Institute of Clinical Dentistry/Faculty of Health Science, Tromsø 2014

The Influence of *bis*-EMA Content on the Leachability of Dental Composite Resins

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Masteroppgave i Integrert masterprogram i Odontologi Juni 2014

Abstract

Objective:

Composite resin based materials (CRM) are widely used in dentistry for its aesthetics and ability to preserve tooth structure. The main ingredients are monomers that during polymerization undergo crosslinking to form the resin matrix, bonding the material together. However, not all the monomers will react during polymerization, and remain in the matrix as free residual compounds that can elute. This is of clinical importance as monomers and their degradation products are known to cause allergic reactions, and there are concerns regarding the potential toxic effects of these eluted compounds. The objective of this study was to investigate how different ratios of *bis*-GMA *versu*s *bis*-EMA relative to each other would influence the leakage of TEGDMA from the composite.

Materials and methods:

Storage solutions of experimental composites with known concentrations of *bis*-EMA, *bis*-GMA, TEGDMA and UDMA were used, with storage time 24 h, 7 days and 30 days. Three randomly collected solution samples from each group (n=36) and time interval was chosen. The analysis was performed by using GC-MS.

Results:

TEGDMA was detected in all samples. The results showed a trend where the group containing the most *bis*-EMA eluted less than the groups containing *bis*-GMA at the time intervals studied.

Conclusions:

Within the limitations of the present study the following conclusions were drawn:

- a trend was shown toward less leakage from the composite containing the most *bis*-EMA compared with the composites containing *bis*-GMA at the time intervals studied. The null hypothesis was therefore considered as rejected.
- leakage of TEGDMA was found for all composites tested, however the quantification was uncertain. More research on the subject is needed to determine if there is a significant difference.

Introduction

With its superior aesthetics and the technique with ability to preserve tooth structure, polymer resin-based materials are widely used in dentistry, as composite resin-based materials (CRM), prosthetic materials and composite resin-based cements.

The main ingredients in CRMs are monomers, *e.g.* bisphenol-A glycidyldimethacrylate *(bis*-GMA), ethoxylated bisphenol-A dimethacrylate (*bis*-EMA), triethyleneglycol dimethacrylate (TEGDMA), urethane-dimethacrylate (UEDMA) (Figure 1) and filler particles. The latters are used for reinforcement, while the first mentioned are aromatic or aliphatic dimethacrylates that undergo crosslinking and polymerization during light-curing, forming the resin matrix, bonding the material together. The aromatic compounds contain conjugated aromatic rings, which make them more stable than the flexible chains in aliphatic compounds (1,2).

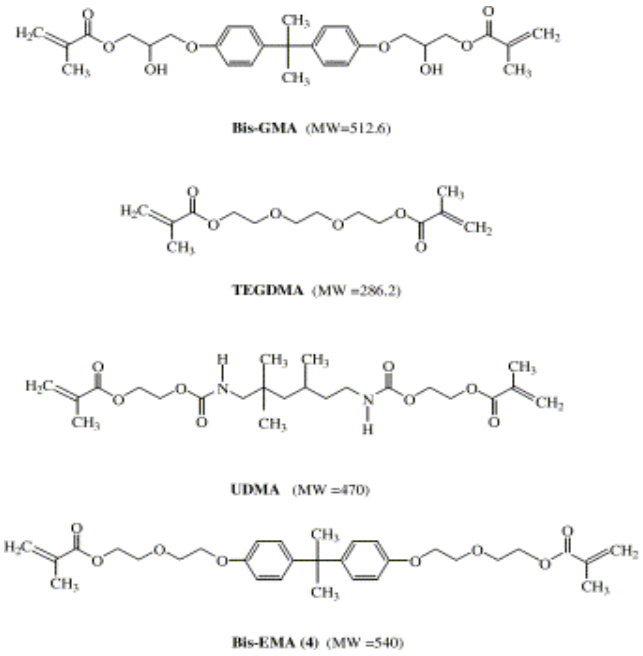


Figure 1: Molecular structure of *bis*-GMA, TEGDMA, UDMA and *bis*-EMA.

