Determination of Residual Monomers in Dental Pit and Fissure Sealants Using Food/Oral Simulating Fluids

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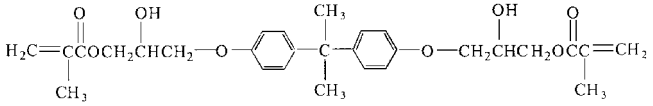
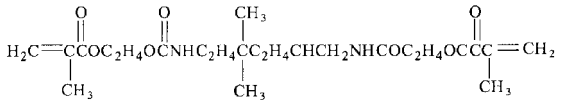
Specimens were cured by using a 1 mm (thickness) × 5 mm (diameter) teflon mold, and were immersed in artificial saliva and in 75% ethanol for 1, 7, 14, 21 and 28 days in order to quantify and to identify toxic components and to determine any degradation byproducts of Bis-GMA that might be released from five commercially available resin-based dental sealants. In artificial saliva, the only released component was triethylene glycol dimethacylate (TEGDMA). In 75% ethanol, TEGDMA, 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl]propane (Bis-GMA) and urethane dimethacrylate (UDMA) were released highly at the initial stage, indicating that the amount of component released is not linearly correlated with the immersion time. The amount of released TEGDMA was found to be much higher in 75% ethanol than in artificial saliva. Importantly, bisphenol-A (BPA) was detected from all the uncured sealants tested, suggesting that all the sealants tested contain BPA as a contaminant.

Introduction

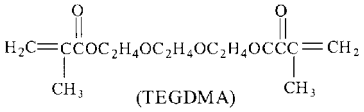
2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl] propane (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacylate (TEGDMA), benzoyl peroxide and methyl methacrylate are the major components of resin-based dental materials.[1] During light-curing of the resin-based materials, Bis-GMA, UDMA and TEGDMA have been suggested to form a three-dimensional network structure. [1a] However, unreacted monomers may be released and may enter human body via skin, oral and gastrointestinal mucosa, dentin and pulp.[2a]



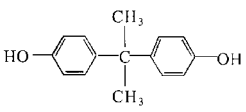
(Bis-GMA)



(UDMA)



(TEGDMA)



(BPA)

Bis-GMA is a methacrylic ester based on bisphenol-A (BPA), which is the most commonly used matrix material for dental polymers because of its acceptable chemicalstability, mechanical properties and ability to simulate natural tooth. BPA, a known endocrine disrupter that mimics the female hormone, estrogen, has been associated with estrogenicity and has been known to have potential to interfere with the body's own hormones. Therefore, it can cause a wide range of health problems ranging from infertility and cognitive impairment to enlarged prostate glands and cancer.[2]

The high viscosity of Bis-GMA necessitates admixture with lower molecular weight dimethacrylate monomer to achieve a suitable viscosity. TEGDMA, ethylene glycol dimethacrylate (EGDMA) and bisphenol-A dimethacrylate are added to Bis-GMA as diluents to change the rheology of the resin phase. Because of favorable stereochemistry, long-chain flexible dimethacrylate glycols such as EGDMA and TEGDMA have been found to exhibit relatively high degree of conversion of the methacrylate double bonds. However TEGDMA has been suspected to be propitious to bacterial growth.[3] Also, under clinical conditions, esterase present in saliva has been reported to attack the ester linkage of Bis-DMA, leading to the formation of BPA.[4] Bis-GMA based dental materials are known to be highly susceptible to chemical softening.[5] A recent study revealed that an estrogenic chemical, bisphenol-A (BPA) is present as an impurity in dental Bis-GMA based materials.[4,6]

In the oral environment, it has been assumed that food ingredients and chemical environments as well as saliva and dental plaque may affect dental polymers. According to the Food and Drug Administration (FDA) Guidelines of the United States, 75% ethanol is recommended as a food/oral simulator and might be considered clinically relevant.[7] Upon exposure of Bis-GMA to ethanol, it has been reported that hydro-peroxidation and transesterification may occur within the polymer matrix, which could affect the properties of polymeric materials.[8]

Several studies have been performed to extract undesirable components from Bis-GMA based sealants.[9] However these studies were performed for a relatively short extraction

period up to a maximum of one week. Furthermore, the fluids used in the previous studies were water, ethanol or other solvents unlike the oral fluids. Therefore, we employed artificial saliva or food/oral simulating liquids such as 75% ethanol suggested by FDA as extraction solvents, and kept the extraction period much longer, e.g., up to 28 days.

