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REVIEW

Bioanalysis as a powerful tool in Dentistry: the case of short-term and long-term release of Monomers from dental Composites

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ABSTRACT

Novel materials used in preventive and restorative dentistry contain monomers with endocrine or cytotoxic properties, which can cause minimum or even severe damage to human body, when found in specific concentrations. The degradation of resin composite restorations after aging and/or storage in different solutions is associated with leaching monomers, like bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), Urethane dimethacrylate (UDMA), Bisphenol A (BPA), which are potentially leading to toxicity and mutagenicity effects or cause allergic reactions. These monomers may cause health issues to patients, therefore their determination both in-vitro and in biological fluids e.g. saliva, blood serum/plasma and urine is significant. Moreover, analytical methods are necessary to investigate the rate of elution, as well as the conditions that mainly affect the mechanism of short-term and long-term release of monomers from dental composites.
In this review article we present some of the techniques and methods used to determine the short-term and long-term release of these monomers from modern dental materials and prove that analytical chemistry and especially bioanalysis can be a powerful tool in dentistry

KEYWORDS: bisphenol-A (BPA), bisphenol A-glycidyl methacrylate (Bis-GMA), dental composites, dimethacrylate monomers, triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA).

INTRODUCTION

Dental materials

Since 1819, when an English chemist, Joseph Bell invented amalgam, dentists have been widely using dental amalgams for carious posterior teeth restoration. Dental amalgams are wear-resistant and less sensitive, while being processed, than composites. They also have good compressive strength. However, they have several negative impacts on human health, due to mercury leakage and its toxic properties, causing damage to neurons and kidneys. Another disadvantage is the

risk of tooth fracture on account of poor tooth reinforcement and last but not least the unnecessary partial removal of tooth structure to increase mechanical retention [1].
All disadvantages mentioned above, led to their gradual replacement from safer composite dental materials. In 1939, Charles Goodyear invented the vulcanization of rubber. That material was used in denture bases for the next 100 years. In 1868, the invention of cellulite, by the Hyatt brothers was directly adapted to denture manufacturing [2,3]. However, these materials were also replaced when the first acrylic resin, poly-(methyl methacrylate) (PMMA), was introduced in 1937. Due to their stability in various conditions and lower water absorption, acrylic resins were immediately accepted by dentists [4,5]. In 1962, the first resin composite was introduced, when Bowen discovered the monomer Bis-GMA (bisphenol A-glycidyl methacrylate), in an attempt to improve the properties of acrylic resins [3]. Those composites, whose properties have improved over the years, are still used nowadays. They consist of a polymeric matrix and inorganic (ceramics, glass-ceramics, or glasses), organic or composite fillers to reinforce the matrix [6].
Resin based restorative materials are tooth-colored, they have good mechanical and compressive strength, they are suitable for the replacement of natural tooth tissue and the removal of healthy tooth tissue, leading to weakening of the remaining tooth structure, is not necessary [1,7]. However, the matrix of these materials consists of monomers, which are likely to elute into the immediate environment, when not fully polymerized or due to thermal, chemical or mechanical factors [8]. These liquid monomers, are polymerized into a solid when cured either chemically or by light. If the polymerization process is not complete, or if the dental material starts to decompose some of the unbound liquid monomers will inevitably elute into the oral cavity.
Some of the monomers used in dental resins, are bisphenol A (BPA) derivatives, such as Bis-GMA (Bisphenol A-glycidyl methacrylate) and its ethoxylated form Bis-EMA, Bis-DMA (Bisphenol A-dimethacrylate), while others, are not. The most non-BPA-based monomers used in dental resins, are TEGDMA (triethylene glycol dimethacrylate), UDMA (urethane dimethacrylate), DMA (N,N-dimethyl acetamide) and HEMA ((hydroethyl)methacrylate)). Dental resins are composed primarily of BPA derivatives, rather than pure BPA, because moisture from saliva could cause hydrolysis of its hydroxyl groups. BPA may however be found as an impurity due to manufacturing process, or as a degradation product [9][10]. Several studies have addressed that BPA is detected in saliva as a result of hydrolysis of Bis-DMA by salivary enzymes [11-14].

BPA-based monomers

The BPA-based monomers have been identified as endocrine disruptors. This means they can mimic and interfere with hormone receptors, such as thyroid, androgen or estrogen receptors and immune system receptors, causing trouble on thyroid hormone concentrations, low fertility on both men and women and trouble on gene expression [15]. Other health outcomes of BPA-based monomers include immune function, oxidative stress and inflammation, obesity, cardiovascular diseases and diabetes [13].
In 1988, the US Environmental Protection Agency (EPA) set the tolerable daily intake (TDI) dose of BPA at 50 μg/kg body weight per day (bw/day). In 2015, the European Food Safety Authority (EFSA) revised this TDI to a lower level of 4 μg/kg bw/day, based on the results of scientific studies showing that BPA can cause health issues on concentration levels even lower than 10 μg/kg [9]. Although this level was supposed to be temporary and it was expected to be revised in 2017, it still has not been changed and EFSA claims that the studies regarding the TDI dose of BPA will be completed by 2020. The most common BPA-based monomers used in dental materials are shown in **Figure 1**.

Non-based BPA monomers

It has not been proved that non-BPA-based monomers act like endocrine disruptors, but some toxicological tests suggest that they are cytotoxic. Firstly, molecular and cellular

