

Evaluation of TEGDMA leaching from four resin cements by HPLC

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ABSTRACT

Objective: The aim of this study was to evaluate the elution of TEGDMA from dual cured resin cements, used for bonding of ceramic restoration by high performance liquid chromatography (HPLC).

Methods: Forty freshly extracted caries and restoration free molar teeth used in this study. Standardized Class I preparations were prepared in all teeth. Ceramic inlays were cemented with one of the dual cured resin cements (Variolink II, Rely X ARC, Rely X Unicem and Resilute). After cementation, specimens were stored in 75% ethanol solution. HPLC was used to analyze the amounts of TEGDMA in different time intervals. Two-way ANOVA and Tukey HSD tests were used to evaluate the results ($P < .05$).

Results: The amount of TEGDMA eluted from Resilute was the highest and the amount of TEGDMA eluted from Rely X Unicem was the lowest ($P < .05$). The total amount of monomers was the highest after 21 days ($P < .05$).

Conclusion: In the case of resin cements, elution of TEGDMA was the highest in Resilute and lowest in Rely X Unicem. The amount of TEGDMA eluted from resin cements was influenced by the time. (Eur J Dent 2012;6:255-262)

Key words: Residual Monomer; TEGDMA; resin cements; HPLC; ceramic; inlay; ethanol

INTRODUCTION

In recent years, many luting agents and resin cements have been introduced to clinicians with the claim of clinically better performance than existing materials due to improved characteristics. The luting of indirect restoratives to abutments is

critical in achieving proper performance of indirect restorations.¹

Luting materials must undergo a chemical reaction to harden. This reaction can be initiated in one of three main manners: (1) mixing two or more different components of the material, which is improperly termed chemical polymerization; (2) activating photosensitive molecules of the material in visible light polymerization; or (3) a combination of both methods, called dual polymerization. It is relevant to clinicians that the type of polymerization greatly influences three important aspects of the luting procedure: its control, its pace, and access.²

Dimethacrylate monomers are widely used in dentistry and form an essential ingredient in dentin bonding agents, restorative dental composites,

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luting agents and fissure sealants.^{3,4} The selection of the monomer strongly influences the reactivity, viscosity, and polymerization shrinkage of the monomer, as well as the mechanical properties, water uptake, and swelling by water of the resin.⁵ The resin matrix includes one or more so-called 'heavy' monomer systems (e.g. Bisphenol A glycidylmethacrylate, Bis-GMA, urethanedimethacrylate, UDMA) and 'light' co-monomer systems (e.g. triethyleneglycoldimethacrylate, TEGDMA, hydroxyethylmethacrylate, HEMA) to reduce the viscosity of monomers and to enhance bonding strength to dentine.⁶

Acrylates, mainly methacrylates, were found to cause cytotoxic effects. Evaluation of the cytotoxicity of 39 acrylates and methacrylates that were used in dental resin materials showed a relationship between their structure and the degree of cytotoxicity.¹ The mechanism of cytotoxicity induced by TEGDMA in human fibroblasts has been recently studied.⁷ TEGDMA was recently found to be moderately mutagenic in V79 cells in subtoxic concentrations,⁸ and might promote the proliferation of the important cariogenic microorganisms *Lactobacillus acidophilus* and *Streptococcus sobrinus*.⁹

The determination of the quality and quantity of the residual monomers is usually performed by using high-performance liquid chromatography (HPLC),¹⁰ as it is a very powerful and commonly used separation method. It is preferred to gas chromatography because it provides a greater level of control over the separation process, in this case because the monomers are soluble in the mobile phase.¹¹

The elution process of monomers released from dental resin composites has been widely studied in the literature.¹²⁻¹⁹ The main concerns are the amount of leachable monomers and duration of time needed for the complete elution, but there is little information about the roles of the size and chemical characteristics of the monomers. In addition, there is contradictory information about the time needed for the complete elution of the extractable amount of unreacted monomers.²⁰ Some studies have indicated that the elution is completed in 1 to 7 days, while other studies have found that it lasts for a longer period of time.^{17,19} There is interest in identifying resin cements as a potential source of unreacted monomers in oral and other environmental tissues.