Bis-GMA and *bis*-EMA are high molecular weight (MW) monomers with similar structures. They both contain two aromatic groups linked to two aliphatic chains. The aliphatic chains in *bis*-GMA contain hydroxyl groups which the aliphatic chains in *bis*-EMA do not have and thus *bis*-GMA is less flexible than *bis*-EMA. The strong intermolecular bonds of *bis*-GMA result in a decreased degree of conversion and crosslinking compared to *bis*-EMA (3, 4). In addition, because of the lack of flexibility, monomer solutions of *bis*-GMA are more viscous

than those of *bis*-EMA (5).

The viscosity of the monomer solution (i.e. matrix) is important and depends on type of monomers used, their molecular weight and flexibility. It will affect both the polymerization process and cross linkage as well as the amount of filler particles that can be incorporated (4-7). Monomers such as low-MW TEGDMA and high-MW UEDMA are often added into the matrix to reduce the viscosity of the blend. Even though UEDMA has a high-MW it is more flexible than *bis*-GMA. The flexibility is due to its aliphatic structure, giving it a higher molecular mobility and reduced viscosity. However, adding diluting monomers, especially TEGDMA, will also increase polymerization shrinkage (7). Lately *bis*-EMA has been brought to attention due to its lower viscosity and higher degree of conversion compared to *bis*-GMA (3,4).

The degree of conversion (DC) is the percentage of methacrylic (*i.e.*vinyl) groups converted by polymerization. Regardless the type of composite material, the DC will never be 100 %, but usually between 50 and 80 % (4,7,8). This is due to the structural properties of the CRMs and the rate or speed of polymerization. As the polymerization proceeds, the monomers will be trapped within the cross-linked network without any methacrylic groups to react with. As a result, 20-50 % of methacrylic groups will remain unreacted (4). Most of the unreacted methacrylic groups are part of dimethacrylate molecules that have reacted on one end, making elution impossible. However, there are unreacted "residual" monomers diffusing in the cross-linked polymer and capable of eluting from the composite. Previous studies have shown that less than 10% of the residual monomers are elutable (9,10). The rate of elution is depending on factors such as diffusion and the cross-linkage of the network (11).

It has been shown that smaller molecules and ions have a greater rate of elution than larger molecules (12). TEGDMA has been detected as one of the main eluted monomers from CRMs (10,13-16). Larger molecules have a lower tendency to leach because of their higher weight, especially in shorter time periods, however their degradation products (*e.g.* methacrylic acid, metacrylates) can be detected as eluates (16-18).

The release of low-MW monomers is preferably investigated by using Gas Chromatography in combination with Mass Spectrometry (GC/MS) (16, 19). When analysing the elution of high-MW monomers however, Liquid Chromatography/MS is more suitable (18). GC-MS

enables sensitive and selective analysis of volatile organic compounds. The analytes are dissolved in an organic solvent and a small volume (≈ 1µl) of the solution is injected into the injector of the GC. Due to high temperature of the injector the solvent and the analytes are spontaneously converted to gas phase. An inert gas (usually helium) at a constant flow rate transfers the analytes from the injector and into the separation column. Due to differences in boiling point and differences in interaction with the stationary phase of the column, different analytes travel through the column with different velocity and are thus separated. The result is usually presented in a chromatogram. When the different analytes elute from the GC column they are transferred (in gas phase) to the ion source of the mass spectrometer. In the ion source the analyte molecules collide with a beam of 70eV electrons (EI ion source). The following reaction will occur:

$$M + e^- \rightarrow M^{+\bullet} + 2e^-$$

The $M^{+\bullet}$ is called the molecular ion. Some of the molecular ions will contain enough internal energy to fragment further. Typically, MS analysis therefore provides information both about the MW of a molecule and mass of fragments of a molecule. The result is called the mass spectrum of the actual compound. EI-spectra are easy to reproduce, they are not instrument-dependent and hence it is easy to build libraries of EI spectra of a large array of organic compounds.

Great concerns have been raised regarding the potential for cytotoxic, genotoxic and oestrogenic effects of the eluted monomers and degradation products (e.g TEGDMA, HEMA, bisphenol-A etc.) (16, 22). Uncured or insufficiently cured CRMs are also known to be able to cause allergic contact dermatitis and toxic reactions in dentists or patients due to their eluates and/or degradation products (23). It is assumed that there are only small amounts of eluates released from cured CRMs. However, little knowledge exists about the effects of low exposure from these eluates on patients.