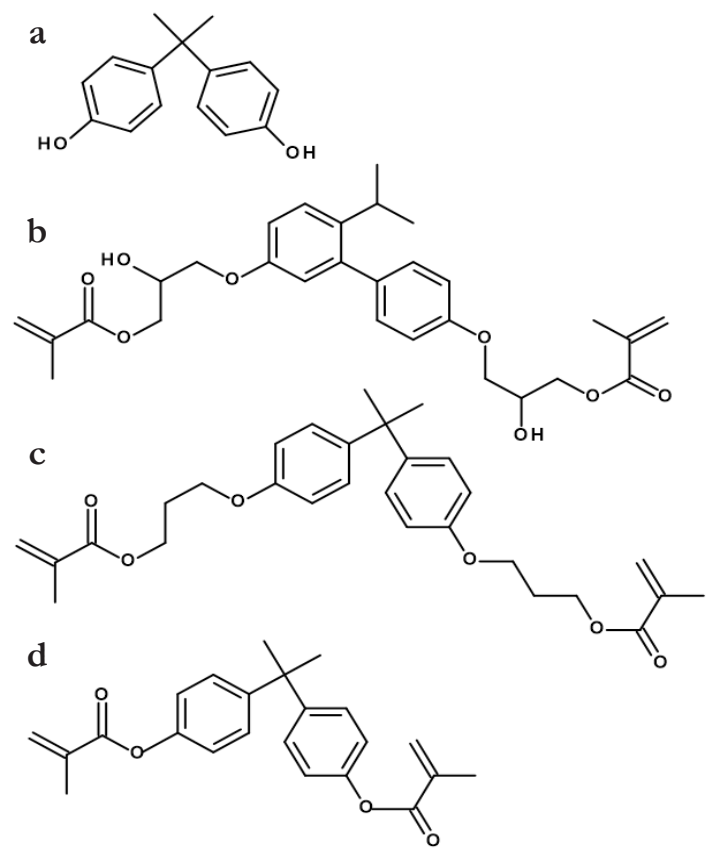

Figure 1. BPA-based monomers; (a) BPA; (b)Bis-GMA; (c) Bis-EMA; and (d) Bis-DMA.

mechanisms of cytotoxicity are initiated by these unreacted monomers, leading to pulp alteration and retraction of the gingival margin. Secondly, they provide a quite good substrate for cariogenic bacterial strains, causing the formation of secondary caries and long-term degradation of the polymers, leading eventually to the failure of the restoration. Thirdly, they can be related to local and systemic allergic reactions, in general [16].
TEGDMA (triethylene glycol dimethacrylate) is one of the most frequently used monomers in dental composite resins. The effective dose (ED_{50}), for TEGDMA, assessed by studies carried in human dental pulp is 0.08 mg/mL [17]. When TEGMA exceeds that value, some of the negative effects mentioned above may occur. Some of the non-BPA based monomers are given in **Figure 2**.

Computer aided design/computer aided manufacturing (CAD/CAM) materials

During the 20th century, dental materials as well as dental technologies have made a remarkable progress. Nowadays, computer-aided design/computer-aided manufacturing (CAD/CAM) technology has been used worldwide in dentistry. It offers several advantages, such as increased quality, automation of fabrication procedures, minimized inaccuracies and faster delivery. The curing part is also not required for CAD/CAM Resin composite blocks (RCB) as they are pre-polymerized into ready-to-mill blocks [18]. Although ceramic blocks have been the most used materials for CAD/CAM, the advantages of RCB led to their development as viable alternative [19, 20].
The most commonly used monomers are triethylene glycol dimethacrylate (TEGDMA)

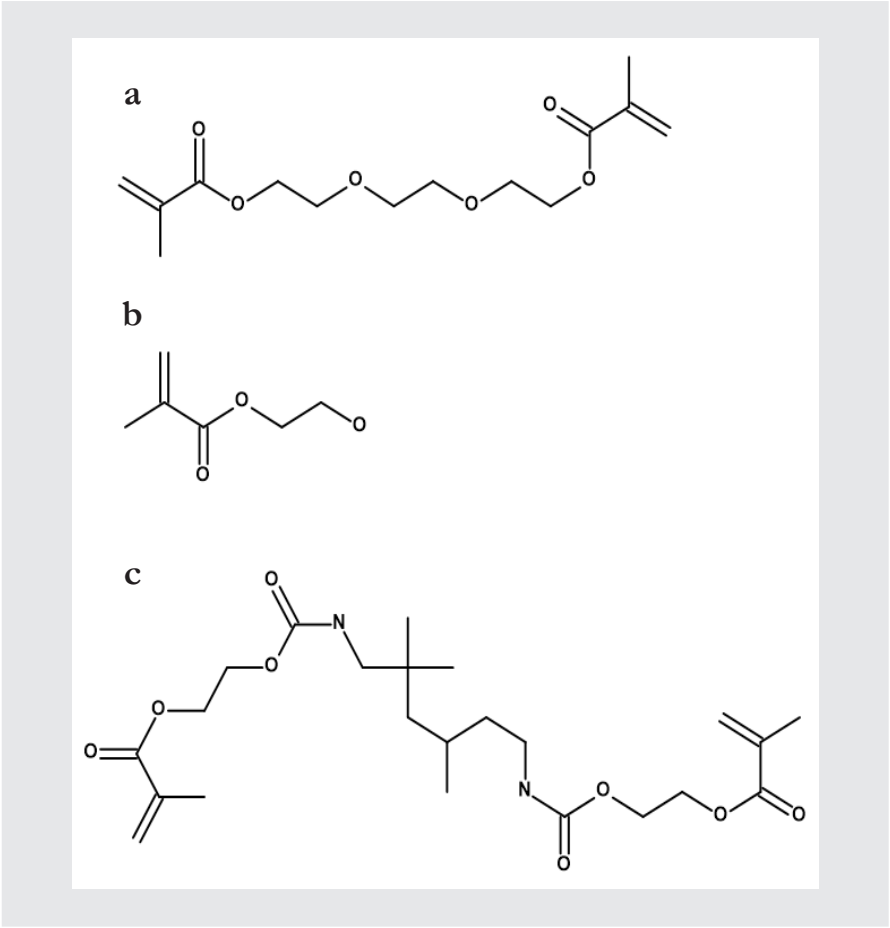

Figure 2. Non-BPA-based monomers; (a)TEGDMA; (b) HEMA; and (c) UDMA.

or urethane dimethacrylate (UDMA). However, there are also composite resin CAD-CAM blocks that contain BPA-glycidyl dimethacrylate (Bis-GMA) or other monomers including bisphenol A-ethoxylate dimethacrylate (Bis-EMA) or N,N-dimethylacrylamide (DMA). These molecules could be released, due to incomplete polymerization process, or degradation of the material and are important because they are related to material toxicity [20, 21]. In this technology, a digital camera is used to get the digital print of teeth, whilst the construction of the restoration is done by using a computer, which allows the use of high quality and high endurance materials. Those are industrially constructed, under excellent conditions, in comparison with those that are constructed by conventional ways. The result is that the restoration is highly resistant through time and they simulate very well the natural dental tissues [20].

