Use of Liquid Matrices for Matrix-Assisted Laser Desorption Ionization of Polyglycols and Poly(dimethylsiloxanes)

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Received December 12, 1995; Revised Manuscript Received September 9, 1996[⊗]

ABSTRACT: Liquid matrices were developed and studied for application in the MALDI analysis of polyglycols and poly(dimethylsiloxane). Characteristic positive ion MALDI-TOF mass spectra were obtained from over 20 liquid matrices. These systems are characterized by rapid and simple preparation and exhibited good vacuum stability. The spot-to-spot and point-to-point reproducibility of signal intensity and molecular weight values are indicative of the homogeneous environment created by liquid matrices. The best performance was achieved when the chromophore, 2-cyano-5-phenyl-2,4-pentadienoic acid, was used in the analyte or analyte/mediator systems (mediator = nonabsorbing liquid) at or near saturation concentrations. Internal standards, including silver and low molecular weight polyglycols, have been successfully employed for mass calibration. Comparison of MALDI molecular weight data with those obtained from SIMS and GPC shows some systematic disparities. Possible reasons for these differences are discussed. Excellent agreement is seen between molecular weight values obtained with liquid and solid MALDI matrices. However, conventional solid MALDI matrices generally exhibit better resolution and can be applied for analysis of higher molecular weight materials. This may be related to the higher laser intensity required to produce ions from liquid matrices.

Introduction

Polymer characterization has been successfully performed using a number of mass spectrometric soft ionization methods including secondary ion,[1−6] laser desorption,[7−9] field desorption,[10−12] fast atom bombardment,[13−15] electrohydrodynamic,[16,17] plasma desorption,[18,19] thermospray,[20] and electrospray ionization[21,22] mass spectrometry. However, with the exception of electrospray, which produces complex spectra, all of the above suffer from an upper mass limit.

The development of the soft ionization method, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS),[23,24] has considerably increased the accessible mass range for analysis of synthetic and biological polymers. The technique, when combined with a time of flight analyzer (MALDI-TOF-MS), offers the advantages of high mass accuracy (0.01%),[25] wide mass range (up to 10^6 Da),[26] and simple and rapid sample preparation (usually minutes). Although the major application of MALDI has been to biopolymers, analysis of many synthetic polymer classes has been demonstrated.[27−41] Spectra of the oligomer distribution in the quasi-molecular ion form, with little or no fragmentation or adduct attachment, provide an accurate and reproducible tool for measurement of molecular weight as well as information on molecular structure and purity.

However, most MALDI matrices have been developed for investigation of materials of biological interest, thus necessitating significant solubility in aqueous media. Much of the synthetic polymer work has also been performed using these solid, polar matrix materials. Application of MALDI to a diverse set of analytes, such as synthetic polymers, requires the preparation of analytes in the proper matrices. Until recently, little work has been devoted to the development of matrices for use with less polar materials, a class including a large percentage of synthetic polymers.

Furthermore, most solid matrices suffer from inconsistencies in crystal formation, leading to sample heterogeneity that adversely affects signal reproducibility, rapid destruction of the area of laser interaction leading to temporal signal degradation, and low miscibility with less polar materials. The performance of MALDI matrices in synthetic polymer analysis is impacted by the need for higher analyte concentrations. This requirement leads to lower matrix/analyte ratios and can be detrimental to formation of matrix crystals.

As opposed to solid matrices, liquid matrices offer the advantage of a homogeneous sample environment, thus improving point-to-point signal reproducibility. Liquid matrices create a continuous source of analyte, hence improving shot-to-shot reproducibility and increasing the number of shots per point. The use of liquid matrices may also increase the miscibility of hydrophobic analytes, a feature particularly important for polymer applications. Liquid matrices are commonly employed in fast atom bombardment (FAB).[42] Liquid matrices have also been applied to the UV MALDI analysis of biomolecules[43−47] and porphyrins,[48] and for MALDI analysis in the infrared.[49−51] The use of glycerol mixed with ultrafine cobalt powder as a MALDI matrix for the analysis of polyglycols has been reported.[52] The neat liquid matrix *o*-nitrophenyl octyl ether (*o*-NPOE) produced MALDI spectra showing polystyrene distributions.[29] However, liquid matrices are less commonly employed than their solid counterparts. The limited application of liquid matrices to MALDI can be traced to inferior vacuum stability, relative to solids, and the lack of a suitable liquid phase chromophore absorbing appreciably at the nitrogen laser wavelength.

The present study reports the performance of a series of novel, vacuum stable liquid phase systems applied as matrices for the characterization of synthetic polymers using UV MALDI-TOF-MS. The absence of resonant absorption at the nitrogen laser wavelength by most of these compounds was overcome by the addition of a strongly absorbing organic chromophore. A series of polyglycol standards and a poly(dimethylsiloxane) were analyzed to evaluate the mass range of ion detection, ionization pathways, fragmentation, repro-

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[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1996.

ducibility, and resolution. Results for the polyglycols are compared with those produced with solid MALDI matrices. Molecular weight values calculated from liquid matrix MALDI spectra are also compared with those obtained with time of flight secondary ion mass spectrometry (TOF-SIMS) and gel permeation chromatography (GPC).

