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## 12.1 Introduction

Components of composite materials might be released under different circumstances in the oral cavity having consequences on the human health and on the materials themselves. The evaluation of the elution of substances from restorative materials presents an important parameter on the estimation of their biocompatibility. The mechanisms of cytotoxicity of composite materials concerning the released substances, as proposed by Goldberg [1], are determined firstly as their short-term release during the monomer-polymer conversion, taking place in the first hours after polymerization and secondly as the release of substances that can take place as a result of erosion and degradation over time.

Release of substances is a critical issue for dental materials. It can lead to important loss of material and probably also to failure of the restoration. Wear presents a progressive loss of the material or changes of the material surface after coming in contact with substances. Fatigue, abrasive, and/or chemical wear might result in changes in material structure and/or material surface. The

eluted monomers might react with the bacteria in the oral cavity, which can have some biological implications after coming in contact with the pulp and/or gingival tissues, or exert systemic effects on human health [1]. Different routes of systemic intake of the released monomers are described including ingestion of the substances in the gastrointestinal tract, diffusion to the pulp through dentinal tubules, and uptake in the lungs [2]. Most research papers [3–11] are focused on the elution of monomers from composite materials; however the release of other components like filler particles should also be considered as they are not without negative consequences. Besides these, various additives, photoinitiators, and co-initiators have been shown to be eluted under different experimental conditions [3, 10].

## 12.2 Elution of Monomers from Composite Materials: What Is Eluted?

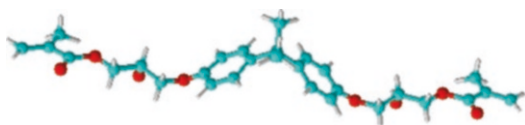
The content of the organic matrix of the composite materials plays an important role on the potential release of monomers. The composition and the amount of the eluates depend on the composition of the composite materials, their “chemistry,” and the extraction medium used [12, 13]. Several additional factors influence the final amount of the eluted monomers. According to Ferracane [3], the elution of monomers from

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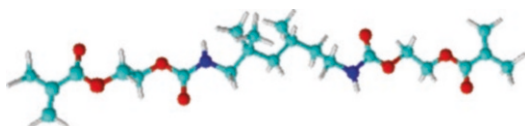
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composite materials relates to the following: the polymerization reaction, the kind of solvent used, and the size and kind of the eluted substances. Smaller molecules elute easier than the larger ones as the former show higher mobility.

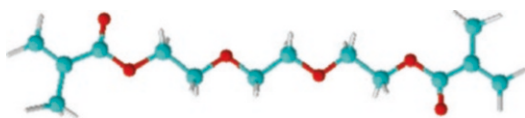
More than 30 different compounds have been shown to be extracted from polymerized dental composites, and among those, major monomers, co-monomers, various additives, and reaction products have been detected [2, 3, 7, 10]. BisGMA (bisphenol-A glycol dimethacrylate Fig. 12.1), UDMA (urethane dimethacrylate, Fig. 12.2), and TEGDMA (triethylene glycol dimethacrylate, Fig. 12.3) are the most often investigated and detected monomers to be released from dental composites [2, 14–16]. In several studies it is reported that BisGMA was shown to be released in high rates [16–19], although it is a large molecule. It is suggested that this high elution rate of BisGMA is due to the fact that it is a rigid and highly viscous monomer with high transition temperature, resulting in its lower degree of conversion during polymerization [20]. Although HEMA does not present a component of modern composite materials nowadays, as it shows high cytotoxicity, it has been stated to be eluted from composite materials [2, 21]. The presence of HEMA has been explained by the authors



**Fig. 12.1** BisGMA (bisphenol-A glycol dimethacrylate)



**Fig. 12.2** UDMA (urethane dimethacrylate)



**Fig. 12.3** TEGDMA (triethylene glycol dimethacrylate)

either as a part of the organic resin matrix of the composite materials or more probably as a by-product by the degradation of UDMA by the analytical process [22].

BisEMA and other derivatives of bisphenol A have been reported to release. Of high interest is the detection of small amounts of bisphenol A itself in the eluates of composite materials. This point will be discussed in detail in the following section. The release of degradation products of the monomers might also take place [7, 23, 24]. The eluted monomers might react with each other creating new by-products [25], which results in crucial problem in their analytical detection. Although it is mentioned that usually 5–10% of the unbound monomer is eluted [3], the eluted amount varies among the experimental studies, as the elution depends on the composition and structure of the composite material, on the curing process performed and additionally on the analytical method used.

### 12.2.1 Elution Rate of Monomers Over Time

In the past, it was thought that 50% of the eluted substances are extracted in water within 3 h, while in the same time 75% of the eluted monomers are extracted in 75% ethanol solution [14]. According to these, it was concluded that within 24 h almost all leachable substances are eluted and after that no further release of monomers takes place over time. It is definitely a fact that the elution of monomers is not linear over time. Some authors observed that the highest release takes place in the first week after polymerization [11, 26], while other authors have shown that the release of monomers continues over time, up to 30 days [16, 17, 19, 27] or even for 1 year after polymerization [15].

## 12.3 Parameters that Influence the Elution of Monomers

As it is mentioned above, reasons that can affect the release of monomers can be found in the material properties itself, the kind of storage media

used, and the conditions in the environment of the oral cavity that might affect the composite filling surfaces.

