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Synthesis and characterization of neutral and anionic cellulosic amphiphiles

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ABSTRACT

Polymer design and selection are crucial to the development of amorphous solid dispersions (ASD) for solubilization of otherwise poorly water-soluble drugs. The matrix polymer is required to interact strongly at the molecular level with the drug to prevent recrystallization, but must also be able to release the drug at an adequate rate upon entering the absorptive portion of the digestive tract. Herein we report versatile syntheses of a non-ionic, water-soluble cellulosic polymer family, cellulose trioxodecanoates, containing a hydrophilic oligo(ethylene oxide) side chain. This series of cellulose derivatives is designed for both adequate stabilization of amorphous drugs with high crystallization tendency, and timely release of those drugs. Alternatively, these polymers can be rendered anionic by also appending a pH-responsive ω-carboxyalkanoate group. Detailed structural information and structure–property relationship characterization of these amphiphilic polymers are described, which will permit evaluation of these materials as ASD polymers for enhancement of drug solubility and bioavailability.

1. Introduction

Renewable, plant-based cellulose materials are of considerable current interest for pharmaceutical applications, including for drug delivery (Curatolo, Herbig, & Nightingale, 2013; DiNunzio, Miller, Yang, McGinity, & Williams, 2008; Friesen et al., 2008; Kai, Akiyama, Nomura, & Sato, 1996; Kushida, Ichikawa, & Asakawa, 2002; Shelton et al., 2009). Hydroxypropylmethyl cellulose acetate succinate (HPMCAS) and hydroxypropylmethyl cellulose phthalate (HPMCP) are already commercially available as important drug delivery vehicles. Oral drug administration is favored by patients due to its unsurpassed convenience and low expense, and is therefore frequently the preferred method of drug delivery. Cellulose derivatives are best suited for oral drug delivery, since humans lack the cellulase enzymes needed to degrade and clear cellulose from the circulatory system. Recently, cellulose derivatives have been used to form miscible dispersions with drugs, trapping the drugs as high-energy amorphous solids to address pharmaceutical issues like poor drug aqueous solubility and bioavailability (Ilevbare, Liu, Edgar, & Taylor, 2012a). These intimate drug/polymer mixtures are termed amorphous solid dispersions (ASDs).

Due to the absence of crystal lattice energy, ASDs create higher drug solution concentrations and often higher dissolution rates. ASD may be more advantageous than other solubilization approaches including cyclodextrin complexation, micellar formation and co-solvents, because the generation of supersaturated solutions enhances drug permeation across the gastrointestinal (GI) epithelial membrane, resulting in increased drug flux and exposure in the circulation (Alonzo et al., 2011; Miller, Beig, Carr, Spence, & Dahan, 2012; Miller, Beig, Carr, Webster, & Dahan, 2012; Miller et al., 2011). However, this formulation strategy can fail due to the strong driving force for recrystallization of metastable amorphous drugs from ASDs and/or from supersaturated solutions. In order to resolve this stability issue, cellulosic polymers have been purpose-designed to interact sufficiently with the drug at the molecular level, and to possess high glass transition temperatures to prevent drug mobility and resulting crystallization. Even at high storage temperature and humidity, properly designed ASDs can be stable against crystallization for years (Kennedy et al., 2008; Newman, Knipp, & Zografi, 2012). It has been found that the effectiveness of cellulosic polymers is based on the type and strength of their intermolecular interactions with specific drugs (Wegiel, Mauer, Edgar, & Taylor, 2013). For example, for a drug containing strong hydrogen bond (H-bond) donor groups, the best ASD

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stabilization might be observed with a polymer containing strong H-bond acceptor groups.

In order to sustain supersaturation in the GI tract for a time sufficient for permeation to occur, crystallization of amorphous solids from solution, involving nucleation and subsequent crystal growth, must be prevented. Well-designed cellulosic polymers not only can prevent crystallization in the solid state, but also prolong supersaturation after ASDs are dispersed in the aqueous medium of the GI tract (Alonzo, Zhang, Zhou, Gao, & Taylor, 2010). In our previous screening study, the polymers that were effective nucleation and crystal growth inhibitors were deduced to have a moderate level of hydrophobicity relative to the drug molecule and to contain functional groups that can form specific polymer–solute intermolecular interactions, thereby disrupting the reorganization of solute molecules back into a crystalline structure (Ilevbare, Liu, Edgar, & Taylor, 2012b, 2013a, 2013b; Ueda, Higashi, Yamamoto, & Moribe, 2013). The goal is to prolong supersaturation rather than to increase thermodynamic solubility, in order to maximize permeation.

In the previous approach, cellulose ω-carboxyalkanoates showed outstanding ability to inhibit drug nucleation and crystal growth from solution (Kar, Liu, & Edgar, 2011; Liu et al., 2014). In collaboration with the Taylor group at Purdue University, our research group has studied ASD of a series of flavonoids with carboxylated cellulose derivatives including in-house synthesized cellulose acetate adipate propionate (CAAdP), commercially available cellulosic polymers carboxymethylcellulose acetate butyrate (CMCAB) and hydroxypropylmethylcellulose acetate succinate (HPMCAS), compared with the synthetic ASD polymer poly(vinylpyrrolidone) (PVP) (Li, Harich, Wegiel, Taylor, & Edgar, 2013; Li et al., 2013b,c; Li, Wegiel, Taylor, & Edgar, 2013). For all flavonoids studied, maximum concentrations achieved followed the sequence PVP > HPMCAS > CMCAB ≈ CAAdP, which is exactly the sequence of decreasing polymer solubility. The low flavonoid concentrations obtained from CMCAB and CAAdP ASDs were attributed to poor release from these very hydrophobic polymer matrices.

We became interested in preparing hydrophilic but not ionic derivatives of cellulose in order to address these release problems, and also to provide a direct test of the hypothesis advanced in these earlier studies that specific interactions of carboxyl groups with (often amine-containing) drugs are crucial to stabilization against crystallization (Ilevbare et al., 2013a, 2013b). Therefore we propose here an approach to synthesis of polymers of interest for ASD; preparation initially of a series of water-soluble cellulosic esters with varying degrees of substitution, then acylation of the remaining hydroxyl groups with either hydrophobic or pH-responsive moieties to control the hydrophilic–hydrophobic balance as well as the charge of the final product. By deepening our understanding of these structure–property relationships, we hoped to learn how to enhance drug release from ASDs without sacrificing the ability to stabilize the drug against recrystallization.

