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Ionic liquids enable accurate chromatographic analysis of polyelectrolytes

Kosuke Kuroda and Hiroyuki Ohno

Molecular weight distribution of polyelectrolytes were analysed with high performance liquid chromatography using ionic liquids as eluents to get information on molecular weight distribution, because there were no electrostatic repulsion. A mixed sample of polycation and polyanion was also analysed to detect independently.

Due to its fascinating characteristics, polyelectrolytes open a lot of applications in such different fields as medicine, water treatment, mineral separation, paper-making processes, paint and food industries, cosmetics, and pharmacy. In these applications, polyelectrolytes play a critical role, but their mechanism is highly complicated. To obtain better insight into these mechanisms, fine characterization of polyelectrolytes is an overriding issue. Among many characteristics, molecular weight distribution (MWD) is of special importance. A MWD of polyelectrolytes has been usually investigated by gel permeation chromatography or size exclusion chromatography (their principle is the same), but the analysis is known to face discrepancies.^{1,2} Since polyelectrolytes have highly large hydrodynamic volume in water or polar organic solvents due to intra- and intermolecular charge repulsion, they elute at low retention volume (in the void volume). To prevent this, simple electrolytes are generally added to the eluent. Relatively high concentration ($> 1 \text{ mol L}^{-1}$)¹ is empirically known as the lowest concentration for complete suppression of repulsion of intra-molecular charges. However, addition of slightly high concentration of electrolytes ($> 0.1 \text{ mol L}^{-1}$)¹ simultaneously provides other problem; a new interfering peak (called salt exclusion peak) is induced due to Donnan effect,³⁻⁶ and the

peak often overlaps with the peak of polyelectrolytes. Therefore, it is obviously impossible to satisfy both conditions (i.e., salt concentration: over 1 mol L^{-1} and less than 0.1 mol L^{-1}), and one was obliged to increase the simple electrolyte concentration as high as possible to enable the observation of accurate peak. This conflicting condition is not solved until now.

To resolve this problem, we have paid an attention to ionic liquids (ILs)⁷⁻⁹ as eluents. ILs are liquids fully composed of ions, and therefore they provide ion concentration quite high enough to prevent inter- and intra-molecular charge repulsion. Furthermore, ILs are expected to diminish interfering salt exclusion peak in spite of their extremely high salt concentration. Since ILs contain no water molecules ideally, Donnan effect should be negligible in them.

As the second serious issue in chromatographic analysis of polyelectrolytes, we can point out that the universal condition applicable for a variety of polyelectrolytes has never been suggested. This is attributed to such the situation as suitable conditions of eluent (concentration of salt, pH, species of polymer used as filler, pore size of filler materials) are different significantly depending on the species of polyelectrolytes. This further leads two serious problems. One is the difficulty to fix an appropriate condition for individual sample. There is only a semi-empirical knowledge for the selection. The other issue is more serious, i.e., there is no way to analyse MWD of polyampholytes and/or polyion complexes. There is no common eluent applicable to both polycation and polyanion, and therefore no potential technique for polyampholytes. MWD of polyion complexes composed of polycations and polyanions have never been analysed. According to the literature², dilute solution of neutral salts like NaCl ($0.05 - 0.3 \text{ mol L}^{-1}$) near pH = 7 is the first choice for polyanions. On the other hand, concentrated solution of neutral salts like Na_2SO_4

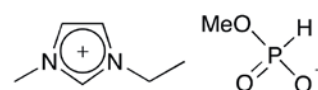


Fig. 1 Ionic liquid used as an eluent in this study.

^a Department of Biotechnology, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo, 184-8588, Japan
E-mail: ohnoh@cc.tuat.ac.jp

^b Functional ionic liquid laboratories, Graduate School of Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo, 184-8588, Japan

[‡] Present address: Division of Material Engineering, Kanazawa University, Kakumamachi, Kanazawa 920-1192, Japan

[†] Electronic Supplementary Information (ESI) available: Chromatograms of the sample with different feed amount of NaPSS; Chromatogram of diallyldimethylammonium salt/maleic acid copolymer; Chromatograms of gelatin and silk. See DOI: 10.1039/x0xx00000x

(0.3 – 0.8 mol L⁻¹) with acid like acetic acid or hydrochloric acid (0.5 mol L⁻¹) was reported for polycations. By comparing these candidates, it seems impossible to manage chromatographic analysis of both polycations and polyanions with only eluent of single-species.