Experimental Section

I. Instruments. MALDI spectra were acquired using a modified LAMMA 1000 (Leybold-Heraeus GmbH) (TOF-MS) equipped with a N_2 laser (VSL 337 ND: Laser Science Inc., Boston, MA). The instrument has been described elsewhere.[53] Laser irradiance was controlled by diaphragm defocuing the beam in the sample plane. The positive ions desorbed by irradiation of a spot (30×50 to 100×150 μm) on the sample were accelerated to 4 keV and space focused using an Einzel lens. Detection efficiency is enhanced by postacceleration of ions to 15 keV before detection by a discrete dynode type secondary electron multiplier (SEM). The signal from the SEM was amplified and stored in a 200-MHz 8×32K channel transient recorder (LeCroy, Inc., Spring Valley, NY) subsequent to transfer to an IBM compatible PC terminal. Typically, 50−500 shots were averaged to produce experimental spectra.

A TOF-SIMS III (Ion-Tof GmbH, Münster, Germany) was used to obtain positive ion comparison spectra of lower molecular weight polymers. The instrument consists of a pulsed primary Ar^+ ion source, a high-resolution first-order reflectron, and a 2-m time-of-flight analyzer. Secondary ions were postaccelerated (10 keV) prior to detection by a channel plate−scintillator−photomultiplier combination. The TOF-SIMS III has been described in more complete detail elsewhere.[54,55] Data processing of both MALDI and SIMS spectra was performed using in-house generated software (“GOOGLY” by Andrew Proctor).

GPC measurements were performed in THF at 35 °C at a flow rate of 0.35 mL/min maintained by a Waters 590 HPLC pump. The instrument was equipped with two Phenomenex narrow bore columns, a Waters 410 differential refractometer detector, and a Waters 745 data module. Calibration was performed using Waters polystyrene standards, at a typical sample concentration of 25 mg/mL.

II. Sample Preparation. Room temperature nonabsorbing liquids used as **mediators** were *m*-nitrobenzyl alcohol (*m*-NBA), 1,2,4-butanetriol (BTT), and 4′-pentyl-4-biphenylcarbonitrile (PBC) from Aldrich Chemical Co., thioglycolic acid (TGA) from Sigma Chemical Co., *o*-nitrophenyl octyl ether (*o*-NPOE) from Fluka, and poly(isobutylene) (PIB) and poly(ethylene glycol) (PEG 586) from Polysciences, Inc.

The **UV-absorbing liquid** *N*-(4-methoxybenzylidene)-4-butylaniline (MBBA) was purchased from Aldrich Chemical Co. **Solid absorbers** were 2-cyano-5-phenyl-2,4-pentadienoic acid (CPPA), *trans*-3-indoleacrylic acid (IAA), sinapinic acid (SA), and 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) from Aldrich Chemical Co. and α-cyano-4-hydroxycinnamic acid (α-CHCA), ferulic acid (FA), and 2,5-dihydroxybenzoic acid (DHB) from Sigma Chemical Co.

Analytes were poly(ethylene glycols) (PEG 586, PEG 1450, PEG 8160, and PEG 15000) from Polyscience, Inc., poly(propylene glycols) (PPG 440, PPG 1080, PPG 2160, and PPG 3100) from Scientific Polymer Products, and poly(dimethylsiloxane) (PDMS) from Bayer Corp.

A flow diagram of sample preparation for liquid or solid polymers with **liquid matrices** is presented in Figure 1. Solid analytes, organic absorbers, and other additives such as internal standards or salts were dissolved in mutually miscible solvents, usually water, ethanol, tetrahydrofuran, acetone, toluene, or mixtures of these solvents. These solutions were then mixed with liquid mediators or analyte in microcentrifuge tubes using sonication or vortex mixing. The mixtures were vacuum centrifuged using a Savant Speed Vac Plus to remove volatile solvents. Typically, 1−2 μL of the analytical solution was deposited on a piece of substrate material (stainless steel, etched silver, or cellulose) mounted on a glass slide.

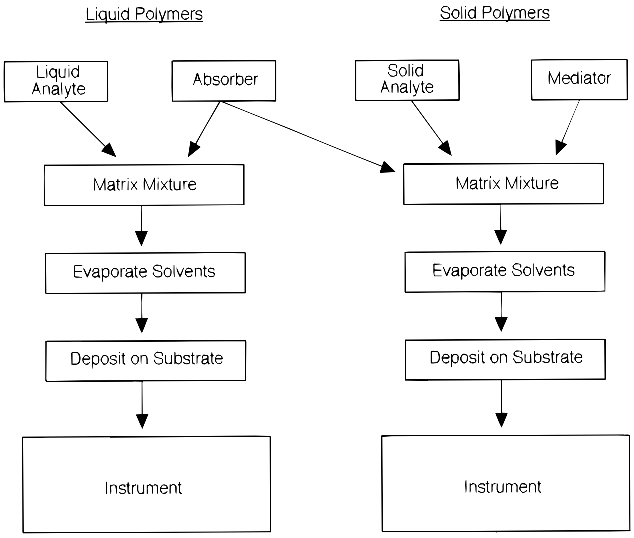

Figure 1. Sample preparation scheme for liquid matrices.