The aim of this study was to evaluate with HPLC *in vitro* elution of TEGDMA from resin cements (Variolink II, Ivoclar Vivadent AG, Schaan, Liechtenstein; Rely X Unicem, 3M ESPE, Seefeld, Germany; Rely X ARC, 3M ESPE, Seefeld, Germany; Resilute, Pulpdent Co. Watertown, USA) with dual polymerization mechanisms used for bonding of ceramic inlay restorations. The first research hypothesis was that after polymerization of resin cements there would be elution of residual monomers in ethanol/water solution. The second research hypothesis was that the number of residual monomers would increase with time.

MATERIALS AND METHODS

Forty freshly extracted caries and restoration free molar teeth were used in this study. All teeth were embedded in acrylic molds. Then standardized box-shaped Class I inlay preparations were prepared with 5-degree conical burs (no. S6845KR, Komet Dental, Gmungen, Austria) and 5-degree micro fine conical diamond burs (no. 8845KR, Komet Dental, Gmungen, Austria.) in a high-speed hand piece mounted on standard cavity machine (Nova mcm, Nova Ltd, Konya, Türkiye). Each inlay preparation was, 6 mm in length, 3 mm in width, and 2 mm in depth and had 5-degree convergence of the walls.

Impressions were made of all teeth preparations with polyether impression material (3M ESPE AG, Dental Products, Seefeld, Germany) and poured in a vacuum mixed polyurethane die material (Alpha Die MF, Schültz-Dental GmbH, Rosbach, Germany) according to the manufacturer's instructions. To fabricate IPS Empress Esthetic (Ivoclar, Vivadent, Schaan, Liechtenstein) inlays, polyurethane dies were painted with 2 coats of light polymerized die spacer (Renfert USA inc, Illinois, USA) to produce 40 µm space on lateral walls for resin cement. Inlay restorations were waxed (Yeti Dental Produkte GmbH, Engen, Germany) and sprued. Ceramic inlays were pressed after investment. All procedures were performed with IPS Empress Esthetic materials and protocol. After divestment, ceramic inlays were finished with diamond burs (no. 863-11-016, Komet Dental, Gebr Brasseler, Germany) and glazed. The adaptation of the inlay to the tooth cavity was evaluated by means of silicone replica technique. The replica specimens were sectioned bucco-lingually and

mesio-distally and the inlays were investigated for occlusal and proximal marginal gap width under a light microscope (AxioTech Vario/100 HD, Zeiss, Jena, Germany) at 200x magnification. Measurements of the marginal gap widths were performed one location and at three points at occlusal margins resulting in six measurements of occlusal location and totaling 6 measurements of each replica. At occlusal the marginal gap width was measured as the shortest distance between the edge of the inlay and the tooth structure. Mean value of gaps were obtained at $43.23 \pm 8.4 \mu\text{m}$.

Before cementation acrylic blocks were fixed to the mounting plate of a slow-speed diamond saw sectioning machine (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The roots were removed from the remaining crown approximately 1 to 2 mm below the cemento-enamel junction. Then the aperture of root sealed with amalgam (KerrAlloy, Kerr Dental, Orange, CA, USA) to prevent extra monomer leaching from pulp chamber.

The all ceramic inlays were treated with fluoridic acid (Ceramic Etchant, Ceramco, Burlington, NJ) for 1 minute and neutralized (Ceramic Etchant Neutralizer, Ceramco) in accordance with the manufacturer's instructions. For luting samples with Variolink II resin cements, Silane (Monobond S, Ivoclar) was first applied with a brush to the ceramic inlays for 60 seconds, and then a bonding agent (Heliobond, Ivoclar) was applied. After the teeth were etched, primer (Syntac Primer, Ivoclar) was applied to the tooth surface for 15 seconds, adhesive (Syntac Adhesive, Ivoclar) for 10 seconds, and then a bonding agent (Heliobond, Ivoclar) with a brush. Resin cement, comprising a combination of 50% white base, and 50% catalyst was hand-mixed following the manufacturer's directions, and applied to both prepared teeth and the ceramic inlays. For Rely X Unicem, capsules of cements were mixed 15s by Rotamix (3M ESPE, Seefeld, Germany) and applied on surfaces of restorations and dentin cavity walls. For Rely X ARC, Silane (Ceramic Primer; 3M ESPE) was first applied with a brush to the ceramic inlays for 60 s and dried for 5s. The cavity walls was etched for 15s with Scotchbond etchant (3M ESPE) and rinsed. After consecutive coats of 3M Single Bond adhesive was applied on the cavity wall and dry for 5s, cement was mixed 10s applied on surface of cavity walls and restorations. For Resilute, the adhesive sys-