The aim of this study was to quantify and to identify toxic components such as BPA, TEGDMA, UDMA, Bis-GMA from dental sealants in liquids similar to those in the oral environment, and to identify any degradation byproducts of Bis-GMA in sealants.

Experimental Section

Materials. Five commercially available light-cured resin-based dental sealants were studied (Table 1). As shown in Table 1, TEGDMA is used commonly as a diluent in all the sealants studied. Bis-GMA and UDMA are the main component of sealant I, II, III and IV except V. Stock solutions of BPA (Aldrich, Chemical Co., USA), TEGDMA (Aldrich Chemical Co., USA), Bis-GMA (Polysciences Inc., USA) and UDMA (Ajac Inc., USA) were prepared in 10 ml volumetric flasks, by dissolving 0.100 mg of each compound in 99.99% ethanol (Merck, Germany).

Specimens and solutions. Three disks of each material were prepared in teflon molds with a diameter of 5.0 mm and a thickness of 1.0 mm. The mold was positioned on a mylar strip on a glass slab, and was filled with each of the sealants listed in Table 1. After then, the filled mold was covered with a mylar strip and pressed with a glass slab. Sample in the mold was light cured for 40 seconds from the top and the bottom surfaces with a light-curing device (Visilux II, 3M, USA). After curing, specimens were weighed (Sartorius, Germany, readability ± 0.01 mg) and then immediately immersed in two solutions of artificial saliva and 75 % aqueous ethanol. The artificial saliva used in this study was prepared by mixing 30 mL of glycerin, 150 mL of 1.1% sodium carboxymethylcellulose solution (Na-CMCS) and 150 mL of 0.9% isotonic sodium chloride solution. Na-CMCS were prepared in a 1000 mL volumetric flask, by dissolving 11.0 g of sodium CMC, 1.0 g of methyl parabenzoate, 60 mL of glycerin and 5 mL of 99.99% ethanol in 0.9% sodium chloride solution. Each specimen was placed in a glass vial containing 10 mL of artificial saliva or 75% ethanol at 37 °C for specific periods (1, 7, 14, 21 and 28 days). The vial was sealed with paraffin to prevent evaporation of volatile materials. The eluates were secured at 4 °C until analysis.

Analysis of eluates. Eluates of the sealant specimen were analyzed by reversed-phase HPLC using a 600 E system controller liquid chromatograph, equipped with a Photodiode Array Detector Waters 990, 712 auto sampler, and a column of Waters Nova Pak (4 μm, 3.9 mm i.d. × 150 mm length). The flow rate was 1 mL/min at 37 °C. The eluent was the mixture of distilled water and acetonitrile. Identification and quantitative analysis of components were performed by comparison of the elution time and the integration of absorption peak area of the eluates with those of the authentic sample.

BPA is practically not soluble in water, but highly soluble in alcohol. Therefore, additional experiment was performed using Gas chromatography and Mass spectroscopy for a comparison purpose. A Hewlett Packard 6890 Gas Chromatography (GC) fitted with a split-splitless injector for capillary columns and a 5973 Mass Spectroscopy (Mass) were used for detection of BPA. The samples for this study were prepared from uncured sealants immersed in 99.99% ethanol for 4 minutes. GC was performed under the following experimental conditions: column, 25 m × 0.2 mm i.d. × 0.33 μm film thickness; detector, Flame Ionization Detector; injection port temperature, 310 °C; column oven temperature, programmed to 100-230 °C at 30 °C/min, and 230-310 °C at 5 °C/min; carrier gas, 25 mL/min, helium. Mass spectroscopy was performed on a gas-liquid chromatography-quadrupole mass spectrometer-computer data system, under the following conditions: electron energy, 70 eV; ion source temperature, 250 °C; current, 60 μA.