In order to impart the desired release characteristics in a neutral cellulose derivative, we elected to build on the chemistry of cellulose trioxodecanoates pioneered by the groups of Zhang and Heinze. Zhang's group reacted cellulose with the diethylene glycol diether derivative, 3,6,9-trioxodecanoyl chloride, in DMAc/LiCl solution, to obtain degrees of substitution in the range from 0.11 to 3.0 (Zhou, Zhang, Okamura, Minoda, & Miyamoto, 2001). The Heinze group explored reaction of cellulose with trioxodecanoic acid activated by reaction with *p*-toluenesulfonyl chloride in DMAc/LiCl (Heinze & Schaller, 2000) or in ionic liquid activated by reaction with 1,1′-carbonyldiimidazole (Dorn, Pfeifer, Schlufter, & Heinze, 2010). However, methods for synthesis of cellulose esters with hydrophilic oligo(ethylene oxide)-containing side chains have not been systematically studied, and further derivatization of water-soluble cellulose trioxodecanoates with hydrophobic or ionizable moieties to afford amphiphilic polymers has not been reported, nor has their use in ASDs been reported.

The non-ionic, water-soluble cellulose ether HPMC has been reported to inhibit both nucleation and growth of felodipine crystals even at ppm levels (Alonzo et al., 2012), and is generally considered to be an effective ASD polymer. With their oligoethylene glycol-containing side chains, cellulose trioxodecanoate esters may further maximize molecular interactions with drugs containing H-bond donor groups. Herein we describe an in-depth investigation of methods of trioxodecanoylation of cellulose, and thereby generate families of charged and uncharged amphiphilic cellulose mixed esters for ASDs designed for oral drug delivery applications and bioavailability enhancement.

2. Experimental

2.1. Materials

Microcrystalline cellulose (Avicel® PH-101, Fluka, DP 260) was dried under reduced pressure at 50 °C overnight prior to use. *N*,*N*-Dimethylacetamide (DMAc), 1,3-dimethyl-2-imidazolidinone (DMI) and *N*,*N*-dimethylformamide (DMF) were purchased from ACROS Organics and dried over 4 Å molecular sieves. Adipic anhydride was synthesized according to a reported procedure (Liu, Kar, & Edgar, 2012). Other purchased reagents were used as received. Oxalyl chloride (98%), 1,1′-carbonyldiimidazole (CDI, 97%), *p*-toluenesulfonyl chloride (TosCl, 99%), 4-dimethylaminopyridine (DMAP), pyridine (anhydrous, 99.0%), succinic anhydride (99%), phthalic anhydride (99%) and regenerated cellulose dialysis tubing (MW cut-off 3500 Da) were purchased from ACROS Organics. Lithium chloride, sodium hydroxide solution (0.1 N, certified) and hydrochloric acid solution (0.1 N, certified) were purchased from Fisher Scientific. 2-[2-(2-Methoxyethoxy)ethoxy]acetic acid (3,6,9-trioxodecanoic acid, TODA, technical grade) and propionic anhydride (97%) were purchased from Sigma–Aldrich.

2.2. Analytical measurements

2.2.1. NMR spectroscopy

^{1}H NMR spectra were acquired on Bruker Avance 500 spectrometers. Samples were analyzed as solutions in $CDCl_3$, DMSO-d_6 or pyridine-d_5 (*ca.* 10 mg/mL) at 25 °C in standard 5 mm o.d. tubes. A drop of trifluoroacetic acid was added to shift the water peak downfield from the spectral region of interest. 16–32 scans were obtained for each sample. ^{13}C NMR spectra were obtained on a Bruker Avance 500 MHz spectrometer using 6400 scans in D_2O (*ca.* 50 mg/mL) at 70 °C.

2.2.2. Thermogravimetric analysis (TGA)

TGA was performed on a TA Q500 thermogravimetric analysis system (TA Instruments, New Castle, DE). Thermal decomposition of ~10 mg dry samples was carried out at a heating rate of 15 °C/min from ambient temperature to 650 °C. Thermogravimetric (TG) and differential thermogravimetric (DTG) curves were plotted by Universal Analysis 2000 software.

2.2.3. Differential scanning calorimetry (DSC)

DSC analyses were performed on a TA Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE). Dry powders (8 ± 2 mg) were loaded in Tzero™ aluminum pans (TA instruments, New Castle, DE). Each sample was equilibrated at 0 °C and then heated to 180 °C at 20 °C/min, then held at 180 °C for 3 min. The sample then was cooled at 100 °C/min to −50 °C and held at −50 °C for 3 min. During the second heating cycle, the sample was heated to 220 °C at 20 °C/min. For the modulated method, the sample is

equilibrated at −50 °C, held isothermally for 5 min, then heated at 5–7 °C/min to 220 °C with modulation of ±1 °C every 40 s. All DSC measurements were verified in duplicate.

2.2.4. Ester saponification and back-titration

Cellulose trioxodecanoate (~200 mg for degree of substitution (DS) < 1 samples, ~250 mg for DS > 1 samples) was carefully weighed and charged into a 50 mL three-neck flask. An accurately measured volume of NaOH (20 mL for DS < 1 samples, 25 mL for DS > 1 samples) was added and stirred vigorously in a closed system at room temperature for 24 h.

The mixture was then titrated with 0.1 N HCl solution. Potentiometric and conductometric probes were inserted in the two sides of the flask. Each measurement was carried out by adding 100 μL aliquots of HCl solution, allowing the mixture to stir for 20 s and then collecting the pH and conductivity data points.

The precipitates were isolated by filtration, washed extensively with water, vacuum dried and then analyzed by infrared spectroscopy in attenuated total reflection (ATR) mode. 64 scans with a resolution of 4 cm^{-1} were obtained for each spectrum.

2.2.5. Elemental analysis

Cellulose trioxodecanoate propionates were washed extensively with water and vacuum dried at 40 °C for 8 h prior to elemental (C, H) analysis, carried out by Atlantic Microlab, Inc. Isocontent equations were established using Mathematica® software to convert %C and %H to DS values, and the graph was plotted using solved equations (see Supporting Material).

2.2.6. Solubility testing

Dried sample (~10 mg) was added to a 10 mL glass vial, then 2–3 mL of solvent was added. The mixture was subjected to vortex mixing for 5 min (moderate heating was applied) and placed on a roller overnight; the solubility was determined by visual examination.

2.2.7. Cloudy/clear point measurements

Solutions of cellulose trioxodecanoates (3 mL) were prepared in deionized water at 1 wt% and were stirred at room temperature until all polymer dissolved. Turbidity of the solution was measured by the transmission of red light through the sample vial as a function of the temperature while stirring at 700 rpm, using a Crystalline Particle Viewer station developed by Avantium Technologies B.V. The samples were measured from 25 to 90 °C with a heating rate of 0.5 °C/min followed by cooling to 25 °C at a cooling rate of 0.5 °C/min. The cloud points were reported as the 50% transmittance temperature during the heating scan and the clear points were identified as the temperatures where transmittance returned to 100%.

2.2.8. Size exclusion chromatography (SEC)

Molecular weight determination was by SEC in *N*-methyl-2-pyrrolidone (NMP) containing 0.05% LiBr at 50 °C on a Waters Alliance model 2690 chromatograph. Mono-disperse polystyrene standards were used to establish a universal molecular weight calibration curve. A Viscotek refractive index detector and a viscometer were used for the data collection. We report number average molecular weights relative to polystyrene standards.

