SATURATION REDUCES IN-VITRO LEAKAGE OF MONOMERS FROM COMPOSITES

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ABSTRACT

INTRODUCTION: Accurate knowledge of the quantity of released monomers from composites is important. To evaluate the elution of monomers from composites after polymerization *in vitro*, composite samples are typically immersed in an extraction solvent.

OBJECTIVE: To determine whether the volume of extraction solvent and the immersion time influences monomer leachability from dental composite materials.

MATERIALS AND METHODS: Composite disks of two commercial composites, (Filtek Supreme XTE, 3M ESPE and Gaenial Universal Flow, GC) were prepared. The disks (n=10) were placed in a glass vial with 1ml, 2ml or 3ml of extraction solvent (100% ethanol with deuterated diethylphthalate as internal standard). After either 7 or 30 days at 37°C, the supernatant was collected and the amount of released monomers (BisEMA, BisGMA, UDMA, TEGDMA) and Bisphenol A was measured with liquid chromatography mass spectroscopy.

RESULTS: For both tested composites, the highest amount of released monomers was measured after sample incubation in 3ml, while the lowest amount was measured in 1ml of extraction solvent. Furthermore, 30 days did not result in much more monomer release compared to 7 days, and for most monomers, there was no statistically significant difference in release between 7 and 30 days.

CONCLUSION: Release kinetics in *in-vitro* experiments are also influenced by saturation of the extraction solvent with the leached monomers. This is important as it is unlikely that saturation can be reached in an *in-vivo* situation, where saliva (or pulpal

fluid) is continuously refreshed. Saturation of the extraction solvent can be avoided *in vitro* by refreshing the extraction medium after equal time intervals.

KEYWORDS

Elution, leachability, saturation, equilibrium, composite materials

INTRODUCTION

In spite of being the standard restorative material nowadays in most countries, composite is controversial due to the release of compounds into the oral environment. Not only do composite restorations release residual monomers due to incomplete polymerization, they may also release molecules such as photoinitiator, co-initiators polymerization inhibitor and photostabilizers. [1]. After light polymerization of a composite material in a cavity, a crosslinking reaction takes place resulting in a polymer network. Initially 50 – 70 % of the functionalized methacrylate groups remain unpolymerized [2,3], but this level decreases further within the first 24h after irradiation to 30-40 %, thanks to the so-called post-irradiation polymerization. Fortunately, not all of these monomers with a remaining methacrylate group will be able to elute from composites, as most of these detectable methacrylate groups have already reacted only on one end and are thus attached to the polymer [4,5]. Nevertheless, it is estimated that around 10% of the monomers with an unpolymerized methacrylate group is actually capable to leach out in the oral environment [6,7].

As most of the composite ingredients have been associated *in vitro* with allergic reactions and with biotoxic effects such as cytotoxicity, genotoxicity and reproductive toxicity, the release of these compounds into the environment is a cause of concern.[8–11] However, accurate toxicological assessment should always be based on accurate exposure measurements. [12]

To identify and quantify compounds released from dental composites, researchers typically use an *in-vitro* method, suggested by the Food and Drug Agency (FDA) and also described by the ISO standards [13], where a composite sample is immersed in an

extraction solvent for a specified period of time at 37°C. After the extraction period, compounds emanated from composite can be identified and quantified in the extract can be analyzed with one of the chromatography methods such as gas chromatography (GC) or liquid chromatography (LC) combined with mass spectroscopy (MS). While the first method is based on separation of the compounds based on their physical properties, MS allows identification of the compounds by analyzing the mass of ionized daughter ions.

Even when these *in-vitro* conditions only weakly resemble the oral environment, where a filling is subject to mechanical, chemical and enzymatic challenges, the results of such extraction experiments can give an indication of the potential exposure to compounds released from dental composites. However, the large heterogeneity in the methodology and test set-up of these quantification studies has a great impact the outcomes of these studies. In a meta-analytical review, it was shown that the quantity of released monomers could even vary with a factor up to 100.000 between different studies. Of course, this large variation can be explained by the fact that different composite materials were tested, but also the type of extraction solvent (absolute ethanol, mixture of ethanol and water, artificial saliva, etc.), the incubation conditions (temperature, incubation time), and the analytical method play an important role. [1]

However, in the previously mentioned meta-analytical research, a weak but significant correlation was observed between the amount of extraction solvent and the amount of released monomers: the larger the volume of extraction solvent, the higher the concentration of monomers. It was suggested that this may be due to saturation of the extraction solvent with the emanated monomers. This finding could be important for future exposure research, especially with regard to long-term release. Reaching of an

equilibrium in the solvent could actually mean that the previously registered quantities in *in-vitro* experiments may underestimate the *in-vivo* situation, where it is unlikely that an equilibrium can be reached due to continuous removal of saliva. However, there are no studies evaluating the optimal amount of an extraction solvent and incubation time necessary to perform *in-vitro* studies for qualification and quantification of leached compounds from dental composites.

