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Application of solid phase microextraction on dental composite resin analysis

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ABSTRACT

A direct immersion solid phase microextraction (DI-SPME) method was developed for the analysis of dentin monomers in saliva. Dentine monomers, such as triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) and 2,2-bis-[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl]-propane (Bis-GMA), have a high molecular weight and a low vapor pressure. The polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber with a medium polarity was employed for DI-SPME, and 215 nm of detection wavelength was found to be optimum in the chromatogram of HPLC measurement. The calibration range for DI-SPME was 0.30–300 μg/mL with correlation coefficients (r) greater than 0.998 for each analyte. The DI-SPME method achieved good accuracy (recovery 96.1–101.2%) and precision (2.30–8.15% CV) for both intra- and inter-day assays of quality control samples for three target compounds. Method validation was performed on standards dissolved in blank saliva, and there was no significant difference ($p > 0.2$) between the DI-SPME method and the liquid injection method. However, the detection limit of DI-SPME was as low as 0.03, 0.27 and 0.06 μg/mL for TEGDMA, UDMA and Bis-GMA, respectively. Real sample analyses were performed on commercial dentin products after curing for the leaching measurement. In summary, DI-SPME is a more sensitive method that requires less sample pretreatment procedures to measure the resin materials leached in saliva.

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1. Introduction

Due to the adverse health effect of ingesting and the environmental pollution of mercury in dental amalgams, dental composite resin has been widely employed as the restorative material [1,2]. Poly(methylmethacrylates) exhibits significant merits in physical, esthetic, and handling properties, and therefore has been frequently used as denture base materials in the past decades. The most commonly used dimethacrylate composite monomers are triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA) and 2,2-bis-[4-(2-hydroxy-3-methacryloyloxypropoxy) phenyl] propane (Bis-GMA). Their chemical structures are shown in Fig. 1.

Bis-GMA is a difunctional monomer with high viscosity, and TEGDMA is most often used as a diluent. However, abrasion resistance, water sorption and polymerization shrinkage may have significant effects on the defects of dental resin composites [3,4]. It has been found that several components are leachable through degradation and/or erosion from resin based dental material into the saliva [5]. In addition, the salivary esterases can degrade the surfaces of composite resins, which may then result in the liberation of methacrylic substances.

The leachable substances from resin based material can induce local effects in oral tissues, *i.e.*, pulp, gingival, oral mucosa as well as adverse systemic reactions that are either allergic or toxic [6]. Ratanasathien et al. indicated that increasing the exposure time period of culture cells to the tested monomers led to increased cytotoxicity. They also found that exposure time and interactions between dentin components may be important factors affecting the cytotoxicity of dentin composites [7]. Prior to a battery of *in vitro* and *in vivo* tests, the amount and species released by dental composite resins should be determined.

Sample preparation in biomedical analysis is commonly performed by liquid–liquid extraction (LLE) and solid phase extraction (SPE), and measurement is mainly followed by gas chromatography and/or liquid chromatography [8–11]. However, the wet chemical sample preparation of LLE and SPE is considered time consuming, SPE cartridges plugging and toxic solvent exhausting involve high economic cost and hazardous organic solvent pollution concerns. Especially it is difficult to work on the small amount of sample using these conventional sample treatment techniques while the large volume of saliva sample is not practically collected.

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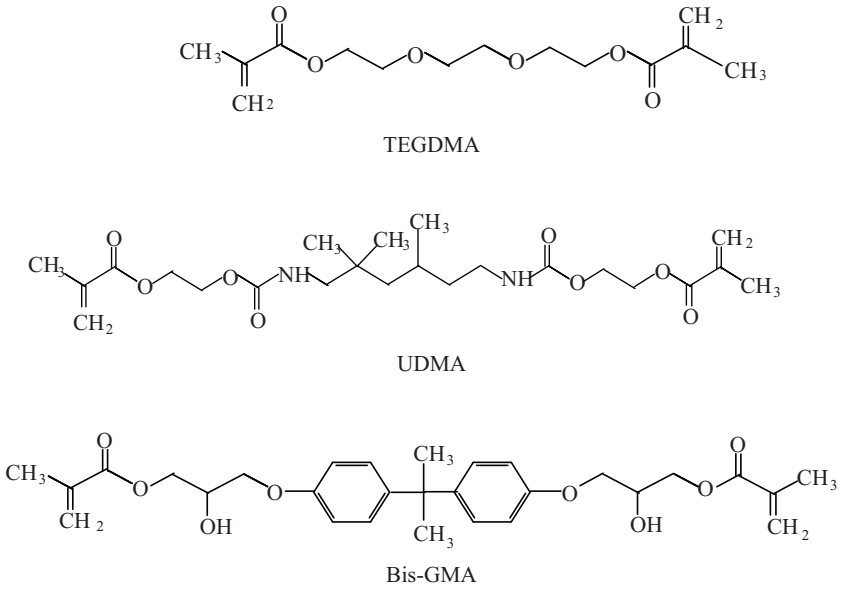

