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Original paper

Analysis of Major Components and Bisphenol A in Commercial Bis-GMA and Bis-GMA-based Resins Using High Performance Liquid Chromatography

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The purpose of this study was to examine the quality of commercially available Bis-GMA because concerns about Bis-GMA and Bis-GMA-based resins have been recently expressed in dentistry. Four major components and bisphenol A, which is a compound of recent controversy, were quantitatively analyzed using high performance liquid chromatography (HPLC) in three commercial Bis-GMA and six Bis-GMA-based composite resins. The contents of genuine Bis-GMA, Iso-bis-GMA, Bis-GMA-H, and Bis-GMA-M as well as the total content of the four monomers were 45.7-57.5%, 19.9-26.2%, 1.8-5.0%, 0.6-15.0% and 83.7-85.6% in the commercial Bis-GMAs, or 3.8-9.1%, 1.7-4.3%, 0.1-0.5%, 0.1-2.0% and 5.8-14.0% in composite resins, respectively. There were some differences in the composition of the major components between domestic and foreign materials. Bisphenol A contents in the unpolymerized composite resins were 1.5-10.2 μg/g resin.

Key words : Bis-GMA, Bisphenol A, Composite resin

INTRODUCTION

Bisphenol A diglycidyl methacrylate (Bis-GMA) has been widely used in dental composites and sealants without characterizing its components even though commercially available Bis-GMA is a mixture of various compounds. However, with the recent concerns and controversy about Bis-GMA and Bis-GMA-based resins in dentistry[1−3], it is important to examine the quality of commercial Bis-GMA.

We previously demonstrated[4,5] that one selected commercial Bis-GMA was composed of many minor components and four major components, and whose chemical structures are presented in Fig. 1. In the present study we analyzed the major components contained in three commercial Bis-GMA and six Bis-GMA-based composite resins using high performance liquid chromatography (HPLC). In addition to the major components, the quantity of bisphenol A (BPA), which is possibly present as an impurity in commercial Bis-GMA, was also determined because BPA is a potentially estrogenic compound of recent concern and its release from Bis-GMA-based resins is controversial in dentistry[1,2,6−10].

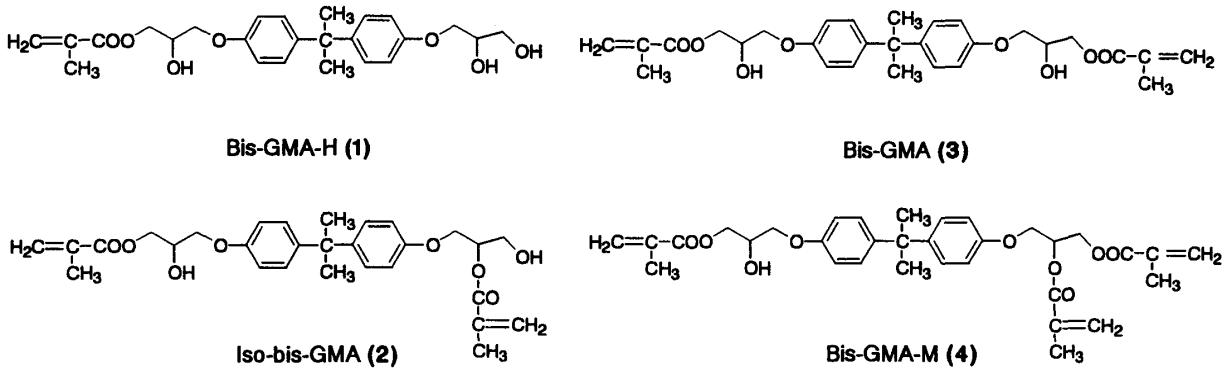

Fig. 1 Chemical structures of four major components contained in commercial Bis-GMA.
Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)phenyl]propane.
Iso-bis-GMA: 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(3-hydroxy-2-methacryloyloxy-1-propoxy)]diphenylpropane, isomer of Bis-GMA.
Bis-GMA-H: 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dihydroxy-1-propoxy)] diphenylpropane with a partially hydrolyzed Bis-GMA structure.
Bis-GMA-M: 2,2-[4-(2-hydroxy-3-methacryloyloxy-1-propoxy)-4'-(2,3-dimethacryloyloxy-1-propoxy)]diphenylpropane with a methacrylated Bis-GMA structure.