The aim of this study was thus to investigate the effect of the amount of extraction solvent and the incubation time on the released quantity of monomers from composite samples. The null hypothesis of this study was that the amount of solvent and the incubation time do not have an influence on the amount of released monomers from composites.

MATERIALS AND METHODS

Specimen preparation:

A composite with a paste-like consistency (Filtek Supreme XTE, Seefeld, Germany) and a flowable composite (Gaenial Universal Flo, GC, Tokyo Japan) were selected. (Table 1). Disk-shaped samples were prepared using a Teflon mold (h=2 mm; d=5.75 mm). Before polymerization, the composite material was covered with a glass plate to prevent the formation of an inhibition layer. The sample was polymerized for 40s, as per manufacturers' instruction with a light curing unit (Bluephase 20i, Ivoclar-Vivadent, Schaan, Liechtenstein) with an output $>1000 \text{ mW cm}^{-2}$ (high power mode).

Immediately after polymerization, the composite samples were then immersed in the extraction solvent (1 ml, 2 ml or 3 ml). The extraction solvent consisted of pure ethanol

(99.99%, VWR, Haasrode, Belgium) mixed with D4-diethylphtalate and D16-bisphenol A as internal standards. (Fig. 1). The first group of samples containing either 1 ml, 2 ml and 3 ml of extraction solvent (n=10) was incubated for 7 days, whereas the second group was incubated for 30 days at 37° C. The supernatants were then transferred to LC/MS vials (ref 548-0611, VWR, Haasrode, Belgium; Teflon caps, ref 548-0582, VWR, Haasrode, Belgium) and the quantity of released monomers was determined by ultra-pressure liquid chromatography mass spectroscopy (UPLC-MS/MS).

Stock solutions of BisGMA, BisEMA(3), BisEMA(6), BisEMA(10), UDMA, TEGDMA and BPA were prepared for preparation of calibration curves were prepared by dissolving commercial reference standards in the extraction solvent. Stable-isotope-labeled derivatives (D4-diethylphtalate and D16-bisphenol A) were added to overcome potential sample manipulation losses.

UPLC-MS/MS analysis:

Liquid-chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm particle size) using an Acquity UPLC system from Waters. Tandem mass analysis was carried on Micromass Quattro Premier™ Mass Spectrometer (Waters, Manchester, UK). The compounds were determined using multiple reactions monitoring (MRM), with argon as the collision gas.

The separation of BisGMA, BisEMA(3), BisEMA(6), BisEMA(10), UDMA and TEGDMA was performed at 40°C with a flow rate of 0.35 mL/min. The mobile phase was composed of water (eluent A) and acetonitrile (eluent B) containing 0.1% formic acid. The chromatographic separation program started at 10% eluent B and gradually increased to 100% in the next 2 min. This composition was held until 30s before

returning to the initial conditions. The analysis was performed using electrospray ionization (ESI) in positive mode and the quantification specific MS/MS parameters are shown in Table 2.

The mobile phase for separation of BPA consisted of water (eluent A') and methanol (eluent B') containing 0.5% ammonium hydroxide. A flow rate of 0.5 mL/min was applied. The chromatographic separation program started at 5% eluent B', followed by linear increase to 95% in next 3 min; this composition was held for 42s before returning to the initial conditions. The analysis was performed using electrospray ionization (ESI) in a negative mode.

The peak area was determined for each monomer and plotted versus the concentration using linear regression analysis for BisGMA ($r^2=0.988$), BisEMA(3) ($r^2=0.971$), BisEMA(6) ($r^2=0.9709$), BisEMA(10) ($r^2=0.9711$) UDMA ($r^2=0.891$), TEGDMA ($r^2=0.981$) and BPA ($r^2=0.987$). The calibration standards were used to quantify the monomer concentration in the sample solutions. The monitored ion transitions, the applied cone voltage, the collision energy for each transition and the limit of quantification (concentration giving a signal-to-noise (S/N) ratio ≥ 10) are reported in Table 4.

