

## Research Article

# Effect of Polymerization Time on Residual Monomer Release in Dental Composite: In Vitro Study

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Light activated resin-based composites are the most accepted and used materials among clinicians. The aim of this study is to determine the amount of residual monomer released from nanofiller composite resins for different polymerization times and storage periods in vitro. To this purpose, Tetric Ceram (Ivoclar, Liechtenstein), Clearfil Majesty Posterior (Kuraray, Japan), Grandio (VOCO, Germany), and Filtek Ultimate Universal (3M, USA) were used as nanofiller resin composites samples. Four groups ( $n = 40$ , diameter: 5 mm, thickness: 2 mm) of each material were fabricated, and each group was exposed to three different polymerization time (10, 20 and 40 sec). High-performance liquid chromatography (HPLC) was used to measure the amount of monomers released over 1, 15, and 30 days. The highest amount of monomer release was seen in Tetric EvoCream composite, while the least monomer release was seen in Clearfil Majesty composite. Regardless of the polymerization time, material, or storage period, the highest amount of eluted monomer was Bis-GMA. It is observed that there is no statistically significant difference between various polymerization times. Monomer release reached its highest level on the 15th day and decreased on the 30th day for all composites. Polymerization time did not affect the monomer release from the composites, but the type of the monomers and concentration of the filler used in the composites affected the amount of released monomers. The use of TEGDMA (co)monomer reduced the monomer release.

## 1. Introduction

Methacrylate-based polymers have different uses in the human body, one of which is dental composites. Dental composites are stabilized by forming polymer networks. To stabilize these networks, liquid polymers are usually converted into a solid or gel form by the formation of crosslinks [1, 2]. Chemical crosslinking provides mechanical stability and insolubility of the polymer by restricting the movement of the chain. It improves the mechanical properties of hydrogels [3]. Dental resin-based restorative materials are complex polymers. The main organic components such as bisphenol A glycol dimethacrylate (Bis-GMA), urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA), methacrylate 2-hydroxyethyl methacrylate (HEMA), and ethoxylated bisphenol A-dimethacrylate (Bis-EMA) crosslink with smaller monomers to form a solid polymer network during polymerization [4]. Bis-GMA is the main component

of resins as it has good mechanical properties, chemical stability, and the ability to mimic natural tooth color. UDMA has advantages such as lower viscosity, additive to adhesion, higher hardness due to the flexibility of urethane bonds, and biocompatibility, used as an alternative to Bis-GMA [5]. TEGDMA is a diluent that increases fluidity and decreases viscosity, providing higher inorganic content in the resin. It also reduces glass transition temperatures, mesh stiffness, and increases polymer conversion rate [6]. The diffusion within the network is restricted, and the conversion of monomers to polymers is limited (degree of conversion (DC) 45-70%) during crosslinking progress; so, the optimal (100% polymerization) cannot be reached [7, 8]. Residual monomers may remain in the post polymerized material or may dissolve and slowly release from the polymer, especially in a wet environment, due to insufficient polymerization [9, 10]. Incomplete polymerization relates to some factors such as light intensity, curing time, and material thickness [11].

Another reason for releasing is that reactive sites (double bonds) undergo hydrolysis or oxidation, causing the deterioration of the material [10]. The harmful residual monomers and small and hydrophilic substances, which are absorbable by cell culture medium or artificial saliva [9, 12], can easily spread throughout the body by the body fluids. Different studies have shown that the release of residual monomers can have local and systemic side effects for the human body [12–15]. This toxicity depends on the amount of the residual monomers and structural and chemical composition [16] of the polymerized material, the type, duration, and intensity of the application of light sources [17].

In the last 10 years, one of the most important innovations in this area has been the introduction of nanotechnology into composite resins. Nanotechnology is the creation of macroscale structures by various processes of materials in the size of 0.1–100 nm (nanometers) [18]. In the new generation hybrid composites, there are filler particles of 0.04–20 nm in size. Using particles of this size changes the content and formulation of the conventional matrix. The developments are not only limited to this, but also the traditional monomer types are changing. This brings a more homogeneous matrix distribution with smaller particles and reduces the monomer matrix volume. As a result, the negative properties of the composite such as residual monomer release and polymerization shrinkage are reduced [19].

The main aim of this study is to assess the amount of the released monomers' elution between four different nanofiller composite materials after different polymerization times (10, 20, and 40 sec) in different polymerization periods (day 1, 15, and 30).