MATERIALS AND METHODS

Materials

Commercial Bis-GMA was obtained from Shin-Nakamura Chemical Co. (D-GMA, Wakayama, Japan, Lot No. 061K), Polysciences, Inc. (Warrington, PA, USA, Lot No.456653), and Freeman Chemical Co. (Port Washington, WI, USA, Lot No.819-045-9). Bisphenol A diglycidyl ether (BPDGE)(product of Shell Co.; Lot No.unknown) was obtained through Shin-Nakamura Chemical Co. Triethylene glycol dimethacrylate (TEGDMA, Lot. No.GG01) and bisphenol A (BPA, Lot No.GG01) were obtained from Tokyo Kasei, Tokyo, Japan.

The composite resins used were: Clearfil AP-X (Lot No.0048) and PhotoClearfil A (Lot No.000001) from Kuraray Co., Kurashiki, Japan, Palfique Estelite (Tokuyama Co., Tokuyama, Japan, Lot No.2B-986), Z100 (3M, St. Paul, MN, USA, Lot No.2AH), Tetric (Vivadent, Schaan, Liechtenstein, Lot No.615849) and Charisma (Kulzer, Wehrheim, Germany, Lot No.77).

HPLC analysis

Reversed-phase HPLC analysis was performed using a JASCO system (GULLIVER 1500, JASCO, Tokyo, Japan), equipped with a CAPCELL PAK C18 column (UG120, 4.6 mm×250 mm, Shiseido, Tokyo, Japan), a diode array detector (MD-1510) (detection at $\lambda = 230$ nm), and a fluorescence detector (FP-1520S, at excitation wavelength 275 nm and emission wavelength 300 nm). The fluorescence detector was used only for the analysis of BPA.

Each solution for the analysis was prepared by dissolving 20 mg of commercial Bis-GMAs and BPDGE or 100 mg of composite resins in 10 ml acetonitrile followed by

filtration through a Millipore filter of 0.45μm pore size. Ten μl of the sample solution was injected and analyzed at 40℃ with a flow rate of 1.0 ml/min of an acetonitrile/water gradient programmed from 50/50% to 100% for 55 min, followed by 100% acetonitrile for 5 min.

Standard acetonitrile solutions of Bis-GMA, Iso-bis-GMA, Bis-GMA-H and Bis-GMA-M, each of which was isolated from a commercial Bis-GMA of Shin-Nakamura Chemical Co. according to the procedure reported previously[4,5], at 0.1 mg/ml as well as those of BPA at 5-50 ng/ml concentrations were also analyzed under the same conditions described above for the quantitative analyses. The detection limits of the standard four Bis-GMAs detected by UV and BPA detected by fluorescence were 50-100 ng/ml (0.5-1.0 ng) and 3 ng/ml (30 pg), respectively.

The concentration of each component was calculated by integration of the component absorption peaks, using the standard solutions as references. Analysis was performed in duplicate and the means are reported. The content of each component was expressed as the percentage of each component contained in commercial Bis-GMA and composite resin pastes in mass%.

RESULTS AND DISCUSSION

Fig. 2 shows a typical chromatogram of two commercial Bis-GMAs and two commercial Bis-GMA-based composite resins. Figs. 2a and 2b show five and four major peaks and Figs. 2c and 2d show six and five peaks, respectively. The major peaks at the retention times of 7.9 min (1), 17.0 min (2), 17.3 min (3), and 30.1 min (4) were

