HPLC ANALYSIS OF BIS – GMA AND TEG–DMA RELEASED FROM DENTAL MATERIALS

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Introduction

Dental composite materials are today widely used not only because of their aesthetic properties but for the ability to adhere to tooth substance. Dental composite materials consist mainly of filler particles and a polymer matrix based on different monomers, dimmers and/or oligomers of methacrylates and/or acrylates, together with additives[1]. The release of organic degradation products is dependent on the size of the molecules, where smaller molecules are presumed to have enhanced mobility and should therefore be eluted faster than larger molecules[2, 3].

Örtengren used HPLC for detection of Bis – GMA and Teg – DMA in the storage water [1]. Quantifiable amounts of both substances were registered in the storage water for the studied materials. In the work of Pulgar the oligomer Bis – GMA leached from composites and sealants before and after polymerization was confirmed by HPLC analysis[4]. Eight monomers extracted in five solvents were analyzed from different resins from Shintani by HPLC[5].

In this work, the leaching of bisphenol A diglycidylmethacrylate (Bis - GMA) and triethilenglycol dimethacrylate (Teg - DMA) was investigated. Residual monomers were determined using reversed phase high performance liquid chromatography (HPLC) with UV diode array detector. The aim of our work was to identify and quantify Bis - GMA and Teg - DMA released from dental materials through dentin in aqueous medium.

Experimental

Reagents. Acetonitrile (HPLC grade) from Aldrich was used. Reference standards: Bis - GMA (FW - 512.21) and Teg - DMA (FW - 286.148) were provided from Vivadent, Liechtenstein. Stock solutions were prepared in ethanol.

Dental composites. Two composite materials were examined: Tetric (Vivadent, Liechtenstein) and Esthet – X (Dentsply, De Tray). The basis of these composites consists of the monomers Bis - GMA and Teg - DMA. The universal dentin-adhesive "Exite" (Vivadent, Liechtenstein) was used.

Sample preparation. Two groups of samples for each of the two commercial dental materials (Tetric and Esthet) were prepared with and without adhesive polymerized for 20 s as abbreviated time of polymerization often used in practice and for 40 s as recommended time of polymerization. The dental composites were deposited in a cavity (6 mm wide and 2 mm deep) of extracted teeth from patients aged 18-25 years. The so prepared teeth were in contact with water (4 mL) from the pulp side. After 24 hours, 7 and 14 days, the water solutions were analyzed for detection and determination of the monomers Bis - GMA and Teg - DMA.

HPLC analysis. The analysis of the monomers was carried out with a Varian HPLC system equipped with a ternary pump Model 9012 and UV Diode Array Detector Model 9065. The column used was C - 18 Lichrospher 60 RP Select B (250 x 4 mm, particle diameter 5 μm, Merck) and the mobile phase consisted of CH_3CN and H_2O (60 : 40, *V/V*). The flow rate was 1 mL/min and the sample volume: 20 μL. The elution was performed at room temperature and monitored in the whole UV range. For quantitation, detection at $\lambda = 220$ nm was performed for both analytes.

Results and Discussion

Identification of the analytes Bis - GMA and Teg - DMA was made by comparison of the retention times and UV – spectra of the registered peak and the reference peak obtained for standards. The retention time obtained for Bis - GMA was 4.394 min and for Teg - DMA 6.401 min.

For quantitative analysis, calibration curve was constructed using standard solutions of Bis - GMA and Teg - DMA in the concentration range from $1 \cdot 10^{-3}$- $5 \cdot 10^{-5}$ mol/L. Detection was performed at the wavelength of 220 nm for both analytes because they exhibit significant absorption, which gives a good sensitivity of the method. The parameters obtained for the linear dependence peak area/concentration for the two analytes are the following:

Bis – GMA	$A = 1.2714 \cdot 10^6 \cdot c + 1.8198 \cdot 10^4$	$R^2 = 0.9988$	$\sigma = 2.8007 \cdot 10^4$
Teg - DMA	$A = 1.5127 \cdot 10^6 \cdot c + 3.9870 \cdot 10^4$	$R^2 = 0.9992$	$\sigma = 2.7740 \cdot 10^4$

The limits of detection (LOD) and quantification (LOQ) were determined using calibration in the low concentration region ($5 \cdot 10^{-6}$ - $5 \cdot 10^{-5}$ mol/L) for both analyzed compounds, which is presented in Table 1.

Table 1. *Results obtained for LOD and LOQ for both analytes*

Analyte	Regression equation	R^2	σ	LOD/ mmol/L	LOQ/ mmol/L
Bis – GMA	$A^* = 1.2753 \cdot 10^6 \cdot c - 2.9232 \cdot 10^3$	0.9968	2921.81	0.0076	0.0229
Teg - DMA	$A = 1.7235 \cdot 10^6 \cdot c + 1.6457 \cdot 10^3$	0.9928	6428.16	0.0123	0.0373

A^* - peak area

This procedure was then used for analysis of the residual monomers, which had penetrated in the water solution through the dental barrier after 24 hours, 7 days and 14 days. Chromatograms of the analytes Bis - GMA and Teg - DMA obtained for samples and standard solution are given in Figure 1.