The limits of quantification (LOQ) were determined based on the lowest calibration levels analyzed in pentaplicates and corresponding to the following performance criteria: accuracy within the interval 80-120% of the target level and repeatability with a relative standard deviation lower than 25% (Table 2).

The correlation coefficients, R^2 , of the regression equations exceeded the value 0.9564, demonstrating a good correlation between the measured response (peak area) and the concentration of the target compounds (Table 3.) The limits of quantification were

determined based on the lowest calibration levels analyzed in pentaplicates and corresponding to the following performance criteria: accuracy within the interval 80-120% of the target level and repeatability with a relative standard deviation lower than 25% (Table 2).

The absolute amount of each monomer in the extract was calculated in μmol .

Statistical analysis:

In order to assess the release of monomers from tested dental composites, a linear model, was constructed using statistical software (R 3.1.12.12.1, R Foundation for Statistical Computing, Vienna, Austria). In this model, the tested material, the amount of extraction solvent and the incubation time of the sample were considered, along with their first order interaction. All tests were performed at a significance level of $\alpha=0.05$.

Differences in the total monomer release from dental composites was evaluated by Tukey's multiple comparison test ($\alpha=0.05$).

RESULTS

Irrespective of the incubation time or amount of extraction solvent, all tracked monomers could be detected in Filtek Supreme XTE samples, whereas BisGMA and BisEMA10 were not detectable for Gaenial Flo (Fig.1).

A significantly higher amount of BisGMA could be observed in 3 ml of extraction solvent compared with 2ml and 1ml after a 7-day incubation of Filtek Supreme XTE (Fig. 2). Even though the total amount of the investigated compounds (BisEMA3, BisEMA6, BisEMA10, UDMA, TEGDMA) differed depending on the amount of solvent, the same release pattern could be observed for the other monomers. Similar

observations were done after 30-days of incubation, as there was a significant difference between the 3ml group, compared with the 2ml and 1ml groups. Assessment of leached compounds from Gaenial Universal Flo, showed a similar extraction pattern, with the highest extraction of compounds in the largest amount of extraction solvent (3ml) and gradual decrease with the lower volume, 2ml and 1ml.

Surprisingly, in both composites, 30 days did not result in much more monomer release compared to 7 days. There was no difference between 7 and 30 days of incubation for most monomers. Only for UDMA and TEGDMA, a significantly higher amount was released after 30 days (Fig. 2).

Even though there was no substantial dissimilarity for Filtek Supreme XTE in the amounts of released BPA in the different volumes of extraction solvent, the incubating time played significant role (Fig. 2). The release of BPA from Gaenial Universal Flo was lower than the LOQ, and therefore the released quantities cannot be taken as an accurate amount.

Evaluating the total monomer release from both composites, a significant increase was observed between the 1ml, 2ml and 3ml groups as well as between 7- and 30-days incubation. (Fig. 3) Only for Filtek Supreme XTE, there was no difference in total amount of released monomers in 1ml after 7 or 30 days.

DISCUSSION

For a long time, there has been concern about the compounds released from composites in the oral environment [8,9]. However, a well standardized *in-vitro* protocol that is as clinically relevant as possible, is essential to estimate more accurately the quantities of ingredients that can be released from composites, especially when evaluating the long-term release. From previous studies, it was already clear that the type of extraction solvent has an important influence on the amount of released compounds – significantly more is released in organic solvents – but in this study, we also found that the absolute amount of released monomers significantly varies depending on the quantity of extraction solvent in which a composite sample is immersed. More monomers will emanate in larger volumes of extraction solvent, which should be attributed to the fact that smaller extraction volumes will sooner be saturated. In addition, it was clear that the release of some monomers can reach a chemical equilibrium in the solvent and that leaving a composite sample a longer time in the solvent does not per se leads to more release. Both findings, which both can be explained by saturation of the extraction solvent, are very important as saliva, which is the extraction solvent in clinical situations is continually refreshed. [14]

