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Characterization of hydroxypropylmethylcellulose (HPMC) using comprehensive two-dimensional liquid chromatography

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ABSTRACT

Various hydroxyl-propylmethylcellulose (HPMC) polymers were characterized according to size and compositional distributions (percentage of methoxyl and hydroxyl-propoxyl substitution) by means of comprehensive two-dimensional liquid chromatography (LC × LC) using reversed-phase (RP) liquid chromatography in the first dimension and aqueous size-exclusion chromatography (aq-SEC) in the second dimension. RP separation was carried out in gradient-elution mode applying 0.05% TFA in water and 1-propanol, while 0.05% TFA in water was used as mobile phase in aqueous SEC. A two-position ten-port switching valve equipped with two storage loops was used to realize LC × LC. Detection of HPMC was accomplished by charged-aerosol detection (CAD). Data processing to visualize chromatograms was carried out using Matlab software. The significant influence of the LC × LC temperature on (the retention of) HPMC was studied using a column oven which allowed accurate temperature control. Due to the phenomenon of thermal gelation, which is a result of methyl and hydroxypropyl substitution of anhydroglucose units from the cellulose backbone, we were able to obtain additional, specific information on compositional characteristics of various HPMC samples. As the retention behaviour of gelated and non-gelated polymer proved to be different, the fraction of the polymer that is gelated in the chromatographic column could be monitored at different temperatures. Moreover, the temperature at which half of the polymer is gelated could be correlated with the cloud-point temperature. As a result, differences in inherent cloud points of modified cellulose can be used as a further distinguishing property in "temperature-responsive" LC × LC.

1. Introduction

Cellulose, the most abundant polymer in nature, is derived from D-glucose units, which condense through β(1 → 4)-glycosidic bonds. The multiple hydroxyl groups of the polysaccharide backbone can be partially or fully reacted with various reagents to create derivatives with useful properties. Cellulose ethers are the most important commercial materials, including methylcellulose (MC) [1], ethylcellulose (EC) [2], hydroxypropylcellulose (HPC) [3] and hydroxypropylmethylcellulose (HPMC) [4–7]. Their hydrophilic, polymeric, non-toxic and biodegradable nature as well as their almost unlimited availability allows diverse applications, ranging from viscosity modifiers [8], gelling, binding and foaming agents [9,10] to excipients for controlled-release drug tablets [5–7].

Due to this broad scope of usage, reliable and robust analysis techniques have to be established to characterize modified celluloses. The detailed molecular characterization of these "chemically improved polysaccharides" (ChImPS) according to their (distribution in) size, degree of (methyl) substitution (DS) and molar (hydroxypropyl) substitution (MS) is of the utmost importance in particular for pharmaceutical applications as drug excipients, since structure–property-relationship studies [11] and controlled-drug-release materials [12] require extensive knowledge of the average molecular structures and molecular distributions.

The great variety of possible molecular structures of these cellulose ethers, however, makes it very difficult to fully characterize them by simple analytical techniques. In particular, heterosubstituted polysaccharides (such as HPMC) represent highly complex materials exhibiting a number of molecular property distributions. These include size distribution (variation of the length of the linear cellulose backbone) and compositional variations in terms of methyl- and hydroxypropyl substituents (Fig. 1). Commonly used analytical techniques for the determination of these properties,

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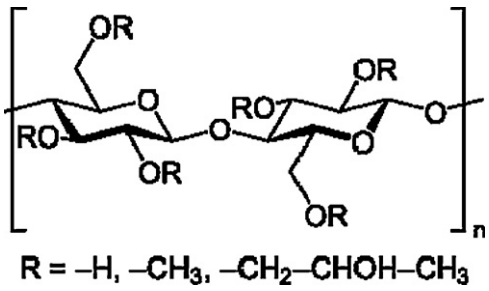

Fig. 1. Typical chemical structure of hydroxypropylmethylcellulose (HPMC) consisting of the polymeric backbone of cellulose, a natural carbohydrate that contains a basic repeating structure of anhydroglucose units, and methyl and hydroxypropyl substitutions.

such as laser-light scattering and ^{1}H-NMR, only result in averaged data and yield no information on the sample dispersity. To determine any distribution, a prior separation has to be achieved. Multiple distributions require the utilization of advanced, multi-dimensional separation techniques [13].

Comprehensive two-dimensional liquid chromatography (LC × LC) [13–16] entails the convenient on-line combination of two different ("orthogonal") separation mechanisms with negligible loss of sample and separation efficiency in the interface between the two columns. LC × LC saves time compared to the labor intensive off-line fractionation and re-injection. Comprehensive coupling can easily be realized by using an appropriate switching valve equipped with two storage loops [13]. The combination of interactive LC and size-exclusion chromatography (SEC) is the most commonly used comprehensive two-dimensional separation method for polymers [13,17]. It represents an appropriate instrumental setup for investigations of chemical-composition and molecular-size (or mass) distributions within a sample. SEC is a well-established technique for the determination of polymer-molecular-weight distributions [18]. Separation of variously sized analytes is based on differences in accessible pore volume in a stationary bed. In case of HPMC samples, relative molecular-weight (M_r) distributions can be measured by aqueous SEC (aq-SEC) methods. Compositional distributions of HPMC (percentages of methoxy and hydroxypropoxy functional groups) can be assessed by applying reversed-phase liquid chromatography (RPLC) under gradient conditions, since the degree and the nature of substitution affect the hydrophobicity of modified cellulose. Retention mechanisms are, however, not fully orthogonal. It is expected that the molecular weight affects the RPLC retention.