The null hypothesis tested is that the amount of monomer released from the different nanofiller composite eluting below the clinical suitability ranges over the entire selected polymerization period and not showing a difference in various monomer elution between the selected polymerization times.

## 2. Material and Methods

**2.1. Sample Preparation.** Four hybrid [Tetric Ceram (Ivoclar, Liechtenstein), Clearfil Majesty Posterior, (Kuraray, Japan), Grandio, (VOCO, Germany), and Filtek Ultimate Universal (3M, USA)] resin composite were examined. Used materials are given in Table 1. Standard Teflon cylinder molds with 5 mm diameter and 2 mm height were prepared. Composites were placed inside the molds. All composites were polymerized with Woodpecker LED B brand light source (Guilin Zhuomuniao Medical Devices, China). Three different polymerization times were tested: 10 s, 20 s, and 40 s. In total, 30 samples were prepared from each composite ( $n = 120$ ). Medium coarse polishing discs (Super-Snap Buff Disk, Shofu Dental GmbH, Germany) were used to eliminate the oxygen inhibition layer that could form on the material surface.

120 sample discs were used in total, which were placed in 20 mL amber-colored glass bottles with a vacuum cap, containing 75% ethanol/25% water solution (Merck). All bottles were kept in an oven at 37°C until the measurements were made. The storage medium was renewed after 1, 15, and 30

days. At the time of measurement, 1 mL samples were taken from these solutions with the help of Eppendorf's and transferred to the opaque 1.5 mL amber-colored glass vials. Thus, by taking samples from stock solutions in three different periods, a total of 360 injections were made suitable for entering the HPLC device.

**2.2. HPLC Analysis.** For the calibration of the Agilent 1260 Infinity II Quaternary LC HPLC device (Agilent Technologies, USA), stock solutions of monomers were diluted. 6 different solutions (0.1, 1, 10, 100, 500, 1000 parts per million (ppm)) were prepared for each monomer, and these were injected into the HPLC system to calibrate the device. Retention times for Bis-GMA, UDMA, and TEGDMA standard monomers (Sigma Aldrich, USA) were 5.3, 4.9, and 4.4 minutes, respectively.

Residual monomer measurements, C18 reverse phase analysis column (Agilent ZORBAX Eclipse XDB columns-C18, Agilent Technologies, USA), and diode array detector (Diode Array Detector, Agilent Technologies, USA) with a particle size of 5  $\mu\text{m}$  attached to the HPLC device with dimensions 25 cm  $\times$  4.6 mm were carried out. The analysis was performed at room temperature, and the flow rate of the mobile phase was 1 mL/min. At this stage, 80% acetonitrile/20% water solution (ACN Merck) was used as the mobile phase. 20  $\mu\text{L}$  of fluid was injected into the column from each sample to obtain 204 nm wavelength chromatograms where monomers exhibit maximum absorption.

**2.3. Statistical Analysis.** The repeated measures analysis divides ANOVA factors into two types: between subjects' factors and within subject factors. Two between subjects' factors which are the composite resins, and the irradiation times, also three within subjects' factors which are measurement times of released monomers (1st day, 15th day and 30th day) were analyzed. Three different monomers (Bis-GMA, UDMA, TEGDMA) released per composite resins under the same between subjects' factors combination in the remaining solvent for different periods were measured. After completing the repeated measurement analysis, then TUKEY multiple comparison tests were used for obtaining significance among between subject factors levels. These analyses were performed for all monomers. Finally, paired  $t$ -test was used to compare the amount of released monomers with each other (Bis-GMA, UDMA and TEGDMA) in the remaining solvent for each treatment combination.

## 3. Results

The amounts of monomer released from the composites are given in Table 2.

The Bis-GMA is the most released monomer in all the different polymerization times, with no statistically significant difference in its release in different polymerization times ( $p \geq 0.05$ ). The UDMA is the second most released monomer in all the different polymerization times, with no statistically significant difference in its release at different polymerization times ( $p \geq 0.05$ ). TEGDMA is the least released monomer in all the different polymerization times, and it did not show a

TABLE 1: Composite materials used.

