Identification of Residual Acrylates Leached from Six Resin Cements by HPLC and GC/MS

6 Farklı Rezin Simandan Salınan Rezidüel Akrilatların HPLC ve GC/MS ile Saptanması

Subutay Han ALTINTAŞ,[a]
Serdar KILIÇ,[a]
Bora BAĞIŞ,[b]
Ahmet YAŞAR,[c]
Aslıhan ÜŞÜMEZ[d]

[a]Department of Prosthodontics, Karadeniz Technical University Faculty of Dentistry, Trabzon,
[b]Department of Prosthodontics, İzmir Kâtip Çelebi University Faculty of Dentistry, İzmir
[c]Department of Analytical Chemistry, Karadeniz Technical University Faculty of Pharmacy, Trabzon,
[d]Department of Prosthodontics, Bezmiâlem Vakıf University Faculty of Dentistry, İstanbul

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Yazışma Adresi/*Correspondence:*
Serdar KILIÇ
Karadeniz Technical University Faculty of Dentistry,
Department of Prostodontics, Trabzon, TÜRKİYE/TURKEY
serdarklic@gmail.com

ABSTRACT Objective: This study was conducted to identify residual acrylates and other products from six different resin cements to obtain information about the composition and occurrence of sensitizing acrylates. The hypothesis of the study was that the analytical results would show differences regarding the information provided in material safety data sheets (MSDSs) of resin materials. **Material and Methods:** The resin cements tested were Variolink II, Rely X ARC, ResiLute, Nexus 2, Rely X Unicem, and SuperBond C&B (VII, RXARC, RL, N2, RXU, SBC&B). Sixty disc shaped specimens (5 mm diameter x 2 mm thickness, n=10) made from each materials were polymerized by light curing according to manufacturers' instructions and cured samples were stored in ethanol/deionised water solution for 3 days. Residual acrylates of the resin cements in solution were identified using gas chromatography with mass-selective (GC/MS) detection and high performance liquid chromatography (HPLC) with ultraviolet detection. Among detected components were monomers and co-monomers; identification was confirmed with reference substances. **Results:** There were some residual acrylates of each material that were not given in their MSDS. From elution in ethanol/deionized water, 3 acrylates from VII, 7 acrylates from RXU, 2 acrylates from RXARC, 5 acrylates from RL, 5 acrylates from N2 and 4 acrylates from SBC&B were identified. **Conclusion:** Material safety data sheets of resin materials need to be improved so that the health risks for patients and dental personnel can be assessed reliably.

Key Words: Resin cements; chromatography, high pressure liquid; gas chromatography-mass spectrometry

ÖZET Amaç: Bu çalışmanın amacı, 6 farklı rezin simandan salınan rezidüel akrilat ve diğer ürünlerin saptanması ile bunların komposizyonu ve oluşumlarının incelenmesidir. Hipotez ise, rezin simanların materyal güvenlik bilgi formlarındaki (MSDS) verilerin, çalışmanın analitik bulguları ile farklılık göstermesidir. **Gereç ve Yöntemler:** Testlerde kullanılan rezin siman çeşitleri Variolink II, Rely X ARC, ResiLute, Nexus 2, Rely X Unicem ve SuperBond C&B'dir (VII, RXARC, RL, N2, RXU, SBC&B). Her materyalden 10 adet olmak üzere toplam 60 disk şeklindeki örnek (5 mm çap x 2 mm kalınlık), üretici firma önerileri doğrultusunda ışık ile polimerize edildi ve 3 gün boyunca etanol/deiyonize su solüsyonu içerisinde bekletildi. Solüsyon içerisinde bulunan rezin simanların rezidüel akrilatları, gaz kromatografi-kütle spektrometresi (GC/MS) ve ultraviole dedektörlü yüksek performans sıvı kromatografisi (HPLC) ile saptandı. Ölçüm sonucunda tespit edilen monomer ve ko-monomer salınımları referans maddeler yardımı ile doğrulandı. **Bulgular:** Her materyal için MSDS'de belirtilmeyen bazı rezidüel akrilatlar tespit edildi. Etanol/deiyonize su solüsyonu içerisine olan salınım incelendiğinde MSDS'da bulunmayan 3 akrilat VII'den, 7 akrilat RXU'den, 2 akrilat RXARC'den, 5 akrilat RL'den, 5 akrilat N2'den ve 4 akrilat SBC&B'den saptandı. **Sonuç:** Rezin esaslı materyallerde MSDS formlarının daha detaylı açıklanması, hasta ve dental personelin sağlık riskinin daha güvenilir bir şekilde belirtilmesi açısından önemlidir.

