Effect of nano-bioceramics on monomer leaching and degree of conversion of resin-based composites

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The aim of this laboratory study was to evaluate the monomer leaching and degree of conversion (DC) from experimental bioactive resin composites (RBCs) and to do comparison with commercial bulkfill and packable resin composites. Experimental dimethacrylate-based resin composites were reinforced with silanated nano-hydroxyapatite (30 and 45 wt%). The ion leaching and DC of these resin composites were compared and contrasted with SDR™ and Filtek P60™ by using the high performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FTIR), respectively. A significant difference was found in elution of monomer between the resin composites. SDR™ showed significantly high monomer elution and structural changes compared to other resin composites. The DC of bioactive RBCs showed the highest conversion rate after polymerization. Resin composite with nano-hydroxyapatite with the presence of a bioactive component might provide biomimetic approach for the material. Moreover, a low concentration of nano-hydroxyapatite nano-fillers have shown better properties than micro-fillers based resin composites.

Keywords: Bioactive dental composites, Bulkfill composites, Monomer leaching, Degree of conversion, HPLC

INTRODUCTION

Resin-based dental composite materials have remained in the focus of intense research in recent years with the goal of improving restoration performance by changing the type of resin monomers, fillers and their concentrations, coupling agents, and by developing novel polymerization techniques. In addition to micro/nano-hybrid resin composites, bulkfill and flowable resin composites were introduced due to the rising demand of professionals and patients. Some recently introduced bulkfill resin composites are flowable (non-viscous) so that they can adapt easily and smoothly to the cavity walls. Low viscosity of these resin composites is achieved by increasing the resin monomer content from 20 to 40 wt% and decreasing the filler load i.e., down to ca. 60–68 wt%.

Attaining bioactivity in dental resin composite restorations represents one of the big achievements in the field of modern biomaterial science and research. Bioactive fillers and some fibers have been incorporated to enhance adhesion between tooth tissues and restorative material to prevent micro leakage and bacterial ingression. Among bioceramics, hydroxyapatite (HA) has gained popularity in dental restorative science. The homogenous dispersion of nano-HA (n-HA) with the organic matrix of dental resin composite at the microscopic level (and silanization of fillers) can improve the physical and mechanical properties.

It has been observed that after photo-curing of resin composite restorative material the polymerization reaction is not complete and methacrylic compounds (rest monomers) are released into the oral cavity tissues and biological fluids (over certain time) where they may cause local adverse effects. Eluted (co)monomers can reach such concentrations that might induce necrotic or apoptotic cell death in the human gingival fibroblasts. The presence of unpolymerized monomers is linked with the degree of conversion (DC) of resin composites. This said, it has always been observed that the DC of resin composite is never complete (i.e., 100%) and uncured residual monomers are left after polymerization. The DC of various resin composites ranges between 35 and 77% and residual components are in the form of monomers, dimers, and oligomers. The uncured monomers act as plasticizers: thereby properties such as wear resistance, hardness, and tendency to degrade in the oral conditions are negatively affected. The qualitative and quantitative analysis of elution products can be performed by the high performance liquid chromatography (HPLC), gas-liquid chromatography, mass spectrometry, and electrospray ionization/mass spectrometry. The HPLC analysis is usually performed for high molecular weight resin...
monomers because they are decomposed in gas-liquid chromatography, which greatly affects the results. However, HPLC cannot provide the information about presence of residual monomers within the molecular structure of resin composite. The study of DC in dental resin composites by spectroscopic technique provides a better understanding of polymerization process of these resin composite materials. Fourier transform infrared spectroscopy (FTIR) is one of the most widely used fingerprint techniques for analysis and measurement of DC and residual monomers (RM) in dental resin composites.

The resin composite SDR™ (Dentsply, Konstanz, Germany) has recently been introduced and also used in clinics, whereas very little data is available regarding its release of residual monomers and the effect of aqueous media on the structural changes. Information about release of TEGDMA and urethane modified monomer is limited. Previously, the elution was analyzed after 72 h, however, it is expected that maximum leaching occurs in first 24 h. Even so, it is required to find out the in-depth information about leaching of monomers from this newly developed/widely used composite.

