# SPECTROSCOPIC, CHROMATOGRAPHIC AND MORPHOLOGICAL STUDY OF PHOTOPOLYMERIZED DENTAL COMPOSITES

# YORDANKA UZUNOVA<sup>\*</sup>, LUDMIL LUKANOV, PENKA VASILEVA<sup>a</sup>, IVAN FILIPOV<sup>b</sup>

Medical University-Plovdiv, Faculty of Pharmacy, Department of Chemistry and Biochemistry, 15a Vassil Aprilov Blvd., 4002- Plovdiv, Bulgaria,

<sup>a</sup>University of Sofia "St. Kliment Ohridski", Faculty of Chemistry and Pharmacy, Department of General and Inorganic Chemistry, Laboratory of Nanoparticle Science and Technology, 1, J. Bourchier Blvd., 1164-Sofia, Bulgaria

<sup>b</sup>Medical University-Plovdiv, Faculty of Dentistry, Department of Operative Dentistry and Endodontics, 3 Christo Botev Blvd., 4002-Plovdiv, Bulgaria

The goal of the present study was to characterize the morphology, determine the degree of conversion of light polymerized resin-based dental composites and to quantify the leachable portion of unreacted monomers in water solution. Two microhybrid composites - Charisma (Heraeus Kulzer, Germany) and Filtek Z 250 (3M, USA), colour A2, one microfilled composite - Durafil VS and one condensable composite - Solitaire2 (Heraeus Kulzer, Germany)) were polymerized with LED light curing unit. The surface morphology of the composite films and their cross-sections were determined using scanning electron microscopy (SEM). The degree of polymerization was evaluated by Fourier transform infrared spectroscopy (FTIR). The leaching of unpolymerized monomers from photo curable composites was measured by High- performance liquid chromatography (HPLC). It was found that the degree of polymerization was highest for Filtek Z 250 (58%) and lowest for Durafill VS (42%). HPLC results showed elution of basic monomers of their organic matrix. These results are consistent with the SEM observation. A relatively smooth surface and homogeneous cross-section structure with irregular shaped zirconia-silica particles was observed in the microhybride and packable composites, while the microfilled composite Durafill VS is characterized by significant roughness probably due to the large aggregates made of silica microfillers embedded in a prepolymerized organic matrix.

(Received October 9, 2012; Accepted November 16, 2012)

Keywords: Dental composites, Degree of conversion, Leachable monomers, Morphology

#### **1. Intorduction**

The compounds 2,2-bis[4-(2-hydroxy-3-metacryloxypropoxy) phenylpropane (bis-GMA), 1,6-bis(methacryloxy-2-ethoxycarbonylamino)-2,4,4-tetramethylhexane (UDMA) and triethyleneglycol dimethacrylate (TEGDMA) are commonly used as base monomers in dental composite formulations. When cured, these systems do not reach complete double bond conversion after polymerization [1-3]. The formation of network during the polymerization

<sup>\*</sup> Corresponding author: d\_anny@abv.bg

process causes the reduction of the mobility of monomers and oligomeric molecules resulting in pendant methacrylate groups and unreacted monomer molecules trapped in the material.

The degree of conversion (DC) is strongly influenced by factors such as material composition, total light energy, irradiation times, wavelength of the light source [4, 5]. On the other hand, the degree of conversion is related to final physical, mechanical and biological properties of composite resins [6]. It has been demonstrated that composite properties tend to improve as the degree of conversion attained during photo-polymerization is increased [7]. In addition, increased cure may result in a lower amount of non-polymerized, potentially leachable monomer, leading to a more biocompatible restoration [8]. Moreover, uncured functional groups can act as plasticizers, reducing the mechanical properties of the composite [9].

Techniques such as Fourier transformed infrared (FT-IR) spectroscopy [10-12], Raman spectroscopy [13, 14] electron paramagnetic resonance (EPR) [15], nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC) [16] and differential thermal analysis (DTA) [17] have been used to determine the degree of conversion. Among these, FT-IR spectroscopy is the most frequently used technique [18].