For the solution of above-mentioned issue, ILs are also hopeful to be an eluent. ILs are expected to prevent intra- and inter-molecular charge repulsion regardless of charging characteristics of polyelectrolytes. Previously, ILs have been applied to some analytical systems¹⁰⁻¹⁹ due to their unique properties. We have also developed high performance liquid chromatography with ILs as eluents, so-called HPILC, and analysed MWD of insoluble polysaccharides in most molecular liquids.^{20, 21} In this study, HPILC was examined as a potential method to analyse MWD of polyelectrolytes.

We used a dried IL, 1-ethyl-3-methylimidazolium methylphosphonate²² [C₂mim][(MeO)(H)PO₂], see Fig. 1) as an eluent. This IL has very high proton-accepting ability to dissolve many kinds of polymers even cellulose, low viscosity (25 cp. at 55 °C), and relatively small formula weight (206.2). The concentration of neat [C₂mim][(MeO)(H)PO₂] is calculated to be 5.8 mol L⁻¹ (density: 1.19 g·cm⁻³), which is higher than those of aqueous salt solutions investigated as eluents until now. Sodium polystylenesulfonate (NaPSS) was selected as a strong polyelectrolytes typically showing polyelectrolyte characteristics such as intramolecular chain expansion.²³ NaPSS standard samples with *M_w* from 3,000 to 900,000 were used to confirm the usefulness of ILs as eluents for HPILC analysis in a broad range of *M_w*.

NaPSS samples with different *M_w* were individually dissolved in [C₂mim][(MeO)(H)PO₂] and their HPILC profiles were measured (Fig. 2). At the void volume (2.2 ml), no peaks were found. Columns used in this study allow to detect the *M_w* lower than one million (details later). In addition, there were no signals which overlap with those of NaPSS because there were no salt exclusion peaks in spite of quite high salt concentration. In these ILs, there is no Donnan effect observed. While solvent peak was found at over 4.5 ml as a

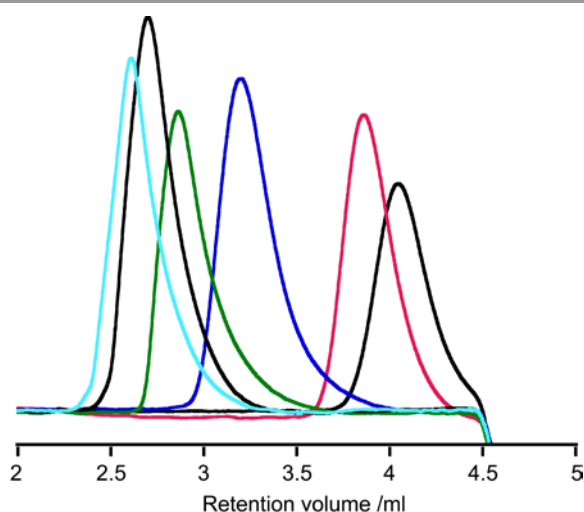


Fig. 2 Chromatograms of NaPSS. *M_w* of NaPSS: 900k, 300k, 80k, 30k, 7k, and 3k (from left to right).

negative peak, the peak was never overlapped with those of polymers. The retention volume (4.5 ml) was revealed to be larger than that of low molecular weight compound (glucose, in the previous experiment).²⁰ It should be noted here that appearance of the negative peak in the chromatogram of non-ionic polymer rationalises that it is not a salt exclusion peak but a solvent peak.

The shape of polyelectrolyte peaks was found to be unimodal, showing general peaks as polymer standards. Their peaks were observed in different retention volumes, indicating that the *M_w* profiles of NaPSS are easy to be analysed.

The relation between *M_w* and retention volume of NaPSS is plotted in Fig. 3. The relation was a typical curve for column chromatography. The detectable *M_w* range was about one million to five thousand. The difference in resolution between low and high *M_w* regions is attributed to connected two columns utilised for high *M_w* and low *M_w* materials. It is therefore possible to expand the detectable *M_w* range by selecting suitable columns. The relation between *M_w* and retention volume of pullulan (non-charged polymer) was also depicted as a reference. The data for pullulan are plotted on a typical curve, i.e., governed by only hydrodynamic size. The curve for NaPSS almost overlapped with those for pullulan. This clearly shows that HPILC profiles for NaPSS simply reflect their *M_w* without the effect of electrostatic interaction.