For the last two decades, HA has been investigated in resin-based dental composites and only very few researchers have studied the role of n-HA in these composites. However, the authors could not find any study, where elution of monomers i.e., Bis-GMA, UDMA and TEGDMA were investigated qualitatively or quantitatively on n-HA-based composites. HA is opaque against visible light and when added in higher concentration to resin composites it adversely affects the curing reaction. That said, it is important to address the role of HA in dental resin composites, which ultimately determines the longevity of dental resin composites.

In this laboratory study, experimental resin composites were prepared by using silanized n-HA as the filler and their concentration (wt%) was optimized as flowable and packable composites. Previously, we have found that low concentrated silanized n-HA based resin composites showed better physical and mechanical results than high concentrated micro-fillers based commercial composites. This said, the objectives of this study were to prepare two experimental resin composites based on relatively low concentrations of n-HA as filler and to compare and contrast them with high concentrated micro-fillers based resin composites. The quantitative and qualitative analysis of monomer leaching from experimental, commercial resin composites were evaluated periodically to investigate the degree of conversion, residual monomer percentage, and structural changes after water immersion. It was hypothesized that uniformly distributed silanized n-HA based resin composites would show less monomer leaching due to utilization of lower ratio of diluent monomers and would show higher degree of conversion.

**MATERIALS AND METHODS**

All chemicals and precursors used in this study were of analytical grade and were used as such. Nano-hydroxyapatite (n-HA) was synthesized by using microwave irradiation technique and the process of silanization of the n-HA particles has been described in detail previously.

**Synthesis of experimental resin-based composites**

For experimental resin composite synthesis, all resinous materials were purchased from Sigma Aldrich, St. Louis, MO, USA. The resin monomer matrices (Bis-GMA: UDMA: TEGDMA) were mixed in a mass ratio of 40:35:25 by stirring continuously for 30 min in dark and under controlled environment (23°C±1°C and 55% humidity) and stirring was maintained at 200 rpm. Next, camphorquinone (0.5 wt%) was added to be used as an initiator and ethyl 4-dimethylaminobenzoate (0.5 wt%) was used as a diluent solvent. The experimental flowable and packable dental resin composites were prepared by incorporating 30 and 45 wt% of silanized n-HA particles in the resin matrix mixture with the help of acetone, respectively. The resin composite mixture was left to overnight under dark light for continuous stirring to achieve complete homogenous mixing of nano-fillers and resin matrix and to evaporate the acetone. The resulting experimental resin composites with 30 and 45 wt% were denoted as “Exp-RBC30” and “Exp-RBC45”, respectively. Two types of commercial dental resin-based composites i.e., bulkfill flowable resin composite SDR™ (Dentsply; shade A3) and micro-hybrid 3M Filtek™ P60 (3M ESPE; Seefeld, Germany; shade A3.5) were used in this study as controls. The type and composition of materials are listed in Table 1.

**Sample preparation**

Sample discs of both experimental and commercial resin composites were prepared in 4×4 mm Teflon molds. The specimens were light-cured from both sides by using a light curing unit (LED, Woodpecker, wavelength 420–480 nm, irradiance level 800 mW/cm²) for 60 s. These samples were covered with Mylar strips to prevent the formation of oxygen inhibition layer and to achieve smooth surface of specimens. The tip of light curing device was as close as 1.0 mm to the surface of material. After curing, samples were polished by using grit papers 1200, 2000, and 4000 to make the surface smooth. The surfaces were visually analyzed under a light microscope (×100). Before immersing the samples in media, they were placed in a desiccator with silica gel for 22 h at 37°C and the initial weight was measured and then kept for further 2 h in a desiccator. The mass was weighed with the precision of ca. 0.0001 g. These preparatory measures and storage of samples were established according to ISO 4049 and further processed for FTIR and HPLC analysis.