The significant impact of methyl- and hydroxypropyl substitution of cellulose on the solution behaviour is commonly used in pharmaceutical applications for the control of hydration of the excipient and can be examined by the phenomenon of thermal gelation [19–22]. An aqueous solution of HPMC starts to gel when heated, at temperatures that are specific for each HMPC type. This process is reversible, since these gels will liquefy again upon cooling. The precipitation temperature, gelation temperature, and gel strength of these aqueous HPMC solutions were found to be a function of molecular weight, degree of methyl- and hydroxypropyl substitution, concentration, and presence of additives [22]. It will be shown, that this unique effect can be exploited for advanced characterization of HPMCs by LC × LC, if appropriate column temperatures are applied.

In this contribution we accomplish the characterization of HPMC samples provided by two different manufacturers by means of RPLC × SEC. Furthermore, we demonstrate for the first time how thermal gelation-phenomena of modified cellulose can be visualized in RPLC × SEC and how this reveals information on the compositional distributions of HPMC. RPLC × SEC can be used as an additional distinguishing characteristic between different HPMC batches.

2. Experimental

2.1. Chemicals

Hydroxypropylmethylcellulose (HPMC) samples with different molecular weights and chemical compositions (Table 1) were obtained from Dow (Terneuzen, The Netherlands) and ShinEtsu (Tokyo, Japan). Each HPMC standard used for this study was dissolved in a mixture containing 13.5% of 1-propanol and 0.05% TFA in water under gently stirring for 4 h at room temperature to a final concentration of 10 mg/mL. HPMC standard solutions were stored at 4 °C. 1-Propanol (>99.5%) was purchased from Biosolve (Valkenswaard, The Netherlands) and filtered with a 0.45 μm filter before use. Trifluoroacetic acid (>99%) was obtained from Sigma–Aldrich (Steinheim, Germany). All water used was purified using an Arrium 611UV nanopure unit from Sartorius (Goettingen, Germany) and filtered with a 0.45 μm filter.

2.2. Instrumentation

For gradient-LC analysis in the first dimension two Shimadzu LC-10ADvp solvent-delivery units (Shimadzu, 'sHertogenbosch, The Netherlands) delivering a total flow rate of 25 μL/min were connected to a 5-μL high-pressure gradient mixer (Sulpelco, Zwijndrecht, The Netherlands). The mobile phase for RP gradient elution consisted of 0.05% TFA in H_2O (A) and 0.05% TFA in 1-propanol (B). Linear gradient elution was realized from 13.5% (B) to 38% (B) in 100 min. A narrow-bore RP column (Zorbax 300SB-C8, 2.1 × 150 mm, 3.5 μm particles) from Agilent Technologies (Waldbronn, Germany) was used for reversed-phase gradient elution. The aqueous SEC system consisted of a Shimadzu LC-10ADvp solvent-delivery unit providing a constant flow of 1.25 mL/min of 0.05% TFA in H_2O. HSPgel AQ column (6.0 × 150 mm, 4.0 μm particles,

Table 1
Supplier's data on seven HPMC samples including grade, manufacturer, molar hydroxypropyl substitution (MS), degree of substitution (DS), percentage of hydroxypropoxy (HPO) and methoxy (MeO) functional groups, molecular weight (M_r) and cloud-point (CP) for a 2% aqueous solution at 95% light transmission.

HPMC #	Manufacturer	Molar hydroxypropyl substitution (MS)[a]	Degree of substitution (DS)[a]	HPO [%]	MeO [%]	M_r [g/mol]	Cloud point (CP)[b] [°C]
1	ShinEtsu	0.17	1.50	6.6	24.1	98 800	63.5
2	ShinEtsu	0.29	1.50	11.0	23.3	129 000	59.0
3	Dow	0.24	1.50	9.0	23.6	130 000	62.5
4	Dow	0.33	1.57	12.2	24.0	137 000	59.6
5	Dow	0.25	1.46	9.6	23.0	126 000	65.4
6	ShinEtsu	0.29	1.52	10.9	23.6	328 000	60.2
7	Dow	0.33	1.50	12.4	23.1	344 000	61.0

[a] Calculated from ^{1}H-NMR data.
[b] For a 2% w/w aqueous solution at 95% light transmission.

