Characterization of sodium carboxymethyl cellulose by comprehensive two-dimensional liquid chromatography

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ABSTRACT

Two series of sodium carboxymethyl celluloses (NaCMC) with average degrees of substitution (DS) ranging from 0.45 to 1.55 were synthesized from low molecular mass Avicel cellulose (Avicel samples) and from high molecular mass cotton linters (BWL samples). The samples were characterized by online two-dimensional liquid chromatography using gradient liquid adsorption chromatography in the first and size exclusion chromatography (SEC) in the second dimension. This method allows the simultaneous determination of the chemical composition (DS) and the molar mass distribution within the individual samples. Moreover information was obtained on the dependence of the elution volume in gradient chromatography on molar mass. As expected, evidence for a stronger influence of molar mass on gradient elution volume was found for the low molecular mass NaCMC as compared to the high molecular mass BWL samples. Finally the applicability of the method for the simultaneous separation of blends heterogeneous with respect to chemical composition (DS) and molar mass was demonstrated. Such blends cannot be efficiently separated by either SEC or gradient chromatography alone, nor by simply combining the results of both methods. Only the complete two-dimensional chromatogram can reveal the complexity of such blends, since it reveals the correlations between molar mass and chemical composition.

1. Introduction

Sodium carboxymethyl cellulose (NaCMC) is one of the most important cellulose derivates in terms of sales (approx. 230 000 t per annum (Thielking & Schmidt, 2006) up to now). NaCMCs are produced commercially by polymer-analogous reaction of alkali cellulose with monochloracetic acid or its sodium salt (slurry process) in aqueous NaOH and an organic liquid (Heinze, 2005). Due to the molar mass distribution of the parent celluloses and the statistical nature inherent in the substitution reactions, the products obtained are supposed to be heterogeneous in terms of chemical composition on different structural levels and in molar mass. These heterogeneities and also the correlation between molar mass and chemical composition can influence application properties of NaCMC products. Evidence on the influence of chemical heterogeneity of NaCMC on the monomer (AGU, anhydroglucose unit) level on application properties like the solubility in water, the gel formation in salt containing solutions etc. are given in the following

articles (Heinze, 2005; Ramos, Frollini, & Heinze, 2005; Saake et al., 2000; Stigsson, Kloow, & Germgard, 2006). It is highly probably that also the heterogeneity on the polymer level/s influences the properties of the products.

It should be mentioned that the term homogeneous is often used in the field of cellulose derivatives to describe a random distribution pattern of the AGUs which is in agreement with the random distribution of the Spurlin model, while the term heterogeneous indicates a deviation from this model. However, a sample yielding a random substitution pattern of the AGUs is not really homogeneous, as the different AGUs will differ in their distributions along and among the different chains. In the following, the term narrowly distributed denotes a fraction or material in which all chains have the nearly same ratio of substituents to AGUs, irrespective of the placement of the substituents within the AGUs or along the chains.

Due to their impact on application properties knowledge on the above mentioned heterogeneities and their correlations is essential for establishing and understanding structure-property relationships. The molar mass distribution of NaCMCs can be obtained by SEC (Eremeeva & Bykova, 1998; Hoogendam, Keizer, Stuart, & Bijsterbosch, 1998; Kath, Lange, & Kulicke, 1999; Kulicke, Kull, Kull, & Thielking, 1996; Rinaudo, Danhelka, & Milas, 1993; Shakun

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et al., 2013b), while the chemical composition distribution (DS-distribution) of intact NaCMC chains can be determined using gradient liquid adsorption chromatography as described recently (Shakun et al., 2013a). However, no information on the correlation between the structural parameters DS and molar mass for individual samples is available by these one-dimensional methods.

Two-dimensional (2D) chromatography allows the simultaneous separation according to chemical composition and molar mass. In two-dimensional chromatography, the sample is first separated by one structural parameter, e.g. chemical composition. The resulting fractions are then subjected to another chromatographic separation with respect to another structural feature, e.g. by SEC, rendering the molar mass distribution of chemically homogeneous or at least narrowly distributed fractions (with respect to chemical composition). By this procedure the correlations between different structural features can be identified. Ideally the coupled separation methods should be orthogonal to each other, which mean that each separation is independent of the separation parameter in the other dimension. However, complete orthogonality is not mandatory. In many cases it suffices if each chromatographic dimension separates predominantly according to one parameter and the influence of the second parameter is much weaker. Even when both chromatographic methods are not orthogonal information on the correlations between both structural features can be obtained.

A variety of successful applications of two-dimensional separations on synthetic polymers have been reported (Gerber & Radke, 2005; Ghareeb & Radke, 2013; Glöckner, 1984; Greiderer, Steeneken, Aalbers, Vivo-Truyols, & Schoenmakers, 2011; Jiang, Horst, Lima, & Schoenmakers, 2005; Raust, Brüll, Moire, Farcet, & Pasch, 2008). However, applications of two-dimensional chromatography to polysaccharides and especially to cellulose derivatives are rarely found in literature. Greiderer et al. (2011) described online two-dimensional characterization according to the size and the composition of hydroxypropyl celluloses. Ghareeb and Radke (2013) applied offline two-dimensional liquid chromatography to separate cellulose acetates with respect to degree of substitution (DS) and molar mass. However, no application of two-dimensional chromatography to NaCMCs has been reported yet.