**Preparation of standard solutions for HPLC**

The HPLC reference standards of TEGDMA, UDMA, Bis-GMA (Sigma-Aldrich) were taken from the stock solutions of these monomers. The solutions were diluted to yield a final calibration solution of standards ranging from 1–1,000 µg/mL (with a step difference of 10 µg/mL).
The peak areas of each of standard solution were plotted with their concentrations while linear regression analysis was done to help in the determination of specimen’s unknown concentration of leachable monomers. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated from the signal to noise ratio of 3 (S/N=3) and 10 (S/N=10) for LOD which has already been reported to be 0.022 µg/mL for TEGDMA, 0.12 µg/mL for UDMA, and 0.07 µg/mL for Bis-GMA. LOQ was 0.075 µg/mL for TEGDMA, 0.4 µg/mL for UDMA, and 0.23 µg/mL for Bis-GMA, respectively.

Acetonitrile was used as a solvent to prepare the standard solutions of monomers. A calculated amount of monomers (Bis-GMA, UDMA, and TEGDMA) was weighed separately by using an analytical balance (Shinadzu, Kyoto, Japan). Acetonitrile equivalent to the calculated amount of the monomers was measured with the help of a pipette and added in these vials. The solutions were stirred manually for 5 min and diluted with the solvent by the serial dilution method. Given this, initially these solutions were labeled as 1.0 mg/mL, then, 1.0 mL was removed from each solution and poured in the other empty glass vial and the remaining 9.0 mL acetonitrile was added to get standard solutions of 0.1 mg/mL. By applying the same iterative method, it was further diluted to obtain the final 0.001 mg/mL of each monomer’s standard solution.

**HPLC analysis**

All the standard solutions were assessed by Hitachi Primaide HPLC system (Tokyo, Japan) with a CNW Athena C-18-WP column (5 µm, 100 Å) having an internal diameter of 4.6 mm and length of 250 mm. The mobile phase contained acetonitrile and water (70:30, v/v). The flow rate of 10 µL solution was 1.0 mL/min and the eluents were monitored at λ<sub>max</sub>=230 nm. The proposed method was successfully applied for the qualitative and quantitative analysis of resin monomers leaching from all four materials by comparing the elution peak area with those of standard solution.

A total of 36 samples were prepared as described above for the HPLC study and each sample was placed in 10 mL deionized water at 37°C. The sample solutions were taken out periodically, i.e., on day 1, 7, and 28. The peak concentration areas of leachable monomers from each samples had the same retention time with those of standard compounds under same HPLC conditions. The analysis was carried out by contrasting and comparing the peak area of the known concentration as the reference standard of TEGDMA, UDMA, and Bis-GMA. The calibration curves of the standards were prepared based on the known concentrations of the TEGDMA, UDMA, and Bis-GMA standard solutions expressed in µg/mL. Linear regression analysis of these standard calibration curves was carried out.

The eluents were analyzed by using Hitachi Primaide HPLC system with Primaide 1,110 pump, Idex 7725i manual sampler with 20 µL fixed loop, 50 µL Hamilton syringe, Primaide 1310 column oven, Primaide 1410 UV/Vis detector, and data acquisition software (version 1.0). The mean values and standard deviation were calculated for commercial (SDR and Filtek P60) and experimental (Exp-RBC30 and Exp-RBC45) resin composites. One way ANOVA and the post hoc Tukey’s tests were performed to compare and contrast the monomer release among the study groups and the significance value was p≤0.05. To find out the structural changes in samples after periodical immersion in deionized water, same samples were also assessed with FTIR at each interval.

