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## Correlation between the degree of conversion and the elution of leachable components from dental resin-based cements

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**Abstract:** This study examined the possible correlation between the degree of conversion (*DC*) and the amount of substances eluted from three commercial cured resin-based cements. The *DC* of the various resin-based cements was measured by Raman spectroscopy, while the quantity of unreacted monomers released from the cement matrix (triethylene glycol dimethacrylate, TEGDMA, urethane dimethacrylate, UDMA, 2-hydroxyethyl methacrylate, HEMA and bisphenol A) was determined by high pressure liquid chromatography (HPLC). The obtained results, after multiple statistical evaluation (one way ANOVA, LSD post hoc test), showed no significant differences in the *DC* values between the resin cements. On the contrary, the results of the HPLC analysis depicted statistically significant differences between the three materials with respect to the amount of leached monomers. In addition, no correlation between the *DC* and the amount of eluted substances from the tested cured composite cements was found.

**Keywords:** dental resin-based cement; degree of conversion; eluted monomer.

### INTRODUCTION

Contemporary prosthodontics promotes new all-ceramic systems and different resin-based luting cements. Adhesive composite cements belong to a relatively small but specific group of dental composite materials. They are highly responsible for the final appearance and functional capacity of cemented ceramic crowns in the mouth environment.<sup>1,2</sup>

Although, the composition of luting resin-based dental composites is basically identical to other composite materials (filling composite and core build-up materials), there are some differences. Resin-based cements must provide the

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practitioners with optimum handling features. Therefore they have modified rheological properties (higher flow capacity and lower viscosity) resulting from a reduced filler content (fine particle hybrid type). In addition, the different polymerization initiation system of resin cements is responsible for the participation of additional components: chemical polymerization or/and light-curing initiators. Newer self-adhesive resin cements contain acidic monomers with several methacrylate and phosphate groups.<sup>3</sup>

The setting of resin-based cements belongs to a radical-chain type of polymerization. Most frequently, a material sets through the light or chemical initiated polymerization of matrix monomers by the opening of double bonds at both methacrylate residues of the monomers and the resulting cross-linking process. Ideally, dental resins would have all their monomers converted to polymer during the polymerization process. However, all dimethacrylate monomers exhibit residual unsaturation in the final product, mainly in the form of pendant methacrylate groups, due to steric hindrance.<sup>4,5</sup> The percentage of reacted C=C bonds is defined as the degree of conversion (*DC*). This value, which varies between 35 and 77 % (88 %) among different composite materials, affects many properties including mechanical features, solubility, dimensional stability and biocompatibility of resin-based materials.<sup>6–8</sup> The degree of conversion may be assessed directly or indirectly. Micro-Raman spectroscopy and Fourier transform infrared micromultiple internal reflectance spectroscopy (FT-IR) are the available direct methods frequently used for measuring the *DC* values of the composites. Infrared spectroscopy is the most sensitive as it detects the C=C stretching vibrations directly before and after curing of the material, but it is a time consuming and costly method.<sup>9</sup> On the other hand, FT-Raman spectroscopy is a nondestructive technique which provides for investigation of samples of any thickness and geometry. Physical property test such as hardness, flexural strength and modulus of elasticity are indirect methods used to evaluate the conversion of double bonds in a composite.<sup>9–11</sup>

The elutable residual monomer content must be differentiated from the degree of conversion. Residual monomers are those components that are released from the resin into various media. The results concerning the correlation between *DC* and eluted residual monomers in water are strongly controversial.<sup>8,12</sup> Approximately, one tenth of the non-reacted methacrylate groups exist as residual monomers,<sup>8</sup> or 0.2–3 mass% is the measured elution of monomer from the polymer matrix of various resin composites.<sup>13,14</sup> The main analytical methods used for the determination of substances eluted from cured composite materials are gas chromatography (GC) and high performance liquid chromatography (HPLC). If necessary, they are combined with mass spectroscopy (MS), whereby different forms and masses of the eluted species can be identified.<sup>15–17</sup>

The present study was performed as a part of a comprehensive investigation of the biocompatibility of dental resin luting composites. The biological characteristics of the resin-based cements were not investigated as much as the properties of the filling composites or dental adhesives (similar materials with different applications and purposes). Literature data very rarely reveal the correlation between the quantity of eluted substances and the degree polymerization of composite cements. This correlation significantly influences the clinical success of cementation procedures, because cement hardens through prosthetic restorations. Therefore, the aim of this study was to determine the correlation between the degree of conversion and quantity of leachable monomers of three commercial resin-based cements.

#### EXPERIMENTAL

The three different cure dental resin-based cements included in the study are presented in Table I. They differ from each other with respect to the resin matrix and the filler loading (Table I). Their compositions, according to the manufactures, are not available in detail, due to the rights of the manufacturers to keep the composition a secret.

TABLE I. Resin-based cements tested in this study

Brand	Manufacturer	Shade	Filler content, mass%	Size of filler particles, $\mu\text{m}$	Chemical composition
Panavia F2	Kuraray Medical	White	78	0.01–3.5 Avg. 0.5	Bis-GMA <sup>a</sup> , TEGDMA <sup>b</sup> , MDP <sup>c</sup> , quartz glass, barium glass, silanated silica, silanated colloidal silica
Variolink 2	Ivoclar, Vivadent	Transparent	71.2	0.04–3.0 Avg. 0.7	Bis-GMA, UDMA <sup>d</sup> , TEGDMA silica, barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass
Nexus 2	KerrCorp.	White	68	0.02–2.8 Avg. 0.6	Bis-GMA, TEGDMA, UDMA, Ba-Al-borosilicate glass, SiO <sub>2</sub>

<sup>a</sup>Bisphenol A–glycidyl dimethacrylate; <sup>b</sup>triethylene glycol dimethacrylate; <sup>c</sup>10-methacryloyloxydecyl dihydrogen phosphate; <sup>d</sup>urethane dimethacrylate

#### *Specimen preparation*

Ten specimens of each material were prepared in accordance with the manufacturer's instructions and divided into two groups with five specimens in each group.