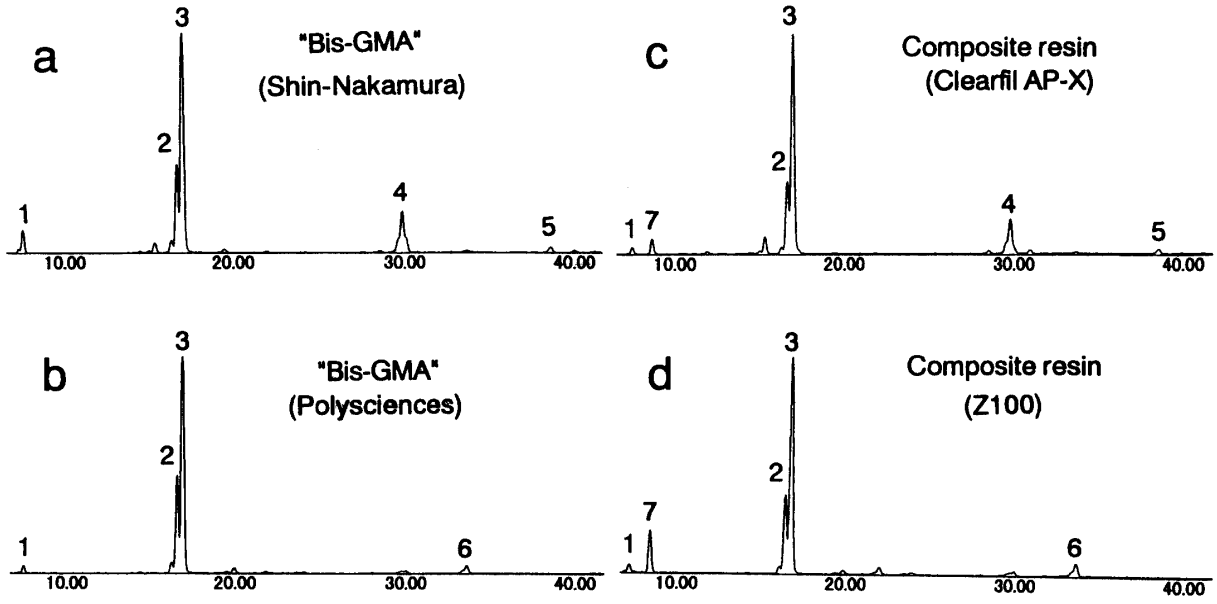

Fig. 2 Typical chromatograms obtained by HPLC for commercial Bis-GMAs and composite resins.
1, Bis-GMA-H; 2, Iso-bis-GMA; 3, Bis-GMA; 4, Bis-GMA-M; 5, 6, unknown; 7, triethylene glycol dimethacrylate

assigned to Bis-GMA-H, Iso-bis-GMA, Bis-GMA, and Bis-GMA-M, respectively. A peak with a retention time of 9.0 min (7) in the composite resins was assigned to triethylene glycol dimethacrylate (TEGDMA). Two peaks with retention times of 34.0 min (6) and 38.9 min (5) remained undesignated.

Three peaks occurred commonly at retention times of 7.9, 17.0 and 17.3 min in all nine materials analyzed. In addition to these three peaks, two major peaks at 30.1 (4) and 38.9 min (5) and one major peak at 34.0 min (6) were observed in four domestic and five foreign materials, respectively. These findings demonstrate that the quality of commercial Bis-GMA is different between the manufacturers.

Regarding minor peaks, approximately 30-35 peaks were detected for the three commercial Bis-GMA by injecting 20μg Bis-GMA to the column and by setting a peak detection limit above 500μAU (absorbance unit). Most of the minor peaks appeared to originate from a raw material used for the synthesis of commercial Bis-GMA because commercially available bisphenol A diglycidyl ether (BPDGE) used as a starting material for the synthesis is not a pure compound but contains many impurities. A total of 27 peaks were detected in addition to one major peak at the retention time of 15.7 min assigned to BPDGE in a commercial BPDGE under conditions similar to the analysis of commercial Bis-GMA described above.

Therefore, it is likely that minor components will further increase by the reaction of commercial BPDGE and methacrylic acid, which is a typical preparative method of Bis-GMA. Considering the four identified components shown in Fig. 1 and the presence of the unreacted BPDGE, the following methacrylates will possibly be included as minor components: one trihydroxy monomethacrylate (isomer of Bis-GMA-H), two epoxy monomethacrylates, one dimethacrylate (isomer of Iso-bis-GMA), one trimethacrylate (isomer of Bis-GMA-M) and tetramethacrylate.

Table 1 shows the findings of the analysis of commercial Bis-GMA. The contents of genuine Bis-GMA, Iso-bis-GMA, Bis-GMA-H and Bis-GMA-M as well as the total content of the four monomers were 45.7-57.5%, 19.9-26.2%, 1.8-5.0%, 0.6-15.0%, and 83.7-85.6%, respectively. Although the composition of the four monomers identified in the commercial Bis-GMA was different between the products, the total content for the four monomers showed a very similar value of approximately 85%.