Very different data has been reported for the degree of conversion of dental composites. Knejevic et al. have found a degree of conversion from 45% to 76% for hybrid dental composites [19], while Bala [10] was reported DC from 47.4 to 61.1%. Significantly lower polymerization degree of 42-44% was reported for nanofil composites [11]. Composites based on bis-GMA and polymerized for 40 seconds with LED curing unit were studied by Tarle et al. [20]. Using FT-IR spectroscopy they have found a degree of conversion from 54.9% to 68.6%. Determined DC by Yoon et al. ranges within 36.9% - 62.2% [21]. Similar values close to those published by Tarle et al. was reported by Rojas and Obici [22, 23].

By HPLC and gas chromatography it was found that the leaching of monomers is a process lasting several days to several months, and most monomers are released during the first 24 hours after polymerization [24-26]. According Polidorou [27] the leaching of monomers depends mainly on the degree of polymerization, while other authors consider that the chemical structure of monomers is essential for their release from the polymer [24]. Shajii opines that the ratio organic matrix/ inorganic fillers also affects leachability of monomers [28].

The objective of the present study was to determine the degree of conversion of different type light polymerized resin-based dental composites and quantify the leachable portion of non-reacted monomers from UDMA-based composites in water solution. Along with the spectroscopic and HPLC measurements morphological observations were performed.

## 2. Materials and methods

#### **Degree of conversion**

Four light-cured resin-based composites were studied. All of the used composites were colour A2. The list of the composites including their type and composition are given in Table 1. To measure the degree of conversion, the unreacted pastes were placed between two polyethylene films and pressed to form a thin film. IR spectrum of each non-polymerized dental composite (n=5) was taken on Thermo Nicolet spectrophotometer (64 scans, resolution 4 nm, spectral range 400-4000 cm<sup>-1</sup>). Each composite was irradiated with Bluedent LED Smart device (800 mW/cm<sup>2</sup>, D & A Electronics, Bulgaria). The exposure time was 20, 40 and 60 seconds. IR spectrum of polymerized specimens (n=5) was recorded at the same conditions. The percent of unreacted carbon-carbon double bonds was determined from the ratio of absorbance intensities of aliphatic C=C (band at 1635 cm<sup>-1</sup>) against the internal standard aromatic C=C (band at 1608 cm<sup>-1</sup>) before and after curing of the specimen. Degree of conversion was calculated according to Eq. (1):

$$DC,\% = [1 - \frac{(\frac{C_{1635}}{C_{1608}})_{\text{polymer}}}{(\frac{C_{1635}}{C_{1608}})_{\text{composite}}}] \times 100$$
(1)

where  $C_{1635}$  is the area of absorption band at 1635 cm<sup>-1</sup> for the aliphatic C = C bond and  $S_{1608}$  is the area of absorption band at 1608 cm<sup>-1</sup> for the aromatic C = C bond. The area values were calculated using baseline technique. Data were analyzed using one-way ANOVA and Tukey test. Significance level was set to 0.05.

Type of composite	Organic matrix	Inorganic fillers, %
Charisma microhybrid	Bis-GMA, TEGDMA	64 w% Ba-Al-F silicate glass 0.02-2 μm SiO <sub>2</sub> 0.02-0.07 μm
Solitaire 2 packable	Bis-GMA, TEGDMA	75 w%* SiO <sub>2</sub> and Ba-Al-F glass 2-20 μm
Durafill VS microfilled	UDMA, TEGDMA,Bis- GMA	50 w% SiO <sub>2</sub> 0.02-0.07 μm Prepolymeized particles 10-20 μm
Filtek Z250 microhybrid	Bis-GMA, UDMA, bis- EMA, TEGDMA	78.5 w%* Zirconia/silica, nonsilanized 0.01-3.5 μm

Table 1. Type and composition of resin based composites used in this study.

