

Influence of Sample Preparation Method and Silver Salt Types on MALDI-TOFMS Analysis of Polybutadiene

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Abstract: Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis of nonpolar polymeric materials is affected by the sample preparation as well as the matrix and cationizing agent. This study examined the influence of silver salt types on the MALDI analysis of polybutadiene (PB). Silver trifluoroacetate (AgTFA), silver benzoate (AgBz), silver nitrate (AgNO₃), and silver *p*-toluenesulfonate (AgTS) were used as the silver salts to compare the MALDI mass spectra of PB. The mixture solution of PB and 2,5-dihydroxybenzoic acid (DHB), as a matrix dissolved in THF, was spotted on the sample plate and dried. A droplet of the aqueous silver salt solution was placed onto the mixture. The mass spectrum with AgBz showed the clear [M + Ag]⁺ ion distribution of PB while the mass spectrum with AgTFA did not show [M + Ag]⁺ ions but only silver cluster ions. The mass spectra with AgNO₃ and AgTS did not show a clear [M + Ag]⁺ ion distribution. The difference in the formation of [M + Ag]⁺ ions of PB depending on the silver salts was attributed to the silver cation transfer reaction between the silver salt and the matrix (DHB). The mass spectrum showed a clear [M + Ag]⁻ ion distribution of PB when the conjugate acid of the silver salt was less acidic than the matrix.

Keywords: MALDI-TOFMS, polybutadiene, silver salt, sample preparation.

Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been used to characterize a wide range of polymers such as biopolymers and synthetic polymers.¹⁻⁵ MALDI-TOFMS is suited for polymer analysis because of the simplicity of the mass spectra which show mainly single-charged molecular ions and TOF analyzer in which very high molecular weight polymers can be analyzed. The critical procedure in a MALDI experiment is sample preparation. There are various sample preparation techniques depending on the nature of sample to be analyzed. The commonly established techniques are the dried droplet method, fast evaporation (fast solvent drying) method, two-layer method, and solid/solid compressing method. The dried droplet method⁶ is most simple and common. The mixture solution of matrix and analyte is placed a droplet on the sample plate and dried at room temperature. Procedure of the fast evaporation method⁷ allows the matrix and the analyte to be applied onto the sample plate in independent steps. The two-layer method⁸ is that the first layer on the sample plate is the densely packed matrix microcrystals formed by fast solvent evaporation of a matrix solution

and a mixture solution containing both matrix and analyte is then deposited onto the first layer to form uniform analyte/matrix microcrystals. This technique is suited for analysis of complex peptide and protein mixtures. The solid/solid compressing method⁹ is used for hard to dissolve sample.

A typical MALDI sample preparation for nonpolar polymers uses a suitable solvent and combines the matrix with salts of soft Lewis acid metal ions. The metal ions act as cationizing agents by interacting with the soft Lewis bases such as olefinic double bonds and phenyl rings present in analyte polymers. When metal salts are used as cationization reagents for nonpolar polymers, pseudomolecular ions [M + Cation]⁺ of the analyte are produced in the ionization process. Many metal cations have been compared.¹⁰⁻¹² Deery and coworkers¹⁰ reported that Li⁺, Na⁺, K⁺, Ag⁺, Cu²⁺, and Zn²⁺ were good cations for analysis of polystyrene with dithranol as a matrix, while Rashidezadeh and coworkers¹² reported that Ag⁺, Cu²⁺, and Pd²⁺ were good cations.

