SPME and GC–MS Analysis of Triethylene Glycol Dimethacrylate Released from Dental Composite

Djavanshir Djozan[1,*], Saeed Jozan[2], Roya Aminian[2], and **Tahmineh Baheri[1]**

[1]Faculty of Chemistry, University of Tabriz, Tabriz-Iran and [2]School of Dentistry, Shahid Beheshti Medical Science University, Teheran-Iran

Abstract

A solid-phase microextraction (SPME) coupled with gas chromatography followed by mass spectrometry was developed for the determination of triethylene glycol dimethacrylate (TEGDMA) in aqueous media originated from cured dental composite. Cylindrical specimens of a common dental composite were cured and immersed for 48 h in 3 mL portions of human saliva and also some non-biologic media e.g., pure water and Ringer's solution. The extraction was carried out by direct SPME for 15 min. The efficiency and reliability of some commercially available and modified pencil lead fibers were evaluated for the extraction of interest compound from aqueous media. Some effective and experimental parameters of SPME and gas chromatography procedures were examined and optimized. The obtained results reveal that the direct SPME using the modified pencil lead is very effective and can extract TEGDMA with a good selectivety from among various compounds such as 2,6-di-*tert*-butyl-4-methyl phenol (buylated hydroxy toluene) (BHT), 2-propenoic acid, 2-methyl-oxybis (2,1-ethanediyl oxy-2,1-ethanediyl) ester (TEEGDMA), 3,5-di-t-butyl-4-hydroxy benzaldehyde, benzoic acid 4 (dimethyl-amino)-ethyl ester (DMA BEE), 2-propenoic acid, 2-methyl-dodecyl ester (methacrylic acid, dodecyl ester), 2-ethoxy ethyl methacrylate, and drometrizole (TINP). The results obtained also prove that the studied composite releases 11.0, 13.4, and 28.3 µg/mL TEGDMA into distilled water, Ringer's solution, and saliva, respectively, at 48 h of the exposition.

Introduction

All dental composites contain considerable amounts of diluents to make the products applicable for dental purposes. Actually, the most abundant group of diluents and/or co-monomers consists of ethylene-glycol compounds, mainly triethylene glycol dimethacrylate. $CH_2 = C(CH_3)COO(CH_2CH_2O)_3 COC(CH_3) = CH_2$ (TEGDMA) is characterized by molecular weight of 286.32, boiling point of 162ºC at 760 mm Hg and density 1.07. This compound is one of the most important resinous sensitizers of dental origin in patients and personnel (1) and has a considerable cytotoxic potency in comparison with other resinous monomers or additives (1–4). It has also been observed that this compound can penetrate cell membranes and, subsequently, may react with intercellular molecules (5). It causes large deletions of DNA sequences and can result in high mutation frequency (6). Also it has been shown that TEGDMA induces dental pulp inflammation and necrosis (7,8). In addition, it has been documented that this compound may promote the proliferation of the important carcinogenic microorganisms probably leading to recurrent caries and pulp alterations (9,10). Furthermore, TEGDMA induces apoptosis in vitro, which is a major form of cell death in biological systems as well as in chemically induced injury (11). Monitoring of persisted non-polymerized TEGDMA in human saliva therefore becomes of increased interest in toxicology and is necessary for better knowledge about formulation and safety of composites.

A great number of articles on determination of these compounds in non-biological fluids like Ringer's solution (9.0 g/L NaCl, 0.42 g/L KCl, 0.25 g/L $CaCl_2 \cdot 2H_2O$ in redistilled water with pH adjusted at 7.4) have been published using high-performance liquid chromatography (HPLC), gas chromatography (GC), and gas chromatography coupled with mass spectrometry (GC–MS) methods (12). The complexity of saliva interferes with accurate analysis of these components of the composite. Also, conventional methods for analyzing these chemicals involve tedious and solvent consumptive procedures. So, it is necessary to improve the methods of extraction and instrumental techniques.

