacic acid (SA) at 60 °C.

The reactions kinetics (Fig. 1) showed completion of derivative formation within 40 min for all three diacids. The kinetic profiles were monotonically asymptotic. Chromatographic analyses showed that the retention time (t_R) of all corresponding products were independent of mobile phase pH in a range from 2.9 to 7.0. In addition, phenacyl ester derivative kinetics remained stable beyond 40 min for reactions carried out to 2 h. Although theoretically a mixture of both monoester and diesters products can be formed, no biphasic kinetic profile of the reaction products was observed corresponding to monoester formation, typical of A → B → C kinetics with B corresponding to the monoester and C the diester. In view of the pH independence of the reaction products, these results indicate that the phenacyl derivatives were diesters.

3.2. Chromatography and selectivity

Typical chromatograms obtained from spiked standards prepared with Maltrin M40 as the background, a blank

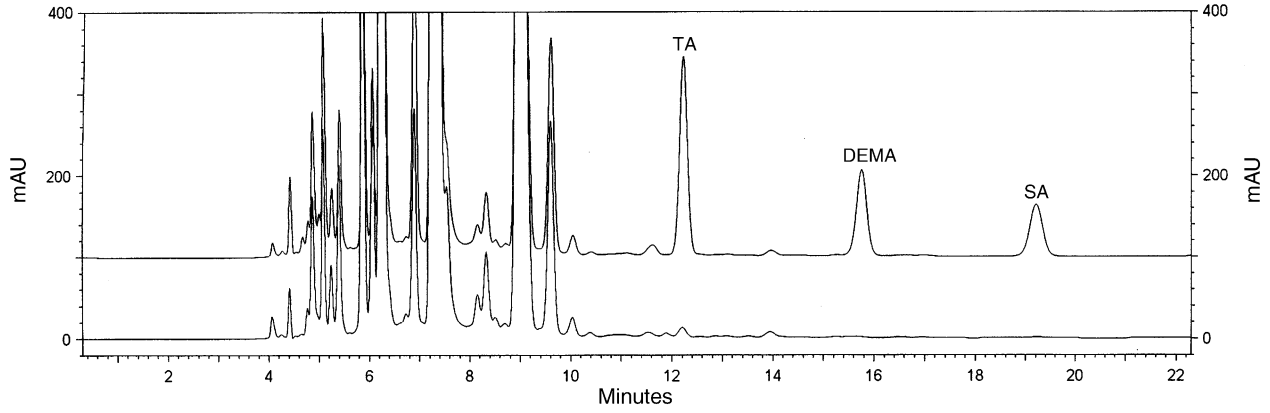

Fig. 2. Chromatogram of dicarboxylic acid ester derivatives from a sample spiked with terephthalic acid (TA), diethylmalonic acid (DEMA), and sebacic acid (SA) corresponding to 10 μg/ml of each in hydrolysate with a Maltrin M40 (maltodextrin from corn starch) matrix background (*upper trace*); blank sample containing Maltrin M40 matrix background (*bottom trace*).

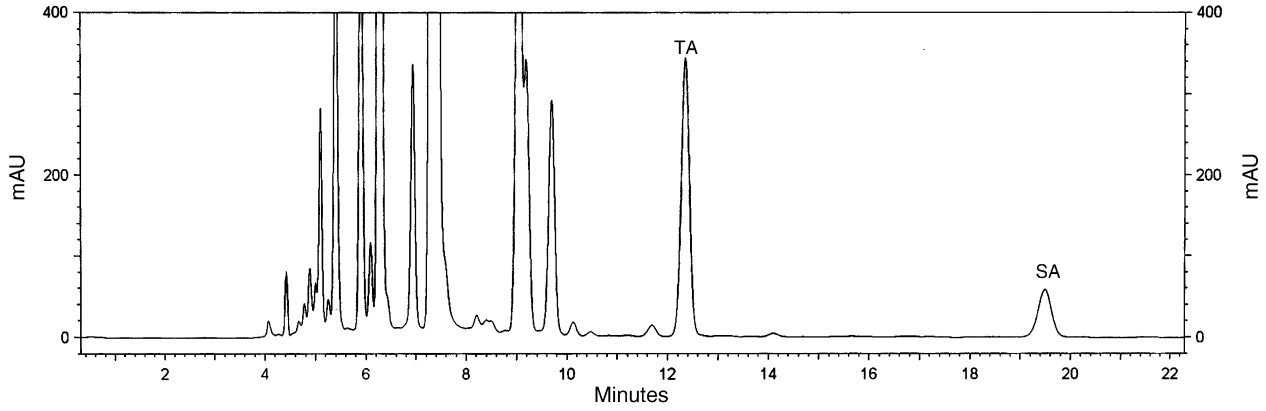

Fig. 3. Chromatogram of an authentic sample resulting from the hydrolytic degradation of a carbohydrate polyester cross-linked with terephthaloyl chloride; sebacic acid (SA) is the internal standard; terephthalic acid (TA) is the diacid hydrolysis product.

sample with Maltrin M40, and authentic Maltrin M40 polyester cross-linked with terephthaloyl chloride are illustrated in Figs. 2 and 3.