**Degree of conversion analysis**

To evaluate the degree of conversion (DC) total five freshly prepared pre-cured samples of each group were used. The samples were placed in Teflon mold (5×2 mm) and were analyzed with FTIR before and immediately after curing. After taking the readings of uncured composites, the samples were cured for 60 s from both sides with high intensity blue light. A FTIR instrument (Thermo Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA) with a diamond attenuated total reflectance (ATR) accessory was used in this study. It was maintained that samples should closely attached to diamond window. The spectra were registered and collected over the region 4,000–400 cm<sup>-1</sup> at a resolution of 8 cm<sup>-1</sup> and with averaging 256 scans. The data were analyzed by using the OMINIC software.

The DC was measured according to ISO 4049 and
it was calculated by assessing the changes in the ratio of the absorbance intensities of the aliphatic C=C peak at 1,638 cm\(^{-1}\) and that of an aromatic C=C at 1,608 cm\(^{-1}\) of the uncured and cured resin composite samples. Due to the lack of aromatic C=C group in the SDR resin composite, the internal standard peak at 1,602 cm\(^{-1}\) was used. For Bis-GMA and TEGDMA, the same peaks were used as the internal standard. However, for UDMA, 1,638 cm\(^{-1}\) (aliphatic) and 3,330 cm\(^{-1}\) (amide) bands were used as the internal standards. The following formula has been used to find out DC:

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

where:
- \(R_{\text{polym}}\) = ratio of polymerized aliphatic and aromatic carbon bonds
- \(R_{\text{unpolym}}\) = ratio of unpolymerized aliphatic and aromatic carbon bonds.

To determine the residual monomers (RM) before and after immersion in deionized water, the quantities of carbon-carbon double bonds were recorded periodically, \textit{i.e.}, immediate after curing, then on day 1, day 7, and day 28. The changes in the numbers of double bonds were recorded using the N-H absorbance peak at 3,370 cm\(^{-1}\), the aromatic absorbance peak at 1,608 cm\(^{-1}\) and the aliphatic peak at 1,638 cm\(^{-1}\) as an internal standard. In the case of SDR, 1,602 cm\(^{-1}\) was used instead of 1,608 cm\(^{-1}\) peak. The amounts of residual monomers (RM\%) were calculated using the following equation as described by Nomoto and Hirasawa\(^{19}\):

\[
RM(\%) = \left[\frac{(C-E)\times(B\times C\times A\times D)\times 100}{C}\right]
\]

where:
- A=C=C (1,638 cm\(^{-1}\)) of uncured resin composite
- B=N-H (3,370 cm\(^{-1}\)) or C=C (1,608 cm\(^{-1}\)) of uncured resin composite
- C=C=C (1,638 cm\(^{-1}\)) of cured resin composite before immersion
- D=N-H (3,370 cm\(^{-1}\)) or C-C (1,608 cm\(^{-1}\)) of cured resin composite before immersion
- E=C=C (1,638 cm\(^{-1}\)) of cured resin composite after extraction from media.

**RESULTS**

**HPLC**

Appropriate aliquots of standard stock solutions of the resin monomers were taken and collected in different volumetric flasks and diluted to form a final calibrated solution of standards ranging from 1.0 to 1,000 µg/mL. The aim of regression analysis was to establish the equation that shows the linear relationship between the y and x-axes values, \textit{i.e.}, \(y = mx + c\), where “m” denotes the gradient of line and “c” denotes the intercept of gradient with the y-axis. The correlation coefficient \(R^2\) values for Bis-GMA, UDMA and TEGDMA were 0.994, 0.989 and 0.981, respectively.

The LOD and LOQ are two important characteristics that describe the smallest concentration of the analyte that can be reliably measured by an analytical procedure. The HPLC results showed that both commercial (Fig. 1) and experimental (Fig. 2) resin composites released TEGDMA, UDMA and Bis-GMA monomers, where the maximum release was observed on day 1 which was subsequently reduced on day 7 and negligible release was observed on day 28. The one-way ANOVA test revealed

![Fig. 1](image-url) Monomer leaching profile of bulkfill SDR and microhybrid packable Filtek P60 on day 1, 7, and 28, where B, U, and T represents Bis-BMA, UDMA, and TEGDMA respectively.
Fig. 2  Monomer leaching profile of Exp-RBC30 and Exp-RBC45 on day 1, 7, and 28, where B, U, and T represents Bis-BMA, UDMA, and TEGDMA respectively.

