Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Mass Spectrometry 241 (2005) 43–48
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Quantitative analysis of polypropyleneglycol mixtures by desorption/ionization on porous silicon mass spectrometry

Shoji Okuno[a,*], Yoshinao Wada[a,b], Ryuichi Arakawa[a,c]

[a] *Japan Science and Technology Agency, Innovation Plaza Osaka, Wada Project Laboratory, 3-1-10 Technostage, Izumi, Osaka, Japan*
[b] *Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan*
[c] *Department of Applied Chemistry, Kansai University, 3-3-35 Yamatecho, Suita, Osaka 564-8680, Japan*

Received 7 September 2004; accepted 28 October 2004
Available online 8 December 2004

Abstract

Mixtures of diol and triol types of polypropyleneglycol (PPG) bearing two and three hydroxyl end groups were analyzed quantitatively by matrix-assisted laser desorption/ionization (MALDI) and desorption ionization on porous silicon (DIOS) with the conventional dried droplet method. The reproducibility of MALDI mass spectra depended on the factors regarding sample preparation such as the analyte/matrix ratio, and the type of solvent and/or chemical matrix employed. For DIOS, the analyte concentration and the selection of solvents were important for good reproducibility. Optimization of these factors allowed reliable quantification of the polymer mixtures. Under optimized conditions, DIOS would be suitable than MALDI for this purpose.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Matrix-assisted laser desorption/ionization (MALDI); Desorption ionization on porous silicon (DIOS); Quantitative analysis; Polymer mixture

1. Introduction

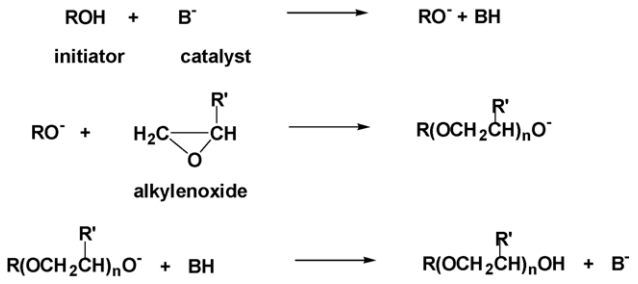
Polyethers such as polyethyleneglycol (PEG), polypropyleneglycol (PPG), and polytetrahydrofuran (PTHF) are currently used widely in industry as lubricants, stabilizers, removers, antifoaming agents, or raw materials for polyurethane. Polyethers are usually obtained, as shown in Scheme 1 , by the reaction between alkyleneoxide and an initiator with active hydrogen. While conventional analytical methods such as IR and NMR provide general profiles of the overall structures of polymers, it is necessary to obtain the structural information more in detail to understand the physical properties of these polymers.

Matrix-assisted laser desorption/ionization (MALDI) [1–4] is a promising ionization method for the analysis of biopolymers and synthetic polymers [5–11]. Since Tanaka et al. reported the successful ionization of PEGs by their unique soft laser desorption method [4], a variety of polyethers has been analyzed by MALDI–MS [12–22]; e.g., Li and coworkers reported characterization of complex polyethers [19] and Hercules and coworkers analyzed PTHF, a soft block in a polyurethane, after chemical degradation of the urethane bond by ethanolamine [20].

The quantitative analysis of polyether constituents is essential, since the polymer products are usually mixtures of various types and/or molecular weights of polyethers, of which the mixing ratio defines the properties of the products. The determination of the mixing ratio of different types of polymers using MALDI–MS is not easy [23]. End group ionization efficiency is the most important factor in determining the relative intensities of peaks in the MALDI mass spectra of mixtures of nylon 6 and polybutyleneterephthalate [24]. Mixtures of polystyrene and poly(α-methylstyrene) were quantitatively analyzed by hyphenated combination of size-exclusion chromatography and MALDI–MS [25]. This technique can be applied to a polymer mixture composed of similar chemical structures and molecular weights.