\* According to reference [6]

#### **HPLC** analysis

To determine the leachable portion of non-polymerized monomers, five cylindrical samples with a diameter of 8 mm and a height of 2 mm were prepared. Samples were covered with Mylar strip and irradiated only on the upper side for 40 seconds and placed in 5 mL of deionised water at 37 ° C for 7 days. The amount of the monomers leached out of polymer after 24, 72 and 168 hours was monitored by chromatographic analysis. A Varian HPLC system consisted of a ProStar 230 solvent delivery module and ProStar 335 photo diode array detector equipped with reversed phase column Omnisphere 5 C18 ( $250 \times 4.6$  mm) Varian was used. Sample injection volume was 20 µL. Leached monomers from Durafill were quantified on methodology described in [29]. UDMA was eluted with a gradient of acetonitrile and water (60% to 100%) at a flow rate of 0.75 mL/min. Monomers eluted from Filtek Z 250 (Bis-GMA, TEGDMA, UDMA) were eluted with mobile phase water/acetonitrile (65%/35% to 100%) at a flow rate 1 mL/min. Detection of the monomers was at 205 nm. Results were compared with t-test.

#### SEM study

Surface and cross-section micrographs of the composite films were acquired with a Scanning electron microscopy (JEOL JSM 5510) operating at 10 kV in vacuum on samples gold coating. The composite films were broken after cooling in liquid nitrogen for the observations of cross-section morphology.

## 3. Results and discussion

Commonly used method evaluating DC in light activated resin based composites is infrared spectroscopy. It was suggested that it is most sensitive mode for evaluating depth of cure in light-activated dental composites [19].

Infrared spectra of the composites were recorded in the range 400-4000 sm<sup>-1</sup>. This spectral range comprises characteristic frequencies of the monomer functional groups (C=O, C=C<sub>aliphatic</sub>, O-H, C=C<sub>aromatic</sub>). Since the composites are very complex systems of monomers (with complex molecules) and inorganic fillers, the spectrum is complicated, but it can be defined by absorption bands of stretching vibrations of C=C aliphatic double bond at 1635 cm<sup>-1</sup>, and absorption bands of skeletal vibrations of the benzene ring at 1608 cm<sup>-1</sup> and 1583 cm<sup>-1</sup>.

observed at 1720 cm<sup>-1</sup> and 1537 cm<sup>-1</sup> (less pronounced) are attributed to the stretching vibration of C=O group and N-H bond, respectively.

IR spectra of polymerized composite were recorded within the same spectral range. Figure 1 shows only the spectral range of interest for DC determination.



Fig. 1. Infrared spectra of non-polymerized and polymerized composites in the range 1500cm<sup>-1</sup> - 1750 cm<sup>-1</sup>: a) Charisma, b) Durafill VS, c) Filtek Z250, d) Solitaire 2;1-composite paste,2-20 sec irradiation, 3-40 sec irradiation, 4-60 sec irradiation

The reduction of absorption intensity at 1635 cm<sup>-1</sup> is a result of decreasing the concentration of aliphatic C=C double bonds during the polymerization. Meanwhile a change in the intensities of absorption of the other groups is not observed. Since the concentration of aromatic C=C and N-H bonds does not change during polymerization, the absorption bands at 1608 cm<sup>-1</sup> and 1537 cm<sup>-1</sup> can serve as an internal standard for determining the degree of polymerization. The mean values of DC at different irradiation times are listed in Table 2

Irradiation time, s	Degree of conversion, %			
	Charisma	Solitaire 2	Durafill VS	Filtek Z 250
20	$49.3 \pm 1.4$	$43.1 \pm 2.9$	$42.6 \pm 1.4$	$43.4 \pm 3.2$
40 60	$52.5 \pm 2.0$ $53.7 \pm 2.5$	$51.3 \pm 0.6$ $54.1 \pm 1.4$	$52.0 \pm 2.9$ $52.4 \pm 2.4$	$58.4 \pm 1.4$ $58.6 \pm 1.6$

Table 2. Mean values of degree of conversion for different composites studied.

