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Identification and Quantification of Monomers Released from Dental Composites Using HPLC

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ABSTRACT

The aim of this study was to detect and quantify the main residual monomers released from composites, using high performance liquid chromatography (HPLC). Discs were made with dental composites (Herculite XRV, Tetric Ceram and Filtek Z250) and immersed in deionized water at 37°C for 28 days, with water changes in 1, 7, 14 and 21 days. The mean concentration of residual monomers were subject to the Kruskal-Wallis test ($p<0.05$). Tetric Ceram exhibited significantly higher concentrations of leached monomers. Bis-GMA was the monomer released in lower concentrations for all the materials. There was no statistical difference between the amounts of TEGDMA and UDMA. Most of the monomers demonstrated maximal concentration at the 7-day period. The HPLC analysis identified Bis-GMA, TEGDMA and UDMA in detectable quantities for all the tested composites.

Key words: Dental composites, monomers, HPLC, TEGDMA, Bis-GMA, UDMA

INTRODUCTION

Restorative composite resins have in their composition monomers, inorganic filler particles, initiators, diluents and a coupling agent which is responsible for the bonding between the filler particles and the resin matrix (Ferracane, 1994). Since the early 60s, the most commonly used monomers in composites are dimethacrylates such as bisphenol-A glycol dimethacrylate (Bis-GMA) and urethane dimethacrylate (UDMA). Recently, other types of monomers have been introduced in order to reduce the polymerization shrinkage and water sorption (Sideridou et al., 2003). Some monomers are very viscous and require a diluent to be workable. The most commonly used diluent is triethyleneglycol dimethacrylate (TEGDMA).

Despite of being considered highly stable structures, dental composites are susceptible to degradation (Geurtsen, 1998) due to the incomplete polymerization and the influence of the aqueous oral environment (Oysaed and Ruyter, 1986). When a composite material is immersed in water, some of the components, such as unreacted monomers (Inoue and Hayashi, 1982; Oysaed et al., 1988; Tanaka et al., 1991; Ferracane, 1994; Örtengren et al., 2001), filler particles (Söderholm, 1983) and other components (Lygre et al., 1999) are leached out of the material. This can be assessed as solubility or leaching. These products can be released into salivary fluids and contact the muccosa tissues, and even reach the pulp via

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dentinal tubules (Ferracane, 1994). Thus, studies on the elution of the unbonded components would be important, since they have demonstrated that residual monomers and additives eluted from composites have a wide range of toxic potencies (Wataha et al., 1999; Wada et al., 2004; Al-Hiyasat et al., 2005).

The size of the molecules is an important factor affecting the elution of components of dental composites. Smaller molecules are presumed to have increased mobility and might therefore be eluted faster than larger molecules. It has been shown that TEGDMA is the main component released from cured dental composites (Geurtsen, 1998; Örtengren et al., 2001), although small amounts of other substances can also be eluted into water (Örtengren et al., 2001).

As the detection and behavior of these leachable components are crucial to understand the degradation process of polymer-based materials, the aim of this study was to detect and quantify the main residual monomers released from hybrid composites after different storage times, using high performance liquid chromatography (HPLC).

MATERIAL AND METHODS

Thirty discs were prepared with dental composites using a stainless steel mold (15.0 ± 0.1 mm diameter and 1.0 ± 0.1 mm thickness). The technical profiles of the hybrid composites used in this study are presented in Table 1.

The polymerization of the specimens was carried out with the LEDemetron 1 (Demetron Research Corp, Danbury, CT, USA). The mean output intensity of the light source was 600mW/cm^2, as assessed by the radiometer attached to the curing unit. The discs were irradiated with an 11mm diameter tip positioned on one central and four peripheral points for 20 seconds each, leading to a 100 seconds exposure time for both top and bottom surfaces of each specimen.