To some up, the new materials, including CAD/CAM materials, used both in preventive and restorative dentistry contain monomers with endocrine or cytotoxic properties, which can cause minimum or even severe damage to human body, when found in specific concentrations. So, it is essential to investigate and specify their degree of elution from dental materials. In this review article we present some of the techniques and methods used to determine the short-term and long-term release of these monomers from modern dental materials, in biofluids such as saliva, blood plasma/serum and urine, as well as in various solvents and conditions in order to investigate the mechanism and degree of their degradation. Another aim of this review article is to prove the pivotal role of analytical chemistry and especially bioanalysis in dentistry [22, 23].

Analytical techniques

Most of the techniques used in monomer analysis currently are chromatographic. In this section there are presented some of the methods developed for the determination and quantification of monomers released from dental materials. Many methods have been developed *in-vitro*, whilst only a few were applied to biological fluids. In the following studies the elution of monomers was investigated in different curing conditions and times and different storage periods, in order to evaluate the effect of those factors on the amount of eluted monomers. The presented studies are divided based on the separation technique (Liquid and Gas Chromatography) and the detector used (Mass Spectrometer or Ultraviolet).

Liquid chromatography methods

LC-MS/MS

In a study carried in 2009 by Polydourou et al. [24], liquid chromatography tandem mass spectrometry was used to investigate the elution of Bis-GMA, TEGDMA, UDMA and BPA from two light-cured (nanohybrid and organically modified ceramics) and a chemically-cured resin composite materials, in different curing times and different storage periods. Each specimen was stored in 1 mL of 75% v/v ethanol.

Limit of quantification (LOQ) were: 1 μg/mL for UDMA, 0.5 μg/mL for TEGDMA, 1 μg/mL for Bis-GMA, and 0.5 μg/mL for BPA. Values lower than these levels could not be quantified. Solvent gradient of 0.1% w/v formic acid and acetonitrile were used for the analysis.

The results showed that the amount of monomers released from the organically modified ceramic was significantly lower than from the respective from the other two materials.

Concerning the curing time, for the nanohybrid, less monomers was released after increasing the curing time. For the organically modified ceramic, 80 s of curing time resulted a higher degree of monomers release.

The elution of TEGDMA was decreased after storage for 28 days and 1 year. However, a similar amount of Bis-GMA was released at each storage time, even after 1 year.

Lastly, this study showed that the organically modified ceramic released a very small amount of monomers compared to the other materials.

A combination of LC and MS in the form of LC-MS/MS can be very helpful to identify other substances and degradation products that could be released from the composite materials, besides the studied monomers.

LC-QTOF-MS

In a study carried in 2018 by Vervliet et.al [25], a variety of commercially available dental materials was analyzed by liquid chromatography coupled to a QTOF instrument. For the optimization, a triple quadrupole mass spectrometer, equipped with an electrospray ionization source, was used.

There were several mobile phases tested, such as acetic acid (0.1% v/v) and ammonium acetate buffers (pH 3.7). However, the use of ammonium fluoride as a mobile phase proved to be an improvement for the sensitivity for detection of monomers. The analysis time was less than 10 minutes and the relative standard deviation (RSD%) of the method was 14.4 %.

For analysis of dental resin materials, unpolymerized sample was dissolved in methanol, vortexed, sonicated and centrifuged. The supernatant was analyzed after addition of internal standard solution. Besides the monomers that were present in the materials as fillers, degradation products and impurities of Bis-GMA and TEGDMA were detected in several samples and they were able to be identified thanks to the MS system. In total, 39 compounds were able to be detected, identified and quantified in dental materials.

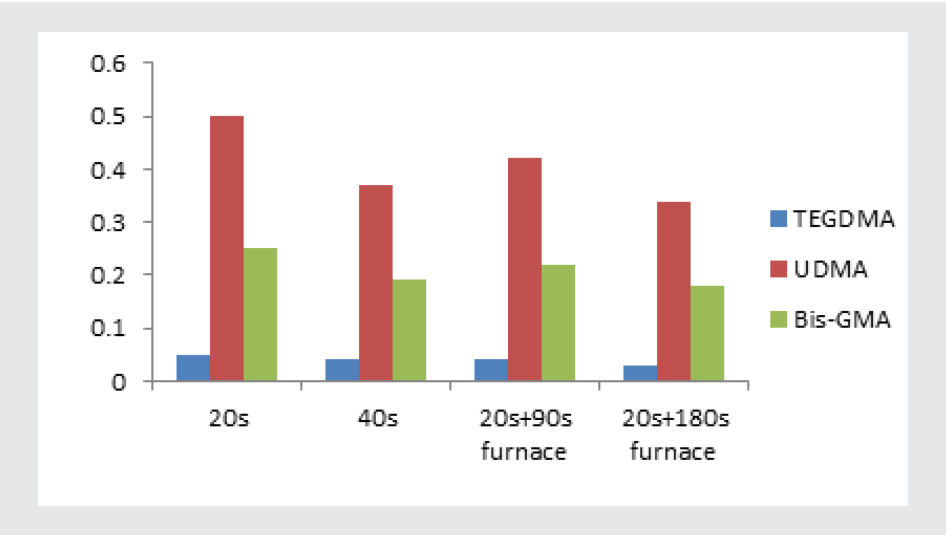

Figure 3. Amount of eluted monomers from composite cured with different exposure time (μg monomer/mg composite)