Data presented in Table 2 show that the degree of conversion for all the composites studied increases with increasing the exposure time. The values of DC for microhybrid composites ranging from 43.4% to 58.6% are close to those reported by Bala [9]. Charisma and Solitaire 2 are composed of identical monomers (TEGDMA and bis-GMA), but their percentage content in the composites is different as the content of inorganic fillers is different, too.

1828

Regardless of this, the achieved DC values at the longer time of irradiation are similar (52.5% and 53.7% for Charisma vs. 51.3% and 54.7% for Solitaire 2). The condensable composites contain more fillers than hybrid ones, which are able to scatter and refract the falling light to a greater extent. This is the most probable reason for almost the same polymerization degree of these two composites. Furthermore, the increased content of fillers may limit the mobility of free radicals in the polymer matrix which results in pendant methacrylate groups and decreased DC. Although the manufacturer of Charisma claims that exposure time of 20 sec is sufficient for adequate polymerization, it appears that prolonged exposure increases the degree of polymerization. Bis-GMA presents in Filtek Z 250 along with UDMA, TEGDMA and bis-EMA. This polymer matrix has a low concentration of double bonds, which provides more efficient polymerization at the same other conditions. This finding could be a probable reason for highest DC value (58.4%) for Filtek Z 250 composite. Durafill VS contains UDMA, TEGDMA and bis-GMA. Its degree of conversion is lower than the other composites studied but is no statistically different from their DC at 20 s irradiation (excluding Charisma). Even with the lowest content of fillers Durafill VS does not show a higher degree of polymerization compared to composites with more fillers. One possible explanation could be the assumption that prepolymerized fillers includes non-reacted species [17].

It is generally assumed that the greater the extent of polymerization the lower will be the level of unconverted monomers available to be leached. Although cross-linked dimethacrylate resins are virtually insoluble, water and some solvents are able to penetrate the polymer chain, promoting extraction of non-reacted components [26].

The present study demonstrated that the microfilled and microhybrid composites evaluated underwent degradation during its stay in water. The concentrations of leaked monomers found in the water are summarized in Table 3.

Composite	Time	Monomer concentration, µg/mL			
		Bis-GMA	TEGDMA	UDMA	
Durafill VS	24 h	ND	$44.67 \pm 1.53$	$5.24 \pm 0.13$	
	72 h	ND	$63.53 \pm 2.47$	$6.18\pm0.21$	
	168h	ND	$85.22 \pm 2.49$	$6.79\pm0.27$	
Filtek Z 250	24 h	ND	$2.63 \pm 0.81$	$4.91 \pm 0.65$	
	72 h	$0.29\pm0.04$	$3.12 \pm 0.22$	$5.39\pm0.36$	
	168 h	< LOQ	$3.98 \pm 0.32$	$6.66 \pm 0.38$	

*Table 3. Concentration of unreacted monomers* ( $\mu g/mL$ ) *leached out in water.* 

*ND-not detectable. LOQ – limit of quantification* 

The monomers TEGDMA and UDMA are leached from Durafill VS and Filtek Z 250. The concentration of both leached monomers increases with time, confirming our previous study [28], but the largest amount is leached for the first 24 hours. The concentration of TEGDMA is much larger than that of UDMA, which is due to its greater mobility and hydrophilicity. Previously reported concentrations of TEGDMA released from microhybrid and packable composite was two and three times less respectively [28]. Surprisingly very low concentration was registered for Filtek but this result is in agreement with findings of Manojlovic [26]. Although Durafill VS shows lower DC compared to Filtek Z 250, the leached amount of UDMA is not statistically different.

Information about composition of organic matrix of Solitairte, enclosed in material data safety sheet [30], does not mention presence of UDMA as was marked in [6] and [10]. From FT-IR spectrum of the composite (Fig. 1c) is seen that less pronounced band at 1537 cm<sup>-1</sup> (N-H bond) exist. Additional experiment should be done to find out whether this material leaches out UDMA in water solution.

