Polymer 54 (2013) 2632–2638

Contents lists available at SciVerse ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Separation of cellulose acetates by degree of substitution

Hewa Othman Ghareeb, Wolfgang Radke*

Fraunhofer-Institut für Betriebsfestigkeit und Systemzuverlässigkeit LBF, Bereich Kunststoffe, Schlossgartenstraße 6, D-64289 Darmstadt, Germany

ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form
20 March 2013
Accepted 21 March 2013
Available online 28 March 2013

Keywords:
Cellulose acetate
Degree of substitution
Degree of substitution distribution

ABSTRACT

A liquid chromatographic method was developed allowing separating cellulose acetates (CA) with respect to degree of substitution (DS). The normal-phase gradient separation, which is based on an adsorption–desorption mechanism, uses a multi-step gradient of dichloromethane (DCM) and methanol (MeOH) on a bare silica column as stationary phase. Applicability of the developed multi-step gradient allows separating CAs within the DS-range DS = 1.5–2.9, which is the target DS-range for most CA applications. To the best of our knowledge the developed method for the first time allows determination of the DS-distribution of intact CA chains over a wide DS-range.

1. Introduction

Due to the architectural complexity of cellulose derivatives, analytical methods for comprehensive analysis of the molecular heterogeneities have become increasingly important in cellulose research field. Cellulose derivatives are complex copolymers being heterogeneous at least in molar mass and chemical composition. These heterogeneities need to be characterized as they critically affect many properties of these derivatives such as adhesion strength, solubility, viscoelasticity and drug release from hydrophilic tablets just to name a few [1–7]. The heterogeneity in chemical composition of cellulose derivatives includes the distribution of the substituents within the individual anhydroglucose units (AGUs), i.e. the partial degree of substitution (DS) of O-2, O-3, and O-6 atoms, as well as the distribution of the substituents among the polymer chains (heterogeneity of 1st order) and along the polymer chains (heterogeneity of 2nd order). DS refers to the average number of substituted hydroxyl groups per AGU. Therefore, DS can take values between 0 and 3 for unbranched cellulose derivatives.

Today cellulose derivatives are mainly characterized in terms of their molar mass and molar mass distribution (MMD), their average DS and of the distribution of the substituents on both the monomer and oligomer levels. NMR techniques are applied to obtain a first insight in the partial DS within the monomer units [8–16]. Alternatively complete acidic chain degradation and subsequent determination of the resulting differently substituted AGUs can be applied to characterize the composition of cellulose derivatives [17–20]. In another characterization pathway, the polymer chains are completely or partially degraded using acids or enzymes. The partial degradation of the chains results in a mixture of monomers and/or oligomers present in different molar ratios. These mixtures are separated and characterized subsequently in detail using various analytical techniques, alone or in combination, such as size exclusion chromatography (SEC) [21–24], anion-exchange chromatography (AEC) [23–28], gas–liquid chromatography (GLC) [21,27,29,30] and mass spectrometry (MS) [26–35]. This gives information on the monomer composition as well as the substituent distribution on the oligomeric levels. Such data can be compared with the product distribution expected for a specified monomer distribution along the chain at a certain DP, e.g. a completely random monomer distribution. This procedure therefore yields information on the distribution of the differently substituted AGUs along the polymer chain (2nd order heterogeneity).

In general, any or at least a large amount of information on whether the different monomeric or oligomeric units result from the same or from different chains is lost, if the sample is fully or partially degraded. Therefore, establishing methods to characterize the chemical heterogeneity of cellulose derivatives on the level of intact polymeric chains is still a highly challenging task.

Gradient HPLC has been shown to be a powerful tool for separating (co)polymer molecules according to chemical composition [36,37]. Such separations allow determining the chemical composition distribution (CCD) of the copolymers, i.e. the distribution of the monomer units among the different polymer chains. Strictly, cellulose derivatives have to be regarded as complex polymers

* Corresponding author.
E-mail address: wolfgang.radke@lbf.fraunhofer.de (W. Radke).

0032-3861/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.polymer.2013.03.041

composed of at least eight different monomer units, which are the differently substituted AGUs. Today, it is hardly possible to separate intact cellulose chains of such complexity by the fractions of the differently substituted AGUs. In our somehow more naïve picture we regard cellulose derivatives as being composed of only two kind of structural units, the AGUs and the substituents. A separation with regard to DS is therefore similar to a separation of binary copolymers according to chemical composition. Thus, the DS-distribution is the analogue to the chemical composition distribution of binary copolymers. The efforts on separation and characterization of cellulose derivatives, particularly CAs, with respect to the DS-distribution are very limited and some of the attempts described in literature are briefly discussed here:

SEC with MALLS/RI/UV detections has been used to gain insight on the substitution distribution along the chains of CA [38]. The procedure requires the presence of substituents that can be detected by an UV-detector in relation to the molar mass determined by MALLS/RI. It was found that DS varies with molar mass, with the lower molar mass fractions being less substituted than the higher molar mass fractions. However, the DS gradient over the MMD curve does not represent a true separation in terms of DS, because SEC separation is based on size, not on chemical composition. Furthermore, since CAs do not contain UV-active groups, they had to be modified to the respective amide derivatives. Thus, the modification procedures have to be quantitatively performed in order to achieve reliable results.