The most frequently followed protocol by many researchers, dealing with elution from dental materials, has been described in the ISO 10993-13:2010 standard. It is proposed that the minimum ratio between the mass of the test sample and the volume of the test solution should be 1g:10ml in order to achieve an optimal extraction.[13] Additionally, as composite materials are considered to be permanent restorative materials, the leachability should be assessed after 7 and 60 days of incubation according to the ISO standards, with possible additional test periods.[13] Several elution studies, reported

that the most intense elution of compounds takes place immediately during the first days after immersing a composite sample in an extraction solvent, followed by a slow decrease in elution and a plateau within a couple of days. [7,15] Ferracane *et al.* explained this phenomenon by the fact that after a couple of days, maximal diffusion has taken place and that the release of monomers was complete. Muncksgaard *et al.* showed that the release of monomers could be estimated by use of Fick's law of diffusion, which poses that the main driving force for diffusion is the concentration gradient.[16] On the other hand, Manojlovic *et al.* explained this leaching pattern by a first high release of compounds at the surface of the specimen, followed by slower release of compounds within the specimen, which can be released only after swelling of the polymer chains [17]. They also succeeded in mathematically describing this release process by combining two exponential regression functions based on the first order kinetics law.

In our study, we observed a similar leaching pattern, where the release was complete within the first days, as for some monomers, there was not much additional release after 30 days compared to 7 days. However, we believe that the observed decrease in monomer release does not necessarily imply that all leachable compounds are removed from the composite. Our results – the fact that the volume of extraction solvent plays a role and that almost similar release is obtained for some monomers after 7 and 30 days – indicate that another factor with regard to elution kinetics needs to be taken into consideration when setting up *in-vitro* leaching experiments: namely the limited solubility of methacrylate monomers in the solvent. Indeed, saturation of the extraction solvent will also halt the release of monomers. In larger volumes, saturation of the solvent will take more time to reach. As a result, the plateau phase in the released

amount of compound may be the result of (1) the decrease of the concentration gradient between the sample and the solvent, and/or (2) saturation of the extraction solvent by the monomer.

The fact that only for BisGMA and BisEMA(3) and BisEMA(6) no additional release was observed after 30 days, but that the release of UDMA and TEGDMA after 30 days was significantly higher than after 7 days, shows that reaching saturation of the solvent is highly depends on the individual monomer. UDMA and TEGDMA are indeed more solvable in ethanol than BisGMA and BisEMA, which are rather hydrophobic monomers [1]. Nevertheless, it was also clear that most release of all monomers took place in the first week, which gives an indication about the elution rate.

The findings of this study may also partially provide an explanation for the large variation in the observed quantities of released monomers from composites in previous studies. The protocols in these studies not only included different types of composite material with varying sizes, shapes and preparation protocol, but also the type and volume of extraction solvent varied significantly [1].

Ferracane and Condon stated that among several factors influencing process of elution from resin based dental materials, several factors play a major role [18]. First, the amount of residual monomers, which depends on the rate of polymerization. Second, the chemical properties (hydrophilicity and differences in solubility parameter between polymer and the solvent) and the structure (crosslinking density and porosity) of the polymer network determines the which extent a polymer can be affected by the solvent. When the solubility parameter (δ) of a solvent and the polymer are similar, the effect of the solvent is greatest. It is, however, important to understand that a polymer network

does not actually dissolve in a “good” solvent, yet it swells. The internal forces between the polymer chains are overpowered by the forces between a solvent and polymer chains. Due to this phenomenon the spaces between polymer chains will become enlarged, which will allow unreacted monomers to leach out. The results of this study reveal another important factor influencing the release from composites: the release kinetics – i.e. amount of released compounds and elution rate - will be influenced by the maximum solubility of a monomer in the extraction solvent, and once the maximum concentration in the solvent is reached or in other words, once the solvent is saturated with the compound, no further release should be expected.

In our study we have used absolute ethanol as an extraction solvent. So far, it has been shown that different types of solvents can have different effects on a polymer network. [19,20] However, organic solvents such as ethanol are proven to be the best solvents for residual monomers from composite materials since they can easily penetrate in the resin matrix and swell a polymer network. Even if artificial saliva or water would be more clinically relevant as an extraction medium, organic solvents alone or in a mixture with water represent the worst case scenario of possible leachability from dental materials. [1,21,22] Moreover, ethanol solvents have been proposed by the FDA with intention to simulate exposure to dietary products like water, light beverages, alcohol, candy, syrups, wine and beer. [19]

Considering that saturation of the extraction solvent may take place, strategies to prevent this should be taken into consideration, especially for long-term testing. When Polydorou *et al* tested the long-term release, they tested the release after 24h, 7 days, 28 days, and then after 1 year, with refreshing the extraction solvent after each time point. The fact that the concentrations of monomers after 28 days and 1 year were very

similar also corroborate our findings. However, in order to prevent that saturation of the solvent is reached while testing long-term elution from composites, either a very large volume of solvent should be used or the solvent should be refreshed regularly. Probably, the latter strategy is preferable, because large volumes of solvent may yield very low concentrations that are well below the detection limit of current analytical methods.

