

Investigation of Polystyrene, Polyisoprene, and Poly(2-vinylpyridine) using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Hyuneui Lim, Yeonhee Lee,^{†,*} Seunghee Han,[†] Youngsook Yoo,[‡] and Kang-Jin Kim

Department of Chemistry, Korea University, Seoul 136-701, Korea

[†]Advanced Analysis Center, Korea Institute of Science and Technology, Seoul 136-791, Korea

[‡]Doping Control Center, Korea Institute of Science and Technology, Seoul 136-791, Korea

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MALDI-TOF-MS is a soft ionization technique that has the potential to determine the molecular weight for various compounds, particularly for large biomolecules.¹⁻³ Recently, considerable interest has been focused on the analysis of synthetic polymers by MALDI-MS.⁴ Compared with the traditional methods of polymer analysis, MALDI-MS can provide more abundant information on molecular weight, repeat unit, end group, additives, impurities, and side products for pure polymers, and even on complex copolymers with recently improved resolution and mass accuracy.⁵⁻⁷

Although this new method is fast, popular, and instrumentally very simple, MALDI results obtained by different groups for polymer analysis have revealed different conclusions.^{8,9} This comes from considerable variations in sample preparation and instrumental limits.^{10,11} Recently, Schriemer groups reported the detection of very high molecular weight narrow polydisperse polystyrene by careful formulation of the matrix/analyte preparation and by using a sensitive MALDI detection system.¹²

This paper discusses the matrix effect, cationization agent effect, and solvent effect with the different molecular weight polystyrenes used as GPC standards. UV absorption and fluorescence properties of matrices are studied to get the fundamental understanding about the MALDI mechanism of ion formation using an UV laser. This work also demonstrates that MALDI can be a useful tool for polyisoprene and poly(2-vinylpyridine) molecular weight determination using proper matrix selection and sample preparation.

Experimental Section

Sample preparation. All reagents were obtained commercially and used without further purification. Polystyrene standards were supplied from Aldrich (Milwaukee, WI, USA) with molecular weight information. Polyisoprene and poly(2-vinylpyridine) standards were supplied from Polyscience (Warrington, PA, USA). The matrices, silver salts, and solvents except toluene (Merck, Darmstadt, Germany) were purchased from Aldrich.

To study the effect of matrices, silver salts and solvents, polystyrene 3,000 were dissolved in tetrahydrofuran, chloroform, dichloromethane, and toluene at a concentration 2×10^{-3} M. Matrices were prepared to a 0.15 M concentration in each solvent, and silver salts were saturated in ethanol. Matrix, cationization agent, and polymer solution were

mixed with a volume ratio of 1 : 1 : 1. A 0.7 μ L of the mixture was spotted on the probe. The samples were allowed to vacuum-dry. As the polymer molecular weight increases, an increasing number of matrix molecules is used to prepare polymer/matrix solution. NaCl for poly(2-vinylpyridine) was also saturated in ethanol.

For UV absorption and fluorescence spectra, the solution of matrices with a concentration of 10^{-3} M~ 10^{-5} M was prepared by dissolving in chloroform.

Instrumental Methods. Mass spectral data were collected on HP G2025A MALDI-TOF mass spectrometer equipped with a pulsed nitrogen laser. This instrument was operated in positive mode using 28 kV ion acceleration. Spectra were acquired by averaging spectra from 10 shots for low molecular weight polymers to 100-150 shots for high molecular weight polymers. The collections of spectra and the calculation of molecular weight were processed by HP supporting software.

UV absorption spectra were acquired from HP 89531 UV spectrometer. The correction of background was performed with chloroform. Fluorescence emission study of matrices was done with Aminco Bowman Series 2 Luminescence Spectrometer. Samples were excited at 337 nm, which was the employed wavelength for the laser beam in this work.

