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Long Term Monomer Elution of Bulk-Fill and Conventional Resin Composites

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ABSTRACT

The process of elution of unreacted monomers from dental resin composites can adversely affect their biocompatibility and longevity. In this regard, it is of interest to monitor relies on unreacted monomers from SonicFill, especially since this new type of bulk-fill composite materials entered dental practice with their simplified and shortened application protocol. This study aimed to assess long term monomer elution from bulk- fill (SonicFill) and conventional low shrinkage resin composites (FiltekP60 and FiltekUltimate), applied in 2mm layer thickness and stored in different extraction media - 6-month period exposure in artificial saliva, followed by 24 hours exposure in 75% ethanol solution. Analysis of monomer elution was performed using HPLC-MS. Monomers detected in artificial saliva were: UDMA and EDB from the conventional composites. No eluted monomers were detected from SonicFill. In just 24 hours of exposure in 75% alcoholic solution, components were extracted from all the materials that were not extracted for 6 months in the artificial saliva: Bis-GMA, TEGDMA, EDB from SonicFill; Bis-GMA from Filtek P60, and Filtek Ultimate. There was a new peak in the release of UDMA from Filtek P60 and Filtek Ultimate. Data reveal, that the extraction medium is extremely important for the separation of monomers. The degree of polymerization cannot completely influence the release of monomers into the surrounding environment. Internal factors, concerning materials composition, are more important, than the ability to control clinically degree of polymerization by intensity and duration of light exposure.

Keywords

SonicFill, Monomer elution, Biodegradation.

Abbreviations

HPLC-MS: High-Performance Mass Spectrometry in combination with ultra-high-performance chromatography; SEM: Scanning Electron Microscopy; UDMA Urethane dimethacrylate; EDB: Ethyl-4-dimethylamino benzoate; Bis-GMA: Bisphenol A glycidyl dimethacrylate; TEGDMA: Ttiethyleneglycol dimethacrylate.

Introduction

Worldwide, dental resin composites become the main material for direct restorations in both the frontal and distal areas of the dentition [1,2]. Their application brings better aesthetics; less invasive preparation due to the micromechanical and adhesive bonding with hard dental tissues; provides strengthening of the tooth structures remaining after the preparation [1,2].

Dental resin composite materials consist of four main components: 1) organic polymer matrix (dispersed medium) usually containing dimethacrylate resins; 2) inorganic filler (dispersed phase) fillers and tins; 3) coupling phase that adheres the matrix to the filler particles (silanes); 4) activators and inhibitors of the polymerization process [2,3].

Resin-composites have improved significantly since they were first introduced, to enhance their fiscal-mechanical properties and polymerization shrinkage. Improvements were mainly

in terms of the changes involved in the inorganic filler phase [1]. The resin matrix constitutes about 20–40 wt% of a resin composite material and is composed of dimethacrylate monomeric compounds including mainly Bis GMA (Bisphenol A Glycidyl Dymethacrylate), UDMA (Urethane Dimethacrylate), TEGDMA (Triethylene Glycol Dymethacrylate), Bis EMA (Ethoxylated Bisphenol A Glycidyl Dymethacrylate) [3,4]. Variable combinations and proportions of these monomers are included in current resin composite materials resulting in different copolymer systems.

Studies, concerning conventional dental resin composites showed, that complete conversion of monomers does not occur during the formation of a polymer structure. The level of conversion reaches 40-75% [5-7]. 25-50% of the methacrylate groups can remain unreacted [5]. Some of them present like pendant groups in the polymer structure, others exist like free monomer molecules, trapped in the polymer network. These free unreacted monomers represent 2-10% and can diffuse into the surrounding structures - dentin, dental pulp, gingiva, oral cavity [3,4]. This process can adversely affect the biocompatibility of the materials and the longevity of restorations. Scientific analyzes indicated allergic, cytotoxic, and genotoxic effects of residual monomers and biodegradation products of dental composites [4,9-12]. Little is still known about the metabolism, systemic distribution, and possible future harmful effects on human health [4]. Scientific investigations found more than 30 chemical substances released from dental resin composites into different storage media through the processes of diffusion and swelling: monomers, oligomers, initiators, catalysts, biodegradation products, impurities, metal ions [3,13].

The degree of polymerization of dental composites depends on internal and external factors. Internal factors include resin matrix composition, filler composition, photoinitiator system, and their proper balance. The most important external factors are the conditions of polymerization – the power of the light source and the duration of light exposure [2,3]. These conditions can be influenced clinically. The energy of the light decreases significantly when transmitted through a resin composite [2,14]. For that reason, an incremental placement technique in layers with a thickness of 2 mm or less has been the standard to sufficiently convert monomers [15].

