http://dx.doi.org/10.5012/bkcs.2013.34.6.1708

MALDI-TOF Analysis of Polyhexamethylene Guanidine (PHMG) Oligomers Used as a Commercial Antibacterial Humidifier Disinfectant

Hyo Jin Hwang, Jungjoo Nam, Sung Ik Yang,[†] Jung-Hwan Kwon,[‡,*] and Han Bin Oh[*]

Department of Chemistry, Sogang University, Seoul 121-742, Korea. [*]E-mail: hanbinoh@sogang.ac.kr
[†]Department of Applied Chemistry, Kyung Hee University, Yongin, Gyeonggi 446-701, Korea
[‡]Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-713, Korea
[*]E-mail: junghwankwon@korea.ac.kr
Received February 27, 2013, Accepted March 14, 2013

Polyhexamethylene guanidine (PHMG) polymers used as an active ingredient in an antibacterial humidifier disinfectant were reported to cause harm to the human health when inhaled, although physical contact with this material was known to present low toxicity to humans. It is therefore necessary to develop an optimal analysis method which enables detection and analysis of PHMG polymers. MALDI-TOF investigations of PHMG are performed with a variety of matrices, and it is found that CHCA and 2,5-DHB are excellent matrices which well reflects the polymer population even at high mass. For the provided PHMG sample, the number-average (M_n) and weight-average (M_w) molecular masses were determined to be 744.8 and 810.7, respectively, when the CHCA was used as a matrix. The rank of the matrices in terms of averaged molecular weight was CHCA ~2,5-DHB > 5-NSA > DHAP, THAP > ATT > IAA ~ super-DHB ~ HABA. In addition, PSD of the PHMG oligomer ions exhibited a few unique fragmenation characteristics. The formation of *a*- and *c*-type fragments was the major fragmentation pathway, and the 25-Da loss peaks generally accompanied *a*- and *c*-type fragments.

Key Words : MALDI-TOF, Matrix, Polyhexamethylene guanidine (PHMG), Polymer, Mass spectrometry

Introduction

Electrospray ionization (ESI)[1-3] and matrix-assisted laser desorption/ionization (MALDI)[4,5] mass spectrometry (MS) have opened a route for analysis of non-volatile organic molecules, including biopolymers and synthetic polymers. In particular, MALDI-MS offers an easy, robust, versatile, sensitive, and tolerant sample introduction tool for analysis of peptides,[6,7] proteins,[8,9] oligonucleotides,[10] DNA,[11] polymers,[12-14] small molecules,[15,16] and so on. With the goal of optimizing MALDI-MS analysis, extensive efforts have been made to investigate matrix effect,[17,18] matrix additives,[19] sample-matrix preparation methods,[20-22] and its mechanism.[23-26]

MALDI-TOF (time-of-flight) MS has become a standard analysis tool for the structural analysis of synthetic polymers. MALDI-TOF MS analysis of synthetic polymers readily provides polymer characteristic information such as the average molar mass (M_W), molar mass distribution (MMD), repeat unit structure, copolymer sequence, and the end-group.[12] Since MALDI mass spectra of synthetic polymers are sensitive to a variety of factors such as the matrix, mixing ratios of matrix/polymer/cationization agent, and the sample preparation method, careful selection of such experimental factors is needed. For example, the matrices commonly used in the analysis of polymers are a little different from those used for peptides, and the optimal matrices for specific polymers are well documented.[27,28]

In 2011, a large number of casualties related to pulmonary disease similar to acute interstitial pneumonia including at least four adult deaths and affecting a total of 28 patients were announced by the Korea Center for the Disease Control and Prevention.[29,30] An epidemiological toxicology investigation using rats led to the conclusion that the cause of this lung injury was from the long-term exposure to active ingredients (AI) of commercial disinfectants used in humidifiers.[31] The chemical disinfectants were put in the water-tanks of humidifiers for the prevention of microbial, mold, and algal growth.[30] The patients likely inhaled droplets containing the AIs of the disinfectants, which were sprayed into the air by the household humidifiers. The AIs of the chemical disinfectants used in the household humidifiers were identified as polyhexamethylene guanidine (PHMG: CAS RN 89697-78-9) and oligo (2-(2-ethoxy)ethoxyethyl guanidinium chloride.[30,31] These macromolecules have been reported to have high antibacterial activity, and are widely used as disinfectants and biocides in water systems, topical wounds, and the environment.[32-35] Their biocidal effects are due to the ability to disrupt the cell membrane.[32,36] Interestingly, these materials were reported to present low toxicity to humans,[37] but as described above, when these molecules are inhaled, they seem to cause harm. In general, hazardous chemicals that could potentially cause damage upon inhalation are required by the Korean law for hazardous chemicals management to undergo acute inhalation toxicity tests. However, the modified use of PHMG and related materials as a disinfectant for humidifiers was uncharted territory with regard to regulation and enforcement. The manufacturers sold the disinfectants without the submission of any data on

inhalation toxicity or risk evaluation.[30]

It is therefore necessary to develop an optimal analysis tool that enables detection and analysis of the AIs in commercial disinfectants. The AIs in humidifier disinfectants are known to be of polymeric nature with a hexamethylene guanidine repeat unit. As described above, MALDI-TOF MS is a robust and sensitive tool for detection and analysis of synthetic polymers. Thus we have attempted to examine a variety of matrices with the aim of determining the optimum matrices for simple detection and analysis of these hazardous chemical disinfectant AIs in commercial products. Furthermore, to confirm the structure of PHMG oligomers, post-source decay (PSD) was carried out for isolated PHMG ions.