Anahtar Kelimeler: Rezin simanları; kromatografi, yüksek basınçlı sıvı; gaz kromatografi-kütle spektrometri

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Resin materials consist of a resin matrix, inorganic fillers, and a coupling agent.[1] A resin matrix can contain one or more monomers, for example Bis-GMA and/or UDMA, co-monomers (TEGDMA), and various additives, such as an initiator, co-initiator, and inhibitor of polymerisation, and a photostabiliser.[1,2] After polymerisation, a significant amount of organic compound residues remain unbound in the cured material. The degree of conversion (DC) has been found to be between 46% and 80% in composite materials.[3,4] Of the methacrylate groups, 25-60% may have unreacted residues, but less than 10% of the available methacrylate groups are free to diffuse out.[5] The ingredients and degradation products of resin materials are known to be allergenic, cytotoxic, and even genotoxic.[2,6-12] Acrylates, mainly methacrylates which are widely used in dentistry, were found to cause cytotoxic and allergenic effects. Evaluation of the cytotoxicity of 39 acrylates and methacrylates that were used in dental resin materials showed a relationship between their structure and the degree of cytotoxicity. Some ingredients of resin materials may exert direct effects on the DNA genotoxicity which may be transferred to the next generations of the organisms (mutagenicity). These effects may occur at subtoxic concentrations.[13,14]

Little is known about the uptake, distribution, metabolism and final fate of the components of resin materials. Because manufacturers' information about the ingredients in the materials is often incomplete, studies of the materials are necessary to better understand the amounts and types of potentially harmful residual monomers. Dental clinics should review information about any hazardous compounds in the restorative materials they use in product Material safety data sheets (MSDSs).[15-17]

Material data sheets are presumed to provide information regarding potential hazardous chemicals in products. However, a recent product analysis revealed incomplete MSDSs regarding resin materials.[18] Because of the frequency of incomplete information about the ingredients in dental materials, further studies are needed to provide information about potentially harmful compounds that may be elute from dental materials.[1,18-21]

The aim of the present study was to identify residual acrylates from six different resin cements to obtain information about the composition and occurrence of sensitising acrylates. The null hypothesis of the study was that the analytical results would show differences regarding the information provided in MSDSs of resin materials.

MATERIAL AND METHODS

RESIN MATERIALS PREPARATION

Six different resin materials were investigated in this study. The tested resin cements were Variolink II (Ivoclar) (Low viscosity), Rely X ARC (3M ESPE), ResiLute (Pulpdent), Nexus 2 (Kerr) , Rely X Unicem (3M ESPE) and SuperBond C&B (Sun Medical) (VII, RXARC, RL, N2, RXU, SBC&B). Information given by the manufacturers about the acrylates of the resin materials investigated is given in a table (Table 1).

Ten disc-shaped specimens for each material was prepared in teflon molds at a thickness of 2 mm with a diameter of 5 mm. The molds were filled with uncured material and covered with a mylar strip to protect the resin cement from the oxygen inhibition zone, and the materials were polymerised by light curing (Hilux Utra Curing Unit, Benlioglu Dental, Ankara, Turkey) at 550 mW/cm² (with a light tip to specimen distance of 0 mm) for 40 s. The samples prepared from SBC&B which is a single self-curing dental adhesive resin cement used in the recent study, were polymerised for 10 min., immediately after mixing polimer powder with activated liquid (Monomer and Catalyst mixture) according to manufacturers' instructions.

