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Analysis of Polysaccharide Derivatives by Aqueous Size Exclusion Chromatography

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ABSTRACT: Aqueous size-exclusion chromatography (SEC) equipped with refractive index (RI) and multiangle laser light scattering (MALLS) detectors were applied to evaluation of charged molecules content in a series of polysaccharide derivatives with different average molar mass (MM) values. It was shown that a minor amount of residual charged groups in the polysaccharide chains caused a polyelectrolyte expansion effect and neutral fractions of larch arabinogalactans (AG) were separated from neutral molecules in various concentrations of the injected samples. Electrostatic effects in separation of carboxymethylcelluloses (CMC) were demonstrated by concentration dependence of retention volume at various injected sample concentrations.

KEYWORDS: size exclusion chromatography, molar mass, arabinogalactan, carboxymetylcellulose, polyelectrolyte expansion, concentration effects.

I. INTRODUCTION

Size-exclusion chromatography (SEC) is one of the most powerful analytical techniques for investigation and determination of molar mass distribution of polymers [1,2]. The chromatographic behavior of solutes separated by SEC can be described by the general chromatographic equation:

$$K_{SEC} = (V_R - V_o) / (V_t - V_o),$$

where V_R is the measured peak elution volume, V_t the total column volume, and V_o the exclusion (or void) volume. Industrial polysaccharides include, but are not limited to, such materials as native and modified starches, dextrans, glucans, pullulans, modified celluloses, pectins, carrageenans, and gums from microbial and plant seed sources. Information as to size, structure and conformation is useful in order to better understand the solution behavior, intra- and intermolecular interactions, rheology, and function [3]. The aim of this study was to conduct investigation of the electrostatic interactions, such as polyelectrolyte expansion effect of arabinogalactan (AG) and carboxymetylcellulose (CMC) in aqueous SEC. Many of hydrophilic polymers are polyelectrolytes and, therefore, their elution properties in SEC are 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 element so as to decrease the degree of dissociation of ionic groups both in the macromolecular chain and on the sorbent surface.

II. EXPERIMENTAL

The SEC system was composed of an Agilent 1100/1260 Series chromatograph with a quaternary pump with degasser (G1311B), an auto sampler (G1329A), two Ultrahydrogel Linear columns (300 x 8 mm) from Waters (USA) connected in series, a differential refractometer (RID10A, Shimadzu), and a three angle light scattering detector (Mini DAWN TriStar, Wyatt Technology Corporation, USA) with a semiconductor laser diode at 690 nm. Larch AG (Ara: Gal) = 15:85 was obtained from Megazyme International Ireland Ltd., Ireland. Industrial CMC provided by CARBONAM LTD, Namangan, Uzbekistan.

III. RESULTS AND DISCUSSION

Fig. 1 presents the combined elution profiles of larch AG at three injected sample concentrations 1, 2, and 4 g/L. The chromatograms consist of at least three distinct fractions and two of them are early-eluted peaks belonging to ionic species of AG in water. The decrease in retention volume of early eluted multiple peaks with reduction of injected sample concentration indicated the presence of polyelectrolyte expansion effects in the system. The decrease of the concentration in the injected sample leads to an increase of the size of the macromolecules and to their early elution,

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correspondingly the retention volume will be decreased. Intermolecular electrostatic interactions or polyelectrolyte expansion effects in SEC of charged polymers are seen in the concentration effects as a decrease in the retention time (V_t) with reduction of injected sample concentration *C*.

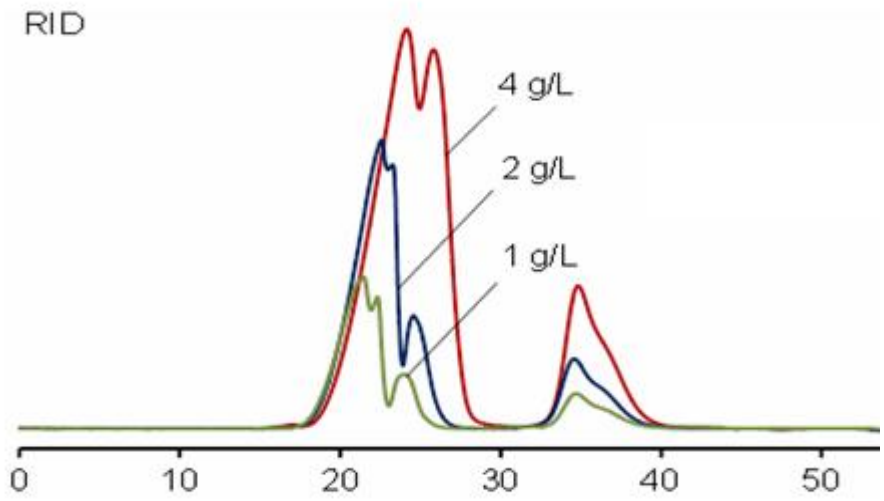

Fig.1 – Elution profiles of larch arabinogalactan (Megazyme) Mw=40 kDa at 3 injected concentrations. Eluent: H_2O. Detector: RI