New composite materials entered the dental practice. The developments aimed to reduce the polymerization shrinkage and the generated polymerization stress along with simplifying and shortening the application protocol. As a result, composite resins, named "bulk- fill" were created. Improved translucency and the photoactive groups, included in the methacrylate matrix, allows better control on polymerization kinetics and makes possible polymerization of the composites to a depth of 4mm using the bulk/monoblock technique [16,17]. The main concern, regarding bulk-fill materials, is whether they can polymerize sufficiently in layers of 4-5 mm at shorter periods, recommended by manufacturers [16]. The studies, included investigations of the polymerization rate of bulk-fill resin composites are controversial. Some of the studies stated that bulk-fill composites polymerized sufficiently in layers of 4 - 5mm [18,19,20], others stated that the prescribed by manufacturers time is not sufficient for reaching an adequate degree of conversion on a layer of 4-5 mm [21]. A high degree of polymerization is very important for achieving optimal mechanical and physical properties of the materials. An insufficient degree of polymerization can adversely affect the biocompatibility of the restoration. It was shown, that the number of eluted monomers of bulk-fill composites is comparable to that of conventional materials, despite their increased layer thickness of 4 mm [13,22]. The number of eluted monomers increases with the elution time [23,24].

It is supposed, that the use of incremental placing technique in thinner layers, may help to overcome some concerns and shortcomings, by achieving an adequate light penetration to the bulk-fill composites. Studies involving different types of bf composites have shown a higher level of conversion of materials in layers of 2 mm compared to application in layers of 4 mm and more [26]. In this regard, it is of interest to monitor relies on unreacted monomers from bulk-fill composites polymerized in 2mm layer thickness.

The group of bulk-fill composites is very heterogenous, their laboratory properties vary largely between individual products and they have to be compared with clinically established conventional resin composites.

The present study aimed to identify *in vitro* elution products (qualitative analysis) of bulk-fill composite SonicFill (Kerr) and two well approved conventional composites - Filtek Ultimate (3M-ESPE) and Filtek P60 (3M-ESPE), applied in 2mm thickness, for 6 months exposure in artificial saliva, followed by 24 hours exposure in 75% ethanol solution.

Materials and Methods

The main characteristics of the investigated dental materials according to the manufacturer's data are described in the table below (Table 1).

Preparation of samples for testing

Six discs were made of each material (3 x 6 =18). For this purpose, the materials were applied in equal metal cylinders with a diameter of 5 mm and a height of 2 mm. The upper and lower surfaces of the cylinders were free. The cylinders were placed on a glass base and the material was applied from the free upper surface. The application was done at once until the entire cylinder was filled (2 mm). After filling the cylinder, the free surface was covered with a glass slide (1mm thickness) and the material was polymerized. Polymerization of composites covered tightly with a glass slide prevents the formation of an oxygen-inhibited layer. The polymerization was performed with an Elipar Freelight II (3M-ESPE) with light output power 1200 mW/cm^2, and a soft start

Table 1: The main characteristics of the investigated dental materials.

Material (producer)	**Organic matrix**	**Material type** and filler loading	**Inorganic filler**
Filtek P60 (3M - ESPE)	Bis-GMA; Bis-EMA; TEGDMA; UDMA	Condensable; Filled 80 wt %	silica filler, zirconia filler
Filtek Ultimate (3M - ESPE)	Bis-GMA; Bis-EMA; TEGDMA; UDMA	Nanocomposite; Filled 78.5 wt %	silica filler, zirconia filler, zirconia/silica cluster filler
SonicFill (Kerr)	Bis-GMA; Bis-EMA; TEGDMA; (patented modifiers of polymerization process)	Bulk-fill; Filled 83.5 wt %	silicon dioxide filler, glass oxide filler

of photoactivation mode for 20 sec. This was followed by inversion of the disk to the other free side and direct polymerization (through 1mm glass base) in the same mode for another 20 sec (40 sec in total). After polymerization, the disks were removed from the cylinders, grouped by materials tested.

Time course of the experiment in artificial saliva

The disks of each material were placed (3 materials x 6 samples) individually in a 6-well plate, then 5 ml of artificial saliva was added to each well. At the zero points from the beginning of the experiment, 100 µl were taken from the liquid in each well, which was used in subsequent experiments.

Throughout the experiment, the test materials were stored in the dark at a constant temperature of 25 ± 1 °C. At certain time intervals (24 hours, 48 hours, 5 days, 10 days, 14 days, 1 month, 2, 3, 4, 5, 6 months) 100 µl aliquots of the liquid were taken and the artificial saliva was replaced with new 5 ml/pit. The aliquots of artificial saliva taken during the time course of the experiment were stored at - 20 °C in the freezer before LC/ MS analysis.