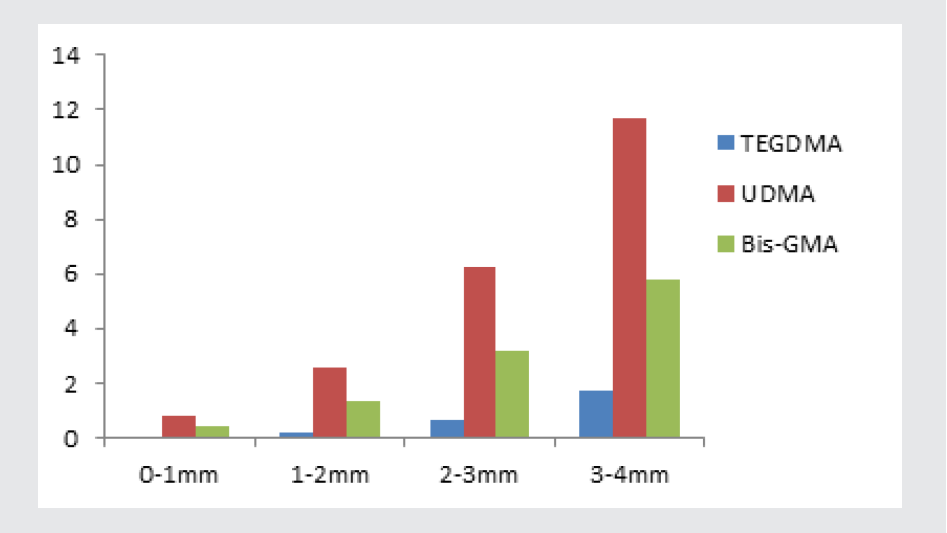

Figure 4. Amount of eluted monomers from different layer depth of composite (μg monomer/mg composite)

HPLC-Micro Raman spectroscopy

The aim of the following study was used to determine the correlation between the quantity of eluted monomers from dental composites, using HPLC and the degree of conversion, using micro-Raman spectroscopy. For this purpose, Lempel et al. [26], used a Bis-GMA/UDMA/Bis-EMA/TEGDMA-based composite resin material, which was stored in a 75% v/v ethanol/water solution for 72 hours. Separation was achieved with gradient elution. Eluent A consisted of ACN/distilled water (40/60% v/v) and eluent B contained ACN/distilled water (95/5% v/v).

The LOQ of the method for each monomer was: 4.4 pmol (1.3 ng) for TEGDMA, 6.7 pmol (3.1 ng) for UDMA and 2 pmol (1.0 ng) for Bis-GMA. The results showed that there was a significant increase in the degree of conversion and decrease in monomer elution, when

the energy used for the polymerization was increased from 20 to 40 J/cm^2. If the variable is the depth of the polymerization (the applied resin layer thickness), the ratio between degree of conversion and monomer elution is 1:3. This means that 1% increase in degree of conversion provides 3% decrease in monomer elution. It should be mentioned that an increase in depth from 1 mm layer to 3 mm led to 10% decrease in the degree of conversion (and 30% increase in monomer elution). The results are summarized in **Figures 3 and 4**.

HPLC-MS

The purpose of this study carried by Ruwaida Z. Alshali [27], was to assess monomer elution from bulk-fill and conventional resin composites stored in water, 70% v/v ethanol/water solution and artificial saliva, for 24 h, 1 month and 3 months. All storage media contained caffeine as an internal standard. All composite materials were cured for 20 s using a LED light-curing unit under standard curing mode. The solutions were analyzed with high performance liquid chromatography coupled with mass spectrometer.

All monomers showed a variable extent of elution into 70% v/v ethanol/water solution with significantly higher amounts than those detected in water and artificial saliva. Significantly higher elution was detected from UDMA-BisEMA based composites compared to Bis-GMA and Bis-GMA/Bis-EMA based systems in 70% ethanol/water solution. The rate of elution into different media varied between different monomers and was highly dependent on the molecular weight of the eluted compounds.

Elution from bulk-fill resin-composites is comparable to that of conventional materials despite their increased increment thickness. Monomer elution is highly dependent on the hydrophobicity of the base monomers and the final network characteristics of the resin-matrix.

The objective of the following study by Putzeys E. et al. [28], was the quantification of the long-term elution of various compounds, including TEGDMA, Bis-GMA and UDMA, from resin-based dental composites, during a year. The materials were immersed in water, artificial saliva or ethanol and stored in the dark at 37°C. The extraction solutions were refreshed weekly. The composites were cured for 20 s with a LED light-curing unit. The samples were analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Chromatographic separation was achieved using a gradient mobile phase consisting of 2 mM of ammonium acetate buffer (pH 5.6) and methanol. The LOQ of the method was: 10 ng/mL for Bis-GMA, 50 ng/mL for BPA, 5 ng/mL for TEGDMA and 5 ng/mL for UDMA.

The results showed that depending on the composite and extraction solution, certain monomers (Bis-GMA and UDMA) were able to continuously elute from the materials, up until 52 weeks after initial immersion. The elution was higher when ethanol was used as extraction solution. The tested materials continued to release small quantities of monomers over longer periods when a continuous refreshing protocol was used. BPA could not be quantified as its level was lower than the method's LOQ.

HPLC-UV

In a study carried out by Y. Uzunova et al. [29], an HPLC method was developed for the determination and quantification of Bis-GMA, TEGDMA and other monomers (Bis-DMA, Bis-GA, GMA) in polymer network of fillings. The mobile phase was a gradient prepared from acetonitrile and water. To obtain satisfactory separation mobile phases containing different proportions of ACN and water were tested (ACN/water 50/50% or 0/100% v/v). For the detection a UV detector was set at 205 nm and 275 nm simultaneously. The two composites studied were polymerized with a Bluedent LED Smart light curing unit for 40 s and 20 s respectively only from the upper side. The composites were immersed in deionized water and were kept at 37°C for 7 days. The elution was investigated after 24 h, 72 h (3 days) and 168 h (7 days).