Experimental Details

Materials. A PHMG sample was obtained from an anonymous Korean company who sold a commercial humidifier disinfectant. The humidifier disinfectant was recalled by the company and is now no longer available on the market. However, the polymer synthesis procedure and any information with respect to the degree of polymerization were not provided by the humidifier disinfectant manufacturer. For MALDI-TOF MS analysis, the following matrices were used: 2,5-dihydroxybenzoic acid (DHB, Aldrich), α-cyano-hydroxycinnamic acid (CHCA, Aldrich), 5-nitrosalicylic acid (NSA, Fluka), 2,4,6-trihydroxyacetophenone (THAP, Fluka), super-DHB (Fluka),[38,39] 6-*aza*-2-thiothymine (ATT, Fluka), 2,4-dihydroxyacetophenone (DHAP, Fluka), 1,5-diaminonapthalene (DAN, Aldrich), sinpinic acid (SA, Fluka), 2-(4-hydroxyphenylazo)-benzoic acid (HABA, Fluka), *trans*-3-indoleacrylic acid (IAA, Sigma), 5-aminosalicylic acid (ASA, Sigma), 5-formylsalicylic acid (FSA, Aldrich) and 1,8-dihydroxyanthracen-9(10*H*)-one (dithranol, Aldrich). All the solvents used in the present study were of HPLC grade (Fluka).

Sample Preparation. The solid PHMG sample was dissolved in distilled water at a concentration of 0.5 mg/mL. The PHMG sample was purified and concentrated by solid phase extraction using a spin-column (C-18, Ultra-Micro SpinColumn, Harvard Apparatus, Holliston, MA, USA). This procedure was crucial for getting good signal intensity on the MALDI-TOF spectrum. The spin-column was pre-wetted and equilibrated by eluting with 150 μL of Buffer B (50% CH_3CN/50% H_2O/0.1% trifluoroacetic acid) at 5,000 rpm and 150 μL of Buffer A (98% H_2O/2% CH_3CN/0.1% trifluoroacetic acid) at 5,000 rpm three times each. The PHMG sample solution (150 μL) was eluted for 15 min through the spin-column at 5,000 rpm. The spin-column loaded with the PHMG sample was then washed with Buffer A (150 μL). Finally, the PHMG sample loaded within the spin-column was eluted using Buffer B (150 μL) solution. The eluted solution was evaporated to dryness using a vacuum centrifuge. The dried-down PHMG samples were resuspended in 10 μL of the saturated matrix solution (in methanol). A 1 μL of the sample-matrix mixture was spotted onto the sample plate.

Mass Spectrometry. MALDI-TOF mass spectra were acquired using a Bruker Autoflex Speed Series mass spectrometer (Bruker Daltonics, Leipzig, Germany) equipped with a 355 nm Nd:YAG laser. Mass spectra were acquired at 500 Hz in positive reflection mode and averaged over 1,000 laser shots. PSD fragmentation of the precursor ions of interest was carried out using the LIFT technique of the Bruker MALDI-TOF mass spectrometer.[40] Precursor ions were isolated with a 10 Da isolation widow. MALDI-TOF spectra and PSD spectra were obtained after careful calibration. MALDI-TOF calibration was done with the standard peptide calibration kit using a manufacturer-supplied instrument setting file.

Results and Discussion

Matrices. Figure 1 shows the MALDI-TOF mass spectra of PHMG oligomers obtained with a variety of different matrices; (a) CHCA, (b) 2,5-DHB, (c) 5-NSA, (d) THAP, (e) super-DHB, (f) ATT, and (g) DHAP. For each matrix, UV laser power was optimized in order to obtain a MALDI spectrum that showed a good intensity distribution, particularly in the high *m/z* region. In these mass spectra, a large number of peaks were observed in high abundance. In general, MALDI-TOF mass spectra for synthetic polymers show a molecular peak distribution with an identical mass difference between the peaks. However, the MALDI-TOF spectra in Figure 1 did not show such a simple distribution. The overall intensity distribution could not be described with a single polymer profile, and the *m/z* intervals between the neighboring peaks were irregular. These unusual peak distribution characteristics are due to the fact that PHMG oligomers can have a variety of *iso*-forms. PHMG polymers can take a number of different molecular structures, the so-called types A-G (see Scheme 1).[32] Three of them, *i.e.*, types A, B, and C, have a linear form, and types D and F-G are in a branched form and a cyclic form, respectively. The branched and cyclic structures are known to be generated by an intramolecular poly-condensation, and the contents of these forms can be controlled kinetically by reducing the molar quantity of hexamethylenediamine during the synthesis procedure.[32,37]