In detail, the incomplete polymerization of the composite resins [28, 29], presenting usually a conversion rate, which varies between 35% and 77% [30–32], plays a role in the elution of monomers. This is due to the fact that a part of the monomers which remain unreacted after the polymerization reaction can be eluted from the polymerized structure. The degree of conversion is an important factor influencing the mechanical properties of the composite materials and additionally their biocompatibility [3, 33]. The composition of the composite materials in addition to the layer technique and the polymerization conditions used can influence the degree of conversion.

For a long time it was believed that the degree of conversion determines the elution of monomers from restorative materials. In some cases, a strong correlation was observed between the degree of conversion and the amount of eluted monomers for conventional materials, as the highest amount of eluted monomers (TEGDMA and BisGMA) was observed to be correlated to the lowest degree of conversion [34]. However, for some materials, the filler content was found to correlate to the elution of monomers so that materials with high filler content could show lower release of monomers, suggesting that the degree of conversion is not the only parameter that can influence the monomers' release [34]. Therefore the release of monomers cannot be predicted solely due to the degree of conversion. Pongprueksa et al. [26] observed no correlation between the degree of conversion and the elution of monomers from the composite materials tested. This is in accordance with other findings [17] in the literature. The authors [17] found that the composite and polymerization type (photo, chemical, or dual) are more important parameters for this elution. Materials with the same degree of conversion showed different elution rates, according to the kind of composite material. Dual-cured composite material revealed higher amounts of BisGMA and TEGDMA than the photo-cured material

[17]. Interesting are the findings of studies where a correlation among the degree of conversion and monomers' release was found in the cases of suboptimal polymerization [35] or when changes on the composition of the materials took place [36]. Changes in the photoinitiator system were shown to affect the degree of conversion and the elution of monomers [37], verifying the strong inverse correlation among them. Replacing camphorquinone with Lucirin-TPO and using short curing times increased the degree of conversion and the elution of monomers remained stable or decreased.

According to literature data, it can be concluded that although the amount of the residual monomers might be influenced and correlated to the unreacted amount, the elutable residual monomer must be clearly differentiated from the amount of monomers that is not converted during the polymerization reaction, meaning that although the conversion rate might influence the eluted amount of monomers, it does not definitely determine it. Therefore, a direct correlation between the degree of conversion and elution of monomers is nowadays not justified, as the elution mechanism is also related to the molecular weight and the hydrophobicity of the substances as well as the filler content [34] and depends on the composite material used and the network characteristics of the resin matrix [26, 38].

### 12.3.1 Material Effect on the Elution of Monomers

Although there have been a great development concerning composite materials over the past decades and improvement of the conversion rate, as mentioned above, the elution of monomers is still a present problem. The composition of the composite materials has been shown to be more important for the polymerization success than the used polymerization unit or the kind the polymerization method used [39, 40]. A great effort has been performed by the industry to manufacture composite materials with a more complex network in order to prevent the direct

elution of substances. It has been shown that the amount of monomers released from nanohybrid composite materials is higher than the one of ormocers [27]. The authors commented that a main part of the elution of substances takes place in the first 24 h. After this, the elution process becomes slower, while additional time is necessary where the swelling of the polymer chain takes place and then a further “extraction” of monomers happens. Storage media are shown to attack composite materials differently, depending on their chemistry. For materials with more complex chemistry, for example, ormocers, even if they do not show a better conversion rate compared to the hybrid composite materials, they do show less release of monomers due to the reduced initial contained amount and especially due to their complex three-dimensional network which does not allow the unbound monomers to elute easily [16, 17, 27]. Susila et al. [41] could show that ormocer and silorane materials elute less monomer and exert less cytotoxicity than conventional dimethacrylate-based composite materials.

In evaluating the data existing on the newest category of composite materials, the bulk-fill materials, the findings are controversial, as they state an inhomogeneous group of composite resins. In some cases [34], the amount of monomers (BisGMA and TEGDMA) released from the bulk-fill materials was lower than the one of the conventional materials, and in other cases [42], it was comparable with the conventional ones. High elution of monomers from bulk-fill materials was stated up to 30 days after polymerization and the elution was found to increase over time [43]. It is supposed that the use of the incremental application technique of composite materials may overwhelm some of their shortcomings, by achieving an adequate light penetration. For all conventional composite materials, the application of 2-mm layers is accepted as the best solution. In the case of using bulk-fill composite materials, a respective adequate depth cure is shown to be achieved by applying layers of 4 mm. By reducing the layer thickness of bulk-fill flowable materials, the released amount of substances can decrease

[34]. However, the kind of composite material used was shown to be more important than the technique used. By increasing the filler/matrix ratio, it is demonstrated that the conversion decreases, as the presence of fillers states a problem for the polymeric chain propagation [44]. Flowable materials elute higher amount of their unreacted monomers [18] independently of the application method used (bulk-fill or layer technique) [26]. In addition to the filler amount in the composite materials, the light permeability of the fillers together with the monomer composition, the kind and amount of initiators, and the inhibitor/accelerator systems influence the degree of conversion of the composite resins [44]. The adhesion strength of the fillers in the organic matrix, due to the coupling agents, exerts an effect on the possible “washout” of the filler particles in the oral cavity.

