

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry for Polymer Analysis: Solvent Effect in Sample Preparation

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The success of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry for the characterization of polymer structures and for the determination of average molecular weights and distributions depends on the use of a proper sample/matrix preparation protocol. This work examines the effect of solvents, particularly solvent mixtures, used to prepare polymer, matrix, and cationization reagent solutions, on MALDI analysis. It is shown that the use of solvent mixtures consisting of polymer solvents does not have a significant effect on the molecular weight determination of polystyrene 7000 and poly(methyl methacrylate) 3750. However, solvent mixtures containing a polymer nonsolvent can affect the signal reproducibility and cause errors in average weight measurement. This solvent effect was further investigated by using confocal laser fluorescence microscopy in conjunction with the use of a fluorescein-labeled polystyrene. It is demonstrated that sample morphology and polymer distribution on the probe can be greatly influenced by the type of solvents used. For sample preparation in MALDI analysis of polymers, it is important to select a solvent system that will allow matrix crystallization to take place prior to polymer precipitation. The use of an excess amount of any polymer nonsolvent should be avoided. (J Am Soc Mass Spectrom 1998, 9, 1303–1310) © 1998 American Society for Mass Spectrometry

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been shown to be a very useful tool for polymer characterization [1–13]. It can be used to determine average molecular weights and molecular weight distributions of narrow polydispersity polymers [14]. It can also provide structural information on end-group, repeat unit, and chemical modification of a polymeric system, if oligomer resolution is attained [15–17]. However, the success of this technique for polymer analysis is very much dependent on the availability of a suitable sample/matrix preparation. Sample preparation involves polymer dissolution, followed by mixing with the matrix solution and a cationization reagent. Great care must be taken in developing sample preparation protocols to ensure the generation of accurate and precise results. Several studies have shown that a number of factors in sample preparation can affect the MALDI results [2, 18–23]. Among them, the type and quality of solvents can greatly influence the MALDI analysis. For example, we have shown that the dryness and purity of tetrahydrofuran (THF) used to prepare polymer samples play a central role in the success of detecting high molecular weight polymers [13]. Recently, it has been shown that the use of certain

binary solvent systems can cause mass discrimination [23], although the reasons for such an error were not investigated.

Accurate determination of the average molecular weights of a polymer by MALDI requires the instrument and the sample preparation method to provide a large dynamic range of ion detection as well as true mass spectral representation of the relative intensities of oligomers in a polymer distribution. As has been shown in MALDI biopolymer analysis, analyte distribution in matrix crystals can significantly affect the signal reproducibility, detection sensitivity, and relative intensities of individual components in a mixture [24]. Analyte distribution can be affected by the solvent system used for preparing the analyte and matrix. However, unlike biopolymer analysis where a common solvent can often be found to dissolve both the analyte and matrix, the choice of a solvent system for polymer analysis by MALDI is much more critical. In particular, solvents used to dissolve polymers may not be compatible with the matrix or cationization reagent. The current practice (and still recommended) is that, whenever possible, a single solvent system should be sought to prepare the polymer/matrix sample. However, for a number of applications, the use of a solvent mixture cannot be avoided. In this case, the choice of solvents becomes an important issue in the development of a useful sample/matrix preparation protocol.

In this work, we report a detailed investigation of the

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solvent effect on MALDI analysis of polymers. It is demonstrated that the solvent effect follows a systematic pattern. It is hoped that understanding this solvent effect will clarify or avert any possible misinterpretation of the MALDI results and aid in the development of optimal sample preparation methods for polymer analysis by MALDI-TOF MS.

Experimental

Instrumentation

Mass spectral data were collected on a linear time-lag focusing MALDI-TOF mass spectrometer with a 1-m linear flight tube [25, 26]. The ions are generated using the 337 nm laser beam from a nitrogen laser, having a pulse width of 3 ns (model VSL 337ND, Laser Sciences, Newton, MA). Laser fluence was maintained slightly above ion detection threshold in all analyses. A micro-channel plate (MCP) detector was used for ion detection and a Hewlett-Packard MALDI data system was used for mass spectral recording and data processing. This data system is a modified version of the software used for the HP Model G2025A MALDI-TOF MS, in which the instrument control features have been disabled. All data were further processed using the Igor Pro software package (WaveMetrics, Lake Oswego, OR). No correction of $1/(dm/dt)$ was applied to the mass spectra during the conversion of the time domain to the mass domain. The dm/dt term is the derivative of the calibration equation used for converting time, t , to mass, m [27]. Average molecular weights (M_n , M_w) were determined directly from the time domain according to the following equations:

$$M_n = \Sigma(N_i M_i) / \Sigma N_i$$

$$M_w = \Sigma(N_i M_i^2) / \Sigma N_i M_i$$

where N_i and M_i represent signal intensity in peak area and mass for the oligomer containing i monomers, respectively. The polydispersity, PD, was determined from the ratio of M_w to M_n . Average molecular weights were corrected for the contribution of the cation. In general, mass spectra from 100 laser shots were summed to produce a final spectrum. All mass spectra shown in the figures are the smoothed spectra using 15-point Savitzky-Golay smoothing. No baseline correction was performed. The sloping baseline generally observed in the MALDI spectra of polymers becomes noticeable when the detection sensitivity of the analyte ions decreases and the background ion intensity, particularly in the low mass region, increases.