Solid MALDI matrices were prepared using IAA, HABA, and α-CHCA. A 0.2 M solution of IAA in acetone was vortex mixed with an equal volume of the analyte (1 mg/mL) in acetone. A 2−3 μL aliquot of the mixture was slowly pipeted onto a stainless steel substrate, allowing rapid formation of matrix crystals.[30]

HABA was prepared by dissolving 1.5 mg/mL in a 1:1 mixture of acetonitrile/water. This solution was vortex mixed with an equal volume of polymer solution (1 mg/mL); then ca. 3 μL was deposited onto a stainless steel substrate and allowed to air dry.

Polycrystalline films, similar to those used for protein analysis,[56] were prepared by first pipeting 5 μL of 0.1 M α-CHCA in acetone onto a stainless steel substrate. After air drying, the matrix was crushed using a flat glass surface and the loose material was gently brushed away, leaving a thin layer of matrix material adhered to the substrate. A saturated solution of the same matrix in a less volatile solvent (such as 1:1 methanol/water) was vortex mixed with an equal volume of 1 mg/mL analyte. A 5 μL aliquot of this solution was deposited onto the film and allowed to air dry.

Polymers for SIMS analysis were prepared in methanol or acetone to yield a 10^{-3} M solution with respect to the repeat unit mass. A 5 μL aliquot of the solution was pipeted onto a 1 cm diameter etched silver substrate. The silver had been etched with warm 20% nitric acid and then rinsed with deionized water, toluene, and methanol.

Results and Discussion

I. Liquid Matrices. Although *m*-NBA, *o*-NPOE, and glycerol have been employed as matrices for FAB or MALDI mass spectrometric analysis using a probe introduction system; exposure to instrument vacuum in the range 10^{-5}−10^{-6} mbar depletes these materials. Loss of the more volatile components creates a dynamic environment, where the analyte concentration and physical state of the matrix are uncontrollable and unknown. Since the matrix/analyte ratio is a critical parameter[57] in MALDI, degradation of performance during analysis may occur. The search for more suitable liquid matrices required scrutinizing prospective materials with regard to properties similar to those necessary for solid MALDI matrices:[58]

1. Absorption. Suitable matrix material should have a molar extinction coefficient between $\epsilon_\lambda = 10^3$ and 10^5 L cm^{-1} mol^{-1} at the ultraviolet laser wavelength (λ).

2. Vacuum Stability. The material must not appreciably evaporate or sublime at typical instrument source pressures (ca. 10^{-6} mbar).

Table 1. Materials Used To Prepare Liquid Matrices Producing MALDI Polymer Spectra

	structure
Liquid Mediators	
1,2,4-butanetriol (BTT)	$HOCH_2CH_2CH(OH)CH_2OH$
4′-pentyl-4-biphenylcarbonitrile (PBC)	$CH_3(CH_2)_4C_6H_4C_6H_4CN$
thioglycolic acid (TGA)	$HSCH_2CO_2H$
poly(isobutylene) (PIB)	$H[CH_2C(CH_3)_2]_nH$
poly(ethylene glycol) (PEG)	$H[OCH_2CH_2]_nOH$
Solid Absorbers	
α-cyano-4-hydroxycinnamic acid (α-CHCA)	$HOC_6H_4CH{=}C(CN)CO_2H$
2-cyano-5-phenyl-2,4-pentadienoic acid (CPPA)	$C_6H_5CH{=}CHCH{=}C(CN)COOH$
sinapinic acid (SA)	$HOC_6H_2(OCH_3)_2CH{=}CHCO_2H$
ferulic acid (FA)	$HOC_6H_3(OCH_3)CH{=}CHCO_2H$
2,5-dihydroxybenzoic acid (DHB)	$(HO)_2C_6H_3CO_2H$
Liquid Absorber	
N-(4-methoxybenzylidene)-4-butylaniline (MBBA)	$CH_3(CH_2)_3C_6H_4N{=}CHC_6H_4OCH_3$

3. Miscibility. The matrix and analyte should be soluble in a common solvent and remain miscible after the solvent has evaporated, thus allowing homogeneous dispersion of the analyte in the matrix/analyte mixture.

4. Chemical Compatibility. The matrix must not have functional groups that promote chemical reactions causing changes in analyte structure or matrix performance.

5. Induced Ionization. The matrix should have transferable protons (typically from −OH or −NH groups) or allow cation (Na^+, K^+, Li^+, Cs^+, or Ag^+) attachment.