Pongprueksa et al [23] tried to evaluate the long-term elution from different composite materials during a 6 week incubation period. In contrast to the previous studies, [5,25,26] the extraction solvent was refreshed after regular time intervals (every 7 days) and subsequently analyzed using UPLC/MS. Interestingly, they did not observe a plateau in the release during the first weeks. Moreover, some monomers were released in equally high amounts after a several weeks compared to the first week. Moreover, the differences in release kinetics of the compounds may be caused by the fact that some types of monomers tend to elute faster, and reach their maximum in the first days, which might have an effect on the release and reaching a saturation of slower-eluting compounds. Both the fact that refreshing the extraction solvent prevented that saturation was reached and swelling of the polymer which may facilitate the release of monomers deep within the sample, may explain his observations. [7] Therefore, refreshing the extraction solvent could be an important strategy to better estimate the total quantity of residual monomer that can be released.

Last, it is interesting to note that we could track BisEMA, which is a hydrophobic BisGMA analog without free hydroxyl groups, in the extract of both composites.[27] In the extract of Filtek Supreme XTE, both BisEMA(3) and BisEMA(10) were found, even though the manufacturer only mentioned BisEMA(6) in the description of the

composition. We also observed BisEMA(3) and BisEMA(6) in Gaenial Universal Flo samples, even though the manufacturer did not mention them. It is long known that manufacturers keep the exact composition of composites a trade secret and the information that they provide is often not complete. On the other hand, it is also important to understand that BisEMA does not represent a single monomer but that it usually comes as a mixture of different molecular weight BisEMA molecules.[28] The different BisEMA types differ from each other by the length of the ethylene oxide (oxirane) chain, located between monomer core and functional group. As a result, the molecular weight of these monomers differs which results in a different retention time during chromatographic analysis. Therefore, it is important that to include the different chain-length BisEMAs in the analysis to avoid inaccurate interpretation of the results. However, each type of BisEMA including analyzed BisEMA(6) and BisEMA(10) can exist in a form of different isomers which is characterized with different structural formulas as showed in the Figure 4. The real arrangement of ethylene oxide groups on the both sides of the core molecule can not be distinguished by used UPLC-MS/MS method and therefore all types of isomers were quantified together.

To summarize, *in-vitro* monomer release from composites is significantly influenced by the volume of the extraction solvent, with the greatest volumes of extraction solvent yielding the highest amounts of residual monomers. Depending on the monomer, longer extraction times did not result in more release. Both observations should be attributed to the fact that the elution reaction will arrest after saturation of the solvent is reached. This is an important finding as it is unlikely that saturation will be reached in an *in-vivo* situation, where saliva (or pulpal fluid) is continuously refreshed. A possible way to overcome this problem in *in-vitro* testing is refreshing the extraction medium in equal

time intervals, so that saturation of the extraction solvent is never reached. These findings are especially important for optimizing the extraction protocol for future analytical research evaluating the long-term exposure to released compounds from composites.

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FIGURES:

Fig.1:

Fig. 2:

Fig. 3:

FIGURE LEGENDS

Fig. 1: Scheme of the experimental set-up.

Fig. 2: The elution of individual monomers from the two evaluated composite materials, after either 7 or 30 days of incubation for Filtek Supreme XTE (**A**) and Gaenial Universal flo (**B**). Significantly higher amounts of all tracked monomers were extracted when the samples were incubated in a larger amount of extraction solvent. There was no significant difference of extracted compounds between 7 days and 30 days incubation period for all monomers, except for UDMA and TEGDMA for both tested composites, ($\alpha < 0.05$).