Samples and Reagents

Bradykinin, bovine ubiquitin, and equine cytochrome *c* used in the calibration were obtained from Sigma (Milwaukee, WI). The matrix used in their analyses

(sinapinic acid) was purchased from Aldrich (St. Louis, MO). The polymers used in this study include polystyrene (PS) 7000 (Polymer Laboratories, Amherst, MA) and poly(methyl methacrylate) (PMMA) 3500 (American Polymer Standard, Mentor, OH), and fluorescein-labeled polystyrene 7700 (labeled at one end group, according to the supplier) (Polysciences, Warrington, PA). *All-trans*-retinoic acid (Aldrich) was used as the matrix for these polymers. *Trans*-indoleacrylic acid (IAA) (Aldrich) was also used for the analysis of the labeled polystyrene. AgNO₃ and NaCl were reagent grade (Aldrich) and used without further purification. Tetrahydrofuran (THF) (VWR, Toronto, Canada) used in the dissolution of polymers and matrices was pretreated with potassium hydroxide, filtered, then distilled over sodium metal, in the presence of benzophenone as an indicator of dryness.

Sample Preparation

Polymer samples for MALDI analysis were prepared by combining the analyte, matrix, and cationization reagent solutions. The polymers were dissolved in THF to prepare stock solutions with concentrations of approximately 2×10^{-3} M for polystyrenes and PMMA (based on nominal mass for calculation). Retinoic acid was prepared to 0.15 M in THF. Silver nitrate was used as the cationization reagent for polystyrenes and NaCl was used for PMMA. Silver nitrate was dissolved in ethanol to 0.15 M. Sodium chloride was prepared in methanol to a saturated solution.

A 100- μ L sample solution of a known solvent composition was prepared by mixing different volumes of the analyte, matrix, and cationization reagent solutions as well as the testing solvent. For example, the sample solution containing 99.5% THF and 0.5% ethanol was prepared by mixing 5 μ L of the polymer solution in THF, 0.5 μ L of AgNO₃ in ethanol, 44.5 μ L of the matrix solution in THF, and 50 μ L of THF. The sample solution containing 5% water, 94.5% THF, and 0.5% ethanol was prepared by mixing 5 μ L of the polymer solution in THF, 0.5 μ L of AgNO₃ in ethanol, 44.5 μ L of the matrix solution in THF, 45 μ L of THF, and 5 μ L of water. Typically, 1 μ L of the mixture was added to the MALDI probe tip and allowed to air dry.

For the MALDI analysis and confocal microscopic imaging of fluorescein-labeled polystyrene using IAA as the matrix, IAA was prepared to 0.4 M in THF. The polymer was dissolved in THF to 2×10^{-3} M. Saturated silver nitrate solution in ethanol was used. The sample solutions were prepared in the same manner as described above.

Confocal Microscopy

Molecular Dynamics' Multiprobe 2001 confocal laser scanning microscope was used for the generation of all images reported here. An argon/krypton laser operating at 488 nm was used for the excitation of the

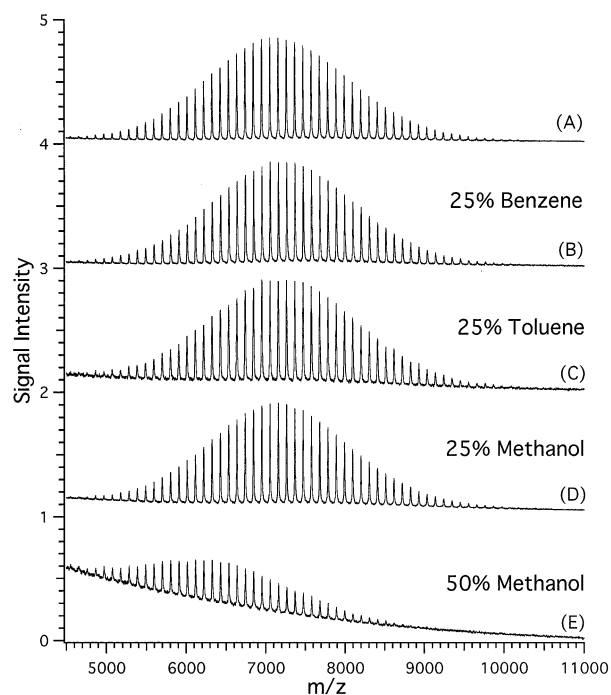


Figure 1. MALDI mass spectra of polystyrene 7000 obtained by using different solvent systems for sample preparation: (A) 99.5% THF/0.5% ethanol, (B) 25% benzene/74.5% THF/0.5% ethanol, (C) 25% toluene/74.5% THF/0.5% ethanol, (D) 25% methanol/74.5% THF/0.5% ethanol, and (E) 50% methanol/49.5% THF/0.5% ethanol. All *trans*-retinoic acid was used as the matrix and silver nitrate was used as the cationization reagent.

fluorescein-labeled polystyrene. The sample was deposited onto a stainless steel MALDI probe. The probe was then placed in the specimen holder in the microscope. The fluorescence image of the analyte as well as the matrix was from one planar image on the surface of the sample layer.