Regarding the effect of material quality on monomer elution, the performance of low cost and not widely known brands of composites was compared to a standard widely used composite material [45]. Low-cost composite materials showed some shortcomings compared to the commonly used material, in terms of their mechanical and physical properties, as well as their monomers’ release and potential cytotoxicity.

### 12.3.2 Effect of Curing Parameters on the Elution of Monomers

The curing mode, light curing time, and curing unit used are widely discussed in the literature concerning their effect on the elution of monomers from composite materials as they affect their polymerization degree. Polymerization of composite materials with different curing units and curing protocols results in different degrees of monomers’ elution and softening in ethanol [46]. Additionally the energy density, power density, and mode of cure affect the softening and the elution of monomers from composite resins [47]. The higher the energy delivered to the material during the polymerization, the lower the degree of softening of the material and the eluted amount of monomers [48]. Different

curing protocols can influence the solubility and water sorption of composite resins [48, 49]. Longer curing times were shown to result in lower solubility of the composite materials [50, 51]. Through the depth of the filling material, the light intensity is radically reduced. It must be taken into consideration that in addition to the altered properties of the materials caused by using shortened curing times, the increased solubility of the superficial layer is also a result of it. In the study of Kim et al. [50], composite materials with the same degree of conversion values showed different solubility values. The authors suggested that the difference in the functionality of the base monomers used among the tested composites, influencing the residual amount of monomers, is the reason of their findings.

The influence of curing time on the elution of monomers from composite materials has been widely studied in the literature [52–55]. A reduction of the curing times of the composite materials compared to the recommended curing times results in increased release of substances [53, 54]. The curing times recommended by the manufacturers were insufficient to minimize the release of substances [52, 53]. An increase of the curing time up to 200 s could not eliminate the eluted substances [53]. Although an increase of curing time is recommended by several studies [53, 55] in order to avoid the negative implication of the eluted substances, the rise of pulpal temperature due to increased curing time should be taken into consideration.

Not only the curing time but also the kind of curing unit and mode is of interest for achieving adequate polymerization. The compatibility of the material composition with the curing unit used seems to be important. The kind of the curing unit (halogen and LED) was not found to exert a determinative effect on the amount of eluted monomers [56], whereas the material itself was found to be more important than the curing unit used [57]. The effect of curing modes on composite materials has been shown to be material dependent, as the composition of the materials seems to be of higher importance concerning the effect on the polymerization reaction than the curing unit used [39, 40].

### 12.3.3 Effect of the Storage Medium on the Elution of Monomers

The release of monomers after polymerization is suggested to be influenced by the incomplete polymerization or additionally caused by the solvents in the oral cavity or the degradation of the materials after the initial polymerization. Regarding the chemical degradation of the composite materials in the oral cavity over time, this is thought to be caused by hydrolysis or enzymatic catalysis [58], through esterases from the human saliva.

The monomer elution is highly dependent on the hydrophobicity of the base monomers and the network characteristics of the resin matrix. Sorption and solubility of the dental composites are affected by factors like the hydrophilicity of the polymers and cross-linking density of the network [59]. Extraction media can be hydrophilic (e.g., physiological saline solution), hydrophobic (e.g., DMSO), or mixed (e.g., 75% ethanol and 25% water). Among the published studies, different media have been used in order to evaluate the elution of substances from composite materials: saliva or water in order to simulate the oral environment, culture media, and ethanol or acetone alone or in combination with saliva. Using ethanol, chloroform, or toluene as extraction medium can result in a high release of monomers [9]. The organic solutions can penetrate through the organic matrix easier than water, and therefore they can increase the elution of unreacted monomers [3]. It has been shown that the removal of leachable components from polymerized composites by the use of organic solvents drastically decreased cytotoxicity [9]. The effect of solvents on the composite materials in the oral cavity simulates some kind of erosion leading to weight loss of the polymer [60, 61]. It is mentioned that the solvent can penetrate the matrix, resulting in a change of the mass and the dimensions of the matrix. If the unreacted monomer is soluble in the solvent, it may be leached out of the polymer mass. Swelling and leaching both result from the process of diffusion. As the immersion solvent penetrates the matrix causing the opening between polymer chains, unreacted

and leachable monomers may diffuse out [13]. Softening of the resin matrix allows the solvent to penetrate easier [60, 61] and might occur due to water sorption in wet oral environment, resulting in release of monomers. Water sorption can accelerate the degradation of dental composites [62, 63], influencing their physical and mechanical properties. First, the filler-resin matrix debonding is assumed [62], while after that the softening of dental resins through the plasticizing action of water occurs.

Food-simulated liquids have softening and hydrolyzing effects on dental composites which attribute to the degradation of the polymer matrix [39, 64, 65]. A lot of studies [13, 15, 16] have used ethanol 75% as extraction medium in order to simulate the clinical conditions in the oral cavity. Although ethanol 75% is thought to be a very aggressive medium, it is supposed by the Food and Drug Administration (FDA) of the USA to be a “food simulator and aging accelerator” [13]. An important factor that affects the release of monomers is the solubility parameters of the solvents. Weathering agents, such as ethanol, can accelerate the degradation of composite materials [66, 67]. Storage of composite samples in ethanol/water solution resulted in higher sorption values compared to artificial saliva and distilled water which both showed similar behavior [68]. Ethanol can penetrate easily into the resin matrix and therefore promote the release of substances. The highest solubility values of experimental composites were observed in ethanol 75%, while the solubility values in artificial saliva were a little higher than the ones found in distilled water [68]. Especially heavy monomers like UDMA and BisGMA are not able to be leached in aqueous media [69]. According to Ferracane [3], the oral environment is represented somewhere between the more aggressive organic solutions and water. Although a lot of media have been tested from time to time in order to evaluate the elution of monomers from composite materials, there is no systematic evaluation of them, which would make the comparison of the results of the different studies easier.

