HPLC analysis of dental resin composites components

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Abstract: Five uncured commercial dental resin composites (two bis-glycidyl methacylate based products and three non-bis-glycidyl methacylate based products) were examined for contamination with bisphenol A, which is a known xenobiotic. After the samples were processed with acetonitrile for extraction of their components, high performance liquid chromatography analysis was performed and the eluted peaks were fractionated for comparison using UV spectra. The results suggested that all the resin composites tested were contaminated with bisphenol A or its derivatives. Theoretically, bisphenol A is not a component of dental resin composite, but it could remain as an impurity of the composite during the synthesis of Bis-GMA. The results suggest that it is necessary to investigate the ability of this impurity and its derivatives in dental resin composites to cause estrogenic effects, as well as to evaluate the release of the impurity from cured resin composites. © 1999 John Wiley & Sons, Inc. J Biomed Mater Res, 47, 374–378, 1999.

Key words: dental resin composites; high performance liquid chromatography analysis; bis-glycidyl methacrylates; bisphenol A

INTRODUCTION

Phenol derivatives have been shown to have hormonal activity in human beings,[1,2] and there have been reports implicating environmental xenobiotics in various diseases such as human infertility and genital tract malformations.[3,4] Recently, concerns about estrogenic xenobiotics have increased. There are many chemicals that act as estrogenic xenobiotics: alkylphenolic compounds, bisphenol A (BPA), phthalates, polychlorinated biphenyls, and organochlorine pesticides. When human beings are directly exposed to BPA, estrogenic activity may occur.[5,6] BPA is used not only as a component of resin materials but also to form derivatives for industrial use. Epoxy resins and polycarbonates synthesized from BPA and other organic chemicals are known to be degraded by autoclaving and release BPA.[5,6] Plastics such as polycarbonates made from BPA might also contain residual BPA monomers that do not polymerize in the production process.

In dentistry, 2,2-bis[4-(2-hydroxy-3-methacrylyloxypropoxy)phenyl]-propane (Bis-GMA), which is synthesized from BPA and glycidyl methacrylates, is commonly used as a major component for restorative materials or fissure sealants because of its acceptable mechanical properties, chemical stability, and ability to simulate natural tooth color. One investigation indicated the presence of the residual BPA in some dental products that are based on Bis-GMA.[7] Furthermore, that study reported the release of the residual BPA, a xenobiotic, into the oral cavity from cured dental sealants.[7] After this report was published, several attempts to determine whether BPA is released from cured dental composites were carried out.[8–11] Almost all of them concluded that BPA is not released.[8–11] There appear to be several reasons for the lack of detection of BPA in these studies: the concentration of BPA was too low to detect, the detection method was not suitable, insufficient time was allowed for BPA release, or the tested products were not contaminated with residual BPA. Solving these problems is important to investigate the presence of residual BPA in the uncured materials. The purpose of the current study was to establish a method that was able to clearly detect residual BPA in uncured dental resin composites. Our method used high performance liquid chromatography (HPLC) and fractionation of components for molecular analysis.

MATERIALS AND METHODS

Selection of solution to extract monomers from dental resins

In the preliminary stage, a mixture of hydroxyethyl methacrylate (HEMA, Wako Pure Chemical Industries, Ltd., Ja-

