Analysis of Major Components Contained in Bis-GMA Monomer

Ayumi OHSAKI and Yohji IMAI

Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University, 2-3-10,
Kanda-Surugadai, Chiyoda-ku, Tokyo, 101-0062, Japan

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The major components contained in commercial Bis-GMA monomer were isolated by thin layer chromatography (TLC) and identified by NMR and high resolution mass spectroscopy. In addition to the two major components already known (Bis-GMA and Iso-bis-GMA), an unknown hydrophilic third major component was isolated and identified as 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dihydroxy-1-propoxy)]diphenylpropane. This compound was designated as BIS-GMA-H, because it has a structure of Bis-GMA with one of which methacrylic ester bond hydrolyzed.

Key words : Bis-GMA, HPLC, NMR

INTRODUCTION

Bisphenol A glycidyl methacrylate, or Bis-GMA, has been widely used in dental composites and sealants. However, the estrogenic effects of Bis-GMA-based resins have recently raised controversy and concern about the safety of the monomer leached out of these materials^{1–3)}. The leached components from resins have generally been analyzed by high performance liquid chromatography (HPLC). Although the chromatogram of commercially available Bis-GMA exhibits four major peaks and many minor peaks at different retention times as shown in Fig. 1, only one peak has usually been assigned to Bis-GMA monomer. It seems that two big peaks at the retention times of 17.3 min and 17.0 min in Fig. 1 have been combined and designated as Bis-GMA. Meanwhile, the third major peak with a retention time of 7.9 min was designated “Unknown I” in a recent paper²⁾. The “Unknown I” substance has been observed in all of six Bis-GMA-based sealants tested²⁾.

The third component seems to have drawn little attention up to the present time. However, it is very important from the viewpoint of leaching characteristics and safety issues, because it is supposed to be more hydrophilic, as judged from its shorter retention time on a reverse-phase ODS column, than the other three components and may therefore leach out more easily into an aqueous environment. While the structures of the two major components have already been determined⁴⁾, the structure of the third component remains undetermined. Thus the purpose of this investigation was to identify the third major component contained in the so-called Bis-GMA monomer.

Fig.1 Chromatogram of commercial Bis-GMA monomer. HPLC analysis was performed using a flow rate of 1 mL/min for an acetonitrile/water gradient programmed from 50/50% to 100% in 55 min.

MATERIALS AND METHODS

Materials

Bis-GMA used in this study was D-GMA, lot No.0601K from Shin-Nakamura Chemical Co., Wakayama, Japan.

HPLC analysis

HPLC analysis was performed using a JASCO system (GULLIVER 1500, JASCO, Tokyo, Japan), equipped with a CAPCELL PAK C_{18} column (UG120, 4.6 mm $\times$ 250 mm, Shiseido, Tokyo, Japan) and a diode array detector (MD-1510) (detection at $\lambda = 230$ nm) and at a flow rate of 1 mL/min at 40°C. Mixtures of acetonitrile (Cica-MERCK, HPLC grade, Kanto Chemical Co. Tokyo, Japan) and water were used as the solvent system, with a gradient starting at 50% acetonitrile and increasing to 100% within 55 min.

Isolation of major three components

To isolate the components, thin layer chromatography (TLC) (methanol/ chloroform: 5/95) was carried out on TLC plates coated with silica gel (0.25 or 0.5 mm) containing a fluorescent indicator (200 mm $\times$ 200 mm, 60 F₂₅₄, Kanto Chemical Co. Tokyo, Japan) and visualized under a UV lamp. Analytically, TLC plates were dipped in a solution of 20% phosphomolybdic acid in ethanol followed by heating. The third

component (3) obtained was purified further by isocratic HPLC on a Waters radial-pack cartridge (8NVC184 μ , 8 mm $\times$ 100 mm, Japan Waters, Tokyo, Japan) using a solvent (acetonitrile/water: 45/55) at a flow rate of 2 mL/min. The retention time of (3) was 3.4 min.

Analyses of the components isolated

^1H and ^{13}C NMR spectra were recorded in CDCl_3 containing 0.05% tetramethylsilane (TMS) on a spectrometer (JEOL, ALPHA 500, JEOL, Tokyo, Japan) operating at 500 MHz and 125 MHz, respectively. Chemical shifts were referenced internally to ^{13}C resonance in CDCl_3 (77.0 ppm) and to TMS for ^1H (0.00 ppm). ^1H resonance was assigned by a combination of one-dimensional and two-dimensional methods; ^1H - ^1H COSY (Correlation spectroscopy)^{5,6}, ^1H - ^{13}C COSY⁶, and HMBC (^1H -Detected Multiple-bond Heteronuclear Multiple Quantum Coherence Spectrum)⁷.