Results and Discussion

Figure 1 showed the spectra of polystyrene 3,000 using four different matrices under same conditions except laser power. Among the matrices, dithranol and *all-trans*-retinoic acid were good matrices to obtain intense signal, whereas 15-crown-5 ether showed poor capability as a matrix. Not shown in the figure, it was observed that spectra obtained with other matrices such as 6,13-pentacenequinone, 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), and 5-chlorosalicylic acid were similar to polystyrene spectrum of 2,5-dihydroxybenzoic acid (2,5-DHB). And 2-nitrophenyl octyl ether (NPOE) matrix gave the same spectrum as 15-crown-5 ether. This discrimination of matrix on MALDI spectra became severe as the molecular weight of polystyrene was increased. With increasing molecular weight of the analyte, larger molar amount of matrices should be added to the probe in order to prevent polymer chain association and to increase energy absorption by matrix. Whereas all matrices yielded MALDI spectra of polystyrene 3,000, we could

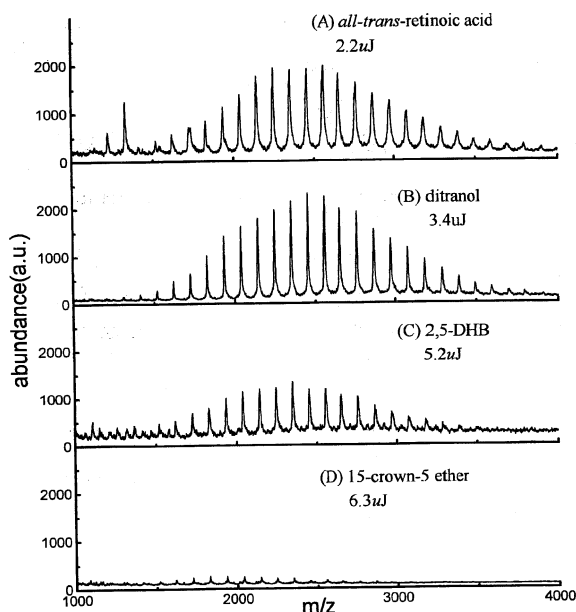


Figure 1. Mass spectra of PS ($M_n = 2,398$, certified by the supplier) with AgNO_3 as a cationization agent and chloroform as the solvent in all sample preparation using (A) *all-trans-retinoic acid*, (B) dithranol, (C) 2,5-DHB, and (D) 15-crown-5 ether.

obtain the reproducible ion signal of polystyrene 13,000 using *all-trans-retinoic acid*, 5-chlorosalicylic acid, 6,13-pentacenequinone, and dithranol, as a matrix. The MALDI results of polystyrene indicated that *all-trans-retinoic acid* and dithranol were an efficient matrix for high molecular weight polymers.

Based on the above investigations, we examined the different molecular weight polystyrenes by simple and reproducible sample preparation method including dithranol or *all-trans-retinoic acid* as a matrix, AgNO_3 as a cationization agent, and chloroform as a solvent. *All-trans-retinoic acid* showed very intense peaks of matrix clusters in low mass region. Their peaks overlapped with low mass oligomer peaks and gave a difficulty to determine the molecular weight for polystyrene 1000. Up to molecular weight 13,000, oligomer peaks were resolved with mass difference of 104 Da. In polystyrene spectra of molecular weight below 100,000, the singly charged molecular ions and clusters such as dimer ($2M^+$) and trimer ($3M^+$) were shown. As the molecular weight increased above 100,000, the multiple charged ions such as M^{2+} , M^{3+} , etc. appeared.

So as to get a general information of matrix, UV absorption spectra and fluorescence spectra of matrices were observed. All matrices had absorption at UV region and fluorescence at visible region. The combined results of UV and fluorescence study with MALDI spectra are listed in Table 1. It implied useful informations about ion formation of polystyrene in MALDI. Matrices which have properties of intense absorbance at 337 nm and weak fluorescence at an excitation of 337 nm showed good resolution and oligomer distribution in MALDI spectra.¹³ It is indicated that the success of the MALDI experiment is more related to nonradiative processes in the matrices' relaxation properties than

Table 1. UV absorption, fluorescence, and MALDI mass spectra of PS by using different matrices

matrix	UV absorption	Fluorescence (λ_{em})	MALDI mass spectra*
POPOP	+++	+++ (420 nm)	●●●
<i>all-trans-retinoic acid</i>	+++	-- (480 nm)	●●●●
dithranol	+++	- (435 nm)	●●●●
6,13-pentacenequinone	++	+ (443 nm)	●●●
NPOE	++	- (400 nm)	●●
5-chlorosalicylic acid	+	+++ (388 nm)	●●●
2,5-DHB	+	++++ (400 nm)	●●●
15-crown-5 ether	-	+ (414 nm)	●

+ : good peak intensity. - : poor peak intensity. ●●●● : good signal intensity and resolution. ● : poor signal intensity and resolution. * : PS spectra with AgNO_3 as a cationization agent and chloroform as a solvent.