Based on these molecular types of PHMG oligomers, a large number of the mass peaks shown in Figure 1 could be assigned and annotated. In our MALDI spectra, only three linear types, *i.e.*, types A, B, and C, were observed. The absence of the branched or cyclic structures, *i.e.*, types D and F-G, indicates that the polymer condensation ceased at a relatively early stage of the polymer chain reactions. If the polymer condensation reactions would have proceeded further, more branched types would have formed and we would have gotten much more complicated MALDI spectra. The type A structure has one guanidine and one primary amine as end-groups, type B has two primary amine groups, and type C has two guanidine groups (see Scheme 1). In Figure 1, a series of type A, B, and C peaks are denoted with

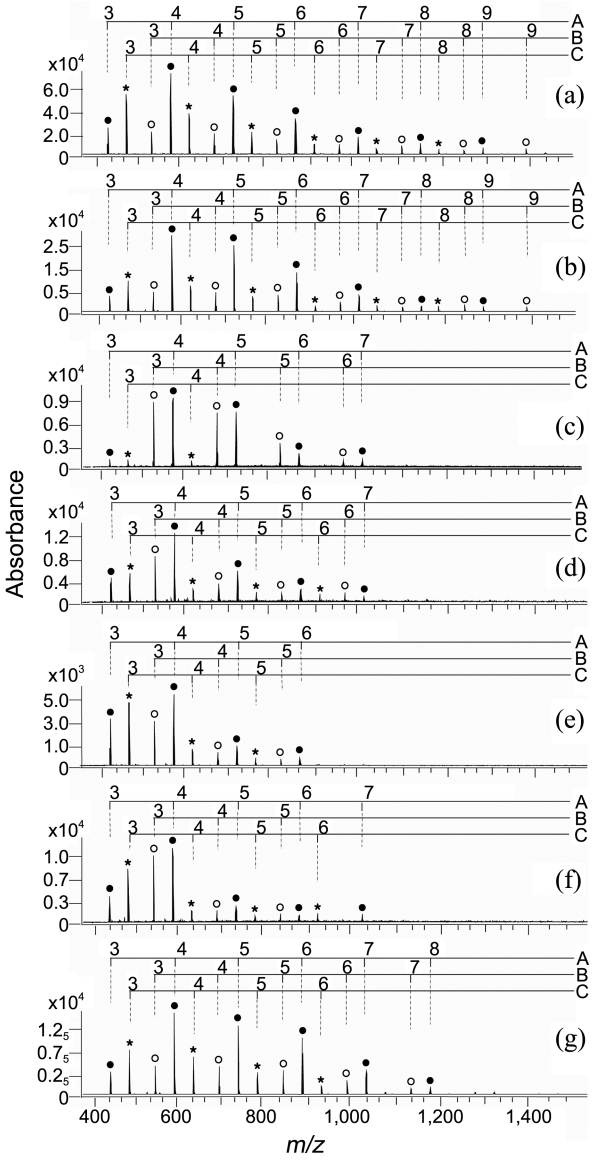


Figure 1. MALDI-TOF mass spectra of PHMG oligomers obtained with (a) CHCA, (b) 2,5-DHB, (c) 5-NSA, (d) THAP, (e) super-DHB, (f) 6-aza-2-thiothymine, and (g) DHAP matrices. Type A, B, and C peaks are denoted with filled (●) and empty (○) circles and an asterisk (∗), respectively.

a filled (●), empty (○) circle, and an asterisk (*), respectively.

