Use of Graphite Plate for Homogeneous Sample Preparation in Matrix/Surface-assisted Laser Desorption and Ionization of Polypropyleneglycol and Polystyrene

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Matrix/Surface-assisted laser desorption/ionization (M/SALDI) mass spectrometry of polypropylene glycol and polystyrene, directly deposited on graphite plate, is demonstrated. Graphite plate is effective both as an energy transfer medium and robust sampling support for LDI of polymers. Mass spectra of polymers can be easily obtained due to homogeneous distribution on graphite surface and their ion signals are long-lived by large effective desorption volume enough to investigate M/SALDI process.

Introduction

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight(TOF) mass spectrometry is a relatively new analytical method[1,2] that has a demonstrated usefulness in the analysis of polymers.[3,4] Compared with conventional methods such as GPC and vapor phase osmometry, it has the advantage of high mass accuracy, wide mass range, and rapid data acquisition. However, despite the great potential of MALDI, its capability is limited by difficulties in homogeneous sample preparation in solid state.[5] Most solid matrices suffer from inhomogeneous sampling due to inconsistencies in crystal formation, leading to low signal reproducibility, and rapid destruction, leading to temporal signal degradation. For these reasons, the fast evaporation method[6-7] and application of liquid matix[8] *etc*. have been suggested, but these methods still require scrupulous care and show low shot-to-shot stability. In other approaches, studies on various substrate have been performed with etched metal plates,[9] self-assembled monolayer[10] and hydrophobic materials[11,12] such as paraffin wax film and poly(tetrafluoroethylene) plate as the sample supports to improve sample uniformity.

Graphite plate is a good candidate for sample support. Graphite itself can be used as a matrix due to its black-body absorption, and its large and effective surface. Evaporation by smearing sample solution into graphite can prevent the heterogeneous concentration in round rim of sample spot caused by the spread of solution on the metal surface. Application of graphite in MALDI has been tried in powder form mixed with absorbing liquid matrix or with non-absorbing liquid matrix.[13,14] These mixtures were capable of producing high-quality mass spectra of peptides, proteins, oligosaccarides and synthetic polymers, even when the liquid matrices had no appreciable absorption at the desorption wavelength (337 nm). However, the use of a mixture of graphite powder and liquid matrix has the disadvantage of being highly sensitive to the size and the mixing ratio of graphite particulate and sample manupulation is not easy. In addition, a mixture of graphite powder and liquid matrix can be used only on the horizontal sample probe because of its fluidity.

This report describes an easy and simple sample preparation method using graphite plate and application to analysis of polypropyleneglycol (PPG) and poylstyrene (PS), which are the prototypes of GPC standards for synthetic polymer. Though graphite plate had been used to investigate the substrate effect in the laser desorption process of small molecules,[15] the application as MALDI sample support has not been reported to our knowledge.

Experimental Section

Instrumentation. A home-built 0.65 m linear TOF mass spectrometer was used, a full description of which can be found elsewhere.[16] The typical background pressure in the vacuum chamber during operation was 1×10^{-7} Torr, using a 4"-diffusion pump (VHS-4, Varrian) for a source region and a turbo molecular pump (V 250, Varrian) for a drift tube. The sample plate was mounted on the repelling plate, which was normally maintained at 15 kV (4.5 kV for PPG2000), and 25 mm from the gridless and grounded extraction plate with a 15 mm hole in diameter. A circular (25 mm diameter) dual micro-channel plate detector (Galileo Electro-Optics) was used to detect ions. The ion signals were digitized using a LeCroy 9354AL (500 MHz) digital oscilloscope and spectra were transferred to an IBM compatible PC for mass calibration and data processing. The laser source was a forth harmonics of a Nd:YAG laser system (Minilite II, Continuum), with a pulse width of 5 ns and a maximum output energy of 5 mJ. The laser beam, at an angle of 45°, was irradiated on the sample plate or was focused by a quartz lens of 25 cm focal length. The power of the laser was attenuated using a neutral density filter.

Sample preparation. Graphite plate (2 mm thickness) for lubrication and machining purposes was used without any treatment unless otherwise noted. In the experiments on dependence of sodium salt content, graphite plates were washed in de-ionized water for 1 hour, using a sonicator to remove the aqueous-soluble metal salts contained and dried in a furnace at 500 °C. All chemicals were used with no further purification as purchased from Aldrich Co. A PPG solu-

tion (1 mg/mL in CH$_3$CN) mixed with the NaCl aqueous solution was deposited on a spot of dithranol (10 mg/mL in THF) on the stainless steel plate or directly on graphite plate. A few μL of Polystyrene (PS) solution (1 mg/mL in THF) mixed with a CF$_3$COOAg solution (10 mg/mL in THF) and a dithranol solution with equi-volume ratio were dropped on the stainless plate and graphite plate.