High resolution mass spectra (HRMS) were recorded on a double focusing high resolution mass spectrometer (JMS-AX505H, JEOL, Tokyo, Japan). Electron impact ionization (EI) was applied with an electron energy of 70 eV.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of the Bis-GMA monomer used. It exhibits four major peaks and many minor peaks at different retention times. The first major peak (1) was assigned to so-called Bis-GMA. The second peak (2) close to Bis-GMA was assigned to Iso-bis-GMA⁴, an isomer of Bis-GMA. The structures of the two Bis-GMAs were identified by NMR analysis as shown in Fig. 2. They agree with those reported in the literature⁴.

The third compound corresponding to the retention time of 7.9 min (3) in Fig. 1 was obtained as a colorless oil and its HRMS produced an m/z of 444.2135 M^+ , suggesting $\text{C}_{25}\text{H}_{32}\text{O}_7$ (formula mass, 444.2148).

Fig. 3 shows the NMR data obtained. The ^1H spectrum showed the presence of two kinds of tertiary methyl group at δ 1.96 ppm (3H) and δ 1.63 ppm (6H), and the presence of aromatic proton signals with ortho-coupling constant ($J=8.6$ Hz) at δ 6.81 ppm (4H) and δ 7.14 ppm (4H), and broad singlet signals of exomethylene protons at δ 5.61 ppm (1H) and δ 6.14 ppm (1H), respectively. All of the remaining protons were revealed to be attached to the oxygen-bearing carbon, since they were observed at δ 4.0-5.0 ppm. In the ^{13}C NMR spectrum, the chemical shifts were similar to those of Bis-GMA (1) except for three oxygenated carbon signals at δ 63.70 ppm (triplet), δ 69.22 ppm (triplet), and δ 70.35 ppm (doublet). Based on these data and the result of extensive NMR examinations (distortionless enhancement by polarization transfer⁵, ^1H - ^1H COSY, ^1H - ^{13}C COSY, and HMBC), the structure of the third component (3) was established as 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dihydroxy-1-propoxy)]diphenylpropane, designated as Bis-GMA-H, because it is a partially hydrolyzed Bis-GMA. The content of Bis-GMA-H was 5.0 wt% in the Bis-GMA used in the present study.

Fig. 2 Structures of Bis-GMAs (1-3)

Fig. 3 ^1H and ^{13}C NMR data of Bis-GMA-H (3)

Bis-GMA-H has a retention time very close to that of bisphenol A (BPA), which is a substance of recent controversy in Bis-GMA-based resins¹⁻³), under a certain HPLC condition as suggested in a recent paper²); "Unknown I" (Bis-GMA-H) and BPA were reported to have retention times of 2.32 and 2.05 min, respectively. Moreover, an unseparated peak for Bis-GMA-H and BPA was observed at the retention time of 4.0 min, for example, in the HPLC system used here when HPLC analysis was performed using a flow rate of 1 mL/min for an acetonitrile/water gradient programmed from 70/30% to 100% in 10 min. This suggests that the peak for Bis-GMA-H may possibly be misunderstood as that for BPA under a certain liquid chromatography

condition. The extremely high BPA contents of composite resins and a sealant reported by Olea and colleagues¹⁾ may probably be examples of this; regarding the curiosity of their HPLC data, one of the present authors has already commented that the data relevant to HPLC analysis presented by Olea *et al.* are not reliable and that these data should be corrected or withdrawn⁸⁾.

In view of the ease of leaching of Bis-GMA-H into an aqueous environment and its presence in all Bis-GMA-based resins⁹⁾, more attention should be paid to the third component contained in commercial Bis-GMA monomers in evaluating its biological effects. Moreover, in HPLC analysis, care should be taken to establish the condition necessary to clearly separate the peaks for BPA and Bis-GMA-H. Identification of the fourth major component remains undetermined, but is now in progress and the result will be reported in the near future.

CONCLUSION

The third major component contained in a commercial Bis-GMA monomer was isolated by TLC and identified by NMR and HRMS as 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dihydroxy-1-propoxy)]diphenylpropane (Bis-GMA-H).

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