Besides ethanol 75%, by trying to evaluate clinically relevant media, artificial saliva,

Dulbecco's Modified Medium (DMEM), and DMEM with 10% fetal bovine serum have been additionally investigated [70]. Although a higher release is usually observed in the case of ethanol 75%, TEGDMA as a small molecule can be eluted also in saliva and DMEM, suggesting that the solubility parameter of these solvents is very close to TEGDMA. Small molecules like TEGDMA have higher mobility and can elute faster than large molecules [11]. However, the elution of TEGDMA in DMEM 10% FCS solution is significantly lower than in saliva. TEGDMA seems to bind to albumin contained in serum resulting in different findings in the analytical process [69].

Authors have used methanol [10, 53] as extraction solvent in order to achieve a higher release of monomers. The extraction of photoinitiator (CQ), (co-)initiators, photostabilizers, and inhibitors took place in the case of methanol. Acetone was also used for evaluation of monomers' elution medium [45]. However, storing the samples for 7 days in acetone, which is a very aggressive medium, far away from the clinical conditions, resulted in a high elution of substances.

The oral cavity environment has an effect on the *in vivo* degradation of composite resins [71]. Human saliva presents the more clinically relevant medium in order to simulate the daily clinical conditions in oral cavity. Comparing human saliva to water and artificial saliva, the amount of monomers eluted is found to be lower when human saliva is used. Additionally the composition of human saliva might affect the elution rate [72]. Due to the presence of enzymes, human saliva seems to be more aggressive than the artificial one, being able to degrade the monomers of composite resins [18]. Components of human saliva (various salts and glycoproteins) have been suggested to bind comonomers of the composite materials [72]. Therefore it is assumed that using artificial saliva and water as extraction media, they do not represent the clinical situation in the oral cavity. The amount of small molecules like TEGDMA detected in human saliva was shown to be similar with the one released in ethanol 75% [16]. This correlation did not account for the larger molecules detected.

In order to evaluate the clinical relevance of the gained findings from studies using different storage media, the parameters used should be also taken into consideration, as they might influence the findings and generate correct or wrong assumptions for the dental composite restorations in the oral cavity. The preparation of the eluates presents an important parameter to affect the findings. Although there are instructions for the preparation of the eluates according to ISO 10993-12:2012 [73], most of the studies are performed under different conditions.

### 12.3.4 Methods for Analysis of the Eluted Monomers

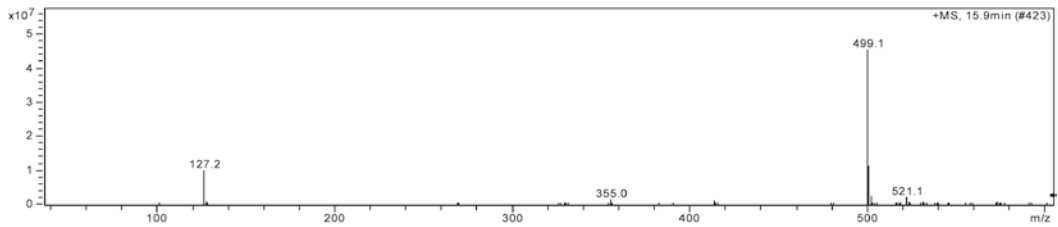
Evaluating the available data in the literature, it is obvious that several different methods have been used from time to time in order to identify and quantify the eluted substances. Although all the available methods are thought to be able to determine the eluted substances, there is a big difference among them concerning their ability to identify unknown substances, to identify substances with high molecular weight (MW), to give the exact MW and amount of the identified substances, and, in the case of by-products or degradation products, to be able to differentiate the substance from which the identified substances came from.

The qualitative and quantitative methods for the analysis of the monomers and degradation products include gas chromatography (GC), high-performance liquid chromatography (HPLC) [7, 20, 74–77], gas chromatography/mass spectrometry [5, 10, 78], and electrospray ionization/mass spectrometry [6, 20].

HPLC analysis is usually preferred to gas chromatography, because it gives a greater level of control over the separation process in this case since the monomers are soluble in the mobile phase. Gas chromatography (GC) is usually used for detection of small molecules. Bulky matrix monomers can be identified by GC only in the case of combining it with mass spectrometry (MS) as GC/MS detects substances by their decomposition products and fragmentation

pattern [10, 79], and therefore it is not suitable for analyzing molecules with high molecular weight [80]. Large base monomers, like UDMA, decompose in GC. The proposed analytical methods used in HPLC techniques may vary between the different studies, due to the conditions established for determination of residual monomers. Although most of the studies have used HPLC as a tool to investigate the elution of monomers from the composite resin materials, it has been pointed out [79] that this method alone is not appropriate.