Esterification of carboxylic acids with phenacyl bromide produce a large number of reaction by-products, having retention times of <10 min in this assay. These peaks are present in chromatograms from neat samples without a carbohydrate background as well as those with carbohydrates. Similar results in other matrixes using this reaction have been reported (Capristo et al., 1999). In developing the chromatography, the intent was to adequately separate analytes of interest by increasing on-column retention relative to the reaction by-products in order to increase selectivity.

Terephthalic acid, diethylmalonic acid and sebacic acid derivative retention times were 12.3 ± 0.11 ($n=25$), 15.9 ± 0.14 (25), and 19.5 ± 0.20 (25) min, respectively. The blank control sample was clean with respect to the diethylmalonic acid and sebacic acid derivatives. Low-level interference with a similar retention time as terephthalic acid was detected in the blank which limited the lower level of quantitation for terephthalic acid to about 0.5 μg/ml. Qualitatively, chromatographic profiles from blank samples using other carbohydrates resulted in virtually identical chromatography.

The suitability of this assay for other dicarboxylic acids including suberic and azelaic acids is demonstrated in Fig. 4. For $\leq C_5$ dicarboxylic acids including succinic acid, retention was inadequate (<10 min) resulting in reaction by-product interference. Since the retention time increases with diacid hydrocarbon length, this assay would be considered to provide adequate selectivity for dicarboxylic acids of $\geq C_6$ hydrocarbon length.

Minimizing soluble polysaccharides in the injected samples was essential. During the development of this method and prior to adding an SPE cleanup step, it was noticed that analyses of an autosampler tray of samples resulted in progressively longer retention times of analytes to values more than double their initial values. Although the Beta Basic 8 column is specified by the manufacturer to be endcapped with a 7% carbon load, significant on-column accumulation of background polysaccharides was found, presumably due to their interaction with the silica support. This accumulation resulted in significant changes in chromatography probably due to mixed mode retention of analytes. Retention of the background polysaccharides

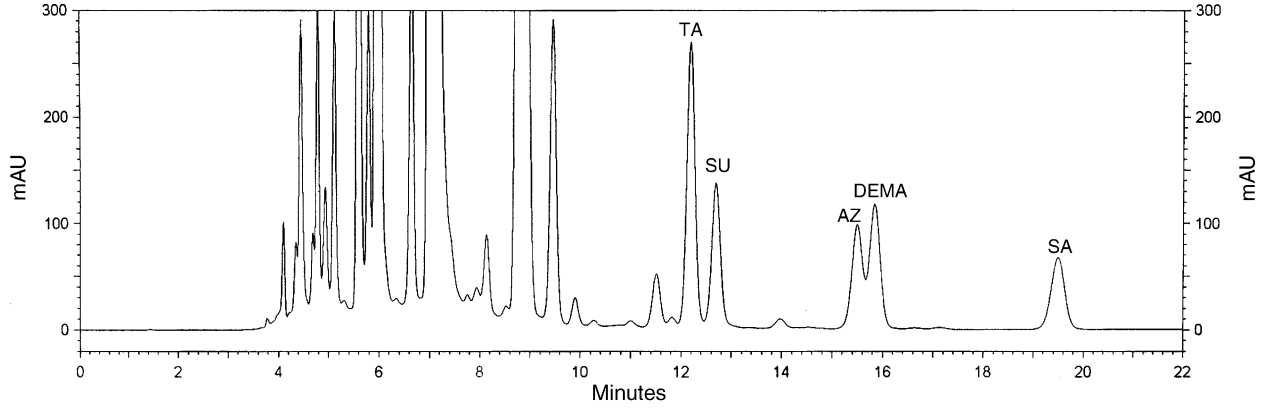

Fig. 4. Chromatogram of $\geq C_6$ dicarboxylic acids demonstrating utility of method for other diacids; terephthalic acid (TA), suberic acid (SU), azelaic acid (AZ), diethylmalonic acid (DEMA), and sebacic acid (SA).