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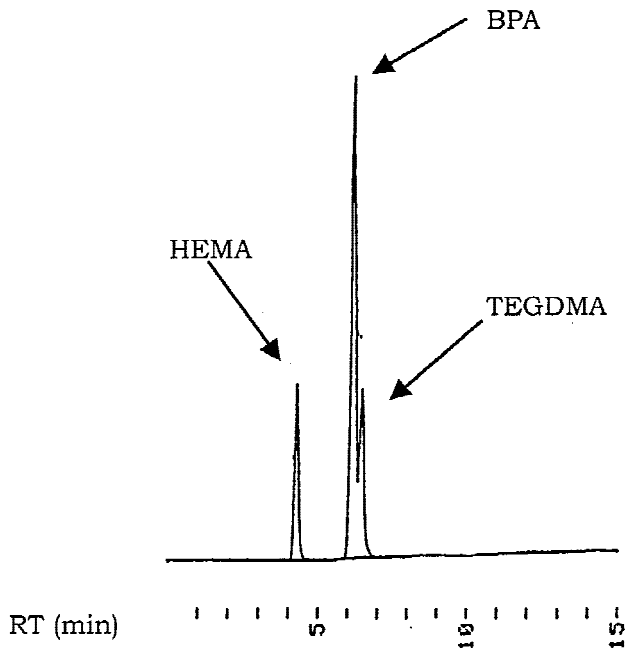

Figure 1. Chromatogram of monomer mixture using methanol solution as the mobile phase; BPA and TEGDMA were not separated under these conditions.

pan), BPA (Wako Pure Chemical Industries, Ltd., Japan), and triethyleneglycol dimethacrylate (TEGDMA, Wako), which were all at a concentration of 0.1 mg/mL in ethanol, was measured with HPLC equilibrated with 70% methanol as the mobile phase. As shown in Figure 1, this method did not separate BPA and TEGDMA. Thus, we selected acetonitrile as the solvent and the mobile phase because BPA, TEGDMA, and Bis-GMA are all hydrophobic. All chemicals in this study except for Bis-GMA were of analytical or reagent grade. Because Bis-GMA is not sold as a standardized reagent, it was provided by a dental manufacturer (Kuraray Co. Ltd., Tokyo). All procedures were performed in glass vessels to avoid contamination by plastics.

Analysis of commercial products

Five products of dental resin composites used in this study are listed in Table I. The products could be classified as Bis-GMA based and non-Bis-GMA based according to the manufacturers' claims. Five hundred milligrams of resin composite of each of the five materials was dissolved with 500 mL of acetonitrile using a laboratory vortex mixer and then centrifuged at 15,000 × *g* for 10 min at 4°C. Ten microliters of the supernatant was applied to the HPLC apparatus (Hitachi Co. Ltd., Japan) under the following conditions. The column used was a μ Bonda Pak C18 (7.8 × 300 mm, Waters Corp.). Samples were eluted at a flow rate of 2.0 mL/min for the first 5 min using a solvent linear gradient of 50% acetonitrile in water to 100% acetonitrile and then eluted at the same flow rate for 10 min with 100% acetonitrile. After that the flow rate was increased to 4.0 mL/min for 5 min with 100% acetonitrile to wash the column. Finally, the flow rate and the concentration of acetonitrile were gradually decreased over 5 min to 2.0 mL/min and 50% acetonitrile in water, respectively. The eluted monomers were detected by absorbance at 205 nm.

The amount of the component suspected BPA was quantified based upon the standard curve of BPA, and the detection level of BPA was determined to be 0.1 μg/mL of the solute extracted.

UV spectra of eluted peaks

We confirmed whether the monomers in the composite mixtures could be fractionated under the above conditions by comparing the UV spectra of the fractions and analyzing their molecular structure. The eluted peaks were collected by a fraction collector (SF-2120, Advantek Toyo Kaisha, Ltd., Japan), and the absorption spectrum was measured between 185 and 340 nm. Some samples, especially Bis-GMA and BPA, had peak absorptions that overlapped in the 200–230 nm range. This overlapping was caused by high concentrations of the samples, and they were therefore diluted with acetonitrile and remeasured.