Release of monomers in 75 % ethanol

Disks from the previous stage of the study were dried on filter paper. and stored in the dark at a constant temperature of 25 ± 1°C for 1 month. After that, the samples were placed in 6-well plates individually and 5 ml of 75% ethanol were added to each well. Then, the aliquot of 100 µl was taken from each individual well, as control. The samples were incubated for 24 hours in dark and constant temperature and a new 100 µl aliquot was taken for LC /MS analysis.

Chemicals and Reagents

Formic acid and acetonitrile of LC-MS grade were obtained from Merck Co. (Germany). All remaining used reagents were of the highest purity available in the laboratory. The artificial saliva was prepared in the laboratory and its composition is presented in table 2.

Table 2: Composition of artificial saliva.

Components	**Concentration [g/L]**
Carboxymethyl cellulose	2.0
Potassium chloride (KCl)	0.62
Sodium chloride (NaCl)	0.87
Magnesium chloride ($MgCl_2$)	0.06
Calcium chloride ($CaCl_2$)	0.17
Dipotassium phosphate (K_2HPO_4)	0.8
Monopotassium phosphate (KH_2PO_4)	0.3
Sodium fluoride (NaF)	0.0044
Sodium bicarbonate ($NaHCO_3$)	0.33

Liquid chromatography-mass spectrometry analysis (LC-MS)

The analyses were carried out on Q Exactive® hybrid quadrupole-Orbitrap® mass spectrometer (ThermoScientific Co, USA) equipped with a HESI® (heated electrospray ionization) module, TurboFlow® Ultra-High-Performance Liquid Chromatography (UHPLC) system (ThermoScientific Co, USA), and HTC PAL® autosampler (CTC Analytics, Switzerland).-

Chromatographic conditions

The chromatographic separations of the analyzed compounds were achieved on Synchronise C18 (100 x 2.1 mm, 1.7 µm) analytical column (ThermoScientific Co, USA). using gradient elution at 300 µl/min flow rate. The used eluents were: A - 0.1 % formic acid in water; B - 0.1 % formic acid in ACN. The analyses were performed using the following binary gradient: 0% B for 2 min; 0–15% B in 3 min, 15–90% B within 30 min; 90% B in 1 min; 90–0% B for 2 min and 0% B for 3 min.

Mass spectrometry conditions

Full-scan mass spectra over the m/z range 100–1200 were acquired in negative and positive ion mode at resolution settings of 140 000. The mass spectrometer operating parameters used in a negative ionization mode were: spray voltage - 4.0 kV; capillary temperature - 320°C; probe heater temperature -300°C; sheath gas flow rate 35 units; auxiliary gas flow 12 units; sweep gas 3 units (units refer to arbitrary values set by the Q Exactive Tune software) and S-Lens RF level of 50.00. Nitrogen was used for sample nebulization and collision gas in the HCD cell. All derivatives were quantified using 5 ppm mass tolerance filters to their theoretical calculated m/z values. Data acquisition and processing were carried out with the XCalibur® ver 2.4 software package (ThermoScientific Co, USA).

Evaluation of LC-MS data

The raw data obtained from mass spectroscopic analysis were processed using the SIEVE 2.2 software package. For the experiment, an own database of components (individual substances) has been created, based on the existing manufacturer's information and data from the scientific literature [3-7]. This database includes 43 individual substances and was used to identify the substances present in the samples. The results obtained for the samples taken in the initial (zero) time of the experiment were used as a control during the relative quantitative and statistical evaluation of the data. The relative quantification of each individual compound was made using integrated peak areas in extracted ion chromatograms because the standard substances were not available during the study.

Results

LC /MS analysis of samples incubated in artificial saliva

All aliquots of artificial saliva taken during the 6 months' time course of study were analyzed by LC/ MS in the positive and negative mode of operation of the mass spectrometer. The raw data obtained during analysis were processed using SIEVE 2.2 software. The raw mass chromatogram was aligned, normalized, and subjected to nonlinear regression analysis to extract, identify, relatively quantify and statistically evaluate each individual compound present in samples. The final result showed the release of two monomeric components - urethane dimethacrylate (UDMA) and ethyl-4- dimethylamino benzoate (EDB) from the samples prepared from Filtek P60 and Filtek Ultimate materials. The relative amount of these compounds during the time course of the experiment are presented in figure 1 and 2, respectively. In the samples prepared of Sonic Fill no release of monomeric compounds was detected.