Results

Polystyrene 7000

Figure 1 shows five MALDI mass spectra of PS 7000 obtained by using different solvents for sample prepara-

tion. The solvent system used for producing the spectrum shown in Figure 1A consisted of 0.5% ethanol and 99.5% THF. With the deposition of 1 μ L of this initial solution on the sample probe, the final solid sample contained 100 pmol of PS 7000, 66.8 nmol of the matrix, and 750 pmol of AgNO₃. For the spectra shown in Figure 1B–D, all experimental conditions were the same as those used for Figure 1A except the solvent systems consisted of 0.5% ethanol, 74.5% THF, and 25% benzene, or toluene, or methanol. Table 1 lists M_n , M_w , and PD values obtained for these samples by MALDI. The relative differences in average weights obtained from different solvent systems are also shown. Table 1 indicates that the use of a solvent mixture containing 25% benzene, or toluene, or methanol does not affect the measurement of M_n and M_w of PS 7000. The differences are well within the statistical errors at the 99% confidence limit.

Another set of MALDI spectra for PS 7000 were obtained by using solvent mixtures containing 0.5% ethanol, 49.5% THF, and 50% benzene, toluene, or methanol. The average weight results from this set of spectra are also shown in Table 1. In the case of using benzene or toluene as the third solvent, similar spectra as those shown in Figure 1B, C were obtained. However, a different spectrum was obtained in the case of using 50% methanol as the third solvent (see Figure 1E). Figure 1E shows a severe mass discrimination at the high mass region of the polymer distribution. As Table 1 illustrates, the M_n and M_w values are reduced by 9.6% and 9.7%, respectively, from those obtained by using the solvent system containing 0.5% ethanol and 99.5% THF. The precisions for M_n and M_w measurements, as indicated by the standard deviations shown in Table 1, are also reduced. It should be noted that, when a mixture of THF and benzene or toluene was used as the solvent, the initial sample solution was transparent. But white turbidity was observed in the sample solution prepared with the solvent mixture containing 49.5% THF and 50% methanol.

The effect of water addition on mass spectral patterns of PS 7000 is even more pronounced. Figure 2 shows two spectra obtained from two different sample

Table 1. MALDI results for the analysis of polystyrene 7000 using different solvent systems for sample preparation^{a,b}

Solvent systems	M_n	M_w	PD	% Diff. ^c
99.5% THF/0.5% ethanol	7057 \pm 22 (7029 to 7085)	7174 \pm 19 (7157 to 7203)	1.017	...
25% methanol/74.5% THF/0.5% ethanol	7085 \pm 21 (7054 to 7107)	7206 \pm 23 (7175 to 7223)	1.017	0.4
25% toluene/74.5% THF/0.5% ethanol	7073 \pm 29 (7035 to 7107)	7196 \pm 19 (7178 to 7225)	1.017	0.2
25% benzene/74.5% THF/0.5% ethanol	7063 \pm 24 (7036 to 7098)	7196 \pm 16 (7162 to 7206)	1.019	0.1
50% methanol/49.5% THF/0.5% ethanol	6377 \pm 140 (6196 to 6512)	6476 \pm 145 (6287 to 6624)	1.016	-9.6
50% toluene/49.5% THF/0.5% ethanol	7056 \pm 23 (7017 to 7075)	7179 \pm 27 (7138 to 7199)	1.017	0.0
50% benzene/49.5% THF/0.5% ethanol	7057 \pm 28 (7029 to 7097)	7174 \pm 33 (7142 to 7224)	1.017	0.0
5% water/94.5% THF/0.5% ethanol	6103 \pm 1464 (3375 to 7340)	6455 \pm 1072 (4334 to 7573)	1.058	-13.5

^aGPC data from the supplier: M_n = 6770, M_w = 6962, and PD = 1.03.

^b M_n , M_w , and standard deviations were calculated from five trials except the solvent system containing water where the results were from nine trials. The ranges for M_n and M_w are shown in parentheses.

^c% Difference in M_n , compared to that obtained by using 99.5% THF/0.5% ethanol as the solvent.

