

UDC 541.64:543.544

SECONDARY EFFECTS IN AQUEOUS SIZE-EXCLUSION CHROMATOGRAPHY OF POLYSACCHARIDES

© A.S. Boymirzaev^{1*}, Sh. Shomurotov², A.S. Turaev²

¹Namangan Institute of Engineering and Technology, Department of Chemical Technology, 7, Kasansai str, Namangan, 160115 (Uzbekistan),
e-mail: azamatsb@gmail.com

²O. Sadikov Institute of Bioorganic Chemistry Uzbek Academy of Sciences,
M. Ulugbek str., 83, Tashkent, 100125 (Uzbekistan), y-mail: ibchem@uzsci.net

This paper's main concern is with the investigation of the elution properties of water-soluble polysaccharides in Size-exclusion chromatography (SEC) using multiangle laser light scattering and differential refractive index detectors in aqueous solvents. Chromatographic data on influence of nature of solvent to the formation of polymer aggregates is presented. We have shown that in pure water as eluent the bimodal and multimodal chromatograms of Na-carboxymethylcellulose (Na-CMC) and arabinogalactans (AG) were received. This phenomenon indicates of presence of intermolecular enthalpic interactions in solutions. We found that aqueous eluents containing 0,1 M NaCl for Na-CMC and 0,14 M NaNO₃ for AG are preferred to prevent of secondary effects distorting of SEC separation mechanism.

Keywords: modified carboxymethylcellulose, arabinogalactan (AG), aggregate formation, polyelectrolyte effects, size exclusion chromatography.

Introduction

Gel Permeation Chromatography or Size-exclusion chromatography is one of the most versatile and powerful analytical techniques for investigation and determination of molecular mass distribution of polymers [1]. Compared to other methods of analysis such as osmometry or static light scattering, it has the advantage that it determines complete distributions of molar masses as opposed to merely an average molecular weight. The chromatographic behavior of solutes separated by SEC can be described by the general chromatographic equation:

$$K_{SEC} = (V_R - V_0) / (V_t - V_0), \quad (1)$$

where V_R is the measured peak elution volume, V_t the total column volume, and V_0 the exclusion (or void) volume.

In practice, interaction between the packing and the solute molecules is observed and, therefore, K_{SEC} can be defined by eq. (2).

$$K_{SEC} = K_D K_P, \quad (2)$$

where K_D is the distribution coefficient for pure size exclusion and K_P is the distribution coefficient for solute/stationary phase interactions [2]. If solute molecules are separated solely by size-exclusion and no enthalpic interactions are observed, then K_P is unity and the retention volume for SEC is given by eq.(1). This has to be checked for new polymers and modified systems.

Boimirzaev Azamat Solievich – Candidate of physico-mathematical sciences, associate professor,
tel.: +998 69 2340943. e-mail: azamatsb@gmail.com
Shomurotov Shavkat – Researcher, candidate of chemical sciences, tel.: +998 71 262-35-40, e-mail: shsha@mail.ru
Turaev Abbaskhan Sabirkhanovich – Head of the Laboratory, Professor, Doctor of Science,
tel.: +998 71 262-35-40, e-mail: ibchem@uzsci.net

SEC of water-soluble polymers has been a field of intense activity in recent years due to the short-time consuming of this separation technique [3]. In contrast to the elution mechanism of polymers in organic media [4], the understanding of the separation mechanisms of hydrophilic polymers in aqueous media demands much more

* Corresponding author.

theoretical and experimental attention, mainly due to the multitude of so-called secondary effects referred to as polymer-gel interactions distorting a pure size-exclusion 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 electrostatic interaction. This is particularly important in SEC of polar molecules such as carbohydrates [3]. Such interaction can be prevented by the use of an electrolyte solution of sufficiently high ionic strength as the mobile phase.

SEC has been and will continue to be an important tool in the characterization and analysis of cellulose and its derivatives [5–10]. One of the most demanding problems in polysaccharide solutions is the tendency for aggregate formation of macromolecules and intermolecular associates. With increasing polymer concentration the hydrodynamic size of molecular aggregates will increase [11]. This aggregation complicates the investigations of structural and molecular mass properties of isolated macromolecules by chromatographic methods. In this paper we studied the influence of aqueous salt solutions on the elution properties of polymeric drug carrier on the basis of modified Na-carboxymethylcellulose (CMC) and arabinogalactans in SEC with dual detection.