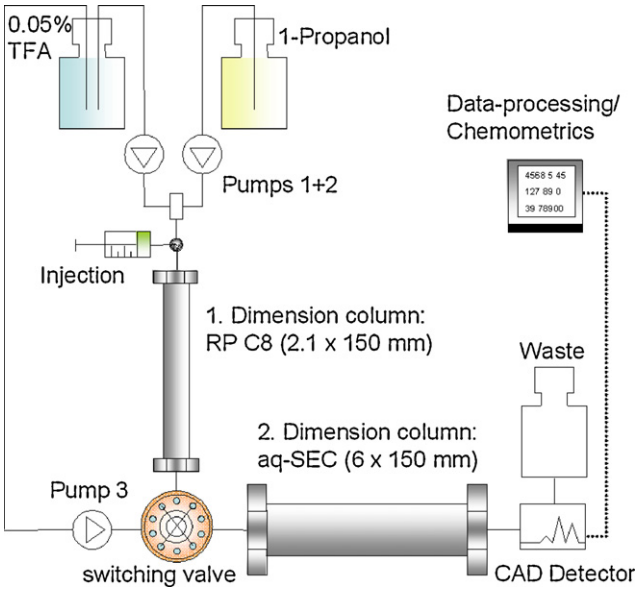

Fig. 2. Schematic illustration of the RPLC × SEC setup for the characterization of HPMC. 0.05% TFA in water and 1-propanol were used as mobile-phase components. A narrow-bore RP-column and an aqueous-SEC column were applied as first- and second-dimension separation media, respectively. A two-position ten-ways valve was operated in symmetrical configuration to ensure comprehensive coupling. Detection of cellulose polymers was achieved by charged-aerosol detection (CAD). Data were processed with Matlab software to visualize 2D chromatograms.

10–400 kDa molecular-weight range) obtained from Waters (Milford, MA, USA) was applied in the second dimension. Degassing of mobile phases was carried out with a DGV-14A on-line degasser from Shimadzu, while a Shimadzu SIL-10ADvp autoinjector with an integrated sample cooler (temperature set to 18 °C) was used for the 3-μL injections. The LC and SEC columns were coupled with a nitrogen-actuated VICI two-position ten-way valve from Valco (Schenkon, Switzerland), which was located in the column oven to guarantee that the RP and SEC columns had the same temperature as the storage loops of the switching valve. This valve was operated in symmetrical configuration [23] using a high-speed switching accessory (switching time of 20 ms) and dual injection loops of equal volume (100 μL). The modulation time was set to 3 min. The impact of the column temperature on the separation of HPMC was investigated using a CTO-10ACvp column oven from Shimadzu, which allowed variation of column temperatures between 10 and 80 °C. Thermal gelation studies were performed at 18, 22, 26, 30 and 38 °C. Detection of the cellulose polymers was accomplished by a charged-aerosol detector (Corona Plus) from ESA (Chelmsford, MA, USA) using nitrogen (250 kPa) at room temperature, corona voltage of 2.24 kV and a current of 1.00 μA.

A schematic illustration of the LC × LC setup used for this study is shown in Fig. 2. Column dimensions, particle size, flow rates, and second-dimension injection volume (i.e. loop size) were selected for the application of HPMC characterization by consulting guidelines from different protocols [23,24]. Mobile-phase composition and gradient elution in the first dimension (RP) were adapted to obtain appropriate LC × LC conditions.

2.3. Instrument control and data processing

Shimadzu Scientific Instrument software and Shimadzu Class-V8^{TM} 7.2.1 (Columbia, MD, USA) were used to control the solvent-delivery system, autoinjection unit, switching valve, column oven and detector. Two-dimensional chromatograms were processed using in-house routines written in a Matlab 7.1 (Natick, MA, USA) software environment.

3. Results and discussion

3.1. HPMC characterization by LC × LC

Fig. 3a exhibits the RP chromatographic profile of HPMC #1 under gradient-elution conditions. It shows the polymer-typical cockscomb shape, as, for example, observed in poly(vinyl alcohol) studies [25]. Fig. 3b illustrates the chromatogram of the aqueous size-exclusion separation of HPMC #1 using 0.1% TFA in water, covering the molecular-weight range from 10 to 400 kDa, while the comprehensive two-dimensional RP × SEC of HPMC #1 applying a modulation time of 3 min (two storage loops of 100 μl each) is displayed in Fig. 3c. All chromatograms in Fig. 3 were recorded at 20 °C using a charged-aerosol detector (CAD).