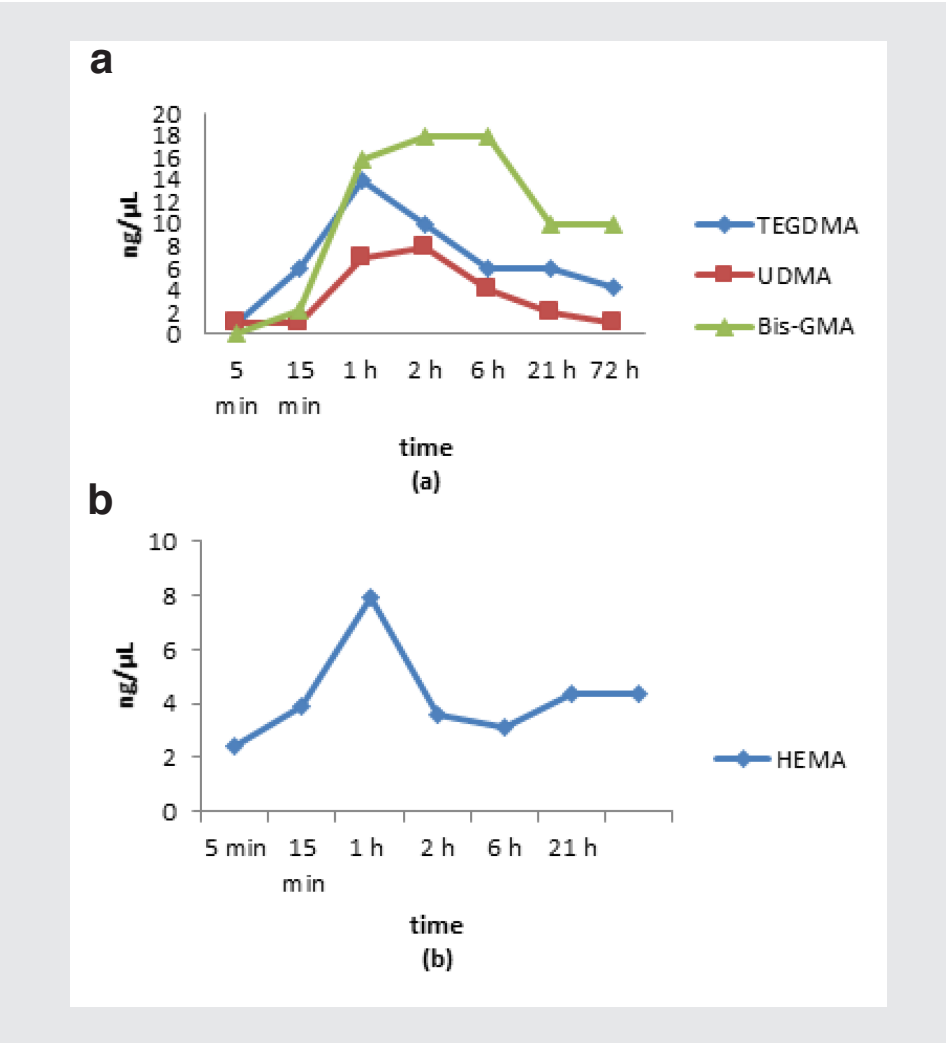

Figure 5. (a) Diagram of the eluted monomers concentrations from variolink cement in relation to time. (b) Diagram of the eluted monomers concentrations from multilink cement in relation to time. Amount of eluted monomers from different layer depth of composite (µg monomer/mg composite)

The Limit of Detection (LOD) was between 0.081 µg/mL (TEGDMA) and 0.180 µg/mL (Bis-GA) for all monomers and the time of the analysis was under 16 minutes.

For the confirmation of the accuracy the parameters studied were the Recovery (%) which was found between 95% and 100% for all monomers and the CV (%), which was found under 3.8% for all monomers.

The highest concentration of eluted compounds was obtained after 168 h (49.52± 6.32 µg/mL). TEGDMA was found in higher concentrations due to its flexibility and high mobility, compared to the other monomers. Lastly, it seems that curing time affects the degree of elution, because the concentration level for each monomer is slightly higher in the composite that was cured for 20 s (Charisma) than in the one cured for 40 s (Solitaire 2).

An HPLC-UV method was used by Phan et al. [19], in order to evaluate the monomer release from high-temperature high-pressure (HT/HP) polymerized urethanedimethacrylate (UDMA). Each specimen was stored in an ethanol/water solution (75/25% v/v), in an oven at 37°C. They were analyzed after 1 day, 7 days, 14 days and 28 days. The mobile phase

consisted of 65/35% acetonitrile/water and the analysis was carried out under isocratic conditions. The UV detector was set at 210 nm.
The accuracy of the procedure was checked using the standard addition method and the results for the recovery, ranging from 102.5% to 105.7%, confirmed that the method was appropriate for quantitative analysis.
The results showed that the release of the monomer increased from 1 day to 28 days. For the three pressure-polymerized materials, monomer release was inferior to LOD (2.62 $x10^{-6}$ mol/L) and LOQ (7.95×10^{-6} mol/L) at all storage times, with the exception 28days release from only one material, which was just above LOQ. The highest amount of UDMA was obtained from the photopolymerized material after 28 days (323.48±39.71 M/g/cm^2) followed by that released from the thermopolymerized material (57.39±1.77 M/g/$cm^{2)}$ after 28 days.
In conclusion, HT/HP reduced monomer release compared to photopolymerization and thermopolymerization. The presence of an initiator was beneficial for the monomer release.