To characterize the commercial Bis-GMAs further, the contents ratios of each of the three monomers to Bis-GMA were calculated because the ratios will give some additional information on the preparation of Bis-GMA. They were 0.44-0.48 for Iso-bis-

Table 1 Content of major components (in mass%) and bisphenol A (in μg/g) in commercial Bis-GMA

	Bis-GMA-H(Ⅰ)		Iso-bis-GMA(Ⅱ)		Bis-GMA(Ⅲ)		Bis-GMA-M(Ⅳ)		Ⅰ+Ⅱ+Ⅲ+Ⅳ	BPA
Manufacturer	Ⅰ(%)	Ⅰ/Ⅲ*	Ⅱ(%)	Ⅱ/Ⅲ*	Ⅲ(%)	Ⅲ/Ⅲ	Ⅳ(%)	Ⅳ/Ⅲ*	(%)	(μg/g)
Shin-Nakamura	5.0	0.11	19.9	0.44	45.7	1.00	15.0	0.33	85.6	130.0
Polysciences	1.8	0.03	26.2	0.48	54.6	1.00	1.1	0.02	83.7	43.4
Freeman	1.9	0.03	25.1	0.44	57.5	1.00	0.6	0.01	85.1	42.6

*Content ratio of each compound to Bis-GMA (Ⅲ)

Table 2 Content of major components (in mass%) and bisphenol A (in μg/g) in composite resins

Composite resin	Bis-GMA-H(Ⅰ)		Iso-bis-GMA(Ⅱ)		Bis-GMA(Ⅲ)		Bis-GMA-M(Ⅳ)		Ⅰ+Ⅱ+Ⅲ+Ⅳ	BPA
	Ⅰ(%)	Ⅰ/Ⅲ*	Ⅱ(%)	Ⅱ/Ⅲ*	Ⅲ(%)	Ⅲ/Ⅲ	Ⅳ(%)	Ⅳ/Ⅲ*	(%)	(μg/g)
Photo Clearfil A	0.2	0.03	2.4	0.38	6.2	1.0	1.7	0.27	10.5	3.5
Palfique	0.3	0.04	2.7	0.38	6.6	1.0	2.0	0.30	11.6	8.6
Estelite	0.3	0.05	2.3	0.41	5.6	1.0	1.6	0.29	9.8	8.6
Z100	0.2	0.05	1.7	0.45	3.8	1.0	0.1	0.03	5.8	2.3
Tetric	0.1	0.03	1.9	0.44	4.3	1.0	0.1	0.02	6.4	1.5
Charisma	0.5	0.05	4.3	0.47	9.1	1.0	0.1	0.01	14.0	10.2

*Content ratio of each compound to Bis-GMA (Ⅲ)

Bis-GMA, 0.03-0.11 for Bis-GMA-H and 0.01-0.33 for Bis-GMA-M. This suggests that very similar addition reactions of methacrylic acid to BPDGE occurred in the synthesis of the three commercial Bis-GMAs because the ratio for Iso-bis-GMA was similar. However, the other two ratios were approximately 3- to 33-fold different between the three commercial Bis-GMA. Normal and abnormal additions of methacrylic acid to the epoxy groups attached to BPDGE are suggested to occur at approximately 1 : 0.45.

Table 2 shows the findings of the analysis of the composite resins. The contents of Bis-GMA, Iso-bis-GMA, Bis-GMA-H and Bis-GMA-M as well as the total content of the four monomers were 3.8-9.1%, 1.7-4.3%, 0.1-0.5%, 0.1-2.0% and 5.8-14.0%, respectively. The contents ratios of each monomer to Bis-GMA were 0.38-0.47 for Iso-bis-GMA, 0.03-0.05 for Bis-GMA-H and 0.01-0.30 for Bis-GMA-M. The ratios for Iso-bis-GMA and Bis-GMA-M in the resins were in a similar range as those observed in the three commercial Bis-GMAs. However, the maximum value of the ratio for Bis-GMA-H decreased from 0.11 in the commercial Bis-GMAs to 0.05 in the resins. Two possible explanations for this are: (1) the lot of commercial Bis-GMA analyzed in the present study contained more Bis-GMA-H by chance than usual; (2) Commercial Bis-GMA supplied by the manufacturer was subjected to some extraction process before formulating the resin monomers by a dental resin manufacturer.