EXTRACTION PROCEDURE OF RESIDUAL MONOMERS

Cured samples were detached from the teflon molds and immediately immersed in light proof glass bottles containing 20 mL 75% ethanol and 25% deionised water after polymerisation of the resin cements and stored at 37°C for 3 days. After this time, the extracts were removed from the bottles, which contained immersed specimens. To avoid contamination from other polymer-based

TABLE 1: Acrylates of the resin materials given by manufacturers.

Resin cement	Name of manufacturer	Chemical composition	Batch No:
Variolink II	Ivoclar Vivadent AG Schaan/ Liechtenstein	Paste A: Bis-GMA,urethane dimethacrylate, TEGDMA, inorganic filler, ytterbium trifluoride, initiator, stabilizer Paste B :Bis-GMA, UDMA, TEGDMA, inorganic filler, ytterbium trifluoride, benzoyl peroxide, stabilizer	J05278
Rely X Unicem	3M ESPE AG Dental Products Seefeld/Germany	Methacrylated Phosphoric Acid Esters Triethylene Glycol Dimethacrylate Substituted Dimethacrylate	221806
RelyX ARC	3M ESPE AG Dental Products Seefeld/Germany	Paste A: Sılane Treated Ceramıc TEGDMA, BADGE Sılane Treated Sılıca Functıonalısed Dımethacrylate Polymer Paste B: Sılane Treated Ceramıc TEGDMA, BISGMA Sılane Treated Sılıca Functıonalısed Dımethacrylate Polymer	20060106
Resilute	Pulpdent Corporation Watertown/ USA	Base+catalyst: Methacrylate	041117
Nexus 2	Kerr Co., Orange, CA, USA	Base: Bis-GMA, camphoroquinon, barium aluminoborosilicate glass Catalyst: Bis-GMA, teriethylene glycol dimethacrylate, barium aluminoborosilicate glass	409177
C&B Super Bond	Sun Medical Co.Ltd. Moriyama, Shiga/ Japan	Tri-n-butylborane (TBB) TBB-O Methyl metacrylate(MMA) 4-methacryloxyethyltrimellitic acid anhydride (4-META) Poly (Methyl metacrylate) (PMMA)	LL2

materials and plastics, gloves were not used. The glass bottles were distilled with ethyl acetate twice and kept at 100°C for at least 12 h before use.

ANALYTICAL PROCEDURE

The residual acrylates of the resin cements in solution were identified by using gas chromatography with mass-selective detection and liquid chromatography with ultraviolet detection. Among the components detected were monomers, comonomers, initiators, stabilisers, decomposition products and contaminants. Identification was confirmed with reference substances, if available (Figure 1).

HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY) ANALYSIS

The analysis of extracts from the resin cements as well as reference solutions of the monomers in water/acetonitrile (25:75) was carried out by High Performance Liquid Chromatography (HPLC) (Agilent Technologies, USA) under the following conditions:

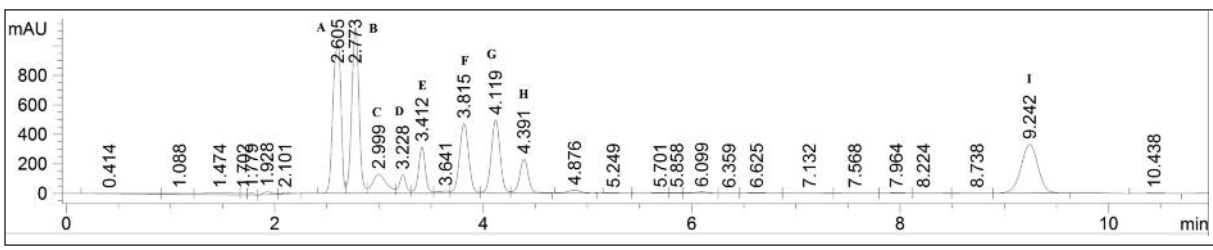

FIGURE 1: Standard chromatograms and retention time of monomers: (A)HEMA, (B) Bisphenol A, (C) Bisphenol A Ethoxylate, (D) MMA, (E) TEGDMA, (F) UDMA, (G) Bis-GMA, (H) BADGE, (I) BisDMA.

