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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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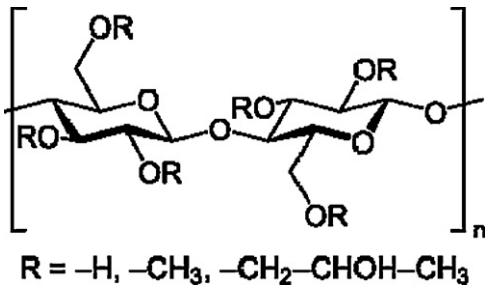

Fig. 1. Typical chemical structure of hydroxypropylmethylcellulose (HPMC) consisting of the polymeric backbone of cellulose, a natural carbohydrate that contains a basic repeating structure of anhydroglucose units, and methyl and hydroxypropyl substitutions.

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.