Results and Discussion

Figure 1 shows the optical microscope images of sample spots of the PPG 2000 with the dithranol and NaCl deposited on the stainless steel plate and graphite plate. On the stainless steel plate, the best sample spot was obtained with difficulty after several trials, and it appeared homogeneous to the naked eyes. However, Figure 1(a) of the optical microscopic view indicates that a number of grains about 100 μm in size were formed by local aggregation of an analyte, matrix and salts. On the contrary, the rim around spot as well as the large grain samples are not seen on the graphite plate as shown in Figure 1(b). The particulates smaller than 10 μm in the microscope image represent the morphology of the graphite surface, not the irregular distribution of sample.

Figure 2 shows the MALDI-TOF mass spectra of PPG2000 obtained at an extraction voltage of 4.5 kV. For a MALDI spectrum of PPG2000 on the stainless steel plate, it was necessary to find "good spots" or to avoid "bad spots" on the
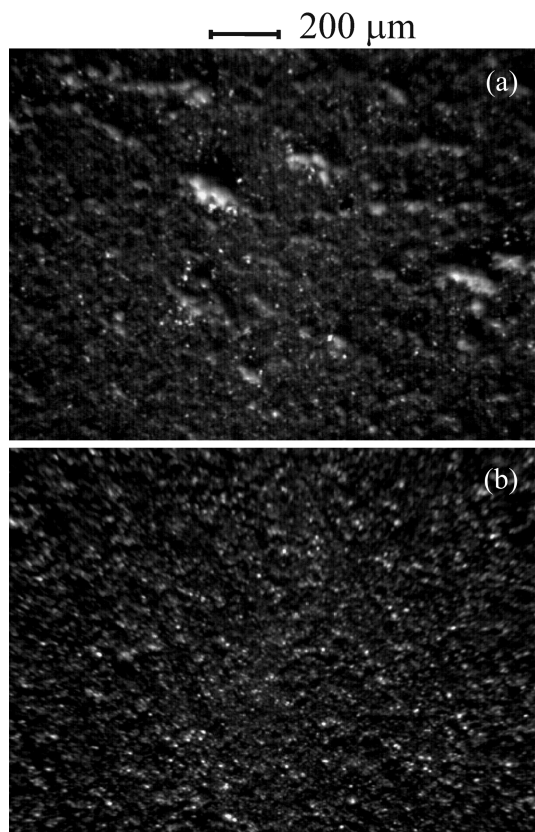

Figure 2. MALDI mass spectrum of PPG 2000. Samples were prepared to same deposition method as explained in Figure 1. Spectra were averaged of 100 laser shots in sampling rate of 100 M/s of the digital oscilloscope. Symbols, + and × represent 58n+81 and 58n+97 mass series of the fragment ions, respectively.

substrate. This resulted in lowering the spot-to-spot reproducibility. When the graphite plate was used as a sample support, the ion signals were more intense and showed a better signal-to-noise ratio than those of the stainless steel plate. More than anything else, the spectrum was more easily obtained in any place of sample spot regardless of the irradiation position of the laser. The mass series of dominant ion signal observed is 58n + 41 (n = 20-50), which is consistent with that of the sodiated PPG molecule, HO-(-CH(CH$_3$)-CH$_2$O-)$_n$-H Na$^+$. The calculated number averaged molecular weight is 2010 ± 10 for both spectra, which is within the error range. The nearly equal mass resolution, 350 of the two spectra, indicates that the roughness of the sample surface is not enough to affect the mass resolution. The graphite plates used in this study are for machining and lubrication, not of chemical grade, so they have a large number of various impurities. These impurities of the low molecular weight are highly congested with hydrogenated carbon cluster peaks fragmented from the graphite surface in the mass region lower than 300 amu, which did not affect the spectra of the polymer in this study. The ion signals shown in the mass region of 500-1200 amu are interpreted as ions fragmented during ionization or desorption. They consist of two mass series; one is the 58n + 81 (n = 10-20, marked with +) series and the other is the 58n + 97 (n = 10-20, marked with ×) series, which are well consistent with the results of Donovan under delayed extraction mode.[17] The signals of fragments are more intense than those in Donovan's result at 337 nm of laser light, and it seems to be caused by the higher photon energy of 266 nm used as a light source in the present study.