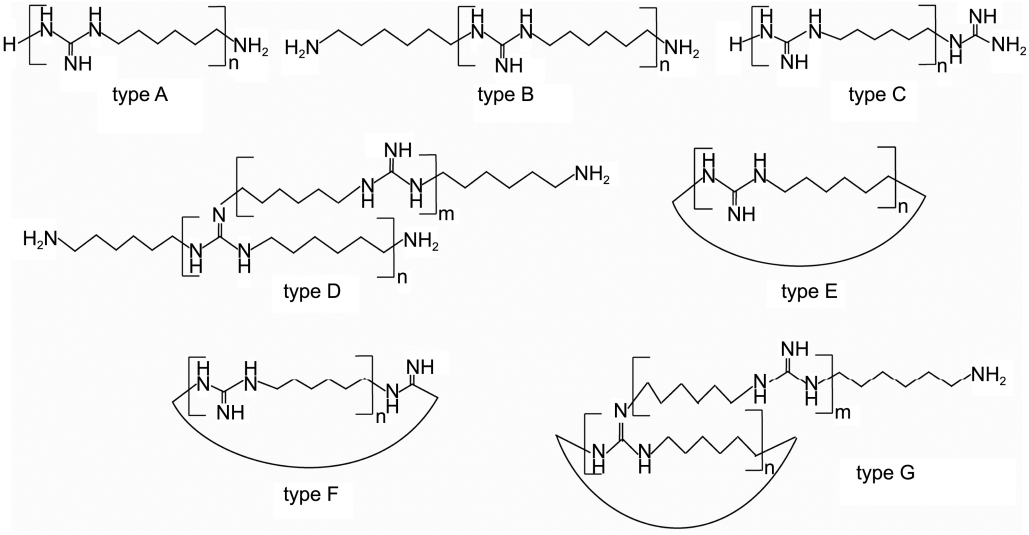
In Figure 1(a), the CHCA MALDI spectrum, a series of type A peaks (●) show a well-shaped intensity distribution in which $(A_4 + H)^+$ with four repeat units is most abundant and the abundance of the larger oligomers (A_n, $n \geq 5$) monotonically decreases. It is also notable that only the protonated form was observed and no alkali-adduct was found. The peaks belonging to the type A PHMG oligomer (●) showed a 141 Da interval between the neighboring peaks, confirming that these peaks represent a family of a certain polymer type. In the case of type B oligomers (○), the overall abundance was much lower than those of type A oligomers; only one third the abundance of type A oligomers. The type B oligomers also shared a 141 Da gap between the neighboring members of the type B oligomer family. Type B and C oligomers showed monotonically decreasing oligomer distributions, with the overall abundance of type C oligomers generally being higher than that of type B oligomers.

The other MALDI spectra in Figure 1(b)-(g) showed similarities to Figure 1(a) with regard to the abundance pattern in two respects. First, for the type A oligomers, the abundance was highest at $n = 4$ and monotonically decreased at $n \geq 5$. Second, the type B and C oligomers showed a monotonically-decreasing abundance pattern. On the other hand, they also showed discrepancies compared with Figure 1(a) in two respects. The relative abundance of the type A, B, and C oligomer peaks in Figure 1(b)-(g) was somewhat different from that in Figure 1(a). As an extreme case, in Figure 1(c), the absolute abundance of type C oligomers was quite low compared with that of the other MALDI spectra. Second, some spectra showed the oligomer peaks up to $n = 9$, *e.g.*, Figures 1(a) and (b), while other spectra did only up to below $n \leq 7$, *e.g.*, Figure 1(e). Among the large number of matrices used for Figure 1, CHCA and 2,5-DHB were excellent matrices in that they showed larger oligomers, *e.g.*, up to $n = 9$, while 5-NSA, THAP, super-DHB, and ATT failed to show these large oligomers. This observation is somewhat in agreement with the previous MALDI-TOF analysis for polyhexamethylene biguanide (PHMB) oligomers, in which only short oligomers could be observed with the ATT matrix.[41]

Matrices other than those used for Figure 1 were also examined, but the acquired MALDI spectra were not very informative with respect to the PHMG oligomer distribution. Figure 2 shows MALDI spectra obtained with (a) 1,5-DAN, (b) SA, (c) HABA, (d) IAA, and (e) dithranol. For these matrices, a good oligomer distribution could not be obtained. Instead, abundant noise peaks appeared in the low *m/z* region, particularly, *m/z* 460-530, and interfered with the oligomer peaks. These peaks were presumably from either matrix itself or fragments of PHMG oligomers. In addition, the peaks arising from oligomers were of low abundance and showed only a short series of oligomers ($3 \leq n \leq 5$).

In the selection of a matrix for MALDI analysis, homogeneous co-crystallization of sample and matrix is crucial. Heterogeneous sample preparation often forces one to search for the MALDI signal sweet spots or does not provide any signal at all. Due to the diverse chemical properties of synthetic polymers, it is often troublesome to find a suitable matrix for MALDI mass spectrometric analysis for polymers. Previously, Hanton and Owens provided some guidelines for matrix selection based on polymer and matrix solubility data.[42] They recommended matching the matrix polarity with the polymer under examination. In this respect, our MALDI-TOF results are consistent with their guidelines. The hydrophilic nature of PHMG allowed the hydrophilic matrices such as 2,5-DHB and CHCA to give excellent MALDI signals, whereas hydrophobic matrices such as IAA and dithranol provided poor results.[27]

PHMG Oligomer Characteristics. From the MALDI spectra shown in Figure 1, the number-average molecular



Scheme 1. Molecular structures of type A-G PHMG. (Scheme reproduced from Wei *et al*., 2009, with permission from Elsevier, copyright 2009).