Three approaches were pursued to develop new liquid matrix systems: (i) The above criteria were used to conduct a search for suitable liquid chromophores. (ii) Liquid phase vacuum stable analytes were mixed with UV absorbers (solid matrix or other material with a high extinction coefficient near 337 nm). (iii) Using approach i, suitable nonabsorbing liquids (mediators) were identified and mixed with a UV chromophore yielding matrix solutions[46] prior to analyte addition. Materials found to produce MALDI spectra from the experimental polymers are listed, along with structures, in Table 1. The application of these materials is detailed below.

A. Analyte/Absorber Mixtures. The organic absorber 2-cyano-5-phenyl-2,4-pentadienoic acid (CPPA) ($\epsilon_{337} = 3.33 \times 10^4$ L cm^{-1} mol^{-1})[59] was found to consistently produce high quality results. MALDI spectra have also been acquired from liquid phase mixtures using DHB, SA, FA, and α-CHCA. The MALDI spectrum obtained by addition of a CPPA solution to liquid PPG 3100 is presented in Figure 2. The oligomer distribution is represented by $(M + Na)^+$ oligomer ions. The cations are separated by 58 Da, which is consistent with the $-[CH_2CH(CH_3)O]-$ repeat unit. The end unit mass was calculated to be 18 Da, indicating −H and −OH termini, by subtraction of the mass sum of *n* repeat units and the cation mass from the oligomer ion mass. The resolution ($M/\Delta M = 610$) was determined using the full width half-maximum (FWHM) definition.

In general, the experimental liquid MALDI mass spectra of synthetic polymers were dominated by Na^+ cationized oligomer ion peaks with the appearance of metal, matrix, matrix fragment, and cluster ion peaks below 500 Da. A lower intensity $(M + K)^+$ oligomer signal was also observed if experimental conditions allowed a sufficiently high S/N ratio. The type of cationization can be determined by examination of the relative position of heterocationized species. The source of the cations in the sample matrix is assumed to be impurities incorporated during manufacture or handling of the polymeric material. The addition of 1−3 μL of saturated solutions of NaCl, KCl, or $AgNO_3$ in methanol generally increased the intensity of that form of cationization, but the S/N was usually lowered and the $(M + Na)^+$ peaks were still dominant. Peaks from fragmentation, protonation $(M + H)^+$, dimers $(2M + Na)^+$, or doubly charged oligomers $(M + 2Na)^{2+}$ were rarely observed.

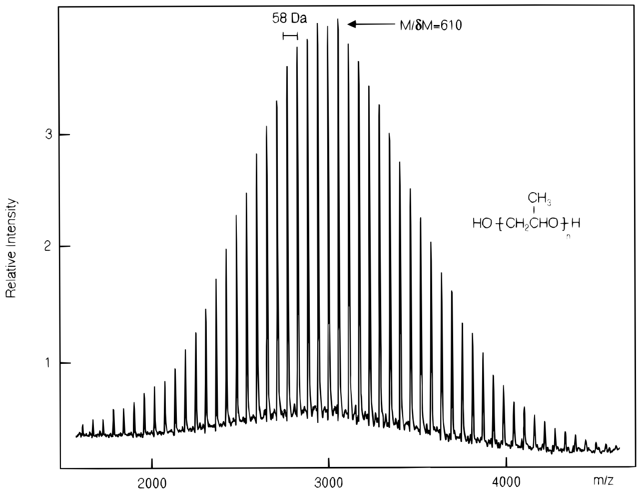

Figure 2. Oligomer distribution of PPG 3100 with CPPA (0.8 M) deposited onto an etched silver substrate. The intense peaks are due to intact Na^+ cationized oligomers.

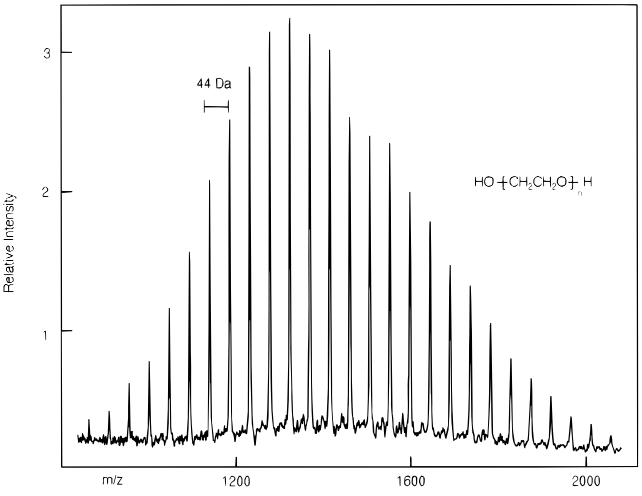

Figure 3. Oligomer distribution of PEG 1450 acquired with a BTT/CPPA (0.8 M) matrix deposited onto a cellulose substrate. The intense peaks are due to intact oligomers cationized with Na^+.