RESULTS

Analysis of standard monomer solutions

Figure 2 shows the chromatogram and UV spectra of the monomer mixture of HEMA, TEGDMA, and BPA; Figure 3 shows the Bis-GMA profile. Relative retention times were 6.3 min for HEMA, 8.3 min for BPA, and 9.4 min for TEGDMA. Bis-GMA exhibited two major peaks at relative retention times of 10.4 and

TABLE I
Resin Composites

Code	Products	Composition	Manufacturer	Batch No.
A	CLEARFIL AP-X	Bis-GMA TEGDMA photoinitiator filler	Kuraray, Japan	0486
B	Restorative Z-100	Bis-GMA TEGDMA photoinitiator filler	3M, United States	5904B3
C	Lite-Fil	UDMA photoinitiator filler	Shofu, Japan	59622
D	ESTIO LC	UDMA Bis-MEPP photoinitiator filler	CG, Japan	941216A
E	PRODIGY		Kerr, United States	706254

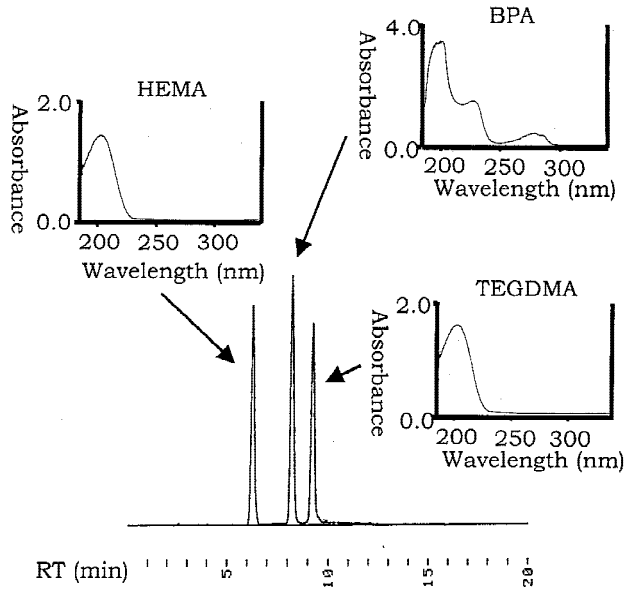

Figure 2. Chromatogram and UV spectra of monomer mixture; BPA and TEGDMA were separated using acetonitrile solution as the mobile phase.

11.7 min. There was also a minor peak at a relative retention time of 8.3 min, which was similar to the retention time of BPA.

Analysis of Bis-GMA based resin composites

Figure 4 shows the chromatogram and UV spectra of a Bis-GMA based commercial dental resin composite. There were three major peaks at 9.3, 10.4, and 11.7 min, which were similar to the retention times of TEGDMA and Bis-GMA. A minor peak at 8.3 min also existed, possibly indicating the presence of BPA or its derivatives. The major peaks at 9.3 and 10.4 min and the minor peak at 8.3 min were fractionated to examine the UV spectra. The UV spectra of the peaks at 9.3 and 10.4 min were identified as those of TEGDMA and Bis-GMA. The peak at 8.3 min absorbed wavelengths at 205, 228, and 278 nm. The pattern of the profile was similar to the UV spectra of BPA and Bis-GMA. Considering the results of the HPLC chromatogram, the peak at 8.3 min could be BPA or its derivatives.

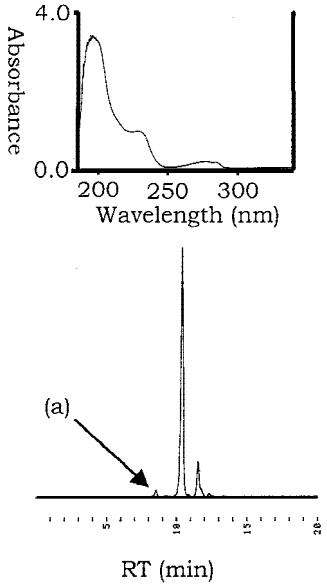

Figure 3. Chromatogram and UV spectra of Bis-GMA; the Bis-GMA used in dental products was analyzed by HPLC. There were two major peaks at 10.4 and 11.7 min. There was also a minor peak at 8.3 min similar to that found in BPA (point a).