Fig. 3  (i) Bulkfill SDR, (ii) Filtek P60, (iii) Exp-RBC30, and (iv) Exp-RBC45 showed comparative spectra (a) before curing, (b) after immediate curing and after immersing in deionized water on day (c) 1, (d) 7, and (e) 28.
that there was a statistically significant difference in the monomer release among all the four study groups. Among resin composites, on day 1, SDR showed the highest release of TEGDMA compared to Filtek P60 and Exp-RBCs and the difference was significant (p<0.05). The sequence of elution of TEGDMA was: SDR>Exp-RBC30>Exp-RBC45>Filtek P60. While on day 7, the sequence of elution of TEGDMA was Exp-RBC45>Exp-RBC30>Filtek P60>SDR and the differences were significant. On day 28, the sequence observed was Exp-RBC30>Exp-RBC45>SDR>Filtek P60. UDMA showed elution in Filtek P60 and SDR on day 1 and the difference was significant, whereas, it did not elute from both experimental resin composites. On day 7, only Filtek P60 released UDMA. However, it was not eluted from any other material. On day 28, none of the materials under study released UDMA. The sequence of elution of Bis-GMA on day 1 was SDR>Filtek P60>Exp-RBC30>Exp-RBC45 and the difference was not significant. While on day 7, the sequence of elution of Bis-GMA was Exp-RBC45>Exp-RBC30>Filtek P60>SDR and the difference was not significant. There was no release of Bis-GMA on day 28 from any commercial and experimental resin composites under study.

**FTIR**

Figures 3 (i–iv) present the FTIR spectra of SDR, Filtek P60, Exp-RBC30, and Exp-RBC45, respectively. The

![FTIR Spectra](image)

**Fig. 4** Spectroscopic peak changes (i) at aliphatic (1,638 cm⁻¹), aromatic (1,602 cm⁻¹), and (ii) at carbonyl (1,730–1,700 cm⁻¹) groups with time interval i.e. before immersion (blue), day 1 (purple), 7 (green), and 28 (red) of (a) Bulkfill SDR, (b) Filtek P60, (c) Exp-RBC30, and (d) Exp-RBC45.
compared to SDR, the highest conversion rate after polymerization respectively. Exp-RBC30 and Exp-RBC45 showed GMA, UDMA, and TEGDMA, was 65, 95 and 88%, respectively. FTIR spectra were collected before curing, immediately after curing, on day 1, 7, and 28. The change in the peak intensities was observed over the time intervals at respective FTIR bands. After immediate polymerization, all resin composites showed characteristic peaks of dimethacrylate based resins. The carbonyl group C=O was observed at 1,716 cm⁻¹ and it was present in Bis-GMA, UDMA, and TEGDMA. The aliphatic peak at 1,636 cm⁻¹ was assigned to the C=C group. The peak at 1,650 cm⁻¹ appeared only in Filtek P60 spectrum and was attributed to amide, CONH₂. The aromatic peak appeared at 1,606–1,608 cm⁻¹, however, for SDR, the peak appeared at 1,602 cm⁻¹. The C-H peaks were observed at 1,500–1,510 cm⁻¹ and 1,295–1,310 cm⁻¹. The =CH plane vibration peaks were observed at 1,450–1,460 cm⁻¹ in all experimental and commercial resin composite samples with variable intensities, according to the degree of conversion and storage over different time intervals. The peaks 1,170–1,300 cm⁻¹ corresponded to C-O-C stretching (aromatic ether and methacrylate). The C-O-C peak at 1,240–1,250 cm⁻¹ was observed only in the experimental and SDR samples. The peaks at 1,050 cm⁻¹ corresponded to the phosphate groups of HA and appeared in experimental resin composites. As shown in the spectra, the peaks became broadened and the intensities decreased linearly with each time interval and mainly the effect of water immersion was observed at the C=O (Fig. 4i) and C-C (Fig. 4ii) region.