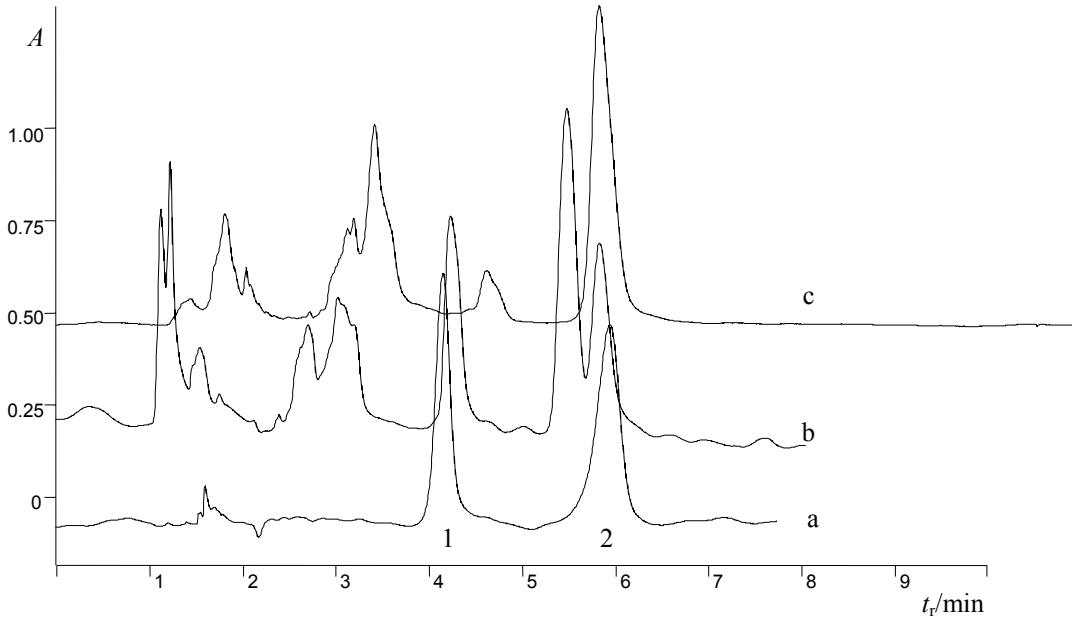

Fig. 1. HPLC chromatograms of (a) a mixture of standard solutions of 1. bis-GMA and 2. TEG-DMA, and samples of (b) Esthet with adhesive and (c) without adhesive polymerized by exposure to light for 20 s after 14 days

After 24-th period, Bis - GMA was detected in all of the commercial samples except Tetric without adhesive polymerized for 40 s and Esthet with adhesive also polymerized for 40 s. After 7 days Bis - GMA was found in all polymerized samples except Esthet with adhesive polymerized for 40 s. This substance was not detected in Tetric sample without adhesive polymerized for 40 s, Esthet sample with adhesive polymerized for 40 s and Esthet sample without adhesive polymerized for 40 s by exposure to light for 40 s. In Tetric sample with adhesive polymerized for 40 s Bis - GMA was detected in quantities close to limit of detection after 24 hours and between limit of detection and limit of quantification after 7 and 14 days. The results for Bis - GMA are given in Table 2.

Table 2. *Results from the determination of penetrated Bis – GMA in water through the dental barrier after 24 h, 7 and 14 days in 8 different samples of polymerised dental material*

Sample	*c*(Bis-GMA)/mmol/L after 24 h	*c*(Bis-GMA)/mmol/L after 7 days	*c*(Bis-GMA)/mmol/L after 14 days
Tetric 20 s* with adhesive	0.0358	0.0847	0.0975
Tetric 40 s* with adhesive	~LOD	between LOD and LOQ	between LOD and LOQ
Tetric 20 s without adhesive	~LOD	~LOD	~LOD
Tetric 40 s without adhesive	/	0.0768	/
Esthet 20 s with adhesive	0.1394	0.2598	0.2708
Esthet 40 s with adhesive	/	/	/
Esthet 20 s without adhesive	between LOD and LOQ	0.0451	0.0760
Esthet 40 s without adhesive	0.0269	0.0344	/

*20 s and 40 s is the time of exposure to halogen light

No detectable quantities of Teg - DMA were observed in Tetric and Esthet samples prepared with and without adhesive in that period, except in the sample of Esthet prepared without adhesive by exposure to light for 20 s, in which 0.0777 mmol/L Teg - DMA were determined

The HPLC analysis showed the highest concentration of monomers after 14 days in most samples. It is shown that the composites and sealants are unstable and that to a greater or lesser degree, depending on the aggressiveness of the medium it is always possible to detect the elution of monomers, olygomers and precursors. These findings confirm the reports that *in vivo* polymerization is not complete and that free monomers can be detected by different analytical methods[5, 6, 7, 8]. In this work, leaching of residual monomers Bis - GMA and Teg – DMA in water through dentin was measured employing polymerization by exposure to light for 20 s and 40 s. The higher quantities of monomers obtained for reduced exposure to light show that the recommended exposure time (40 s) should be used. Also, lower results for residual monomers obtained for samples when an adhesive is used recommend employing adhesives together with the suggested exposure time for complete polymerization.

The proposed HPLC method for analysis of residual monomers in polymerized dental composites is simple, rapid and sensitive. It is suitable for identification and quantification of the monomers Bis - GMA and Teg - DMA from various samples.

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