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HPLC Analysis of Monomer Release from Conventionally and High Temperature High-Pressure Polymerised Urethane Dimethacrylate Intended for Biomedical Applications

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Abstract

Since monomer release poses significant biocompatibility concerns, the aim of this study was to determine, using HPLC, and compare monomer release from conventionally and high-temperature high-pressure (HT/HP) polymerized urethane dimethacrylate (UDMA) for biomedical applications. Three polymers were made: a) a control, obtained by conventional thermo-polymerization of UDMA with 0.5% (w) benzoyl peroxide (BPO) as initiator; b) an experimental, obtained by HT/HP polymerization of UDMA with 0.5% (w) BPO; and c) another experimental, obtained by HT/HP polymerization of UDMA without initiator. Bar-shaped polymer specimens were immersed in HPLC-grade 75% ethanol for 1 d, 7 d, 14 d, and 28 d prior to monomer determination by HPLC with an Agilent 1260 Infinity Quaternary LC. A Poroshell 120 EC-C18 (4.6x50 mm; 2.7µm) column and elution solvent consisting of HPLC-grade 65% acetonitrile in water, with a flow rate of 1 µL/min, were used. A calibration curve was constructed using standard UDMA solutions in the range of $1x10^{-5}$ M to $1x10^{-7}$ M. The limits of detection (LOD=$2.62x10^{-6}$ M) and quantification (LOQ=$7.65x10^{-6}$ M) for UDMA were determined. The accuracy of the method was confirmed by standard additions. Monomer release was statistically higher in the control group at all-time intervals; the lowest release was detected in the BPO-containing HT/HP polymerized group. The results suggested that there was a significant reduction in free monomer content in HT/HP polymerized UDMA and that polymers obtained under HT/HP conditions could be more biocompatible.

Keywords: HPLC; Residual Monomers; High-Temperature Polymerization; High-Pressure Polymerisation, Urethane Dimethacrylate (UDMA)

Introduction

Dental resin composites, consisting of a dimethacrylate-based matrix and radiopaque glass/ceramic fillers, have evolved significantly since the pioneering work of Bowen [1-4]. Current aesthetic demands and technological advances have led to an increase in ceramic and resin composite indirect dental restorations produced with computer aided design/computer aided manufacturing (CAD/CAM) technology [5]. Easier machinability, easier accessibility through the restoration for endodontic treatment (if needed), the likelihood of easier repair, and lower cost, render CAD/CAM resin composite blocks (RCB) as an appealing alternative to the more aesthetic CAD/CAM ceramic blocks [6,7]. The first CAD/CAM RCB was introduced by 3 M ESPE (Paradigm), based on their direct restorative resin composite Z100, a Bis-GMA – TEGDMA (bisphenol A-glycidyl methacrylate – triethyleneglycol dimethacrylate) containing material. Biocompatibility concerns related to monomer release from the incompletely polymerized matrix or following the breakdown of the matrix as a result of exposure to the harsh oral environment have been often raised [8-12]. Considering the current concerns (warranted or not [13]) related to any product that may/might release bisphenol-A, and therefore dental composites containing Bis-GMA [8,12], renders urethane dimethacrylate (UDMA) an appealing alternative monomer to Bis-GMA.

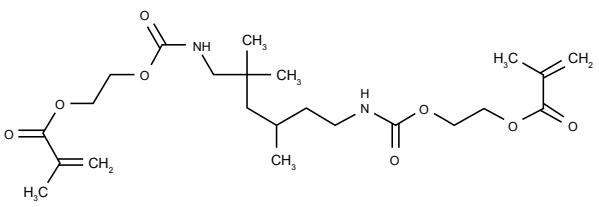

Figure 1: Chemical structure of UDMA (CAS 41137-60-4).