The retention volumes of the asymmetrical early eluted peaks decreased with the reduction of concentration of the solutes and the elution profiles of AG in the chromatograms indicate that part of the polysaccharide molecules are charged and eluted earlier than the neutral fraction.

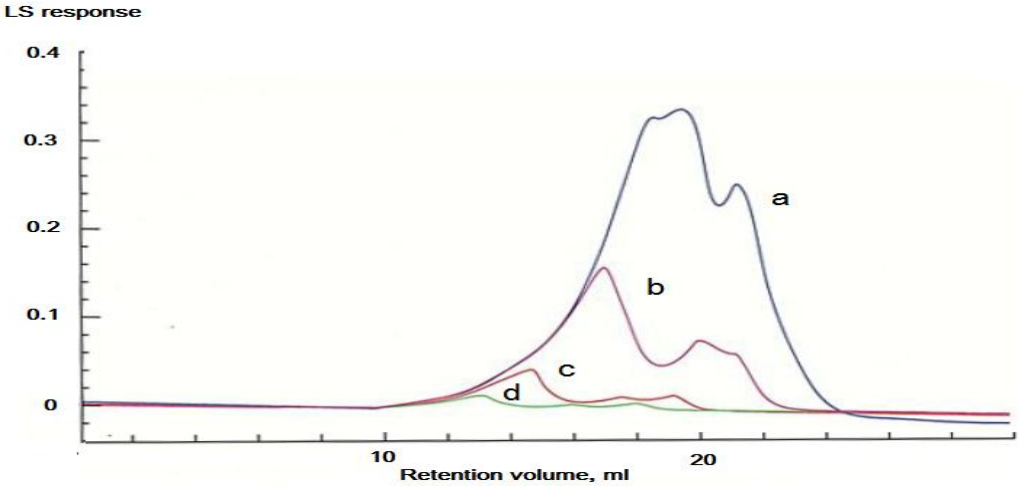

Fig.2. Elution profiles of CMC Mw=48 kDa at various injected concentrations, g/l: a) 5; b) 2,5; c) 0,5; d) 0,1. Eluent: H_2O. Detector: MALLS.

Asymmetric and multimodal distribution profiles received at different sample concentrations of CMC also indicate the polyelectrolyte expansion nature of charged species in macromolecules (Fig.2). Physicochemical properties such as structure, molecular weight and shape or conformation are primary

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factors controlling their functional properties. A typical molar mass sensitive detector is a multi angle laser light scattering (MALLS).