2.3. Methods

2.3.1. Dissolution of cellulose in DMAc/LiCl (referred to in examples as "standard cellulose solution in DMAc/LiCl")

Microcrystalline cellulose (3.00 g, 18.5 mmol) was dissolved in DMAc (112.5 mL) and LiCl (5.63 g) by a procedure reported earlier (Edgar, Arnold, Blount, Lawniczak, & Lowman, 1995).

2.3.2. Reaction of cellulose with 3,6,9-trioxodecanoyl chloride (TODCl)

TODA (35.64 g, 0.2 mol) was first weighed into a 250 mL three-neck round-bottom flask equipped with a dropping funnel and a gas outlet connecting to a 5 M $NaHCO_3$ solution. Thionyl chloride (29.2 mL, 0.4 mol) was added dropwise into the flask with magnetic stirring at room temperature over 30 min. It was allowed to react for 30 min and then heated to 70 °C for an additional 2 h until gas formation stopped. Toluene (50 mL) was added and the unreacted thionyl chloride was co-distilled out by short-path distillation. The residual liquid (TODCl) was checked by ^1H NMR (absence of toluene peaks) and then used for the esterification reaction.

To the standard cellulose solution in DMAc/LiCl pyridine (6.72 mL, 83.3 mmol, 4.5 mol/AGU) was injected all at once, then TODCl (10.92 g, 55.6 mmol, 3 mol/AGU) pre-diluted with DMAc (30 mL) was added dropwise through a dropping funnel at room temperature. The mixture was then heated to 80 °C. After 3 h at 80 °C, the homogeneous solution was cooled to room temperature. The product was precipitated by adding the reaction mixture slowly to 800 mL isopropanol in an ice bath. The product was isolated by filtration and washed with 200 mL isopropanol. Then the waxy compound was redissolved in water, and dialyzed against DI water for 4 days. After the conductivity of the dialysis water reached the same level as that of DI water, the polymer solution was then freeze-dried. The freeze-dried products are amber yellow-colored. Yield: 6.63 g (73%).

2.3.3. Reaction of cellulose with 3,6,9-trioxodecanoic acid (TODA)/1,1′-carbonyldiimidazole (CDI)

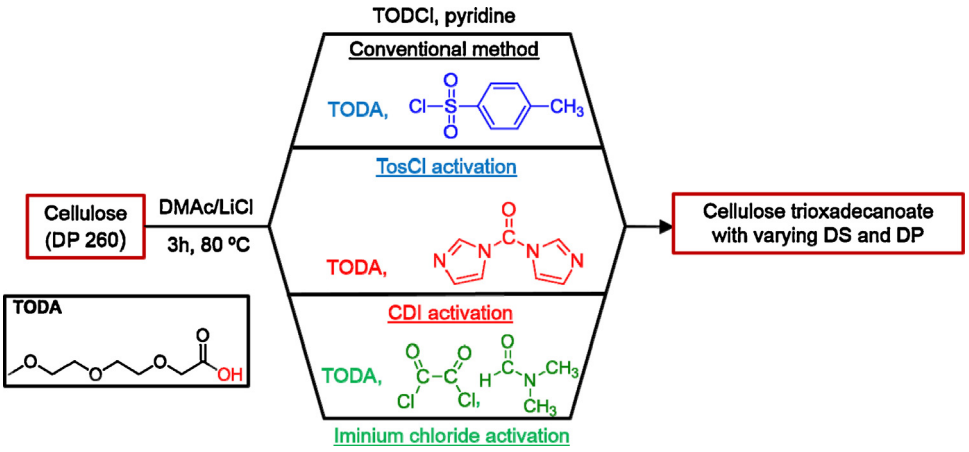
A solution of TODA (9.90 g, 3 mol/AGU) and CDI (9.00 g, 3 mol/AGU) in 30 mL of DMAc was stirred for 30 min at 80 °C. This solution was added to the standard cellulose solution in DMAc/LiCl using a dropping funnel. The mixture was then allowed to stir for 3 h at 80 °C. The homogeneous solution was cooled to room temperature and added slowly to 600 mL ethanol, then the resulting precipitate was isolated by filtration and washed with 200 mL ethanol. The precipitate was redissolved in water, then dialyzed against DI water for 4 days and isolated by freeze-drying. Yield: 5.20 g (74%).

2.3.4. Reaction of cellulose with TODA/p-toluenesulfonyl chloride (TosCl)

A solution of TODA (9.90 g, 55.6 mmol, 3 mol/AGU) and TosCl (10.59 g, 3 mol/AGU) in 30 mL of DMAc was stirred for 30 min at 80 °C. It was added to the standard cellulose solution in DMAc/LiCl using a dropping funnel. The mixture was then allowed to stir for 3 h at 80 °C. The homogeneous solution was cooled to room temperature. The product was precipitated by adding the reaction mixture slowly to 800 mL of isopropanol in an ice bath. The product was isolated by filtration and washed with 200 mL isopropanol. Then the waxy compound was redissolved in water, dialyzed against DI water for 4 days, and then freeze-dried. Yield: 5.40 g (63%).

2.3.5. Reaction of cellulose with TODA/oxalyl chloride/DMF

To prepare the iminium chloride intermediate, to a 100 mL flask equipped with a thermometer, a gas bubbler and a septum, 30 mL of DMF was added and cooled to −20 °C in an isopropanol/dry ice bath. At this temperature oxalyl chloride (4.87 mL, 55.6 mmol,



Scheme 1. Synthesis of cellulose trioxodecanoate by conventional esterification or *in situ* carboxylic acid activation methods.

3 mol/AGU) was added dropwise. During the addition, gas evolution and formation of a white precipitate were observed. After gas evolution ceased, TODA (9.90 g, 3 mol/AGU) was added. The temperature was increased to 0 °C and the mixture was stirred for 20 min. A clear solution was formed.

The obtained iminium chloride solution was added to the standard cellulose solution in DMAc/LiCl dropwise. The mixture was kept for 3 h at 80 °C. The mixture was cooled down and then added slowly to 800 mL of ethyl acetate. The resulting precipitate was isolated by filtration and washed with 200 mL ethyl acetate. Then the gummy compound was redissolved in water, dialyzed against DI water for 4 days, and then freeze-dried. Yield: 6.96 g (82%).

All reactions with 1:1 molar ratio of acylating reagent to AGU followed the same protocol for preparation, isolation and purification.

2.3.6. Perpropionylation

Cellulose trioxodecanoate (750 mg, 1.55–3.16 mmol) was dissolved in 15 mL of pyridine (186 mmol), 15 mL of propionic anhydride (117 mmol) and 75 mg DMAP (0.614 mmol). After stirring for 24 h at 80 °C, the solution was added slowly to 300 mL water. The resulting precipitate was collected by filtration, and then was washed several times with water. The crude product was redissolved in 20 mL of chloroform. This solution was added slowly with rapid stirring to 300 mL of hexanes. After filtration and washing with excess hexanes several times, the sample was dried under vacuum at 40 °C.