	Organic matrix*	Composition
Grandio (VOCO GmbH, Germany)	%2.5-5 Bis-GMA, $\leq$ %2.5 TEGDMA, UDMA	87 wt% or 71.4 vol% inorganic fillers
Filtek Ultimate Universal (3 M, USA)	%1-10 BIS-GMA, %1-10 UDMA, % <1 TEGDMA, < %5 PEGDMA BIS-EMA	78.5 wt% or 63.3 vol% inorganic fillers
Clearfil Majesty Posterior (Kuraray, Japan)	% <3 Bis-GMA, % <3 TEGDMA, UDMA	92 wt% or 82 vol% inorganic fillers
Tetric EvoCeram (Ivoclar Vivadent, Liechtenstein)	%2.5- <10 Bis-GMA, %2.5 <10 UDMA, %2.5- <10 Ytterbium trifluoride %2.5- <10 Ethoxylated Bis-EMA	Prepolymers 34.0 wt%, 75-76 wt%, or 53-55 vol% inorganic

Composition of tested materials provided by manufacturers. \*The specific chemical identity and/or exact percentage (concentration) of this composition has been withheld as a trade secret.

statistically significant difference in its release in different polymerization times ( $p \geq 0.05$ ). There was no statistically significant difference in total monomer release averages according to different polymerization times ( $p \geq 0.05$ ) (Figure 1).

**3.1. BIS-GMA.** The results show that the highest value of Bis-GMA monomer is released from Tetric EvoCream, then the second highest value of it from Grandio. There is also significant difference between them and with the other composites ( $p < 0.001$ ).

The amount of monomer release detected on the 15th day was significantly higher than the amount of monomer release on the 1st ( $p = 0.003$ ) and 30th day ( $p < 0.001$ ) in all groups. The difference of the Bis-GMA monomer released from groups which have 10-, 20-, and 40-second polymerization times was not significant ( $p = 0.612$ ) (Figure 2).

**3.2. UDMA.** The results show that the highest value of UDMA monomer is released from Tetric EvoCream, then the second highest value of it from Filtek Ultimate. There is a significant difference between them, also with the other two composites ( $p < 0.001$ ). Significant difference was found between the 1st and 15th day of UDMA released ( $p = 0.007$ ). The difference of the UDMA monomer released from groups which have 10-, 20-, and 40-second polymerization times was not significant ( $p = 0.954$ ) (Figure 2).

**3.3. TEGDMA.** TEGDMA was not detected in Tetric EvoCeram composite resin samples. The most TEGDMA releasing material was found to be Grandio, the least TEGDMA releasing material was found to be Filtek Ultimate, and there is a significant difference between the composites ( $p < 0.001$ ). The amount of monomer release detected on the 15th day was significantly higher than the amount of monomer release on the 1st ( $p = 0.003$ ) and 30th day ( $p < 0.001$ ) in all groups. No significant difference was found between the 1st and 30th day of TEGDMA released from Filtek, Grandio, and Clearfil ( $p = 0.295$ ). The difference of the TEGDMA monomer released from groups which have 10-, 20-, and 40-second polymerization times was not significant ( $p = 0.525$ ) (Figure 2).

The least elution was observed with the eluents Clearfil Majesty and Grandio composites for all 3 monomers, on all

observation days and with all 3 polymerization times. There was no statistical difference between Clearfil Majesty and Grandio composites ( $p = 0.744$ ). The highest value of monomer release was detected in Tetric EvoCeram composite ( $p < 0.001$ ). The highest released total monomer amount was reached on day 15 for all composites (Figure 3).

## 4. Discussion

Nanofilled composites, produced by the addition of nanosized inorganic fillers to the traditional composites, provide better physical, mechanical, and optical properties: higher fracture resistance, high elasticity modulus, less polymerization shrinkage, high surface processability, high translucency, and closeness to the original color [20]. This study evaluated the release of Bis-GMA, TEGDMA, and UDMA at different polymerization times and storage periods from four nanocomposite resins.

Increasing polymerization times did not lead monomer elution decrease in our study. Monomer release is continued during storage periods (1st, 15th, and 30th days). Therefore, our null hypothesis is not rejected.

Bis-GMA and UDMA were the most released monomers. This may be due to the fact that these are the main monomers used in resins, and that these main monomers are used in the resin with the high ratio. Since the brands of the materials withheld the rates as a trade secret, we cannot interpret the relation clearly. Many researchers such as Sideridou and Achilias, Gorgen, and Polydorou stated that Bis-GMA and UDMA are the most released monomers in their studies [16, 21–23].