Table 1
Calibration curve statistics

Analyte	Peak area ratio			Peak height ratio		
	Slope (SE[a])	Intercept (SE)	r^b	Slope (SE)	Intercept (SE)	r
Terephthalic acid	0.2981 (0.00796)	0.151 (0.0667)	0.992	0.4848 (0.01131)	0.175 (0.0948)	0.994
Diethylmalonic acid	0.2606 (0.01067)	−0.066 (0.0894)	0.981	0.3275 (0.01347)	−0.062 (0.1129)	0.981
Sebacic acid	0.0325 (0.00099)	−0.00137 (0.008380)	0.989	0.0260 (0.00071)	−0.00209 (0.005965)	0.992

[a] Parametric standard error.
[b] Linear correlation coefficient.

relative to the analytes on a C_{18} SPE cartridge, however, was found to be minimal, and analyte extraction was optimized to wash out soluble polysaccharides and elute the purified analytes. Polysaccharide on-column buildup was found to be reversible such that columns could be regenerated by flushing with 8.5 mM aqueous ammonium hydroxide for 30 min followed by distilled water. Routine column cleanup is recommended for assay ruggedness.

3.3. Linearity of calibration curves

Calibration curves were constructed by statistically analyzing 5 calibration levels with 5 replications within each level. All samples contained Maltrin M40 as the background carbohydrate. These curves were evaluated with regard to peak area ratio and peak height ratio of a designated analyte relative to the internal standard. The selected internal standard was added at a concentration level to attain a final concentration of 10 μg/ml after hydrolysis and carbohydrate precipitation. For terephthalic and diethylmalonic acid standard curves, sebacic acid was chosen as the internal standard. For sebacic acid standard curves, diethylmalonic acid was chosen as the internal standard. Least-squares regression analysis used a model with peak area ratio or peak height ratio as the dependent variable and concentration level as the independent variable. Linear calibration curves were established for all three diacids in the 0.5–15 μg/ml calibration range (Table 1). Extended calibration ranges from 0.2 to 40 μg/ml tended to deviate from linearity at the upper end, while the 0.2 μg/ml level was determined to be below the limit of quantitation.

3.4. Accuracy and precision

Accuracy of analyte determinations ranged from 94.4 to 114.1% for all three diacids with the exception of the 0.5 μg/ml sebacic acid concentration level (Table 2). The precision of estimates ranged from 6.1 to 24.8% RSD. UV response limited the lower limit of quantitation for sebacic acid to 1.0 μg/ml, while terephthalic and diethylmalonic acids were sufficiently sensitive to give lower limits of quantitation of 0.5 μg/ml. Although assay reproducibility was considered acceptable, improved precision could be expected based on duplicate sample analysis. This is particularly relevant with regards to diethylmalonic acid.

The effect of different carbohydrate matrixes was evaluated with regards to accuracy based on calibration curves employing Maltrin M40 as the background carbohydrate matrix. Calibration standards containing 10 μg/ml terephthalic acid and maltodextrin from waxy maize starch, high-amylose corn starch, waxy maize starch, hydroxypropylated tapioca starch, and hydroxyethylated corn starch were analyzed. The relative accuracies compared to the assayed 10 μg/ml terephthalic acid concentration level with Maltrin M40 were 93.6% for waxy maize starch, 95.0% for maltodextrin from waxy maize starch, 92.4% for high-amylose corn starch, 101.1% for hydroxyethyl corn starch, and 105.9% for hydroxypropyl tapioca starch. All assayed values were within the 95% confidence interval for terephthalic acid assayed in a Maltrin M40 matrix. These results indicate that the assay is robust with regards to different carbohydrate matrixes and polyesters.

Table 2
Accuracy and precision for the determination of terephthalic, diethylmalonic, and sebacic acids hydrolyzed from carbohydrate polyesters

Analyte	Calibration level (μg/ml)	Assay mean[a] (μg/ml)	SD	Accuracy (%)	RSD (%)
Terephthalic acid	0.5	0.57	0.123	114.1	21.5
	1.0	1.09	0.145	109.0	13.3
	5.0	4.87	0.370	97.3	7.6
	10.0	9.81	1.116	98.1	11.4
	15.0	15.16	0.926	101.1	6.1
Diethylmalonic acid	0.5	0.52	0.093	104.2	17.9
	1.0	1.03	0.154	103.1	14.9
	5.0	4.91	0.651	98.3	13.2
	10.0	10.02	1.268	100.2	12.7
	15.0	15.01	2.314	100.1	15.4
Sebacic acid	0.5	0.65	0.225	130.5	34.5
	1.0	1.09	0.271	109.3	24.8
	5.0	4.93	0.737	98.6	15.0
	10.0	9.44	0.900	94.4	9.5
	15.0	15.39	1.086	102.6	7.1

SD, standard deviation; RSD, relative standard deviation.
[a] $N=5$, calculations based on peak height ratio from appropriate calibration curves.