Figure 3 shows the mass spectra of PPG 2000 without the matrix, dithranol. These spectra indicate that graphite effectively acts as a photon-absorbing material. Even when only the PPG 2000 is adsorbed on the graphite plate untreated, the spectrum with good resolution is acquired as shown in Figure 3(b) though the ion signals are less intense compared with Figure 3(a), and its ion peaks correspond to sodiated propylene glycol oligomers. The attached sodium ions seem

$\longmapsto$ 200 μm

Figure 1. Optical microscope images of sample spots of PPG 2000 deposited on (a) stainless steel plate and (b) graphite plate with dithranol in THF and NaCl in water by layering method.
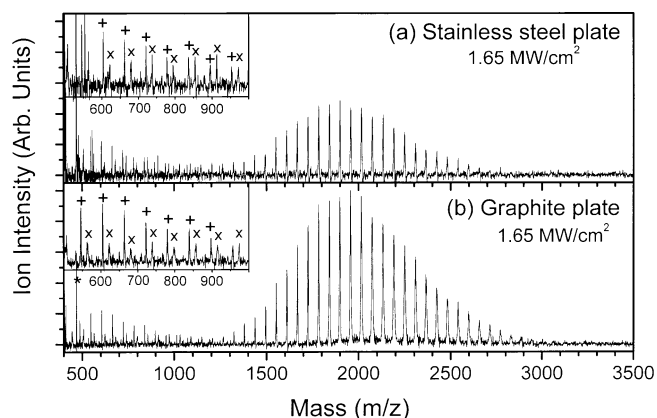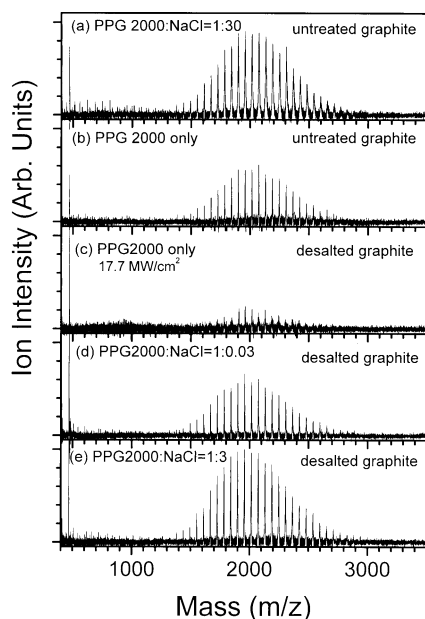
Figure 3. SALDI mass spectrum of PPG 2000 deposited on graphite plate according to variation of amount of sodium salt at laser fluence of 1.65 MW/cm^2 except (b) (17.7 MW/cm^2). Spectra were acquired at sampling rate of 500 M/s of the digital oscilloscope.

Figure 4. SALDI mass spectrum of PPG 5000 on graphite plate according to variation of laser fluence. Symbols, +, × and ○ represent 58n+81, 58n+97 and 58n+65/67 mass series of the fragment ions, respectively.

to have originated from impurities. To investigate the cationization effect of sodium salt, the graphite plates were desalted in deionized water. The ion signal was hardly observed when only the PPG2000 without sodium salt was deposited on the desalted graphite plate as shown in Figure 3(c). As the amount of sodium salt increases, the higher quality mass spectrum is obtained. However, too much sodium salt gives a broadening of the ion peak and fragmentation with lower molecular ion intensity.

This graphite surface-assisted ionization effect was named SALDI (surface-assisted laser desorption/ionization) in the analysis of peptide and protein by Sunner *et al.*,[13] who used 10-150 μm graphite powder. According to Sunner, when using the dry graphite powder without a liquid matrix, the ion intensity was much lower and very short-lived, and the mass resolution was poor, thus both a liquid matrix and graphite powder were essential to obtain the SALDI spectrum. Careful attention is required in the use of powder, and the mixing solution must be mechanically shaken to ensure thorough dispersion of the graphite powder in a liquid matrix.[14] On the contrary, using a graphite plate as photon-absorbing material and substrate ensures the robust sampling. When only the graphite plate without a liquid matrix was used for SADLI in this study, the ion signal existed long enough to investigate the desorption and fragmentation dynamics of PPG under delayed-extraction mode.[16] This shows that the plate form has more available effective volume to desorb the analyte than the powder form.