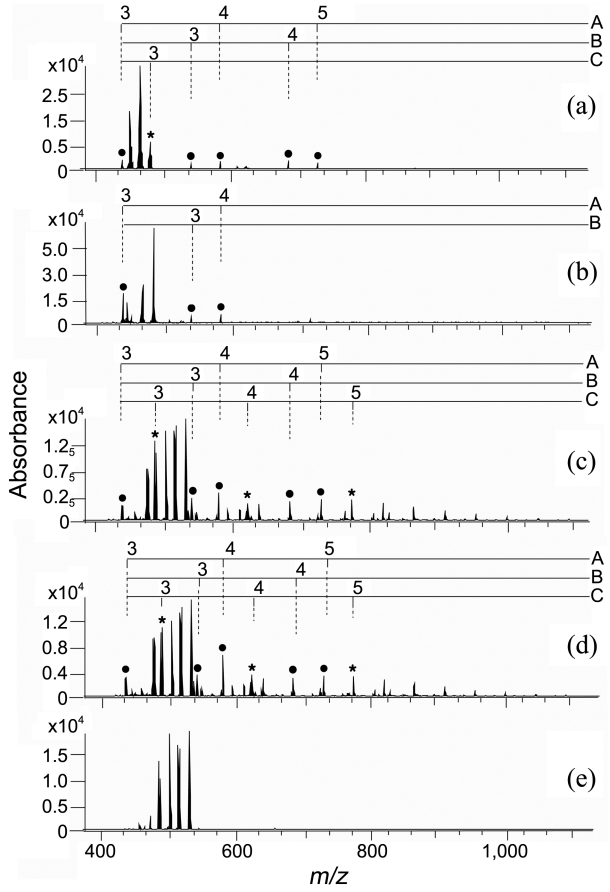


Figure 2. MALDI-TOF mass spectra of PHMG oligomers obtained with (a) 1,5-DAN, (b) SA, (c) HABA, (d) IAA, and (e) dithranol matrices. Type A, B, and C peaks are denoted with filled (●) and empty (○) circles and an asterisk (∗), respectively.

mass (M_n, Eq. (1)), the weight-average molecular mass (M_w, Eq. (2)), and the polydispersity (pd, M_w/M_n) were determined for each matrix using the software '*Polytools*™', and the results are summarized in Table 1.

Table 1. A summary of the number-average (M_n) and the weight-average (M_w) molecular masses, and the polydispersities, determined from the PHMG MALDI-TOF mass spectra shown in Figure 1

Matrix	M_n	M_w	pd
CHCA	744.8	810.7	1.089
2,5-DHB	736.0	781.9	1.062
5-NSA	672.7	686.6	1.021
DHAP	621.2	637.1	1.026
THAP	611.3	633.7	1.037
ATT	559.4	575.1	1.028
IAA	532.9	549.6	1.031
super-DHB	529.0	561.0	1.060
HABA	525.6	542.2	1.032

$$M_n = \frac{\sum_i M_i N_i}{\sum_i N_i} \quad (1)$$

$$M_w = \frac{\sum_i M_i^2 N_i}{\sum_i M_i N_i} \quad (2)$$

The number-average molecular weight values ranged from 525.6 to 744.8 and the weight-average molecular weight values were in the range of 542.2-810.7, depending on the matrix used for MALDI-TOF analysis. The average molecular weight values were rather low. This indicates that the PHMG sample obtained from an anonymous Korean company who used this sample for a commercial humidifier disinfectant consisted of a series of short PHMG oligomers.

Returning to the data in Table 1, in terms of M_n, the CHCA matrix exhibited weight values higher than the HABA matrix by 219.2 Da, which corresponds to approximately two

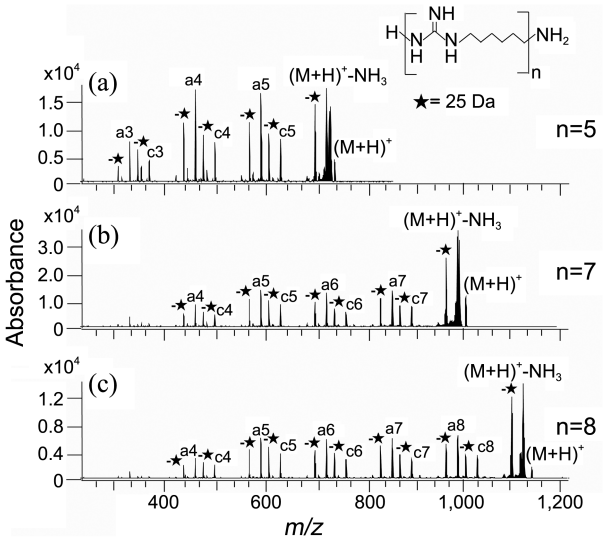
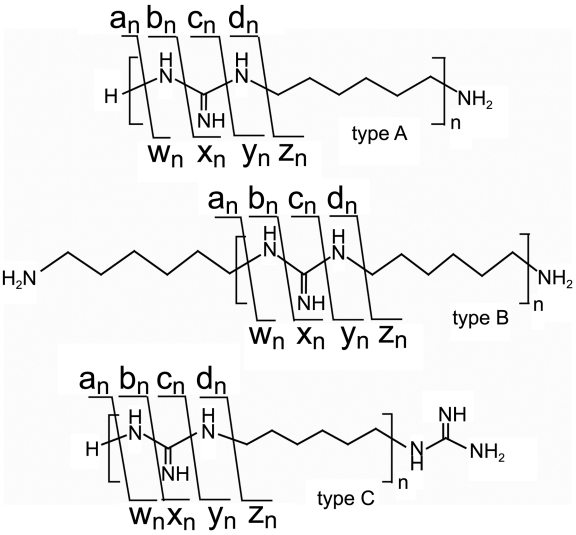


Figure 3. PSD mass spectra of type A PHMG polymers obtained by the LIFT technique: (a) n=5, (b) n=7, and (c) n=8. Note that the symbol −★ indicates the loss of a 25 Da neutral species.