The materials Filtek P60 and Filtek Ultimate have a similar matrix composition. Urethane dimethacrylate (UDMA) is one of the main

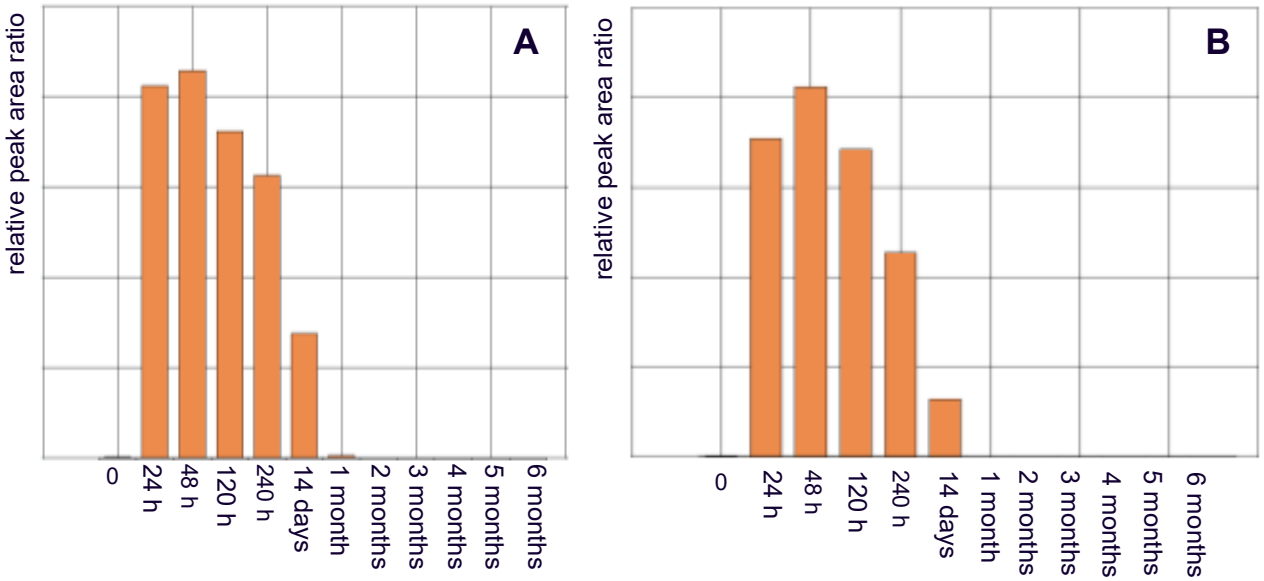

Figure 1: Time course release of Urethane dimethacrylate; (UDMA) from Filtek P60 (**A)** and Filtek Ultimate **(B)** represented as the relative ratio of averaged peak areas obtained by LC/ MS analysis.

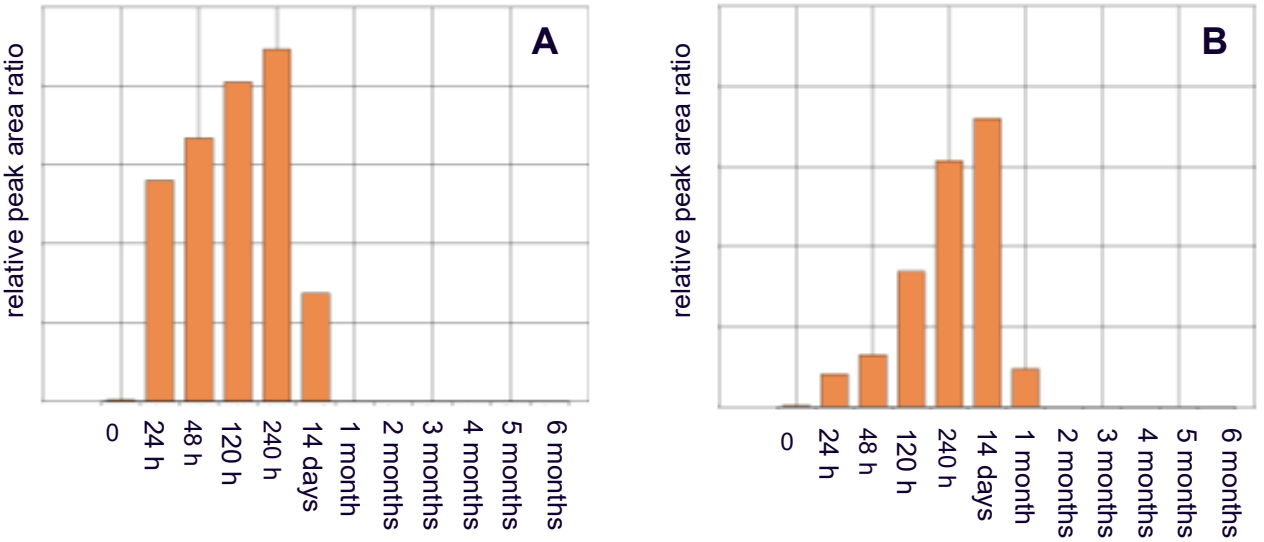

Figure 2: Time course release of ethyl-4-dimethylamino benzoate (EDB) from Filtek P60 (A) and Filtek Ultimate (B) represented as the relative ratio of averaged peak areas obtained by LC/ MS analysis.