In an effort to improve the mechanical and physical properties of RCB, a novel high-pressure high-temperature (HP/HT) polymerization procedure was proposed and successfully used [14]. Besides the significantly improved physical and mechanical properties, it was speculated that HP/HT polymerization would alter the type of polymer network formed resulting in less monomer release. In this study, we set out to test the null hypothesis that there is no difference between monomer releases from conventionally and HT/HP polymerized UDMA RCB. To test the hypothesis, this study employed HPLC to compare monomer release from conventionally and HT/HP polymerized UDMA.

Experimental

Three urethane dimethacrylate (UDMA – Figure 1; MW=470.56; CAS 41137-60-4; Evonik, Essen, Germany) polymers were made:

a) A control polymer, obtained by conventional thermo-polymerization of UDMA with 0.5% (by weight) benzoyl peroxide

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Standard addition	Calculated concentration C_c (mol/L)	Determined concentration C_D (mol/L)	Recovery between C_c and C_D (%)
1	3.400E-04	3.484E-04	102.479
2	3.404E-04	3.555E-04	104.412
3	3.397E-04	3.592E-04	105.725

Table 1: Results from the standard additions method for checking the accuracy of the HPLC method for the determination of UDMA in samples (n=3).

Storage time Group*	1 d	7 d	14 d	28 d
TC	5.25 ± 0.85	14.85 ± 5.80	21.52 ± 5.31	32.89 ± 4.01
HTHPI	0.12 ± 0.05	0.46 ± 0.38	0.81 ± 0.52	0.96 ± 0.58
HTHPNI	2.15 ± 0.18	6.54 ± 0.53	11.31 ± 0.74	19.41 ± 1.43

*** TC**=conventionally thermo-polymerized UDMA with 0.5 % (w) benzoyl peroxide; **HTHPI**=high-temperature high-pressure polymerized UDMA with 0.5% (w) benzoyl peroxide; **HTHPNI**=high-temperature high-pressure polymerized UDMA with no initiator

Table 2: Amount of released UDMA (Mean ± SD, in Mol/g/cm² x 10^{-6}).

(BPO; MW 242; CAS 94-36-0; Sigma Aldrich, Steinheim, Germany) as initiator (group TC);

b) An experimental polymer, obtained by HT/HP polymerization of UDMA with 0.5% (by weight) BPO as initiator (group HTHPI);

c) Another experimental polymer, obtained by HT/HP polymerization of UDMA without initiator (group HTHPNI).

For the control TC group, approximately 100 g UDMA with 0.5% BPO was placed inside a flexible silicone tube (25 mm internal diameter and 1 mm wall thickness) and then kept in a stove (Memmert, Schwabach, Germany) at 90°C and atmospheric pressure (0.1 MPa) for 4 h.

The procedure for HT/HP polymerization has been detailed previously [14]. Briefly, based on the predetermined (160-180)°C thermo-polymerization temperature range of UDMA at atmospheric pressure (0.1 MPa) without initiator BPO, polymerization reactions for the experimental groups were conducted at 180°C. Approximately 100 g of either UDMA with 0.5% BPO (for group HTHPI), or just UDMA (for group HTHPNI), was placed inside a flexible silicone tube. The filled tubes were introduced into an autoclave (custom-built for this study) with pressure and temperature control (LabVIEW version 8.2, National Instruments, USA). A thermocouple was placed in the proximity of the sample to enable accurate monitoring and, via feed-back, control of the temperature. In the first stage, the pressure within the autoclave was increased to 250 MPa at a rate of 1 MPa/sec at ambient temperature. In the second stage, the temperature was increased to 180°C at a rate of 2°C/min. The sample was maintained at 250 MPa and 180°C for 60 min before being cooled off and the pressure released.

The obtained cylindrical polymers were cut, with a diamond disc under water cooling, into approximately (2x2x25) mm bars that were used for mechanical characterization (a separate study [15]) and, thereafter, for the current monomer release study. For the latter, 18 half-bars of each polymer were randomly selected from the tested 3 pb (three point bending flexural test) specimens. Their surface area and weight were precisely measured, recorded and dried, and they were placed into three vials (six half-bars per vial). Each vial was then filled with 10 mL of 75% ethanol [16-19], prepared from HPLC-grade ethanol and HPLC-grade water (Fisher Scientific, Bishop Meadow Road, UK). The vials were sealed and stored in a stove (Memmert, Schwabach, Germany) at 37°C. After 1 d, 7 d, 14 d, and 28 d storage, three 20 µL aliquots of each vial were removed with a micro-syringe for HPLC analysis.