* Corresponding author. Tel.: +81 725 51 3317; fax: +81 725 51 3317.
E-mail address: shoji-okuno@osaka.jst-plaza.jp (S. Okuno).

1387-3806/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2004.10.022



Scheme 1. Method of synthesizing polyethers.

Homogeneity of analyte/matrix crystal is an important factor for quantitative analysis by MALDI–MS. The quality of the cocrystals depends on various factors of sample preparation such as the selection of matrices and solvents, and the matrix/analyte ratio. Siuzdak and coworkers reported a method, laser desorption/ionization on porous silicon, termed DIOS [26]. Porous silicon is a UV-absorbing semiconductor with a large surface area and is produced through electrochemical anodization or chemical etching of crystalline silicon. The applications reported to date cover a wide variety of compounds including peptides, natural products, small organic molecules, and synthetic polymers [26–35]. In DIOS, analytes and cationizing agents are deposited on DIOS chips without using chemical matrix for ionization. Therefore, DIOS is expected to be more suitable for quantitative analysis than MALDI, because it is unnecessary to take the matrix factor into account.

In this report, the applicability of DIOS–MS for the quantitative analysis of PPG mixtures is evaluated in comparison with MALDI–MS. The various factors of the sample preparation such as PPG concentration and solvent selection are investigated to achieve reliable measurements of polymer mixtures by DIOS–MS.

2. Experimental

2.1. Materials

Tetrahydrofuran (THF), ethanol, acetonitrile, sodium iodide, PPGs, 2,5-dihydroxybenzoic acid (DHB), and bovine insulin were purchased from Wako Pure Chemicals (Osaka, Japan). Structures of the PPGs are shown in Fig. 1. The initiator of the triol type PPG [number average molecular weight ca. 3000, TPPG3000] is glycerin and that of the diol type PPG [Mn ca. 2000, DPPG2000] is propyleneglycol. α-Cyano-4-hydroxycinnamic acid (CHCA), was purchased from Aldrich (Milwaukee, WI, USA), and angiotensin I from Bachem AG (Bubendorf, Switzerland).

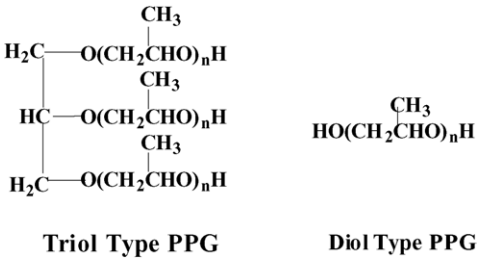


Fig. 1. Structures of triol and diol type PPGs.

2.2. Sample preparations

Two different solvents, H_2O/CH_3CN (1/1, v/v) and THF/C_2H_5OH (1/1, v/v), containing NaI at 1 mg/mL were used as solvents in the present experiments. Solutions of DPPG2000 (10 mg/mL) and TPPG3000 (10 mg/mL) were prepared and they were mixed at varying ratios of 10/90, 25/75, 50/50, 75/25, and 90/10 (v/v). These mixtures were diluted to achieve total PPG concentrations (TPC) of 5, 1, 0.5, and 0.1 mg/mL. CHCA and DHB were dissolved at 10 mg/mL, except for the case of CHCA in H_2O/CH_3CN, for which saturated solution was prepared. Finally, the polymer and matrix solutions were mixed at a 1/1 (v/v) ratio.

Each 0.1 μL aliquot of the polymer solution was deposited on DIOS chips obtained from Mass Consortium (San Diego, CA, USA), and the polymer/matrix solutions (0.5 μL) were spotted onto a stainless sample target and then dried at room temperature.

Micrographs of the polymer/matrix cocrystals were taken using an optical microscope, SZX12 (Olympus, Tokyo, Japan) at a magnifying power of 90.