In our study, TEGDMA used as (co)monomer was the least released monomer. Although TEGDMA is expected to be released more due to its low molecular weight (Bis-GMA mw:512 g/mol, UDMA mw; 470 g/mol, TEGDMA mw; 286 g/mol) [16], it was not the case in this study. The reason for this can be explained by the fact that it is a small monomer with low weight which allows increased polymerization by exhibiting high reactivity in the composite.

The composite releasing the least monomer is the Clearfil Majesty which includes the highest amount of inorganic filler between the composites. As the filler ratio is increased, the amount of monomer release is decreased. We argue that the reason of this decrease is the use of low organic resin matrix.

TABLE 2: Mean values (ppm) and standard deviations of the monomers released.

	Day 1 Monomers	Day 15 Composites	Days	
			Day 30 Polymerization times	Mean $\pm$ SD
<b>Bis-GMA</b>				
Grandio	10 sec	2.506 $\pm$ 0.305	3.637 $\pm$ 0.446	1.934 $\pm$ 0.331
	20 sec	1.877 $\pm$ 0.189	2.623 $\pm$ 0.291	1.370 $\pm$ 0.222
	40 sec	2.231 $\pm$ 1.597	3.281 $\pm$ 2.860	1.421 $\pm$ 0.629
Filtek Ultimate	10 sec	0.727 $\pm$ 0.127	1.126 $\pm$ 0.183	0.733 $\pm$ 0.166
	20 sec	0.902 $\pm$ 0.361	1.457 $\pm$ 0.521	1.065 $\pm$ 0.336
	40 sec	0.814 $\pm$ 0.294	1.251 $\pm$ 0.440	0.885 $\pm$ 0.328
Clearfil Majesty Posterior	10 sec	0.911 $\pm$ 0.081	1.489 $\pm$ 0.122	0.874 $\pm$ 0.082
	20 sec	1.536 $\pm$ 1.110	2.330 $\pm$ 1.762	1.485 $\pm$ 1.086
	40 sec	0.841 $\pm$ 0.214	1.306 $\pm$ 0.311	0.839 $\pm$ 0.214
Tetric EvoCeram	10 sec	5.467 $\pm$ 0.299	11.074 $\pm$ 0.515	8.061 $\pm$ 0.499
	20 sec	4.710 $\pm$ 0.832	9.447 $\pm$ 2.952	7.095 $\pm$ 2.826
	40 sec	4.240 $\pm$ 0.652	9.640 $\pm$ 1.778	7.246 $\pm$ 1.397
<b>UDMA</b>				
Grandio	10 sec	0.329 $\pm$ 0.019	0.321 $\pm$ 0.028	0.176 $\pm$ 0.019
	20 sec	0.325 $\pm$ 0.074	0.292 $\pm$ 0.090	0.169 $\pm$ 0.063
	40 sec	0.402 $\pm$ 0.216	0.428 $\pm$ 0.382	0.271 $\pm$ 0.291
Filtek Ultimate	10 sec	1.487 $\pm$ 0.227	1.978 $\pm$ 0.303	1.257 $\pm$ 0.218
	20 sec	1.794 $\pm$ 0.607	2.745 $\pm$ 0.862	1.935 $\pm$ 0.545
	40 sec	1.616 $\pm$ 0.504	2.345 $\pm$ 0.791	1.598 $\pm$ 0.523
Clearfil Majesty Posterior	10 sec	0.110 $\pm$ 0.000	0.010 $\pm$ 0.000	0.010 $\pm$ 0.000
	20 sec	0.185 $\pm$ 0.110	0.088 $\pm$ 0.193	0.035 $\pm$ 0.107
	40 sec	0.135 $\pm$ 0.041	0.036 $\pm$ 0.043	0.010 $\pm$ 0.000
Tetric EvoCeram	10 sec	7.178 $\pm$ 0.368	12.806 $\pm$ 0.617	8.717 $\pm$ 0.930
	20 sec	6.288 $\pm$ 1.157	11.286 $\pm$ 3.591	7.797 $\pm$ 3.126
	40 sec	5.745 $\pm$ 0.913	11.721 $\pm$ .342	8.436 $\pm$ 1.643
<b>TEGDMA</b>				
Grandio	10 sec	0.819 $\pm$ 0.098	1.186 $\pm$ 0.119	0.197 $\pm$ 0.030
	20 sec	0.553 $\pm$ 0.043	0.938 $\pm$ 0.076	0.139 $\pm$ 0.029
	40 sec	0.667 $\pm$ 0.609	1.394 $\pm$ 1.717	0.330 $\pm$ 0.566
Filtek Ultimate	10 sec	0.000 $\pm$ 0.000	0.051 $\pm$ 0.009	0.040 $\pm$ 0.008
	20 sec	0.005 $\pm$ 0.008	0.095 $\pm$ 0.048	0.056 $\pm$ 0.005
	40 sec	0.000 $\pm$ 0.000	0.078 $\pm$ 0.022	0.038 $\pm$ 0.009
Clearfil Majesty Posterior	10 sec	0.488 $\pm$ 0.055	0.773 $\pm$ 0.073	0.406 $\pm$ 0.141
	20 sec	0.896 $\pm$ 0.737	1.426 $\pm$ 1.148	0.827 $\pm$ 0.641
	40 sec	0.402 $\pm$ 0.120	0.752 $\pm$ 0.179	0.472 $\pm$ 0.115
Tetric EvoCeram	10 sec	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000
	20 sec	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000
	40 sec	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000