Solid-phase microextraction (SPME) is a powerful, simple, fast, and solvent-free sampling method for direct and headspace extraction of volatile and semi-volatile chemicals from various samples through absorption of volatiles on the surface of fiber (9). Fused-silica fibers coated with polydimethylsiloxane, polyacrylate, carbowax, or carboxen are commercially available and are widely used (10,13–17). Most of these fibers suffer from high cost, fragility, and inability to support high temperature. Several new fibers such as anodized aluminum (18), modified pencil lead (19–21), and fibers based on molecularly imprinted polymer (22, 23) have been developed and used as SPME fibers. Within these, modified pencil lead fiber is recently presented and seems to be a useful and a highly suitable fiber for direct and headspace extraction of semi-volatile and volatile compounds from aqueous samples.

This study involves the investigation of SPME efficiency using modified pencil lead fiber for a simple and effective extraction of

*Author to whom correspondence should be addressed: Email djozan@tabrizu.ac.ir.

some organic compounds such as TEGDMA released from cured dental composite into water, Ringer's solution, and saliva. The monitoring of the released and extracted compounds was carried out with GC–MS method.

Experimental

Reagents

Helium (99.999%) was purchased from Air Products (Dubai, United Arab Emirates). TEGDMA was purchased from Aldrich (St. Louis, MO), and other chemicals were in pro-analysis-grade and obtained from Merck (Darmstadt, Germany).

Mother stock solution with a concentration of 100 µg/mL of TEGDMA was prepared by dissolving 25 mg of analyte in water and adjusting to 250 mL. These solutions were used to prepare 25 mL standard solutions containing the required amount of TEGDMA (20 µg/mL) in water, Ringer's solution, or blank saliva. The solutions were stored at 4ºC. For the preparation of real samples, cylindrical specimens of a common dental composite (2 mm thick with a diameter of 6 mm) were prepared and cured for 40 s with a halogen dental curing lamp as requested by the composite producer. Cured samples were immersed for 48 h at 37ºC into 4-mL sample vials, each one containing 3 mL doubly-distilled water, 3 mL Ringer's solution, or 3 mL blank saliva. The vials were sealed with a silicone-rubber septum cap and analyzed after 48 h.

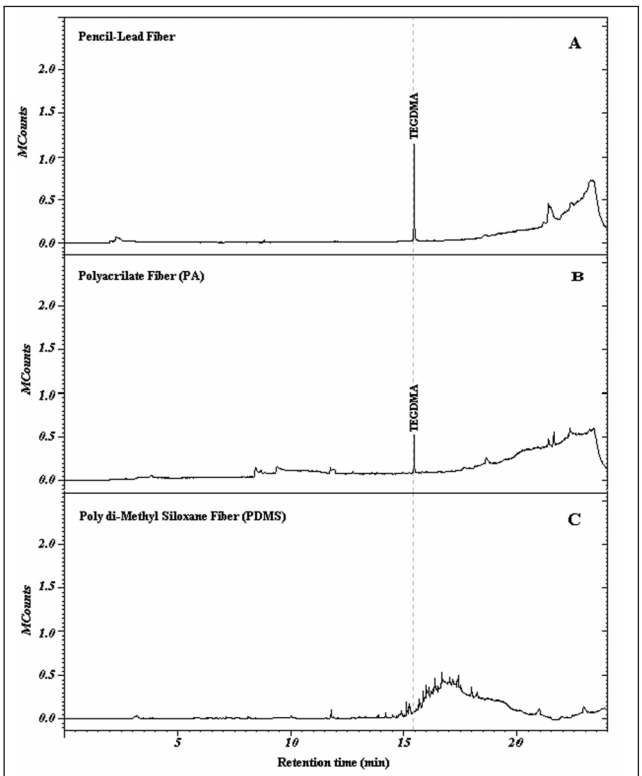

Figure 1. Extraction efficiency of TEGDMA using different fibers: (A) modified pencil lead, (B) poly-dimethyl siloxane (PDMS), and (C) polyacrylate (PA). SPME procedure was carried out from 3 mL of 20 µg/mL model solution at room temperature for 15 min.