After carefully mixing, to minimize the inclusion of air, the resin-based cements were placed into a stainless steel ring mould (thickness, 1 mm, diameter, 8 mm) positioned on a glass slide. Subsequently, a second glass slide and a sheet of polyester film (ION Brand, extra thin, 3M Co., St. Paul, MN, USA) were positioned on the top of mould in order to press the excess material over the brim of the mould and to avoid the contact with oxygen, which is an inhibitor of polymerization. A common practice under clinical conditions is the removal of surfaces which are in contact with air by grinding. The same effect was achieved by covering the surfaces with a polyester film.

The specimens were irradiated with an external halogen light-curing unit (MegaLux soft-start, Mega Physik Dental) in five overlapping sections for 20 s at an intensity of 676 mW m<sup>-2</sup>, emission spectrum 390–490 nm. The diameter of the light tip was 8 mm. The glass slide was removed after the initial twenty second of light activation. Remaining light activation was delivered through a sheet of polyester film to eliminate the potential attenuation of the light intensity through the glass slide. The hard set specimens were released from the mould.

One group of specimens was *DC* tested immediately after specimen preparation. The other groups of specimens were immersed immediately after polymerization in 3 mL of de-ionized water maintained at 37 °C. The specimens were removed from the water after 24 h and extracts (0.5 mL) were analyzed for the qualitative and quantitative determination of the eluted monomers.

#### *Degree of conversion analysis*

The *DC* of the test materials was measured by Raman spectroscopy using a 670 FT-IR FT-Raman spectrometer (Nicolet, USA). The excitation source in the FT-Raman module was an Nd-YAG laser. This emits continuous wave length of 1054 nm (9398 cm<sup>-1</sup>) and has a maximum power level of approximately 1.5 W at the sample. An air-cooled GaAs detector was installed in the FT-Raman module. An XT-KBr beam splitter was used. The employed sample configuration was 180 °C reflective with fully motorized sample position adjustment, with an NMR-tube sample holder. All the FT-Raman spectra were collected in the spectral range from 3701 to 98 cm<sup>-1</sup> and 46 scans. The laser power was 0.7 W.

The *DC* of the dental resin cements was determined by comparison of the ratio (*R*) between the reacted aliphatic C=C bonds and the unreacted aromatic C=C bonds before and after curing. The aliphatic and aromatic C=C bonds have characteristic Raman scattering peaks located at 1638 and 1610 cm<sup>-1</sup>, respectively, as well as infrared absorption peaks but with higher intensities. The peak height (*H*) and the underlying area (*A*) were calculated for each peak, using a standard baseline technique. The ratio (*R*) was determined twice using the mean of the peaks heights and areas to obtain reliable results. The percentage *DC* for each specimen was calculated using the following equation:

$$DC\% = 100(1 - R_{\text{polym}}/R_{\text{unpolym}})$$

where *R* represents peak height at 1640 cm<sup>-1</sup> divided by peak height at 1610 cm<sup>-1</sup>.

#### *Monomer leaching and HPLC*

The unreacted monomers released from the matrix were determined by HPLC.

#### *Chemicals*

All solvents and chemicals used were of analytical grade. The dimethacrylate monomers used were triethylene glycol dimethacrylate, TEGDMA (Sigma-Aldrich, Germany), urethane dimethacrylate, UDMA (Sigma-Aldrich, USA), bisphenol A glycidyl methacrylate, bis-GMA (Sigma-Aldrich, USA), 2-hydroxyethyl methacrylate, HEMA, (Sigma-Aldrich, USA) and bisphenol A (Sigma-Aldrich, USA).

#### *Equipment and chromatographic conditions*

Quantitative analysis was performed on a Surveyor HPLC system (Thermo, USA) consisting of a solvent delivery module, an auto sampler and a photodiode array (PDA) detector. The separation column was reverse-phase Zorbax Eclipse® XDB-C18 (Agilent Technologies, USA), 4.6×75 mm<sup>2</sup> i.d. and 3.5 μm particle size. Before the separation column, a pre-column was installed; 4.6×12.5 mm<sup>2</sup> i.d. and 5 μm particle size (Agilent Technologies, USA). The mobile phase consisted of water (A) and acetonitrile (B). The gradient changed as

follows: 0 min, B 20 %; 15 min, B 70 %; 17 min, B 70 %; 20 min, B 100 %. The initial conditions were re-established and held for 9 min to ensure minimal carry-over between injections. The flow rate was 0.5 mL min<sup>-1</sup> and the injector volume was 10 µL. UV detection was performed at 205 and 275 nm simultaneously. The components were identified by comparing the elution time with that of reference compounds and by their UV spectra. Simultaneously, additional conformation for TEGDMA, UDMA and Bis-GMA was performed using a quadrupole ion trap mass selective detector (LCQ Advantage Thermo, USA). Samples were analyzed using the electrospray ionization technique in the positive mode. The optimal source working parameters for monitoring all ions were as follows: source voltage (4.5 kV), sheath gas (25 a.u.), auxiliary gas (4 a.u.) and capillary temperature (220 °C). Detection of the analytes was based on the isolation of the ammonia adduct [M+NH<sub>4</sub>]<sup>+</sup>, for TEGDMA and bis-GMA and the sodium adduct [M+Na]<sup>+</sup> for UDMA. Subsequent MS<sup>2</sup> and MS<sup>3</sup> fragmentations of the isolated ions were performed using the parameters listed in Table II. Selected reaction monitoring (SRM) or consecutive reaction modes (CRM) were used for identification of all the mentioned monomers.