HPLC-UV-Vis

In a more recent study carried in 2014 by Samanidou et al. [30], a simple HPLC method was used for the determination of five monomers (HEMA, BPA, UDMA, TEGDMA, Bis-GMA) released from resin-based dental restorative materials, through human dentin.
Chromatographic separation was achieved isocratically, within 11 min, with a mobile phase consisting of methanol/acetonitrile/water (60/15/25% v/v/v). For the quantitative analysis, a UV-Vis detector was set at 230 nm.
The specimens were stored in deionized water with 0.02% w/v thymol and were analyzed at 5 minutes, 15 minutes, 1 h, 2 h, 6 h, 21 h and 3 days.
The method was validated regarding to selectivity, linearity, accuracy, precision and sensitivity. Intra-day and inter-day precision revealed RSD values lower than 11%. The LOD ranged between 0.17-0.33 ng/μL.
The results are shown in **Figure 5**. All monomers, except for BPA, were found to be released from resin cements through human dentin into the pulp space.
Another HPLC-UV study, for the evaluation of HEMA, BPA, UDMA and Bis-GMA from a resin cement through human dentin was carried by Kerezoudi et al. [31] in 2016.
For this purpose, 10 human dentin disks were adjusted in a new testing device and the resin cement was added under steady pressure of 25 N, following the manufacturer's instructions. The device was filled with Ringer's solution, the samples were kept at 37°C and were analyzed after 5 min, 20 min, 1 h, 2 h, 21 h, 3 days, 7 days, 10 days and 21 days using a gradient system consisting of CH_3CN/H_2O (45/55% v/v and 88/12% v/v) as a mobile phase. Separation was achieved under 8 minutes. The results showed that only HEMA was eluted and BPA, UDMA and Bis-GMA were not detected in any of the samples. HEMA was detected in all samples from 5 min until 10 days. At 4 of the specimens, HEMA was also detected after 21 days at very low concentrations. An unknown compound was also detected, but could not be identified, at 4.4 min. In general, the highest concentration of HEMA detected was still below the toxic level TC_{50}=468-1300 mg/L.
Samanidou et al. [32] developed an isocratic HPLC method for the determination of BPA, TEGDMA, UDMA and Bis-GMA from dental polymeric materials in artificial saliva. The mobile phased consisted of CH_3CN/H_2O (75/25% v/v) and the separation was achieved within 6 min. When repeatability and between-day precision were examined, the RSD values were found under 11.2% in every case. The LOQ in artificial saliva was calculated at 1.2-3.6 ng/μL.
The dental materials were cured by visible light for 40 s and after that, they were immersed in 25 mL of mixtures of ethanol/water or ethanol/artificial saliva in several volume ratios (75/25%, 50/50%, or 25/75% v/v) and kept at 37°C. The analysis took place after 24 h, 7 days and 14 days.
The extent of elution appears to be greater in an organic solvent (ethanol/water) than

in artificial saliva or water, in general. The highest concentration found, comes from the 14-day elution of UDMA and was calculated as 18 ng/μL. For the rest of the monomers concentration levels were found lower than 9 ng/μL, regarding the solution of immersion and the time of the analysis.

A simple HPLC-UV method was developed by Samanidou et al. [33], for the determination of BPA, TEGDMA, UDMA and Bis-GMA monomers in human blood serum and urine in 2015. The separation was achieved with the use of an isocratic mobile phase of CH_3CN/H_2O 70/30% v/v within 6 min. Intra-day and Inter-day precision revealed RSD % values lower than 13.1% for blood serum samples and lower than 6.6% for urine samples. Another factor examined was recovery, which ranged from 92.6% to 106.1% in blood serum samples and from 95.0% to 106.9% in urine samples. The LOQ as calculated by the calibration curves was 4.2-6.8 ng/μL in blood serum samples and 1.7-3.3 ng/μL in urine samples.

Another aim of the study described above, was to evaluate the stability of those monomers in human blood serum and in urine in terms of long-term storage(at 4°C and -18°C) and in terms of short time storage (room temperature).

Regarding the stability, all monomers studied, were found to be stable at 4°C for 24 h and at -18°C for a week at least and for 3 freeze-thaw cycles, in general.

Gas chromatography methods

GC-MS

The purpose of the following study by Bationo et al. [34], was to characterize monomers released from orthodontic adhesives, using gas chromatography and mass spectrometry. The orthodontic composite samples were light-cured for 20 s and after curing, they were immersed in glass tubes containing Milli-Q water, for 24 h at 37°C. After that, the samples were llyophilized and finally 100 μL of dichloromethane was added.

Most of the compounds found were monomers such as BPA, TEGDMA and HEMA, or their derivatives and additives. Many compounds found and identified in the materials, were not added by the manufacturers, but are residues of the synthesis of the raw material, such as catalysts and stabilizers.

The LOD was 0.02 ppm (μg/mL) and the eluates were able to be identified within the LOD, in the samples. The monomer which was detected in the samples in significant amounts was TEGDMA. On the other hand, the absence of BPA in 3 of the 4 samples suggested that any small quantities of BPA that may have been present, were below the LOD of the analytical method.

In 2007, another study carried by Michelsen et al [35] was published regarding the quantification of nine eluates leached from specimens of four widely used resin-based dental materials. The eluates investigated were: 2-hydroxyethyl methacrylate (HEMA), hydroquinone monomethyl ether (MEHQ), camphorquinone (CQ), butylated hydroxytoluene (BHT), ethyl-4-(dimethylamino)benzoate (DMABEE), triethylene glycol dimethacrylate(TEGDMA), trimethylolpropane trimethacrylate (TMPTMA), oxybenzone (HMBP) and drometrizole (TIN P).

All dental materials were polymerized by visible light for 40 s. After that, they were immersed in ethanol or Ringer's solution (9.0 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2 \cdot 2H_2O$, in distilled water, total volume 1 L, pH adjusted to 7 with NaOH or HCl) and kept at 37°C with constant agitation (200 rpm).

The specimens kept in ethanol were transferred after 24 h to separate glass vials containing ethyl acetate and diethylphthalate as an internal standard. The solutions were evaporated to 200 μL at 60°C and transferred to sample vials to be analyzed by Gas Chromatography and Mass Spectrometry.

The specimens in Ringer's solution were transferred to glass vials containing ethyl acetate and diethyl phthalate as an internal standard, after 7 days. They were agitated for 1 min and rested. They were then, extracted 3 times with ethyl acetate and the extracts pooled

Table 1. Materials and methods of the relevant studies

Studies	Type of dental material	Substance under investigation	Time of polymerization	Solution of immersion	Storage conditions	Time of analysis after immersion or placement	Highest concentration measured	Analytical technique
Polydorou et al. [24]	Nanohybrid, Nano-ceramic, Self-curing composites	TEGDMA, UDMA, Bis-GMA, BPA	Light-cured 0 s, 20 s, 40 s or 80 s or Chemically cured	75% Ethanol	21°C, no light	24h, 7d, 28d, 1y	3.52 ± 0.03 log (μg/mL) (Bis-GMA, nanohybrid, 0 s cured, 24 h)	LC-MS
Vervliet et al. [25]	Resin composites	BPA, Bis-GMA, Bis-EMA, Bis-DMA, TEGDMA, UDMA, EDMAB, TCD-DI-HEA	Unpolymerized	Methanol	-20°C	Not mentioned	Not quantified	LC-QTOF-MS
Lempel et al. [26]	Resin composites	TEGDMA, UDMA, Bis-GMA, Bis-EMA	LED light-cured, 20 s, 40s , 20 s + 90s (+ xenon polymerized) or 20 s + 180 s (+ xenon polymerized)	75% Ethanol/ Water	37°C, no light	72h	0.5 μg/mg (UDMA, 20 s) 11,67 μg/mg (UDMA, 3-4 mm depth of curing)	HPLC-Micro Raman
Alshali et al. [27]	Bulk-fill and conventional resin composites	TEGDMA, UDMA, Bis-GMA, Bis-EMA, DEGDMA, TCD-DI-HEA	LED light-cured, 20s	Water or Artificial saliva or 70 % Ethanol/ water	37°C	24h, 1m, 3m	2822.9 ± 290.4 μg/mL (Bis-EMA, 24 h-1 m)	HPLC-MS