Amount of bis-GMA is very small due to its hydrophobicity. The concentration of leached bis-GMA (0.104  $\mu$ g/mL) is below the detection limit for the first 24 hours. This

concentration increases with the time and becomes  $0.29 \pm 0.04 \ \mu g/mL$  in 72 hours, after that it reduces to levels between LOD and LOQ (0.286  $\mu g/mL$ ) in 168 hours, suggesting chemical transformations. This was not observed in our previous study [29]. Bis-GMA is not detectable in eluates from Duraffil.

HPLC results are consistent with the SEM observations. The morphology of composite films-prepared at 60 s time of irradiation was analyzed by scanning electron microscope and SEM micrographs are presented on Fig. 2 and Fig. 3.



*Fig .2. SEM images of cross sections of the composites studied at 150x and 1500x magnification (a) Charisma, (b) Durafill VS, (c) Filtek Z250, (d) Solitaire 2* 

Cross-section images of Charisma, Solitaire 2 and Filtek Z 250 (Fig 2a, 2c, and 2d) show the dense amorphous structure of polymers formed with a uniform distribution of inorganic fillers. In contrast, Durafill VS (Fig. 2b) is characterized by a significant roughness probably due to the large aggregates made of silica microfillers embedded in prepolymerized organic matrix. From SEM observations of cross-sections it appears that Charisma is the most homogeneous composite with the less prominent features, probably due to the small filler size. Nonsilanized fillers of Filtek Z 250 are also evenly distributed within polymer matrix. Solitaire 2 and Durafill VS show some noticeable features observed in a large extent in Durafill VS.

Top view images show round fillers in different size for Charisma, and more regular ones for Solitaire 2, uniformly distributed on the surface of composites (Fig. 3a and 3d). Large polygonal particles of prepolymerized fillers embedded in polymer matrix along with small ones are observed on the surface of Durafill (Fig. 3b). The surface of Filtek Z 250 is mostly composed of resin and appears more flat than the other composites probably due to the type of fillers (Fig. 3c). Thus, the polymer is more susceptible of water penetration that might alter its microstructure. Charisma and Solitaire 2 are characterized with very closely packed filler particles thus protect the softer resin matrix from solvent incursion.



Fig. 3. SEM images of the different as-cured composites at 5000x magnification: (a) Charisma, (b) Durafill VS, (c) Filtek Z250, (d) Solitaire 2

Durafill and Filtek Z 250 release extremely different amount of TEGDMA and almost equal of UDMA. It can be speculated that composition of material (organic matrix and fillers), achieved DC and homogeneity of the polymer network, influence leaching of monomers in water.

## 4. Conclusions

The results obtained in this study indicate that less filled composite shows comparable DC with more filled at the same irradiation conditions. Irradiation time of 40 seconds seems to be appropriate for sufficient curing of the composites. Polymerized composites are capable of leaching non-reacted monomers irrespective of their DC and morphology. Higher degree of conversion and more filler content does not make necessarily less leached monomer. The latter is related mainly with chemical structure of monomers and morphological features of polymer network - more dense and homogenous structure at higher filled composites with smaller particle size.