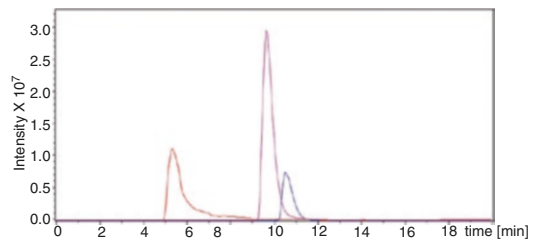
Several parameters involved in the analytical procedure influence the findings of each study, starting from the selection of the substances that are used as external references in order to be able to qualify and quantify the eluted monomers [81]. In the case of HPLC, these pure components are necessary for the identification and quantification of the eluted components. However, as has been reported [79] when HPLC was used alone as analytical method, it has some disadvantages as it is not suitable to give an exact identification of the substances eluted and the references used. In detail, during the analysis of the eluted substances with HPLC alone, the reference standards of the monomers that are expected to be eluted are needed for two purposes: The first is in order to be able to determine the amount of the detected substances through the use of the calibration standard curves of peak areas versus monomer concentration of each substance. A linear curve for each monomer is necessary at known concentrations. Secondly, the chromatogram of each reference substance is of importance in order to determine the mass(es) and the peaks (and their retention time) that are characteristic for each monomer. This step is very important as different peaks for different masses might be present for one monomer by its chromatogram. Usually in most of the published data, it is mentioned that the MW of each expected substance is used for the qualification process. The mass that should be used for the detection of the expected molecule is not necessary the one represented with the higher peak, and in order to be able to give adequate results, it might be necessary to use different peaks for qualification and quantification



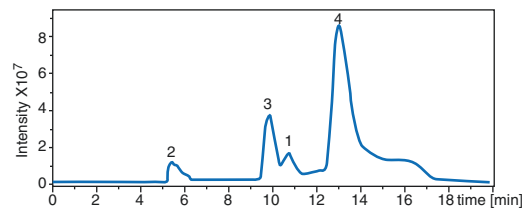
**Fig. 12.4** Identification of substances with HPLC-MS/MS. Here is a given example of the spectrum of one form of UDMA using HPLC-MS/MS (Polydorou O, König A, Hellwig E, Kümmerer K. Urethane dimethacrylate: a mol-

ecule that may cause confusion in dental research. *J Biomed Mater Res B Appl Biomater*. 2009 Oct;91(1):1–4. doi:10.1002/jbm.b.31383)

processes. Additionally, due to the analysis of the reference standards, it is possible to evaluate the stability of respective substances and therefore explain some findings wherein some eluted substances might be unstable after their elution or they might also react with other eluted substances resulting in by-products. In all these cases, the use of HPLC alone to the analytical progress is not adequate to identify all eluted substances. With this method and a very good and appropriate use of the reference standards, it is possible to evaluate only the elution of expected substances. A combination of HPLC with mass spectrometry (LC-MS or LC-MS/MS) (Fig. 12.4) can be used in these cases for a highly specific and accurate identification of the compounds eluted [26, 79, 82]. This method is a very sensitive method being able to identify additional substances to the ones used as references. A mass scanning of the reference standards and the samples prepared for the analysis can give important information about by-products, degradation products, or the possible reaction of eluted substances. In a recent study of Schulz et al. [25], the authors could show that eluted substances can react with each other when they are in the same solution. After mixing three basic monomers, an additional high peak of a molecule with higher mass and lower polarity than the basic monomers was detected (Figs. 12.5 and 12.6) suggesting that some kind of interaction has taken place among the eluted monomers of the composite materials causing difficulties during the analysis process. This can be avoided by selecting the appropriate analytical method and careful performance of the analysis. An additional challenge for the analytical



**Fig. 12.5** Retention time of three standards: [BisGMA (1), TEGDMA (2), and UDMA (3)] (Simon Daniel Schulz, Tobias Laquai, Klaus Kümmerer, Richard Bolek, Volker Mersch-Sundermann, and Olga Polydorou, “Elution of Monomers from Provisional Composite Materials,” *International Journal of Polymer Science*, vol. 2015, ArticleID617407,7pages,2015.doi:10.1155/2015/617407)



**Fig. 12.6** Mixture of three standards (BisGMA, TEGDMA, and UDMA). On the figure are given their peaks with their retention times (1 = BisGMA, 10.6 min; 2 = TEGDMA, 5.3 min; 3 = UDMA, 10 min; 4 = new peak “reaction product,” ~13 min) (Simon Daniel Schulz, Tobias Laquai, Klaus Kümmerer, Richard Bolek, Volker Mersch-Sundermann, and Olga Polydorou, “Elution of Monomers from Provisional Composite Materials,” *International Journal of Polymer Science*, vol. 2015, Article ID 617407, 7 pages, 2015. doi:10.1155/2015/617407)

methods is the decomposing of molecules like UDMA. It can decompose to HEMA, affecting the analytic findings if the appropriate method is not used [22].