TABLE II. Calibration equations, regression correlation coefficients and retention times for the reference compounds at 205 nm

Analyte	Regression equation	R <sup>2</sup>	Retention time, min
HEMA	$Y = 167028x + 205716$	0.9918	4.05
Bisphenol A	$Y = 244316x + 11111$	0.9997	13.48
TEGDMA	$Y = 274101x + 12000$ $Y^a = 159927x + 2000000$	0.9983 0.9992	15.18
UDMA	$Y = 79274x + 12473$	0.9992	18.58
Bis-GMA	$Y = 174038x + 67260$	0.9992	20.10

<sup>a</sup>Calibration equation for the concentration range from 20 to 50 µg mL<sup>-1</sup>

#### Calibration procedure

Stock solutions of reference standards (100 µg mL<sup>-1</sup>) were prepared in methanol. These solutions were stored at 4 °C. Working standards of the analytes were obtained by dilution with methanol to final concentrations at 0.5, 1.0, 5.0, 10.0 and 20.0 µg mL<sup>-1</sup>. For TEGDMA and HEMA, working standards at 30.0 and 50.0 µg mL<sup>-1</sup> were also made. Calibration plots were produced using the standard solutions described above. Calibration curves were obtained by plotting the peak area vs. concentration using linear regression analysis.

#### Optimization of the HPLC and HPLC/MS analysis

Detection and quantification was performed at a wavelength of 205 nm for all the analytes because they exhibit significant absorption. The second wavelength of 275 nm for bis-GMA and bisphenol A detection was used based on their UV spectra. The linearity of the analytes response was established with five concentration levels. The equations obtained, as well as the retention times for reference compounds are also reported in the Table II.

For additional confirmation purposes, the HPLC/MS method was also optimized. In a search for the most appropriate conditions to optimize the mass spectrometric system for analysis, different ionization techniques were examined. The responses of analytes using atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) were compared. Tuning of the instrument, with both ionization techniques, was performed for each tested compound using standard solution prepared at 10 µg mL<sup>-1</sup>. All standard solutions were infused with the syringe pump at 5 µL min<sup>-1</sup> to the mobile phase consisting of 50/50, aceto-

nitrile/water at a flow rate of 0.5 ml min<sup>-1</sup>. Identification of the characteristic ions as well as the choice of the ionization mode for each analyte were performed in the full scan mode by recording mass spectra from  $m/z$  60 to 700 in both the positive and negative mode. For HEMA and bisphenol A, it was not possible to obtain reliable MS spectra with either of ionization sources. The most abundant ions for the remaining three analytes were: ammonia adducts for TEGDMA and bis-GMA and the sodium adduct for UDMA. The response using the ESI technique was 10 times higher than that using the APCI technique. Positive ESI mode was chosen for further analysis.

Furthermore, for TEGDMA, UDMA and bis-GMA, optimization of the isolation width of the chosen parent ion, selection of the optimal collision energy and identification of the most abundant daughter ion were performed in the selected reaction and consecutive reaction modes. The optimized parameters for MS acquisition are given in Table III. The linearity of calibration curves was rather poor, suggesting that MS analysis could not be used for quantification purposes but rather as an additional identification tool for TEGDMA, UDMA and bis-GMA. Sample analyses were performed using the same chromatographic conditions as for the standards.

TABLE III. Optimized MS, MS<sup>2</sup> and MS<sup>3</sup> parameters for TEGDMA, UDMA and bis-GMA identification

Analyte	MS	Isolation width	Collision energy	MS <sup>2</sup>	Isolation width	Collision energy	MS <sup>3</sup>
TEGDMA	303.9 [M+NH <sub>4</sub> ] <sup>+</sup>	2.0	25	286.7	2.0	–	–
	303.9 [M+NH <sub>4</sub> ] <sup>+</sup>	2.0	25	286.7	3.0	20	113.0
UDMA	493.2 [M+Na] <sup>+</sup>	1.0	42	407.1	1.0	–	–
	493.2 [M+Na] <sup>+</sup>	1.0	42	449.6	1.0	–	–
Bis-GMA	512.8 [M+NH <sub>4</sub> ] <sup>+</sup>	2.0	25	512.8	2.0	–	–
	512.8 [M+NH <sub>4</sub> ] <sup>+</sup>	2.0	25	494.7	2.0	–	–

#### Statistical analysis

The data were expressed as mean values with standard deviations. Differences in the continuous variables between the materials (Panavia F2, Variolink 2, Nexus 2) were compared with the parametric one way ANOVA with the LSD *post hoc* test for multiple comparisons.

Correlation (measured as a correlation coefficient,  $r$ ) indicates the strength and direction of a linear relationship between two random variables. Differences were considered significant at  $p < 0.05$  level.

## RESULTS

#### Degree of conversion analysis

Typical FT-Raman spectra recorded for the resin cements are shown in Figs. 1 and 2. The spectra reveals a scattering Raman peak at 1638 cm<sup>-1</sup> corresponding to the reacting aliphatic C=C stretching of the vinyl group in the acrylates and methacrylates. The scattering peak at 1610 cm<sup>-1</sup>, arising from unreacted aromatic rings in the bis-GMA molecule, is commonly used as an internal reference for precise quantitative analysis.<sup>1</sup> For more reliable data, the ratio of the peaks 1638 to 1610 cm<sup>-1</sup> ( $R$ ) was calculated twice, using the means of peaks height and peaks area.

