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Retention Behaviors of Uronic Acid-containing Polysaccharides and Neutral Polysaccharides in HPGPC

Cui Ping LIU, Xing Feng BAO, Ji Nian FANG*

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences,
Chinese Academy of Sciences, Shanghai 200031

Abstract: The chromatographic behaviors of several uronic acid-containing polysaccharides and neutral polysaccharides were investigated in HPGPC for the first time. The effects of sample concentration and ionic strength of mobile phase on retention time were studied. The mechanism for the effects on *Mw* determination results of polysaccharides by HPGPC was also discussed.

Keywords: Polysaccharides, HPGPC, molecular weights.

The determination of molecular weights (*Mw*) of polysaccharides has served as an important process since the physical properties of the polysaccharides are closely related to their *Mw* and/or molecular weights distribution (*MWD*). For *Mw* and *MWD* determinations, high performance gel permeation chromatography (HPGPC) has gained wide acceptance as a preferred method due to its high sensitivity, speed and reproducibility.

In chromatography, it is well known that the molecular weights are determined by GPC on the basis of elution volumes (*Ve*) or retention times (*Rt*) of the test samples. *Ve* has been generally considered to be concentration-independent. However, some studies indicated that sample concentration plays a complex role in such gel determinations[1,2]. Furthermore, in the case of acidic substances, *Ve* was found to be affected by the ionic strength of the mobile phase[3,4,5]. However, the retention behaviors of uronic acid-containing polysaccharides in Ultrahydrogel columns had been little studied systematically before. In this work, the chromatographic behaviors of several uronic acid-containing polysaccharides and neutral polysaccharides were investigated in HPGPC for the first time. The effects of sample concentration and ionic strength of mobile phase on *Rt* were observed, the mechanism for which was also discussed.

Experimental

All chromatographic experiments were conducted on a Waters LC system with RI detector. Two serial GPC columns (7.8×300 mm), Ultrahydrogel^{TM} 2000 and 500 were employed. Millennium^{32} software (version 3.05.01) was used to control the system,

* E-mail: jnfang@mail.shcnc.ac.cn

collect signals and further analyze data. The column temperature was maintained at 30 ±0.1°C. Dextran series were obtained from Pharmacia. Polysaccharides Sal 1, Sal 2, Sal 4, Sal 6, PGL, J 5, J 6 and J 10 were isolated from *Salvia chinensis*, *Ganoderma lucidum* and *Nerium incicum*. The homogeneity was tested by HPGPC. The compositional analytical results are listed in **Table 1**. Sal 1, Sal 6, J 6, PGL and Dextran T-70 were studied at the concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.3125% to investigate the effect of sample concentration. Besides the above samples, Sal 2, Sal 4, J 5 and J 10 were used to probe the effect of ionic strength in mobile phase.

Table 1 Compositional analytical results of samples

	Sugar residue	Molar ratio	Percentage of uronic acid (%)
Sal 1	Rha: Ara: Gal: GalA	1.1: 1.0: 0.8: 1.0	26
Sal 2	Rha: Ara: Gal: GalA	1.0: 3.2: 2.9: 0.9	11
Sal 4	Rha: Ara: Xyl: Gal: Glc: GalA	2.6: 5.1: 2.0: 3.1: 1.0: 1.2	8
Sal 6	Rha: Xyl: Gal: GalA	1.0: 7.0: 1.2: 4.2	31
J 5	Rha: Ara: Gal: GalA	1.0: 2.2: 1.3: 0.5	10
J 6	Ara: Xyl: Man: Gal: Glc	6.8: 1.0: 2.3: 5.2: 5.5	0
J 10	Rha: Ara: Gal: GalA	1.0: 2.3: 1.3: 0.7	13
PGL	Glc		0

Results and discussion

Table 2 shows the *Rt* of Sal 1, Sal 6, T-70, J 6 and PGL at different concentrations with 0.002 mol/L NaAc as eluent at the flow rate of 0.5 mL/min, according to the processed results by LC software. As shown in **Table 2**, Sal 1 and Sal 6, the two uronic acid-containing polysaccharides displayed concentration-dependent behaviors. Their *Rt* increased remarkably, while those of J6, PGL and T-70 kept constant approximately although the sample concentration was increasing. Thus, it was reflected that *Mw* determination of uronic acid-containing polysaccharides would be affected by sample concentration in such gel determinations provided that Dextran series were employed to perform calibration plot. In order to quantify the effect, a calibration curve was performed with Dextran series under the same chromatographic condition as the samples to