In addition, the selection of the substances needed as references in a study is an important parameter that can influence the findings, as this might result in misleading findings. The selection of the pure chemicals as reference standards gained by the chemical industry is commonly done when planning a study as these are supposed to be used for the manufacturing of the composite materials. However, not all companies use the pure monomers for manufacturing dental composites, but some modifications might take place before their use, resulting in substances with differences in MW and altered chemical structure compared to the original ones. The use of the original monomers taken from the chemical industry as reference standards in analytical studies results in failed detection of eluted substances when modified monomers are contained in the materials that are under examination. Additional confusion is caused by the fact that usually these modified substances continue to be labeled like the original monomer. Appropriate evaluation of the composition of the examined composite materials is necessary in order to be able to give adequate and correct findings. The ideal situation is the use of monomers given by the company manufacturing the composite materials that are under evaluation.

Additional attempts have been made to improve the analytical methods for better detection. High-temperature GC (HT-GC) in combination with MS has also been suggested [35] to detect BisEMA homologues in the eluates of composite materials. The use of the high-temperature columns, which can be heated up to 400 °C, was suggested to be able to vaporize the low volatile BisEMA homologues. However, the difficulties of using the appropriate reference standards remained as limitation. The use of ultra UHPLC in combination with tandem MS detector as suggested in new studies appears to be an adequate sensitive method to analyze the eluted substances [83, 84] in different extraction solvents like ethanol, water, and artificial saliva.

Summarizing the data in the literature, the combination of HPLC-MS/MS presents at the moment the most accurate method of performing precise analysis of the eluted substances, under

the condition that appropriate reference materials have been used and a clinical relevant preparation of the samples has taken place. Actual data are promising for a more standardized qualification in the near future.

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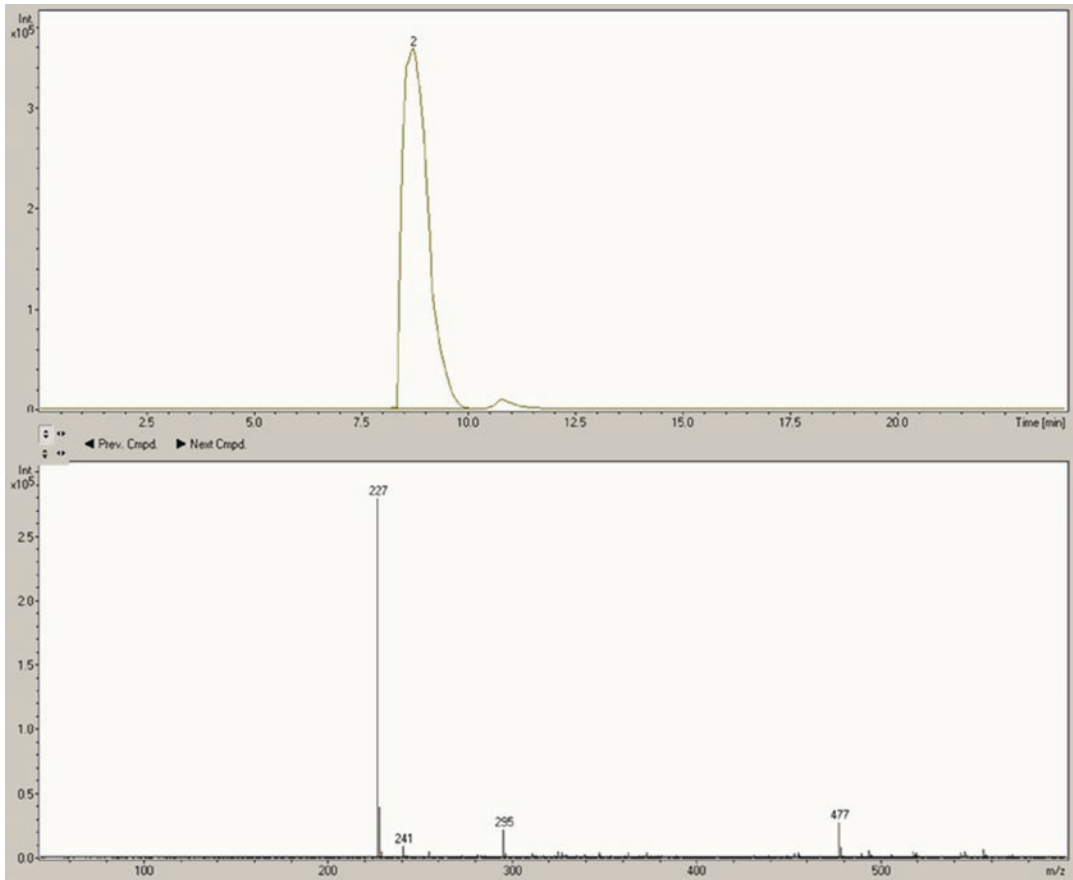
## 12.4 Elution of Bisphenol A from Dental Composite Materials

Bisphenol A (BPA) is the common name for 2,2-bis(4-hydroxyphenyl)propane. It is an organic compound, produced by the reaction of two phenols with one acetone catalyzed by a cation exchange resin. BPA is a xeno-estrogen, a known endocrine disruptor showing similar estrogenic activity like the normal estrogens after binding to human estrogenic receptors. BPA and its release from dental composite materials are widely discussed in literature. The discussion and concern about BPA have been induced due to one of the first reports [85] evidencing the release of BPA from dental materials under different circumstances and commenting the possible estrogenicity of dental composite and sealants. This report has been discussed and commented widely up to date concerning the method and the conditions used in the study, like the hydrolysis in alkaline (pH = 13) and acid media (pH = 1) after heating to 100 °C for 30 min.