Scheme 2. New nomenclature for the PHMG fragments introduced in the present study in order to identify the fragments in the PSD spectra shown in Figures 3-5.

monomer units (2 × 141 Da). From Table 1, it appears that CHCA and 2,5-DHB are the best matrices that well reflect the populations of the high mass polymers. The rank of the matrices in terms of averaged molecular weights given in Table 1 is in good agreement with that visually determined from Figure 1: CHCA ~2,5-DHB > 5-NSA > DHAP, THAP > ATT > IAA ~ super-DHB ~ HABA.

Post-source Decay (PSD) MS/MS. As described above, PHMG polymers can take a number of different molecular structures, *i.e.*, the types A-G. So, in order to clearly identify the PHMG polymers, it is necessary to understand the fragmentation pattern of the PHMG polymers since in some cases the measured *m/z* values of polymeric species themselves are not sufficient to confirm the identity of the observed polymers.[43,44] Post-source decay (PSD) was therefore carried

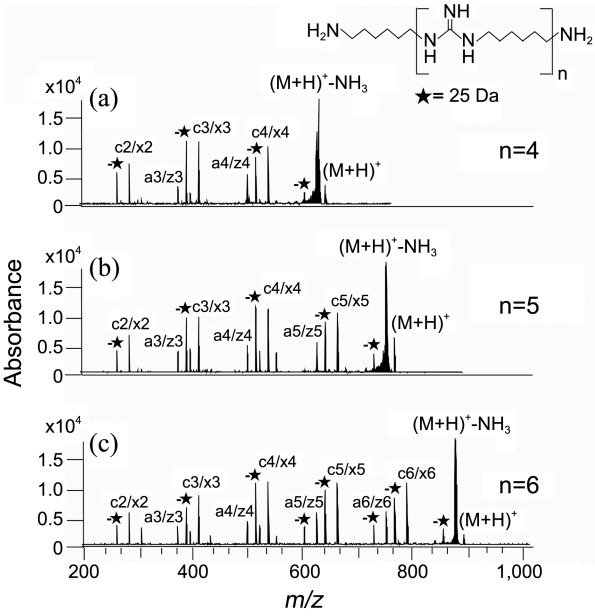


Figure 4. PSD mass spectra of type B PHMG polymers obtained by the LIFT technique: (a) n=4, (b) n=5 and (c) n=6. Note that the symbol −★ indicates the loss of a 25 Da neutral species.

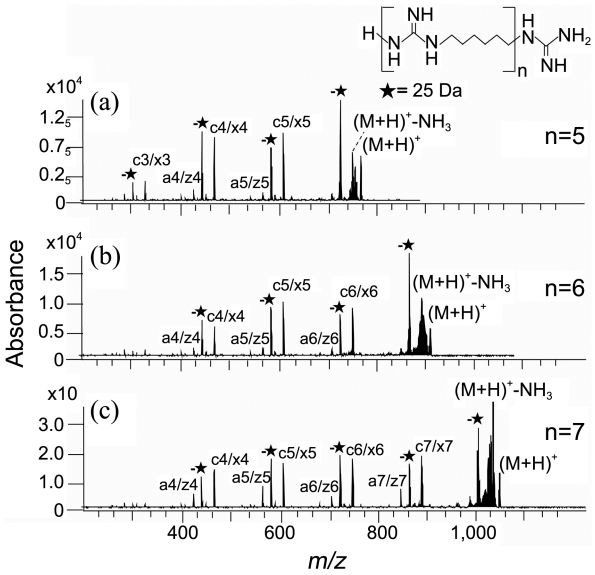


Figure 5. PSD mass spectra of type C PHMG polymers obtained by the LIFT technique: (a) n=5, (b) n=6 and (c) n=7. Note that the symbol −★ indicates the loss of a 25 Da neutral species.

out using the LIFT technique for some polymeric species shown in Figure 1(a).[40]

Figure 3 shows the resulting PSD spectra obtained for the type A polymer species of $(M_n+H)^+$, n = 5 (a), 7 (b), and 8 (c). In Figure 3, in order to facilitate a comparison between the PSD spectra, each spectrum is shown in the same *m/z* region. In addition, new nomenclature for the PHMG fragments was devised to identify the fragments and is shown in Scheme 2. Examination of the three PSD spectra in Figure 3 reveals three fragmentation characteristics. First, *a*- and *c*-type fragments were major fragments in all three spectra.