2.3. Mass spectrometry

Mass spectra were acquired in positive linear mode using a Voyager-DE Pro Time-of-flight (TOF) mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a pulsed nitrogen laser (337 nm). Prior to sample analysis, an external mass calibration was performed using a peptide standard containing 1 μM angiotensin I and 8 μM bovine insulin for the MALDI–MS and DIOS–MS measurements. In all cases, mass spectra from 300 laser shots were accumulated. For DIOS, a thinner stainless stage was prepared in-house to offset the thickness of the chip, and the DIOS chip was taped onto it.

3. Results and discussion

Triol type PPG has three OH end groups, while diol type PPG has two OH end groups (Fig. 1). It is intriguing to analyze mixtures of diol and triol type PPGs and to compare the effects of DIOS–MS to those of MALDI–MS, since the ionization efficiency of the end group is an important factor for quantitative analysis by MALDI–MS [24].

Mixtures of DPPG2000 and TPPG3000 in a sodiated solution of H_2O/CH_3CN or THF/C_2H_5OH were analyzed by MALDI–MS and DIOS–MS, giving the $[M + Na]^+$ ions in the mass spectrum. Typically, the DIOS mass spectra obtained

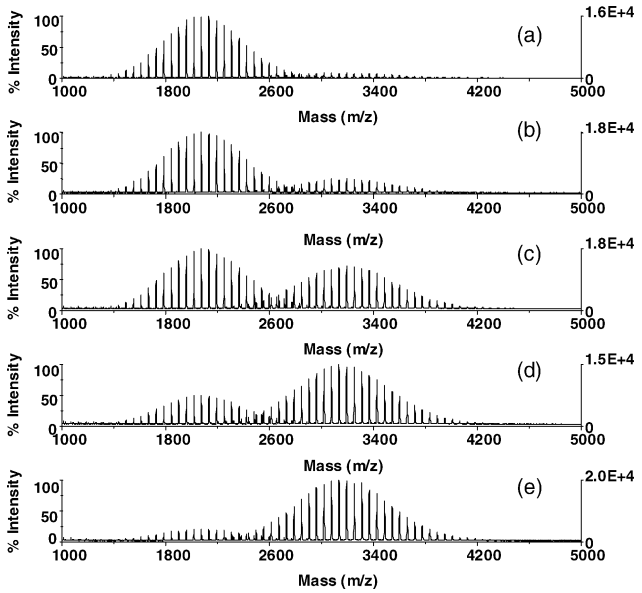


Fig. 2. DIOS–MS spectra of PPG mixtures. (a) TPPG3000 10 wt%, (b) 25 wt%, (c) 50 wt%, (d) 75 wt%, and (e) 90 wt% at TPC 1 mg/mL using THF/C_2H_5OH as the solvent.

using THF/C_2H_5OH as a solvent are shown in Fig. 2. The Na^+ adduct ions of DPPG2000 are observed at mass-to-charge ratios (*m*/*z*) of 1317–3118, and those of TPPG3000 at *m*/*z* 2146–4296. The mass of the repeating unit of PPG is 58 Da. The signals for DPPG2000 and TPPG3000 never overlap, because the molecular weights of the initiators of the polymers are different: Mw 76 for the initiator propyleneglycol of DPPG and Mw 92 the initiator glycerin of TPPG.

The *P* values defined by Eq. (1), where I_{3000} and M_{3000} were the peak intensity and the *m*/*z* value, respectively, of a Na^+ adduct ion of TPPG3000 and I_{2000} and M_{2000} were those of DPPG2000, were calculated to represent the relative ion intensities of DPPG2000 and TPPG3000 in the MALDI and DIOS mass spectra. The *P* values were calculated by taking the seven largest Na^+ adduct ions at *m*/*z* 1900, 1958, 2016, 2074, 2132, 2190, and 2248 for DPPG2000 and at *m*/*z* 3019, 3077, 3135, 3193, 3251, 3309, and 3367 for TPPG3000. Peak intensity represents the number of molecular ions. Thus, the *P* value should correlate with the weight percent of TPPG3000. The *P* values represent the average of the calculations from five mass spectra generated from different sample spots. The standard deviations (σ_{50}) of the *P* values at TPPG300050 wt% are shown in Table 1.