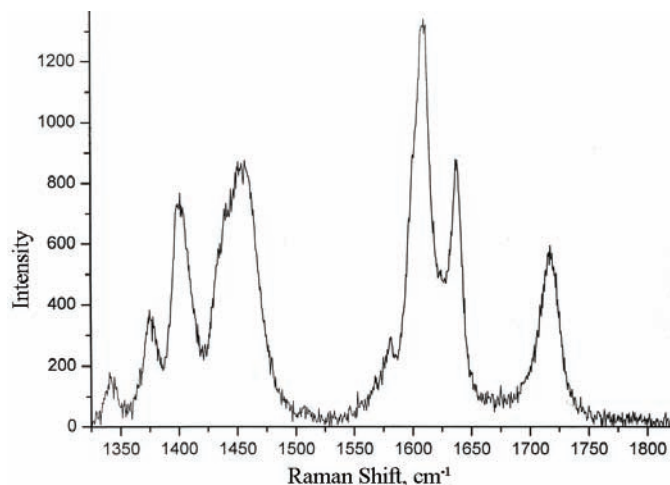


Fig. 1. Typical FT-Raman spectrum of the cured resin cement Variolink 2.

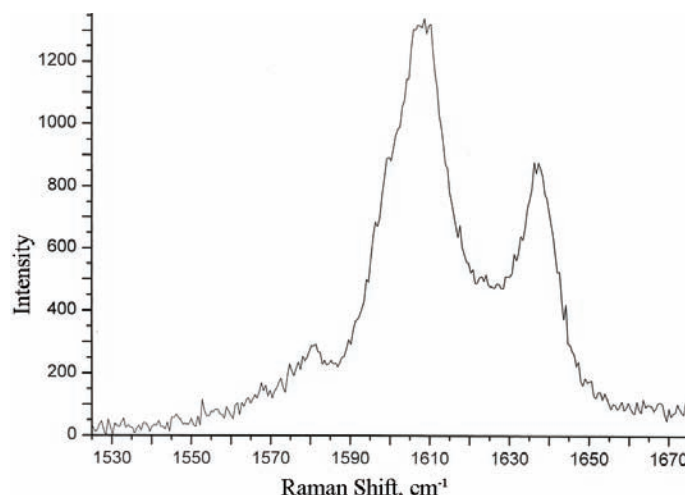


Fig. 2. Magnified section from the FT-Raman spectrum of the cured resin cement Variolink 2.

According to the results of statistical analysis, Variolink 2 showed the highest degree of conversion, whereas Panavia F2 showed the lowest (Table IV). The ANOVA revealed no significant differences in the *DC* between the tested luting resin cements ( $F = 1.925$ ;  $p > 0.05$ ).

#### *Monomer leaching and HPLC*

Concentrations of detected monomers are presented in Table V (means and standard deviation). There was a statistically significant difference between the three materials with respect to the amount of leached monomers ( $p < 0.001$ ) (Table VI).

TABLE IV. Percentage degree of conversion of the materials included in the study ( $n = 5$ ) with standard deviation in parentheses (the means were not significantly different at the  $p = 0.05$  level)

Material	Degree of conversion, %
Panavia F2	66.84 (3.41)
Variolink 2	71.16 (3.73)
Nexus 2	68.46 (3.39)

TABLE V. The monomer concentrations in  $\mu\text{g mL}^{-1}$  eluted from the tested materials in 24 h with the standard deviations in parentheses (the means were significantly different at the  $p = 0.05$  level)

Monomer released	Material		
	Panavia F2	Variolink 2	Nexus 2
HEMA	0.82 (0.32)	0.42 (0.18)	27.20 (1.54)
Bisphenol A	7.24 (0.65)	0.05 (0.34)	1.22 (0.26)
TEGDMA	1.73 (0.63)	30.92 (2.00)	42.90 (0.88)
UDMA	–	1.13 (0.33)	0.96 (0.43)

TABLE VI. Correlation values between degree of conversion ( $DC / \%$ ) and monomer leaching

Material	$R$	Significance, $p$
HEMA	0.103	0.714
Bisphenol A	0.430	0.110
TEGDMA	0.278	0.315
UDMA	0.201	0.473

The released monomers which were detected in the sample Panavia F2, were HEMA, bisphenol A and TEGDMA, while UDMA and bis-GMA were not detected. The highest concentration found was for Bis phenol A, the MS identification of which was not possible, but the UV spectrum of bisphenol A is quite characteristic, allowing the identification of bisphenol A with a high degree of certainty. The chromatogram for the Panavia F2 sample is shown in Fig. 3.

In samples Nexus 2 and Variolink 2, the released monomers HEMA, bisphenol A, TEGDMA and UDMA were detected. In both samples, the highest concentrations found were for TEGDMA. Confirmation of TEGDMA and UDMA presences/absence for both samples was achieved with MS analysis. The chromatogram for sample Variolink 2 is presented in Fig. 4.

In sample Nexus 2, besides TEGDMA, HEMA was also found at a high concentration. Confirmation of identity for HEMA was based only on the retention time and UV spectra. The chromatogram obtained for the Nexus 2 sample is presented in Fig. 5. Bis-GMA was not detected either by UV or MS analysis in any of the examined samples under the employed experimental conditions.