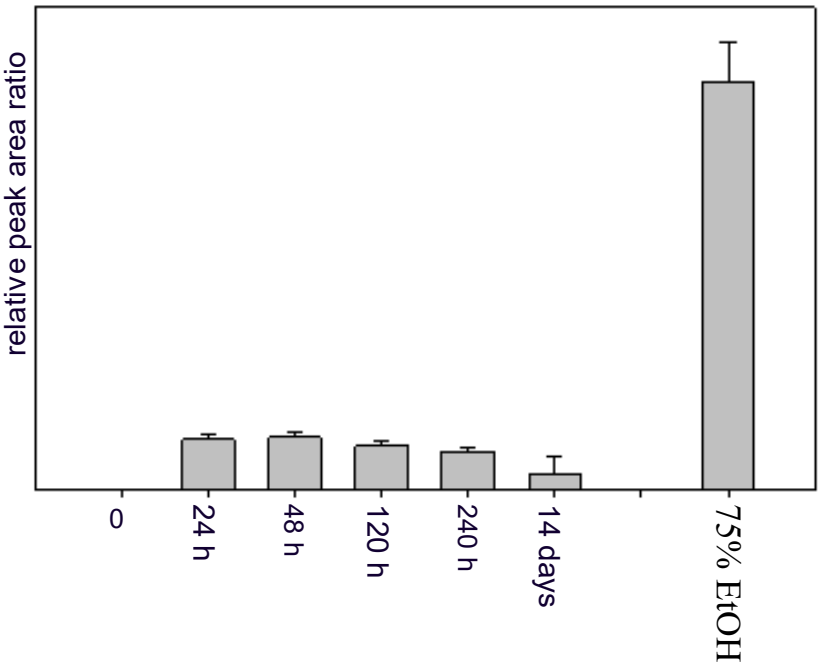

Figure 3: The comparison of averaged peak areas obtained by LC/ MS analysis of the release of urethane dimethacrylate (UDMA) from Filtek P60 after incubation in artificial saliva and 75% ethanol.

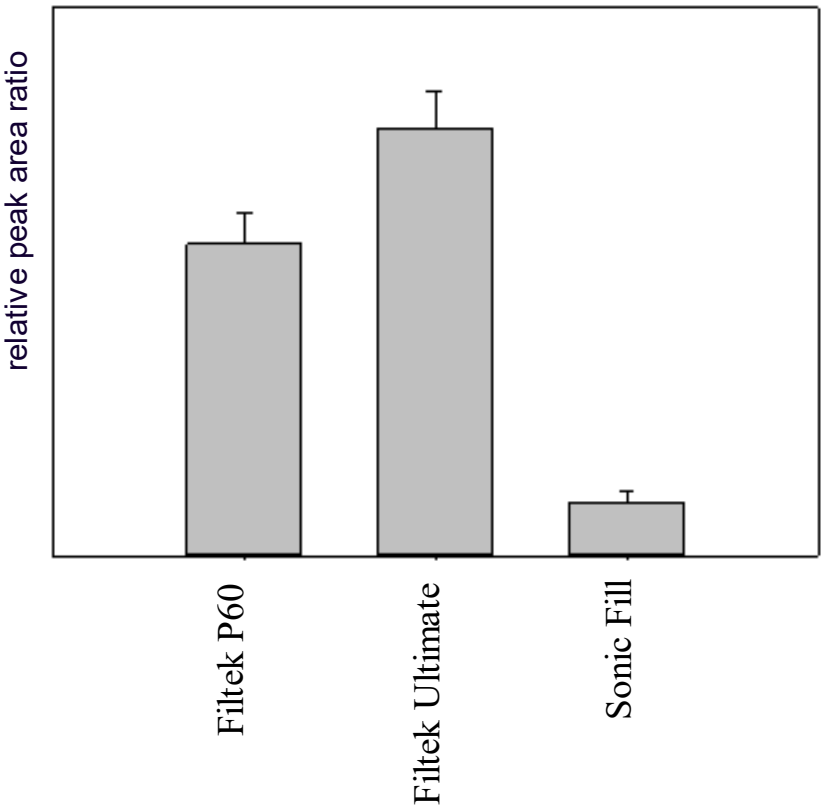

Figure 4. The comparison of averaged peak areas obtained by LC/ MS analysis of the release of Bisphenol A glycidyl dimethacrylate (Bis-GMA) in different materials after incubation in 75% ethanol.