Dependence of concentration of NaPSS was investigated (see Fig. S1 in the supporting information). NaPSS of 0.5 or 2 mg was dissolved in 0.20 g of [C₂mim][(MeO)(H)PO₂], and 5 µL of sample solution was injected. No shift of maximum and minimum retention volume was found. In usual eluents, there is a shift to higher retention volume side as increasing polyelectrolyte concentration. In the case of HPILC, the concentration of ions is obviously sufficient to suppress the intramolecular charge repulsion. Shift of the peak molecular weight (called *M_p*) was observed due to macromolecular

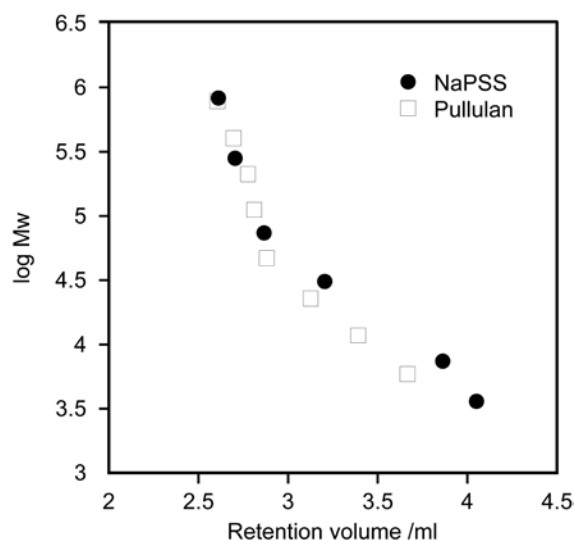


Fig. 3 Calibration curves of (●) NaPSS and (□) pullulan between retention volume and log *M_w*. *M_w* of NaPSS: 900k, 300k, 80k, 30k, 7k, and 3k. That of pullulan: 790k, 400k, 210k, 110k, 47k, 23k, 12k, and 6k.

crowding and viscous fingering; it was also observed in chromatograms of non-charged polymers.

To evaluate further potential of HPILC, we challenged to analyse the mixed samples of oppositely charged polyelectrolytes, which form polyion complex in an aqueous solution. Polystyrenesulfonic acid (PSS) and polyallylamine (PAA) were respectively dissolved in $[C_2mim][[(MeO)(H)PO_2]]$ and mixed. They cannot form polyion complex due to extremely high salt concentration. The HPILC profile of the mixture is seen in Fig. 4. The chromatogram of the mixture was bimodal and the two peaks corresponded to the peak of each component polyelectrolyte. In each chromatogram for PSS and PAA, unimodal peak was found as also seen in Fig. 4, and there was no peak at the void volume. This clearly shows that the mixed sample of polyelectrolytes was analysed independently even in the case of their combination strong enough to form polyion complex. The analyses were repeated for several times to get the same results confirming the reproducibility. Thus, HPILC is extremely potential tool for intact investigation on many charged polymers and their mixtures in nature.

We also analysed MWD of random copolymer of anionic- and cationic monomers so-called polyampholytes (see Fig. S2 in the supporting information). Their small solubility in water has also hitherto prevented from analyses. However, this HPILC system is a potential method to cancel the effect of electrostatic interaction, and is applicable to both anionic and cationic polyelectrolytes as mentioned above. As clearly seen in Fig. S2, no peak was observed at the void volume, strongly indicating that ILs suppress the effect of both positive and negative charge. Therefore we believe that it was analysed appropriately but we do not have any supporting data relating to accurate molecular weight of this copolymer because its MWD has never been analysed until now. HPILC is also expected to be a powerful method for

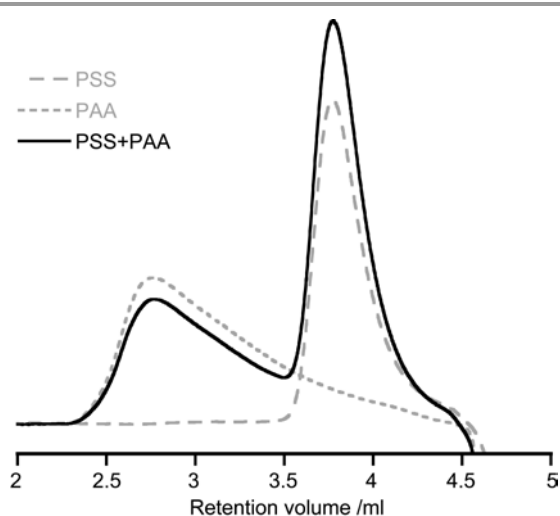


Fig. 4 Chromatograms of anionic polyelectrolyte (PSS) and cationic polyelectrolyte (PAA) and their mixtures (PSS+PAA).

block copolymers from these results.

As typical biopolymers, silk and gelatin (positively and negatively charged protein, respectively) were measured as shown in Fig. S3 in the ESI. From these results, HPILC is effective for not only synthetic polyelectrolytes but also charged biopolymers regardless of their charging characteristics.

We strongly suggest here that the HPILC is a powerful method to analyse M_w and MWD of polyelectrolytes, their mixtures, and polyampholytes.

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