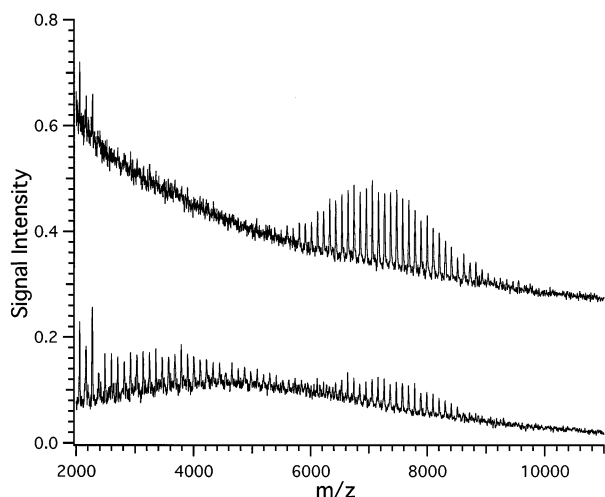


Figure 2. MALDI mass spectra of polystyrene 7000 obtained from two different regions of the same sample prepared by using a mixture solvent containing 25% water/74.5% THF/0.5% ethanol. All *trans*-retinoic acid was used as the matrix and silver nitrate was used as the cationization reagent.

spots using the same sample preparation conditions as those in Figure 1 except the solvent mixture consisting of 0.5% ethanol, 74.5% THF, and 25% water. In addition to the change of spectral patterns, the MALDI spectra were not reproducible and great variations from spot to spot were noted. Table 1 lists the M_n and M_w results obtained from nine trials. As Table 1 shows, the precisions for M_n and M_w measurements in this case are very poor.

Poly(methyl methacrylate) 3750

Figure 3 shows the mass spectra of PMMA 3750 obtained using retinoic acid as the matrix and NaCl as the cationization reagent. The spectrum shown in Figure 3A was obtained with the use of 99.5% THF and 0.5% methanol as the solvent for solution preparation. The solvent mixtures used for obtaining the spectra shown in Figure 3B–D consist of 74.5% THF and 0.5% methanol with the addition of 25% benzene, or 25% toluene, or 25% methanol. In all cases, the final solid sample on the probe, with 1- μ L sample solution deposition, contained 100 pmol of PMMA 3750, 66.8 nmol of the matrix, and an undetermined amount of NaCl. Table 2 lists the MALDI results obtained using different solvent systems for sample preparation. Note that, in the case of using 25% toluene as the co-solvent, a more severely sloping baseline can be observed in the MALDI spectrum (Figure 3C). However, as it can be seen from Table 2, this change of baseline does not affect the M_n and M_w measurements and their precisions.

The addition of a small amount of water to the solvent mixture can affect the determination of the average molecular weights of PMMA 3750. For example, Figure 3E shows a mass spectrum of PMMA 3750

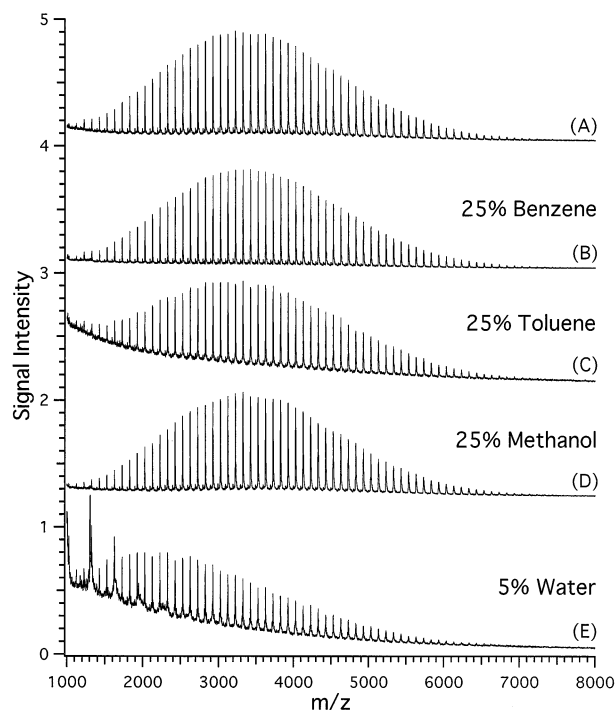


Figure 3. MALDI mass spectra of PMMA 3750 obtained by using different solvent systems for sample preparation: (A) 99.5% THF/0.5% methanol, (B) 25% benzene/74.5% THF/0.5% methanol, (C) 25% toluene/74.5% THF/0.5% methanol, (D) 25.5% methanol/74.5% THF, and (E) 5% water/94.5% THF/0.5% methanol. All *trans*-retinoic acid was used as the matrix and NaCl was used as the cationization reagent.

obtained with the use of 5% water, 95.4% THF, and 0.5% methanol as the solvent for sample preparation. Compared to the spectra shown in Figure 3A–D, this spectrum illustrates a significant mass discrimination against the high mass tail of the polymer distribution.