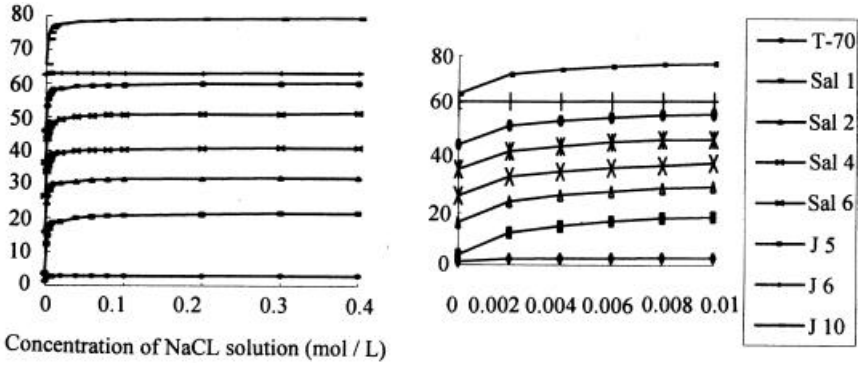
Table 2 *Rt* and corresponding *Mw* of polysaccharides at different concentrations

Sample Cocentration (%)	Sal 1		Sal 6		J 6		PGL		T-70
	Rt (min)	*Mw*	*Rt* (min)	*Mw*	*Rt* (min)	*Mw*	*Rt* (min)	*Mw*	*R t*(min)
0.3125	34.971	54601	29.183	924248	39.750	10124	33.900	84752	34.497
0.625	35.145	50967	29.300	861855	39.733	10179	33.867	85964	34.507
1.25	35.217	49541	29.487	771414	39.756	10105	33.867	85964	34.503
2.5	35.489	44594	29.858	622119	39.745	10140	33.867	85964	34.533
5.o	35.898	38164	30.102	542061	39.774	10044	33.790	86541	34.583

*The molecular weights were evaluated according to the standard curve performed with Dextran series. The equation was Log Mol*Wt* = 2.89e+001-1.54e+000T^1+3.39e-002T^2-2.72e-004T^3. R=0.998016, R^2=0.996037, Standard error = 6.047726e-002

evaluate *Mw* of Sal 1, Sal 6, J 6 and PGL. The results are also shown in **Table 2**. It could be observed that the estimated *Mw* of Sal 1 and Sal 6 reduced distinctly with increasing concentration, while no change took place in *Mw* of J 6, PGL according to the evaluation by GPC software. In addition, for Sal 1 and Sal 6, the 50% decrease in concentration corresponded to more than 10% increase in their estimated *Mw* within the range of 0.625~5%. However, when lower than 0.625%, the 50% decrease in concentration arose less than 10% increase in *Mw*.

Figure 1 Retention behaviors of polysaccharides as a function of ionic strength



(Retention time (min) *versus* concentration of NaCl solution)

Figure 1 shows the retention behaviors of Sal 1, Sal 2, Sal 4, Sal 6, J 5, J 6, J 10 and T-70 as a function of ionic strength in eluent. Due to the severe overlap, they were translated along Y-axis for different distance. It was clear that within the entire experi-mental concentration range of mobile phases, the curves for T-70 and J6 seemed to be straight lines parallel to X-axis. Only a slight increase in the *Rt* of T-70 was observed in 0~0.01 mol/L, which could be ascribed to the mild effect of NaCl on the conformation of T-70[6]. However, a sharp increase in *Rt* was found for all uronic acid-containing polysaccharides in the ionic strength region of 0~0.002 mol/L NaCl. When the concentration was between 0.002~0.1 mol/L, their *Rt* still increased slowly with the increase of ionic strength of eluent. Therefore, ionic strength in mobile phase affected remarkably the *Rt* of uronic acid-containing polysaccharides within 0~0.1 mol/L, while slightly on those of neutral polysaccharides.

In conclusion, ionic strength took an important role in *Mw* determination of uronic acid-containing polysaccharides. The greater the ionic strength of eluent, the smaller the estimated *Mw* of uronic acid-containing polysaccharides with HPGPC, which should be attributed to electrostatic interactions between uronic acid and ionic groups on polymethylacrylate, including ion exchange, ion exclusion, ion inclusion and absorption *etc*[7]. On the contrary, when within the concentration range of 0~0.002 mol/L, the estimated *Mw* of neutral polysaccharides increased slightly with increased ionic strength if Dextran series were employed as standards. When over 0.002 mol/L, the effect of ionic strength could be ignored. In order to determine *Mw* of polysaccharides meaningfully, the concentration of polysaccharides and standards should be adjusted to

within the best range and detected by RI detector, and ionic strength of the eluent (*e.g.* NaCl) should be about 0.1 mol/L in such gel determinations.

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