Fig. 1. Chemical structures of dentin materials.

Zafra et al. [12] proposed a GC–MS method for detection and determination of bisphenol-A, Bis-GMA, Bis-DMA and TEGDMA in human saliva. Their method showed a detection limit as low as 0.3–10 μg/L for the analytes. However, tedious procedures, such as protein precipitation, acidification, evaporation and dissolution, were conducted for the sample preparation prior to injection into the GC–MS. Zafra and his coworkers found that Bis-GMA could not be directly detected by the GC–MS method because of its low volatility and thermal degradation at an injector temperature higher than 250 °C. Four chromatographic peaks, however, appeared for Bis-GMA at 250 and 300 °C, which may be due to thermal degradation of Bis-GMA with the increments in the injector temperature.

The solid phase microextraction (SPME) method was introduced in the 1990s and can be applied to various matrices of gaseous, liquid, solid and biological samples. There are two variants for SPME fiber extraction. Headspace SPME, which involves extracting the analytes in the headspace of the sample matrix, is good for volatile compounds. Direct SPME involves extracting the analytes by dipping the fiber into the aqueous sample and generally working for low vapor pressure and high molecular weight analytes. Direct SPME is often employed for the assay of drugs and related analytes in plasma and urine [13]. Monomers of dental resins, such as TEGDMA (MW 286), UDMA (MW 470) and Bis-GMA (MW 512), have a high molecular weight, and only decomposed products are detectable in the gas chromatography [12]. Therefore, high performance liquid chromatography (HPLC) is a practical method for the measurement of co-monomers with a large molecular weight [14].

Several studies indicated that monomers of TEGDMA, UDMA and Bis-GMA leach out from sealants in minor quantities [8,9]. Therefore, there is a need to develop a more sensitive method which requires less sample pretreatment procedures for the dentin monomer analysis. This study presents the application of direct SPME extraction followed by HPLC analysis for dentin materials of TEGDMA, UDMA and Bis-GMA. The sample throughput, quality of the analytical method, the sample pretreatment and operation procedures, and the analytical parameters were the major aspects being studied. In addition, the diffusion coefficients of analytes in the SPME fiber were determined using a solution-diffusion model, which can be beneficial to determine the equilibrium time for direct extraction using SPME, and facilitate the application of polymeric fibers in the SPME technique.

2. Experimental

2.1. Reagent and materials

In this study, the target dentin resin materials were TEGDMA (Sigma–Aldrich, Steinheim, Germany), UDMA and Bis-GMA (Shinnakamura Kagaku, Wakayama, Japan). The standards of dentin materials were dissolved in ethanol (SHOWA, Tokyo, Japan) as stock solution. The individual test sample as well as working standards were prepared daily in a supernatant of a centrifuged (1670 × *g*) blank saliva matrix collected with the consent of healthy adults, ages 22–25 years, who did not have false teeth. A mixture (65:35, v/v) of acetonitrile (HPLC grade, Fisher Scientific, New Jersey, USA) and D.I. water (NANOpure Infinity™, Barnstead/Thermolyne, IA, USA) was used as the HPLC elution and desorption solvent. A hot plate with a magnetic stirrer (Barnstead/Thermolyne, IA, USA) was employed for the preparation of standards. The centrifuge apparatus was purchased from Kubota (model 5800, Tokyo, Japan).