The HPLC system consist of a Agilent quatpump (USA), with an autoinjector model G1313A and a G1314A UV-vis dedector. The HPLC chromatograms of resudual monomer were recorded by Agilent CAG Bootp Server software. The steel column (Waters Corporation, Milford Massachusetts, USA) 250 mm in length, 4.6 mm in diameter and particle size of 5 μm was used.

STANDART SOLUTION AND SAMPLE PREPARATION

Stock solutions of monomers were prepared by dissolving the monomer ethanol-water to a final concentration of 1000 μg/mL. From these stock solutions, calibration standard solutions were prepared in ethanol-water containing: 5.0, 10.0, 20.0, 40.0 and 80.0 μg/mL.

A 200 μL aliquot of the appropriated solution was transferred to autosampler vials and 10 μL were injected onto the HPLC system. The separation was performed using acetonitrile-water (75:25) as eluent, at a flow rate of 1 mL/min. The monomers were detected by their UV absorbance at 208 nm.

GC/MS (GAS CHROMATOGRAPHY WITH MASS-SELECTIVE DETECTION) ANALYSIS

GC/MS analysis was performed using an Agilent-5973 Network System and a mass spectrometer with an ion trap detector in full scan mode under electron impact ionisation (70 eV). The chromatographic column used for the analysis was a HP-5 capillary column (30 m, 0.32 mm i.d., film thickness 0.25 lm) and the carrier gas used was helium, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. One microlitre of essential oil solution in hexane (HPLC grade) was injected and analysed with the column held initially at 60°C for 2 min and then increased to 260°C with a 5°C/min heating ramp. The injections were subsequently kept at 260 °C for 13 min and the relative percentage amounts of the separated compounds were calculated from the total ion chromatograms by a computerised integrator.

RESULTS

The qualitative identified residual acrylates of the resin cements are summarised according to their occurrence in the resin materials (Table 2). The identified residual acrylates, except for the composition of resin cements, in the MSDSs were: Methyl Methacrylate (MMA), Bisphenol A diglycidyl ether (BADGE), Bisphenol A for VII, MMA, BADGE, Urethan dimethacrylate (UDMA) for RXARC, MMA, UDMA, BADGE, HEMA, TEGDMA, 2,2-bis-[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (Bis-GMA) for RL, MMA, UDMA, TEGDMA, HEMA, BADGE, Bisphenol A dimethacrylate (BisDMA), Bis-GMA for N2, Bis-GMA, UDMA, BADGE, Bisphenol A, Bisphenol A Ethoxylate (BisEMA), HEMA, MMA for RXU, HEMA, UDMA, BADGE, Bis-GMA for SBC&B. Bis-GMA, BADGE, and BisDMA could only be detected by HPLC. The residual acrylates of each tested resin cement material were not given in the MSDS's of the manufacturers.

TABLE 2: The identified residual acrylates of resin cements by HPLC and GC/MS.

Variolink II	Rely X Uicem	Rely X ARC	Resilute	Nexus 2	Super Bond
Bis-GMA	Bis-GMA*	Bis-GMA	Bis-GMA*	Bis-GMA	Bis-GMA*
UDMA	UDMA*	UDMA*	UDMA*	UDMA*	UDMA*
TEGDMA	TEGDMA	TEGDMA	TEGDMA*	TEGDMA	BADGE*
BADGE*	BADGE*	BADGE	BADGE*	BADGE*	HEMA*
MMA*	MMA*	MMA*	MMA	MMA	MMA
Bispheno A*	Bispheno A*		HEMA*	HEMA*	
	Bisphenol A Ethoxylate*			BADGE*	
	HEMA*			BisDMA*	