$$P = \frac{\sum I_{3000} M_{3000}}{\sum I_{3000} M_{3000} + \sum I_{2000} M_{2000}} \quad (1)$$

In MALDI analysis using H_2O/CH_3CN, the σ_{50} values constantly exceeded four, indicating low spot-to-spot reproducibility. Microscopic observation disclosed that two different kinds of deposits, large needle-like crystals and thin layer ones, were intermingled in the DHB/PPG cocrystals (Fig. 3a). Similarly, in the case of CHCA, two or more different kinds of crystals were formed (Fig. 3b). Uneven crystallization often occurs in the dried droplet sample preparation method, especially when the slowly evaporating water-rich solvents are used [36,37]. The heterogeneity of the analyte/matrix cocrystal impairs the reproducibility of mass spectra. In DIOS measurements, the σ_{50} values are unexpectedly high (σ_{50} = 3.2–4.3). Siuzdak and coworkers have recently reported that sample homogeneity is necessary for the quantitative analysis of peptides and amino acids even by DIOS–MS, which is free from chemical matrix [33]. In the present experiments, the polymer and cationizing agents distributed uneven in the spot on the DIOS sample target.

Table 1
Standard deviation (σ_{50}) of *P* values at TPPG3000 50 wt%

TPC (mg/mL)	H_2O/CH_3CN			THF/C_2H_5OH		
	DHB	CHCA	DIOS	DHB	CHCA	DIOS
10	–	–	4.31	–	–	2.44
5	–	4.28	3.22	3.33	3.23	3.00
1	4.51	5.02	3.93	2.28	1.84	0.37
0.5	5.43	5.31	3.73	2.58	3.19	1.31
0.1	20.52	5.34	–	3.89	6.47	3.38

Data points were obtained in five replicate experiments.
TPC, total PPG concentration; –, intense molecular ions of PPGs were not obtained.

Homogeneity of sample/matrix crystals is greatly affected by solvent kinds. When THF/C_2H_5OH was used as the solvent, small needle-like crystals or homogeneous thin layer crystals for DHB or CHCA, respectively, were formed as shown in Fig. 3c and d. The σ_{50} values (σ_{50} = 0.37–6.47) are smaller than those of H_2O/CH_3CN solvent (σ_{50} = 3.22–20.52). This result indicated that the THF/C_2H_5OH solvent supported the homogeneous cocrystal formation and thus improved the spot-to-spot reproducibility compared in the case with H_2O/CH_3CN. The calculated *P* values of TPPG3000 were plotted against weight percents in Figs. 4–6, where the *P* value was proportional to the weight percent of TPPG3000. With DHB (Fig. 4), the σ_{50} value at TPC 1 mg/mL was 2.28, and a linear curve fit

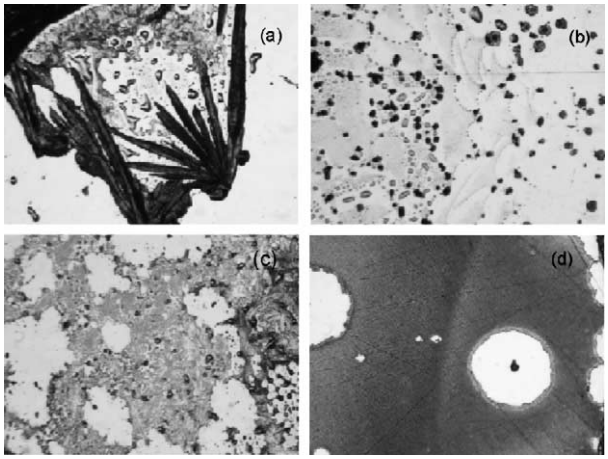


Fig. 3. Micrographs of polymer/matrix cocrystals (×90) (a) DHB matrix using H_2O/CH_3CN, (b) CHCA matrix using H_2O/CH_3CN, (c) DHB matrix using THF/C_2H_5OH, and (d) CHCA matrix using THF/C_2H_5OH.