To the knowledge of the authors, the research regarding leakage from CRMs containing *bis*-EMA seems limited. This could be due to the fact that *bis*-EMA is a relatively new monomer compared to *bis*-GMA. It was therefore of interest to investigate changes in the amount of eluates from experimental composites based on different rates of *bis*-EMA *versus bis*-GMA. *Bis*-EMA has been shown to increase the DC and have a positive effect on water susceptibility (3,4), but little is known about *bis*-EMA's influence on the leachability.

Low-MW substances will be the first to elute. Analysis of the leakage behaviour of TEGDMA would therefore provide an indication of structural changes in the polymer, together with characterization and quantification of other low MW-monomers eluted.

The aim of this study was therefore to do a quantitative and qualitative analysis of TEGDMA eluted from experimental CRMs. In addition detection of other low-MW monomers was performed.

The null hypothesis of the present study was that changes of the content of *bis*-GMA *versus* *bis*-EMA in the CRMs would not change the amount of leaching.

Materials and methods

Samples

For the present study, storage solutions from a recently published study on water susceptibility on experimental composites were used (Table 1) (4). The production of samples and the sorption/solubility test was performed according to ISO 4049:2009 (24). The samples were cured using a LED curing device (Celalux 2, VOCO, Germany) with power density of 850 mW/cm^2. The intensity was controlled before curing by a radiometer (Bluephase, Ivoclar/Vivadent, Schaan, Lichtenstein). Six samples were made for each group. The samples were placed in individual glass containers, and 10 mL of water (Grade II, ISO 3696:1995 (25)) was added to each container. The storage periods for each group were 24 h, 7 d and 30 d, at 37 ˚C ± 1. The solutions were collected after each storage period and each solution sample placed in individual containers and immediately frozen at a temperature of (-18 ± 1 ^{o}C) until the present study was performed.

Table 1: Monomer composition (in percentage mol/weight) for the different monomers

Groups	**Monomers (wt%)**			
	Bis-EMA	Bis-GMA	UEDMA	TEGDMA
Group A (control)	-	60	20	20
Group B	60	-	20	20
Group C	45	15	20	20
Group D	30	30	20	20

Initiator: Camphorquinone (wt%:0.1)/dimethylaminoethylbenzoate (wt%:0.2)
Inhibitor: buthylhydroxytoluene (wt%: 0.05)
Fillers: Size: 0.7μm; total (wt%: 72.8) [dental glass (wt%:66.4); fumed silica (wt%: 6.4)]

Chemicals

The chemicals used are shown in table 2.

Table 2: Substanses used, their supplier and CAS Registry Number

Organic substance	Supplier	CAS Registry Number
TEGDMA	Sigma-Aldrich, Oslo, Norway	109-16-0
DEPH	Sigma-Aldrich, Oslo, Norway	84-66-2
EtAc	Merck, Whitehouse Station, USA	141-78-6

Solutions

Samples of destilled water, TEGDMA and ethyl acetate (EtAc) (Merck, Whitehouse Station, USA) were analysed with GC/MS to make sure they did not contain compounds interfering with the analysis. All procedures were performed with non-contaminated glassware, and plastic gloves were avoided to minimize the contamination from other polymer-based materials. Before use, all glassware was rinsed with EtAc twice to avoid contamination. EtAc is a good solvent for organic materials and was therefore chosen as rinsing agent.

To make quantification possible, reference samples of TEGDMA were made in known concentrations by dilution of TEGDMA (20.25 mg) in EtAc; 0,1 µg/ml, 1 µg/ml, 5 µg/ml and 10 µg/ml, all with internal standard (IS) in concentration of 1.07 µg/ml.