Table 1. Cont'd

Studies	Type of dental material	Substance under investigation	Time of polymerization	Solution of immersion	Storage conditions	Time of analysis after immersion or placement	Highest concentration found	Analytical technique
Putzeys et al.[28]	Resin-based composites	Bis-GMA, TEGDMA, UDMA, HEMA, CQ, Bis-EMA3, Bis-EMA6, Bis-EMA10, TCD-DI-HEA	LED light-cured, 20 s	Water, or artificial saliva or ethanol	37°C	1, 2, 3, 4, 6, 8, 10, 12, 14,16,20,24,28, 32, 36, 40, 44, 48, 52 w	~ 1000 log(nmol) (HEMA in ethanol, 52 w)	UHPLC-MS-MS
Uzunova et al. [29]	Composite fillings	TEGDMA, Bis-GMA, Bis-GA, Bis-DMA, GMA	LED light-cured, 20 s or 40 s	Deionized water	37°C	24 h, 3 d, 7 d	49.52±6.32 µg/mL (TEGDMA, 7 d, 20 s cured)	HPLC-UV
Phan et al. [19]	HT/HP UDMA-based dental materials	UDMA	Photopolymerized (with initiator), Thermopolymerized (with or without)	ethanol/ water (75/25% v/v)	37°C	1 d, 7 d, 14 d, 28 d	323.48 ±3 9.71 $M/g/cm^2 \times 10^{-6}$ (photopolymerized with initiator, 28 d)	HPLC-UV
Samanidu et al. [30]	Resin- based restorative materials on human dentin	HEMA, BPA, UDMA, TEGDMA, Bis-GMA	Dually cured as set by manufacturers	Deionized water with 0.02% w/v thymol	37°C	5 min, 15 min, 1 h, 2 h, 6 h, 21 h, 3 d	18 ng/mL (TEGDMA, 6 h)	HPLC-UV-Vis
Bationo et al. [34]	Orthodontic adhesives	BPA, TEGDMA, HEMA, their derivatives and additives	Visible light, 20 s	Milli-Q water	37°C	24 h	Above LOQ (0.06 µg/mL) for TEGDMA	GC-MS

Table 1. Cont'd

Studies	Type of dental material	Substance under investigation	Time of polymerization	Solution of immersion	Storage conditions	Time of analysis after immersion or placement	Highest concentration found	Analytical technique
Michelsen et al. [35]	Resin composites	HEMA, MEHQ, CQ, BHT, DMABEE, TEGDMA, TMPTMA, HMBP, TINP	Visible light, 40 s	Ethanol or Ringer's solution	37°C, constant agitation	24 h (immersed in ethanol) 7 d (immersed in Ringer's solution)	3.28 μg/mm^2 (TMPTMA in ethanol)	GC-MS
Reichl et al. [36]	Adhesives	TEGDMA, HEMA	LED curing light, as set by manufacturers	Methanol or distilled water	37°C	1 d, 2 d, 5 d, 10 d, 20 d, 30 d	Lower than those causing cytotoxicity (1000-10,000 times)	GC-MS
Moreira et al.[37]	Resin composites	BPA	Halogen light-curing, 60 s	Ethanol/ water 75/25% v/v)	37°C	30 min,1 h, 1 d, 7 d, 30 d	Lower than the tolerable daily dose	GC-MS
Kerezoudi et al. [31]	Resin cement through human dentin	HEMA, BPA, UDMA, Bis-GMA	Dually light cured, 60 s	Ringer's solution	37°C	5 min, 20 min,1 h, 2 h, 21 h, 3 d, 7 d, 10 d, 21 d	Lower than TC_{50}= 468-1300 mg/L	HPLC-UV
Samanidou et al. [32]	Dental composites	BPA, TEGDMA, UDMA, Bis-GMA	Visible light, 40 s	Ethanol/ water, or ethanol/ artificial saliva	37°C	24 h, 7 d, 14 d	18 ng/μL (UDMA, 14d, ethanol/water 75/25% v/v)	HPLC-UV
Samanidou et al. [33]	Resin Composites	BPA, TEGDMA, UDMA, Bis-GMA	Not mentioned	Blood-urine from patients deproteinized and reconstituted to methanol	4°C, -18°C	1 h, 5 h, 24 h, 2 d, 7 d (stability)	Not quantified	HPLC-UV

for each sample. The pooled extracts were transferred to glass vials, evaporated to 200 µL at 60°C and transferred to sample vials to be analyzed by Gas Chromatography and Mass Spectrometry.

The LOD varies between different substances and was between 0.01 and 1 µg/mL. Low weight molecules needed higher concentrations to be detected and LOQ was between 0.1 and 1 µg/mL.

Within-day precision measured as relative standard deviation (RSD%) was between 0.018% and 0.451%. Between-day variation measured as RSD% ranged from 0.019% to 0.512% for all compounds with a slightly higher RSD for the higher concentrations investigated.

The results showed that the eluted amounts were higher in ethanol compared to those in Ringer's solution for all substances except one (MEHQ) from one dental material. The highest amount of a substance (TMPTMA) eluted from one specimen was 3.28 µg/mm^2, eluted in ethanol from only one dental material.