The aim of this paper is to investigate of polymer-gel interactions in SEC of Na-CMC and arabinogalactan in order to determine of the suitable aqueous eluent for true size-exclusion separation mechanism of these macromolecules.

Materials and Methods

SEC was performed with the following components: isocratic pump Merk-Hitachi L-6000A model, Shodex RI-101 refractive index detector, multiangle laser light scattering detector DAWN DSP $\lambda=632,8$ nm (Wyatt Technology Santa Barbara, USA), manual sample injector Rheodyne 2104, with loop volume 100 μL , eluent degasser and two chromatographic columns PL Aquagel-OH Mixed, I.D.: 7,8 mm, length: 300 mm (Agilent Technologies) connected in series. Data acquisition and processing were carried out using the ASTRA IV software (Wyatt technologies). The aqueous eluents having NaN_3 , NaCl or NaNO_3 dissolved in the water at a concentration of 0,1 mol/l and samples were filtered through a 0,1 μm and 0,45 μm hydrophilic PTFE membrane (Omnipore; Millipore, USA) correspondingly before use. SEC conditions were as follows: the sample concentration of 0,05–0,2% (w/V), injection volume of 100 μL , flow rate of 0,8 mL/min and the column temperature of 25 $^\circ\text{C}$. The detector cells of MALLS and RI were kept at ambient temperature. Synthesis of modified Na-CMC and arabinogalactan from larch wood were described in [12, 13], respectively.

Results and discussion

In most cases, SEC of polymers in organic media occurs without any enthalpic interactions in the polymer-gel system. SEC of water-soluble polymers exhibits a number of specific features. The point is that many hydrophilic polymers are polyelectrolytes and, therefore, their elution properties is complicated by various non-exclusion effects, such as ion exclusion (IE), polyelectrolyte expansion (PE), molecular adsorption, and aggregate formation, which distort the normal SEC separation mechanism. The IE and PE 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 [14]. To suppress molecular adsorption, organic solvents, such as methanol, acetonitrile, etc., are added to aqueous eluents.

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. However, to make full use of this advanced characterization method it is necessary to develop an interaction free SEC method, which relies on an optimum stationary phase (column material) and a matching eluent system. Analysis of CMC by SEC in 0,1 M NaNO_3 solutions were complicated by the presence of low amounts of associates formed due to intermolecular interactions [15, 16]. Hoogendam [16] demonstrated that aggregates can be avoided by dissolving the Na-CMC in a first step in pure water and subsequently add NaNO_3 to a final concentration of 0,1 M. For CMC we have received chromatograms from MALLS detection in SEC analysis with a bimodal distribution when NaNO_3 with a concentration of 0,1 mol/l in water was used as eluent (Fig. 1a). The same result was obtained, when we used 0,1 M NaN_3 in water as eluent. However, when 0,1 M NaCl was used as eluent, the first peak in the chromatogram disappeared indicating that the formation of molecular aggregates is suppressed. Further investigations of cellulose derivatives were conducted by SEC using 0,1 M NaCl in water (Fig. 1b). The presence of microgels as a result of small but significant amounts of very high molecular weight CMC was revealed using a MALLS detector. While

the MALLS signal is very sensitive towards the very high molecular mass, the microgel was detected by the refractive index (RI) detector only as very small peak due to its less sensitivity than MALLS. The concentration of the sample affects the SEC analysis in different ways. First, elution volume shifts with changes in sample concentration. The shift in elution volume is large even at low concentrations (Fig. 2).

Chromatograms were obtained with sample concentrations of 0,2, 0,8, 2, or 4 g/l respectively. The asymmetric shape of the chromatogram of the sample can be explained as follows. The polymer solution is injected into a narrow zone of the chromatographic column. The zone smears as it migrates along the column together with the eluent flow. The polymer concentration at the center of the chromatogram is always higher than at its edges. Therefore, molecules located in the region of the leading edge of the zone unfold to a higher degree due to a decrease in the concentration and, consequently, move faster than molecules located at the center of the zone. Molecules located at the trailing edge also unfold to a higher extent because of a decrease in the concentration and, therefore, catch up with slower molecules at the center of the chromatographic zone. This phenomenon occurs only if the PE effect is present, when the stationary phase (sorbent) is uncharged while the polymer is charged positively or negatively. If the polymer and sorbent surface have same charge polarity, the PE effect is superimposed with the ion exclusion effect, and both effects acting in the same direction.