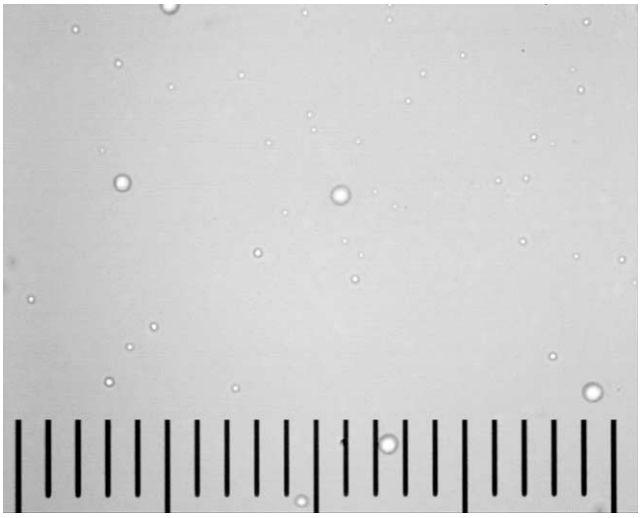

Fig. 5. Optical micrograph of nanocapsules fabricated from the emulsion polyesterification of terephthaloyl chloride and corn starch. Nanocapsules are suspended in 100 mM phosphate buffered saline. Each division of the micrometer is 1 μm.

3.5. Carbohydrate polyester degree of substitution

The cross-linking efficiency of various polyesterified starches was analyzed to relate molar degree of substitution to material properties required for nanoparticulate drug delivery systems, and to evaluate the application suitability of this method. In general, all carbohydrates tested reacted to form three-dimensional structured nanocapsules (Fig. 5). The molar degree of substitution per anhydroglucose unit ranged from 0.044 to 8.32% (Table 3).

Table 3
Estimation of bifunctional acid cross-linking efficiency in various carbohydrate polyesters

Carbohydrate	Diacid halide cross-linker	Diacid mass fraction of polyester (%)	Molar degree of substitution per anhydroglucose unit (%)
Maltodextrin from corn starch	Terephthaloyl chloride	0.39	0.58
Maltodextrin from corn starch	Diethylmalonoyl dichloride	0.041	0.044
Maltodextrin from waxy maize starch	Terephthaloyl chloride	0.57	0.84
Waxy maize starch	Terephthaloyl chloride	1.41	2.09
Corn starch	Terephthaloyl chloride	7.85	8.32
High amylose corn starch	Terephthaloyl chloride	2.49	3.69
Hydroxyethyl corn starch	Terephthaloyl chloride	1.73	2.56
Hydroxypropyl tapioca starch	Terephthaloyl chloride	1.40	2.08

Several structural variables can be identified from this preliminary study which contribute to the efficiency of polyesterification and formation of nanocapsules. Based on the molar degree of substitution per anhydroglucose unit, higher molecular weight starches are 2.5–14.3-fold more reactive when compared to maltodextrins when reacted at equivalent mass concentrations of carbohydrate and using the same cross-linker. This increased reactivity suggests that the tertiary structure of high-molecular weight polysaccharides contribute to either the accessibility or reactivity of glucose alcoholic groups. Higher molecular weight starches tended to have increased wall thickness compared to maltodextrins by microscopic observation and a reduced number of broken casts after rehydration and suspension in phosphate buffered saline. The more sterically hindered diethylmalonoyl dichloride was 10-fold less reactive than terephthaloyl chloride as a cross-linker.

Of the higher molecular weight starches, no differentiation in gross physical attributes was observed between the hydroxyethyl and hydroxypropyl substituted corn starches and unmodified corn starch.

4. Conclusion

It can be concluded that a sensitive and selective HPLC method has been developed suitable for the analysis of $\geq C_6$ dicarboxylic acids hydrolyzed from carbohydrate polyesters. Both aromatic and non-chromophoric diacids can be analyzed using a single assay. This assay can be used for investigation of detailed structural assessment of carbohydrate polyesterifcation requirements for producing nanoparticulate drug delivery systems.

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