2.3.7. Reaction of cellulose trioxodecanoate with phthalic/succinic/adipic anhydrides

In a typical example, cellulose trioxodecanoate (250 mg, 1.05 mmol) was dissolved in 10 mL anhydrous DMI, then pyridine (0.38 mL, 4.74 mmol, 4.5 mol/mol AGU) and phthalic anhydride (0.47 g, 3.16 mmol, 3 mol/mol AGU) were added. The homogeneous solution was stirred at 80 °C for 20 h. After the reaction, the solution was cooled, placed into a dialysis bag, dialyzed against water for 2 days and then freeze-dried. Yield: 302 mg (74%).

It is worth noting that the reaction of cellulose trioxodecanoate with adipic anhydride was performed immediately after fresh, pure adipic anhydride was synthesized (Liu et al., 2014). Cellulose trioxodecanoate (250 mg, 1.05 mmol) was pre-dissolved in 10 mL anhydrous DMI, followed by the addition of adipic anhydride (0.14 g, 1 mol/mol AGU). To prevent adipic anhydride homopolymerization, no pyridine was added.

3. Results and discussion

3.1. General description and comparison of different esterification approaches

Synthesis of cellulose trioxodecanoates is potentially complicated from several perspectives, including difficult isolation of water-soluble products, complicated analysis (overlap of trioxodecanoate ^{1}H NMR signals with cellulose backbone signals), and efficiency of the cellulose/acylating reagent reaction. Therefore we began by systematically exploring several reagent/solvent/catalyst systems for synthesis of a range of cellulose trioxodecanoates (Scheme 1). We first explored the classical reaction of cellulose in DMAc/LiCl solution with an acid chloride; in this case TODCl was separately synthesized by reaction of TODA with thionyl chloride just prior to the cellulose derivatization. Thionyl chloride must be removed by co-distillation (we used co-distillation with the higher-boiling toluene), otherwise residual thionyl chloride (invisible by ^{1}H NMR) leads to temporary solidification during the esterification process, and much lower degrees of substitution.

TosCl and CDI are very effective reagents for *in situ* activation of carboxylic acids. In the case of CDI activation, the reaction has to be performed in two stages. That is, the carboxylic acid imidazolide should be preformed before esterification, otherwise addition of CDI can lead to crosslinking by carbonate formation in the presence of the polyol cellulose (Bamford, Middleton, & Al-Lamee, 1986). First-stage activation was performed within ≤30 min in our experiments, to prevent imidazolide decomposition that occurs after longer activation times, resulting in decreased substitution values (Dorn et al., 2010). No crosslinking was observed upon combining the imidazolide with cellulose, confirming that CDI was mostly consumed within the 30 min activation time. To facilitate comparison with the CDI method, a mixture of TosCl and TODA in DMAc was heated up to 80 °C for 30 min before being added dropwise into cellulose solution, even though there is usually no need for this separate activation when TosCl is used. It is believed that mixing carboxylic acid and tosyl chloride affords a mixture of anhydrides and acid chloride, accounting for the high reactivity observed upon TosCl activation (Heinze, Liebert, & Koschella, 2006).

The most efficient *in situ* activation method in our hands was the application of iminium chloride. Iminium chlorides are readily synthesized by reaction of *N*,*N*-dimethylformamide with a variety of chlorinating agents such as oxalyl chloride. Cellulose esterification was achieved by adding a pre-formed solution of the acid iminium chloride to cellulose dissolved in DMAc/LiCl. Purification of cellulose trioxodecanoate from these reaction mixtures was even simpler because most of the by-products are gaseous and reformed

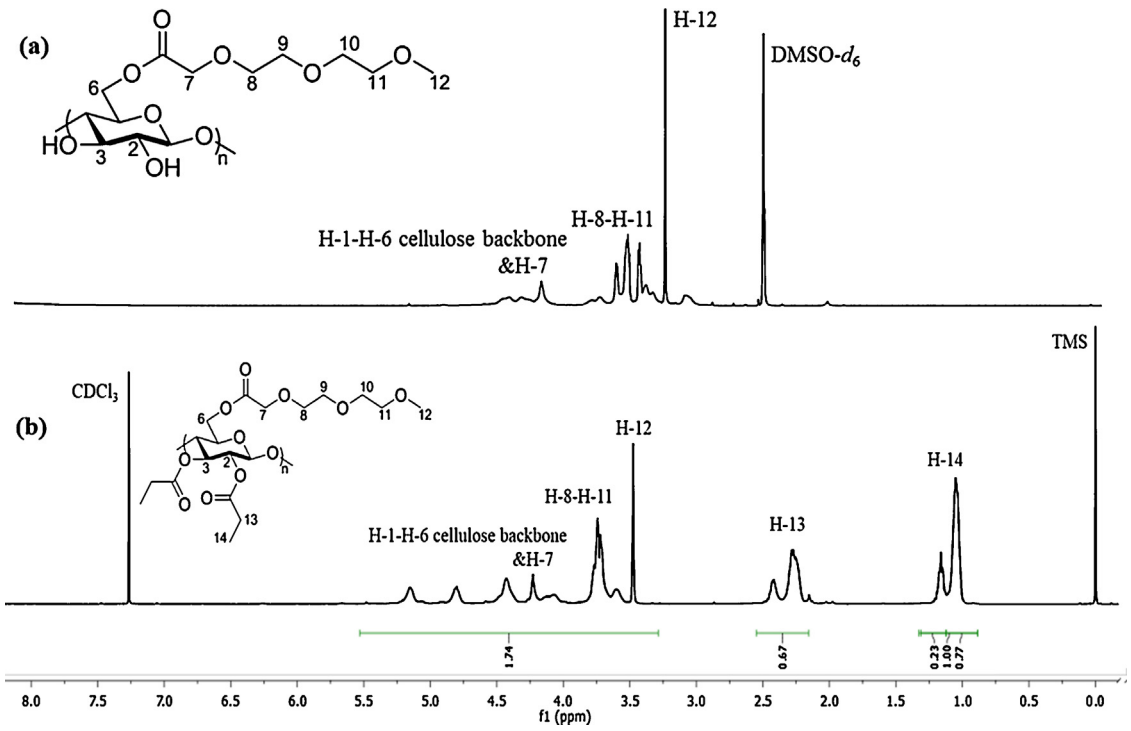


Fig. 1. Representative ^{1}H NMR spectra of cellulose trioxodecanoate (a) and cellulose trioxodecanoate propionate after peracylation (b).

DMF can be readily removed together with DMAc. After precipitation, filtration and vacuum-drying, all the cellulose trioxodecanoate products were again dissolved in water, and dialyzed against water for 4 days to remove various impurities that were prone to being entrapped in the sticky crude polymers.