B. Matrix Solutions. The MALDI spectrum obtained for PEG 1450 using a matrix solution is presented in Figure 3. The liquid matrix was comprised of the moderator BTT mixed with the absorber CPPA

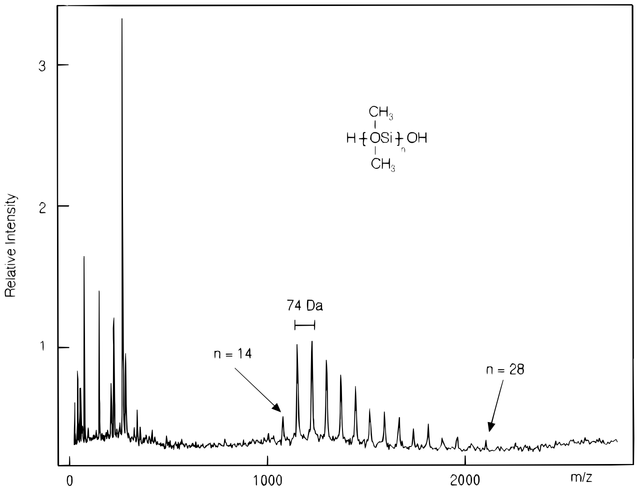

Figure 4. MALDI mass spectrum of PDMS mixed with a MBBA matrix deposited onto a stainless steel substrate. The peaks above 1000 Da represent intact oligomers cationized with Na^+.

(0.8 M) and the analyte to a final concentration of 10 mg/mL. The oligomers are represented by $(M + Na)^+$ and low-intensity $(M + K)^+$ ions, and the spectrum yields the expected value of 44 Da for the $-[CH_2CH_2O]-$ repeat unit. The end unit mass was found to be 18 Da, consistent with the expected −H and −OH attachment.

Matrix solutions yielded spectra from all polyglycol standards tested below molecular weight 15 000. Over 20 combinations of the mediators and absorbers (Table 1) produced liquid MALDI spectra. Depending on the mediator, an absorber concentration of 0.2−1.0 M was used to create the matrix solution. Above this range, saturation of the mixture was observed. Instrument source pressures during analysis ranged from 5×10^{-6} mbar for BTT to 5×10^{-7} mbar for low molecular weight PEG or PIB and decreased in the order of the mediator listing in Table 1. Elevated instrument pressures were indicative of higher volatility of the mediator. The application of less polar materials such as PBC, PIB, or MBBA may allow improvement in the analysis of hydrophobic polymers.

C. Neat Liquids. A neat liquid matrix found suitable for polymer analysis was *N*-(4-methoxybenzylidene)-4-butylaniline (MBBA). This liquid crystal has high absorbance ($\epsilon_{337} = 1.86 \times 10^4$ L cm^{-1} mol^{-1})[60] and is vacuum stable, giving an instrumental source pressure of $<6 \times 10^{-7}$ mbar. Figure 4 displays the spectrum of PDMS in a 1:1 v/v mixture with MBBA. The peaks above 1000 Da represent the sodium cationized $n = 14-28$ oligomers and allow the determination of a repeat unit and end unit mass of 74 and 18 Da, respectively, consistent with that expected for the PDMS structure. The peaks in the 0−500 Da range resulted from metal, matrix, and matrix fragment ions. This matrix has also been used to produce spectra from lower molecular weight polyglycols but may, depending on instrumental conditions, produce cluster ions up to $m/z \approx 1200$ Da. MBBA was the only neat liquid matrix found to produce MALDI spectra.

II. Laser Intensity. It was observed that MALDI analysis using these liquid matrices necessitated high laser fluence, typically 4−6 times that necessary for solid matrices. The need for higher laser intensity has also been noted in the liquid MALDI analysis of polymerized terthiophene derivatives using a mixture of NPOE and 4-nitrophenol.[61] One possible reason for this is a lower effective absorption of laser energy by these matrices relative to their solid counterparts. The mixtures used in liquid MALDI generally have a 0.1−1.0 M absorber concentration relative to the analyte or mediator. To obtain good spectral quality, solid matrices are prepared with typical matrix/analyte ratios of greater than 1000. This is essentially one order of magnitude higher in absorbing molecules per unit volume. These materials, due to the irregularities inherent in crystal growth, also have larger surface areas than the relatively smooth surface of liquids. Assuming that desorption is predominantly a surface process, this yields a much larger number of chromophores per irradiated area in solid state matrices and, hence, higher absorbance compared with liquid matrices. The effect may be somewhat compensated by the longer radiant path length in the liquid, thus allowing absorption of incident laser energy below the surface of the liquid matrix. However, in the droplet interior the desorption process would have to overcome both attractive interactions in the bulk and the surface energy of the liquid. The above reasoning was supported by the observation that the two chromophores with the highest extinction coefficient, e.g. CPPA and α-CHCA, gave better performances than those exhibiting lower absorbances.

III. Molecular Weight Determinations. The masses of the oligomer peaks from calibrated spectra, combined with relative intensities (height or area), allow accurate calculation of the **number average molecular weight** (M_n), the **weight average molecular weight** (M_w), and the **polydispersity** (M_w/M_n), which serves as an indication of the width of the mass range of the oligomer distribution. M_n and M_w are defined as

$$M_n = \sum N_i M_i / \sum N_i$$

$$M_w = \sum N_i M_i^2 / \sum N_i M_i$$

where N_i is the molar fraction of the molecular ion with a degree of polymerization i and M_i is the mass or, without resolution of the isotopic distribution, the average mass of the ith oligomer. Since MALDI produces little or no fragmentation, accurate molecular weight values may also be determined from the distribution envelope when individual oligomers are not resolved.