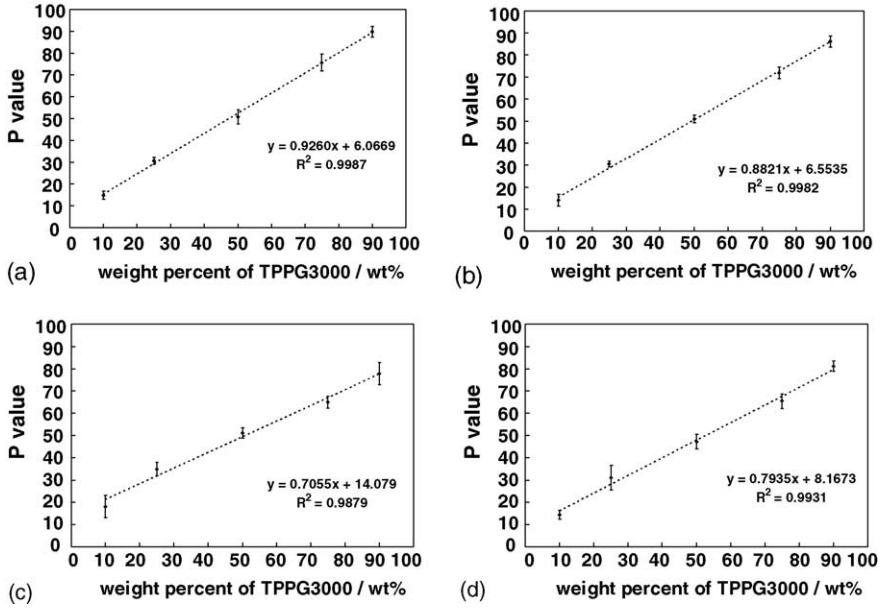


Fig. 4. The plots of P values vs. weight percents of TPPG3000 obtained from MALDI–MS measurements with DHB matrix using THF/C_2H_5OH at TPC 5 mg/mL (a), TPC 1 mg/mL (b), TPC 0.5 mg/ml (c), and TPC 0.1 mg/mL (d).

($y = 0.8821x + 6.5535$, where y represented P values and x represented weight percents of TPPG3000) having a coefficient of variation (R^2) of 0.9982 was obtained. At TPC 5 mg/mL, the R^2 value was 0.9987 ($y = 0.9260x + 6.0669$), and the σ_{50} value was 3.33. Also with CHCA (Fig. 5), good linear curve fits were obtained at TPC 1 mg/mL, the R^2 value was 0.9997 ($y = 0.9701x + 1.0609$) and the σ_{50} value was small ($\sigma_{50} = 1.84$). At TPC 5 mg/mL, the R^2 value was 0.9990 ($y = 0.9355x + 3.7933$), while the σ_{50} value became slightly larger ($\sigma_{50} = 3.23$). The linearity of the approximated curve and the spot-to-spot reproducibility were slightly impaired at low PPG concentrations as 0.1 and 0.5 mg/mL for either matrix. This was probably due to uneven distribution, at a submicroscopic level, of crystals at low PPG concentrations. These results indicated that optimization of the matrix/analyte ratios and the selection of both matrix molecules and the solvent allows good qualitative analysis of PPG mixtures by MALDI–MS.