3.2. Degree of substitution (DS) determination

The identities of the water-soluble cellulose trioxodecanoates with varying DS obtained using these different approaches were established first by FT-IR; it was observed that in all cases a characteristic ester absorption band at 1753 cm^{-1} was present. A number of analytical techniques have been used to characterize the DS of cellulose esters for structure–property studies, including ^{1}H and quantitative ^{13}C NMR, saponification followed by back titration of the alkali excess, pyrrolidinolysis followed by GC analysis (Samaranayake & Glasser, 1993), elemental analysis (Vaca-Garcia, Borredon, & Gaseta, 2001), alkaline hydrolysis followed by the derivatization of the extracted products to be analyzed by gas chromatography (Freire, Silvestre, Pascoal Neto, & Rocha, 2005) and transesterification with trimethylsulphonium hydroxide to afford acid methyl esters for GC analysis (Peydecastaing, Vaca-Garcia, & Borredon, 2009).

^{1}H NMR is usually convenient and is widely used for DS determination of cellulose derivatives, but in the present case it is difficult to determine DS of cellulose trioxodecanoates by ^{1}H NMR directly. Peaks for trioxodecanoate methylene and methoxy end groups at around 3.3–4.5 ppm overlap with signals for the protons of the cellulose AGU in the range 3.5–5.1 ppm (Fig. 1a). Therefore the DS of trioxodecanoate can only be calculated by difference, subtracting DS of propionate from 3.0 after perpropionylation. Complete propionylation is achieved by reaction of cellulose esters with large excess of propionic anhydride in pyridine, using DMAP as catalyst, in which ester exchange reactions have already been confirmed to be negligible (Tezuka & Tsuchiya, 1995). The mixed esters are all readily soluble in $CDCl_3$, therefore the regioselectivity of substitution can be inferred from the ^{1}H NMR spectrum (Fig. 1b), which shows peaks for the propionate CH_3 groups at 0.98–1.12 ppm (substitution at C2 and C3), and 1.12–1.23 ppm (substitution at C6). One key assumption is that perpropionylation goes to completion; in our experience this is a reasonable assumption, but small variances from full acylation cannot be rigorously excluded. Another assumption is that total possible DS is 3.0; the actual maximum potential DS will increase with decreasing degree of polymerization (DP) due to end group effects (*e.g.* it is 3.20 at DP 10 *vs.* 3.02 at DP 100). A sample DS calculation is included in Appendix 1.

Trioxodecanoate substitution occurs preferentially at the O-6 position by all methods, with the highest O-6 preference observed in TosCl activation. For example, propionylated sample PTos-1 (derived from Tos-1) showed a partial DS(TOD) at O-6 of 0.42, while only slight O-2 and O-3 substitution was observed (total DS_{2+3}(TOD) 0.06). The ^{13}C NMR spectrum of Tos-1 before perpropionylation contained only a single TOD group carbonyl resonance at 172.5 ppm, and a single C-1 resonance at 103 ppm, suggesting that only O-6 functionalization took place within the limits of detection of ^{13}C NMR (Supplementary Material, Fig. S1). With higher molar ratio (3 mol reagent per mol AGU), the ^{1}H NMR spectrum of PTos-3 (derived from Tos-3) showed almost no peak from propionyl esterification at O-6 (Supplementary Material, Fig. S2), suggesting that trioxodecanoylation at O-6 was nearly complete.

Alternatively, saponification of the ester groups with aqueous NaOH and back-titration of the excess base has long been used for DS determination of polysaccharide esters (Malm, Genung, Williams, & Pile, 1944). Complete hydrolysis of ester groups is a prerequisite for quantitative determination. In some previous studies, severe hydrolysis conditions (*e.g.* 0.5 N NaOH in aqueous ethanol, 24 h at 80 °C) had to be applied for saponification of long chain aliphatic esters due to their steric bulk and hydrophobic character (Freire et al., 2005).

Complete hydrolysis of cellulose ester derivatives can be confirmed by infrared spectroscopy (Supplementary Material, Fig. S3). Decreased intensities of the aliphatic C—H stretching band (2800–3000 cm^{-1}) and bending vibrations (900–1200 cm^{-1}) are indications of the occurrence of the ester hydrolysis. More

Table 1
Reaction conditions and DS values determined by three analytical methods.

Sample	Reaction conditions		Cellulose trioxodecanoate		
	Reagents	Molar ratio[a]	DS[b] ^{1}H NMR	DS[c] Titration	DS[d] El. Anal.
RCl-1	TODCl	1	0.46	0.55	0.56
RCl-3	TODCl	3	2.04	2.15	2.14
CDI-1	TODA/CDI	1	0.39	0.45	0.50
CDI-3	TODA/CDI	3	1.40	1.50	1.62
Tos-1	TODA/TosCl	1	0.48	0.59	0.59
Tos-3	TODA/TosCl	3	2.02	2.11	2.10
Imin-1	TODA iminium chloride	1	0.58	0.60	0.68
Imin-3	TODA iminium chloride	3	1.88	2.02	2.35

[a] Mol reagent/Mol AGU.
[b] Determined by ^{1}H NMR after perpropionylation.
[c] Determined by saponification and back-titration.
[d] Determined by elemental analysis (%C) after perpropionylation.

importantly, disappearance of the ester carbonyl (C=O) band at 1753 cm^{-1} suggested that complete saponification of all cellulose trioxodecanoates could be achieved under mild conditions (0.1 N aq NaOH, 25 °C, 24 h). The hydrophilic nature of cellulose trioxodecanoates facilitated the deacylation process, overriding any problems caused by steric bulk. The optimized alkali concentration and mild temperature reduced the likelihood of side reactions.

Back-titration with 0.1 N HCl solution was used to measure the excess sodium hydroxide, and the volume of added HCl was plotted against the pH change (Supplementary Material, Fig. S4, DS calculation Appendix 2). The peak in the derivative graph indicated the equilibrium point and it coincided with the inflection point in conductometric measurements (Supplementary Material, Fig. S5).

Even under these mild conditions, the cellulose trioxodecanoate sample might undergo side reactions during saponification. In the absence of a DS method of certain reliability, it was difficult to establish with high confidence whether such side reactions were occurring, or their extent if they did occur. DS values by titration were consistently higher than those measured by ^{1}H NMR of the perpropionates, averaging approximately 10.7% higher. This would be consistent with overestimation of DS by titration due to a minor side reaction that consumed NaOH.

We also attempted determination of DS of the mixed cellulose esters by elemental analysis. Using this method, DS values of the two ester types can be calculated with precision of ±0.10 if the accuracy of the hydrogen measurement is within ±0.1% (Vaca-Garcia et al., 2001). However, %H determinations on laboratory scale do not typically reach that level of accuracy (%H accuracy is typically *ca.* ±0.3%), which could result in inaccurate DS values as shown in Fig. S6 (Supplementary Material). These polymers are quite hygroscopic (displaying a prominent water peak in "anhydrous" $CDCl_3$ in the ^{1}H NMR spectrum), probably contributing to variation during elemental analysis. Thus two-dimensional DS calculation by elemental analysis was not applied; instead, to reduce uncertainty, only %C data were used for calculation of DS (TOD) calculation (DS calculation Appendix AAppendix 3), presuming that the perpropionylation is complete and relying on the fact that DS calculations based on %C are less sensitive than those based on %H assuming the same level of measurement error, as shown in Fig. S6. For instance, +0.05% variations in measured values of %C and %H for a cellulose trioxodecanoate sample of actual DS 2.0 would result in calculated DS values of 1.95 and 2.44, respectively.