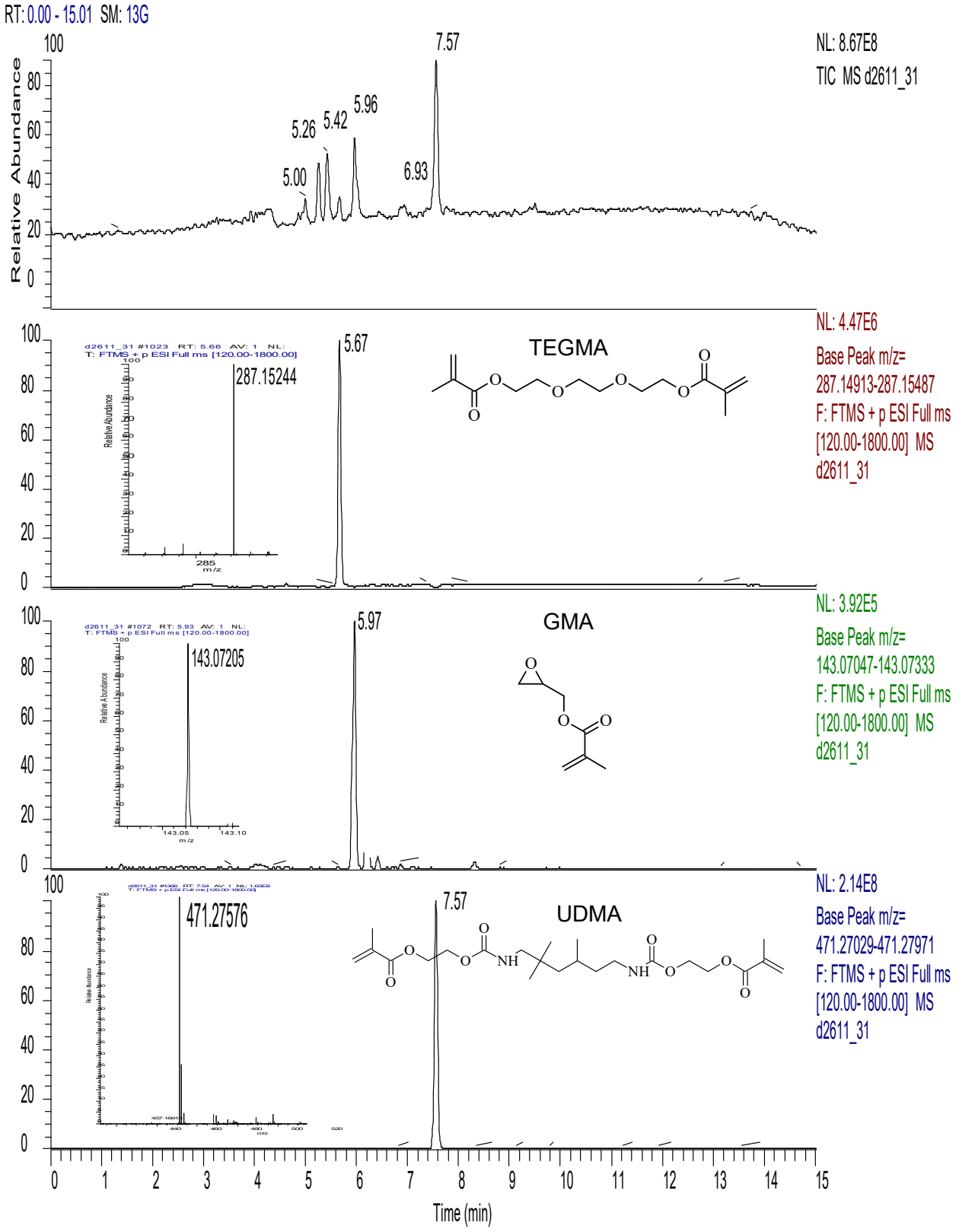

Figure 5: Total ion chromatogram and extracted ion chromatogram of LC/ MS analyses of Sonic Fill sample incubated in 75% ethanol.

monomers present in both. Its release begins at 24 hours, reaches its maximum after 48 hours, and is still present until the 14th day of the experiment. UDMA was not detected in samples taken after the 1st month of study.

The ethyl-4-dimethylamino benzoate (EDB) is the co-initiator of the polymerization process. Release of EDB was detected from both conventional resin composites at 24 hours of incubation. From Filtek P60 amount increases gradually, reaching its apex on the 5th day. In samples of Filtek Ultimate the apex of the release of EDB is on the 14th day. The compound was detected in samples until 2 months of incubation in both materials.

It is not wrong to mention, that on the mass chromatograms collected during the study are present peaks of unknown compounds that could not be identified. Most probably, these compounds are the results of side reactions during the process of polymerization of materials. Additional studies are needed to clarify the problem.