After removal from the molds, the specimens were transferred to a desiccator containing silica gel, maintained at 37°C for 22 h and then transferred to another desiccator at 23°C ± 2°C for 2 h. The specimens were weighed to a precision of 0.0001g using an analytical balance (Bel Mark U210A, Bel Engineering, Monza, MI, Italy). This cycle was repeated until a constant mass was achieved. The specimens were then stored individually in glass tubes filled with 10mL of deionized water at 37°C. The preparation and storage of the specimens were based on ISO 4049 recommendations for solubility tests. The specimens were maintained in this condition for 28 days, with water changes in 24 hours, 7, 14 and 21 days. After each period of water storage, the solutions were transferred to a refrigerator.

Table 1 - Technical profiles of the composites evaluated.

Composite	**Manufacturer**	**Batch number**	**Polymer**	**Filler content % vol**	**Filler content % wt**
Herculite XRV	Kerr Manufacturing, Orange, CA, USA	14546	Bis-GMA, TEGDMA	59	79
Tetric Ceram	Ivoclar AG, Liechtenstein	G18572	Bis-GMA, TEGDMA, UDMA	62	81
Filtek Z250	3M Dental Products, St. Paul, MN, USA	4LU	Bis-GMA, TEGDMA, UDMA, Bis-EMA	60	80

Bis-GMA- bisphenol A glycol dimethacrylate
UDMA-urethane dimethacrylate
TEGDMA- triethyleneglycol dimethacrylate
Bis-EMA- bisphenol A polyethyleneglycol diether dimethacrilate

The analysis of the released monomers was carried out by high performance liquid chromatography - HPLC (Shimadzu Class VP, Kyoto, Japan). The equipment had a C18 5 μm, 250 mm length, 4.5 mm diameter column (Supelco, Inc., Bellefonte, PA, USA), with an UV/DAD detector, using a mobile phase of CH_3CN / H_2O (7:3), at a flow rate of 1 mL/min with 20 μL injection at room temperature (Örtengren et al., 2001). All the measurements were performed three times for each of the extracts. For the analysis of the eluates, standard solutions of Bis-GMA, UDMA and TEGDMA (EssTech, Essington, PA, USA) were prepared by dissolving each monomer in varied concentrations (0.0015 to 1.25 mg/mL). The calibration curves were made within the quantification limits of 0.0015 mg/mL for UDMA and TEGDMA, 0.0010 mg/mL for Bis-GMA. Retention times of each monomer were obtained and the calibration curves were made relating eluted peak area to known concentrations of the standard monomers. The coefficients (R) obtained by a linear regression analysis for Bis-GMA, UDMA and TEGDMA were 0.99958, 0.99994 and 0.99992, respectively.

The lixiviation of the monomers, after each storage time, was detected by HPLC. The mean concentrations of each monomer were plotted using the Origin Scientific Graphing and Analysis 7.5 (OriginLab Corp., Northampton, MA, USA).

The statistical analysis of data was performed by Kruskal-Wallis analysis of variance, for each composite and monomer ($p < 0.05$). Kruskal-Wallis range test was used to determine which comparisons were different.

RESULTS

Figs. 1, 2 and 3 demonstrate the chromatograms of the composites Herculite XRV, Tetric Ceram and Filtek Z250, respectively. The mean and standard deviation of leached monomers from each composite during different periods of storage in water are presented in Table 2. Among the tested composites, Tetric Ceram was the one with significantly higher concentrations of leached monomers ($p=0.001$). Herculite XRV and Filtek Z250 did not demonstrate significant differences from each other. The monomer Bis-GMA was the substance released in lower concentrations for all the materials, with significant differences when compared to the other monomers. There was no statistical difference between Tetric Ceram and Filtek Z250 regarding UDMA monomer release. TEGDMA demonstrated significantly higher leaching in Herculite XRV, when compared to Filtek Z250. However, there was no statistical difference between TEGDMA and UDMA for the composites that were composed by both these monomers