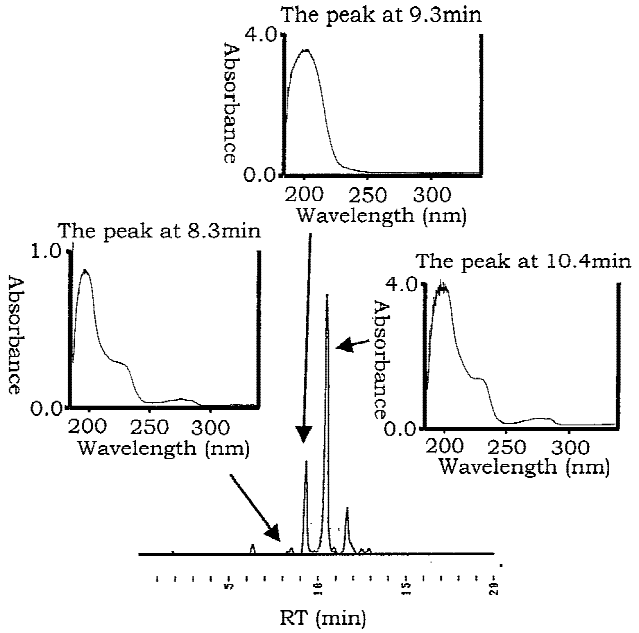

Figure 4. Chromatogram and UV spectra of a Bis-GMA based resin composite. There were three major peaks at 9.3, 10.4, and 11.6 min, which are similar to the retention times of TEGDMA and Bis-GMA. The profiles of UV spectra of the peaks at 9.3 and 10.4 min were identical to those of TEGDMA and Bis-GMA. The peak at 8.3 min was similar to the time of BPA, and the profile of UV spectra was also similar to the profile of BPA.

Analysis of various products

Figure 5 shows the chromatograms of Bis-GMA based products. Both products have BPA suspected peaks. Figure 6 shows the chromatograms of non-Bis-GMA products. The three non-Bis-GMA products also had peaks with retention times similar to that of Bis-GMA, and all three products exhibited smaller peaks eluting where BPA or its suspected derivatives were eluted. The amount of the component suspected to be BPA in the resin composites was estimated from the standard line as follows: A, 1.2 ng/mg raw resin; B, 2.2

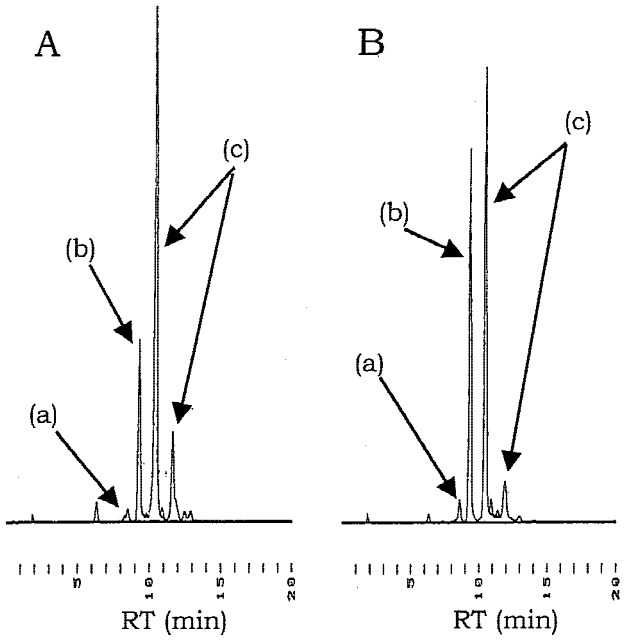

Figure 5. Chromatograms of Bis-GMA based resin composites. There were three major peaks similar to the retention times of TEGDMA (point b) and Bis-GMA (point c) in both products. Both products had peaks of suspected BPA (point a).