tem of Variolink II was used according to manufacturer's instruction with cement. Resilute Part 1 and Part 2 was mixed and applied on surface of cavity walls and restorations. The samples were weighted before and after cementation. The eluted mean amount of Variolink II, Rely X Unicem, Rely X ARC, Resilute respectively were $22.9 \pm 3.2 \text{ mg}$, $23.58 \pm 4.8 \text{ mg}$, $17.5 \pm 3.3 \text{ mg}$, $17.1 \pm 4.1 \text{ mg}$.

The ceramic inlays were placed on the prepared teeth with light finger pressure. 21 Photopolymerization was performed with the light-polymerizing unit (Hilux Ultra Curing Unit, Benlioğlu Dental Inc., Ankara, Türkiye) at 550 mW/cm^2 (with a light tip to specimen distance of 0 mm) for 40 seconds for occlusal, lingual, and buccal surfaces. After undergoing light polymerization, excessed cements were removed by using 15 μ diamond bur (852EF.314.014, Komet Dental, Gmungen, Austria) and cleaned with a rubber cup (9402204030, Komet Dental, Gmungen, Austria) on a slow-speed hand piece for 15 s. The chemical composition is listed in Table 1.

All specimens were immersed immediately in light proof glass bottles containing 75% ethanol, 25% deionized water^{15,22} after polymerization of resin cements and stored at 37°C. The extracts were taken off for every time interval without refreshing (10 minutes., 1 hour, 24 hours, 3 days, 7 days, 14 days, 21 days) from bottles which have immersed specimens. Residual monomer (TEGDMA) which eluted from resin cement in ethanol solution were analysed with HPLC.

HPLC analysis

The analysis of extracts from the resin cement as well as reference solutions of the monomers in water/acetonitrile (25:75) was carried out by HPLC (Agilent Technologies, USA) with the following conditions:

Column: steel column (Waters Corporation, Milford Massachusetts, USA),

250 mm in length, 4.6 mm in diameter, and particle size of 5 μm .

Mobile phase: CH₃CN 75%/H₂O 25% (Acetonitrile)

Flow speed: 1 mL/min.

Detection: UV: 208 nm for TEGDMA

Injection: 10 μL loop at constant room temperature

All measurements were performed 3 times

for each of the extracts. Calibration curves were made relating eluted peak area to known concentrations of TEGDMA. The elution time for TEGDMA was 3.446 min. The concentrations of the leaching monomers from resin matrix were calculated by using the coefficients obtained by a linear regression analysis of the results from the Standard series. Linear calibration equation for TEGDMA is shown in Table 2.

The data of eluted residual monomer from resin cement in time intervals were analysed by two way analysis of variance (ANOVA) (residual cements and time intervals) and Tukey HSD test. The data of residual monomers eluted in different time intervals were analyzed by one way ANOVA and Tukey HSD tests.

RESULTS

The two way ANOVA indicated that amount of residual monomer values vary according to the materials (TEGDMA) ($P<.01$) and time intervals (10 min, 1 hour, 24 hours, 72 hours, 7 days, 14 days, 21 days) ($P<.01$) (Table 3). Mean and standard deviations of groups are presented in Table 4.

While the amount of eluted TEGDMA from Resilute was significantly higher than other three cement, the amount of eluted TEGDMA from Rely X Unicem was significantly lower than other ce-

ments ($P<.01$) and there were no statistically significant differences among Variolink II and Rely X ARC ($P=.57$)

In all resin cements investigated, the amount of TEGDMA in 10 minutes and 1 hour and 1 day were significantly lower than the amount in 21 days ($P<.01$). There were no statistically significant differences among 3, 7, 14 and 21 days ($P=.05$) (Table 4) (Figure 1).