Fluorescein-Labeled Polystyrene 7700

Since retinoic acid gives a very strong fluorescence signal after excitation at 488 nm, *trans*-3-indoleacrylic acid (IAA) was used instead in the imaging experiments. IAA is a very good matrix for low molecular weight polystyrenes [2]. The MALDI spectra of fluorescein-labeled PS 7700 obtained by using either retinoic acid or IAA as the matrix display oligomer peaks not fully resolved to the baseline (not shown). This is likely because of the presence of different end groups (e.g., labeled polystyrene mixed with a small amount of the unlabeled one). The solvent effect for fluorescein-labeled PS 7700 using retinoic acid or IAA was found to be the same as that of PS 7000 shown above. The addition of benzene or toluene to 99.5% THF and 0.5% ethanol did not alter the mass spectral patterns, whereas the addition of a large amount of water or methanol resulted in mass spectral changes.

Table 2. MALDI results for the analysis of PMMA 3750 using different solvent systems for sample preparation^{a,b}

Solvent systems	M_n	M_w	PD	% Diff. ^c
99.5% THF/0.5% methanol	3593 ± 19 (3573 to 3623)	3938 ± 24 (3917 to 3976)	1.096	...
25.5% methanol/74.5% THF	3621 ± 28 (3573 to 3638)	3968 ± 11 (3951 to 3979)	1.096	0.8
25% toluene/74.5% THF/0.5% methanol	3697 ± 26 (3674 to 3737)	4093 ± 9 (4080 to 4101)	1.107	2.9
25% benzene/74.5% THF/0.5% methanol	3623 ± 12 (3606 to 3636)	3974 ± 16 (3957 to 3990)	1.097	0.8
50.5% methanol/49.5% THF	3773 ± 27 (3738 to 3812)	4114 ± 20 (4087 to 4140)	1.090	5.0
50% toluene/49.5% THF/0.5% methanol	3775 ± 12 (3763 to 3790)	4162 ± 29 (4141 to 4203)	1.103	5.1
50% benzene/49.5% THF/0.5% methanol	3615 ± 29 (3588 to 3647)	3966 ± 29 (3928 to 3996)	1.097	0.6
5% water/94.5% THF/0.5% methanol	3418 ± 194 (3152 to 3629)	3718 ± 191 (3459 to 3922)	1.088	-4.9

^aGPC data from the supplier: $M_n = 3750$, $M_w = 4100$, and PD = 1.09.

^b M_n , M_w , and standard deviations were calculated from five trials. The ranges for M_n and M_w are shown in parentheses.

^c% Difference in M_n , compared to that obtained by using 99.5% THF/0.5% methanol as the solvent.

Solubility Test

There are many polymer solubility data published in the literature [28]. These data can provide a guide in choosing a suitable solvent for dissolution of a particular type of polymer. However, solubility depends on a number of factors including molecular weight of the polymer; it is preferable to test the solubility of a given polymer to be analyzed by MALDI. In this study, the solubility test involved weighing out a known amount of the polymer, followed by the gradual addition of various volumes of the solvent. The turbidity of the solution was visually observed and compared. At room temperature, it was found that PS 7000 and fluorescein-labeled polystyrene do not dissolve in water, methanol, or ethanol. Clear solutions at high concentrations (up to about 0.2 M) can be prepared for both polymers in THF, toluene, or benzene, indicating that these three solvents are good solvents for the dissolution of the polymer. For PMMA 3750, a concentration of up to 0.4 M can be made in THF, toluene, or benzene. PMMA 3750 does not dissolve in water, but it dissolves in methanol at a concentration of up to 0.1 M.

Discussion

The above results reveal the salient feature of the solvent effect: a solvent mixture containing a polymer nonsolvent gives rise to poor reproducibility and erroneous average weight results. An ideal polymer nonsolvent is characterized by its inability to dissolve any amount of polymer at any temperature under atmospheric pressure [28]. For instance, water is a nonsolvent for polystyrenes and PMMA. Methanol is a nonsolvent for polystyrenes. It can be readily observed that the addition of an excess amount of water or methanol in the THF sample solution of polystyrene can cause turbidity.

In MALDI analysis, solvent evaporation takes place after the sample solution is deposited onto the probe. If a solvent mixture is used for preparing the initial sample solution, the solvent composition is expected to change during the solvent evaporation process because of the differences of their volatility. This can result in the change of solubility of the polymer. If a solvent

mixture consists of a solvent and a nonsolvent and the nonsolvent is less volatile, one would expect that its content in the final sample solution on the probe prior to the formation of matrix crystals will be much higher than that in the initial solution. In this case, there is a good possibility that the polymer may precipitate before the matrix crystal formation. Because the precipitation of polymer is often a function of molecular weight, mass discrimination can occur in the sample preparation stage. Specifically, any polymer ions detected in MALDI are from the oligomers incorporated into the matrix crystals. The relative contents of the oligomers in the polymer distribution may be altered because of the mass-dependent precipitation. In this work, the term "polymer precipitation" refers to precipitation of the polymer before the formation of matrix crystals, whereas the term "polymer incorporation" refers to the event that involves the cocrystallization of polymer, matrix, and cationization reagent (where applicable). It is obvious that polymer incorporation to take place prior to polymer precipitation is desirable in MALDI.