LC /MS analysis of samples incubated in 75% of ethanol

The samples used in artificial saliva experiments after a long time of drying were incubated in 75% ethanol for 24 hours and liquids were analyzed by LC/ MS as described above. The obtained raw data were processed as previously using SIEVE 2.2 software. The obtained results showed, that in Filtek P60 and Filtek Ultimate compound UDMA is still released. The comparison of averaged peak areas obtained by LC/MS analysis of the release of urethane dimethacrylate from Filtek P60 after incubation in artificial saliva and 75% ethanol is presented in figure 3.

All studied material extricate the Bis-phenol A glycidyl dimethacrylate (Bis-GMA), a compound that was not detected during the experiment in artificial saliva. The relative ratio of Bis-GMA in different materials is presented in figure 4.
It was also found that SonicFill releases a trace amount of Ttiethyleneglycol dimethacrylate (TEGDMA).

In figure 5 total ion chromatogram and extracted ion chromatogram of LC/ MS analyses of Sonic Fill sample incubated in 75% ethanol is presented.

Discussion

High-performance mass spectrometry in combination with ultra-high performance chromatography was used as an analytical method in the present study. This is a highly sensitive and reliable analytical method considered to be extremely suitable for the identification of products eluted from dental polymers [20,30]. Both high molecular weight monomers and low molecular weight components, activators, stabilizers, and inhibitors of the polymerization process could be found through it [27]. Delayed inorganic components cannot be identified.

In the literature most common are the studies determining the release of products from composite materials for short periods [22,28]. There are few studies targeting longer periods: 3 months [13,24]; 6 months [29]; 12 months [27]. According to Ferracane [5], the elution of 50% of the unreacted monomers from composites occurs during the first 3 to 24 hours after polymerization. Usually, at that time post-irradiant polymerization of resin composites has to be completed and the materials are supposed to reach their stability. There are indications that this process continues for longer periods [13,24,27,29]. For the bulk-fill resin composites, there is evidence that the elution of unreacted monomers is not completed at 3 mounds, but even rises in the alcoholic medium [13,23].

The first part of our experiment was taken for 6 months in artificial saliva. For this period, we did not detect any elution components from SonicFill – the bulk-fill material. We attribute this fact to the high degree of conversion of the material, which occurred after polymerization for 40 seconds at a layer thickness of 2mm, because of its enhanced photosensitivity. It can be found data, that SonicFill reaches a conversion rate of 77.2% when polymerized in a 2 mm layer for 20 sec and that the conversion decreases to 63% when the layer thickness increases to 4 mm [25]. At the same, time the filler content is found to correlate with the elution of monomers - in particular materials with higher filler content could show a lower release of monomers [24,28]. SonicFill is filled to 83.5% of its weight and possessed the highest filler content from materials included in the study.

Alshali et al. [30] investigated qualitative and quantitative characterization of monomers of unreacted bulk-fill and conventional dental composites using liquid chromatography/ mass spectrometry. SonikFill was included in the investigation. They found significantly higher amounts of the hydrophobic base monomers BisEMA and BisGMA in the composition of uncured resin compared to other materials investigated. These monomers are of high molecular weight, hydrophobic, and rarely extracted in aqueous media and artificial saliva [3,13]. This is another factor, explaining the lack of extracted monomers from SonicFill in the present study.

For the conventional low-shrinkage composites Filtek P60 and Filtek Ultimate we found the release of the monomers UDMA and EDB in artificial saliva. The release of monomers started at 24 hours. The peak in UDMA release was at 48 hours for both materials. UDMA is present as a basic monomer in the composition of both of the materials. UDMA is easier to extract and is found in aqueous solutions and artificial saliva solutions due to the incomplete polymerization of the composites [29,31], the presence of hydrophilic bonds in UDMA, and the lower molecular weight of the monomer compared to Bis-GMA (MW = 512 g / mol; Bis-GMA; MW = 470 g / mol UDMA) [3]. The monomer is depleted in the salivary medium on the 14th day for Filtek Ultimate and the first month for Filtek P60 and is not detected until the 6th month.

Ethyl-4-dimethylamino benzoate (EDB) is an amine synergist, co-initiator, and stabilizer of the polymerization process. It is often used in combination with camphor quinone [30]. According to

some scientific publications, these lipophilic, lower molecular weight components have an affinity for cell membranes and can exert cytotoxic effects when accumulated in larger quantities [33]. EDB is depleted after the 14th day for Filtek P60 and after the first month of artificial saliva for Filtek Ultimate. EDB was not identified in the alcoholic extracts of Filtek P60 and Filtek Ultimate, indicating that this component was completely depleted after staying in artificial saliva. Activators and stabilizers of the polymerization process are added in small amounts to the composite materials (about 1% of the composition). They have a low molecular weight and are more mobile as molecules, which we believe is the reason for their depletion in 14 days to 1 month in a salivary environment for conventional resin composites.