Specifically, in Figure 3(a) for $(M_n+H)^+$, n = 5, a_i and c_i, i = 3-5 were observed; n = 7(b), a_i and c_i, i = 4-7; n = 8, a_i and c_i, i = 4-8. Second, the 25-Da loss peaks denoted with –★ always accompanied the *a*- and *c*-type fragments. This 25-Da loss is speculated to be associated with the loss of CN (–26 Da) from the guanidine unit and the concomitant H atom transfer (+1 Da) to the detected fragment. Further detailed study seems to be needed to clearly understand the origin of this 25-Da loss. Third, somehow only the fragments with the left-side guanidine end group were observed and no fragment with the right-side primary amine end group was detected.

For the type B and C PHMG polymers, some of the fragmentation characteristics that were observed from the PSD spectrum of the type A polymers were also identified (see Figures 4 and 5).[45] For these types of PHMG, *a*- and *c*-type fragments were still major fragments, but the abundance of *a*-type fragments was always lower than that of the nearby *c*-type fragments. The 25-Da loss peaks also appeared for the type B and C polymers, but the neutral loss peaks arose only from the *c*-type fragments, not from the *a*-type fragments. These shared fragmentation features among the PHMG polymers are clearly good indicators for uniquely differentiating them from other polymers.

Conclusions

MALDI-TOF studies of a PHMG sample revealed that CHCA and 2,5-DHB, which are the two most widely used MALDI matrices, are also good matrices for the molecular mass analysis of PHMG samples. In particular, for the PHMG sample that was used as a commercial humidifier disinfectant by an anonymous Korean company, the number-average (M_n) and weight-average (M_w) molecular masses were determined to be 744.8 and 810.7, respectively, when its molecular mass distribution was determined using the CHCA matrix. In a previous report, a PHMG sample caused lung injury in rats when they were exposed to the long-term inhalation of sprayed PHMG polymers.[31] The rank of the matrices in terms of the averaged molecular weights was CHCA ~2,5-DHB > 5-NSA > DHAP, THAP > ATT > IAA ~ super-DHB ~ HABA. In addition, PSD of the PHMG oligomer ions exhibited a few unique fragmentation characteristics. The formation of *a*- and *c*-type fragments was the major fragmentation pathway, and the 25-Da loss peaks generally accompanied *a*- and *c*-type fragments. In future work, more detailed MS/MS studies, particularly, ESI-MS/MS experiments, will be executed in order to understand the origin of the ubiquitous 25 Da loss peaks. In addition, a methodology for quantitative MALDI-TOF analysis of the PHMG sample will be developed in the near future, which is expected to be very important in regulating the use of PHMG.

Acknowledgments. The Korea Ministry of Environment supported this work through "The Environment Health Action Program". HBO is grateful for the support of the Sogang University Research Grant 20110072.