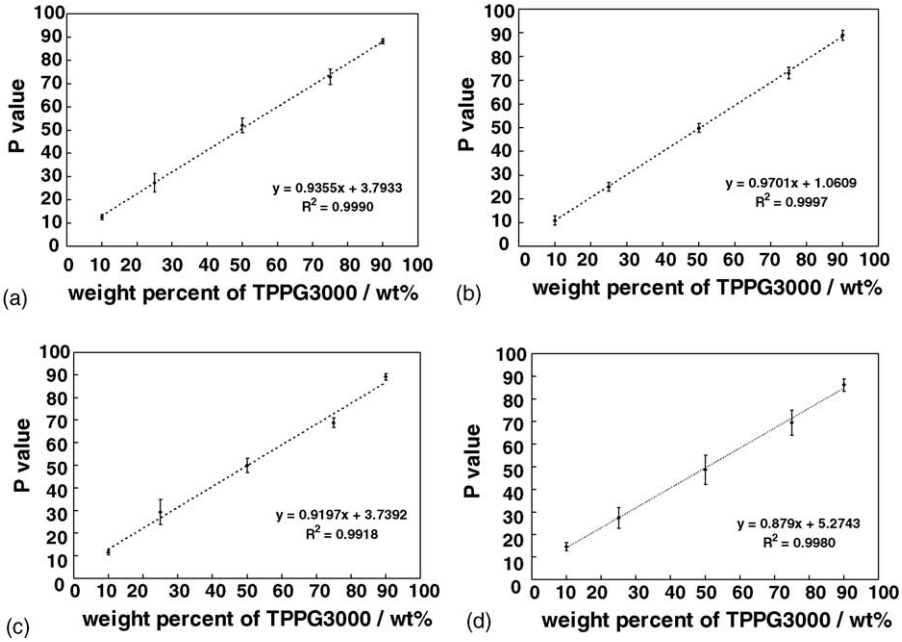


Fig. 5. The plots of P values vs. weight percents of TPPG3000 obtained from MALDI–MS measurements with CHCA matrix using THF/C_2H_5OH at TPC 5 mg/mL (a), TPC 1 mg/mL (b), TPC 0.5 mg/ml (c), and TPC 0.1 mg/mL (d).