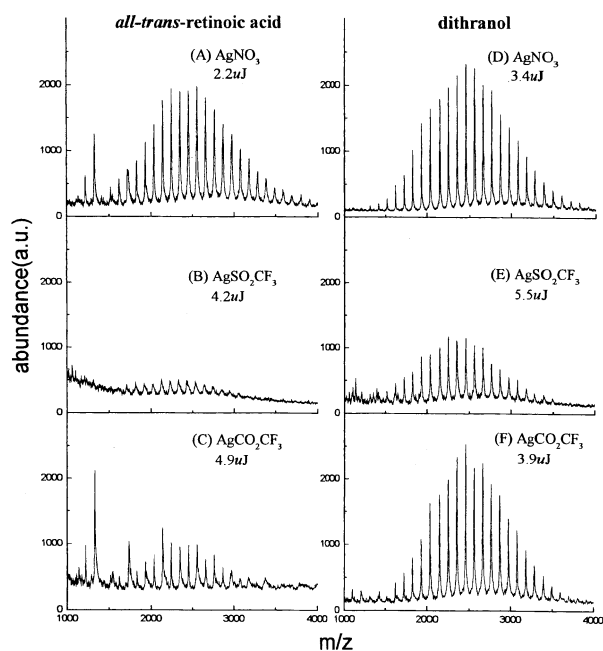


Figure 2. Mass spectra of PS ($M_n = 2,398$) with (A) (B) (C) *all-trans-retinoic acid*, (E) (F) (G) dithranol as a matrix in chloroform.

radiative processes.

Unlike biomolecules in which its ionization is generally achieved by protonation, synthetic polymer ions by MALDI experiment are produced by adduction of monovalent cations such as K^+ , Na^+ , Ag^+ , Cu^+ , etc.^{14,15} We investigated the effect of metal salt on the MALDI spectra, especially the counter ion. Figure 2 showed this effect was very significant for some matrices. In the case of dithranol, well-resolved oligomer signals were generated using AgNO_3 or AgCO_2CF_3 as a cationization agent. When *all-trans-retinoic acid* was used as a matrix only AgNO_3 could produce well defined oligomer distributions which gave the information on the molecular weight and structure.

Similar results were acquired from the different matrices. 15-crown-5 ether and 5-chlorosalicylic acid generate better oligomer distribution in combination with AgNO_3 . In the cases of NPOE and POPOP, the use of AgCO_2CF_3 yields

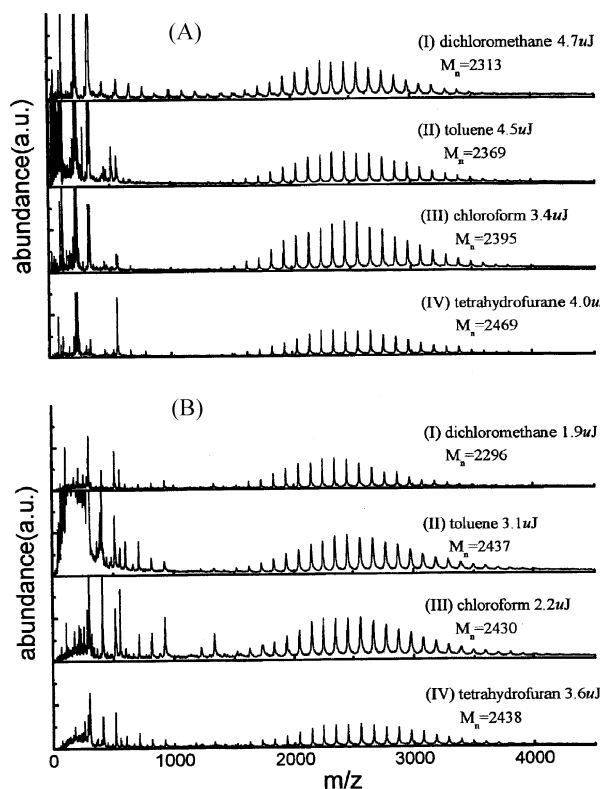


Figure 3. Mass spectra of PS ($M_n = 2,398$) with (A) dithranol, (B) *all-trans*-retinoic acid as a matrix in different solvents. AgNO_3 was a cationization agent.

greater analyte signals in the spectra than others. MALDI spectra using AgSO_3CF_3 display poor peak intensities with all matrices studied because of strong reactivity of SO_3CF_3^- ion. Counter ions have influence on the interaction between matrix and silver salt. Thus, successful cationization agent in polymer analysis can be found by selection of metal ion in conjugation with the use of optimal counter ion.