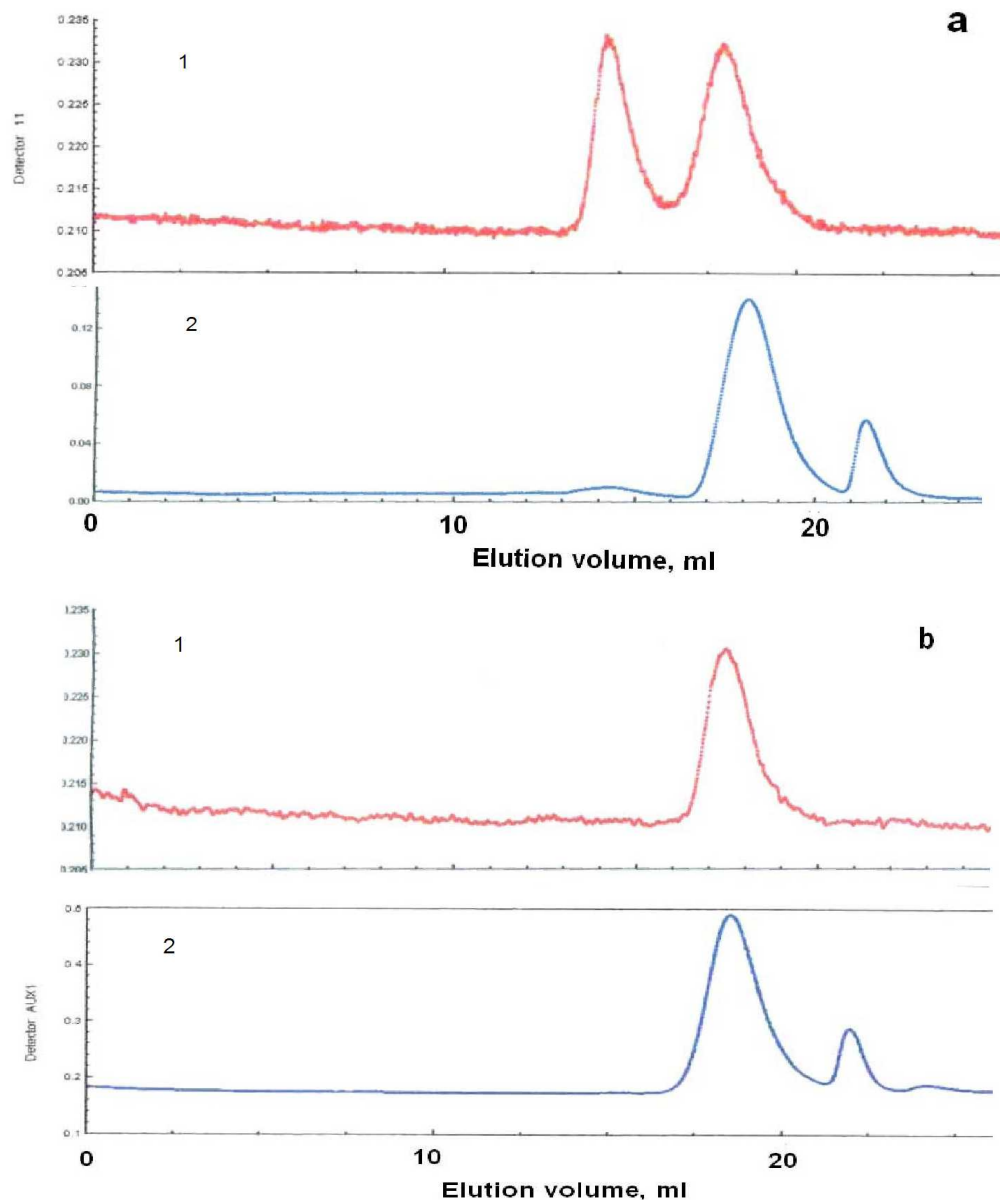


Fig. 1. Gel chromatograms of the modified Na-CMC having $M_w=2,18 \cdot 10^4$ g/mol detected by MALLS (1) and RI detector (2) in 0,1 M NaNO_3 (a) and 0,1 M NaCl (b)