Several studies have been performed concerning the possible implications of BPA on human health [86–89]. BPA has been shown to have estrogenic activity even at concentration lower than 1 µg/m<sup>3</sup> [90]. Recently the European Food Safety Authority (EFSA) has lowered the tolerable daily intake (TDI) from 50 µg/kg/bw/day (microgram/kilogram/(body weight/day)) to 4 µg/kg/bw/day [91].

BPA is used in the manufacturing process for polycarbonate plastic products, food packaging, as inner coating of many food cans, and is also used for synthetization of monomers like BisGMA and BisEMA used in composite materials. The main exposure of the population is definitely through the plastics and food packaging.

Among the published data in the literature, the findings concerning the release of BPA from dental



**Fig. 12.7** Bisphenol A by LC-MS. The upper part presents the chromatogram for BPA (mass 228) in negative mode (MW: 227). The lower part gives the mass spectrum

of BPA (Polydorou O, Trittler R, Hellwig E, Kümmerer K. *Elution of monomers from two conventional dental composite materials.* *Dent Mater.* 2007 Dec;23(12):1535–41)

composite resins are controversial. The problem of identifying BPA in the eluates (Fig. 12.7) is usually because of the analytical method used. A very sensitive precise method with very low qualification/quantification limit is necessary as in the case that BPA is eluted, it will happen in very small amounts. Due to the different methods used, it happens very often that different findings are mentioned for the same composite materials causing confusion on this serious subject of BPA. The release of BPA from composite materials was thought to be due to these monomers, and a possible degradation of them was assumed. However, this is not totally accepted as the degradation of these monomers is not really documented [92]. Another suggestion was that the eluted BPA from composite materials comes through residual

amount as impurity in the basic monomer during its manufacturing process [93].

The elution of BPA has been stated in several studies [16, 26, 70, 94, 95]. Mainly this release was shown in ethanol 75%. The released amount of BPA was material dependent, and in most cases, the maximum amount was after 7 days of storage [26, 94], while a longer release up to 28 days after polymerization has been also mentioned [95]. As far as the importance of the eluted amount is concerned, it is suggested [2] that the released amount of BPA in a period of 24 h is probably important in the case of several large fillings in the oral cavity. However, the present studies [2, 26] mention that the detected amount of BPA in composite materials' eluates is lower than the tolerable daily intake

determined by EFSA assuming that these are safe for the human health.

Interesting is the finding of Yin et al. [96], who evaluated the associations of blood mercury, inorganic mercury, methyl mercury, and BPA with dental restoration in US population. Significant decreases were found in urinary BPA for both evaluated periods 2003–2004 and 2011–2012, especially for individuals under the age of 12 years. This signalizes the efforts to reduce the BPA exposure over the last years. No significant association was found between urinary BPA and dental surface restorations. Based on their findings, the authors suggested that the use of composite materials for dental restorations does not have any effect on the long-term urinary amount of BPA. Other daily used products should be reconsidered as the sources for the BPA exposure. However, no information exist up to date concerning the short-term effect of BPA on human health directly after the application of the filling material as other published data reveal a significant increase in the urinary or salivary BPA in short time after the filling application [97, 98]. Further research is necessary in order to be able to evaluate the estrogenic potential of the composite materials' eluates, under the consideration that BPA is a substance that has been shown to exert effects by low-dose exposure. Development of materials free of substances with estrogenic potential should be considered.

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## 12.5 Elution of Particles from Composite Materials

Besides the elution of monomers, the solubility of filler particles containing zinc, barium, strontium, silicon, boron, and sodium in aqueous solutions was also reported [99, 100]. In 1990 [101], it was reported that leaching of silica and barium did not decrease in the evaluation time period of 6 months. It was suggested that among other filler compositions, the filler content and the filler treatment might influence the amount of eluted substances [101].

During the development of composite materials, in the area of filler particles, much effort

has been made in order to produce new composite materials with better properties. Although the use of nanoparticles in composite materials is not new, their use was extended over the past years, using also ceramic nanofiller resins in order to improve their physical/mechanical properties developing materials with better esthetics, better surface properties, and increased strength and durability.

Although the use and incorporation of nanoparticles/fillers in the composite materials seems to improve their properties having better clinical performance, the nanoscale size has the potential to alter physicochemical properties from the bulk form of any substance [102]. The size of the filler particles contained varies among the different categories of composite materials however; a lot of modern composite materials contain nanosized particles. Modern composites might contain high amounts of nanoparticles (<100 nm). Although modern composite materials nowadays contain radio-opaque glass fillers (with elements like barium, zirconium, strontium, or ytterbium) which are larger than 0.4–1  $\mu\text{m}$ , they additionally contain smaller particles (<100 nm) in order to improve the properties of the materials. Only few data exist in the literature concerning the release of particles, especially nanoparticles, from dental composite materials, as it might take place during the abrasive procedures like cutting, shaping, finishing, or also removing of composite restorations.