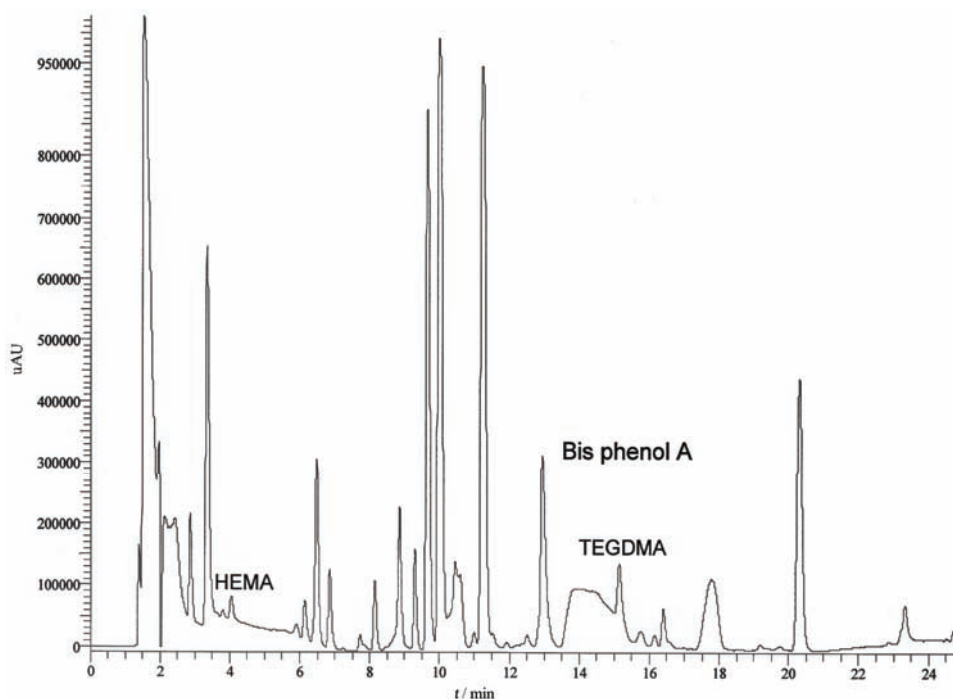


Fig. 3. Chromatogram obtained for Panavia F2 sample at 205 nm.

Obtained results indicate that the percentage of conversion is a rather poor predictor for monomer leaching, as Fig. 6 illustrates.

#### DISCUSSION

The materials used in the present study were chosen for their differences in particle size, and types and weight fractions of filler additives. It is known that the transmission of the curing light is determined by the material and by the size of the filler particles. Different filler particles have different refractive indexes, causing different transmission properties of the composites with respect to wavelength. This fact is probably responsible for the differences in the *DC* of the tested resin-based cements and may lead to different releasing rates of unreacted toxic components.

It is hard to obtain the exact composition of the materials because the manufacturers guard them as company secrets.

The degree of polymerization is one of the important factors which affect the clinical performance of resin cements. Among the methods which determine the degree of conversion (*DC*) of resin materials, Raman spectroscopy has mostly been used. This method relies on the scattering of the IR signal at the surface of a

material with the primary advantage that thick specimens can be analyzed simply in transmission.<sup>18</sup>

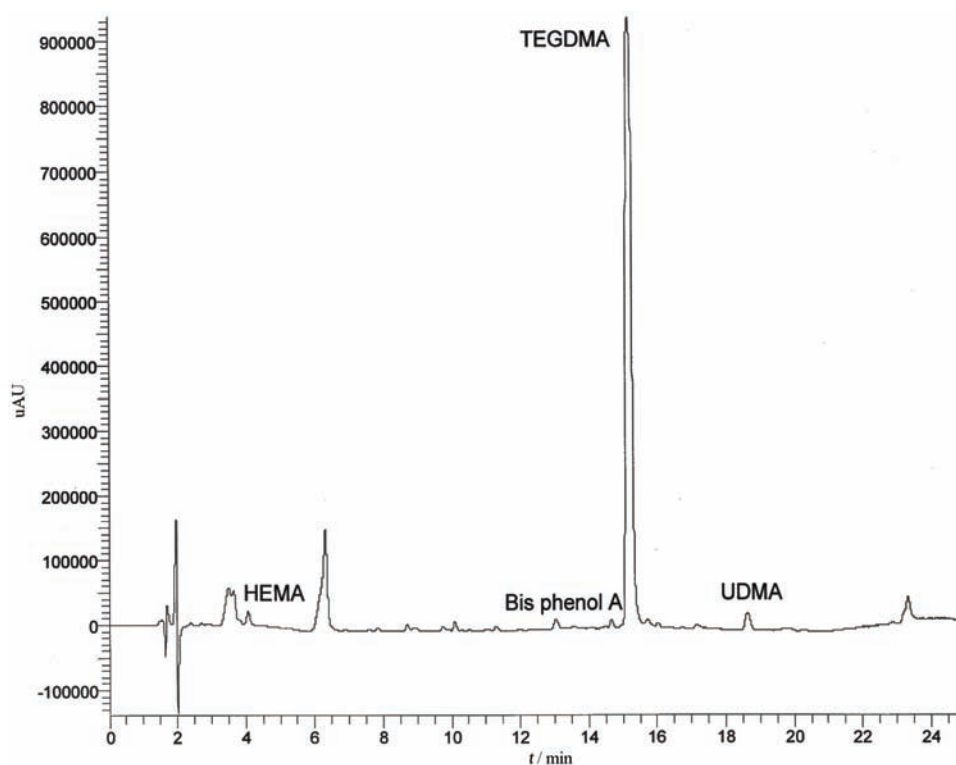


Fig. 4. Chromatogram obtained for the Variolink 2 sample.