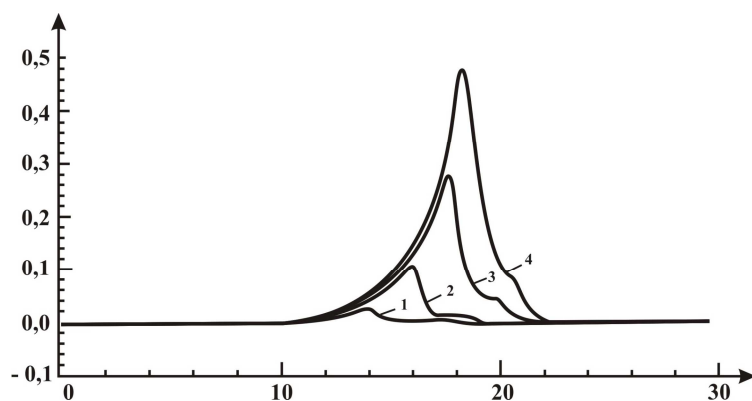


Fig. 2. Chromatograms of Na-CMC at various injected sample concentrations, g/l: 1 – 0,2; 2 – 0,8; 3 – 2; 4 – 4. Eluent: Water

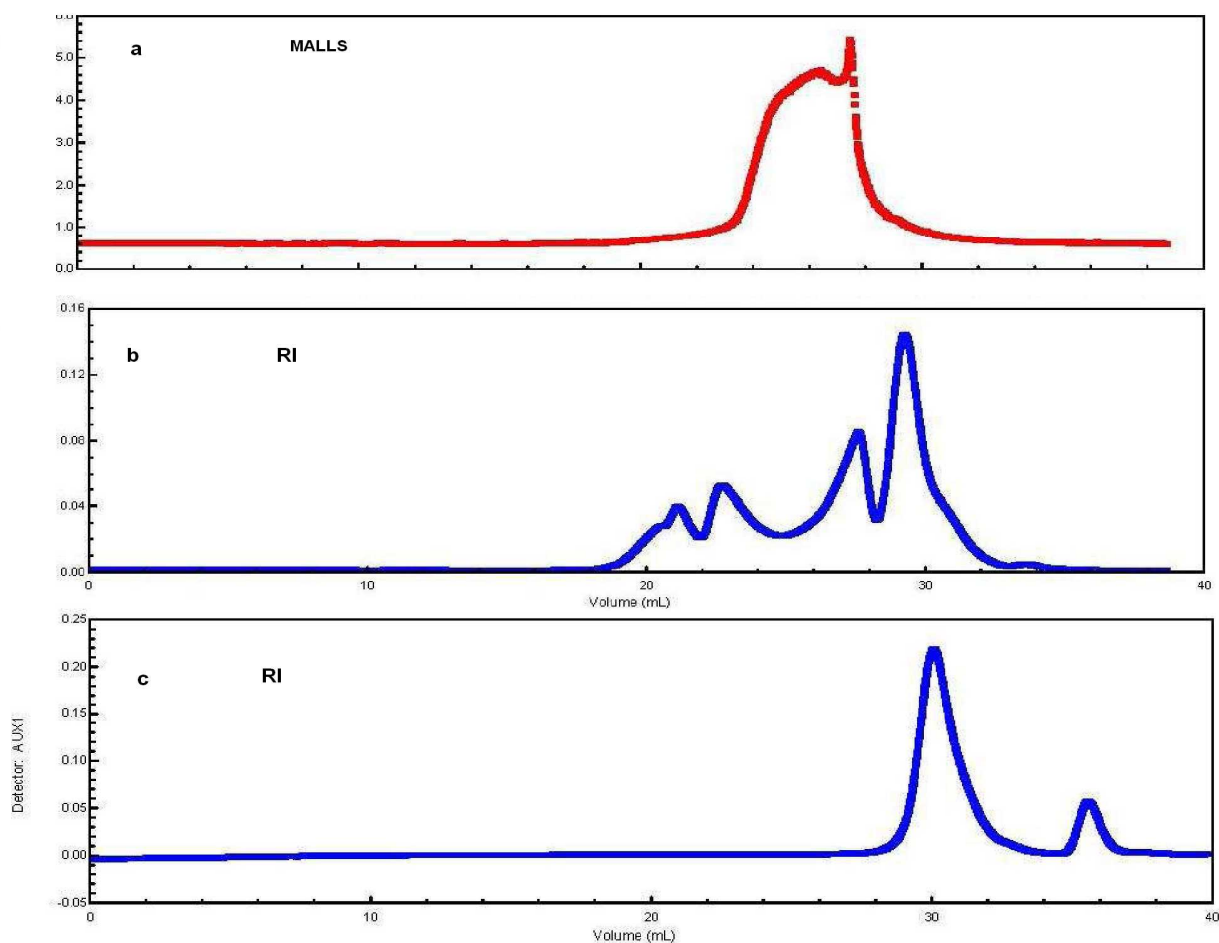


Fig. 3. Chromatograms of arabinogalactan in water (a,b) and 0,01 M Phosphate buffer (pH 7,4) with 0,14 M NaNO_3 (c)