The HPLC analysis was conducted using an Agilent 1260 Infinity Quaternary LC (Agilent Technologies, Waldbronn, Germany), equipped with a quaternary pump (model G1311B) and a UV diode array detector (model G4212B). The column used was Poroshell 120 EC-C18 (Agilent Poroshell, USA), with internal diameter of 4.6 mm, length of 50 mm, and filler particle size of 2.7 µm. The solvent was HPLC-grade 65% acetonitrile (Fisher Scientific, Bishop Meadow Road, UK) in HPLC water, used in isocratic conditions with a flow rate of 1 µL/min. The elution was performed at room temperature and monitored in the whole UV range. For quantification, the 210 nm spectra, where UDMA exhibits significant absorption, were used. Identification of the analyte, UDMA, was made based on the retention time of the UDMA peaks registered for the standard solutions (1.327 min).

A 10 mL $1x10^{-3}$ mol/L UDMA stock solution was prepared by dissolving the appropriate amount of UDMA in 75% ethanol. For the calibration curve, seven 10 mL solutions, (10^{-3}; 10^{-4}; 10^{-5}; $5x10^{-6}$; 10^{-6}; $5x10^{-7}$; and 10^{-7}) mol/L, were obtained from the stock solution by dilution. These solutions were stored at ambient temperature. Linearity of the calibration curve, based on the quantitative determination of UDMA in the seven solutions, was assessed by linear regression analysis. The parameters obtained for the linear peak area/concentration for the analyte (UDMA) are as follows:

A=1.58012 x 10^7 x c, with a correlation coefficient R^2=0.99991 and σ=167.06112, where A=peak area [12,13].

Limit of detection (LOD) and limit of quantification (LOQ) were calculated from the calibration curve obtained in the low concentration region ($1x10^{-7}$, $5x10^{-6}$, $1x10^{-6}$, $5x10^{-5}$ to $1x10^{-5}$ mol/L) according to the formulae:

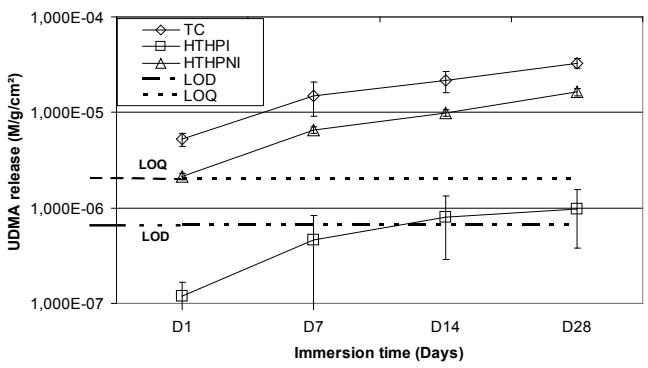
LOD=3.3 σ/S and LOQ=10 σ/S

where σ=standard residual deviation of intercept and S=the slope.

The accuracy of the procedure was checked using the standard addition method. One real sample was spiked with appropriate volumes of a standard solution of UDMA and satisfactory results for the recovery, ranging from 102.48% to 105.72% (Table 1), confirmed that the method was accurate and appropriate for quantitative analysis.