Sample preparation for GC-MS analysis

Three randomly collected solution samples from each group and time interval was chosen (n=36). The samples were stored in room temperature for approximately twelve hours prior to extraction. Each sample was thoroughly vibrated to make sure that potential leakage products were evenly distributed, and 5mL of the eluate was transferred to a test tube. One ml of EtAc containing IS was added to each test tube. Since aqueous samples are not compatible with GC-MS analysis, extraction of organic substances with ethyl acetate was performed. The test tube was stirred, rested and the separated ethyl acetate fraction was collected with a pasteur pipette and transferred to glass vials. The IS, diethyl phthalate (DEPH) (Sigma-Aldrich Chemistry, Oslo, Norway) was used to correct for errors that might occur during the extraction and evaporation. Preparation of IS: 21.37 mg of (DEPH) was dissolved in ethyl acetate to a concentration of 1.07 µg/ml. The eluted samples were further extracted two times

with 1 ml EtAc. Within each extraction the test tube was thoroughly vibrated. The EtAc fractions from each sample were pooled and evaporated down to approximately 0.2 ml before they were transferred to glass vials and analysed with GC-MS.

Analysis

Qualitative and quantitative analysis were performed by using a Gas Chromatography/Mass Spectrophotometry (GC-MS) (Agilent Technologies 7693 Network GC system combined with a Quattro micro^{TM}GC Waters Mass Spectrometer, Agilent Technologies, Santa Clara, USA). The GC was equipped with an autosampler (Agilent 7693A, Automatic Liquid, Sampler, Agilent Technologies, Santa Clara, USA). MassLynx 4.1 SCN 805 (Waters Corp., Milford, US) was the software used for controlling the instrument and data handling. A capillary column with the following specifications was used for chromatographic separation: Wall-coated open tubular (WCOT) low bleed fused silica capillary column with the length of 30 m, 0,25 mm inner diameter and a film-thickness of 0,25 μm (DB-5MS, J&W Scientific, Folsom, USA). Carrier gas was Helium with a flow rate of 1 ml per min, constant flow. Splitless injection was used, the injector temperature was 240°C and purge flow of Helium gas was 70 ml/min.

The temperature was set to start point at 50 ˚C and rise to a maximum of 240°C in two phases; first phase with an increase of 10°C/min to the temperature of 120°C/min, and a second phase with an increase of 20°C/min to the final temperature. Hold time was 1 min at 240°C, in total 24 minutes. The injection syringe was rinsed with EtAc before and after every injection.

The first run of each sample the MS was set to full scan mode for identification of the chemical components from each group and time interval. The analytes were identified by comparing the mass spectra with the mass spectra library, NIST (National Institute of Science and Technology, Gaitherburg, MD, USA). Furthermore, reference substances analysed with the same procedure confirmed the retention time and mass spectra. The quantitative analysis was performed by using Selected Ion Monitoring (SIM) in order to obtain better selectivity and lower detection limits for trace components. Integration of responses was done and standard curve and response factor was computed for TEGDMA with different concentrations and IS in concentration of 1.07 μg/ml. Linearity of aera ratio *versus* quantities was confirmed

for TEGDMA, with a R^2 of 0.98. Mass fragments from SIM mode used for quantification were for TEGDMA 113 and 69, and for IS 149 and 177. Validation: Accuracy was tested in 4 different concentrations (from 0.1 to 10 µg/ml) within and between days. Limit of detection (LOD) for TEGDMA was below 0.1 µg/ml. The peaks of each specific fragment were integrated and the areas compared with the area of IS.

Results

Analyses of IS, reference samples and the water used showed no signs of impurities from the preparation process. All groups of experimental composite showed leakage of organic substances, and TEGDMA was detected in all samples.

The reference samples of TEGDMA were used to make a linear graph with a R^2 value of 0.98 (figure 2), and a dynamic range between 0µg/ml and 10µg/ml. The percentage of TEGDMA *versus* IS was calculated and the values plotted on the reference curve (figure 2) for quantification of TEGDMA in the samples analyzed (figure 3).