Unit of all released monomers' volume is in ppm. 1 ppm = 0.001 mg/mL.

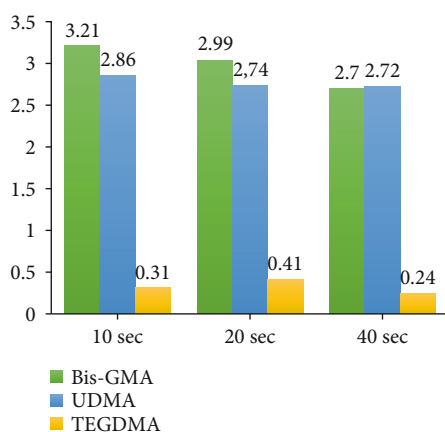


FIGURE 1: The total amount of residual monomers released. \*\*Unit of all released monomers' volume is in ppm. 1 ppm = 0.001 mg/mL.

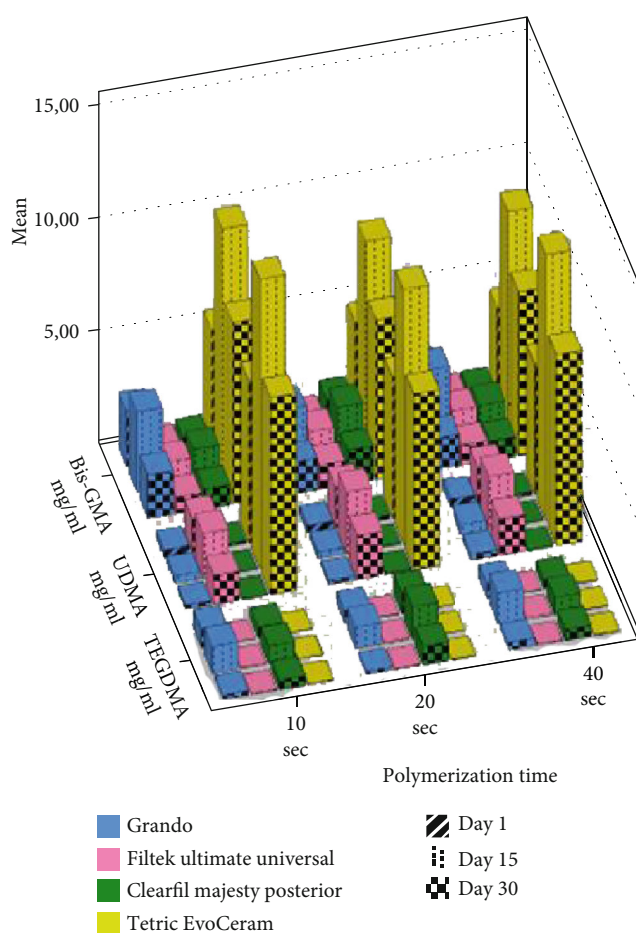


FIGURE 2: Residual monomer released from composites.