3.2. Impact of column temperature on LC × LC of HPMC

The family of hydroxypropylmethylcelluloses consists of products that vary chemically and physically, depending on their desired properties. Molecular differences between HPMCs, such as differences in molecular weight (M_r), degree of methoxyl substitution (DS) and the molar hydroxypropoxyl substitution (MS) affect vital properties, including molecular size, viscosity, solubility in organic solvents and thermal gelation temperature of aqueous solutions. In particular the unique phenomenon of reversible bulk thermal gelation of HPMCs demonstrates the significant impact of chemical modification on the performance of these cellulose ethers in hydrophilic matrix systems. Degrees and ratio of methyl and hydroxypropyl substitution of the anhydroglucose ring represent the main factors affecting bulk thermogelation properties of the polysaccharide polymer in aqueous solution. They gel at temperatures that are highly specific for each HMPC type. The reversible thermogelation phenomenon of aqueous HPMC solutions has been postulated to be primarily caused by the hydrophobic interaction between the cellulose ethers [22]. At lower temperatures, the polymer is well hydrated and there is little polymer–polymer interaction. As the temperature increases, the polymer molecules in solution gradually loose their water shell and as soon as insufficient hydration of the cellulose molecules occurs, they start to gel and form infinite network structures as reflected by a distinct increase in viscosity. RPLC × SEC chromatograms recorded at different temperatures shown in Fig. 4 confirm this assumption. A significant increase of retention is observed, which may be either due to a decrease of analyte solubility (polymer–polymer interactions) or an increased interaction with the hydrophobic reversed-phase surface (polymer–stationary phase interaction). Both factors imply that the modified cellulose becomes more hydrophobic and thus increasingly retained with increasing temperatures.

The phenomenon of thermal gelation is also indicated in the second (SEC) dimension, since the polymer does not undergo separation solely based on size-exclusion effects at higher temperatures (Fig. 4, see separations at 26 °C, 30 °C and 36 °C for HPMC #1). Uncommonly sharp peaks at 3.2 min represent the cellulose polymers eluting from the column after the total permeation time (around 2.75 min). This untypical retention behaviour raises some questions.

One possible explanation may be remarkable adsorption interactions between the hydrophilic SEC stationary phase and the HPMC molecules. As previously discussed, HPMC polymers become more hydrophobic with increasing temperatures due to poorer hydration. Interactions of the increasingly hydrophobic analyte with the stationary phase provide a reasonable explanation for the increased retention, but do not explain the remarkable sharpness of the polymer peak.

A possible explanation uses the model of a solvent/analyte plug passing through the column. This mechanism was first described by