In SEC of arabinogalactans in water, multimodal chromatograms were found: the first peak appears at the void volume of the column (fig. 3a). As stated in reference [17] this phenomenon can be related to the formation of aggregates due to inter/intramolecular interactions of the chain of AG or a partial ion-exclusion effect. These interactions were suppressed by using 0,01 M Phosphate buffer (pH 7,4) with 0,14 M NaNO_3 as eluent (fig. 3b). The chromatograms in Fig. 3 (a) were received in the following polymer concentrations: 0,3; 0,75; 1,5; 3; 6 g/l and no concentration effect was observed in separation of AG in water. With decreasing concentrations of injected polymer from 6 to 0,3 g/l retention volumes of samples remained constant. Shapes of chromatograms did not change with the concentration of the sample.

Conclusion

Specific polymer-solvent interactions of polysaccharides like CMC and AG in aqueous solution can lead to formation of aggregates and polyelectrolyte effects. Dual detection in SEC allows determining and evaluating the degree of aggregation. In SEC of CMC low amount of aggregates were detected in 0,1 M NaNO₃, but to eliminate of aggregates and realize pure SEC separation mechanism using of 0,1 M NaCl as eluent can be recommended. In case of AG 0,01 M Phosphate buffer (pH 7,4) with 0,14 M NaNO₃ allows to prevent of polymer-solvent interactions and SEC analysis of molecular weight distribution of AG can be performed.

Acknowledgement

The authors thanks to Dr.G.P. Alexandrova from Irkutsk Institute of Chemistry, Russian Academy of Sciences for providing the arabinogalactans for investigation.

References

1. Mori S., Barth H. Size Exclusion Chromatography, Springer Verlag Berlin Heidelberg, 1999. 234 p.
2. Strlic M., Kolenc J., Kolar J., Pihlar B. *J. Chromatogr. A.*, 2002, vol. 964, pp. 47–54.
3. Churms S.C. *J. Chromatography Library*, 2002, vol. 66, pp. 267–303.
4. Elias H.-G. *Macromolecules*. Vol. I. Principles: Structure, Synthesis and Properties, 5th ed., Hüthig & Wepf, Basel, Switz, 1990.
5. Horner S., Puls J., Saake B., Klohr E.-A., Thielking H. *Carbohydrate Polymers*, 1999, vol. 40, pp. 1–7.
6. Beister J. Zur Erlangung des Doktorgrades des Fachbereiches Chemie der Universität Hamburg: Dissertation, 2001.
7. Kulicke W-M., Clasen Ch., Lohman C. *Macromol. Symp.*, 2005, vol. 223, pp. 151–174.
8. Porsch B., Andersson M., Wittgren B., Wahlund K-G. *J. Chromatogr. A.*, 2002, vol. 946, no. 1–2, pp. 69–81.
9. Melander M., Vuorinen T. *Carbohydr. Polym.*, 2001, vol. 46, no. 3, pp. 227–233.
10. Conner A.H., Wu Chi-san *Handbook of Size Exclusion Chromatography*. Chromatographic Science Series, 1995, vol. 69, pp. 331–352.
11. Vilenchik L.Z., Barman S., Pathak C.P. Strategies in size- exclusion chromatography. ACS Symposium Series, 1996, vol. 635, chapter 17, pp. 328–346.
12. Shomuratov Sh.A., Murodov E.A., Turaev A.S. *Chemistry and pharmacia*, 2005, no. 4, pp. 61–64.
13. Medvedeva E.N., Babkin V.A., Ostroukhova L.A. *Khimiia rastitel'nogo syr'ia*, 2003, no. 1, pp. 27– 37. (in Russ.).
14. Rinaudo M., Tinland B. *J. Appl. Polym. Sci.*, 1991, vol. 48, pp. 19–31.
15. Picton L., Merle L., Muller G. *Int. J. Polym. Anal. Charact.*, 1996, no. 2, pp. 103–113.
16. Hoogendam C.W., A. de Keizer, Cohen Stuart M.A., Bijsterbosch B.H. *Macromolecules*, 1998, vol. 31, no. 18, pp. 6297– 6309.
17. Medvedeva S.A., Alexandrova G.P., Tantsirev A.P. *Izvestiia vuzov. Lesnoi zhurnal*, 2002, no. 6, pp. 112–119.

Received June 6, 2012