The commercially available types of fiber coating compatible with the HPLC instrument were prospectively selected for analytical performance evaluation in this study. The target dentin materials are polar compounds with a high molecular weight. Therefore, the SPME fiber coatings of polydimethylsiloxane/divinylbenzene (PDMS/DVB 60 μm StableFlex), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS 50/30 μm StableFlex), carbowax/templated resin (CW/TPR 50 μm StableFlex), and polyacrylate (PA 85 μm) with various characteristics were employed for evaluation herein. All SPME fiber assemblies and holders were purchased from Supelco Co. (Bellefonte, PA, USA). Each fiber was conditioned according to the manufacture's recommendations prior to use.

2.2. Instrumentation

The high performance liquid chromatography (Perkin Elmer Series 200 HPLC) with an on-line UV/VIS detector (785A, Perkin Elmer, Conn, USA) was used in this study. The reversed phase column (516 C 18 HPLC column, 25 cm × 4.6 mm × 5 μm, Supelco, Bellefonte, PA, USA) with a mobile phase of acetonitrile–water (65:35, v/v) at a flow rate 1 mL/min was employed in HPLC analysis. The regular HPLC injection port was modified and replaced

by HPLC-SPME interface (Supelco, Bellefonte, PA, USA) where the injection loop was replaced by a fiber desorption chamber.

The HPLC-SPME interface consists of a six-port HPLC valve and a fiber desorption chamber with a volume of 200 μL installed in place of the sample loop. This interface was functioned for fiber desorption and for the introduction of analytes into the HPLC column [15]. The static mode was used in this study while the extracted compounds on fiber were desorbed into the stagnant eluent solvent filled in the chamber for a specific time period, and the mobile phase was then subjected to on-line flushing of desorbed compounds into the HPLC column for measurement.

The leaching test on commercial dentin composites was regularly identified by the retention time on HPLC chromatogram. Prior to HPLC analysis, liquid chromatography mass spectrometry (LC–MS) with a positive mode (LCQ DECA XP ^{plus}, Thermo Finnigan, Milan, Italy) for qualitative confirmation as compared with the standard of each target compound was applied. The LC–MS method for the determination of TEGDMA, UDMA and Bis-GMA in the saliva sample was published elsewhere [16].

2.3. SPME procedure

A 4 mL vial was completely filled with an aliquot of a saliva sample with zero headspace. A magnetic stir bar (8 mm × 3 mm, Hong-Yu Co., Taiwan) was placed in the vial sealed with a PTFE-lined rubber septum aluminum cap. The material sorbed onto the fiber was performed on a manual SPME holder while the fiber was inserted into the vial, which was placed in a sample vial rack on the stirrer/heater at a temperature of 40 °C. At the end of the extraction, the fiber was immediately processed through static desorption on the modified injection chamber. The desorbed analytes were then swept on-line into the inlet of the HPLC column by the mobile phase for analysis. Prior to the next analysis, the SPME fiber was conditioned in the injection chamber by the stream of the mobile phase for 2 min. This effort was made to ensure that the fiber was free of contaminants and carryover.

2.4. Standard solution and sample preparation

The calibration standards and test samples were all prepared consistently throughout the entire study. Dentin standards were dissolved in ethanol as a stock solution and followed by dilution with a blank saliva matrix to prepare the working standards and samples. All standards and test samples were prepared in 4 mL vials and stock solutions of dentin standards were spiked into a blank saliva matrix. The blank saliva was checked before each experiment to make sure there was no interference. The prepared stock standards were kept in a −70 °C freezer until used. Prior to analysis, the frozen standards and samples were defrosted and equilibrated to room temperature. The quality control samples were prepared separately and analyzed along with the test samples.

2.5. Method validation

The working calibration range of HPLC was verified by employing optimized SPME parameters in this study for each standard analyte spiked into the blank saliva matrix at five different concentrations. The limit of detection (LoD) was performed by spiking a pre-tested lowest detectable amount of each component into the blank saliva matrix in seven replicates and analyzing according to the established method. The LoD of each component was determined by the corresponding concentration of three times the standard deviation of the seven replicates. The precision (CV%) and accuracy (recovery%) of the method were evaluated through quality control samples of both intra- and inter-day assays while individual investigation was performed.

The method validation was performed in parallel between the established DI-SPME-HPLC method and the direct liquid injection method employed from previous reports [13,17,18]. In the direct liquid injection method, standards of individual target resins were prepared in ethanol–water (75:25, w/w) solution. A specific amount of stock standard was mixed well with the supernatant of blank saliva at a 1:1 ratio, and was centrifuged at 1670 × *g* for 5 min. The centrifuged aliquots were then ready for analysis.