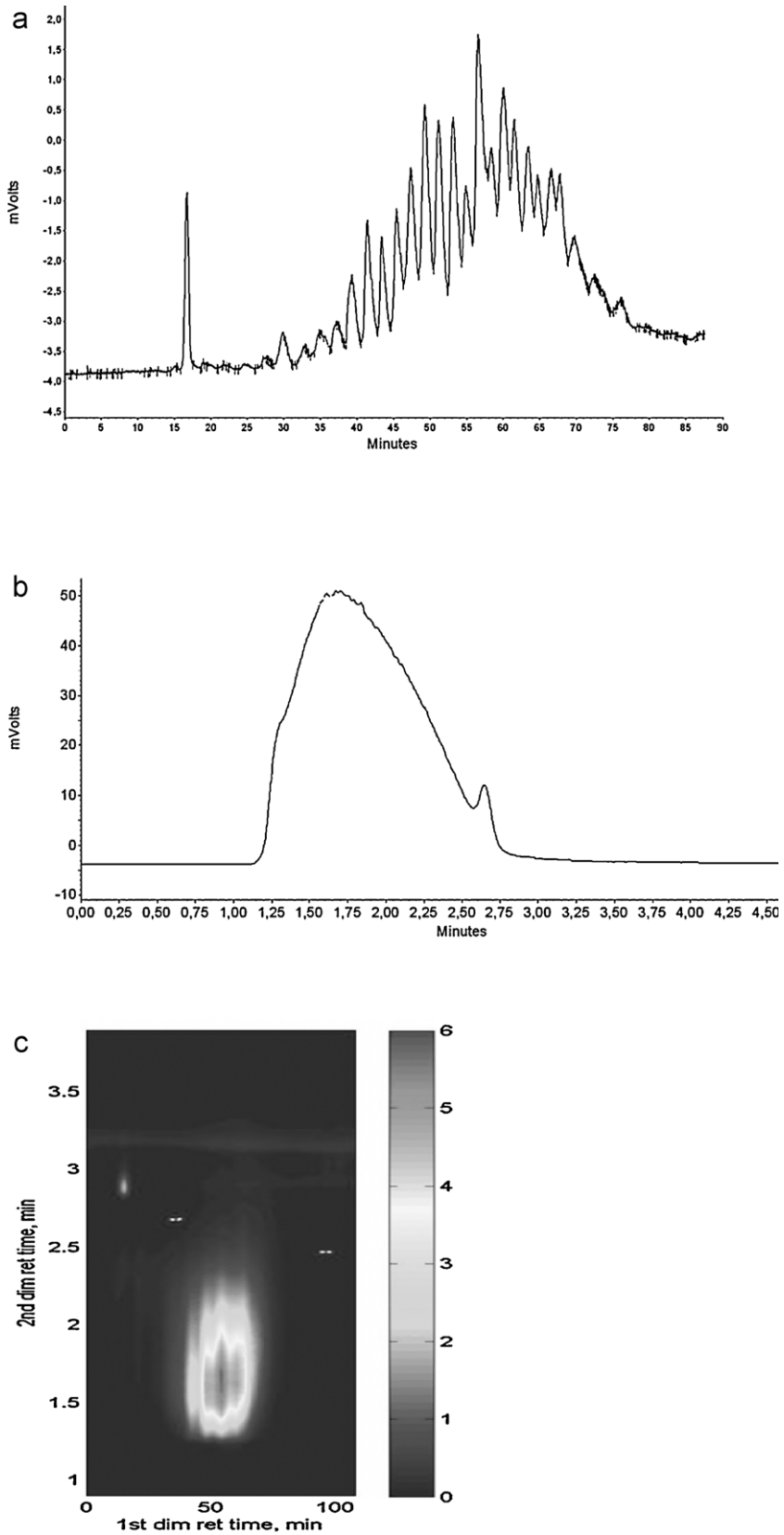

Fig. 3. (a) Reversed-phase gradient elution of HPMC #1. Chromatographic conditions: solvent: (A) 0.05% TFA in H_2O, (B) 1-propanol; gradient: 13.5–38% (B) in 100 min; flow: 25 μL/min; sampling: 3 μL of HPMC #1 (10 mg/mL); column temperature: 20 °C. (b) Size-exclusion chromatography of HPMC #1. Chromatographic conditions: solvent: 0.05% TFA in H_2O, flow: 1.25 mL/min, column temperature 20 °C, CAD detection. (c) RPLC × SEC of HPMC #1. First dimension (*x*-axis) illustrates reversed-phase retention (C_8); second dimension (*y*-axis) represents size-exclusion chromatography (molecular-weight range 10–400 kDa). Signal intensity is indicated by the colour bar on the right side of the chromatogram. RPLC conditions in first dimension: solvent: (A) 0.05% TFA in H_2O, (B) 1-propanol; gradient: 13.5–38% (B) in 100 min; flow: 25 μL/min; sample: 3 μL of HPMC #1 (10 mg/mL); column temperature: 20 °C. Modulation time of switching valve: 3 min; storage loops: 100 μL each. SEC conditions in second dimension: solvent: 0.05% TFA in H_2O, flow: 1.25 mL/min, column temperature: 20 °C; CAD detection under constant nitrogen flow.