Fig. 3: The total monomer release from Filtek Supreme XTE (**A**) and Gaenial Universal flo (**B**) composite samples. **A:** There was a significant increase in released monomers between the 1 ml-, 2 ml- and 3 ml-group, taking into account both evaluated incubation periods. Furthermore, a significant difference was noted in the 2 ml- and 3 ml-group between 7 days and 30 days of incubation. **B:** Significantly increased amounts of total released monomers were noted for larger volumes of extraction solvent, as well as for 30-days of incubation. The increase after 30 days was however, mild, compared the increase in larger volumes of extraction solvent.

TABLES

Table 1: Composite materials used in the research:

Brand	Manufacturer	Classification	Resin Matrix	Filler type	Filler loading
Filtek Supreme XTE	3M ESPE, Seefeld, Germany	Nano-composite (non-flowable)	BisGMA (1 – 10 wt%), BisEMA(6) (1 – 10%), UDMA (1 – 10 wt%), TEGDMA (< 5 wt%)	• SO ₂ (20nm) • Zirconia - silica clusters (0,6-1,4µm) with primary particles of 5-20nm	78 % wt 59.5 % vol
G-aenial Universal Flo	GC, Tokyo, Japan	Nano-hybrid (flowable)	UDMA (15 – 20 wt%), TEGDMA (5 – 10 wt%), Bis-MEPP (5 – 10 wt%)	• SO ₂ (16nm) • Strontium glass (200nm)	69 % wt 50 % vol

Abbreviations: BisEMA, ethoxylated bisphenol A glycol dimethacrylate; BisGMA, bisphenol A diglycidyl dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; Bis-MEPP, γ-Methacryloxypropyltrimethoxysilane

Table 2: Compounds used in this study

Molecule	Name of compound	Molecular weight	Molecular formula	Purity (%)	Manufacturer	CAS number
Bis GMA	Bisphenol A diglycidyl dimethacrylate	512.6	$C_{29}H_{36}O_8$	No information	Sigma-Aldrich, St. Louis, USA	1565-94-2
BisEMA(3)	Ethoxylated bisphenol A glycol dimethacrylate (n+m=3)	496.58	$C_{29}H_{36}O_7$	> 87 %	ESSTECH Inc, PA, USA	41637-38-1
BisEMA(6)	Ethoxylated bisphenol A glycol dimethacrylate (n+m=6)	628.7	$C_{35}H_{48}O_{10}$	> 87 %	ESSTECH Inc, PA, USA	41637-38-1
BisEMA(10)	Ethoxylated bisphenol A glycol dimethacrylate (n+m=10)	805	$C_{43}H_{64}O_{14}$	No information	ESSTECH Inc, PA, USA	41637-38-1
UDMA	Urethane dimethacrylate	470.56	$C_{23}H_{38}N_2O_8$	> 97 %	Sigma-Aldrich, St. Louis, USA	72869-86-4
TEGDMA	Triethylene glycol dimethacrylate	286.32	$C_{14}H_{22}O_6$	> 95 %	Sigma-Aldrich, St. Louis, USA	109-16-0
BPA	Bisphenol A	228.29	$C_{15}H_{16}O_2$	> 99 %	Sigma-Aldrich, St. Louis, USA	80-05-7

Table 3 – UPLC-MS/MS parameters for the identification and quantification of each targeted molecule

Molecule	RT¹ (min)	QT²	Electro spray	Cone (V)	Collision Energy (V)	LOQ³ (nmol/ml)	R² value
BisGMA	2.04	535.3>535.5	+	50	15	0.69	0.9886
BisEMA(3)	2.42	558.2>291	+	30	20	0.26	0.971
BisEMA(6)	2.4	602.5>291	+	20	15	0.397	0.9709
BisEMA(10)	2.32	778.5>335	+	25	30	0.152	0.9711
UDMA	1.95	493.7>493.7	+	50	10	1.57	0.9612
TEGDMA	1.71	309.6>309	+	35	10	1.358	0.9891
BPA	2.20	226.9>211.8	-	40	20	0.0147	0.99

¹ Retention time

² Quantifier Transition

³ Limit of quantification

Table 4. Release of endocrine disruptor bisphenol A in a different volumes of solvent and incubation time

BPA release (nmol)	Filtek Supeme XTE			Gaenial Universal Flo		
	1ml	2ml	3ml	1ml	2ml	3ml
7-day incubation	0.06±0.01	0.08±0.03	0.06±0,02	0	0	0
30-day incubation	0.13±0.03	0.14±0.02	0.12±0.02	0.014±0.002	0.013±0.004	0.018±0.005

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