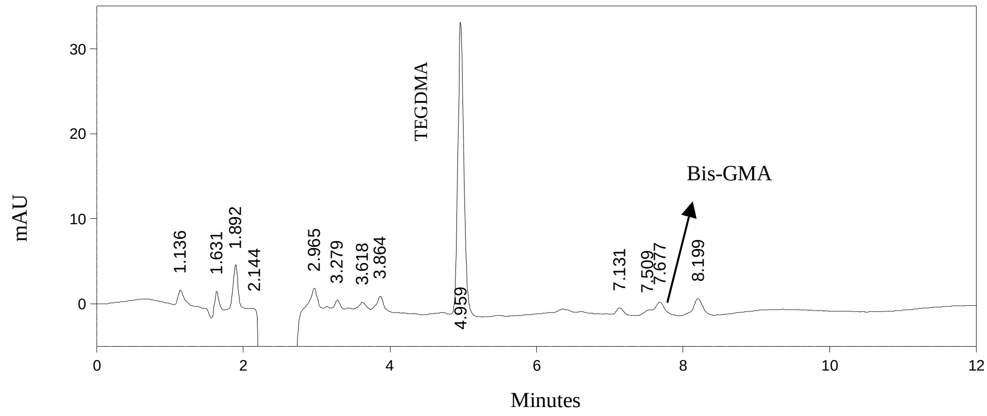

Figure 1 - Typical chromatogram of composite Herculite XRV obtained by HPLC with reference peaks of TEGDMA and Bis-GMA monomers.

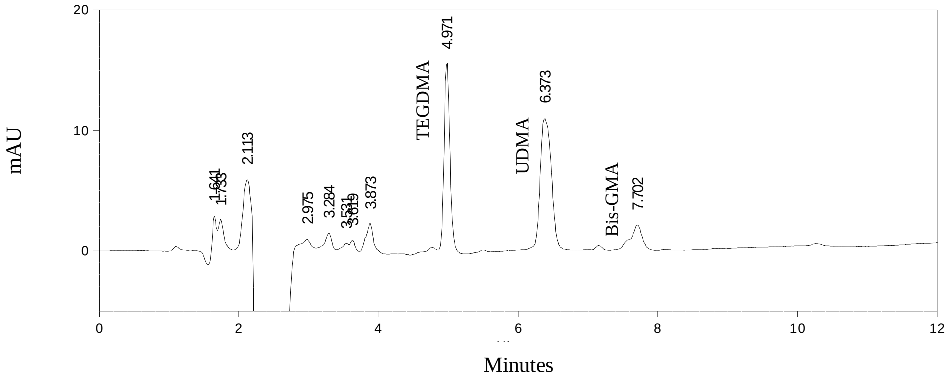

Figure 2 - Typical chromatogram of composite Tetric Ceram obtained by HPLC with reference peaks of TEGDMA, UDMA and Bis-GMA monomers.

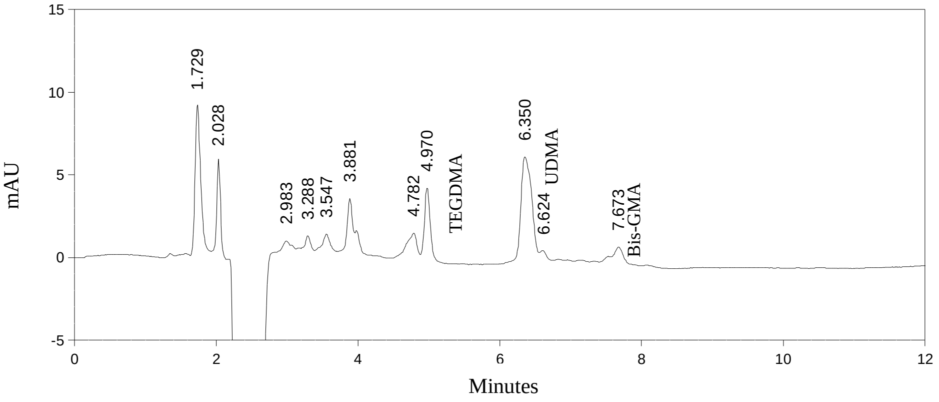

Figure 3 - Typical chromatogram of composite Filtek Z250 obtained by HPLC with reference peaks of TEGDMA, UDMA and Bis-GMA monomers.