* The residual acrylates of each tested resin cement material that had not been provided in their material data sheets (MSDS)

DISCUSSION

In the current study, identification of residual acrylates from six different resin cements was performed in order to obtain information about the composition and occurrence of sensitising acrylates, which were evaluated using HPLC and GC/MS. The residual acrylates of each tested resin cement material were not given in the MSDS's of the manufacturers. According to the findings of the present study, from elution in ethanol/deionized water 3 acrylates from VII, 7 acrylates from RXU, 2 acrylates from RXARC, 5 acrylates from RL, 5 acrylates from N2 and 4 acrylates from SBC&B were identified except for the composition of resin cements, in the MSDSs, therefore the obtained results supported the research hypothesis that the analytical results would show differences in the information given in the MSDSs of materials. Based on studies of leachable components from resin materials, some researchers have concluded that all ingredients of dental resin materials should be declared by manufacturers, with the aim of identifying substances that could cause adverse side effects in patients and/or dental staff.[1,18,21] However, few studies have analysed organic leachables from resin materials with the aim of comparing the results to information provided by MSDSs.

A combination of gas chromatography (GC) and mass spectrometry (MS) has been used to separate and identify organic leachables arising from resin materials.[1,16,21] However, molecules of high molecular weight, for example base monomers such as BIS-GMA and UDMA, may decompose in GC and only decomposed products may be detectable.[1,17,20] This is a difficult process and is rarely done with GC. Most studies on large molecules have used HPLC analyses.[13,22-24] In the current study, the universal analytical separation techniques HPLC and GC/MS were used to detect the quality of residual monomer leaching from the resin materials. High-molecular-weight molecules, such as Bis-GMA, BADGE and BisDMA, could only be detected by HPLC, consistent with previous reports.[10,15,24]

The greater the polymerisation reaction, the fewer residual monomers there are to leach.[13] After polymerisation, a significant quantity of organic compound residues is unbound in the cured material. The DC of carbon-carbon double bonds (C=C) also depends on the type, duration and intensity of the light source, and some properties of the resin system, such as the depth of the resin material.[5,22,25] Quartz-tungsten halogen (QTH) and light-emitting- diode (LED) are the most popular light sources that are used to cure dental resins. The QTH presents a broad wavelength spectrum, which allows efficient activation of different photo-initiators that are used as an alternative to camphorquinone, the most common initiator among light curing dental resins. Some researchers concluded that the QTH lamp promotes better values on the degree of conversion within the nanofilled composite resin than the LED lamp.[26] Furthermore, increased irradiation time, from 30 to 50 s, results in a significant decrease in residual monomer content and the quantities that are released into water.[27] Also some studies indicated that the enhanced irradiation time -10, 20 or 40 s- has only little influence the initial decrease of C-C double bonds and the time of curing (20 s and 40 s) did not influence the degree of conversion.[26,28] In the present study, the resin cements were polymerised according to the manufacturers' instructions with a halogen light source for 40 s.[24] The viscosity of composites and filler loading may interfere in the monomer conversion, because they could restrict the mobility of monomers and the progression of polymerization reaction. A previous study pointed that the DC of resin cements was higher when the light-cured and low-viscosity versions of resin materials were used than when self-curing mode and the high-viscosity resin cements were tested.[29] Therefore, low viscosity version of resin cements were used in the present study.

Several studies have been performed to determine the influence of solvent type and duration on the release of substances from resin materials.[20,30] Some organic solvents, such as ethanol, methanol, or mixtures of these solvents with water, are preferred for simulating oral conditions.[25,27] Organic solvents have the ability to penetrate and swell the polymer network, facilitating the liberation of un-

reacted and leachable monomers. As the solvent penetrates the matrix and expands the openings between polymer chains, oligomers can diffuse out.[25] The intraoral fluids probably represent conditions somewhere between the more aggressive organic solvents and water. The United Stated Food and Drug Administration indicates that a 75% ethanol-water solution as a food/oral simulating liquid is clinically relevant.[5] Thus, in the present study, 75% ethanol/25% deionised water was used as the extraction medium for measuring monomer release.