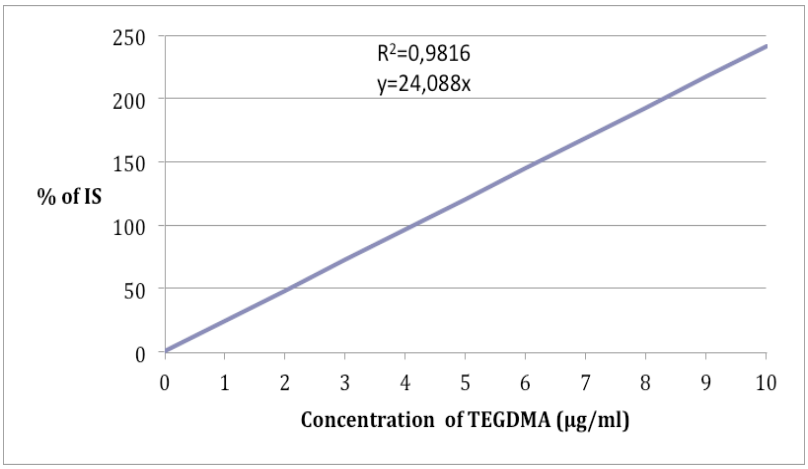


Figure 2: Regression line showing concentration of TEGDMA in relation to internal standard.

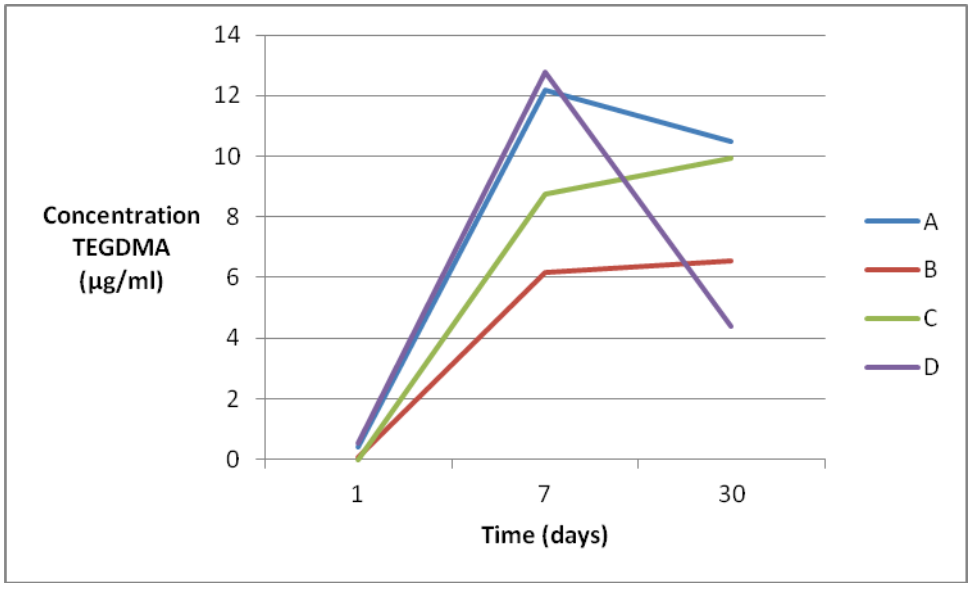


Figure 3. Concentration of TEGDMA in the different groups after 1, 7 and 30 days.

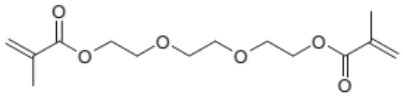
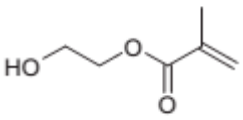
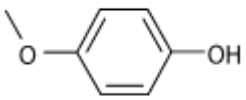
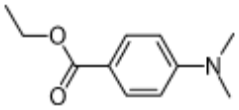
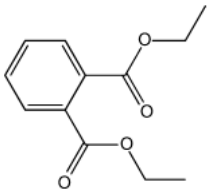
As shown in figure 3 the leakage of TEGDMA was greatest during the first 7 days in all the groups. Group B and C showed only minor differences in leakage between 7 and 30 days. The results also indicate that group A and D had a reduction in the amount of TEGDMA in the same period. The detected maximum concentration of TEGDMA was higher than 10 µg/ml, which was above the dynamic range. Through estimation, it was found to be approximately 12 µg/ml for group A and 13 µg/ml for group D after 7 days. Group B and C had a maximum concentration of 6 µg/ml and 9 µg/ml, respectively after 7 days.

The substances detected are listed in table 3, and their functions/characteristics are shown in table 4. These substances were identified by comparison with the reference library. In total, each of the four groups showed leakage of the same substances, however variations between the different samples in each group were found.