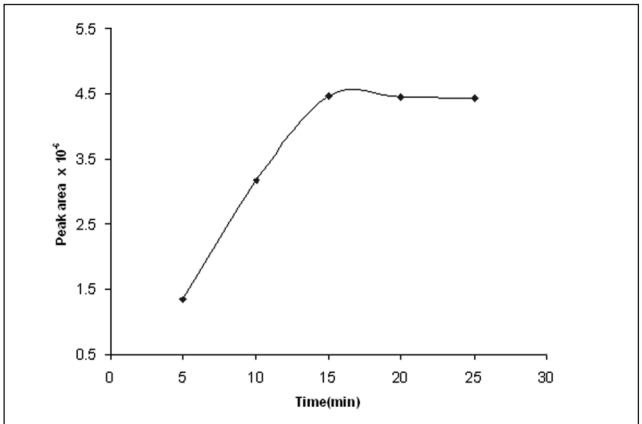

Figure 2. Optimization of extraction time: The experimental conditions were as given for Figure 1.

Apparatus

Monitoring of released organic compounds was performed by GC–MS model Saturn 2000 (Varian, Palo Alto, CA). The chromatographic column used was a Chrompack CP-Sil5 CB (30 m × 0.25 mm × 0.25 µm) (Raritan, NJ). A split/splitless injector model 1077 Varian was used. A SPME manual sampling holder (Supelco, Dorset, UK), a polyacrylate (PA), and a polydimethylsiloxane (PDMS) fiber with 100-µm film thickness were purchased from Supelco. Pencil lead type HB (diameter 0.35 mm, length 60 mm) from Rotring Company (Hamburg, Germany) was modified as described below and was mounted in the homemade SPME device, and the exposed fiber was trimmed to 2 cm. Carbolite furnace (Bemaford, Sheffield, England) was used for thermal conditioning of pencil lead fibers. Four-milliliter sample vials sealed with a silicone-rubber septum cap were purchased from Supelco.

Preparation of pencil lead fibers and analytical procedures

Pencil lead fibers were modified as described in our previous paper (19) by heating at 600°C with water vapor for 60 min in the furnace and then conditioned inside GC injection port for 15 min at 270°C under helium flow.

SPME was run using different fibers. The fiber was plunged into 4-mL sample vial and exposed on 3 mL standard and real samples for 15 min until equilibrium was reached. Monitoring of extracted organic compounds was carried out with GC–MS.

GC–MS operating conditions

In this study, the column temperature was initially 50°C, programmed at 50°C/min to 120°C (held for 5 min), increased to 280°C with a rate of 10ºC/min (held for 1 min). The carrier gas velocity was 25 cm/s. The injection port at 270°C in the splitless mode with a 1-mm internal diameter glass liner was used, and the splitter was opened after 1 min. The ion source of mass spectrometer was maintained at 220ºC for electron impact ionization (EI).

Results and Discussion

Optimization of chromatographic conditions

Chromatographic procedure was run under various tempera-

ture programs cited in literature. The results obtained showed that at column oven temperature program cited in the experimental section the highest peak capacity in a reasonable time would be achieved.

Selection of SPME fiber

In these studies, three types of fiber were used for the extraction of TEGDMA from model solutions: (A) modified pencil lead fiber, (B) fused silica coated with PA, and (C) fused silica coated with PDMS. The efficiency of these fibers for extraction of TEGDMA from model Ringer's solution is shown in Figure 1. Comparison of peak area reveals that the efficiency of extraction of TEGDMA by modified pencil lead fiber is higher than both commercially available PA and PDMS fibers. These results prove also the higher ability of modified pencil lead fiber for the extraction of studied compound from aqueous media.

Optimization of extraction time

Extraction procedures were carried out in stirred solutions repetitively at different times ranging from 5 to 25 min with 5 min intervals followed by thermal desorption and GC–MS analysis of analyte. The results obtained are presented as the variation of peak area of TEGDMA versus exposure time in Figure 2. From these results, it was concluded that extraction equilibrium was reached in 15 min. Further extractions were therefore performed at 15 min.