To further understand the competing processes of polymer precipitation and matrix crystal formation, we have used confocal microscopy to examine the analyte distribution on MALDI samples prepared in the same manner as that used for actual MALDI MS experiments. In this method, the analyte used is a fluorescein-labeled polystyrene. Because the MALDI matrix crystals (IAA) fluoresce at the wavelength used for excitation, albeit very weakly compared to the analyte, the matrix as well as the analyte image can be readily obtained by operating the confocal microscope in the fluorescence mode. The contrast image of the analyte and matrix can be used to provide information on the analyte distribution in the matrix crystals.

Figure 4 shows several images of the MALDI samples prepared by using different solvent systems. To illustrate the heterogeneity of sample distribution, images from two different regions of the same sample preparation are shown in two side-by-side panels. When 99.5% THF and 0.5% ethanol are used as the solvent system, the confocal image shown in Figure 4A displays a relatively uniform analyte distribution on

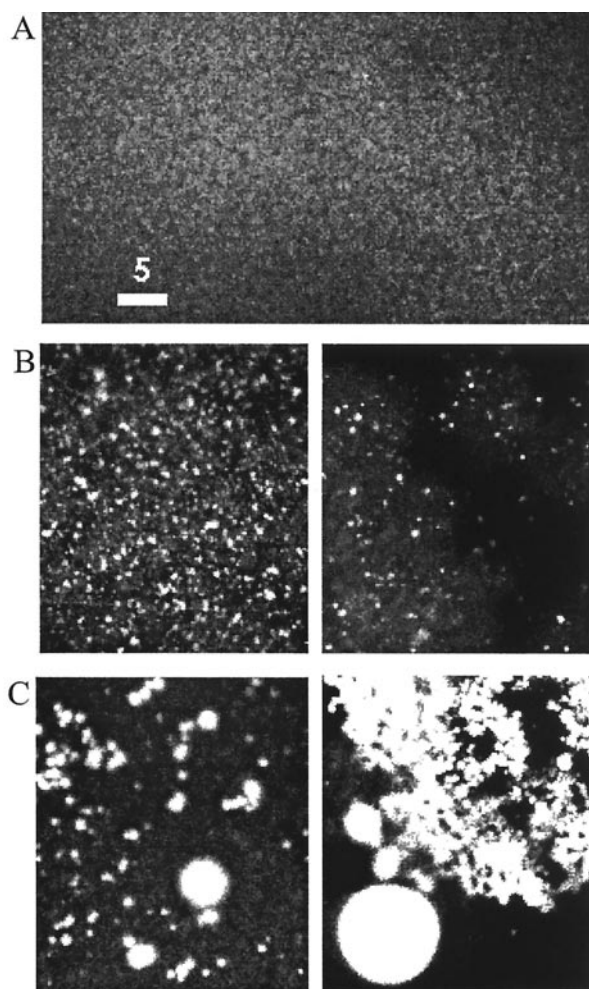


Figure 4. Confocal fluorescence microscopic images of the samples of fluorescein-labeled polystyrene 7700 prepared by using different solvent systems: (A) 99.5% THF/0.5% ethanol, (B) 5% water/94.5% THF/0.5% ethanol, and (C) 25% water/74.5% THF/0.5% ethanol. *Trans*-3-indoleacrylic acid was used as the matrix and silver nitrate was used as the cationization reagent. The scale bar shown is 5 μm .

microcrystals. By adding various amounts of water to the THF/ethanol solvent system, the sample morphology changes as illustrated by the representative images shown in Figure 4B, C. When the water content in the solvent mixture is low (e.g., 5% in the case of Figure 4B), microcrystals are still formed, but they are not uniformly distributed across the entire sample probe. This can be clearly seen on the right panel of Figure 4B. In addition, Figure 4B shows bright spots from the polymer sample, indicative of polymer precipitation. This notion is supported by the systematic increase of the particle size as the water content increases. The images shown in Figure 4C illustrate the formation of large particles as well as particle clusters in some regions of the sample layer, when the solvent mixture contains 25% water. A control experiment was also performed in which the solvent systems used were the same as those shown in Figure 4B, C except no analyte was added. No

bright spots or particle clusters such as those shown in Figure 4B, C were observed. In addition, without adding the matrix to the initial sample solution, similar types of polymer particles were observed.