A. Calibration. Accurate and reproducible mass calibration of MALDI polymer spectra requires the use of internal standards. Use of low molecular weight cations, such as Na^+ or K^+, etc., did not provide accurate calibration of higher molecular weight analytes. While matrix peaks may be utilized, these may vary in position and intensity with changes in laser intensity and are difficult to identify in the noisy low mass region. The necessity of using higher laser fluence for polymer analysis using liquid matrices resulted in excessive fragmentation of well characterized biomolecules, thus limiting their use as internal standards. Calibration of lower molecular weight oligomer distributions can be accomplished by using the $^{107}Ag/^{109}Ag$ peaks resulting from addition of a silver salt or utilization of a silver substrate. More universal and reproducible signals, suitable for mass calibration, were obtained when a low molecular weight polyglycol is included in the matrix mixture as an internal mass standard. Both liquid PEG 586 and PPG 440 have been well characterized in terms of cationization and peak position of the oligomers on the mass scale. These materials allowed the use of up to 10 or more peaks for calibration.

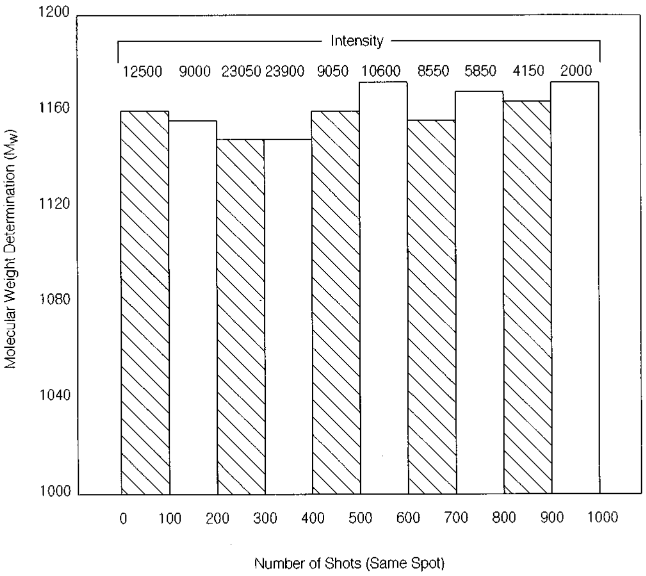

Figure 5. Shot-to-shot reproducibility of a PPG 1080/CPPA (1.0 M) mixture. The intensities represent $\sum I_i$ of the $n = 13$ through $n = 27$ Na^+ oligomers of each 100 shot sequence.

Table 2. Point-to-Point Reproducibility of the Molecular Weight and Intensity of PPG 1080 in a 1:1 Mixture with MBBA

point	M_n	M_w	M_w/M_n	intensity[a]
1	1085	1135	1.05	13 100
2	1105	1155	1.05	14 350
3	1100	1150	1.05	14 500
4	1140	1195	1.05	10 350
5	1090	1135	1.04	9 850
av	1105	1155	1.05	12 430
STD	20	25	0.00	2 200

[a] $\sum I_i$ of $n = 13$ to $n = 27$ oligomers.

B. Reproducibility. The ability to simultaneously and reproducibly measure the relative intensity of a distribution of molecular ions is crucial to the accurate determination of molecular weight values. Most solid matrices are inherently heterogeneous and suffer from poor shot-to-shot and point-to-point reproducibility, as well as temporal signal degradation.[53] MALDI reproducibility can be improved by the use of liquid matrices. This is evidenced by the molecular weight determinations and total oligomer signal intensity from 10 successive 100 shot samplings on one spot of a PPG 1080/CPPA mixture (Figure 5). Up to 1000 successive laser shots can be made on one point of this liquid matrix without serious degradation of signal. The average molecular weight value (M_w) for 1000 shots was determined to be 1160 ± 10; with no significant deviations outside this range. Although signal intensity decreased over the final 300 shots, the ability to acquire structural and molecular weight information from these spectra was not adversely affected.

The point-to-point reproducibility of the molecular weight values and signal intensity acquired from five successive laser positions on a 1:1 PPG 1080/MBBA mixture is shown in Table 2. The homogeneity of this liquid matrix preparation is attested by the consistency of the signal intensity at various points on the matrix. The molecular weight determinations were reproducible and generally fall within the range defined by the standard deviation.

C. Comparison of MALDI Techniques. The performance of liquid matrices may be examined by comparison with results obtained from solid MALDI matrices. The molecular weight determinations for PEG 1450 using 11 matrix preparations are presented in Table 3. The average M_w value was found to be 1450 ± 35 with only the PEG/CPPA (0.8 M) matrix producing results significantly outside this range. Good agreement is seen between the values obtained using the eight matrix solution preparations and the three solid matrices prepared using polycrystalline, dried droplet, and rapid crystallization techniques.