Results and Discussion

Elution from commercial sealants. The retention time of HPLC peaks of the standard solutions of BPA, TEGDMA, UDMA and Bis-GMA was found to be 2.28, 3.37, 6.18 and 7.39 minutes, respectively in the present experimental condition. The average weight (μg) of each released component per mg of sealant was summarized in Tables 2 and 3.

As shown in Table 2, all specimens exposed to artificial saliva released TEGDMA. However, BPA, UDMA and Bis-GMA were not detected. The amount of TEGDMA released

Table 1. Commercial resin-based dental light-cured sealants used in this study

Sealant[a]	Main components	Manufacturer
I	TEGDMA, Bis-GMA, UDMA	Bisco, USA
II	TEGDMA, Bis-GMA, UDMA	Bisco, USA
III	TEGDMA, Bis-GMA, UDMA	Voco, Germany
IV	TEGDMA, Bis-GMA, UDMA	Ultradent, USA
V	TEGDMA, Bis-GMA	3M, USA

[a]I=Pit & Fissure Sealant, II=Elite, III=Fissurit F, IV=Ultraseal XT Plus, V=Concise.

Table 2. Mean weight (μg) of released TEGDMA per mg of each sealant in artificial saliva as a function of immersion period[a]

Sealant[b]	1 Day	7 Days	14 Days	21 Days	28 Days
I	3.13	3.13	3.13	3.13	3.13
II	3.39	3.39	3.39	3.39	3.39
III	0.13	0.13	0.13	0.13	0.13
IV	1.01	1.01	1.01	1.01	1.01
V	3.29	3.29	3.29	3.29	3.29

[a]Estimated uncertainty is ± 5% based on replicate experiments. [b]I=Pit & Fissure Sealant, II=Elite, III=Fissurit F, IV=Ultraseal XT Plus, V=Concise.

Table 3. Mean weight (μg) of released TEGDMA, UDMA, Bis-GMA and BPA per mg of each sealants in 75% ethanol as a function of immersion period[a]

Component	Sealant[b]	1 Day	7 Days	14 Days	21 Days	28 Days
TEGDMA	I	4.19	4.80	4.86	4.89	4.91
	II	6.53	6.59	6.62	6.65	6.66
	III	0.36	0.37	1.15	1.18	1.20
	IV	1.98	2.01	2.13	2.15	2.17
	V	7.18	7.36	7.40	7.44	7.46
UDMA	I	6.91	12.50	13.24	13.81	14.38
	II	5.98	7.05	8.23	8.38	8.53
	III	11.22	19.64	21.58	22.54	23.50
	IV	1.03	1.34	2.12	2.13	2.15
	V	c	c	c	c	c
Bis-GMA	I	7.71	14.08	16.28	17.29	18.30
	II	27.50	36.33	38.27	39.55	40.83
	III	15.41	28.85	32.67	34.80	36.93
	IV	2.93	4.07	4.64	4.93	5.22
	V	8.45	13.34	15.48	16.41	17.30
BPA	I	1.40	3.00	3.58	3.77	3.96
	II	d	d	d	d	d
	III	0.13	0.16	0.16	0.17	0.18
	IV	d	d	d	d	d
	V	d	d	d	d	d

[a]Estimated uncertainty is 5% based on replicate experiments. [b]I=Pit & Fissure Sealant, II=Elite, III=Fissurit F, IV=Ultraseal XT Plus, V= Concise. c: Not contained in specimens. d: Not detected.

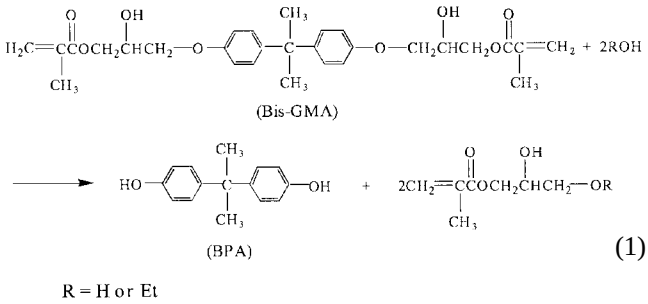
from specimens ranges from 0.13 to 3.39 μg per mg of each sealant, e.g., sealant III releases *ca.* 0.13 μg of TEGDMA per 1 mg of sealant, while sealant I, II and V release *ca.* 3.2 μg of TEGDMA per 1 mg of sealant. Interestingly, no difference in the released amount of TEGDMA can be seen upon increasing the immersion period from 1 day to 28 days, indicating that one day immersion period is long enough for TEGDMA to be released in the artificial saliva.