The above findings can be rationalized by considering the changes in solvent composition during the MALDI sample drying process. THF with a vapor pressure (V_p) of 21.6 kPa at 25 °C is expected to evaporate at a much faster rate than water (V_p 2.3 kPa at 20 °C and 4.2 kPa at 30 °C). The rapid evaporation of THF results in a solvent system with an increasing amount of water (a nonsolvent for polystyrene) in the drying sample. This will result in the precipitation of polystyrene prior to the incorporation of all polymers into the matrix crystals. When a larger percentage of water is used in making the original sample solution, polymer precipitation is expected to occur at an earlier stage of the drying process. The longer duration of precipitation favors the formation of larger polymer particles. On the other hand, if the amount of nonsolvent is sufficiently small that the solvent composition at the onset of matrix crystallization is still not favorable for polymer precipitation, little or no effect on MALDI analysis is expected. This is likely the case for the solvent systems containing 0.5% ethanol and/or 0.5% water. Ethanol (V_p 7.9 kPa at 25 °C) is a nonsolvent for polystyrene, but reproducible results were obtained when the solvent mixture containing 0.5% ethanol and 99.5% THF was used. The use of a solvent mixture containing 0.5% water, 0.5% ethanol, and 99% THF also did not affect the mass spectral patterns in MALDI and the sample image was not changed either.

The effect of the solvent mixture containing methanol on MALDI sample preparation of fluorescein-labeled polystyrene was investigated and several images are shown in Figure 5. The comparison between the images shown in Figures 4A and 5A, B reveals a striking difference in sample morphology. The methanol-THF-ethanol system produces a thin matrix/analyte film. The crystal size is much less than 1 μm in diameter and cannot be measured by the confocal microscope. Overall, the analyte seems to uniformly distribute over the entire sample layer. Similar observations were obtained for the solvent system containing either 5% or 25% methanol. However, when the methanol content is increased to 50%, the sample image (Figure 5C) is entirely different from those shown in Figure 5A, B. Small polymer particles are observed. In this case, methanol is a nonsolvent. The volatility of THF is only slightly higher than methanol (V_p 16.9 kPa at 25 °C). When the solvent mixture containing 74.5% THF, 25% methanol, and 0.5% ethanol was used, the change in solvent composition during the sample drying process is not so great as to cause polymer precipitation. However, when the methanol content is too high, such as in a solvent mixture containing 49.5% THF, 50% methanol, and 0.5% ethanol, polymer precipitation takes place even in the initial sample solution. In the MALDI analysis of the labeled polystyrene, the addition of 25%

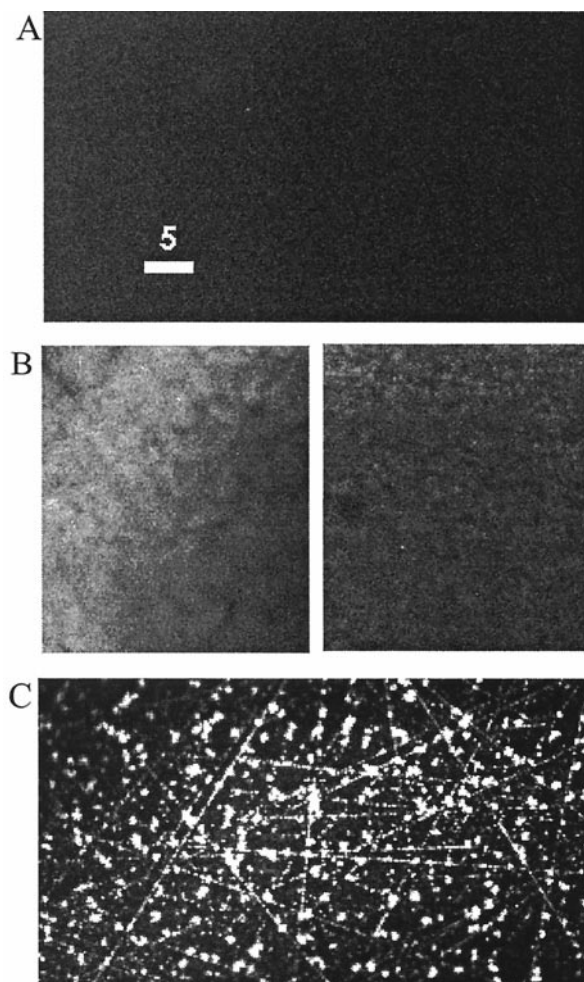


Figure 5. Confocal fluorescence microscopic images of samples of fluorescein-labeled polystyrene 7700 prepared by using different solvent systems: (A) 5% methanol/94.5% THF/0.5% ethanol, (B) 25% methanol/74.5% THF/0.5% ethanol, and (C) 50% methanol/49.5% THF/0.5% ethanol. *Trans*-3-indoleacrylic acid was used as the matrix and silver nitrate was used as the cationization reagent. The scale bar shown is 5 μm .

methanol in the solvent mixture did not alter the mass spectral patterns; but the solvent system containing 50% methanol did change the patterns. This was also the case for PS 7000 (see Table 1).