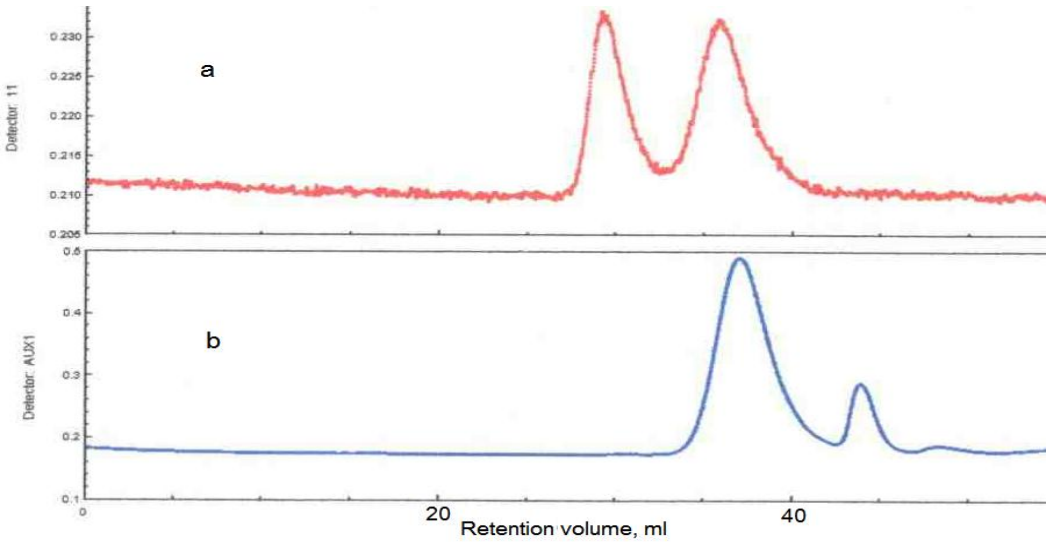

Fig.3. Elution profiles of CMC in 0,1 M $NaNO_3$. Detector: a) MALLS, b) RI.

This detector has the advantage of providing structural information in addition to the molar masses.

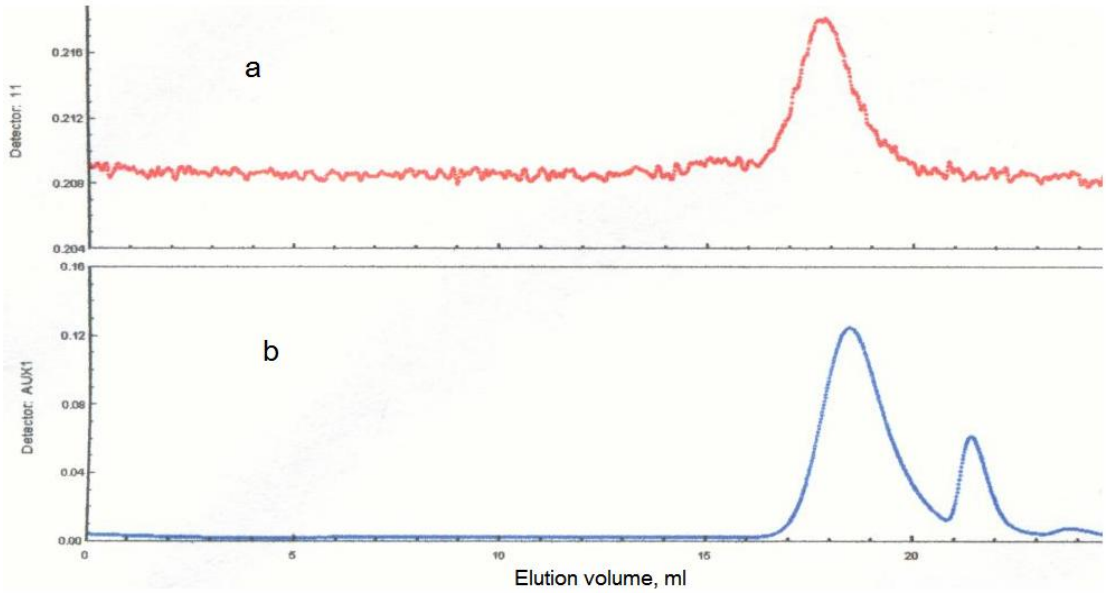

Fig.4. Elution profiles of CMC in 0,1 M NaCl. Detector: a) MALLS, b) RI.

Analysis of CMC by SEC in 0,1 M $NaNO_3$ solutions were complicated by presence of the low amount associates forming due to intermolecular interactions [4]. To avoid of the aggregates of macromolecules Hoogendam C.W [5] demonstrated that the solutes Na-CMC in first step were prepared in pure water, after

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0.1 M $NaNO_3$ were added to sample solution. We have received bimodal chromatograms of CMC from MALLS detector in SEC analysis when used of water consisting $NaNO_3$ with concentration 0,1 mol/l **(Fig.3).** *Same result was occurring, when we used 0,1M NaN_3 in water as eluent. But when 0,1M NaCl was used first peak in the chromatogram is disappeared indicating that formation of molecular aggregates not realized and further investigations on SEC of cellulose derivatives were conducted using 0,1M NaCl in water* **(Fig.4).** *Second, the presence of microgels as a result of small but significant amounts of very high molecular weight CMC was detected using a MALLS detector as first peak in chromatogram.*

IV. CONCLUSION

Elution properties of polysaccharides have been studied by SEC/MALLS/RI. In pure water as eluent acidic species in content of arabinogalactans and CMC will lead to polyelectrolyte effects, particularly polyelectrolyte expansion and in result to early elution of charged molecules. Neutral fractions of AG are separated from charged fractions according to size-exclusion mechanism in H_2O.

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