Table 3. MALDI Molecular Weight Determinations for PEG 1450

matrix	preparation	M_n	M_w	M_w/M_n
BTT/CPPA	matrix solution	1355	1440	1.06
BTT/CPPA[a]	matrix solution	1330	1415	1.06
PBC/CPPA	matrix solution	1375	1460	1.06
PBC/α-CHCA	matrix solution	1280	1405	1.10
PEG 586/CPPA	matrix solution	1445	1540	1.07
PIB/CPPA	matrix solution	1355	1470	1.08
TGA/CPPA	matrix solution	1375	1460	1.06
TGA/MBBA	matrix solution	1430	1475	1.03
α-CHCA[b]	"polycrystalline"	1295	1425	1.10
DHB[b]	"dried droplet"	1305	1455	1.11
IAA[b]	"rapid crystallization"	1370	1430	1.04
	av	1355	1450	1.07
	STD	50	35	.03

[a] Cellulose substrate. [b] Solid matrix.

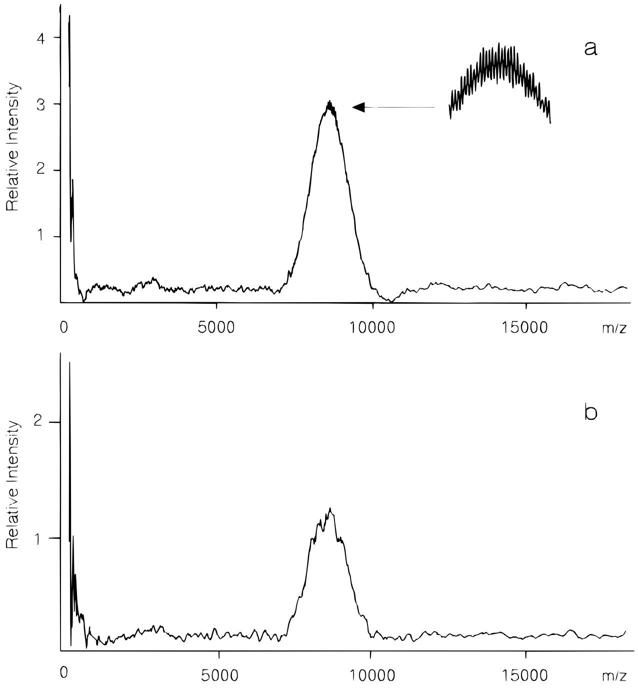

Figure 6. MALDI mass spectra of PEG 8610 with (a) IAA solid matrix and (b) BTT/CPPA (0.8 M) matrix solution. Both from a stainless steel substrate.

Figure 6 compares the spectra obtained from MALDI analysis of PEG 8160 using the solid matrix IAA and a matrix solution BTT/CPPA. Both preparations yielded spectra representative of the molecular weight distribution of the polymer. However, better resolution was obtained with IAA. Table 4 presents a comparison of the results obtained from higher molecular weight PEG using matrix solutions and solid matrices. The molecular weight values were reproducible with both liquid and solid matrix techniques; providing an average M_w of 8635 ± 30 for the analyte. Similar agreement between MALDI methods is seen in the values obtained for PEG 15 000.

Table 5 compares of the molecular weight values acquired from three identical preparations of both matrix solutions and solid MALDI matrices. Excellent

Table 4. MALDI Molecular Weight Values for PEG 8160 and PEG 15000

	M_n	M_w	M_w/M_n
	PEG 8160[a]		
liquid matrix solution BTT/CPPA 0.8 M	8500	8610	1.01
liquid matrix solution PEG 586/CPPA 0.8 M	8610	8680	1.01
“polycrystalline film” α-CHCA	8560	8640	1.01
“rapid crystallization” IAA	8495	8615	1.01
	PEG 15 000[a]		
liquid matrix solution PEG 586/CPPA 0.8 M	12880	12950	1.01
“rapid crystallization” IAA	12700	12770	1.01
“dried droplet” HABA	12820	12950	1.01

[a] Value listed by manufacturer.

Table 5. Molecular Weight Determinations for PEG 1450 Using three Repetitive Preparations of BTT/CPPA 0.8 M and IAA MALDI Matrices

	matrix solution (BTT/CPPA 0.8 M)			solid matrix (IAA)		
	M_n	M_w	M_w/M_n	M_n	M_w	M_w/M_n
sample 1	1385	1460	1.05	1365	1450	1.06
sample 2	1335	1415	1.06	1370	1430	1.04
sample 3	1375	1420	1.03	1360	1435	1.06
av	1365	1430	1.05	1365	1440	1.05
STD	25	25	.02	5	10	.01

agreement of results is seen between the two methods and within the data sets acquired using each preparation, thus demonstrating the sample-to-sample consistency of both MALDI methods.

D. Comparison with Other Techniques. The validity of molecular weight determinations from the MALDI spectra of polyglycols and poly(dimethylsiloxane) in liquid matrices can be evaluated through comparison with values derived from other techniques. The experimental results from liquid matrix MALDI, TOF-SIMS, and GPC are listed in Table 6. Although there is reasonable agreement between the three methods, two systematic disparities are observed.