Table 2 - Mean concentration (SD) of the amount of released monomers from dental composites (µg/mL) after 1, 7, 14, 21 and 28 days.

Composites	**Monomers**	**Concentration (µg/mL)**				
		1 Day	**7 Days**	**14 Days**	**21 Days**	**28 Days**
Herculite XRV	TEGDMA	5.661 (0.061)	5.202 (0.067)	3.492 (0.031)	2.546 (0.031)	2.052 (0.031)
	Bis-GMA	2.200 (0.027)	2.012 (0.040)	2.214 (0.035)	1.573 (0.058)	0.943 (0.005)
Tetric Ceram	TEGDMA	5.643 (0.193)	6.394 (0.211)	3.439 (0.078)	2.471 (0.055)	2.029 (0.010)
	Bis-GMA	0.367 (0.070)	0.539 (0.236)	0.239 (0.061	0.427 (0.025)	0.189 (0.020)
	UDMA	3.264 (0.168)	6.239 (0.411)	5.563 (0.377)	4.849 (0.237)	3.871 (0.459)
Filtek Z250	TEGDMA	1.942 (0.021)	1.939 (0.012)	4.082 (0.084)	1.619 (0.063)	0.000 (0.000)
	Bis-GMA	0.339 (0.010)	0.340 (0.000)	0.338 (0.014)	0.334 (0.000)	0.000 (0.000)
	UDMA	2.421 (0.121)	3.739 (0.150)	2.123 (0.201)	2.482 (0.090)	0.503 (0.861)

The kinetic curves of monomers leached from each of the tested composites are presented in Figures 4, 5 and 6. The graphs showed a reduction in monomer concentrations at the end of 28 days of water storage. TEGDMA and UDMA monomers presented the highest rate of release at the first 7 days, with the exception of TEGDMA in the Filtek Z250 composite, which presented a releasing peak at 14 days. For the Herculite XRV, the highest rates of lixiviation of the Bis-GMA monomer occurred at 24 h and 14 days, whereas in Tetric Ceram and Filtek Z250, most of the leaching rate was at 7 days.

DISCUSSION

The mechanical properties of dental composites are largely affected by an aqueous environment (Osayed and Ruyter., 1986; Söderholm and Roberts., 1990). The water absorption has been shown to be diffusion controlled occurring mainly in the resin matrix (Braden et al., 1976). The presence of water inside the composite facilitates the elution of unreacted monomers and other components. Several factors contribute to this process of elution, such as the chemistry of the solvent, the size and chemical composition of the elutable species and the extension of the polymerization reaction (Ferracane, 1994).

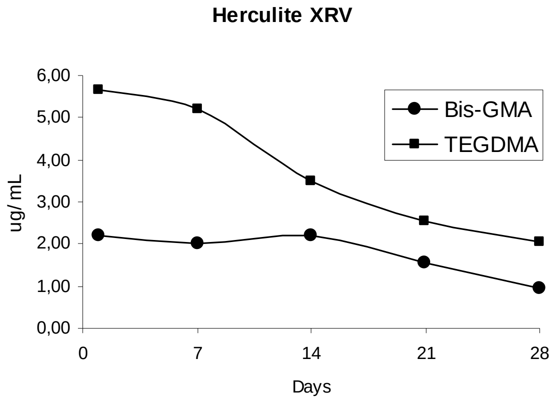

Figure 4 - Concentration profiles of monomers TEGDMA and Bis-GMA released from Herculite XRV into water, plotted against time.