#### References

- [1] Lovell LG, Berchtold KA, Elliot JE, Lu H, Bowman CN. Polym Adv Technol.
- **12**, 335 (2001)
- [2] Sideridou I, Tserki V, Papanastasiou G. Biomaterials. 23, 1819-1829. (2002)
- [3] Dickens SH, Stansbury JW, Choi KM, Floyd CJE. Macromolecules 36, 6043-6053 (2003)
- [4] Halvorson RH, Erickson RL, Davidson CL. Dent Mater 18: 463-469 (2002)
- [5] Rastelli ANS, Jacomassi DP, Bagnato VS. Laser Phys 18, 408-414 (2008)
- [6] Hubbezoglu I, Bolayr G, Dogan O.M, Dogan A, Ozer A, Bek B. Dental Mater J. 26(6), 845-853 (2007)
- [7] Lovell LG, Lu H, Elliot JE, Stansbury JW, Bowman CN. Dent Mater.17, 504-511 (2001)
- [8] Yap AU, Soh MS, Han TT, Siow KS. Oper Dent. 29, 410-415 (2004)
- [9] Asmussen E., Peutzfeldt A J Dent Res. **80(6)** 1570-1573 (2001)
- [10] Bala O, Olmez A, Kalayci S. J Oral Rehabil. 32, 134-140 (2005)
- [11] Atai M, Motevasselian F. Clin Oral Investig: 13, 309-316 (2009)
- [12] Stansbury J.W., Dickens S.H. Dental materials. 17, 71-79 (2001)

1832

- [13] Silva Soares L.E, Martin A.A, Barbosa Pinheiro A.L. J Clin Laser Med Surg. 21, 357-360 (2003)
- [14] Santos GB, Medeiros IS, Felows CE, Muench A, Braga RR. Oper Dent. 32, 79-83 (2007)
- [15] Sustercic D, Cevc P, Funduk N, Pintar MM. J Mater Sci Mater Med. 8, 507-510 (1997)
- [16] Morgan D.R., Kalachandra S., Shobha H.K., Gunduz N., Stejskal E.O. Biomaterials. 21, 1897-1903 (2000)
- [17] Imazato S, McCabe JF, Tarumi H, Ehara A, Ebisu S. Dental Materials. 17, 178-183 (2001)
- [18] Moraes LGP, Rocha RSF, Menegazzo LM, de Araújo EB, Yukimitu K, Moraes JCS. J Appl Oral Sci. 16(2), 145-9 (2008)
- [19] Knezevic A, Tarle Z, Meniga A, Sutalo J, Pichler G, Ristic M. J Oral Rehabil. 28, 586-591 (2001)
- [20] Tarle Z, Meniga A, Knezevic A, Sutalo J, Ristic M, Pichler G. J Oral Rehabil. 29, 662-667 (2002)
- [21] Yoon T.H, Lee Y.K, Kim S.W. J Oral Rehabil. 29, 1165-1173 (2002)
- [22] Rojas S.S, Frigo G.J.M, Bernardi M.I.B, Rastelli A.N. de S, Hernandes A.C, Bagnato V.S. J Therm Anal Calorim. 99, 263-268 (2010)
- [23] Obici A.C, Sinhoreti M.A.C, Frollini E, Sobrinho L.C, Consani S. Polymer Testing. 24, 814-818 (2005)
- [24] Michelsen VB, Lygre H, Skalevik R, Tveit AB, Solheim E. Eur J Oral Sci. 111, 263-271 (2003)
- [25] Ortengren U, Wellendorf H, Karlsson S, Ruyter IE. J Oral Rehabil. 28, 1106-1115 (2001)
- [26] Manojlovic D, Radisic M, Vasiljevic T, Zivkovic S, Lausevic M, Miletich V. Monomer elution from nanohybrid and ormocer-based composites cured with different light sources Dent Mater. 27, 371-378 (2011)
- [27] Polydorou O, Trittler R, Hellwig E, Kummerer K. Elution of monomers from two conventional dental composite materials Dent Mater.23,1535-1541 (2007)
- [28] Shajii L, Santerre J.P. Effect of filler content on the profile of released biodegradation products in micro-filled bis-GMA/TEGDMA dental composite resins Biomaterials.
  20 1897-1908 (1999)
- [29] Uzunova Y, Lukanov L, Filipov I, Vladimirov S. High-performance liquid chromatographic determination of unreacted monomers and other residues contained in dental composites. J Biochem Biophys Methods. 70, 883-888 (2008)
- [30] Materials safety data sheets, http://heraeus-dentalus.com/media/webmedia\_local/msds\_\_\_dfu/msds/Solitaire\_2\_MSDS.pdf