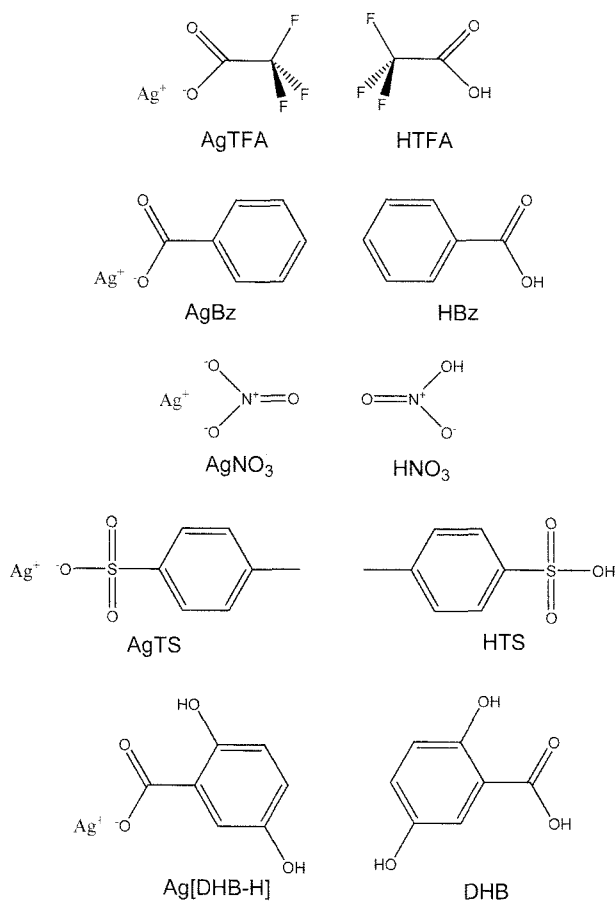
Polybutadiene (PB) is one of the commonly used rubbers and has lots of olefinic double bonds in the backbone.¹³ MALDI analysis of PB has been performed using various matrices and metal salts.¹⁴⁻¹⁶ *all-trans*-Retinoic acid (RTA) is usually employed as the matrix for analysis of PB and a silver or copper salt is commonly used as the cationizing agent. Ionization efficiency of metal cation for MALDI

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analysis of synthetic polymers has been studied, but the counter anion, which has the same metal cation but different anion, effect was not sufficiently investigated. In the present work, we studied the influence of kind of silver salts having different counter anions on the ionization efficiency of PB and silver cluster formation in MALDI analysis. Properties of metal salts depend on the counter anions as well as the kind of metal cations. Silver trifluoroacetate (AgTFA), silver benzoate (AgBz), silver nitrate (AgNO₃), and silver *p*-toluenesulfonate (AgTS) were employed as the silver salts.

Experimental

AgTFA, AgBz, AgNO₃, and AgTS of Aldrich Co. were employed as silver salts. 2,5-Dihydroxybenzoic acid (DHB) purchased from Aldrich Co. was used as matrix. Scheme I shows chemical structures of the silver salts and DHB. Ricon131 of Sartomer Co. was used as PB and its number average molecular weight (M_n) is 4,500. THF of Aldrich Co. was used as solvent. PB solution was prepared by dissolving 1.5 mg in 1.0 mL THF. DHB was dissolved in THF and the concentration was 0.1 M. The silver salts were dissolved in distilled water and the concentrations were 0.01 M. The



Scheme I. Chemical formulae of the silver salts, their conjugate acids, and DHB.

MALDI analysis sample was prepared as follow (two step droplet method). The PB and DHB solutions with the volume ratio of 1 : 5 were mixed. The mixture solution of 1 μ L was spotted onto the sample plate and dried. The silver salt solution of 1 μ L was placed a droplet onto the mixture of PB and DHB.

MALDI mass spectra were obtained with Axima-LNR MALDI-TOFMS (Kratos-Shimadzu Co. of Japan). Ions were produced by irradiation of the sample with nitrogen laser (337 nm). Profiling of product ions was achieved in the positive mode using linear TOF. The accelerating voltage was 20 kV. The sum of 50 shots was collected for each spectrum.

Results and Discussion

In general, for MALDI analysis of nonpolar polymers using metal salts, the fast evaporation method and dried droplet method were employed.¹⁶ It is usually possible to use the fast evaporation method when the solvents for nonpolar polymer, matrix, and metal salt are the same. Nonpolar polymers are dissolved in organic solvents such as THF and toluene but some metal salts are not dissolved in the organic solvents. For example, AgNO₃ can not be dissolved in organic solvents since it is a pure ionic compound. Therefore, metal salts which are not dissolved in organic solvents are not suitable for the fast evaporation method and dried droplet method. In this study, we introduced a new sample preparation method named "two step droplet method" to investigate the influence of silver salt types on the MALDI analysis of PB because some silver salts can not be dissolved in organic solvents. In order to avoid the solvent effect, all the silver salts were dissolved in distilled water since solvents used in MALDI can affect the ionization of analyte.¹⁷ The two step droplet method is that the mixture of PB and DHB dissolved in THF is spotted a droplet on the sample plate and dried and then the second drop of silver salt solution dissolved in water is placed on the first mixture. AgTFA, AgBz, AgNO₃, and AgTS were employed as the silver salts and their MALDI-TOF mass spectra were compared.