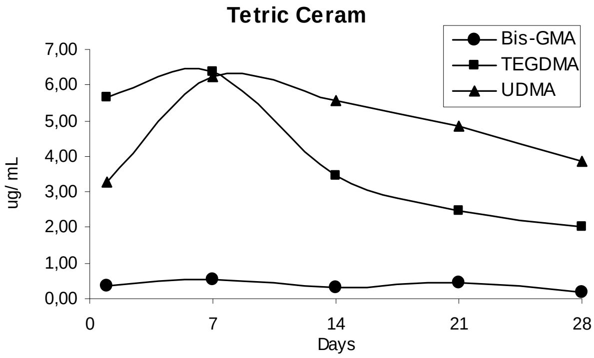

Figure 5 - Concentration profiles of monomers TEGDMA, Bis-GMA and UDMA released from Tetric Ceram into water, plotted against time.

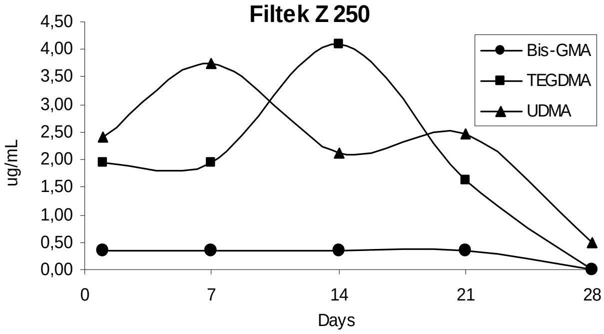

Figure 6 - Concentration profiles of monomers TEGDMA, Bis-GMA and UDMA released from Filtek Z250 into water, plotted against time.

The interaction between the material and the surrounding environment is affected by the nature of the solvent in which a composite is immersed. Laboratory studies have used different storage substances, as water, artificial saliva, alcohol, and acid or basic solvents (Ferracane , 2006). The rate and extent of elution appear to be greater in organic solvents, as compared with elution into pure water. This difference can be attributed to the greater ability of the organic solvent to penetrate and swell the polymer network, facilitating the liberation of unreacted monomers and promoting a stronger degradative effect (Ferracane, 1994). Considering the oral environment, the use of an aqueous solution appears to be reasonable clinically, but it might be insufficient to disclose the potential release of hydrophobic materials (Noda et al., 1999). Although using water as solvent, this study demonstrated that HPLC was able to identify detectable quantities of leachable monomers of the tested composites. This was confirmed by the linearity of the method obtained by the concentration interval and the quantification limits for the three monomers.

The monomers Bis-GMA and TEGDMA were found in all the three composites used in this study. The lowest elution rate among all the monomers was attributed to Bis-GMA, which might be explained by the increased molecular weight and size, reducing the mobility of the molecule and its ability to diffuse out (Spahl et al., 1998). Smaller molecules are expected to leach more and faster than larger molecules (Thompson et al., 1982; Spahl et al., 1998; Pelka et al., 1999; Örtengren et al., 2001). This was observed in the present study by the higher concentration of TEGDMA in all tested composites, when compared to Bis-GMA, which was in agreement with other studies (Tanaka et al., 1991; Spahl et al., 1998; Pelka et al., 1999; Örtengren et al., 2001).

The composites Tetric Ceram and Filtek Z250 exhibited high elution of UDMA. A possible reason for this might be the amount of this monomer in the resin matrix that could promote a higher rate of unreacted monomers. The fact that Tetric Ceram was the composite with the highest monomer lixiviation could be due to the increased UDMA release of this material, whereas Herculite XRV and Filtek Z250 demonstrated lower amounts of monomer leaching. According to a recent study (Sideridou et al., 2003), composites based on a Bis-GMA/TEGDMA resin matrix presented lower solubility and higher cross linking density when compared to other monomer combinations.