In the past years, the possible health risks by using nanoparticles have been widely discussed. Usually particles under 100 nm are thought to present a risk for human health [103–105]; however, it must be taken into consideration that particles up to 300 nm exhibit nanospecific properties and should be treated as nanoparticles. Additionally, nanoparticles might build agglomerates and/or aggregates (>100 nm) which have reactive single particles on their surface [106]. Nanoparticles with a diameter of 50 nm, for example, are able to penetrate the cell membrane [107] increasing the considerations for their possible negative health impacts. Although inhalation of silica might take place by dental personnel, the size of the considered particles

is thought to be larger than the dangerous size [108]. A recent perspective about the toxicity of nanomaterials claims that there is actually up to date no evidence that nanomaterials exhibit novel toxic mechanisms [109, 110], and similar conventional particles should be evaluated as far as their toxicology is concerned [109, 110].

Van Landuyt et al. [111] evaluated the release of “dust” from composite materials. Under standardized *in vitro* conditions to simulate the worst case clinical situations, the authors could detect micro-particles ( $<1\ \mu\text{m}$ ) and nanoparticles ( $<100\ \text{nm}$ ), suggesting that without water cooling, dust inhalation might be hazardous for the human health. The detected amount of the sub-micron particles differed among the composite materials. The same authors in another study [112] with more clinically relevant conditions were able to identify high concentrations of nanoparticles ( $<100\ \text{nm}$ ) to be set free in the air after grinding composite materials with dental burs. Although the present literature data are very scarce, they indicate that the dental procedures might result in increased release of nanoparticles which can be inhaled. The use of water cooling during composite grinding, finishing, and polishing might release this potential. However, no data exist up to date about the toxicological potential of such particles.

### 12.5.1 Methods to Evaluate the Release of Particles from Composite Materials

By evaluating the release of particles from dental composite materials, the determination of their size is of great importance, but besides this, their identification plays an important role in order to be able to certify their possible impact on human health.

In literature, the most suggested methods for the analysis of nanoparticles are the nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), scanning electron microscopy (SEM), size exclusion chromatography (SEC), gel electrophoresis, asymmetrical flow field-flow fractionation (AF4), and analytical ultracentrifugation (AUC) [113–116]. DLS is a powerful and accessible tool

and has become the preferred technique to routinely determine the size of nanoparticles. It has the advantage to detect small amounts of large particles. NTA is an innovative technique for sizing particles from about 30 to 1000 nm, with the lower detection limit being dependent on the refractive index of the nanoparticles, being able to identify and track individual nanoparticles moving under Brownian motion and relate the movement to a particle size [113]. Besides this, a scanning mobility particle sizer (SMPS) was suggested for measuring submicron particle distributions in combination with an electrostatic precipitator (ESP) for electron microscopic characterization [112]. The chemical analysis of the released particles can be performed by SEM and electron dispersive X-ray (EDX).

## 12.6 Conclusions and Future Perspectives

Taken into consideration the data in the literature, it is to assume that composite materials release respective amounts of monomers over time, stating this way a potential risk for human health, in the case that the detected amounts of eluted substances would represent the real clinical situation. Even if the *in vitro* studies are performed in respect of the clinical circumstances, they are not able to imitate the *in vivo* situation representing usually the worst case scenarios. Great differences exist in the sample preparation among studies. Using thin layers of composite materials like 1 mm definitely influences their polymerization degree and the elution of substances. Not only the thickness but also the diameter of the composite samples can affect clearly these properties. Diameter of samples larger than the tip of the polymerization unit will definitely negatively affect the elution of monomers. Although the information gained from these studies is worth full presenting an important screening of the potential release of monomers from the available composite materials, it is not clearly proven up to date that the eluted amounts can generally cause detrimental effects on human health. A direct correlation of the detected amounts of substances

with the biological reactions caused is of great importance. The present data give some evidence of possible effects on human respective cell cultures however; the interpretation of these data in the in vivo situation needs further investigation. Of importance is the presence of BPA in the eluates of composite materials, as this substance exerts biological effects after low-dose exposure. Further research in this area is necessary concerning the uptake of BPA of composite materials and the factors that can influence it in addition to the evaluation of the estrogenic potential of the composite eluates in order to be able to develop appropriate materials.

Summarizing the data cornering the parameters that can influence the monomers' release, the composition and the chemistry of the composite are the most important parameters. Factors like the degree of conversion; curing times, curing modes, and curing units; and also handling modes of the restorative materials seem to be important, being able to improve in some cases the performance of the applied materials; however, they seem not the ones that can regulate the elution of monomers. In addition to these, it has to be mentioned that it is not possible to generalize the findings of the composite materials evaluated in the literature for all composite materials existing nowadays, as due to the rapid development in material science, modern restorative materials with improved properties and chemistry are available nowadays which have been shown to be beneficial concerning the monomers' release. Systematic and standardized screening of modern materials is always necessary in order to be able to estimate their real potential risk.

Besides monomer elution, scarce literature data reveal a release of nanoscale particles from composite materials through grinding and cutting procedures, independently of their composition. Although particles in this size are thought to exert biological effects, parameter size solely is not the one determining the potential effects, as the characterization of the particles is of great importance. Clinical relevant risk assessment of the released particles should be addressed before the composite materials are accused of having detrimental effects on patients and dental practitioners.

The release of monomers and nanoparticles is not only of great importance in respect of the oral cavity and their effects directly on human individuals, but additionally the environmental aspects should be taken strongly into consideration.

The potential environmental pollution through restorative materials is beyond amalgam. The release of BPA and nanoparticles in the wastewater through dental daily clinical procedures will probably become a topic in the future, and it would be in interest of all to act earlier preventing additional environmental pollution.

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