### Table 2

| Materials     | Day 1 |          | Day 7 |          | Day 28 |          | Statistical difference |
|---------------|-------|----------|-------|----------|--------|----------|------------------------|
|               | C=C   | N-H      | C=C   | N-H      | C=C   | N-H      |                        |
| SDR™          | 30.63±2.16 | 32.91±1.85 | 20.60±1.12 | 23.90±0.98 | 20.30±0.87 | 22.78±1.25 |                        |
| Filtek P60™   | 32.18±1.34 | 34.56±1.22 | 27.62±1.87 | 28.56±1.06 | 26.90±1.28 | 27.86±0.83 | p≤0.05                 |
| Exp-RBC30     | 27.34±1.96 | 30.12±0.95 | 22.46±1.10 | 28.23±0.52 | 22.12±0.99 | 27.79±0.97 |                        |
| Exp-RBC45     | 29.13±2.33 | 32.07±1.65 | 26.52±0.87 | 28.67±1.14 | 25.99±1.13 | 28.12±1.28 | p≥0.05                 |

### DISCUSSION

The HPLC analysis was used to evaluate residual monomer release from dimethacrylate based commercial and experimental resin composites. The current laboratory study focused on three widely applied resin monomers Bis-GMA, TEGDMA, and UDMA, and the effect of n-HA inclusion of these resin matrices. Moreover, the current study aimed to evaluate the release of Bis-GMA and UDMA from bulkfill resin composite, which were not addressed previously. The results of the experiment revealed that UDMA did not leach in aqueous media from experimental resin composites. However, it eluted in a very small concentration from SDR™ and in a larger concentration from Filtek P60™. On day 1 and day 7 Bis-GMA was leached out and the concentration was not significantly noticeable but, on the other hand, no release was observed on day 28 from any of the resin composite materials. TEGDMA was the main monomer which was released in considerably higher quantity, in comparison with the other monomers.

Differences between leaching of monomers can be related to the molecular structure of respective monomers. Previous studies have also reported that TEGDMA leached out more due to its less bulkiness and low molecular weight than compared to higher molecular weight monomers such as Bis-GMA and UDMA. It is assumed that larger and bulkier Bis-GMA molecules eluted less than compared to small molecules of TEGDMA with enhanced mobility. The size and chemical structure plays a vital role in the elution mechanism⁹. That said, TEGDMA with low-viscosity characteristics contributes to high mobility during polymerization and its tendency to cyclization leads to higher conversion, but not necessarily contributes to the network formation. The concern is related to SDR™, as this resin composite showed maximum leaching and currently, the trend among dentists has been to use increasingly bulkfill resin composites. On the one hand, the claim of one time curing instead of incremental curing might save some time and effort, but on the other hand, it can affect the longevity of such materials. In addition, it can create systemic effects due to the release of unreacted monomeric contents. The degree
of conversion and % elution are inversely related with each other and free monomers leach more where there is less degree of conversion of monomer\textsuperscript{20}. The results of this laboratory study also showed considerably higher concentrations of residual monomers in commercial resin composites compared to the experimental resin composites. Unfortunately, the exact ratio of Bis-GMA, TEDGMA, and urethane modified monomer in SDR is not reported for commercial resin composites. However, in the experimental resin composites of the current study, the presence of a higher ratio of Bis-GMA (high molecular weight) than other monomers could hinder the complete conversion of low molecular weight monomers, \textit{i.e.}, TEGDMA and UDMA. UDMA showed the minimum release from resin composites and it was observed through FTIR analysis that the DC of this monomer was almost 95\%, which was relatively higher than for TEGDMA and Bis-GMA. As UDMA contains the amide band at 1,500–1,600 cm\textsuperscript{−1} and 3,330 cm\textsuperscript{−1}, therefore, either to select the stretching (3,330 cm\textsuperscript{−1}) or bending (1,540 cm\textsuperscript{−1}) peaks as an internal standard may be justified for the quantitative analysis. In the current study, the authors would like to recommend that for UDMA based dental resins, the stretching peak of FTIR should be considered to use as an internal standard as the stretching peak exhibits higher frequencies than bending peaks.