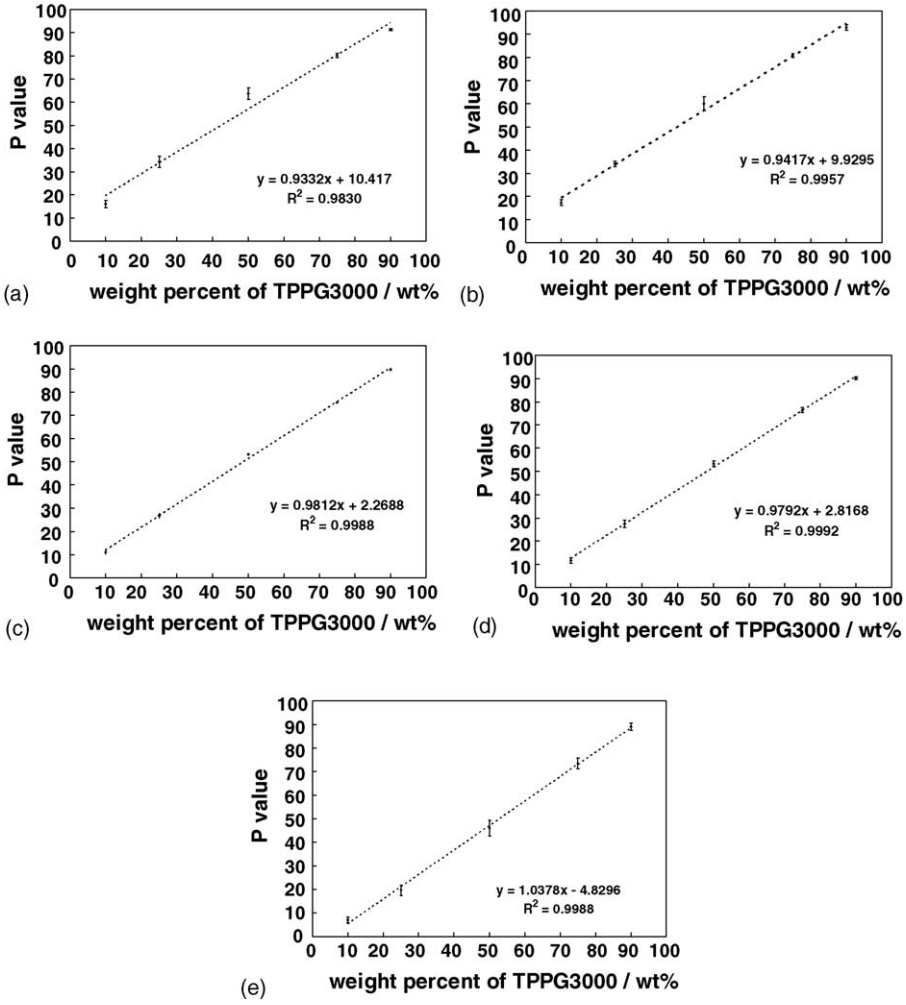


Fig. 6. The plots of P values vs. weight percents of TPPG3000 obtained from DIOS–MS measurements using THF/C_2H_5OH at TPC 10 mg/mL (a), TPC 5 mg/mL (b), TPC 1 mg/ml (c), TPC 0.5 mg/mL (d), and TPC 0.1 mg/mL (e).

In the DIOS–MS measurement, at TPC 0.1 mg/mL, the ion intensities were too weak to give adequate reproducibility ($\sigma_{50} = 3.38$). At concentrations over 5 mg/mL, the linearity of the approximated curve was not optimal, and the σ_{50} values became larger ($\sigma_{50} = 3.00$ at TPC 5 mg/mL, $\sigma_{50} = 2.44$ at TPC 10 mg/mL). High polymer concentrations might exceed the ionization ability of the DIOS chip. On the other hand, excellent linear curve fits were obtained at TPC 1 and 0.5 mg/mL with R^2 values of 0.9992 ($y = 0.9792x + 2.8168$) and 0.9988 ($y = 0.9812x + 2.2688$), respectively. More interestingly, the σ_{50} values ($\sigma_{50} = 0.37$ at TPC 1 mg/mL, $\sigma_{50} = 1.31$ at TPC 0.5 mg/mL) were much smaller than those obtained by MALDI–MS. This result indicated that DIOS was more suitable for quantitative analysis than MALDI. Siuzdak and coworkers have recently utilized an electrospray deposition method to make a homogeneous thin layer of sample molecules [33]. Our results indicated that optimization of various factors allows excellent qualitative estimation by DIOS–MS even with the conventional dried drop method of sample preparation.

4. Conclusion

Mixtures of different kinds of PPGs were analyzed by MALDI–MS and DIOS–MS using the dried droplet method. The reproducibility of MALDI mass spectra was dependent on the analyte/matrix ratio, and the type of solvent and/or chemical matrix. In DIOS measurements, the analyte concentration and solvent selection were important for good quantitative estimation. Optimization of these factors allows reliable quantitative measurements of polymer mixtures. DIOS–MS would be superior to MALDI–MS for this purpose.

References

[1] M. Karas, D. Bachmann, F. Hillenkamp, Anal. Chem. 57 (1985) 2935.
[2] M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, Int. J. Mass. Spectrom. Ion Process 78 (1987) 53.
[3] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.