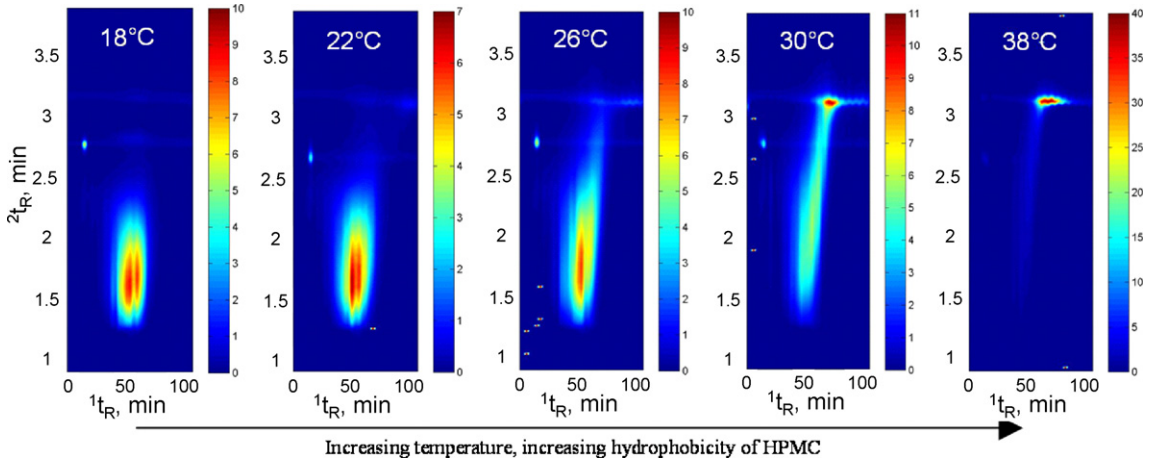

Fig. 4. Study of the influence of the column temperature on the RPLC × SEC separation of HPMC #1. First dimension (*x*-axis) illustrates reversed-phase retention (C_8); second dimension (*y*-axis) represents size-exclusion chromatography. Signal intensity is indicated by the colour bar on the right side of the chromatogram. Chromatographic conditions as in Fig. 3, except column temperature: 18, 22, 26, 30 and 38 °C, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Reingruber et al. [26]. The HPMC is injected on the second dimension (SEC) column dissolved in an aqueous solution that contains at least 13.5% 1-propanol (see RP conditions in the experimental section). This 1-propanol/water/HPMC injection plug passes through the column that is being flushed with SEC mobile phase (0.05% TFA in water). The aqueous mobile phase will not rapidly mix with the injection-plug. Depending on the temperature, the conditions on the second-dimension column may or may not correspond to size-exclusion conditions. At elevated temperatures (starting at 26 °C in Fig. 4) 0.05% TFA in water is no longer an ideal solvent. With increasing temperature the 1-propanol/water mixture becomes a better solvent for the increasingly hydrophobic HPMC than the SEC mobile phase. Analyte molecules that stray to the front of the 1-propanol plug will be retained until they are re-dissolved in the better solvent, resulting in a sharp (focused) peak at the leading edge of the 1-propanol plug. Analyte molecules that lag behind the 1-propanol plug will be re-dissolved in the next injection plug from the first dimension sampling. This interpretation is supported by the sharp band in the second-dimension chromatogram (with ^1t_R between 80 and 100 min) in the 2D chromatograms at 26, 30 and 38 °C; see Fig. 4. This hypothesis is also supported by a separate experiment to determine the 1-propanol retention time for the aq-SEC column with an EcoSEC system (Tosoh bioscience GmbH, Germany) under the same chromatographic conditions (flow 1.25 mL/min, 0.05% TFA in water as mobile phase) that were used for LC × LC. For this experiment, the CA detector was replaced by a refractive-index (RI) detector to visualize the 1-propanol plug in the SEC separation. A blank containing 13.5% 1-propanol in water was injected (3 μL) onto the SEC column. A sharp 1-propanol peak (band width ~8 s) was observed at a retention time of around 3.1 min. When the sample (dissolved in 99% propanol) was injected only in the SEC dimension (with a mobile phase containing 0.05% TFA in H_2O), a split of the polymer peak was observed when the temperature was increased. This confirms that the gelation effect is mainly governed by temperature, and occurs independently to the solvent used to dissolve the polymer (99% propanol, as in this later experiment; or 13.5–38% propanol, as it occurs with the 2D experiment).

There is benefit in using a comprehensive two-dimensional separation, opposed to injecting independently the sample in a (one-dimensional) RP system and a (one-dimensional) SEC system. At a fixed temperature, by means of two-dimensional chromatography, one can inspect the dependency of polarity and size of the (polydispersed) sample (which is related to the shape of the peak corresponding to the non-gelated polymer). As this dependency changes with temperature, one can conclude that the interaction effect of polarity and size cannot be neglected when measuring the effects on gelation temperature.

3.3. *Comprehensive LC × LC characterization of HPMC at low temperatures*

Because the column temperature has a significant effect on the hydrophobicity and thus on the solubility of HPMC in an aqueous environment (see previous section), repeatable, reliable, compara-

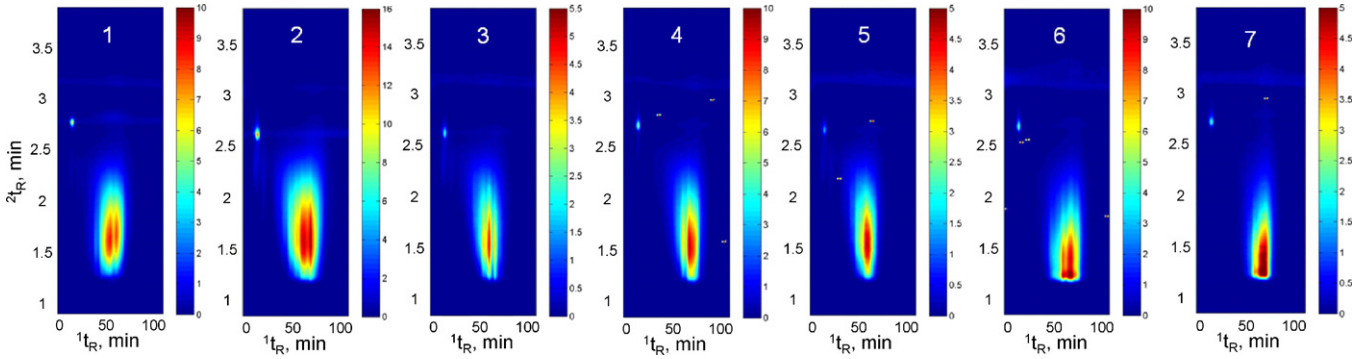

Fig. 5. Comprehensive RPLC × SEC of HPMC #1–7 at 18 °C. First dimension (*x*-axis) illustrates reversed-phase retention (C_8); second dimension (*y*-axis) represents size-exclusion chromatography. Signal intensity is indicated by the colour bar on the right side of the chromatogram. Chromatographic conditions as in Fig. 3, except column temperature: 18 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ble and meaningful chromatographic separations of different HPMC batches can only be obtained if performed at clearly defined, accurate temperatures. A column oven which allows precise control of column temperature is therefore necessary for the characterization of HPMC. The first chromatographic studies of modified cellulose were performed at low temperatures (in our case 18 °C) to avoid thermal gelation. The intention of low-temperature separations of HPMC is to assure complete dissolution in the aqueous eluent and to minimize hydrophobic polymer–polymer interactions. The LC × LC chromatograms of seven different HPMC samples at 18 °C are shown in Fig. 5. As can be observed, only a reversed-phase mechanism (first dimension) and a size-exclusion mechanism (second dimension) are governing the separations.