Furthermore, the change in the peak intensities at 1,638 cm\textsuperscript{−1} and 1,600 cm\textsuperscript{−1} were observed with the immersion time. It is important to determine the concentration of residual monomers which helps to estimate the cross-linked structure, because various properties of polymeric resin composites are significantly dependent upon the cross-linking density and structural quality of the network formed during polymerization. The absence of the polymer network or cross-linking in resin composites decreases the stability of sample\textsuperscript{21}. Fundamentally, the free radical polymerization plays a major role in degree of conversion. Some areas of resin matrix are more cross-linked than the other areas which are loosely cross-linked having a large number of free monomers. These free monomers are trapped between the cross-linked network and they leach out if they are soluble in aqueous media. A similar pattern was observed in the current study\textsuperscript{22}.

The success and longevity of dental resin composites are related to both the quality of the polymer matrix formed and presence of fillers which not only decrease the stress of contraction but also the hydrolytic degradation\textsuperscript{23,24}. The addition of appropriate fillers, not only in wt% but also of their size, surface chemistry, distribution, structure, and presence of hydrophobic resins determine the hydrolytic degradation and increase restoration longevity, along with interaction of fillers with resin matrix. Fillers need silanization to immobilize them in the resinous matrix\textsuperscript{9}. Previously, our group showed that addition of n-HA in the polymeric network and, on the other hand, the presence of hydrophobic matrices increased the degree of wetting and reduced water sorption\textsuperscript{4,25,26}. In the current study, addition of n-HA improved the polymerization process. The focus was given on a uniform distribution of n-HA in the polymeric network and stirring during mixing was maintained for 24 h. Subsequently, it was observed that this method provided an even distribution of nano-particles and there was no sedimentation of filler particles in the base of the storage container. Previously in experimental studies, either spatulation or sonication has been performed for a short period of time, which could affect the uniform distribution of fillers in the resin matrix. The concentration of the nano-particles used in experimental resin composites were 30 and 45 wt%. Given this, if the concentration of filler increased to a certain ratio, then the mechanical properties of resin composites would drop which might be related to agglomeration of fillers. In addition, incomplete curing obviously affects the mechanical properties. In the photo-activation curing systems, the curing process depends on the irradiation output energy received by the photo-initiator system and the penetration of high intensity light in fillers. This said, it depends on the transparency of the matrix resin and fillers towards the irradiation wavelength and the difference between the refractive indices of the matrix and the filler\textsuperscript{27}. HA is opaque against visible light and when added in higher concentrations to resin composites it adversely affects the curing reaction. With 30 and 45 wt% HA loads, interaction between fillers particles and the polymeric network was achieved completely and the polymerization process was not hindered. In addition, the entrapped unbound monomers remained within the structure. In general, fillers are added to enhance the stiffness of dental composite while n-HA may also contribute beneficially to bioactivity.

In the current study, FTIR spectroscopy was utilized as it is a reliable and available finger print method for identifying and evaluating the degree of conversion and structural changes in RBCs. In order to determine the DC and structural changes, the resin composites were analyzed and compared before curing, after curing, after day 1, 7, and 28. The FTIR results complemented the HPLC data and provided a clear picture that unreacted or unbound monomers were eluted by medium immersion and structural changes in the polymeric network of resin composite could be observed within the first day after polymerization. The fact is that the release from resin composite is due to the degree of the polymer conversion that is significantly lower than 100\%. Given this, the DC values of experimental resin composite were higher than commercial composites. To date, no commercial dental resin composite has shown complete polymerization. The presence of residual monomers was evaluated by using two different standards \textit{i.e.}, the C-C and N-H peaks on FTIR\textsuperscript{28}A spectra, as both said groups are present in the resins studied. Previously, it was suggested that the N-H group should be adopted when aromatic groups are not present in the resin\textsuperscript{29}. In the current study, UDMA was used in slightly higher concentration (35\%) in the resin matrix compared to the conventional resin composites, therefore, we considered that it is justified to use the N-H peak as a standard along with C-C. However, the
results revealed insignificant differences in the residual monomer percentages.