It was reported previously that it was possible to obtain the molecular ion signals for only low molecular weight polymers (< 2000 amu.) by direct desorption, where no matrix was utilized.[18,19] Figure 4 shows the SALDI spectrum of

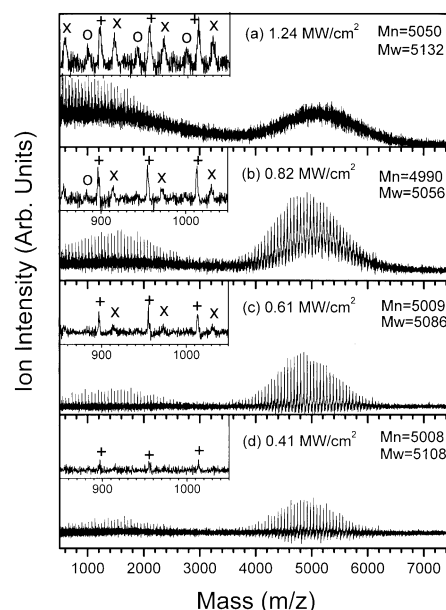PPG 5000 without a matrix observed at an extraction voltage of 15 kV with the variation of laser pulse fluence. A well-resolved mass spectrum of PPG 5000, of which the resolution is *ca.* 600, is obtained at a moderate laser pulse fluence (0.61 MW/cm^2), but further increase in the laser fluence results in the loss of mass spectral resolution with a new mass series, 58n + 65 or 67 of fragmented ion.[17] This can be attributed to the broad distribution of desorption velocity and the initiation of another PPG degradation channel at elevated excess energy. A detailed study is required to interpret the power dependency of fragmentation of PPG.

Figure 5 is the MALDI spectra of a polystyrene standard (PS2500) at a laser fluence of 8.74 MW/cm^2, using the
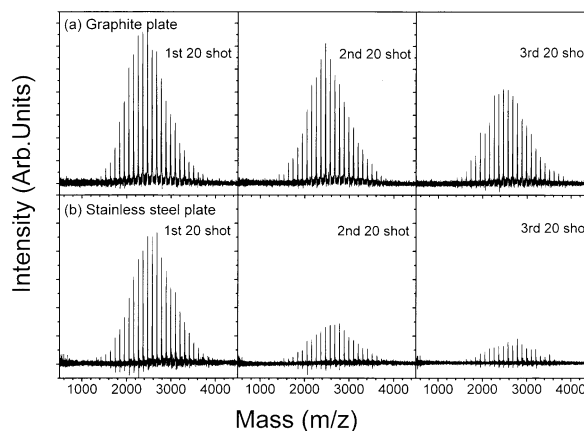

Figure 5. MALDI mass spectra of polystyrene standard (PS 2500) deposited on (a) the treated graphite plate and (b) stainless steel plate at laser fluence of 8.74 MW/cm^2 with dithranol and silver trifluoroactate as matrix and cationization agent, respectively.

treated graphite plate and stainless steel plate. The oligomer distribution is represented by $(M+Ag)^+$ ions. The ion peaks are separated by 104 Da, which is consistent with the -[$CH_2CH(C_6H_5)$]- repeating unit and the mass of end capping group was calculated to be 58, indicating $-C(CH_3)_3$ and $-H$. Polystyrene, which is the prototype of nonpolar synthetic polymer, poses difficulty for the hard sampling on metal surface due to its hydrophobicity.[20] The MALDI spectrum of PS requires the higher energy fluence compared with PPG, and the high irradiated power of the laser deplete the sample on the suface or destroy the surface composition. The ion signals of PS on the stainless steel plate rapidly decrease with an increase in irradiation time on a sample spot and disappear within a few ten shots of the laser. However, on the graphite plate, the ion signals persist longer and show higher spot-to-spot reproducibility. This seems to be the effects of the penetratable surface of graphite and the homogeneous sampling on the graphite plate.

In summary, graphite plate was shown to be effective both as an energy transfer medium and sample support for simple sample preparation. Homogeneous sampling on the graphite plate provides for easy acquisition of mass spectrum and generates a long-lived ion signal by large effective desorption volume. A persistent ion signal is needed to investigate the M/SALDI process which is not well understood. However, the possibilties of the general application of the graphite calls for more studies of the various molecules with high molecular weight and the wavelength dependence.

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