RPLC × SEC chromatograms of HPMC at 18 °C (Fig. 5) demonstrate that some of the 7 samples are visibly distinguishable due to differences in hydrophobicity (see RP retention time on *x*-axis) and size (see SEC retention time on *y*-axis), as well as the widths of their chemical composition (polarity) and molecular-weight distributions (polydispersity). HPMC data provided by the suppliers are listed in Table 1. High-molecular-weight HPMCs >300 kDa (#6 and #7) can be easily differentiated from lower molecular-weight HPMC <150 kDa by comparing SEC retention. Since the degree of (methyl) substitution (DS) is similar for each HPMC (DS around 1.50) in this study, differences in the first-dimension profiles are likely related to variation in the molecular (hydroxypropyl) substitution. Additionally, polymer size may slightly influence the first dimension retention time. There is a clear correlation between molar hydroxypropyl substitution (MS) and retention time. HPMC #1 (MS of 0.17) is containing the lowest fraction of hydroxypropyl groups and exhibits the lowest retention (around 55 min). HPMC #3 and #5 (MS of 0.24 and 0.25) follow with retention times of 55–60 min while HPMC #4 and #7 (MS of 0.33) with the highest MS show up at retention times around 65–70 min in the first dimension. Interestingly, the polydispersity in terms of chemical composition apparently reveals information on the supplier and synthesis process. HPMC #1, #2 and #6 (ShinEtsu samples) possess a noticeable broader distribution of inherent polarity, which can be seen in the first dimension (around 35 min full width) compared to the other five HPMCs from Dow Chemical (around 25 min) under the same chromatographic conditions. The polydispersity in terms of molecular weight on the other hand was found to be relatively similar for all HPMC samples.

3.4. RPLC × SEC of HPMC at different temperatures: relation with cloud point

In contrast to the experiments carried out at lower temperatures, the 2D-separation of HPMC at different temperatures is expected to be affected by thermal gelation effects, as already explained in the context of Fig. 4. The experiments of Fig. 4 were performed for the 7 samples. The goal was to monitor which part of the polymer experiences the gelation effect as a function of temperature. Fig. 6 depicts an example on how this is done for a specific sample at a specific temperature. Each individual polymer-related peak (labelled as purple dots) was integrated and assigned to two different groups. Peaks that have been merged are indicated by the purple line. Group *1 refers to non-gelated polymers, while group *2 relates to gelated polymers. This procedure was applied for all seven HPMC samples at different column temperatures (18, 22, 26, 30 and 38 °C, respectively). For each sample the fraction of non-gelated polymer (*F*) was plotted vs. temperature. This is shown in Fig. 7(a). In a next step, a sigmoid function was fitted for each sample. In this case, the sigmoid function takes the form

$$F = \frac{-1}{1+\exp(-aT+b)} + 1 \quad (1)$$

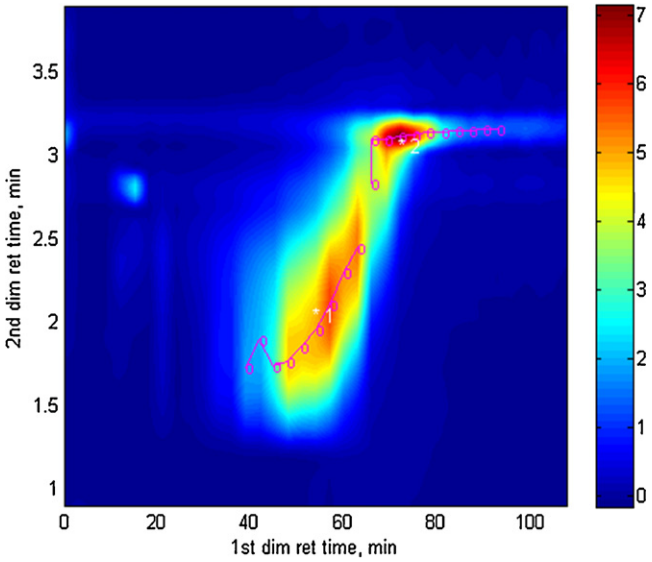

Fig. 6. RPLC × SEC peak volume detection procedure for HPMC #6 at 26 °C. Purple dots represent the individual peaks, purple lines represent the individual peaks that have been merged. White labels refer to non-gelated (1) and gelated (2) fractions of the polymer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