2.6. Leaching test on commercial dentin composites

The commercial dentin composites were employed for the leaching test. Five different specimens of the commercially available resin and resin composite material from different manufacturers were used as the test samples. Each contained a different composition of resin matrices, and all of them had either one or all three constituents of TEGDMA, UDMA and Bis-GMA as the major components. Test specimens (diameter 6.5 ± 0.5 mm × thickness 1 ± 0.1 mm) were shaped in a Teflon mold, and two pieces of cleaned glass micro slide were placed on both the top and bottom surfaces of the mold to assist in making a constant matrix of resin composites. An external visible light source (Visilux 2, Dental Products/3 M) with the diameter of 7 mm light tip and an intensity of 470 mW/cm^2 was used in the polymerization process. Various curing times of 20, 40, 60, 80, and 120 s were preliminarily investigated, and all test specimens were found most completely polymerized at 120 s. Therefore, the curing time of 120 s was employed for the subsequent studies. Sideridou et al. also reported that the irradiation time of 100 s results in decreased amounts of eluted monomers compared to 80 s for resins [14]. After irradiation, the resins were incubated at room temperature for various periods of time at 1, 3, 5 and 7 days for the components' leaching process in both saliva and 75% ethanol–water solution matrices. The DI-SPME method was then applied in these two matrices for sample extraction and followed by HPLC analysis.

3. Results and discussion

3.1. Selection of fiber coating

The headspace SPME was tried for dentin sample extraction at the beginning of the research. However, the measurement results showed low response with large variations for the analysis of HPLC. This result might be due to the high viscosity, high molecular weight and bearing hydrogen bonding formation of the NRH and OH groups in the target analytes. In this study, DI-SPME was found to provide a much better increment of peak area response as well as less deviation on triplicate analyses of HPLC. The wavelength for UV measurement of target compounds from 180 nm to 300 nm was scanned in advance, and 215 nm was found to be optimum, in agreement with the study of Jaffer et al. [19]. The chromatogram of DI-SPME followed by HPLC measurement is shown in Fig. 2.

The stocks of TEGDMA, UDMA and Bis-GMA were spiked in a 4 mL saliva matrix at a concentration of 10 μg/mL for fiber selection study. The most suitable fiber was determined based on the optimum measurement response, reproducibility, stability and less variations. Among the test fibers, PA (polyacrylate) is good for polar compounds, and the other three fibers are bearing with bipolar properties. A previous study used the fiber of DCP (DVB/CAR/PDMS) followed by GC–MS measurement for a dental composite with molecular weight less than 286 g/mol [11]. As shown in Fig. 3, the DCP fiber provided the highest area counts and the largest variations. Fig. 3 also indicates that the fiber coating of PD (PDMS/DVB) revealed the second-highest area responses with smaller deviations as compared with DCP. For the PD fiber coating, PDMS is

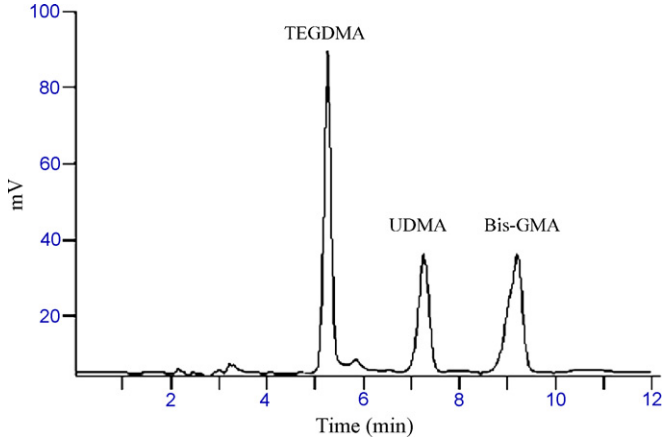

Fig. 2. Liquid chromatogram of dentin materials.

blended with porous solid particles DVB. Therefore, the PD fiber coating has a large specific surface area and can be used to extract polar analytes [20]. In order to achieve better precision, the PD fiber was used for SPME in this study.