Table I. Characteristic Parameters of Calibration Graph and Analytical Features of TEGDMA*

Compound	Calibration equation	LOD (µg/mL)	LDR (µg/mL)	RSD %	R^2
TEGDMA	y = 2.05 × 104 + 2.50 × 105x	0.44	1–50	0.7%	0.999

* The experimental conditions were as given for Figure 1.

Optimization of desorption temperature and time

Preliminary studies were shown that at 270ºC neither septum nor analyte decomposition occurs at detectable levels. Experimental studies reveal that the time required to complete desorption at this temperature is 1 min, and there is no memory effect, and any peak was not observed for successive blank injection of the same fiber.

Repeatability and reproducibility

The results in our previous work (19) proved that the pencil lead modified at 600ºC under water vapor is highly reproducible. To investigate fiber repeatability, one fiber was used in three tests under similar conditions for the extraction of TEGDMA from human saliva. As illustrated in Table I, relative standard deviations (RSD) were below 0.7% (n = 3). These results prove that the proposed home-made fiber is very stable and can be used for several extractions without substantial change in surface properties.

Analytical approach

Quantitative analysis of TEGDMA in standard and model solution from 1 µg/mL to 50 µg/mL was performed by GC–MS after SPME using modified pencil lead fiber. Some analytical performance data in optimum conditions are listed in Table I. From these results, the linearity of calibration graph is excellent in a dynamic range of 1–50 µg/mL. The limit of detection (LOD) for the studied compound is 0.44 µg/mL.

Application

Proposed method was applied for the quantitative analysis of TEGDMA from real samples consisting distilled water, Ringer's solution, and human saliva after 48 h exposition with cured composite. Representative chromatograms were shown in Figure 3. Taking into account the peak areas and using external calibration graph, the quantity of TEGDMA released in the distilled water, Ringer's solution and human saliva were measured to be 11.0 ± 0.5 µg/mL, 13.4 ± 0.5 µg/mL, and 28.3 ± 0.5 µg/mL, respectively.

Mass spectrometric screening of chromatograms shows the presence of many compounds other than TEGDMA, which have been released from cured composite into distilled water, Ringer's solution, and saliva. The main compounds released were illustrated in Table II. Comparing peak areas reveals that TEGDMA released into human saliva is at least 10-fold more than the other compounds.

Table II. Components Released From Dental Composite into Saliva*

	Components	t_R† (min)	Formula	Peak area
1	2,6-Di-tert-butyl-4-methyl phenol (buylated hydroxy toluene) (BHT)	11.55	$C_{15}H_{24}O$	2.31×10^4
2	2-Propenoic acid, 2-methyl-oxybis (2,1-ethanediyl oxy-2,1-ethanediyl) ester (TEEGDMA)	11.90	$C_8H_{15}O_3$	1.37×10^5
3	3,5-Di-t-butyl-4-hydroxy benzaldehyde	12.28	$C_{15}H_{22}O_2$	1.01×10^6
4	Benzoic acid, 4(dimethylamino)-ethyl ester (DMA BEE)	14.03	$C_{11}H_{15}NO_2$	4.56 × 106
5	2-Propenoic acid, 2-methyl- dodecyl ester (meth acrylic acid, dodecyl ester)	14.91	$C_{16}H_{30}O_2$	9.56 × 105
6	2-Ethoxy ethyl methacrylate	15.51	$C_{16}H_{26}O_7$	1.34 × 106
7	2-Propenoic acid, 2-methyl-,1,2-ethandiylbis (oxy-2,1-….) (TEGDMA)	15.80	$C_{14}H_{22}O_6$	9.94 × 106
8	Drometrizole(TINP)	17.99	$C_{13}H_{11}N_3O$	3.17 × 106

* Component numbers correspond to peak numbers in Figure 3. The experimental conditions were as given for Figure 1.
† Retention time.