DC of the monomer is affected by its viscosity and molecular weight. Bis-GMA is a viscous monomer as it has strong hydrogen bonding between hydroxyl groups and aromatic nuclei. UDMA also has high viscosity due to the formation of intermolecular hydrogen bonds between urethane species [24]. Since the viscosities of both monomers

are quite high, it would be better to mix them with comonomers with lower viscosity to dilute them. TEGDMA is mostly used for this purpose. The viscosity level from the highest to lowest is ranked as Bis – GMA (1369 Pa s) > UDMA (28 Pa s) > TEGDMA (0.05 Pa s) [25]. Moreover, TEGDMA is used to improve filler connections, too [26]. In all samples, Tetric EvoCeram dissolves into residual monomer in the medium solution at most. Since TEGDMA monomer is not contained in the Tetric EvoCeram composite, the released monomer amount may be too high (Material Safety Data Sheets (MSDS), Tetric EvoCeram, Ivoclar). Increase in the conversion rate of the polymer is caused by Bis-GMA and TEGDMA strength in forming a three-dimensional polymer network via multiple double bonds. According to Kopperud Tetric EvoCeram composite evaluation research, decrease in the hardness of the material and increase in the residual monomer are caused by heterogeneous distribution of particles/prepolymerized fillers and its agglomeration differences in the composite by varying the scattering and the penetration depth of the polymerization light [27]. Similarly, Sideridou et al. found that a resin matrix composed of Bis-GMA/TEGDMA provides a higher cross-linking and releases less residual monomers than others [16]. In our study, Tetric EvoCeram composite without TEGDMA is found to be the most Bis-GMA and UDMA monomers releasing in all composites. These results are parallel with earlier studies.

In this *in vitro* study, although the measurement of monomers decreased over time, it was still within the detectable range even on the 30th day. Monomer release started on the 1st day, peaked on the 15th day, and tended to decrease on the 30th day. These results agree with the findings of other studies, in which Sideridou and Achilias observed that the initial high monomer release rate decreased over time [16] and in which Polydorou et al. measured the monomer release from composite resins periodically and observed that the most monomer released in the first 24 hours and the amount of release decreased on the 28th day [23]. In another study, it was observed that composite resins have reached the maximum degree of conversion at different times [28]. Since the polymerization continues in the postcuring process, releasing occurs until the degree of conversion reaches its maximum level. The monomer release resulting from the incomplete conversion shows a logarithmic function [29]. Therefore, after the conversion degree is maximized, the releasing of monomers decrease. Accordingly, we believe that it peaks on the 15th day and tended to fall on the 30th day.

According to the manufacturers' instructions, it is sufficient to polymerize the composite for 20 sec with an LED unit to achieve the appropriate hardness. One of the significant findings to emerge from this study is that the effect of the polymerization time on the elution of monomers was not significant, and increasing the polymerization time did not seem to be effective. This might be explained with the initiators and activators of the tested composites being similar and resulting similarly at different light durations. There are studies in the literature showing that different polymerization times do not create significant differences [30, 31]. However, there are studies showing that residual monomer release is negatively affected in short polymerization times [27].