[4] K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 2 (1988) 151.
[5] C. Fenselau, Anal. Chem. 69 (1997) 661A.
[6] R. Aebersold, D.R. Goodlett, Chem. Rev. 101 (2001) 269.
[7] J. Rappsilber, M. Moniatte, M.I.L. Nielsen, A.V. Podtelejnikov, M. Mann, Int. J. Mass. Spectrom. 226 (2003) 223.
[8] G. Montaud, R.P. Lattimer (Eds.), Mass Spectrometry of Polymers, CRC Press, Boca Raton, 2001.
[9] S.D. Hanton, Chem. Rev. 101 (2001) 527.
[10] S.F. Macha, P.A. Limbach, Curr. Opin. Solid State Mater. Sci. 6 (2002) 213.
[11] R.M. Murgasova, D.M. Hercules, Int. J. Mass. Spectrom. 226 (2003) 151.
[12] G. Montaudo, M.S. Montaudo, C. Puglisi, F. Samperi, Rapid Commun. Mass Spectrom. 9 (1995) 453.
[13] A.T. Jackson, H.T. Yates, W.A. MacDonald, J.H. Scrivens, G. Critchley, J. Brown, M.J. Deery, K.R. Jennings, C. Brookes, J. Am. Soc. Mass Spectrom. 8 (1997) 132.
[14] A.M. Belu, J.M. DeSimone, R.W. Linton, G.W. Lange, R.M. Friedman, J. Am. Soc. Mass Spectrom. 7 (1996) 11.
[15] H.M.D. Liu, U.P. Schlunegger, Rapid Commun. Mass Spectrom. 10 (1996) 483.
[16] J.C. Blais, M. Tessier, G. Bolbach, B. Remaud, L. Rozes, J. Guitard, A. Brunot, E. Marechal, J.C. Tabet, Int. J. Mass. Spectrom. Ion Process 144 (1995) 131.
[17] H. Pasch, K. Rode, J. Chromatogr. A 699 (1995) 21.
[18] S. Lee, M.A. Winnick, R.M. Whittal, L. Li, Macromolecules 29 (1996) 3060.
[19] R. Chen, A.M. Tseng, M. Uhing, L. Li, J. Am. Soc. Mass Spectrom. 12 (2001) 55.
[20] J.T. Mehl, R. Murgasova, X. Dong, D.M. Hercules, H. Nefzger, Anal. Chem. 72 (2000) 2490.
[21] A.J. Hoteling, K. Kawaoka, M.C. Goodberlet, W.M. Yu, K.G. Owens, Rapid Commun. Mass Spectrom. 17 (2003) 1671.
[22] K. Shimada, R. Nagahata, S. Kawabata, S. Matsuyama, T. Saito, S. Kinugasa, J. Mass Spectrom. 38 (2003) 948.
[23] M.W.F. Nielen, Mass Spectrom. Rev. 18 (1999) 309.
[24] C. Puglisi, F. Samperi, Macromolecules 35 (2002) 3000.
[25] R. Murgasova, D.M. Hercules, Anal. Chem. 75 (2003) 3744.
[26] J. Wei, J.M. Buriak, G. Siuzdak, Nature 399 (1999) 243.
[27] Z. Shen, J.J. Thomas, C. Averbuj, K.M. Broo, M. Engelhard, J.E. Crowell, M.G. Finn, G. Siuzdak, Anal. Chem. 73 (2001) 612.
[28] J.J. Thomas, Z. Shen, D. Blackledge, G. Siuzdak, Anal. Chim. Acta 442 (2001) 183.
[29] Z. Shen, S. Yao, J.E. Crowell, G. Siuzdak, M.G. Finn, Israel J. Chem. 41 (2002) 313.
[30] W.G. Lewis, L.G. Green, F. Grynszpan, Z. Radic, P. Carlier, P. Taylor, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. 41 (2002) 1053.
[31] R.A. Kruse, S.S. Rubankhin, E.V. Romanova, P.W. Bohn, J.V. Sweedler, J. Mass Spectrom. 36 (2001) 1317.
[32] W.G. Lewis, Z. Shen, M.G. Finn, G. Siuzdak, Int. J. Mass Spectrom. 226 (2003) 107.
[33] E.P. Go, Z. Shen, K. Harris, G. Siuzdak, Anal. Chem. 75 (2003) 5475.
[34] R. Arakawa, Y. Shimomae, H. Morikawa, K. Ohara, S. Okuno, J. Mass Spectrom. 18 (2004) 961.
[35] S. Okuno, Y. Shimomae, K. Ohara, H. Fujiwara, J. Ohyama, M. Ohmoto, Y. Wada, R. Arakawa, J. Mass Spectrom. Soc. Jpn. 52 (2004) 142.
[36] O. Vorm, R. Roepstorff, M. Mann, Anal. Chem. 66 (1994) 3281.
[37] Y. Dai, R.M. Whittal, L. Li, Anal. Chem. 68 (1996) 2494.