Conclusion

The research presented has conclusively demonstrated that SPME with modified pencil lead fiber can be used for effective sampling of trace organic compounds released from dental composite into human saliva. We have demonstrated a simple and not tedious method for preseparation of the studied compounds from

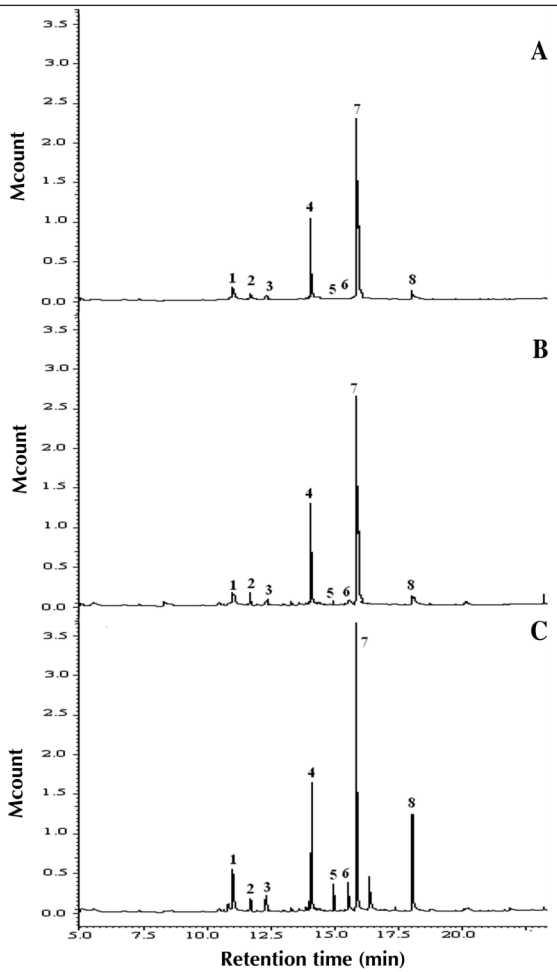

Figure 3. Characteristic chromatograms of organic compounds released from composite into (A) distilled water, (B) Ringer's solution, and (C) saliva. The experimental conditions were as given for Figure 1.

complex matrix prior to GC–MS analysis. The most abundant compound is TEGDMA, which is released in a relatively considerable amount of 28.3 ± 0.5 µg/mL into saliva.

Acknowledgment

We thank the research office of University of Tabriz-Iran for financial support.