AgTFA is a common silver salt used in MALDI-MS and applied for analysis of nonpolar polymers using the dried droplet method. The MALDI mass spectra of PB with AgTFA prepared by the dried droplet method and the two step droplet method were compared. The MALDI mass spectrum of PB with AgTFA prepared by the dried droplet method shows the ions $[M + Ag]^+$ ions of PB and some silver cluster ions in the low mass range as shown in Figure 1. Nonpolar polymers analyzed with silver salts can yield lower quality mass spectral results when interferences due to silver clusters are present.¹⁶ The MALDI mass spectrum of PB with AgTFA prepared by the two step droplet method does not show the $[M + Ag]^+$ ions of PB as shown in Figure 2. The two spectra with different sample preparation methods

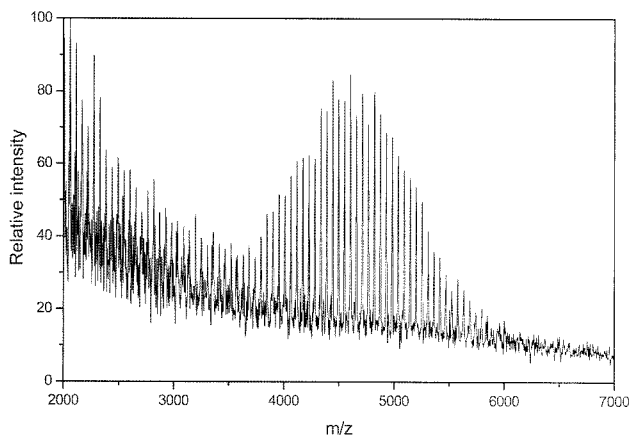


Figure 1. MALDI-TOF mass spectrum of polybutadiene with AgTFA. The sample was prepared by dried droplet method.

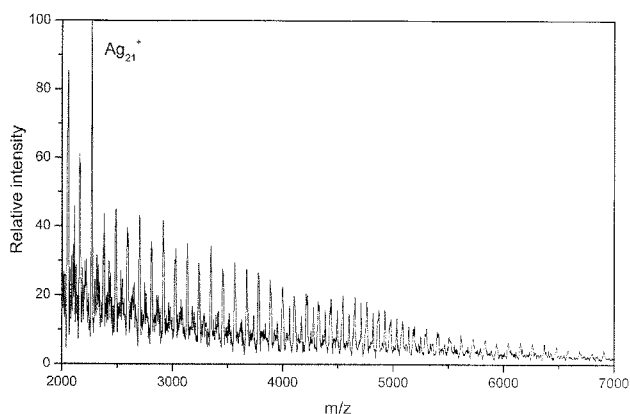


Figure 2. MALDI-TOF mass spectrum of polybutadiene with AgTFA. The sample was prepared by the two step droplet method.

show very different results. The MALDI mass spectrum prepared by the two step droplet method mainly shows the silver cluster ions. The silver cluster ion intensity distribution shows an odd-even alternation pattern and the magic number of $n = 21$ of Ag_n^+ . This is a typical distribution of silver cluster ions produced from silver salts in MALDI analysis.

The difference between the mass spectra obtained with the dried droplet method and the two step droplet method can be explained with the silver cation transfer to the analyte molecule. The silver cation should be transferred from the silver salt to the PB molecule to generate the $[\text{M} + \text{Ag}]^+$ ions of PB. The silver cation transfer procedure can be occurred during the sample preparation, in the desorption/ionization step of the condensed phase, or in the gas phase reactions after the desorption/ionization. If the silver cation transfer procedure can be occurred in the gas phase reactions, the silver cation should transfer from the isolated AgTFA to the PB molecule. This is not possible because the ionic compound of the silver salt hardly react with the non-polar PB in a gas phase. If the silver cation transfer proce-