The required periods after specimen preparation before Raman spectroscopic analysis are not well established. The periods reported can range from immediately after specimen preparation to up to some days after. It can be assumed that luting restoration will be immediately subject to moisture in the mouth. For this reason, in this study, the specimens were *DC* tested immediately after specimen preparation.

The polymerization of resin-based cements depends on several factors: the polymer matrix, the filler particles, coupling between filler and matrix, the type and intensity of the light source, the distance from the curing tip and duration of exposure. In the present study, the type and intensity of the light source, the distance from the curing tip and duration of exposure were kept constant.

The *DC* values for the resin-based cements tested in this study were higher than for composite restorative materials, which typically range from 50 to 60%.<sup>19,20</sup> This finding may be due to the low initial viscosities of the cements com-

pared with those composite restorative materials. The lower viscosity would allow for greater diffusion of free radicals in the polymerization process and for a higher conversion potential.<sup>21</sup>

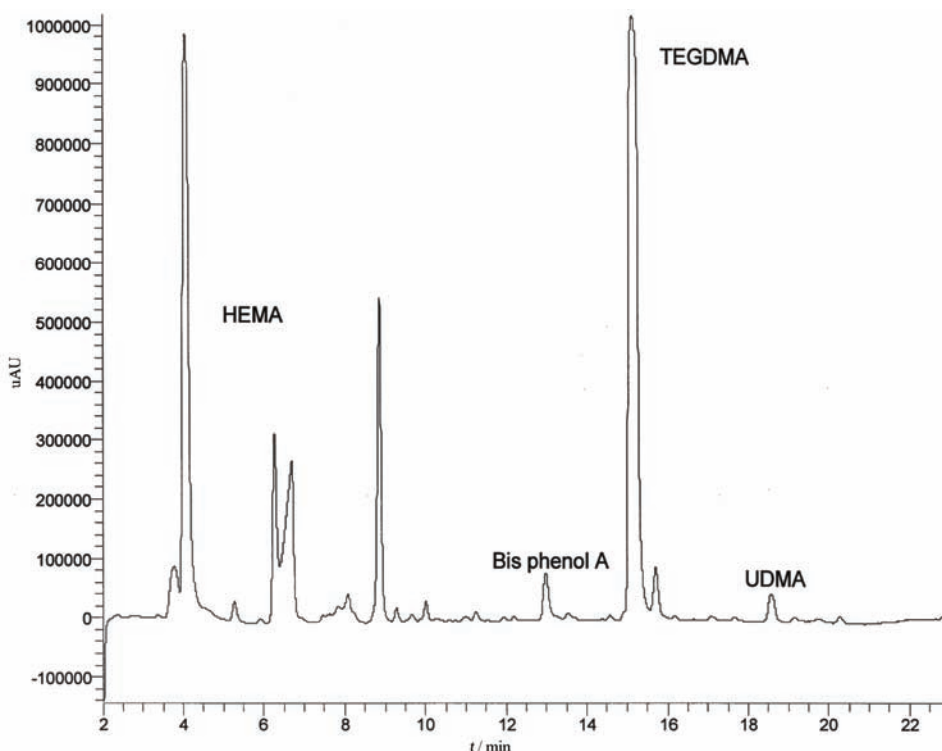


Fig. 5. Chromatogram obtained for the Nexus 2 sample at 205 nm.

However, little has been published about the polymerization of resin-based cements. The results of the present study, mostly agree with those in the literature.<sup>22–24</sup>

In the present study, Variolink 2 showed the highest degree of conversion. This may be due to the content of urethane dimethacrylate (UEDMA), which is known to be a more flexible molecule than Bis GMA because of its urethane linkages. It also has a lower viscosity, which facilitates the migration of free radicals. It is well known that degree of conversion of methacrylate monomers depends on the nature of the polymerizing monomers, more flexible monomer molecules increase the conversion.<sup>25</sup>

However, Nexus 2, with similar chemical composition and filler loading showed a lower degree of conversion than Variolink 2. The general difference between resin-based cements is very difficult to deduce from existing *in vitro* studies because the materials differ in many aspects. A conversion decrease can be

considered from factors that impair the mobility of the reactants. The limited conversion found in many network polymers is due to restricted mobility of the radical chain ends, pendant methacrylate groups and monomer at high cross-link densities.<sup>26</sup>