The solution of ethyl alcohol and water is accepted in scientific circles as a simulator of the aggressive factors in the oral environment (alcohol, sweets, soft drinks). Researchers have established the importance of the solvent for the extraction of residual monomers from composite materials [5]. The solution of 75% ethyl alcohol is very close to the solubility coefficient of Bis-GMA [20]. Bis-GMA was extracted from all materials in the alcoholic solution. The high molecular weight monomer was not detected for 6 months in artificial saliva. When comparing the hydrophilicity and solubility of conventional monomers, they are arranged as follows: HEMA> TEGDMA> UDMA> BisGMA> BisEMA [3].

Degradation of the materials in artificial saliva and the coincidence of solubility coefficient of alcohol and methacrylate monomers leads to maximum softening of the polymer matrix. Alcohol penetrates in-depth, widening the spaces between the polymer chains and allows easier diffusion of unreacted products to the solvent medium. Studies show that this solution extracts residual (co) monomers in 24 hours in higher amounts than those that could be extracted in aqueous solutions [13,20]. These findings were confirmed in the present study: In just 24 hours, components were extracted from the materials that were not excreted for 6 months in the synthetic saliva: Bis-GMA, TEGDMA. Again there is a peak in the release of UDMA from F.P60, F.Ultimate, a monomer that is depleted in saliva samples on day 14 (F.P60) and the first month (F.Ultimate). Evidence for this is the extraction of components from SonicFill in an alcoholic solution (Bis-GMA, TEGDMA, EDB) - from this composite, no components were found separated in synthetic saliva.

According to a study by Lempel et al. [30] TEGDMA has a synergistic effect with the level of conversion of composites. There was no release of TEGDMA from FiltekP60, Filtek Ultimate either in artificial saliva medium or 75% alcohol medium after 6 months. This should mean that an optimization level of material conversion is reached with polymerization of 40 sec per layer of 2 mm. Accordingly, the extraction of TEGDMA from SonicFill in 75% alcohol should mean that no maximum conversion has been achieved, despite the polymerization of 40 sec per layer of 2 mm. In support of this view is the extraction of EDB from the material in an alcoholic environment - a synergist of the photoinitiator of polymerization.

Durner et al. [32] found a significant reduction in monomer release (including TEGDMA) from Filtek Supreme and Tetric Evo Cream samples after increasing the polymerization time from 20 to 40 sec. The photopolymer lamp used by them is analogous to ours, as well as the size of the samples. The extraction of monomers was done in an alcoholic solution. The same inverse relationship has been reported by other researchers [6]. TEGDMA is among the monomers traditionally present in the composition of composites and exhibiting a more pronounced cytotoxic and mutagenic effect. TEGDMA is thought to enhance the proliferation of cariogenic microorganisms (Lactobacillus acidophilus, Streptococcus sorbinus) [32,33].

The group of bulk-fill composites is highly heterogeneous and it is difficult to draw general considerations about them as a whole. That is the reason no coincidence in the literature the conclusions regarding the elution of monomers for bulk-fills are contradictory. An investigation concluded that layer thickness of 4 mm or more can lead to an increased elution of some bulk-fill components, compared to the elution at a layer thickness of 2 mm [34,35]. We can partially agree with these conclusions because in the present study no SonicFill products were found in an environment of artificial saliva for 6 months. However, the study did not include samples of the material polymerized in layers of 4 mm or more.

There is evidence of separation of monomers for 3 months - Dundar et al. [23] found TEGDMA, Bis GMA, and Bis EMA from SonicFill during exposure in alcoholic solution. Alshali et al [13] detected elution of Bis GMA and Bis EMA in 70% ethanol solution again for 3 mounds. The sample thickness of the material in both experiments was 4mm. We found Bis EMA neither in artificial saliva nor in alcoholic solution.

Conclusions

The heterogeneity of resin composite structure, requiring a proper balance of the components and the complexity of the degradation process shows difficulties in *in vitro* simulation of intraoral conditions and behavior of resin composite restorations. The limitations of the present study and obtained data allow us to conclude that:

- No monomers were released from SonicFill for 6 months in an artificial saliva medium, after polymerization of a 2 mm layer for 40 seconds.
- The extraction medium is extremely important for the separation of monomers. The medium of 75% alcohol can extract substances that are not extracted in an environment of artificial saliva for 6 months.
- The level of polymerization cannot completely influence the release of monomers into the surrounding environment. Internal factors concerning materials composition are more important than the ability to influence the clinical degree of polymerization by intensity and duration of light exposure.

The obtained during the presented study results strongly suggest that additional well-designed investigations of a variety of bulk-fill composites in different layer thickness should be carried out to detect exact quantities of eluted monomers and to investigate their cytotoxic effects.

Long-term elution of monomers from resin composites should not be underestimated, because the prolonged release of small amounts of substances can have a cumulative effect and pose a risk to human health.

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