DISCUSSION

In this study, the elution of TEGDMA from resin cements in ethanol/water solution over time is evaluated. The results obtained supported the first and second research hypotheses that after polymerization of resin cements, there would be elution of TEGDMA in ethanol/water solution over certain time intervals and that the amount of residual monomers would increase with time. This *in vitro* study measured the elution of TEGDMA from resin cements which is polymerized by a dual polymerization mechanism used for luting IPS Empress Esthetic ceramic restorations by HPLC. TEGDMA was eluted from the resin cements at all time interval.

The greater the extent of polymerization reactions, the fewer the residual monomers are available to be leached. It is known that degree of con-

Table 1. The chemical composition of resin cements.

Resin cement	Name of manufacturer	Chemical composition
Variolink II	Ivoclar Vivadent AG, Schaan/ Liechtenstein	Syntac primer: maleic acid, TEGDMA, water, acetone Syntac adhesive: maleic acid, TEGDMA, glutaraldehyde, water Heliobond: Bis-GMA, TEGDMA Paste A: Bis-GMA, urethane dimethacrylate, TEGDMA, inorganic filler, ytterbium trifluoride, initiator, stabilizer Paste B: Bis-GMA, UDMA, TEGDMA, inorganic filler, ytterbium trifluoride, benzoyl peroxide, stabilizer
Rely X Unicem	3M ESPE AG Dental Products, Seefeld/ Germany	Methacrylated Phosphoric Acid Esters Triethylene Glycol Dimethacrylate Substituted Dimethacrylate
RelyX ARC	3M ESPE AG Dental Products, Seefeld/ Germany	Ceramic primer: Ethyl Alcohol, Water Scotchbond TM Phosphoric etching gel: Water, Phosphoric, Acid Synthetic Amorphous Silica Paste A: Silane Treated Ceramic TEGDMA, BADGE Silane Treated Silica Functionalised Dimethacrylate Polymer Paste B: Silane Treated Ceramic TEGDMA, BISGMA Silane, Treated Silica Functionalised Dimethacrylate Polymer Single bond: Ethyl Alcohol, Water, TEGDMA, Bis-GMA Dimetakrilat polimerleri, HEMA 2-hidroxy-1.3-dimetakriloksiopropane Copolymer Of Acrylic and Itaconic Acids, UDMA
Resilute	Pulpdent Corporation Watertown/ USA	Syntac primer: maleic acid, TEGDMA, water, acetone Syntac adhesive: maleic acid, TEGDMA, glutaraldehyde, water Heliobond: Bis-GMA, TEGDMA Base + catalyst: Methacrylates

version (DC) of light polymerized resin materials is 55-80%.^{23,24} The DC of C=C also depends on the type, duration and intensity of the light source and some properties of the resin system such as depth of the resin material.²⁵⁻²⁷ According to Rueggeberg and Craig,²⁸ there is an inverse correlation between DC and percent of elution. Reduced irradiation increases solubility significantly.²⁹ Increasing irradiation time from 30s to 50s results in a significant decrease in residual monomer contents and the quantities which are released into water.¹⁹ In the present study, the resin cements were polymerized according to the manufacturers'

instructions with a halogen light source for 40s. Several studies have been conducted to determine the influence of the type of solvent and duration upon the release of substances from resin materials.^{15,19} Various solvents such as distilled water, saliva, ethanol, methanol and acetonitrile have been used in studies for evaluating leaching of monomers. Cross-linked dimethacrylate resins are virtually insoluble but are capable of swelling in good solvents.^{18,27} Because degradation of resins in the oral cavity depends on salivary enzymatic reactions, acidic conditions and erosive factors caused by foods and drinks,³⁰ organic solvents such as ethanol, methanol or mixtures of these solvents with water are especially preferred to simulate oral conditions.²⁷ Organic solvents have the ability to penetrate and swell the polymer network, facilitating the liberation of unreacted and leachable monomers. As the solvent penetrates the matrix and expands the openings between polymer chains, oligomers diffuse out.²⁷ Intraoral fluids represent solvents probably somewhere between the more aggressive organic solvents and water; the US FDA recommends a 75% ethanol-water solution as a food/oral-simulating liquid in order to be clinically relevant.²³ Therefore, in the present study, 75% ethanol-25% deionized water was used