References and Notes

1. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.
2. Kebarle, P.; Verkerk, U. H. *Mass Spectrom. Rev.* **2009**, *28*, 898.
3. Park, S. J.; Jo, K. B.; Oh, H. B. *Analyst* **2011**, *136*, 3739.
4. Karas, M.; Bachmann, D.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Process* **1987**, *78*, 53.
5. Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y.; Yoshida, T. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 151.
6. Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M. *Anal. Chem*. **1996**, *68*, 850.
7. Matsui, N. M.; Smith, D. M.; Clauser, K. R.; Fichmann, J.; Andrews, L. E.; Sullivan, C. M.; Burlingame, A. L.; Epstein, L. B. *Electrophoresis* **1997**, *18*, 409.
8. Mann, M.; Hendrickson, R. C.; Pandey, A. *Annu. Rev. Biochem.* **2001**, *70*, 437.
9. Reyzer, M. L.; Caprioli, R. M. *Current Opinion in Chemical Biology* **2007**, *11*, 29.
10. Bahr, U.; Aygün, H.; Karas, M. *Anal. Chem.* **2009**, *81*, 3173-3179. DOI: 10.1021/ac900100x
11. Kirpekar, F.; Nordhoff, E.; Larsen, L. K.; Kristiansen, K.; Roepstorff, P.; Hillenkamp, F. *Nucleic Acids Res.* **1998**, *26*, 2554.
12. Montaudo, G.; Samperi, F.; Montaudo, M. S. *Prog. Polym. Sci.* **2006**, *31*, 277.
13. So, H. R.; Lee, J. H.; Han, S. Y.; Oh, H. B. *J. Am. Soc. Mass Spectrum.* **2012**, *23*, 1821.
14. Jang, S.; Yang, E. K.; Jin, S. I.; Cho, Y. D.; Choe, E. K.; Park, C. R. *Bull. Korean Chem. Soc.* **2012**, *33*, 833.
15. Cohen, L. H.; Gusev, A. I. *Anal. Bioanal. Chem.* **2002**, *373*, 571.
16. Kim, J.; Han, S. P.; Kim, J. K.; Kim, Y. J. *Bull. Korean Chem. Soc.* **2011**, *32*, 915.
17. Chou, J. Z.; Kreek, M. J.; Chait, B. T. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 10.
18. Gusev, A. I.; Wilkinson, W. R.; Proctor, A.; Hercules, D. M. *Anal. Chem.* **1995**, *67*, 1034.
19. Kjellström, S.; Jensen, O. N. *Anal. Chem.* **2004**, *76*, 5109.
20. Karas, M.; Hillenkamp, F. *Anal. Chem*. **1988**, *60*, 2299.
21. Vorm, O.; Roepstorff, P.; Mann, M. *Anal. Chem*. **1994**, *66*, 3281.
22. Cohen, S. L.; Chait, B. T. *Anal. Chem.* **1996**, *68*, 31.
23. Karas, M.; Kruger, R. *Chem. Rev.* **2003**, *103*, 427.
24. Chang, W. C.; Huang, L. C. L.; Wang, Y. S.; Peng, W. P.; Chang, H. C.; Hsu, N. Y.; Yang, W. B.; Chen, C. H. *Anal. Chim. Acta* **2007**, *582*, 1.
25. Ehring, H.; Karas, M.; Hillenkamp, F. *Org. Mass Spectrom.* **1992**, *27*, 427.
26. Kim, S. H.; Park, S. H.; Song, J. Y.; Han, S. Y. *Mass Spectrom. Lett.* **2012**, *3*, 18.
27. Nielen, M. W. F. *Mass Spectrom. Rev.* **1999**, *18*, 309.
28. Guttman, C. M.; Wetzel, S. J.; Blair, W. R.; Fanconi, B. M.; Girard, J. E.; Goldschmidt, R. R.; Wallace, W. E.; VanderHart, D. L. *Anal. Chem.* **2001**, *73*, 1252.
29. Korea Center for Disease Control and Prevention. Interim report of epidemiological investigation on lung injury with unknown cause in Korea. Public Health Weekly Report KCDC **2011**, *4*, 817-832 (Korean).
30. Lee, J. H.; Kim, Y. H.; Kwon, J. H. *Environ. Sci. Technol.* **2012**, *46*, 2498.
31. Ministry of Health and Welfare Press release. http://english.mw.go.kr/front_eng/al/sal020vw.jsp?PAR_MENU_ID=1002&MENU_ID=100203&page=1&CONT_SEQ=260454&SEARCHKEY=&SEARCHVALUE=(accessed Feb 9, 2013).
32. Wei, D.; Ma, Q.; Guan, Y.; Hu, F.; Zheng, A.; Zhang, X.; Teng, Z.; Jiang, H. *Mater. Sci. Eng. C* **2009**, *29*, 1776. DOI: 10.1016/j.msec.2009.02.005

33. Kim, B. R.; Anderson, J. E.; Mueller, S. A.; Gaines, W. A.; Kendall, A. M. *Water Res.* **2002**, *36*, 4433.
34. Lee, W. R.; Tobias, K. M.; Bemis, D. A.; Rohrbach, B. W. *Vet. Surg*. **2004**, *33*, 404.
35. Woodcock, P. M. in Payne, K. R., Ed., *Industrial Biocides*; John Wiley & Sons: Chichester, 1988.
36. Altert, M.; Feiertag, P.; Hayn, G.; Saf, R.; Hönig, H. *Biomacromolecules* **2003**, *4*, 1811.
37. Feiertag, P.; Albert, M.; Ecker-Eckhofen, E. M.; Hayn, G.; Hönig, H.; Oberwalder, H. W.; Saf, R.; Schmidt, A.; Oskar, S.; Topchiev, D. *Macromol. Rapid Commun.* **2003**, *24*, 567.
38. Addition of 10% 2-hydroxy-5-methoxybenzoic acids to 2,5-DHB.
39. Karas, M.; Ehring, H.; Nordhoff, E.; Stahl, B.; Strupat, K.; Hillenkamp, F.; Grehl, M.; Krebs, B. *Org. Mass Spectrom.* **1993**, *28*, 1476.
40. Suckau, D.; Resemann, A.; Schuerenberg, M.; Hufnagel, P.; Franzen, J.; Holle, A. *Anal. Bioanal. Chem.* **2003**, *376*, 952.
41. O'Malley, L. P.; Hassan, K. Z.; Brittan, H.; Johnson, N.; Collins, A. N. *J. Appl. Polym. Sci.* **2006**, *102*, 4928.
42. Hanton, S. D.; Owens, K. G. *Proc. 46th ASMS Conf. Mass Spectrom. Allied Topics*, Orlando, FL, USA, **1998**, 1185.
43. Jackson, A. T.; Scrivens, J. H.; Williams, J. P.; Baker, E. S.; Gidden, J.; Bowers, M. T. *Int. J. Mass Spectrom.* **2004**, *238*, 287.
44. Han, S. Y.; Lee, S. Y.; Oh, H. B. *Bull. Korean Chem. Soc*. **2005**, *26*, 740.
45. In the case of type B and C PHMGs, due to the centro-symmetric nature of the polymer, the two notations are possible (refer to Scheme 2). For example, in Figure 4(a), *c4* and *a4* can also be assigned as *x4* and *z4*, respectively.