The clinical success of resin materials depends not only on their physical and chemical properties, but also on their biological safety. The organic matrix of dental resin materials contains compounds that cause a wide variety of adverse biological reactions. These analyses have been extensively reviewed in the literature.[14,31-33] Many of these compounds, mostly epoxy resins and acrylic monomers, have been identified as important occupational sensitisers, with an established potential for cross-reactivity. Moreover, work-related adverse effects, such as occupational skin disease (OSD), allergic contact dermatitis, and irritant contact dermatitis, have frequently been reported by dental personnel.[8,18] It has also been hypothesised that components from dental composite materials may alter cytokine secretion in human monocytes if applied at sublethal concentrations. Likewise, other tightly regulated pathways of cellular metabolism, for example the induction of a cellular stress response or changes in lipid metabolism, are also modified.[33,34] The cytotoxicity ranking of the most widely used monomers is Bis-GMA> UDMA>TEGDMA>HEMA>MMA.[35] Furthermore, serious concerns about possible health problems have been raised because compounds such as Bis-GMA, Bis-DMA, and bisphenol A (BPA) have been identified as endocrine-disrupting chemicals, capable of mimicking the effects of natural steroid hormones. These aromatic components are leached from commercial products, such as composites and sealants, at concentrations at which biological effects have been described in experimental models.[33] In addition, Bis-EMA monomers have been shown to have cytotoxic effects analogous to those of TEGDMA.[17] The qualitative identification and diversity of organic elutes from polymerised samples of two composites, one compomer cement and one resin- reinforced glass ionomer cement, have been found to contribute to the potentially hazardous compounds that leach from polymer-based dental filling materials (PBDF);[16] that study reported different compounds that were not declared in the manufacturers' SDSs for these products. Thus, in the present study, the qualitative identification and diversity of products eluted from resin cements were determined to contribute to the improvement of SDS information on resin materials and to identify the aetiology of adverse reactions.

This *in vitro* study was performed under well-controlled laboratory situations. However, the design of the study has several limitations, making it difficult to compare the results with clinical conditions. Furthermore, because only a limited number of resin cements was tested, the results cannot be generalised to other systems. From a clinical viewpoint, there are also limitations concerning the correlation between *in vitro* and *in vivo* tests and clinical usage. However, *in vitro* residual monomer measurements using HPLC and GC/MS are valuable for understanding the leaching ability of organic compounds from these resin cements.[16,19,24] Elution of residual monomers from resin materials is related to their degree of polymerisation, properties of resin composition, and the chemistry of organic solvents *in vitro*.[5] Several factors affect the elution process of residual monomers *in vivo*. One factor is related to the dental personnel who apply the resin materials during treatment. From this point of view, manufacturers' instructions on the application and polymerisation process of their resin materials are of importance. The oral fluids of humans can differ according to their chemical composition, enzymes, and oral stresses. For these reasons, the experimental setup here did not consider the effects of *in vivo* conditions and the elution of residual monomers measured cannot be directly applied to the elution of residual monomers *in vivo*. Thus, further *in vivo* studies evaluating residual monomers and their effects are required.

CONCLUSION

This study focused on the qualitative identification and diversity of the products eluted. Due to differences between the materials, the biocompatibility, including the allergenic potential, may also differ. Some of the identified products were acrylates. Degradation products may be leached into the oral cavity at clinically relevant concentrations. It may be concluded that the extractable quantities of residual monomers should be minimised, either by reducing the mobility within the set restoration by a higher degree of curing or by reducing the release by applying less water-soluble monomers. In addition, all ingredients of a dental composite should be declared by manufacturers. MSDSs of resin materials need to be improved so that the health risks to patients and dental staff can be assessed reliably.

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