A summary of reaction paths, conditions and results is given in Table 1. Together, the analytical methods give a good general picture of product DS, but for the reasons cited above we cannot be certain which method is the most accurate for a given sample. Comparing synthetic methods, reaction with the acid chloride in DMAc/LiCl was overall as efficient as any other method; the CDI method appeared to be least efficient of the three carboxylic acid activation methods, while TosCl and iminium chloride methods had roughly equal efficiency.

3.3. Investigation of physicochemical properties

As previously reported (Zhou et al., 2001), cellulose trioxodecanoates within a certain DS range undergo a phase transition from soluble to insoluble upon heating in water. Among our polymers, cellulose trioxodecanoates with DS (TOD) higher than 1.8 are soluble in water at room temperature, but precipitate out of solution when heated, independent of the activation method used. The phase transition between precipitation and solution in water is reversible, controlled by the temperature (threshold points were determined at around 80–85 °C). Cellulose trioxodecanoate with a slightly lower DS (1.40, ^{1}H NMR, CDI method) does not show thermal aggregation or precipitation behavior, indicating that this phenomenon is highly DS-dependent.

Since ASD polymers are sometimes combined with drugs using thermal extrusion methods (Sarode et al., 2014), we examined the thermal stability of these polymers using TGA. By TGA, cellulose trioxodecanoates and their propionate derivatives are all relatively thermally stable; degradation onsets occur from 277 to 344 °C, close to the thermal degradation temperature of microcrystalline cellulose (starting material).

Lower temperature thermal behavior of ASD polymers is also important; high glass transition temperatures (T_g) help to prevent drug mobility and ensuing crystallization even upon exposure to high humidity and high ambient temperature. DSC (or mDSC) thermograms of cellulose trioxodecanoates with low and high DS(TOD), as well as their respective perpropionates are shown in Fig. 2. For cellulose trioxodecanoate samples with low DS (TOD) (less than 1.40), T_g could not be detected in the temperature range of −50 to 220 °C (Fig. 2a). Perpropionylation eliminated this problem, with T_g readily observed (71–93 °C) for these peracylated products (Fig. 2c). In the case of cellulose trioxodecanoate with DS 2.02, $T_g \sim 91$ °C is measured by mDSC (Fig. 2b). Interestingly, its propionylated product showed characteristic melting and crystallization peaks from the cooling and 2nd heating scans (Fig. 2d). Previous studies also identified this thermal phenomenon in fully substituted cellulose trioxodecanoate and related it to the formation of a columnar liquid crystalline mesophase (Zhou et al., 2001). We did not observe the same thermogram pattern in our highly substituted cellulose trioxodecanoates (DS 2.0), but only in their perpropionylated derivatives. We hypothesize that this thermal behavior is attributable to the crystallization of the oligo(oxyethylene) substituents. Side chain cooperative motions, observed by melting and crystallization transitions, have been reported in waxy esters of cellulose (Lopez-Velazquez, Bello, & Perez, 2004; Sealey, Samaranayake, Todd, & Glasser, 1996). No

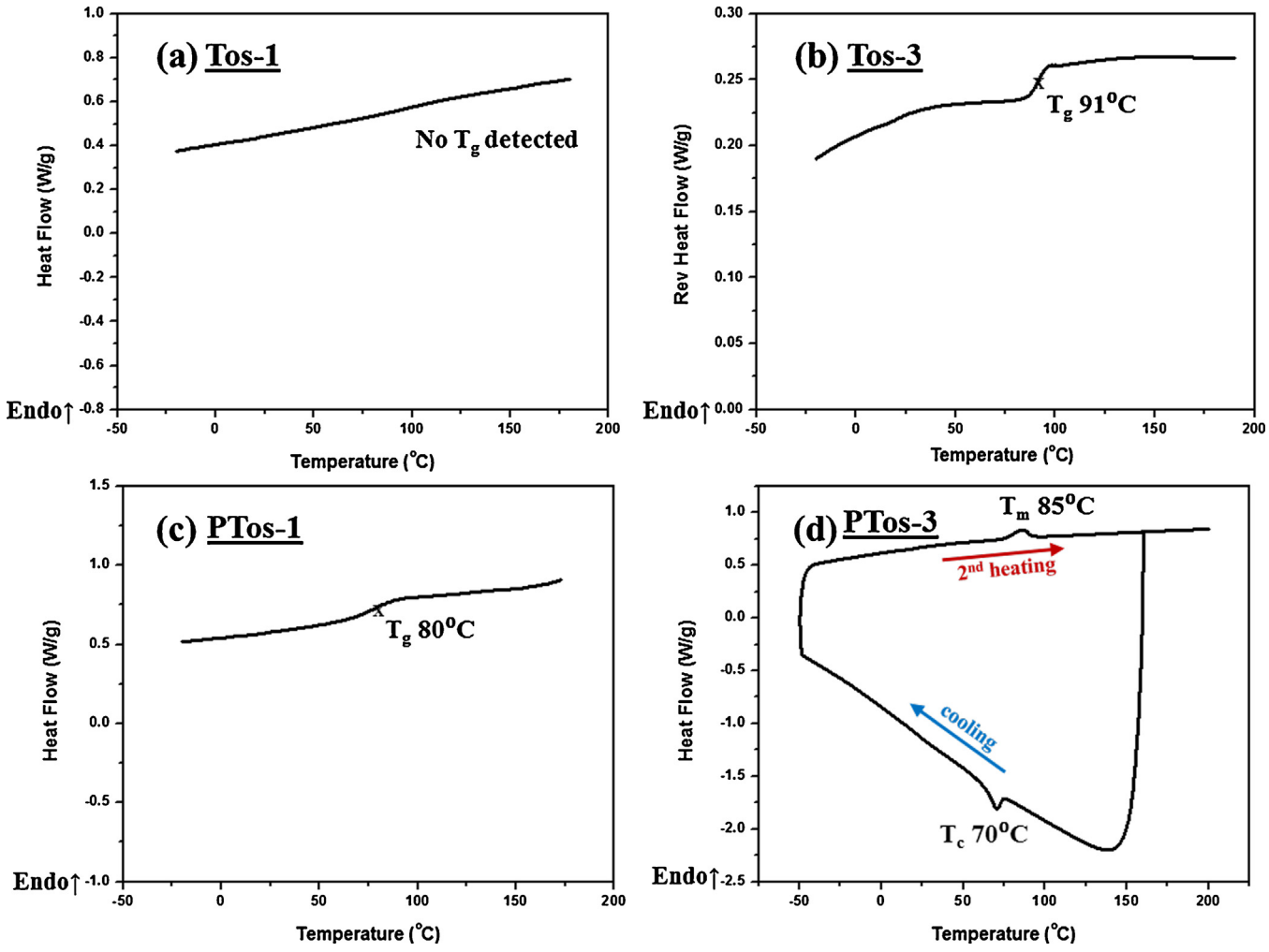


Fig. 2. DSC thermograms of (a) Tos-1 (DS TOD 0.48), (b) Tos-3 (DS TOD 2.02, modulated DSC), (c) PTos-1 (after perpropionylation of Tos-1) and (d) PTos-3 (after perpropionylation of Tos-3).