As shown in Table 3, TEGDMA is released from all the sealants tested. The amount of released TEGDMA varies from 0.36 μg for sealants III to 7.18 μg for sealants V for the 1 day immersion period. The amount of released TEGDMA does not increase significantly upon changing the immersion period from 1 day to 28 days, except sealants III. UDMA was not detected in sealant V, but was detected in sealant I, II, III and IV. The amount of UDMA detected ranges from *ca*. 1 mg for sealant IV to 11.2 mg for sealant III for the 1 day immersion period. Bis-GMA was detected in all the sealants tested. The amount of released Bis-GMA varies from 2.9 μg for sealant IV and 27.5 μg for sealant II for 1 day immersion period. Interestingly, the amount of released UDMA and Bis-GMA increases significantly upon changing the immersion period from 1 day to 7 days, *i.e.*, the amount of released UDMA from sealant I increases from 6.91 μg for 1 day immersion to 12.5 μg for 7 day immersion. One can see a similar result for Bis-GMA. Unlike TEGDMA, UDMA and Bis-GMA, BPA was released only from sealants I and III, but not detected in sealants II, IV and V. Besides, the amount of the released BPA from sealants I and III is much smaller than that of TEGDMA, UDMA and Bis-GMA.

Numerous factors may play a role in the elution process from dental materials. The amount of leachable molecules from dental materials can be affected by the solvent polarity, the degree of polymerization of the material, and the size and chemical composition of the leachable species. Also, the porosity of material and specimen thickness would affect the elution process.[10] Specimens in the present study were fabricated in the same experimental condition (specimen size, curing time and intensity, immersion time and medium).

One can see that the amount of released TEGDMA in 75% ethanol was higher than that in the artificial saliva. Also, Bis-GMA and UDMA were detected in 75% ethanol, while they were not detected in artificial saliva. One can explain the reason as follows: (1) Since the artificial saliva consists mainly of water, and the solubility parameter of water is different from that of Bis-GMA, little matrix softening of sealants was anticipated.[8b] (2) Bis-GMA and UDMA, which are highly soluble in 75% ethanol but practically insoluble in water.[11] (3) 75% ethanol has higher ability to penetrate the cross-linked resin matrix of the sealants than water.[8]

Degradability of Bis-GMA. It has been expected that the released Bis-GMA from sealant may be solvolyzed to BPA as shown in Eq. (1), when exposed in the oral environment for a long time.[4] Such solvolyses would be catalyzed by acids or bases. In order to examine whether Bis-GMA decomposes to BPA by solvolysis in a neutral condition, it was immersed in 75% and in 99.99% ethanol for 30 days at 37 °C. Since no BPA was detected in the reaction mixtures, Bis-GMA is considered to be stable in the present solvent system.



(1)

The uncured sealant extracts obtained after exposure in 99.99% ethanol for 4 minutes at room temperature were analyzed by GC/Mass technique. Samples were scanned in the GC/Mass for a time between 2 and 10 minutes. At a retention time of 8.02 minutes by GC, BPA could be identified based on the fingerprint m/z tracing in the mass (228, 213, 195, 119 and 91 for BPA). BPA was detected from all the extracts of the uncured sealants immersed in 99.99% ethanol even for the short period, 4 minutes. As mentioned above, Bis-GMA does not release BPA by solvolysis in neutral condition even for 30 days. Therefore the BPA detected from the extracts of the present uncured sealants immersed in 99.99% ethanol is considered as a contaminant in the sealants studied.

Conclusions

The present study has allowed us to conclude the following: (1) The majority of monomers such as BPA, TEGDMA, UDMA and Bis-GMA in the sealants are released in 75% ethanol within the first few days. (2) The amount of released TEGDMA was found to be much higher in 75% ethanol than in artificial saliva. (3) All the sealants tested contained BPA as a contaminant.

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