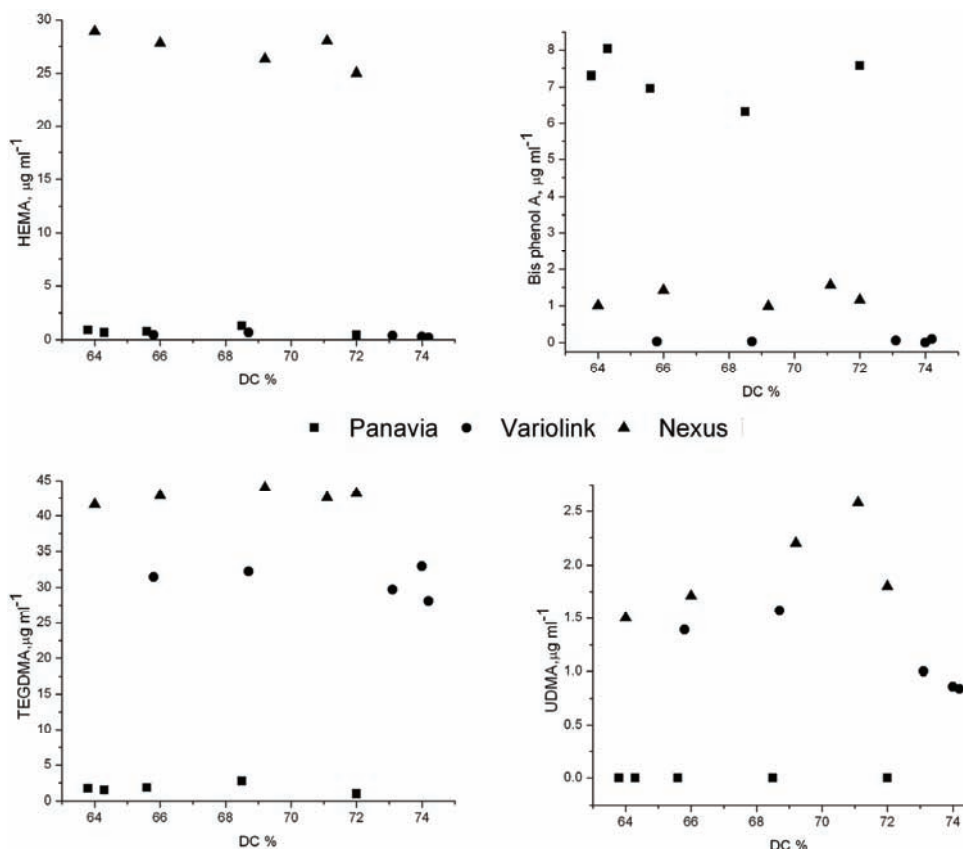


Fig. 6. Monomer leached out as a function of conversion.

On the other hand, Panavia F2 showed the lowest degree of conversion of the studied materials. Furthermore, the relatively higher filler loading of Panavia F2 might contribute to the low *DC* obtained in this study. Incorporation of filler into polymerizable resins was shown to also decrease molecular mobility within the boundary regions extending from the interface of the filler.<sup>27</sup>

The analytical HPLC method used in this study was able to detect monomers that leach out from the investigated resin-based cements (within the limits of the detection system). In this study, the elution of HEMA, bisphenol A, TEGDMA, UDMA and bis-GMA from Panavia F2, Nexus 2 and Variolink 2 into water was followed.

The results showed that bisphenol A was eluted from Panavia F2 in a higher amount than from Nexus 2 and Variolink 2 ( $p < 0.001$ ). Bisphenol A is not an original component of dental composites but a contaminant which could always be expected in resin-based materials containing bis-GMA.<sup>28</sup> Several researchers have studied whether bisphenol A leaches from cured dental restorative or resin-based cements. Many investigations indicated the presence of the residual bisphenol A in some dental products based on bis-GMA.<sup>28–31</sup> However, in some studies bisphenol A was not detected.<sup>14</sup> The lack of detection of bisphenol A in this and similar studies may be due to several reasons: the concentration of BPA was too low to be detected, the detection method was not suitable, or the tested products were not contaminated with residual BPA.

It is possible that the number of polymerized bis-GMA monomers were smaller in Panavia F2 (compared to the other two cements) and some of them were degraded to bisphenol A (bis-GMA monomer precursor), which was detected.<sup>14</sup> Bis-GMA was not found as an eluted substance from any of the tested cements, mostly due to its low solubility in water. Bis-GMA is soluble only in alkaline or acidic aqueous solutions under vigorous conditions, when hydrolysis occurs (100 °C).<sup>15</sup> This process results in the formation of the individual components, *i.e.*, bisphenol A, methacrylic acid and glycerol. The fact that this experiment was performed under ambient conditions and in water leads to the logical conclusion that the bisphenol A in Panavia F2 could be rather an impurity not a degradation product of bis-GMA. Hence, in the case of Panavia F2, the possible explanation for the found concentration of bisphenol A was probably the poor quality of bis-GMA. Bisphenol A exhibits estrogenicity, acts as a steroid hormone and binds to hormone binding sites. Hence, the biocompatibility of Panavia F2 may be questionable and critical, especially if it is used for the cementation of long span fixed restorations and orthodontic appliances.<sup>32–34</sup>

Small, mobile resin monomers, such as TEGDMA, were primarily eluted from Nexus 2 and Variolink 2 in higher quantity compared to Panavia F2 ( $p < 0.001$ ). Due to the low viscosity, relatively high hydrophilicity as well as good water solubility, TEGDMA was always found to leach from polymerized composite, even into aqueous solution.<sup>35</sup> TEGDMA elution depends on the mode of polymerization (an oxygen inhibition layer increases the elution); it easily hydrolyzes at low pH to methacrylic acid, which would be detected as a reaction product.<sup>14</sup>

There are reports which illustrate higher water uptake in composite materials with low filler contents (resin cements are such) compared with materials with a higher filler content. The results of the present study, which are in line with these reports, showed a greater TEGDMA elution from Nexus 2 and Variolink 2, the cements with lower initial filler contents compared to Panavia F2 luting re-

sin.<sup>14,36,37</sup> This leads to the conclusion that the elution mechanism is complex and cannot be explained only by the absorption process.