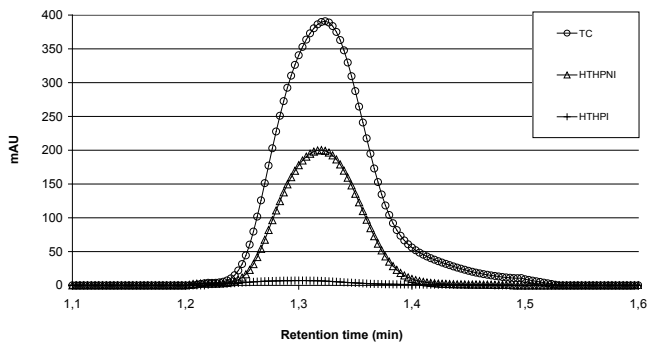
Results

The calculated values for LOD and LOQ were 2.623 x 10^{-6} M and 7.948 x 10^{-6} M, respectively. The results of the quantitative analysis are summarized in Table 2 and in Figure 2. Representative chromatograms



*** TC**=thermo-polymerized UDMA with 0.5 % (w) benzoyl peroxide; **HTHPI**=high-temperature high-pressure polymerized UDMA with 0.5% (w) benzoyl peroxide; **HTHPNI**=high-temperature high-pressure polymerized UDMA with no initiator (M/g/cm² stands for Mol/g/cm²)

Figure 2: Amount of released UDMA vs. storage time – graphical summary of the results*.



* **TC**=conventionally thermo-polymerized UDMA with 0.5 % (w) benzoyl peroxide; **HTHPI**=high-temperature high-pressure polymerized UDMA with 0.5% (w) benzoyl peroxide; **HTHPNI**=high-temperature high-pressure polymerized UDMA with no initiator (mAU stands for milli (10^{-3}) Absorbance Unit)

Figure 3: Representative HPLC chromatograms of 28 d stored samples*

of the analyte UDMA obtained for 28 d stored samples are presented in Figure 3.

After 1 d storage, UDMA was already detected in all the groups and the concentration of released UDMA increased with increasing storage time. The analyte UDMA was detected in quantities between LOD and LOQ after 1 d storage and in quantities higher than LOQ after 14 d and 28 d storage. The quantities of released monomer were statistically significantly different between all three groups at each storage time and between all storage times within each group, with the exception of the 14 d vs. 28 d in the HTHPI group where no difference was detected. The highest concentration of released UDMA at all storage times was detected in the control group (**TC**), while the lowest concentration was detected in the BPO-containing HT/HP polymerized group (HTHPI).

Discussion

Ferracane (1994) stated various factors of importance for the release of unbound substances from polymerized dental composites [20]. Thus, besides the monomer into polymer conversion rate, which determines the quantity of leachable components, the kinetics and mechanism of elution processes depend on the composition and solubility parameters of the solvent used for extractions. Diffusion through the polymer network is determined by the size and the chemical characteristics of the leachable substances.

Free radical thermal polymerization at 90°C and under atmospheric pressure (0.1 MPa) requires the presence of an initiator, which breaks down under these conditions to supply the first free radicals that will initiate the polymerization. The presence of an initiator is not necessary under the HP/HT polymerization conditions used to obtain the experimental composites in this study. The absence of an initiator and any other additives afford the preparation of "pure" polymers/composites [21]. However, under high pressure, monomers lose their mobility and a 12% conversion rate was achieved in a prior study [21]. If high pressure polymerization was conducted under high temperature, the conversion rate increased to 65% [21]. Considering the above, we decided to conduct the polymerization under a relatively high pressure (250 MPa) and at high temperature (180°C). The polymers obtained under these conditions showed a dramatic decrease in free monomer release in comparison with the control thermo-polymerized material. The results also showed that the presence of an initiator further decreases monomer release, suggesting that a higher conversion rate and a higher crosslinking were achieved. This hypothesis should be further investigated.

Polymerization under HP/HT not only affects the degree of conversion and crosslinking, it most likely reduces the size and number of voids [14]. This, in turn, would affect the kinetics of monomer release and contribute synergistically to lower monomer release.

Conclusions

The results of this study suggest that polymerization under HT/HP conditions results in polymers that exhibit a significant (even dramatic) decrease in monomer release. It could be postulated that polymers obtained under HT/HP conditions could be more biocompatible and, thus, less likely to illicit adverse effects.

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