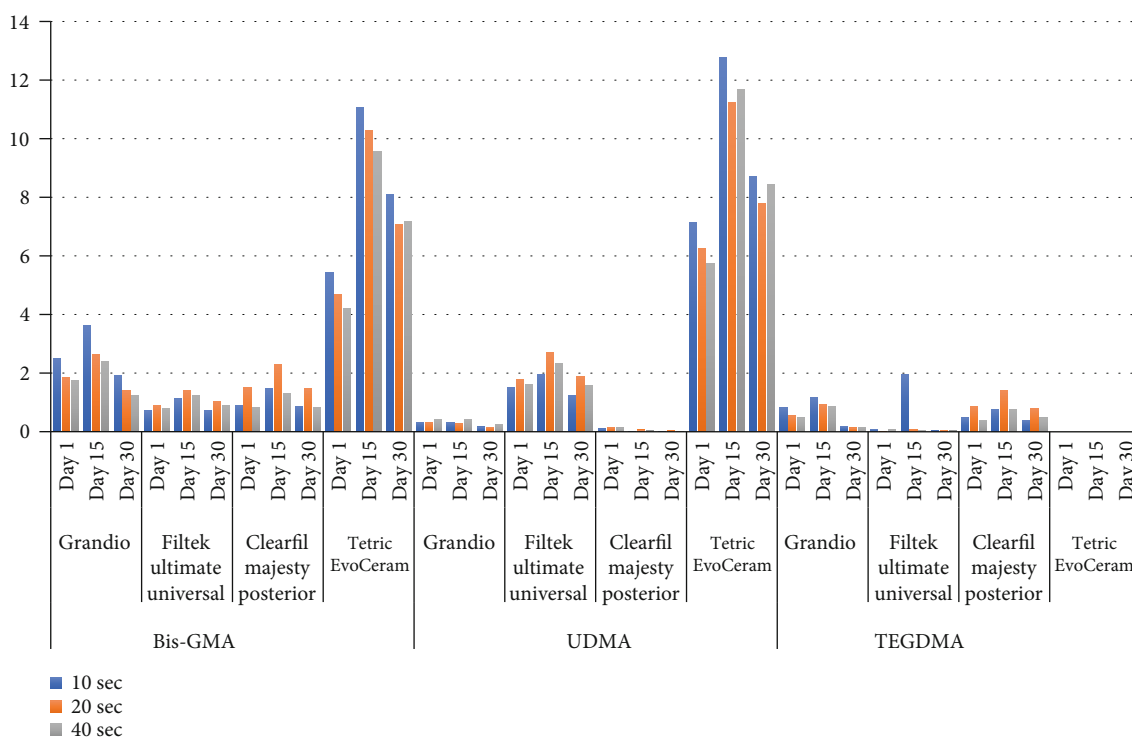


FIGURE 3: The amount of eluted (organic wt%) Bis-GMA, UDMA, and TEGDMA from different composites, at different days and polymerization times. \*\*Unit of all released monomers' volume is in ppm. 1 ppm = 0.001 mg/mL.

Identification of the released components is usually carried out by using HPLC, liquid chromatography-mass spectrometry (LC-MS), and gas chromatography (GC) [32, 33]. Under the normal circumstances, it is recommended to use GC for compounds that can evaporate. However, HPLC and LC-MS are more suitable methods for samples with high molecular weight such as Bis-GMA (mw = 512 g/mol) and UDMA (mw = 470 g/mol) and/or with high potential to deteriorate when it is heated [34, 35]. For this reason, we used the HPLC device in our study.

Apart from the chemical properties of the material, it is observed that the chemistry of the used solvent also affects the elution. Research purpose determines the type of the extraction medium. Ferrakan noticed in 1994 that oral fluids exhibit extractive properties between aggressive organic solvents and water [36]. Therefore, the FDA recommends the use of 75% ethanol/water solution as the liquid corresponds to the medium that exhibits the oral cavity conditions, and this solvent has also been used in many studies [8, 37–40]. Furthermore, Bis-GMA and UDMA did not leach into the aqueous media, while 75% ethanol/water solution is functional. However, TEGDMA was detected in all extraction media [41]. For these reasons, we used the 75% ethanol/water solution recommended by the FDA.

Commonly used dental composite resin matrix consist of BPA-based (bisphenol A) Bis-GMA and Bis-EMA and non-BPA-based TEGDMA and UDMA monomers [42, 43]. These fundamental building blocks can act as endocrine disruptors by imitating and disturbing hormone receptors.

This can result with infertility and gene expression problems in any gender [44]. In the present study, observed maximum mean  $\pm$  standard deviation values of the residual Bis-GMA are  $11.074 \pm 0.515$  ppm in Tetric Evoceram,  $2.33 \pm 1.762$  ppm in Clearfil Majesty,  $1.457 \pm 0.521$  ppm in Filtek, and  $3.637 \pm 0.446$  ppm in Grandio composites. Nontoxic BPA consumption can only be 0.05 milligrams/kilogram of body weight (mg/kg bw/day) according to EFSA (European Food Safety Authority). Average weight of a woman and a man is 50 kg and 70 kg, respectively; so, they can tolerate 2.5–3.5 mg release of bisphenol A (BPA) per day, respectively [45]. Monomers released from four nanocomposites are tolerable by the human system but according to previous studies regarding the cytotoxic effect of eluted monomers, these observed amounts might cause adverse reactions in human pulp in the present study [46–48].

One of our research's limitations is that mimicking the oral environment since 75% ethanol-water elution medium speeds up the expected process. Further examinations are needed to find its actual release process into the saliva.

## 5. Conclusion

Polymerization time did not affect the monomer release when the initiators and activators of the tested materials were similar. However, composite's content, amount, and type change the amount of released monomers. The use of TEGDMA (co)monomer reduced the monomer release. Therefore, the polymer network structure formed as a result of the

monomers' interaction with each other may have changed the amount of released monomer. The high proportion of inorganic filler by volume in the composite reduced the monomer release.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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