crystallization and melting transitions were found during the fast cooling and 2nd heating scan for sample Tos-3 (Supplementary Material, Fig. S7), it is likely that the remaining hydroxyls of partially substituted cellulose trioxodecanoate (DS 2.0) can retard the tendency for oligo(oxyethylene) side chains to crystallize through hydrogen bonding.

In summary, selected physicochemical properties of cellulose trioxodecanoates and their perpropionylated derivatives are listed in Table 2. In designing ASD polymers, a rule of thumb is that recrystallization of the amorphous drug during transportation and storage is best prevented by using a polymer whose T_g is 100 °C or higher, thereby accounting for the plasticizing effects of water (humidity), the drug, and high ambient temperature (Newman et al., 2012), and of course the polymer must not itself crystallize. From this perspective, low DS cellulose trioxodecanoates and their esters, together with some of the high DS cellulose trioxodecanoates are promising ASD matrix candidates.

From the GPC results (Table 2), the use of the mild CDI catalyst provided products with DP values (238–252) similar to that of the starting material (DP 260). TosCl and iminium chloride activations

Table 2
Physicochemical properties of cellulose trioxodecanoates and derived perpropionates.

Cellulose trioxodecanoate (before propionylation)				Cellulose trioxodecanoate propionate (after propionylation)					
No.	DS[a] TOD	Precipitation behavior (cloudy/clear point °C)	T_g (°C)	No.	T_g (°C)	T_m (°C)	T_c (°C)	M_n (kDa)	DP[b]
RCl-1	0.47	No	/	PRCl-1	90	/	/	[c]	[c]
RCl-3	2.04	Yes (85.1/85.7)	/	PRCl-1	22	92	74	[c]	[c]
CDI-1	0.39	No	/	PCDI-1	93	/	/	56.5	252
CDI-3	1.40	No	/	PCDI-3	51	97	/	92	238
Tos-1	0.48	No	/	PTos-1	80	/	/	36.3	152
Tos-3	2.02	Yes (78.5/80.9)	91	PTos-3	21	85	70	82.6	170
Imin-1	0.58	No	/	PImin-1	71	/	/	52.8	207
Imin-3	1.86	Yes (86.4/87.2)	126	PImin-3	/	84	67	96.5	210

/, could not be detected.
[a] Determined by ^{1}H NMR after perpropionylation.
[b] Molecular weight of monosaccharide repeating unit is calculated based on the DS information determined by ^{1}H NMR after perpropionylation.
[c] Dispersities ($Đ$) of the polymers are unusually high (>20), therefore, results are not included.

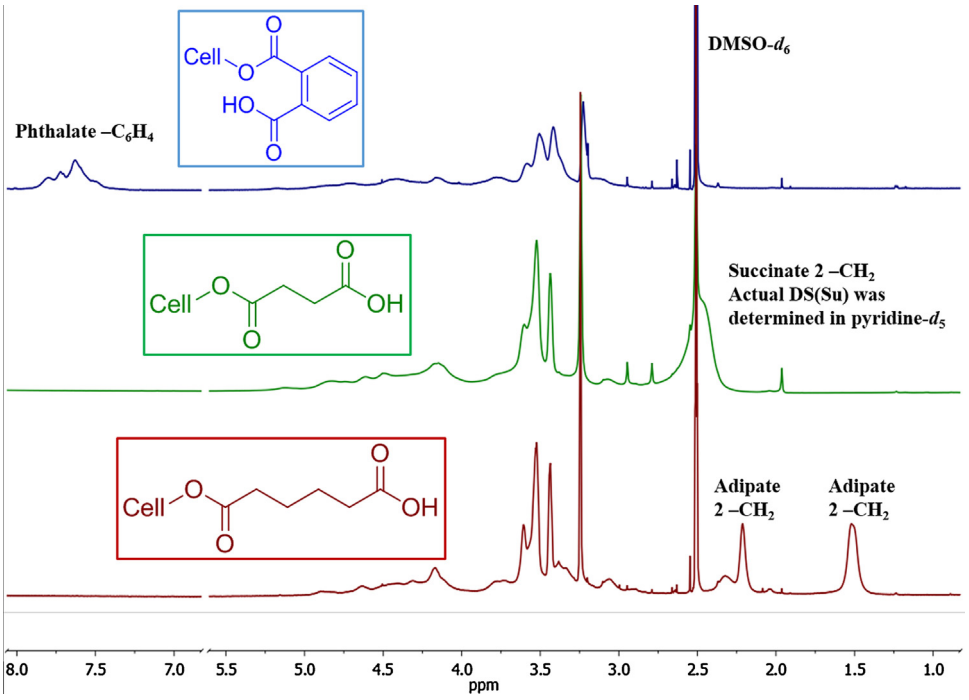


Fig. 3. [1]H NMR spectra of cellulose trioxodecanoate phthalate/succinate/adipate, derived from cellulose trioxodecanoate RCl-1.

caused some chain degradation due to the formation of acid *in situ*, and TosCl more severely reduces molecular weight. In general, dispersities (*Đ*) of those cellulose esters were in the range from 1.5 to 3.5. However, conventional esterifications with TODCl seemed to cause severe chain cleavage, resulting in large dispersities (*Đ* > 20) (see Supplementary Material, Table S1). Solubility evaluations of cellulose trioxodecanoates are summarized in Table 3. All cellulose trioxodecanoates synthesized (DS range 0.39–2.04) are soluble in water and DMSO. Higher DS derivatives (RCl-3, Tos-3 and Imin-3), not only exhibit phase transition behavior in aqueous solution, but also possess significant amphiphilic nature; they are soluble in a wide range of organic solvents including acetone, acetic acid and hot ethanol. This indicates that solution processing of high DS cellulose trioxodecanoates (*e.g.* spray-drying) is possible. Low DS trioxodecanoates can be rendered organic soluble by adding hydrophobic groups such as propionate esters, or functional groups such as ionizable moieties.