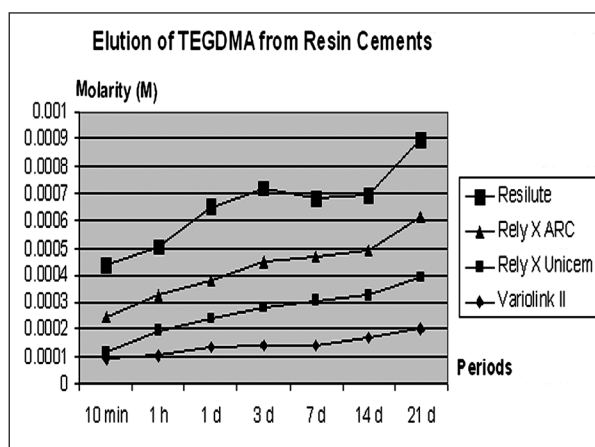


Figure 1. Cumulative monomer leaching values categorized according to resin cements and time intervals. Values are presented as Molarity (M).

Table 2. Linear calibration equations for TEGDMA.

Monomer	λ (nm)	r^2	Equation
TEGDMA	208	9.899	$y = 7.8365E+05x + 1.8544E+02$

Table 3. Two way analysis of variance indicates the amount of residual monomer values vary according to the materials. SS: Sum of square, DF: Degree of freedom, MS: Mean square.

	SS	DF	MS	F	Sig.
Material	4.54E-07	3	1.51E-07	22,09	.000
Period	2.58E-07	6	4.30E-08	6,27	.000
Material & Period	7.80E-08	15	5.20E-09	0,76	.723

*E indicates 10^x

Table 4. TEGDMA concentrations eluted from the resin cements in 7 different time intervals.

Periods	Variolink II	Rely X Unicem	Rely X ARC	Resilute
10 min	$89.4 \pm 42.9E-6^a$	$26.8 \pm 13.7E-6^A$	$130.4 \pm 71.7E-6^A$	$188.32 \pm 55.4E-6^a$
1 hours	$101.5 \pm 65.2E-6^{a,b}$	$93.7 \pm 60.1E-6^{A,B}$	$132.2 \pm 90.8E-6^A$	$178.5 \pm 72.4E-6^a$
1 day	$133.8 \pm 44.7E-6^{a,b}$	$105.9 \pm 73.4E-6^{A,B}$	$138.3 \pm 71.6E-6^A$	$271.2 \pm 115E-6^a$
3 days	$139.0 \pm 37.8E-6^{a,b}$	$146.1 \pm 58E-6^{A,B}$	$160.2 \pm 79.0E-6^A$	$269.5 \pm 91.7E-6^a$
7 days	$142.5 \pm 60.1E-6^{a,b}$	$161.9 \pm 104E-6^{A,B}$	$161.3 \pm 75.5E-6^A$	$216.3 \pm 64E-6^a$
14 days	$173.6 \pm 68.2E-6^b$	$149.5 \pm 122E-6^{A,B}$	$167.1 \pm 79E-6^A$	$205.7 \pm 104E-6^a$
21 days	$203.8 \pm 55.4E-6^b$	$190.2 \pm 130E-6^B$	$219.9 \pm 120E-6^A$	$282.6 \pm 133E-6^a$

*Groups with different type of letters are statistically significantly different.

The concentration values were calculated as M (Molarity).

as an extraction medium to measure monomer release.

Adequate polymerization is crucial in obtaining optimal physical properties and clinical performance of resin composites.³¹ Ideally, a dental restorative resin might have all of its monomers converted to polymers during the polymerization reaction. Dual-cure materials are intended to be more effective in the early stages of polymerization because they contain both photoinitiators and components for a chemically activated reaction. Braga et al³² investigated the early shear strength of porcelain-dentin bonding using dual-cure cements at 10, 30, and 90 min and reported significant differences between 10 and 30 min. Krishnan et al³³ reported that the solubility of visible light-cured dental composite was found to increase with time in their study on the effect of diluents on the properties of a visible light-cured dental composite at specific intervals of 1, 7, 14, 21, 28, and 30 days. In addition, Kavara et al³⁴ investigated the elution of residual monomers by HPLC analysis at time intervals of 1, 3, 6, 12, and 24 hours and 3, 7, and 14 days. To determine early and late elution of monomers from dual-cured resin cement, the time intervals of 10 min, 1 hour, and 1, 3, 7, 14, and 21 days were determined.