Table 3: Detected eluates in the different groups

	Gr. A	Gr. B	Gr. C	Gr. D
TEGDMA	X	X	X	X
HEMA	X	X	X	X
MEHQ	X	X	X	X
DMABEE	X	X	X	X
Tinuvin P	X	X	X	X

Table 4: Organic substances given with their function within the material, the molecular ion, characteristic ions, structure formula and retention time. The same parameters are given for IS.

Organic substance	Function	Molecular ion, m/z	Characteristic ions, m/z	Structure formula	RT (min)
TEGDMA	Monomer	286	69, 113		16,38
HEMA	Monomer	130	69, 87		7,71
MEHQ	Inhibitor	124	109,124		11,07
DMABEE	Coinitiator	193	148, 164, 193		15,47
DEPH	I.S.	222	149, 177		14,65

Discussion

The null hypothesis of the present study was that changes of the content of *bis*-GMA *vs*. *bis*-EMA in the CRMs would not change the amount of leaching. The results showed a trend where the group containing the most *bis*-EMA eluted less than the groups containing *bis*-GMA at the time intervals studied. The null hypothesis was therefore considered as rejected.

The study was designed to make the different groups comparable due to the composition of the materials. The content in the CRMs was known and only two ingredients (*i.e. bis*-GMA and *bis*-EMA) were varied. Different outcomes will therefore reflect changes in the amount of *bis*-GMA and *bis*-EMA, supported by a recent study (4). Most of the previous research has used commercial composites, which leads to an uncertainty regarding the exact content.

In addition to the controlled production of the CRMs used, the sorption/solubility procedure, storage of the eluates and preparation of the samples for the GC/MS analysis, was done according to ISO standard (4049) or frequently used standardized protocols (16, 17, 24).

The risk for the eluates to change during storage in the freezer is limited, due to their chemical structure. The same procedure has been used in previous studies (17, 26, 27) and the method for storage was also established by personal communication (Prof. Ulf Wiel Gedde, School of Chemical Science and Engineering; Royal Technical Institute, Stockholm, Sweden).

The present study used distilled water as a solvent, and the clinical transferability can be questioned. In the oral cavity, CRMs are exposed to temperature changes, saliva proteins and mechanical wear. In addition, saliva flow will prevent equilibrium of the eluates from being established. On the other hand, using standardized distilled water (Grade 2) will give equal conditions for all groups and minimizes the risk of uncontrolled factors to influence the results. In addition, the water used was controlled by GC/MS analysis prior to the other samples to ensure the purity. It has been shown that a solution of 75% ethanol/water is more effective than distilled water for extracting unreacted components from composites, probably because of its enhanced ability to penetrate the polymer network (9). According to Ferracane, the rate and extent of elution within the oral cavity is probably somewhere between that of ethanol and water (9). Ethanol has a greater ability to penetrate and swell the polymer network and could lead to more leakage than would normally appear in the oral cavity.

Investigations with human saliva *in vivo* could be of higher clinical value (26), but the conditions would be more variable and the practical feasibility would be beyond the scope of this study.

For quantification of TEGDMA, reference samples of TEGDMA were made. The regression line made from these reference samples gives a R^2 value close to 1. This indicates a low variability in the results, and thus strengthens the study. However, some of the eluted concentrations found were above the dynamic range, which make precise quantification difficult. In addition, the chromatograms obtained after 7 and 30 days show that samples from all groups contain a higher amount of eluted TEGDMA than the instrument settings allowed for. This is evident by blunt or cut-off peaks on the chromatograms. The result is that quantification of eluates after 7 and 30 days is uncertain, and the actual concentration of eluted TEGDMA after these time periods is higher than the results of the present study indicate. This explains the decrease in concentration for groups A and D after 7 days shown in figure 3, as the cut-off peaks make the amount of elution seem much less than it is in reality. Figure 3 therefore gives the erroneous impression that group A and D, the groups containing the highest amount of *bis*-GMA, give less leakage than the groups containing more *bis*-EMA. In addition, one would expect leakage after 30 days to be higher than or, in the case of saturation, the same as that recorded after 7 days. This would cause the concentration curve of eluted TEGDMA in figure 3 to either flatten out or continue rising. The reason for the limited measuring range is that minimal amounts of leakage were expected, and the samples were diluted accordingly. Should the experiment be replicated to obtain precise quantification, samples would have to be diluted more than was done in the present study.