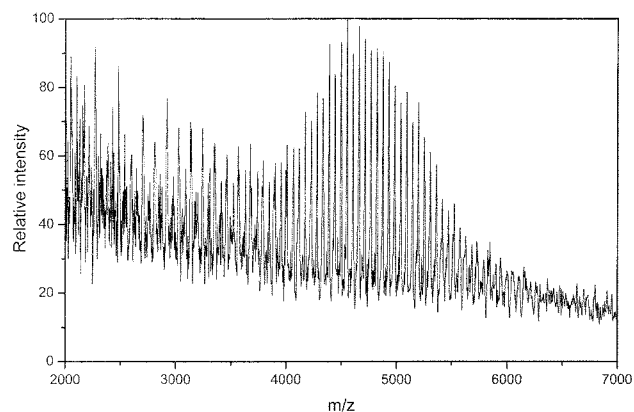


Figure 3. MALDI-TOF mass spectrum of polybutadiene with AgBz. The sample was prepared by the two step droplet method.

dures can be occurred during the sample preparation or in the desorption/ionization step of the condensed phase, the silver salt should be placed near the PB molecule. For the dried droplet method, since the mixture solution containing PB, DHB, and AgTFA in THF was used, AgTFA can be placed near the PB molecule. For the two step droplet method, the AgTFA solution can not be mixed well with the mixture layer of PB and DHB because PB and DHB were dissolved in THF whereas AgTFA was dissolved in distilled water.

Figure 3 shows the MALDI mass spectrum of the PB with AgBz prepared by the two step droplet method and distribution of the $[\text{M} + \text{Ag}]^+$ ions of PB are clearly displayed. The ion distribution is similar to the mass spectrum of the PB with AgTFA prepared by the dried droplet method. The MALDI mass spectrum of the PB with AgNO_3 prepared by the two step droplet method shows the $[\text{M} + \text{Ag}]^+$ ion distribution as shown in Figure 4 but it is not clear compared with Figures 1 and 3. Figure 5 gives the MALDI mass spectrum of the PB with AgTS prepared by the two step droplet method. The $[\text{M} + \text{Ag}]^+$ ions of PB are hardly detected and the silver ion clusters are not also detected.

The experimental results say that the counter anion of the

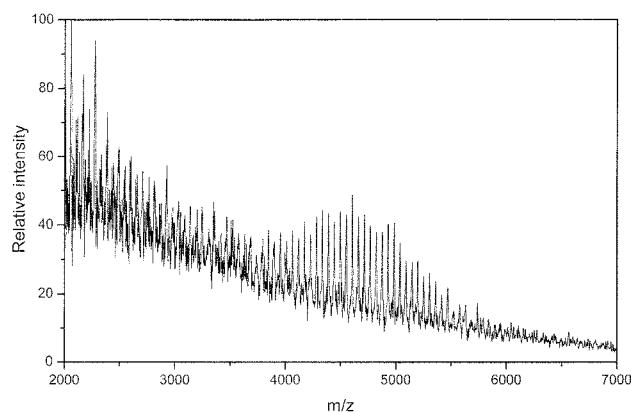


Figure 4. MALDI-TOF mass spectrum of polybutadiene with AgNO_3 . The sample was prepared by the two step droplet method.

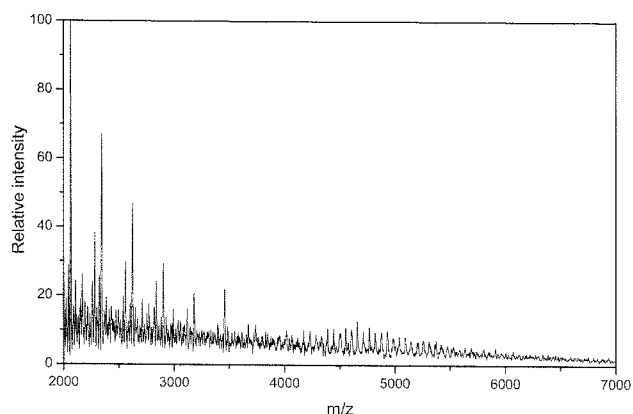
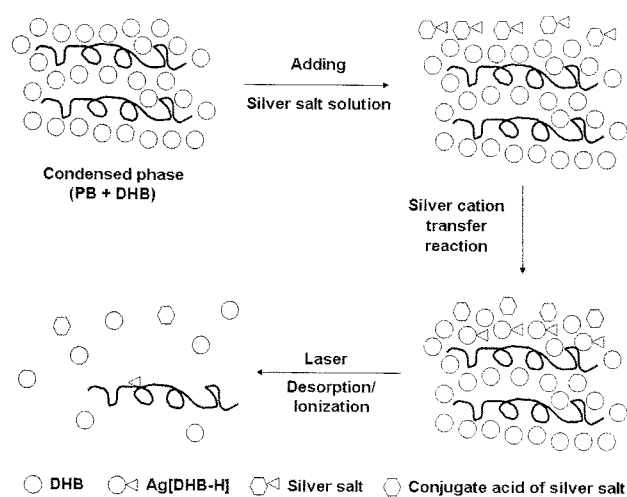