It was noted that the results from MALDI are consistently higher than those obtained by TOF-SIMS analysis. The possible mass dependency of the three processes may contribute to lowering the molecular weight values calculated from TOF-SIMS spectra; sputtering, detector response, and fragmentation.[62] The disappearance cross-section is a measure of the decrease in secondary ion yield as a function of sputtering time and has been observed to increase with increasing PEG oligomer mass regardless of the width of the polymer distribution.[63] Disparities in detection efficiency with oligomer mass can bias TOF-SIMS results toward lower values. TOF-SIMS fragmentation increases at higher molecular weights and it is possible that higher mass oligomer may fragment by a mechanism that produces a lower mass oligomer. It was observed that the intensity of fragments relative to oligomer ions, in the TOF-SIMS spectra, increased dramatically with increasing molecular weight in the series of PPG standards with M_w values of 440, 1080, 2160, and 3100. The ratio of the intensities of the intact oligomer to fragments ions was found to be ∞, 10, 0.3, and 0.01, respectively. Although each of the above factors may bias TOF-SIMS results, experimental observations support the increase of fragmentation with increasing oligomer mass as the major process in lowering TOF-SIMS molecular weight values.

It was also observed that the weight average molecular weight values and resultant polydispersities obtained with GPC are generally higher than those calculated from MALDI spectra. The GPC values shown are from chromatograms calibrated using narrow molecular weight distribution (MWD) polystyrene standards. Since GPC uses size exclusion for separation, calibration requires use of molecules of the same type as the sample to convert the size-sorted chromatograms into molecular weight distribution curves. If the necessary calibration standards are not available, universal calibration provides a method for correction of the differences in the hydrodynamic volume of dissimilar materials. This correction is made using a plot of log($[\eta]M$) vs V_R, where $[\eta]$ is the intrinsic viscosity, M is the molecular weight, and V_R is the retention volume. The value of $[\eta]$ for a linear polymer in a specific solvent and its molecular weight are related by the empirical Mark−Houwink equation $[\eta] = KM^a$, where values of the Mark−Houwink constants K and a vary with polymer type, solvent, and temperature.[64] But this methodology is impractical for most water soluble polymers where the appropriate Mark−Houwink constants are not known or readily available.[65]

It is conceivable that mass discrimination during ion production, transfer, or detection may enhance the signal of lower molecular weight oligomers in MALDI spectra. This possibility was recently studied by measuring the relative ion intensities from equal molar mixtures of the 25 mer and 50 mer ($\Delta m/z = 2500$ Da) of poly(methyl methacrylate) (PMMA).[66] The results indicated there was no observable mass discrepancy in MALDI. Thus, the need for more accurate calibration is the most plausible explanation for the higher GPC values obtained from the relatively hydrophilic polyglycols.

Table 6. Comparison of Polymer Molecular Weight Determinations Using MALDI Liquid Matrices with Values from TOF-SIMS and GPC

	M_n			M_w			M_w/M_n		
polymer[a]	MALDI	SIMS	GPC	MALDI	SIMS	GPC	MALDI	SIMS	GPC
PEG 586	610	540	c	680	595	c	1.11	1.10	c
PEG 1450	1335	1225	1075	1415	1260	1375	1.06	1.03	1.28
PEG 8150	8500	b	10935	8610	b	11045	1.01	b	1.01
PEG 15 000	12880	b	15920	12950	b	16715	1.01	b	1.05
PPG 1080	1085	930	875	1120	980	1140	1.03	1.05	1.30
PPG 2160	2265	1915	2075	2310	1965	2325	1.02	1.03	1.12
PPG 3100	3000	2530	2695	3090	2620	3200	1.03	1.04	1.19
PDMS	1650	1120	c	1870	1180	c	1.13	1.05	c

[a] Nominal molecular weight as listed by manufacturer. [b] Only fragments observed at this molecular weight. [c] Molecular weight below GPC range.

Conclusions

Positive ion MALDI-TOF mass spectra were obtained from over 25 liquid matrix systems applied to three classes of synthetic polymers. Liquid matrices developed were characterized by ease of sample preparation and excellent vacuum stability. The best performance was obtained when CPPA was used at or near saturation concentrations in either mediators or analytes. Less polar materials such as PBC, PIB, or MBBA also produced MALDI spectra.

The sodium-cationized spectra of intact oligomers allowed simultaneous structure determination of lower molecular weight materials, and molecular weight distributions of all experimental polymers. Some disparities were observed in the comparison of liquid matrix MALDI and TOF-SIMS or GPC results. Excellent agreement was seen between molecular weights obtained with liquid and solid MALDI matrices. Liquid matrices were found to provide superior signal reproducibility over solid matrices tested. However, it was observed that solid matrices exhibit better performance in terms of higher resolution and in application to higher molecular weight materials.

Acknowledgment. This work was supported by the National Science Foundation under Grant CHE-9520336.

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MA951829E