References

1. R. Becher, H.M. Kopperud, R.H. Al, J.T. Samuelsen, E. Morisbak, H.J. Dahlman, E.M. Lilleaas, and. J.E. Dahl. Pattern of cell death after in vitro exposure to GDMA, TEGDMA, HEMA and two compomer extracts. *Dent. Mater.* **22:** 630 (2006).
2. F.X. Reichl, J. Durner, R. Hickel, K.H. Kunzelmann, A. Jewett, M.Y. Wang, W. Spahl, H. Kreppel, G.W. Moes, K. Kehe, U. Walther, W. Forth, and W.R. Hume. Distribution and excretion of TEGDMA in guinea pigs and mice. *J. Dent. Res.* **80:** 1412 (2001).
3. C.A. Quinlan, D.M. Zisterer, K.F. Tipton, and M.I. O'Sullivan. In vitro cytotoxicity of a composite resin and compomer. *Int. Endod. J.* **35:** 47 (2002).
4. H. Schweikl, A. Hartmann, K.A. Hiller, G. Spagnuolo, C. Bolay, G. Brockhoff, and G. Schmalz. Inhibition of TEGDMA and HEMA-induced genotoxicity and cell cycle arrest by N-acetylcysteine. *Dent. Mater.* **23:** 688 (2007).
5. J. Volk, G. Leyhausen, S. Dogan, and W. Geurtsen. Additive effects of TEGDMA and hydrogenperoxide on the cellular glutathione content of human gingival fibroblasts. *Dent. Mater.* **23:** 921 (2007).
6. M. Kaga, M. Noda, J.L. Ferracane, W. Nakamura, H. Dguchi, and H. Sano. The in vitro cytotoxicity of eluates from dentin bonding resins and their effect on tyrosine phosphorylation of L929 cells. *Dent. Mater.* **17:** 333 (2001).
7. C. Hansel, G. Leyhausen, U.E. Mai, W. Geurtsen. Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms in vitro. *J. Dent. Res.* **77:** 60–67 (1998).
8. J. Engelmann, G. Leyhausen, D. Leibfritz, and W. Geurtsen. Metabolic effects of dental resin components in vitro detected by NMR spectroscopy. *J. Dent. Res.* **80:** 869 (2001).
9. J. Pawliszyn. *Solid phase microextraction: Theory and practice,* Wiley-VCH, New York. 1997.
10. C.L. Arthur and J. Pawliszyn. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **62:** 2145 (1990).
11. J.T. Samuelsen, J.E. Dahl, S. Karlsson, E. Morisbak, and R. Becher. Apoptosis induced by the monomers HEMA and TEGDMA involves formation of ROS and differential activation of the MAP-kinases p38, JNK and ERK. *Dent. Mater.* **23:** 34 (2007).
12. D. Nathanson, P. Lertpitayakun, M. Lamkin, M. Edalatpour, and L. Lee Chou. In vitro elution of leachable components from dental sealants. *JADA.* **128:** 1517 (1997).
13. M. Chai and J. Pawliszyn. Analysis of environmental air samples by solid-phase microextraction and gas chromatography/ion trap mass spectrometry. *Environ. Sci. Technol.* **29:** 693 (1995).
14. H.B. Wan, H. Chi, M.K. Wong, and C.Y. Mok. Solid phase microextraction using pencil lead as sorbent for analysis of organic pollutants in water. *Anal. Chim. Acta* **298:** 219 (1994).
15. S.B. Hawthorne, D.J. Miller, J. Pawliszyn, and C.L. Arthur. Solventless determination of caffeine in beverages using solid-phase microextraction with fused-silica fibers. *J. Chromatogr.* **603:** 185 (1992).
16. Y. Liu, Y. Shen, and L.L. Lee. Porous layer solid phase microextraction using silica bonded phases. *Anal. Chem.* **69:** 190 (1997).
17. F. Mangani and R. Cenciarini. Solid phase microextraction using fused silica fibers coated with graphitized carbon black. *Chromatographia* **41:** 678 (1995).
18. Dj. Djozan, Y. Assadi, and S. Hosseinzadeh-Haddadi. Anodized aluminum wire as a solid-phase microextraction fiber. *Anal. Chem.* **16:** 4054 (2001).
19. Dj. Djozan and Y. Assadi. Modified pencil lead as a new fiber for solid phase microextraction. *Chromatographia* **60:** 313 (2004).
20. Dj. Djozan, T. Baheri, R. Farshbaf, and S. Azhari. Solid-phase microextraction using pencil lead fiber for in-vitro and in-vivo sampling of defensive volatiles from insect scent gland followed by gas chromatographic analysis. *Anal. Chim. Acta* **554:** 197 (2005).
21. Dj. Djozan, T. Baheri, and M.H. Pournaghi-Azar. Development of electro solid phase microextraction and application for methamphetamine analysis. *Chromatographia* **65:** 45 (2007).
22. Dj. Djozan, T. Baheri, M. H. Pournaghi Azar, and M. Mahkam. Preparation of new fibers on the basis of codeine imprinted polymer. *Mat. Manufact. Pro.* **22:** 758 (2007).
23. Dj. Djozan and T. Baheri. Preparation and evaluation sof solid-phase microextraction fibers based on monolithic molecularly imprinted polymers for selective extraction of diacethyl morphine and analoqous compounds. *J. Chromatoger. A* **16:** 1166 (2007).

Manuscript received February 7, 2008;
Revision received May 11, 2008.