Nevertheless, in the current study, the results showed that the experimental resin composites have higher degree of conversion than SDR\textsuperscript{21}, which exhibits a slightly higher degree of conversion than Filtek P60\textsuperscript{22}. The peak change in the region at the carbonyl=CO region was observed in the spectra of all materials studied. It can be clearly observed in all spectra that the peak broadened and its intensity changed after immersion in deionized water. It can be deducted that the carbonyl group is more susceptible to degradation. In the Exp-RBC30 and Exp-RBC45 spectra, the PO\textsubscript{4} peak of n-HA in the region 1,000–1,050 cm\textsuperscript{-1} was prominent and peak shifting and intensity variation can be observed. The peaks became broader and decreased in intensity linearly with ageing of RBCs.

The presence of n-HA created an effect in ion leaching and hydrolytic degradation of the resin composite. The non-silanized n-HA has tendency to link filler and the silane coupling agent is sensitive, i.e., siloxane bonds (Si-O-Si) are vulnerable to hydrolysis, because this covalent bond has a significantly ionic character\textsuperscript{23}. In contrast, the covalent C-C bond between the polymer matrix and the silane molecule is much more stable to hydrolytic attacks than the covalent Si-O bond. Factors which determine the hydrolytic degradation and structural changes are physico-chemical nature of the silane molecule, silanization methodology, silane layer (molecules in it) orientation that develops, and extent of filler coverage\textsuperscript{5,20-31}. It is noteworthy that silanes with various organo-functional moieties may promote adhesion differently as published on studies with silica-coated\textsuperscript{32} zirconia and titanium substrates\textsuperscript{30-32}.

It is also expected that the mobile and very small water molecules diffuse into the resin matrix-filler particles interphase and break the chemical bond between them by hydrolytic reactions and also cause the degradation of resin matrix and filler particles\textsuperscript{33}. This may lead to dramatic structural changes in resin composites, subsequently decreasing the physical and mechanical properties and affecting the clinical service life of the resin composite cementation (or restoration)\textsuperscript{34}. Water molecules diffuse into the polymer network and occupy positions at the free volume between polymer chains and micro voids, causing plasticization and swelling of polymer matrix and also initiates chains scission causing monomer elution\textsuperscript{29}. The newly synthesized resin composite is a polar material because it contains mainly carbonyl and hydroxyl groups. This is why water molecules can easily penetrate into the polymer network allowing diffusion of unreacted monomers. The results of the study enabled us to know more about the possible toxicity and side (adverse) effects of all restorative materials. This said, there is a need to develop new dental resin composites with ideal clinical properties and minimum release of monomers which would need appropriate interlocking with the matrix chains. The low weight concentration of bioactive nano-fillers based composites have potential to be as a restorative material and they exhibited relatively better results than high concentrated micro-filler particles based composites.

**CONCLUSION**

Release of monomers after polymerization from experimental and commercial resin composites have been evaluated. TEGDMA showed the highest level among the other monomers. Flowable resin composites showed more elution than packable resin composites. Overall, 45 wt% n-HA based experimental composite showed minimal leaching of monomers compared to other composites. The degree of conversion was higher in experimental resin composites and presence of 30 and 45 wt% n-HA exhibited a higher degree of conversion and structurally stable than for commercial resin composites. These unreacted monomers have potential to cause adverse effects on oral tissues.

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