Figure 6 shows several images obtained with the use of solvent mixtures containing toluene, THF, and ethanol. The addition of 5% toluene does not seem to dramatically affect the overall crystal morphology (see the left panel of Figure 6A). In a few region, smaller crystals are observed (see the right panel of Figure 6A). However, when solvent mixtures containing 25% or 50% toluene are used, larger *crystals* are formed as shown in Figure 6B, C. From the macroscopic point of view (i.e., in the context of a typical laser beam of 150 to 200 μm in diameter), the analyte is uniformly distributed in the crystals. No polymer *particles* or *clusters* are observed. It is interesting to note that the intensity of the fluorescence signal in Figure 6C is greater than that of Figure 6B. This is because of the increase of back-

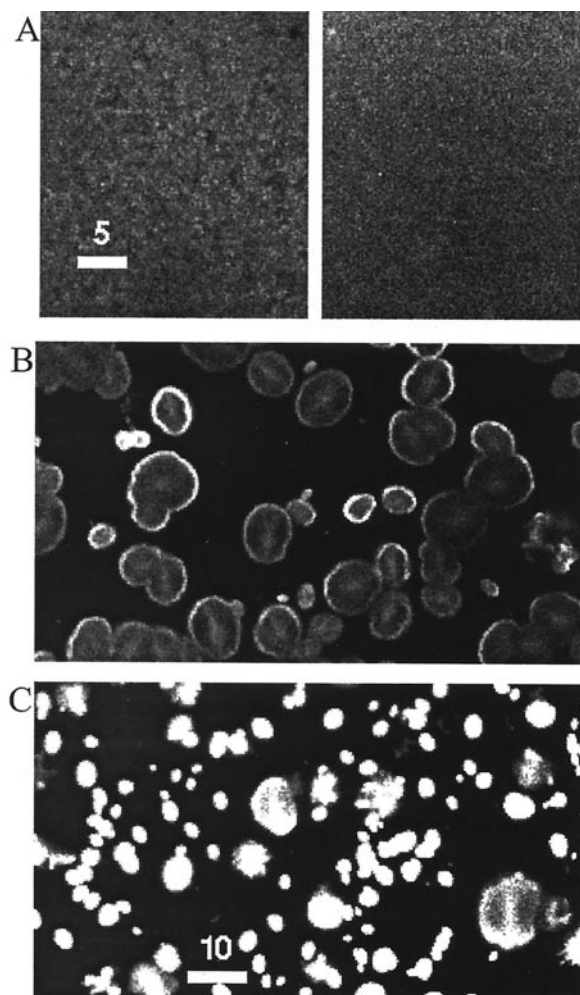


Figure 6. Confocal fluorescence microscopic images of samples of fluorescein-labeled polystyrene 7700 prepared by using different solvent systems: (A) 5% toluene/94.5% THF/0.5% ethanol, (B) 25% toluene/74.5% THF/0.5% ethanol, and (C) 50% toluene/49.5% THF/0.5% ethanol. *Trans*-3-indoleacrylic acid was used as the matrix and silver nitrate was used as the cationization reagent. The scale bar shown is 5 μm .

ground fluorescence signals from the matrix crystals. The reason for this background fluorescence signal enhancement is unknown. However, in a control experiment where no analyte was added to the sample, similar crystal morphology was observed, but fluorescence signals were much weaker. It is clear that the addition of toluene does not cause the precipitation of the polymer.

For the analysis of polystyrenes, a strong correlation between the signal reproducibility or precision of the M_n and M_w measurement and the degree of polymer precipitation was observed. The sample with a uniform distribution without precipitation gives very reproducible spectra from spot to spot and excellent precision (RSD < 1% from three individual runs). The sample prepared with polymer precipitation, such as 50% methanol in Table 1, provides poor reproducibility from spot to spot and sample to sample. In extreme cases

such as samples prepared with the use of an excess amount of water, no useful M_n and M_w results can be obtained.

The MALDI results obtained from the analysis of PMMA 3750 illustrate a different type of solvent effect. The morphology of the sample on the probe under different solvent conditions was found to be similar to that of the labeled polystyrene. For example, a thin film of sample was formed when the solvent mixture containing methanol was used. Unlike polystyrene, PMMA 3750 dissolves in methanol. But the M_n and M_w results obtained by using a solvent mixture containing 50% methanol, 49.5% THF, and 0.5% ethanol is different from that obtained by using 0.5% methanol and 99.5% THF as the solvent for sample preparation (see Table 2). Both data are shifted to higher numbers; precisions are still good. As Table 2 shows, similar findings are observed for two additional solvent mixtures containing 25% and 50% toluene. In these cases, the variation of M_n and M_w can be attributed to mass discrimination in polymer incorporation, ionization, and/or detection [27, 29]. Note that the polydispersity of PMMA 3750 is ~ 1.1 . As the polydispersity increases, the possibility of mass discrimination in MALDI analysis should increase [27, 29].

In conclusion, during the drying of the sample solution on the MALDI probe, polymer precipitation competes with the process of salt and matrix crystallization. Any solvent conditions that favor polymer precipitation will result in possible errors in average molecular weight measurement. It is important to recognize that, when a clear, dilute stock solution is made with a particular solvent system, it does not guarantee that the polymer is still well dissolved at the onset of the matrix crystal formation. If no polymer precipitation takes place, other processes including ionization and detection can still potentially introduce mass discrimination. This is particularly true for broad polydispersity polymers.

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