Molecules of high molecular weight, base monomers such as Bis-GMA and UDMA, however, decompose in gas chromatographs and only the decomposition products of these are detectable.¹⁶ For this reason most studies on large monomers have been analysed by HPLC,^{10,13,16} which is preferred to gas chromatography because it provides a greater level of control over the separation process, in this case since the monomers are soluble in the mobile phase.¹¹ HPLC analysis was used in this study to evaluate monomer release from resin cements because it is a very powerful and commonly used separation method.

The polymer network is composed of cross-linked molecules within which the unreacted monomers reside. As the solvent penetrates the matrix and expands the openings between polymer chains, monomers diffuse out. Although complete saturation of the composite with solvent requires weeks or months as a result of the slow nature of the diffusion of chemicals into the cross-linked resin matrix, elution appears to be completed within days because subsequent weight changes are so

small as to be almost immeasurable. Therefore, although further leaching may occur with time, the majority of the elutable species are extracted from any exposed surface within a matter of hours.²³ A study conducted by Lee et al¹⁴ concluded that the elution of monomers from dental resin composite specimens stored at 37°C for 7, 14, and 30 days increased as a function of time, and the quantity of monomers in 30 days storage was 5-7 times higher than what was produced after 7 days storage. This result is in accordance with the results of the current study, which concluded that the cumulative quantity of residual monomers was the highest after 21 days storage for both resin cements.

Materials are applied to enamel or dentin or both, and most also come into contact with oral fluids after placement. Some adverse effects have been reported following clinical use of the materials, although fortunately these are of low to moderate incidence.²² The cytotoxicity ranking of the most widely used monomers was BisGMA>UDMA>TEGDMA>HEMA>MMA. It was further shown that the cytotoxicity of these substances was related to their lipophilicity.^{35,36} In the present study, the highest mean concentrations of residual TEGDMA from resin cements (Variolink II, Rely X ARC, Rely X Unicem, and Resilute) were 203.8 µM, 219.9 µM, 190.1 µM, and 282.5 µM, respectively. According to other studies³⁷⁻⁴⁰ on the cytotoxic effects of residual monomers, these concentrations of residual monomers in the current study may cause adverse reactions in human cells.

Elution of residual monomers from resin materials involves degree of their polymerization, properties of resin composition, and chemistry of organic solvents *in vitro*.²³ Several factors affect the elution process of residual monomers *in vivo*. One of the factors involves dentists applying resin materials. From this perspective, the application and polymerization process of resin materials according to manufacturers' instructions gains importance. In addition, seating of restorations and the thickness of resin materials between tooth and restoration are important due to contact between the surface of the resin material and oral fluids. The second factor involves patients and their oral environments. Oral fluids of humans can differ according to their chemical composition, enzymes and oral stresses. The third factor involves the steps of the application process of resin materi-

als. With increasing steps, the operational sensitivity may be influenced so that the polymerization of resin materials might be insufficient. Monomer conversion values of dentin bonding agents applied and light-cured alone were not determined in this study. Because the chemistry of the analysed interface changed when the resin was applied and diffused into the uncured bonding agent, direct comparison of conversion values between the light-cured bonding agent alone and that of the mixture of the bonding agent and cement cannot be made. Thus, the significance of these differences is not known.⁴¹ For this reason, the experimental setup did not consider the effects of an *in vivo* situation, and elution of the residual monomers measured cannot be directly applied to the elution of residual monomers *in vivo*. Therefore, *in vivo* studies are needed for the evaluation of residual monomers and their effects.

CONCLUSION

1. Residual monomer (TEGDMA) was eluted from resin cement in all time interval.
2. The cumulative amount of eluted TEGDMA was increased as a function of time.
3. The highest cumulative amount of TEGDMA was detected in 21 days.
4. While the highest cumulative amount of TEGDMA was eluted from Resilute, Rely X Unicem showed the lowest TEGDMA elution.
5. The highest amount of eluted TEGDMA concentration detected was viewed as critical for toxic reactions in human cell.

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