3.2. Effects of agitation and pH on extraction

An effective sample agitation can reduce the radius of the boundary layer around the fiber of SPME, thus, the equilibration time is decreased. In addition, a magnetic stirring is needed for the biological sample using the direct SPME analysis because of a higher viscosity of the sample matrix [13]. The application of magnetic stirring for SPME extraction efficiency was evaluated in this study. Fig. 4 shows that the area counts of HPLC were increased with the stirring speeds of 0–800 rpm for UDMA and Bis-GMA. However, the standard deviations of the area count were significantly increased at stirring speeds of 500 and 800 rpm. For TEGDMA, a maximum area count of HPLC was found at 200 rpm, and the standard deviations were not significantly different ($p = 0.57$) from 0 to 800 rpm. In order to reach a reproducible analytical system, a stirring rate of 200 rpm was employed in the present study.

The pH effect on SPME extraction efficiency was evaluated at pH of 3, 5, 7.65, 9 and 11, respectively. Fig. 5 indicates that the maximum peak area of HPLC was obtained at pH 7.65

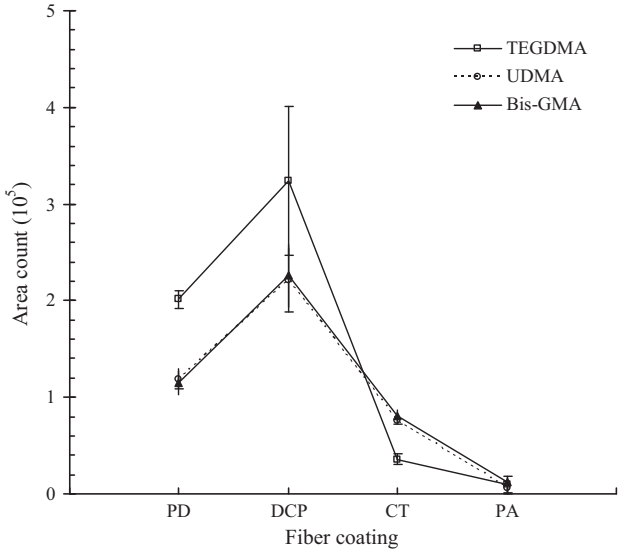

Fig. 3. Selection of fiber coatings for SPME.

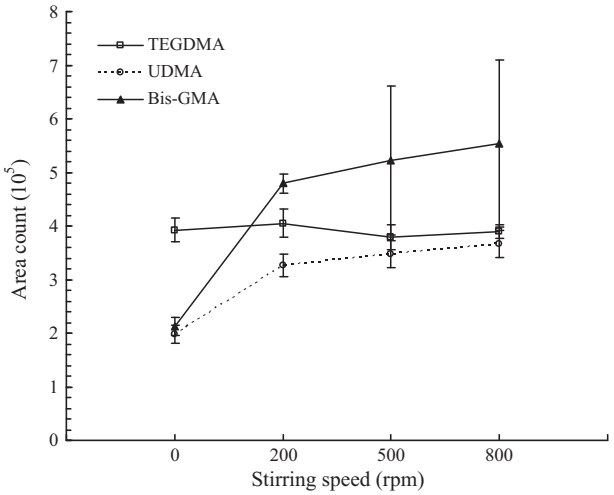

Fig. 4. Evaluation of sample revolution rates.

(the original pH of blank saliva), and the standard deviations were lower as compared with the results with other pH values. However, in the case of the higher pH value, such as 9 and 11, of a more alkaline environment, the peak areas of HPLC dropped tremendously. It is speculated that the sample was dissociated in a basic solution. Furthermore, it should be noted that a more acidic or basic sample matrix might damage the fiber coating of SPME [21]. In this study, the saliva matrix was analyzed without pH adjustment, *i.e.* a nearly neutral environment.

3.3. Extraction and desorption investigation

The sample extraction time for direct immersion of PD fibers was investigated from 5 to 25 min. Fig. 6 shows the average HPLC peak areas for TEGDMA, UDMA and Bis-GMA performed at different SPME extraction times. After 20 min, the peak areas of HPLC became approximately constant, *i.e.*, having less than 5% variations, indicating that SPME reached sorption equilibrium in the samples. Therefore, the SPME extraction time was 20 min for the analytes of this study system.