As mentioned, group B showed less leakage than the other groups. Cornelio *et al.* found that materials containing only *bis*-EMA or having higher amounts of *bis*-EMA than *bis*-GMA resulted in materials with higher DC and also less water uptake (4). It is reasonable to suggest that this can be explained by the difference in binding structure, and hence the difference in elution. *Bis*-GMA contains hydroxyl groups that give strong hydrogen bonds with water molecules, while *bis*-EMA form weaker intermolecular bonds with its ether and carbonyl groups. The hydroxyl groups therefore increase the affinity for water molecules (4, 28). Diffusing water molecules might result in more leakage by cleaving the intermolecular bonds within the polymer network. As discussed by Cornelio *et al.* even small amounts of *bis*-GMA seem to have a negative effect on the DC (4). Material from the present study show a similar

trend, where the group containing no *bis*-GMA (*i.e.* group B) shows less leakage than the group containing 15 % *bis*-GMA (*i.e.* group C). However, the higher DC in materials containing more *bis*-EMA does not necessarily mean that more TEGDMA is incorporated in the polymer network. Figure 3 shows that the leakage is greatest for the material containing the highest amount of *bis*-GMA (*i.e.* group A) and lowest for material containing no *bis*-GMA (*i.e.* group B) during the first 24 hours. This is most likely due to the tighter polymer network in the material with no *bis*-GMA, in addition to reduced formation of hydrogen bonds, which results in lower water uptake and slower diffusion of TEGDMA. This cannot be applied with certainty for the longer storage periods, as the full quantity of leakage is not known due to reasons discussed previously.

A limited amount of HEMA, a monomer not included in our experimental composites, was also detected. It is unlikely that HEMA has been created during our sample preparation, and this finding could therefore indicate impurities from the manufacturer or the supplier. The HEMA found could also be a degradation product from UEDMA. (15, 27, 28)

For exact quantitative analyses reference samples should be made. As this study had a main focus on TEGDMA, only reference samples of this monomer were made.

The clinical consequences of the leakage found remain uncertain. The limit of cytotoxic effect (LOT) of TEGDMA seems to be unknown, making it difficult to draw conclusions regarding potential adverse effects from our study. Potential adverse effects cannot be excluded, but more research needs to be performed.

Conclusions

Within the limitations of the present study the following conclusions could be drawn:
The results obtained showed a trend towards less leakage from the group containing the most *bis*-EMA compared with the groups containing *bis*-GMA at the time intervals studied. The null hypothesis was therefore considered as rejected. Leakage of TEGDMA was found in all groups, however the quantification is uncertain. More research on the subject is needed to determine if there is a significant difference.

Acknowledgements

The authors would like to acknowledge VOCO GmbH, Cuxhaven, Germany, for manufacturing the experimental composites. Many thanks also to Professor Einar Jensen, Department of Pharmacy/Faculty of Health Sciences, Tromsø for help providing the facilities for the leachability analysis, and to Vibeke Michelsen, Associate Professor, Institute of Clinical Dentistry/Faculty of Medicine and Dentistry, Bergen for assistance with design and implementation of the experiments. Both cosupervisors have given valuable guidance during the whole process. At last the authors would like to give a special thanks to supervisor Professor Ulf Thore Örtengren, Institute of Clinical Dentistry/Faculty of Health Science, Tromsø for all his time spent, excellent guidance and for making this project possible.

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Appendix 1: Abbrevations

bis-EMA	Ethoxylated bisphenol-A dimethacrylate
bis-GMA	Bisphenol-A glycidyldimethacrylate
CRM	Composite resin-based material
DC	Degree of conversion
DEPH	Diethyl phthalate
EtAc	Etylacetate
GC/MS	Gas Chromatography in combination with Mass Spectrometry
IS	Internal Standard
LOD	Limit of detection
MW	Molecular weight
SIM mode	An analysis with greater sensitivity and lower detection limits for trace components
TEGDMA	Triethyleneglycol dimethacrylate
UEDMA	Urethane-dimethacrylate
WCOT	Wall-coated open tubular