Concerning the clinical significance of eluted TEGDMA, it is clear that it may be released either through dentin tubules into pulp, or through the surface of a restoration into the oral cavity (mostly TEGDMA from the filling materials). For the first pathway of release, dentin adhesives may be the prime issue of concern, since these materials consist of monomers that are more hydrophilic than monomers typically contained in resin composites. Recent results showed that release of TEGDMA through dentine was 60 % of the direct release; therefore dentin appears to exert its protective effects principally by retarding or “damp-ing” the initial high release of TEGDMA to a substantial degree.<sup>38</sup>

The second pathway of release enables components to be eluted into salivary fluids and brought into contact with mucosal tissues. Depending on the level of irradiation and curing duration, this could play a significant role in the elution action.<sup>39,7</sup>

In addition, there are results of the synthesis of a reliable chemical inhibitor (CI) which can prevent the adverse effects mediated by TEGMA, HEMA and bleaching agents (cells death inhibition, increasing cell viability and function).<sup>40</sup>

The elution of HEMA (2-hydroxyethyl methacrylate) was the highest from Nexus 2 compared to the other two tested cements ( $p < 0.001$ ). It is known that HEMA mediates apoptotic cell death by inducing changes in the cell-surface composition and by activating many critical genes to apoptosis these, in turn, activate endonucleases that cause DNA fragmentation. Additionally, HEMA seems to be a more potent inhibitor of cellular function than TEGDMA.<sup>41</sup>

Urethane acrylates monomer UEDMA was released in small quantities from Nexus 2 and Variolink 2 due to its “hydrophobic” nature and dimensions (bulky and large substance). The main role of UDMA in resin composite materials is to improve their resistance to wear and to reduce water absorption. However, it must be emphasized that particular base monomer, such as UDMA, are more toxic in general when tested individually, compared with TEGDMA co-monomer. However, the elution rate of UDMA was low and only trace amounts leach out into the aqueous media. Some results suggested synergistic cytotoxic effects of TEGDMA and UDMA in combination, while other studies confirmed UDMA as a sensitizing agent.<sup>42</sup> The total replacement of TEGDMA by UEDMA in a resin composition would change the mechanical properties significantly, leading to a decrease in the elasticity modulus, and in the flexure and tensile strength. This is associated with the capacity of urethane acrylate resins to form hydrogen-ionic links with copolymers and thus restrict the mobility of the polymers.<sup>43,44</sup>

The present study shows that it is not possible to predict the elution of monomers based on the manufacture’s declaration. A comparison of the composites investigated showed that, although several compounds were extractable from

each material, the quantities differed between the resin cements, even when they had a similar *DC*. The water extracts of resin cement Nexus 2, especially, revealed a considerable concentration of both TEGDMA and HEMA, but a minor concentration of UDMA. In contrast to Nexus 2, cement Panavia F2 revealed a considerable concentration of bisphenol A, with minor concentrations of HEMA and TEGDMA found. Only, the elution from Variolink 2 was to some extent as expected according to the declared composition. The detected concentration of bisphenol A was negligibly low and concentrations of both HEMA and UDMA were minor. The only eluted monomer found in a considerably high concentration was TEGDMA.

For the particular resin system or composite, the percentage elution can be correlated with the degree of conversion. However, due to differences in monomer composition, type and concentration of adhesive promoters, and the cross-link density of polymer networks, it is not possible to predict accurately the elution for different composites based on their relative degree of conversion.<sup>7</sup> The results in this study are in line with these statements, showing no correlation between the *DC* and the percent of monomers eluted from resin-based composite cements. The great importance of composite cements in every day practice actualizes the existence of controversial opinions in the literature (inverse correlation between the *DC* and the percent elution), confirming that this subject will still be a topic of interest in the future.<sup>12,45</sup>

#### CONCLUSIONS

Within the limitation of the current study, it can be concluded that resin-based cements may show different *DC* depending on their composition, but without significant differences. All the tested luting resin cements resulted in acceptable levels of the degree of conversion with the same polymerization conditions. These results cannot be unreservedly extrapolated across other brands of related materials because of the possibility of minor changes in chemistry and the polymerization conditions causing significantly different responses. It can also be assumed that quantity of eluted monomers from composite cements depends on various factors (the composition and solubility parameters of the solvent-tested media, condition used for light curing, size and chemical characteristics of leachable substances). This research did not confirm a direct correlation between the degree of conversion and elution of monomers among the different commercial resin-based cements.

## ИЗВОД

КОРЕЛАЦИЈА СТЕПЕНА КОНВЕРЗИЈЕ И КОЛИЧИНЕ ОСЛОБОЂЕНИХ СУПСТАНЦИ  
ИЗ ДЕНТАЛНИХ КОМПОЗИТНИХ ЦЕМЕНАТАКОСОВКА ОБРАДОВИЋ-ЂУРИЧИЋ<sup>1</sup>, ВЕСНА МЕДИЋ<sup>1</sup>, МАРИНА РАДИШИЋ<sup>2</sup> И МИЛА ЛАУШЕВИЋ<sup>2</sup><sup>1</sup>Клиника за стоматолошку протезику, Стоматолошки факултет, Универзитет у Београду, Ранкова 4,  
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Рад истражује могућу корелацију степена конверзије (DC) и количине ослобођених мономерних супстанци из три комерцијална препарата композитних денталних цемента. Степен конверзије композитних цемента одређен је методом Раман спектроскопије, док је количина неизреагованих и ослобођених мономера из цементног матрикса детектована помоћу HPLC методе. Резултати добијени након вишеструке статистичке евалуације (one way ANOVA, LSD post hoc test) не показују значајне разлике у степену конверзије различитих препарата композитних цемента. Насупрот томе, резултати HPLC анализе бележе значајне разлике у количини и врсти мономерних супстанци отпуштених из композитних цемента. Такође, статистичка анализа у оквиру ове студије није показала корелацију степена конверзије испитиваних композитних денталних цемента и количине ослобођених супстанци из ових материјала.

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