Other techniques used to obtain insight into the CCD of CAs are fractionated precipitation and thin layer chromatography (TLC). Fractionated precipitation involves dissolving a solid polymer in a good solvent and precipitating the desired fractions stepwise by decreasing the solubility as a result of the controlled addition of a non-solvent. However, this method is laborious and requires large amounts of solvents and samples. In addition it is not very selective and difficult to automate. In most cases, the isolated fractions of CAs varied in DP but were of the same DS [39–43]. Thus, the separations were based on molar mass rather than on chemical composition. Kamide et al. evaluated the use of TLC for the separation of CAs according to DS and molar mass [44,45]. By stepwise changing the eluent composition, they were able to identify experimental conditions where clear dependences of retardation factor (R_f) on either DS or molar mass were observed. The resulting separations according to DS and molar mass were found to be independent of molar mass and DS, respectively. The methods allowed calculating both the DS-distribution and the MMD. However, TLC is difficult to quantify and has a poor reproducibility.

Regarding the application of gradient chromatography, two separation systems for different DS-ranges are reported in literature. Both systems followed reversed-phase liquid chromatography under different conditions. The first system reported by Floyd et al. allowed the separation of cellulose diacetate in the range of DS = 2.3–2.7 [46]. The separation was carried out on a poly(styrene-co-divinyl benzene) based column in a linear gradient from acetone/water/MeOH (4:3:1) to acetone in 15 min at a flow rate of 0.8 mL/min. The samples eluted in the order of increasing average DS. The second system reported by Asai et al. was applied for the separation of cellulose triacetate in the range of DS = 2.7–2.9 [47]. The separation was performed on a Waters Novapak-phenyl column in a gradient from chloroform–MeOH (9/1):MeOH–water (8/1) [2:8] to 100% in 28 min at a flow rate of 0.7 mL/min.

Both systems allow calculating DS-distributions from a linear correlation between DS and retention volume. However, the applicability of both methods is confined to the DS-ranges investigated.

Therefore, the aim of the present paper was to develop a chromatographic method capable of separating CAs according to DS over a wide DS-range.

2. Experimental section

2.1. Materials

CA samples with average DS ranging from DS = 1.5 to DS = 2.9 were used. Five of the samples (sample 4, DS = 1.72; sample 13, DS = 2.42; sample 14, DS = 2.45; sample 15, DS = 2.45; sample 17, DS = 2.92) were kindly provided by Rhodia Acetow GmbH (Freiburg im Breisgau, Germany). The others were prepared in our laboratory by partial saponification of a commercial high DS sample (sample 16, DS = 2.60, Acetati, Italy). Information on the average DS, weight average molar masses (M_w), weight average degrees of polymerization (DP_w) and molar mass dispersity ($Đ_m$) of the samples are summarized in Table 1. The details on the synthesis and characterization of the samples are given elsewhere [48]. Dichloromethane (DCM), dimethyl acetamide (DMAc), dimethyl sulphoxide (DMSO) and methanol (MeOH) (VWR, Darmstadt, Germany) were of HPLC grade and used as received.

2.2. Chromatographic system and conditions

For the chromatographic separations a Shimadzu HPLC system consisting of a DGU-14A degasser, an FCV-10ALvp solvent mixing chamber, an LC-10ADvp pump and an SIL 10ADvp auto sampler were used. For detection an evaporative light scattering detector (ELSD, model PL-ELS 1000, Polymer Laboratories, UK) was added. The detector was operated at a nebulization temperature of 50 °C, an evaporation temperature of 90 °C and a gas flow of 1.0 SLM. The flow rate of the mobile phase was 1.0 mL/min unless mentioned otherwise. Data collection and processing were performed using 'WinGPC Software version 7.0' (Polymer Standards Service GmbH, Mainz, Germany).

The experiments were performed on a pure silica stationary phase Nucleosil, 5 μm particle size, 100 Å pore diameter, 250 mm × 4.0 mm I.D. (Macherey & Nagel GmbH, Düren, Germany). The column temperature was kept constant at 35 °C using a column oven K4 (Techlab GmbH, Erkerode, Germany). The injected sample volume was 15–20 μL with concentrations of 1.5–2.0 g/L, if not stated otherwise.

Table 1
Characterization data of samples used (samples from the industrial source are marked in grey).

Sample name	DS[a]	LS-M_w[b,e] [g/mol]	DP_w[c]	$Đ_m$[d]
Sample 1	1.53	57,600	254	3.0
Sample 2	1.59	72,700	318	3.4
Sample 3	1.66	73,500	317	3.5
Sample 4	1.72	44,600	190	2.6
Sample 5	1.81	68,100	286	3.2
Sample 6	1.87	64,900	269	3.4
Sample 7	1.92	63,200	260	3.2
Sample 8	1.95	76,400	313	3.5
Sample 9	2.09	70,700	283	3.3
Sample 10	2.16	71,900	284	3.2
Sample 11	2.19	71,000	279	3.5
Sample 12	2.27	74,400	289	3.3
Sample 13	2.42	64,400	244	3.2
Sample 14	2.45	64,700	244	3.3
Sample 15	2.45	93,300	352	3.4
Sample 16	2.60	69,800	257	3.5
Sample 17	2.92	175,000	613	4.4

[a] Determined by ^{1}H NMR [48].
[b] Determined by SEC–MALLS in *N*,*N*-dimethyl acetamide/lithium chloride [48].
[c] Calculated from M_w and DS [48].
[d] Based on PMMA-equivalent molar mass [48].
[e] Note: The molar masses of chemically heterogeneous copolymers have to be regarded as apparent molar masses. For the present samples, the error due to chemical heterogeneity was estimated to be of approximately the same size as the experimental error in LS measurement.