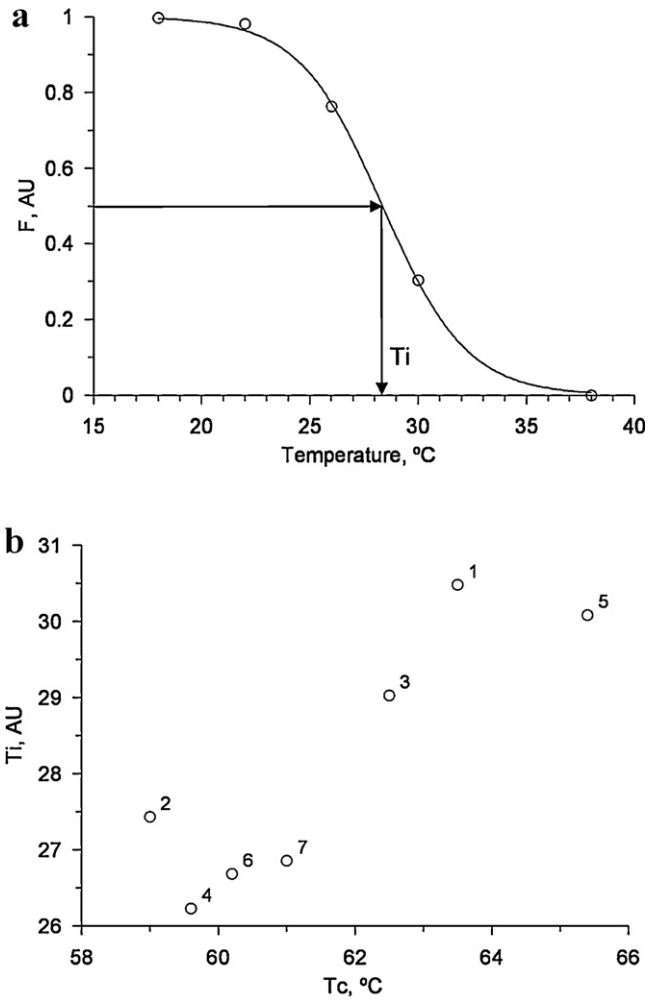

Fig. 7. (a) Proportion of non-gelated component to the total amount of sample detected (*F*) as a function of temperature for HPMC #4. Temperature at the inflection point (T_i) is indicated. (b) Correlation between T_i (temperature of inflection point) and T_c (cloud point temperature) for the seven samples of this study.

where T is the temperature, and a and b are the fitted parameters (the result of the fitted function is overlaid in Fig. 7a). From Eq. (1) one can deduce the temperature as a function of the fraction of the non-gelated polymer

$$T = \frac{-\log(F/1-F)+b}{a} \quad (2)$$

From Eq. (2), the temperature T_i at which half of the polymer is non-gelated ($F = 0.5$) is seen to correspond to in $T_i = b/a$, which coincides with the inflection point of the sigmoid (see Fig. 7a). We call this the "chromatographic gelation temperature" and this can be correlated with the cloud point temperature for the different samples of this study. This is depicted in Fig. 7b. One should take into account that the mechanisms involving the cloud point experiments and the "chromatographic gelation" experiments are not exactly the same, but related to the same phenomena. In particular, the solvent (that places a crucial role in the gelation effects) is different. Hence, the cloud-point temperatures and the chromatographic gelation temperature do not correlate perfectly.

The results of this computation should, however, be taken with care for several reasons. First, the integration of peaks (as displayed in Fig. 6) carries some errors, since sometimes there is ambiguity about to which group (group 1 or group 2) an individual peak belongs. Second, the ELSD is a non-linear detector, and therefore the sum of the individual peaks is not proportional to the total concentration. Hence changes in peak shape (when the total concentration is the same) are translated in changes in total peak area. This latter effect is partially compensated by the fact that the quantities of F (used in Eq. (1)) are not absolute values but fractional values. As a result, the average repeatability of a fractional peak area (measured with 4 samples after 5 repetitions) ranged between 10 and 15%. In summary, differences in cloud points of aqueous HPMC solutions represent an additional, quantitatively valuable property for comprehensive "thermo-responsive" LC × LC characterization. All seven HPMC batches can be distinctively differentiated at a glance by comparing thermal gelation characteristics from the 2D chromatograms with reference to their cloud points.

4. Conclusions

Comprehensive two-dimensional LC, with RP and SEC separation dimensions, represents a useful tool to characterize and distinguish different HPMC polymers in terms of molecular weight (M_r), molecular composition (DS and MS) and chemical distributions (polydispersity). The impact of the column temperature on the retention of HPMC in both RP and SEC separations was found to be significant. Accurate oven temperatures are, therefore, essential for repeatable and comparable chromatographic studies of HPMC. Nevertheless, the unique property of thermal gelation of modified cellulose was found to be helpful to gain specific information on the chemical nature of these polymers. Strong correlation between characteristic cloud-points (CP) of each HPMC and the degree of thermal gelation at appropriate column temperatures may be clearly visualised in comprehensive LC × SEC chromatograms. The reversible phenomenon of thermogelation can be utilized in RPLC × SEC as an additional, distinguishing characteristic of HPMC batches, but can also be associated with compositional differences between modified polysaccharides. This in turn may help to obtain a better understanding of hydration and dissolution effects of HPMC and may be consulted in drug release and structure–properties relation studies.

The study of the influence of the column temperature opens doors to a new dimension in HPMC characterisation by means of (2D-) LC using the phenomenon of thermal gelation of modified cellulose.

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