The values obtained in the present study could be higher if the immersion in water was done immediately after the light polymerization of the specimens. The desiccation of specimens at 37°C was carried out until a constant mass was achieved, and this period could have allowed the post-polymerization of the composites, increasing the degree of conversion and reducing the amount of unreacted monomers (Pearson and Longman, 1989; Ferracane, 1994; Lygre et al., 1999; Ferracane, 2006). A recent study (Mortier et al., 2005) evaluated the solubility of different dental composites with and without initial dehydration and found values up to 8-times higher for specimens not previously desiccated. Another reason for the present results could be the high light intensity of the second-generation LED used in this study associated to an increased time of exposure, resulting in a higher degree of conversion of the composites.

Most of the methacrylate groups remain bound within the polymerized composite and only a small fraction of the unreacted molecules present in a dental composite are capable of being leached (Tanaka et al., 1991; Ferracane, 1994). The use of high performance liquid chromatography (HPLC) in the current study allowed a quantitative analysis of the lixiviation of monomers (µg/mL), resulting in greater accuracy and reliability of the obtained data. However, other studies (Inoue and Hayashi, 1982; Pearson and Longman, 1989; Tanaka et al., 1991; Ferracane, 1994; Spahl et al., 1998; Pelka et al., 1999) reported the amount of leachable components of dental composites as a percentage of the initial weight of the specimen.

The leaching curves of the monomers in this study revealed that most of the monomers were lixiviated within the first seven days. However, detectable amounts of some monomers were found at the 28-day period of storage in water. This could suggest that extended periods of time might be used for detection of eluates from composites. Örtengren et al (2001) investigated the elution of monomers from resin-based materials with storage times ranging from 4 h to 180 days, observing a maximum monomer concentration after seven days. Wataha et al (1999) reported that several commonly used resin-based restorative materials continued to release biologically relevant amounts of mass into artificial saliva even after aging for two weeks. In contrast, Ferracane and Condon (1990) reported that about 85-100% of the elutable species were extracted within 24 h. However, the authors did not desiccate the specimens before weighing and immersion in water or water/ethanol, which could have resulted in higher detectable rates of eluates.

The chemical composition of resin-based restorative materials affects directly the amount of leachable components. Therefore, in order to minimize the extractable quantities of residual monomers and additives from composites, less water soluble monomers and polymerizable additives should be used and all components of dental composites should be declared by the manufacturer (Geurtsen, 1998; Spahl et al., 1998). Additionally, dental clinicians should achieve a high degree of conversion by using light curing units with the proper intensity and exposure time.

The results of the present study demonstrated that HPLC was able to identify Bis-GMA, TEGDMA and UDMA in detectable quantities for the tested composites. TEGDMA and UDMA were the monomers leached in higher amounts, whereas Bis-GMA was found in lower concentrations. Most of the monomers demonstrated peak of leaching at seven days. Tetric Ceram was the composite with the highest concentration of released monomers.

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RESUMO

A liberação de monômeros residuais pode afetar o comportamento clínico e a biocompatibilidade dos materiais resinosos. O objetivo deste estudo foi detectar e quantificar os principais monômeros residuais liberados de resinas compostas, usando cromatografia líquida de alta performance (HPLC). Discos foram construídos de resinas compostas de uso odontológico (Herculite XRV, Tetric Ceram and Filtek Z250) e imersos em água deionizada a 37°C durante 28 dias, com mudanças de água em 24 horas, 7, 14 e 21 dias. As concentrações médias dos monômeros residuais foram submetidas ao teste de Kruskal-Wallis ($p<0,05$). Tetric Ceram apresentou as maiores concentrações de monômeros lixiviados. Bis-GMA foi o monômero liberado em menores concentrações para todos os materiais. Não houve diferença estatística significante entre TEGDMA e UDMA. A maioria dos monômeros demonstrou máxima concentração no período de 7 dias. A análise por meio de HPLC identificou Bis-GMA, TEGDMA e UDMA em quantidades detectáveis para todas as resinas compostas testadas.

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