Figure 5. MALDI-TOF mass spectrum of polybutadiene with AgTS. The sample was prepared by the two step droplet method.

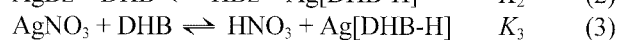


Scheme II. Mechanism for the generation of $[M + Ag]^+$ ions in MALDI analysis with the two step droplet method.

silver salt affects the MALDI analysis of PB. Among the mass spectra of the four silver salts with the two step droplet method, only the mass spectrum of AgBz shows the clear $[M + Ag]^+$ ion distribution. This can be explained with the reactions of the silver salts with the matrix. The reactions depend on the acid dissociation constants of the conjugate acids of the silver salts compared with the acidity of the matrix, DHB. The conjugate acids of AgNO₃ and AgTS (HNO₃ and HTS, respectively) are strong acids. The pK_a s of the conjugate acids of AgTFA and AgBz (HTFA and HBz, respectively) are 0.3 and 4.2, respectively, and that of DHB is 3.0. New silver salt, Ag[DHB-H], can be produced by reactions of DHB and the silver salts as shown in Scheme II. When the pK_a of the conjugate acid of the silver salt is larger than DHB, the Ag[DHB-H] can be produced well.

The condensed phase of PB and DHB is formed by placing and drying of the mixture of PB and DHB solution in THF on the sample plate. When the silver salt solution in water is placed onto the condensed phase, some DHB mole-

cules can transfer to the silver salt solution by dissolving in water but PB molecules can not transfer to the silver salt solution since PB is nonpolar. The transferred DHB molecules can react with the silver salt as shown in the reactions (1)-(4). The equilibrium constants are depending on the difference in the acidities of DHB and conjugate acid. When the acid dissociation constant of the conjugate acid is lower than that of DHB, the equilibrium constant becomes larger. The equilibrium constants of the reactions (1)-(4) have an order of $K_2 > K_1 > K_3$ and K_4 . The Ag[DHB-H] molecule formed by the reaction of the silver salt and DHB can be located near the PB molecule. By absorbing the laser energy of DHB and/or Ag[DHB-H], the PB molecules are desorbed together with DHB and Ag[DHB-H] molecules. The $[M + Ag]^+$ ions of PB can be produced by the silver cation transfer from the Ag[DHB-H] to the desorbed PB molecule. The procedure to generate the $[M + Ag]^+$ ions was demonstrated in Scheme II.



Conclusions

The mass spectrum of PB prepared by the dried droplet method using AgTFA as the silver salt showed a clear $[M + Ag]^+$ ion distribution of PB, while that prepared by the two step droplet method did not show the ion distribution of PB but the silver cluster ion distribution was displayed. The mass spectrum prepared by the two step droplet method using AgNO₃ showed the $[M + Ag]^+$ ion distribution but it is not clear. The mass spectrum using AgTS did not display the PB ion distribution. Among the four silver salts, only the mass spectrum of AgBz displayed a clear $[M + Ag]^+$ ion distribution without the silver cluster ions. The experimental results were explained with the silver transfer reactions between the silver salt and matrix. The reaction of AgBz with DHB is more favorable than those of the other silver salts because the conjugate acid of AgBz is much less acidic than DHB as well as the other conjugate acids.

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