Non-Exclusion Effects in Aqueous Size-Exclusion Chromatography of Polysaccharides

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Abstract

This paper concerned to investigation of aggregate formation processes in aqueous Size-exclusion chromatography (SEC) of Na-carboxymethylcellulose (Na-CMC).

Keywords: SEC, Na Carboxymethylcellulose, Aggregate Formation

1. Introduction

Size-exclusion chromatography is one of the powerful methods for determination and investigation of molecular weight distribution of polymers [1]. In aqueous SEC of polymers [2], the understanding of the separation mechanism demands much more attention due to the enthalpy interactions distorting a pure size-exclusion separation mechanism [1]. Because of the presence of polar, and often anionic, groups in the stationary phases used in SEC, the mobile phase must be carefully chosen to repress polymer-gel and intermolecular interactions. This is particularly important in SEC of polyelectrolytes and polar molecules such as carbohydrates [3]. Suppression of interactions, such as polyelectrolyte expansion, ion-exclusion, molecular adsorption and aggregate formation depends on nature of electrolyte, optimal value of pH and ionic strength of eluent.

The aim of this paper is to investigate of aggregate formation process in SEC of Na-CMC in order to determine of the suitable aqueous eluent for true size-exclusion separation mechanism of macromolecules.

2. Materials and Methods

SEC was performed on the liquid chromatograph, consisting from syringe pump Merk-Hitachi L-6000A model, Shodex RI-101 refractive index detector, multiangle laser light scattering detector DAWN NSP (Watt technology), manual sample injector Rheodine 2104, degasser of eluent and two chromatographic columns PL Aquagel-OH Mixed termostated at 25˚C and connected in series. Synthesis of Na-CMC was described in [4]. SEC analysis were performed using two types of eluent: NaCl and NaNO3 in the water with concentration 0.1 mol/L.

3. Results and Discussion

Many of hydrophilic polymers are polyelectrolytes and, therefore, their elution properties in SEC is complicated by various non-exclusion effects, such as ion exclusion, polyelectrolyte expansion, molecular adsorption, and aggregate formation, which distort the normal SEC separation mechanism. These effects can be eliminated by increasing the ionic strength and changing the pH of the eluent so as to decrease the degree of dissociation of ionic groups both in the macromolecular chain and on the sorbent surface [5]. Physicochemical properties such as structure, molecular weight and shape or conformation are primary factors controlling their functional properties. A typical molar mass sensitive detector is a multi angle laser light scattering (MALLS). This detector has the advantage of providing structural information in addition to the molar masses. Analysis of CMC by SEC in 0.1 M NaNO3 solutions were complicated by presence of the low amount associates forming due to intermolecular interactions [6,7]. To avoid of the aggregates of macromolecules Hoogendam C.W. [7] demonstrated that the solutes Na-CMC in first step were prepared in pure water, after 0.1 M NaNO3 were added to sample solution. We have received bimodal chromatograms of CMC from MALLS detector in SEC analysis when used of water consisting NaNO3 with concentration 0.1 mol/L (Figure 1(a)). Same result was occurring, when we used 0.1 M NaNO3 in water as eluent. But when 0.1 M NaCl was used first peak in the chromatogram is disappeared indicating that
Figure 1. Gel chromatograms of the Na-CMC (Mw = 2.18 × 10^4) received from MALLS (a) and RI detector (b) in 0.1 M NaNO₃.

Figure 2. Gel chromatograms of the Na-CMC (Mw = 2.18 × 10^4) received from MALLS (a) and RI detector (b) in 0.1 M NaCl.

4. Conclusions

Specific polymer-solvent and intermolecular interactions in aqueous SEC can lead to formation of aggregates of Na-CMC in 0.1 M NaNO₃. Dual detection in SEC allows determining and evaluating of degree of formation of the aggregates. In SEC of Na-CMC low amount of aggregates in 0.1 M NaNO₃ was detected. To eliminate of aggregates and realize pure SEC separation mechanism of Na-CMC we are recommend use of 0.1 M NaCl as eluent.

5. References