3.4. Anionic derivatives of cellulose trioxodecanoates

A common observation when using water-soluble polymers as ASD matrices is rapid drug release in the gastric environment; such release can be problematic in some therapeutic situations (Pereira et al., 2013). As an alternative to the hydrophobic alkanoyl groups, we also attempted to append ω-carboxyester moieties to the remaining free hydroxyl groups of the cellulose trioxodecanoate products, in order to impart pH sensitivity, some hydrophobicity from the poly(methylene) chains, and create the potential for specific interactions between the polymer carboxyl groups and drug H-bond acceptors. Cellulosic polymers with good water dispersibility might also serve as rheology modifiers for waterborne coating applications (Edgar, 1993).

Two cellulose trioxodecanoates were chosen (RCl-1 and RCl-3) for this set of experiments, with DS(TOD) of *ca.* 0.5 and 2.0, respectively. Reactions were carried out in the polar, aprotic, non-derivatizing solvent DMI, using pyridine as base catalyst. The only exceptions were the reactions with adipic anhydride; since weak base is known to initiate adipic anhydride homopolymerization (Albertsson, Carlfors, & Sturesson, 1996), no catalyst was used in these experiments. Ring-opening esterifications with RCl-3 were ineffective. DS values achieved were 0.10 (phthalate) and 0.02 (succinate), upon reaction with the corresponding cyclic anhydrides (3 mol/mol of modified AGU). DS of succinate was unexpectedly low, largely due to auto-catalyzed succinate hydrolysis during the dialysis step. The products after freeze-drying all remained soluble in water. Reaction of RCl-1 with the respective cyclic anhydrides (1 mol/mol of modified AGU) provided ω-carboxyester DS of 0.17 (phthalate) and 0.46 (succinate); these products were still soluble in water. However, when 3 equivalents of anhydride was used, the isolated products lost aqueous solubility and formed cloudy dispersions in water. The ω-carboxyester DS values increased to 0.75 for phthalate and 1.02 for succinate, according to ^{1}H NMR analysis (Fig. 3) However, the reaction of RCl-1 with 3 equivalents of adipic anhydride led to crosslinking, with formation of a transparent gel, similar to the crosslinking observed and rationalized in our previous studies (Liu et al., 2012). Reaction of RCl-1 with smaller amounts of adipic anhydride (1 mol/mol of modified AGU), or of RCl-3 with adipic anhydride (3 mol/mol of modified AGU) provided organic soluble products with DS (adipate) of 0.20 and 0.49, respectively. However, the calculated DS values are certainly overestimated to some extent, since adipic anhydride

Table 3
Solubility range of cellulose trioxodecanoates.

No.	DS[a] TOD	Solubility				
		H_2O	Acetone	Acetic acid	Ethanol	DMSO
RCl-1	0.47	+	–	–	–	+
RCl-3	2.04	+	+	+	*	+
CDl-1	0.39	+	–	–	–	+
CDl-3	1.40	+	–	–	–	+
Tos-1	0.48	+	–	–	–	+
Tos-3	2.02	+	+	+	*	+
Imin-1	0.58	+	–	–	–	+
Imin-3	1.86	+	+	+	*	+

+, soluble; –, insoluble; *, soluble with some heating, precipitate out upon cooling
[a] Determined by ^{1}H NMR after perpropionylation.

homopolymerization is still an issue; poly(adipic anhydride) can be observed in the product ^{1}H NMR spectrum but is difficult to quantify due to spectral overlap. The homopolymer could not be easily removed through dialysis, or by additional product washing with non-polar hexanes. Overall these anionic cellulose trioxodecanoate ω-carboxyesters have interesting potential as ASD matrix polymers.

4. Conclusion

A family of water-soluble, non-ionic cellulose trioxodecanoates with DS ranging from 0.39 to 2.04 was prepared by the reaction of microcrystalline cellulose (DP 260) through conventional esterification and three *in situ* carboxylic acid activation approaches. NMR spectroscopy after perpropionylation, saponification followed by back-titration, and elemental analysis were used to determine the DS values, and the benefits and limitations of each method have been described; they are all useful and together give a good approximate picture of substituent DS, but none of the three can yet be confirmed as a gold standard method. Thermal behaviors of these esters are predictably dependent on substituent DS. Extent of cellulose chain degradation using the three activation methods is in the order TosCl > iminium chloride > CDI. The good solubility in water and polar aprotic solvents for most of these materials means that cellulose trioxodecanoates are amenable to solution processing for various applications, and their generally good thermal stability is promising for extrusion applications. Anionic derivatives have been readily synthesized by reaction with cyclic anhydrides; the resulting carboxylate substitution (phthalate, succinate and adipate) will impart pH sensitivity in aqueous solution, and some of these derivatives appeared to be easily water-dispersible.

Although some highly substituted cellulose trioxodecanoate esters (DS ≈ 2.0) exhibit a tendency for crystallization due to cooperative motion of the side chains, properly designed cellulose trioxodecanoates and their derivatives are promising for further studies as ASD polymers for enhanced oral drug delivery. Formulation with lipophilic and highly crystalline drugs, impact of these polymers upon creation of supersaturated drug solutions, and stability of amorphous drugs in both solid and solution phases will be described in forthcoming reports.

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Appendix A.

Appendix 1. DS calculation by ^{1}H NMR spectroscopy after perpropionylation (I: integration)

$$\frac{3(3-\mathrm{DS_{TOD}})}{7+13\mathrm{DS_{TOD}}} = \frac{I_{\mathrm{H,CH_3\ of\ propionyl}}}{I_{\mathrm{AGU+TOD}}}$$

$$\mathrm{DS_{TOD}} = \frac{9I_{\mathrm{AGU+TOD}} - 7I_{\mathrm{H,CH_3\ of\ propionyl}}}{3I_{\mathrm{AGU+TOD}} + 13I_{\mathrm{H,CH_3\ of\ propionyl}}}$$

Appendix 2. DS calculation after saponification and back-titration

$$\mathrm{DS_{TOD}} = \frac{(V_1 - V_2) \times 0.1 \times (162 + 160\mathrm{DS_{TOD}})}{1000 \times m}$$

$$\mathrm{DS_{TOD}} = \frac{16.2(V_1 - V_2)}{1000 \times m - 16(V_1 - V_2)}$$

where V_1, V_2 are the volumes of 0.1 N NaOH and 0.1 N HCl solution (in mL) used during the titration, and m is the mass of added cellulose trioxodecanoate (in g).

Appendix 3. DS calculation by elemental analysis (%C: carbon content percentage)

$$\%\mathrm{C} = \frac{12.011[6 + 7\mathrm{DS_{TOD}} + 3(3 - \mathrm{DS_{TOD}})]}{12.011[6 + 7\mathrm{DS_{TOD}} + 3(3 - \mathrm{DS_{TOD}})] + 1.008[10 + 12\mathrm{DS_{TOD}} + 4(3 - \mathrm{DS_{TOD}})] + 15.999[5 + 4\mathrm{DS_{TOD}} + (3 - \mathrm{DS_{TOD}})]}$$

$$\mathrm{DS_{TOD}} = \frac{180.185 - \%\mathrm{C} \times 330.333}{\%\mathrm{C} \times 104.105 - 48.044}$$

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.07.004.

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