Polystyrene can be dissolved in a variety of solvents. Figure 3 displays eight MALDI spectra of polystyrene using four different kinds of solvents. The molecular weight determined in each spectrum was acceptable value. The selection of solvent is not important if solvent dissolves the polymer and matrix completely.

The sample preparation method established for polysty-

Table 2. Number-average molecular weight (M_n) of PS and PI standards

polystyrene	GPC*	MALDI	polyisoprene	GPC*	MALDI
800	700	884	1,000	560	652
3,000	2,398	2,395	3,000	3,280 ⁺	2,454
4,000	3,400	3,332	10,000	10,600	8,445
13,000	12,600	11,493	30,000	19,300	18,843
35,000	29,100	26,593	70,000	57,726	55,351
50,000	41,200	44,493			
200,000	198,250	205,393			

GPC*: data were certified by supplier. PI 3,280⁺: most probable molecular weight

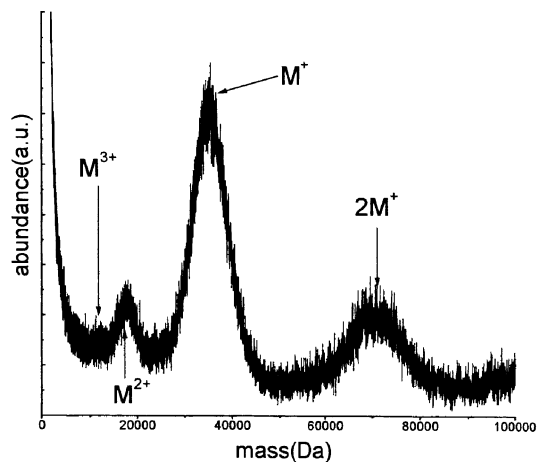


Figure 4. Mass spectra of poly(2-vinylpyridine) ($M_n = 28,000$, $M_w = 30,000$) certified by manufacturer with GPC. Dithranol was used as a matrix with NaCl as a cationization agent in chloroform.

rene was applied to polyisoprene and poly(2-vinylpyridine). Polyisoprene is nonpolar and has double bonds to provide an attachment site for the silver ion. Table 2 lists the data for the number-average molecular weight of polystyrenes and polyisoprenes with GPC and MALDI. Oligomer peaks in MALDI spectra were not resolved in high mass range, so that molecular weight was determined by reading the most intense peaks. The molecular weights obtained by MALDI gave good agreement with GPC results certified by suppliers.

Even though poly(2-vinylpyridine) has similar structure to polystyrene, only Na^+ attached oligomers were observed in MALDI experiment. It seems that hetero atoms in poly(2-vinylpyridine) interact favorably with sodium ion. Figure 4 showed mass spectrum of the poly(2-vinylpyridine) 30,000 with adduction of sodium ion by using dithranol. Multiple charged ions and cluster ion as well as single charged molecular ion were detected.

Conclusion

The MALDI parameters for the analysis of polymer have been evaluated. Optimal matrix, cationization agent, and solvent were established, especially for polystyrene, polyisoprene, and poly(2-vinylpyridine). Generally, matrix with intense absorbance at 337 nm and weak fluorescence performed very well for polystyrene MALDI analysis.

The choice of cationization agent must be considered with both metal and counter ion to prevent side reactions. It was not a critical problem to choose a solvent if the polymer was dissolved completely in the solvent. Dithranol and *all-trans*-retinoic acid were used as a matrix and AgNO_3 as a cationization reagent in order to get the successful MALDI spectra for polystyrene and polyisoprene. This protocol also was applied to poly(2-vinylpyridine) with dithranol and NaCl. The number-average molecular weights measured by MALDI gave a reasonable agreement with those determined by GPC.

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