The purpose of the following study by Reichl et al. [36], was to quantify the amount of TEGDMA and HEMA eluted from several adhesive systems, using Gas Chromatography and Mass Spectrometry. Each adhesive was polymerized by a LED curing light and the period of irradiation was set according to manufacturers' instructions. After this, the vials containing the polymerized adhesives were filled with methanol or distilled water, caffeine was added as an internal standard and they were then stored at 37°C. For measurements, 1 µL of the supernatant was injected into the GC-MS at 1, 2, 5, 10, 20 and 30 days after the beginning of the experiment. The absolute LODs were 0.01 µg for TEGDMA and 0.02 µg for HEMA, respectively. The results showed that the highest concentrations of free TEGDMA and HEMA are seen when the adhesives are stored in methanol, rather than water. The quantities released from adhesives though, are lower than those required to induce cytotoxic effects (85–1000 times for methanol and about 5000–10,000 times for water). These amounts decrease with ongoing experimental time. Lastly, it should be noted that for several adhesives, quantification was not possible because the quantities released were below the method's LOD.

The objective of the following study by Moreira et al. [37] was to quantify in vitro the BPA release from orthodontic composites and to assess in vivo the BPA levels in saliva and urine samples of patients after bracket bonding with orthodontic adhesives.

Each resin was photoactivated for 60 s with a halogen light-curing unit. After polymerization the samples were immersed in an ethanol/water solution (75/25% v/v) and kept at 37°C. They were collected after 30 minutes, 24 h, 1 week and 1 month after immersion, an internal standard was added (BPA-d_{16}; 1 mg/mL) and they were then dried under a vacuum system at 45°C. For the derivatization, BSTFA and TMCS were added to the dry residue. After vortexing and immersion in a thermostatized bath at 37°C the derivatized solution was injected into the gas chromatography system for analysis. For the *in vivo* study, urine samples collected from patients 30 minutes, 24 h, 1 week and 1 month after bracket bonding, were subjected to enzymatic treatment with the addition of a sodium acetate buffer and the diluted enzyme solution. The mixture was maintained at 37°C for 4 h and then the internal standard was added for liquid-liquid extraction. The extraction solvent (MTBE) was added, the samples were vortexed, centrifuged and the supernatant was dried under vacuum. The residue was derivatized as previously described. The saliva samples were treated in the same way as the urine samples with the difference that the enzymatic treatment was not necessary.

The results showed that BPA was released from all composites, but the detected levels were lower than the recommended daily dose. All materials reached peak levels 1 month after bonding. The *in-vivo* experiment showed that bracket bonding led to increased BPA levels in urine and saliva. The levels were also in that case lower than the recommended daily dose. In **Table 1**, we sum up the most important findings of all studies mentioned in this review.

DISCUSSION

All methods mentioned above are suitable for the determination of eluted monomers found in modern dental materials, such as BPA, Bis-GMA, Bis-EMA, Bis-DMA, TEGDMA, UDMA and HEMA, which are the monomers of which the polymeric matrix of dental materials is mainly composed. Methods using UV detectors are precise and accurate but their disadvantage is that unknown peaks found in the chromatograms can't be identified, so it's not possible to determine their origin [19, 29–33]. Gas chromatography methods require extra steps, during sample preparation, thus to convert the studied substances into a form compatible with the method. So, even though they exhibit excellent repeatability and accuracy, they are not preferred by analysts [34–37]. The most preferred methods are those that combine separation by liquid or high-pressure liquid chromatography and mass spectrometer detection [24, 25, 27]. Those methods have an advantage over the ones using UV detectors, because they can identify all peaks, even those not due to the monomers studied. Their advantage over gas chromatography methods is that the sample preparation process is more rapid and less complex.

Most of the methods mentioned in this review article were developed and applied in-vitro. There are only a few methods applied to biological fluids, such as blood, urine and saliva mentioned in literature [33] and given that dental materials, eluted monomers and human health are inseparably connected, more research should be done on this field.

Concerning the amount of the eluted monomers from dental materials cured thermically, by visible light, or LED light it seems to increase during storage and the long-term elution should be further studied. On the other hand, for CAD/CAM materials, polymerization is more adequate, and the released amount decreases during storage. Furthermore, an increase in polymerization time causes a decrease in the quantities released [6, 20].

CONCLUSIONS

As mentioned above dental materials used nowadays are consisted of several monomers that seem to act like endocrine disruptors or have cytotoxic effects on humans. During the curing process of dental materials those monomers might not be completely polymerized, and it is possible that small quantities of them to remain free and unpolymerized causing negative effects on human health. It is even possible for them to be found as impurities as a result to the incomplete and poor construction of dental resins. Lastly, the degradation of dental materials is likely to happen long term causing the elution of free monomers in the oral cavity. Taking into consideration all the above, it is necessary to study the long-term and short-term elution of these monomers from conventional dental materials and to verify whether the eluted quantities can cause troubles on human health or not.

For this purpose, Liquid Chromatography methods coupled with Mass Spectrometers are mostly used, as they seem to be more suitable to identify and quantify the eluted monomers, with excellent precision. UV detectors were also used in some of the investigated studies and the results were as reliable as those from the MS detection, with the difference that not all peaks found on the chromatograms (derivatives, additives, impurities or degradation products) were able to be identified.

Fewer articles for gas chromatography coupled with mass spectrometers methods were found, most likely because more steps during the sample preparation process are required.

In all of the researched studies the highest concentrations found were lower than those required to cause negative effects on humans. For example, the TDI for TEGDMA which is 0.08 mg/mL, was way higher than the measured concentration in every case. The same applies to BPA for which the TDI is even lower than TEGDMA, (4 μg/mL bw/day).These results confirm the assumption that modern dental materials are safer and have fewer negative outcomes on human health, or even none at all. However, those materials should be further studied for the long-term elution of monomers.

Lastly, in literature referring to dentistry and dental material's possible hazardous

properties, the majority of the studies found are chromatographic, either liquid or gas. That proves that analytical chemistry and dentistry are two science fields inextricably linked. Especially bioanalysis is a necessary and powerful tool in the evaluation of short-term and long-term monomer release from dental materials. Analytical chemistry is extremely useful when used to investigate possible negative outcomes of materials concerning human health.

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