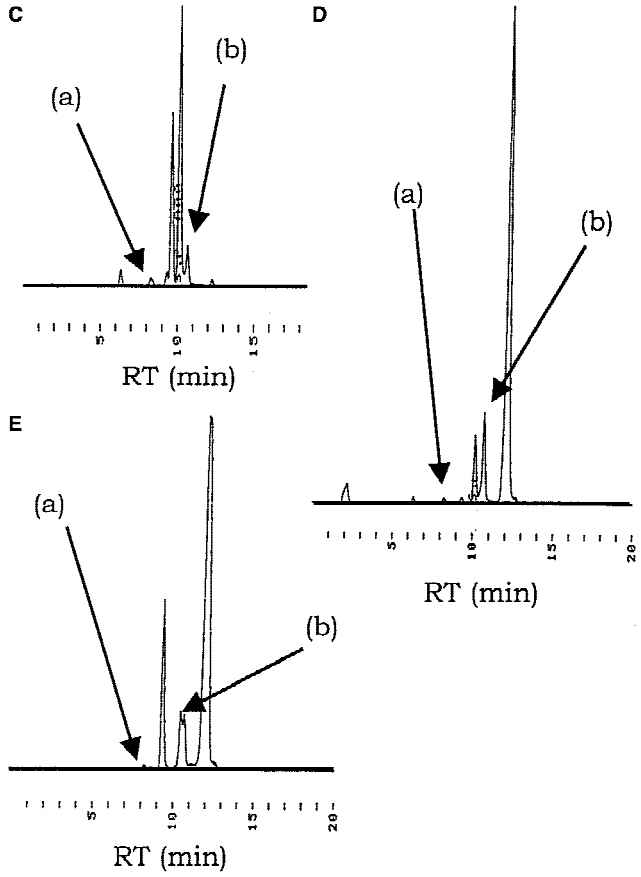

Figure 6. Chromatograms of non-Bis-GMA based resin composites. All three products contained a small amount of Bis-GMA (point b) and exhibited the suspected BPA peaks (point a).

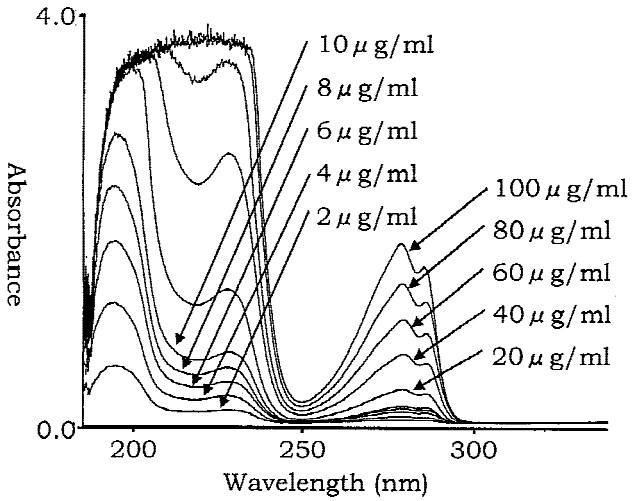

Figure 7. UV spectra of bisphenol A. Bisphenol A was dissolved in acetonitrile at various concentrations. It showed a concentration-dependent absorbance at 195, 228, 278 nm; but absorptions at 195 and 228 nm overlapped in concentrated samples.

ng/mg raw resin; C, 0.1 ng/mg raw resin; D, 0.1 ng/mg raw resin; and E, 0.3 ng/mg raw resin (Figs. 5 and 6).

DISCUSSION

Since Olea et al. reported the possibility of BPA being released from dental sealants,[7] concerns about xenobiotic activity of dental restorative materials and sealants have been raised.[8–13] Many researchers examined this release under various conditions and tried to detect BPA from cured materials. Nathanson et al. reported no release of BPA from cured dental sealants,[9,11] and Cherry et al. reported that BPA was not detected from the serum of dentists who had dental sealants applied to their own teeth.[12] Because BPA is not an original component of dental composites but a contaminant, the amount of residual BPA is probably small. Considering the oral environment, many investigators selected an aqueous solution for studying BPA release, even though BPA does not dissolve well due to its hydrophobic nature. Although selection of an aqueous solution is reasonable clinically, it might be insufficient to disclose the potential release of hydrophobic materials. Additionally, BPA and TEGDMA were not separated using an aqueous solution as the mobile phase in the HPLC analysis. For these reasons the use of an aqueous solution may inhibit the detection of BPA. Therefore, acetonitrile was used for extraction of BPA and the other components and the mobile phase in the HPLC analysis in this study. Furthermore, the samples were centrifuged at higher speeds than those in other reports, perhaps providing better separation.[6–9] The results in our