It was therefore the intention of the present work to couple the recently developed NaCMC separation according to DS (Shakun et al., 2013a) online to SEC using the SEC-method described recently (Shakun et al., 2013b). This was done in order to simultaneously separate NaCMC by DS and molar mass. For this purpose the molar mass dependence of gradient elution volume was studied in the course of the present work. Finally, the applicability of two-dimensional chromatography for the simultaneously separation of samples blends heterogeneously with respect to the chemical composition (DS) and the molar mass was investigated.

2. Materials and analytical methods

2.1. Solvents and samples

Water was deionized through a Milli-Q system (Millipore water). Acetic acid was supplied by Merck KGaA (Darmstadt, Germany). Ammonium acetate (NH$_4$OAc) and methanol were purchased from VWR (Haasrode, Belgium).

2.2. Samples characterization

NaCMC from Avicel und BWL were laboratory samples synthesized at the University of Jena. A typical protocol for the synthesis and the details on the characterization of degree of substitution (DS) and molar mass are given in (Shakun et al., 2013a, 2013b).

Table 1
DS and M_w of the NaCMC samples.

Sample	DS[1]	SD[2]	M_w[3] [g/mol]
Avicel 1	0.45	0.17	481,000[4]
Avicel 2	0.75	0.16	42,000
Avicel 3	0.98	0.17	35,000
Avicel 4	1.23	0.17	39,000
Avicel 5	1.54	0.18	44,000
BWL 1	0.46	0.26	219,000
BWL 2	0.73	0.26	260,000
BWL 3	0.95	0.19	383,000
BWL 4	1.25	0.14	435,000
BWL 6	1.55	0.10	339,000

[1] Determined by acidic hydrolysis followed by HPLC (Shakun et al., 2013b).
[2] Standard deviation of DS determined by gradient chromatography (Shakun et al., 2013a).
[3] Determined by SEC with light scattering detection (Shakun et al., 2013b).
[4] This sample reveals a small prehump at low SEC elution volumes. SEC LS verified that this prehump is due to very high molar mass aggregates (Shakun et al., 2013b).

The characterization data are summarized in Table 1. It should be noted that the molar mass of Avicel 1 is substantially higher that the molar masses of the other Avicel samples. This high molar mass is a consequence of a small prehump of very high molar mass aggregates in the SEC chromatogram. Light scattering measurements in two different solvents (0.1 N NaCl and 0.1 N NH$_4$OAc) revealed approximately 20% difference in the derived molar masses, without completely eliminating the aggregates.

2.3. Chromatographic system

Chromatographic separations in the first dimension (gradient chromatography) were performed using an Agilent 1100 HPLC instrument consisting of a quaternary pump (typ G1311A), degaser (typ G1322A), automated sample injector (typ G1313A) and column oven (typ G1316A). A Luna PFP (2) column (particle size 5 μm, pore diameter 100 Å, column dimensions 250 × 4.6 mm) from Phenomenex (Aschaffenburg, Germany) was operated at 35 °C for the separation by DS.

The one-dimensional gradient separations were performed at a flow rate of 1 mL/min applying a 40 min linear gradient from water/methanol = 95/5 to 65/35. Both solvents contained 100 mmol/L ammonium acetate and 446 mmol/L acetic acid in order to adjust the pH to 4.1. For detection an evaporative light scattering detector ELSD 2100 (Polymer Laboratories, Church Stretton, England) operated at an evaporator temperature of 120 °C, a nebulizer temperature of 40 °C and a gas flow rate 1.5 SLM was used. The injection volumes are given in the legends to the respective graphs. The injection procedure was analogous to the injection procedure used in the two-dimensional separations described below.

For the two-dimensional separations the flow rate in the first dimension was set to 0.034 mL/min. In order to maintain the same gradient slope in the 1000 min gradient as in the one-dimensional gradient separation the eluent composition was changed from water/methanol = 95/5 (v/v) to 69.5/30.5 (v/v). Both, water and methanol contained 100 mmol/L ammonium acetate and 446 mmol/L acetic acid in order to adjust the pH to 4.1. The injection volume was ten times 100 μL. The ten injections were carried out using isocratic conditions of water/methanol = 95/5 at a flow rate of 1 mL/min. During this injection procedure, at which the sample components were adsorbed onto the stationary phase, the effluent of the first dimension was not transferred to the second dimension column but to waste. Following the tenth injection the flow rate in the first dimension was reduced to 0.034 mL/min. After back pressure stabilization the gradient was started. Simultaneously the transfer valve between the both dimensions was activated and the