Marked differences in the monomer ratio for Bis-GMA-M were noticed among the commercial Bis-GMAs and the resins, especially between the domestic and foreign products: their values were 0.27-0.33 and 0.01-0.03, respectively. These differences will have some influences on the physicochemical properties of the resins such as viscosity, consistency, polymerizability, filler loading, water sorption, mechanical properties etc. The possibility that these properties change was suggested by a recent study[11] reporting the synthesis and properties of Bis-GMA-M.

The Bis-GMA-H contained in all the resins was roughly within a similar range and therefore will have little influence on the resin properties. However, Bis-GMA-H is relatively hydrophilic compared with the three other hydrophobic monomers. Therefore, unpolymerized residual Bis-GMA-H contained in cured resins is expected to leach more easily into aqueous media[14].

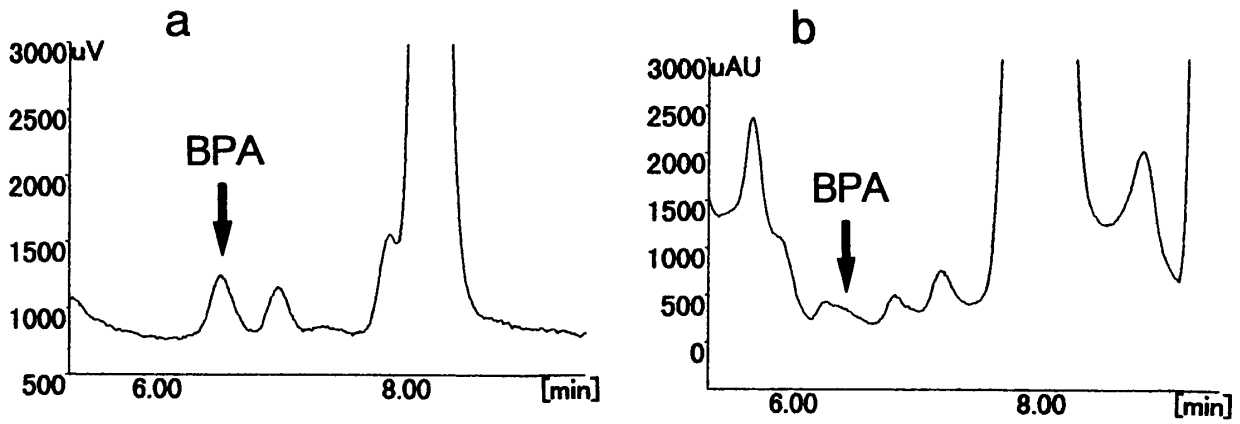

Fig. 3 Comparison of chromatograms in BPA area obtained by HPLC detected by the fluorescence (a) and UV (b) methods after injecting 10μl of a sample solution prepared by dissolving 100 mg of a composite resin (Z100) in 10 ml acetonitrile.

The BPA contents in the three commercial Bis-GMAs and six composite resins are presented in the first right column in Tables 1 and 2. They were 42.6-130.0μg/g and 1.5-10.2μg/g, respectively. The amounts of BPA found in the resins were approximately one tenth of commercial Bis-GMA. This is plausible because the commercial Bis-GMA content in the resins was estimated to be approximately 10%.

Trace amounts of BPA were determined successfully using the fluorescence detector. Fig. 3 shows comparisons of expanded HPLC chromatograms, focusing on the BPA region, of a composite resin paste (Z100) obtained using the fluorescence and the UV detectors. A peak at a retention time of 6.6 min in Fig. 3a was assigned to 0.27 ng of BPA, which corresponded to 2.3μg of BPA/g in the resin paste. Quantitative analysis of trace BPA was difficult using the UV detector, as is clear from the comparison between Figs. 3a and 3b.

BPA contents in unpolymerized dental resins have been reported previously: (1) 4.2 and 5.8μg/g in fissure sealants, determined by HPLC/fluorometry[12] and (2) 15.4 and 20.2μg/g in fissure sealants and 6.4μg/g in a composite resin, analyzed by gas chromatography/mass spectrometry (GC/MS)[13]. Thus, the BPA contents obtained in the present study appear within a similar range to those cited above.

When all the available data are taken together, it suggests that the content of BPA in commercial Bis-GMA-based dental resins is less than 20μg/g resin. Therefore, as discussed previously[14], the amount of BPA released from the cured resins is so minor that little or no long-term estrogenic effect on the body due to leached BPA will be expected.

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