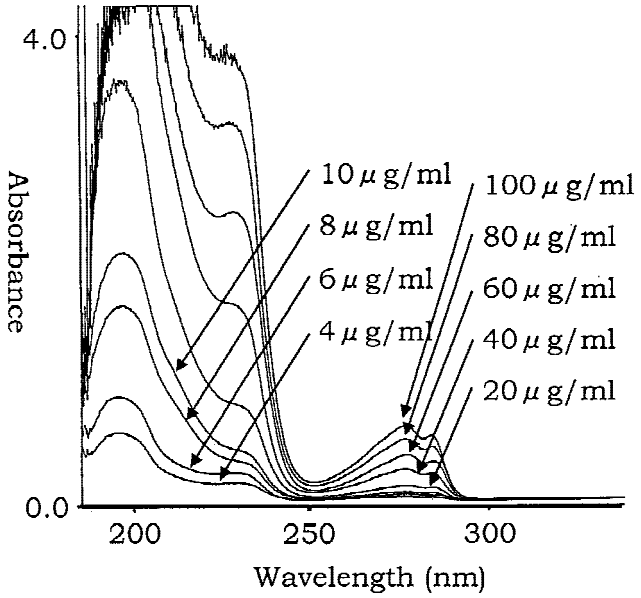

Figure 8. UV spectra of Bis-GMA. Bis-GMA was dissolved in acetonitrile at various concentrations. It also showed a concentration-dependent absorbance at 195, 228, and 278 nm; but absorptions at 195 and 228 nm overlapped in concentrated samples.

study suggested that residual BPA or its derivatives contaminated the resin composites. The BPA-suspected peak was fractionated using this method, which helped to analyze the peak in greater detail. We believe this method is beneficial and recommend it for the study of BPA release from cured materials. Nathanson et al.[9] found a peak similar to BPA in dental sealants and compared the UV spectra of the peak with that of a BPA standard solution. They concluded it was not BPA because the peak showed a different absorption profile at 230 nm when compared with the BPA standard solution. Figures 7 and 8 show the UV spectra of BPA and Bis-GMA solutions at various concentrations. The BPA and Bis-GMA both showed absorption at wavelengths of 195, 228, and 278 nm. But the absorptions of 195 and 228 nm were overlapped and were therefore indistinguishable in concentrated samples. Considering the results of the HPLC analysis, the peak might be BPA or a derivative.

We also investigated supposedly non-Bis-GMA based products (Fig. 6). The results, as expected, showed the major component of the products was not Bis-GMA, but these resins contained minor amounts of Bis-GMA and perhaps contamination from BPA or a derivative. Therefore, all the products examined in this study are suspected to contain BPA or a BPA derivative. Although the presence of BPA in dental resin composites does not directly mean it is released, there is still the possibility of BPA release due to the deterioration of the dental composites over time because BPA does not join the polymer network of the dental composites. Because there are unknown differences between *in vitro* and intraoral clinical conditions, further study about BPA released under various conditions, especially hydrophobic conditions, is needed.

In conclusion, the dental resin composites containing Bis-GMA may be contaminated with BPA or BPA derivatives, although the amount of contaminant appears to be low. Bis-GMA is an important component of dental resin composites because of its chemical and mechanical properties. In fact, there are few resin composites that do not contain Bis-GMA or its derivatives. Therefore, it is important to determine whether the residual impurities could be released from the cured materials over time under various conditions.

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