The optimum desorption solvent for the PD fiber was investigated using the mobile phase (acetonitrile–water in 65:35), acetonitrile, and ethanol, respectively. The analytical precision was the criterion for judgment, and the mobile phase solvent system was found to provide the most stable desorption efficiency and least

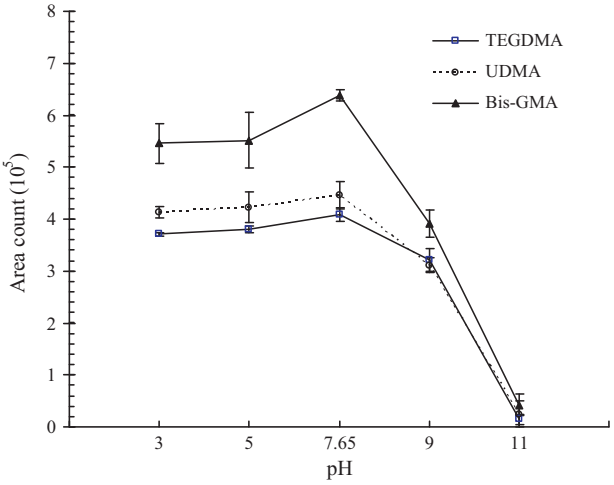

Fig. 5. Effect of pH on extraction efficiency of dentin materials.

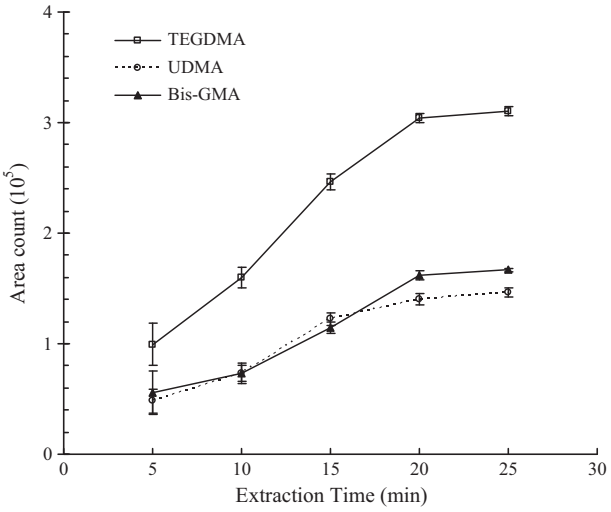

Fig. 6. SPME extraction time of dentin materials for HPLC analysis.

deviation (<5.0% RSD) on triplicate analyses at a concentration of 10 μg/mL for all three dentin components. The mobile phase is an appropriate solution for desorption and can be performed in the dynamic mode with a flowing mobile phase [22]. As a result, desorption solvent employed for sample analyses was the same as the mobile phase solvent system in this study.

After the dentin materials were extracted onto the PD fiber, the appropriate desorption time for HPLC measurement needed to be determined. The static solvent desorption mode was performed online at the injection chamber of HPLC. Desorption time for SPME in HPLC was determined by the desorption efficiency experiments. The extracted analytes onto the PD fiber were analyzed in three consecutive runs. The ratio of area counts from the first run and the sum of all three runs were calculated as the desorption efficiency. The desorption efficiency was evaluated to also investigate whether the carryover was present.

Desorption time periods of 5, 10, 15 and 20 min were conducted. While SPME fiber was desorbed for 15 min, the desorption efficiency for each compound was more than 95%, and no significant peak area was observed from the third run. Therefore, the desorption time of 15 min for PD fibers was selected herein for the SPME followed by HPLC analysis. It should be noted that the leftover analytes in the fiber from the previous run may have been subsequently desorbed and may have given rise to overestimation of the target analyte concentration of the sample. In this study, the fiber conditioning between runs was consistently performed by dipping in the HPLC injection port for 2 min at the end of each analysis to make sure there was no carryover or cross-contamination throughout the whole study.

3.4. Validation of the DI-SPME method

The calibration range for the DI-SPME method was 0.30–300 μg/mL, and the linear regression coefficients (r) were greater than 0.998 for three studied compounds. For quality control assessment of the DI-SPME method, both intra- and inter-day assays were performed at two different concentrations within the calibration ranges of the analytes. As shown in Table 1, the overall average precision (CV %) was in a range of 2.30–8.15%, and accuracy (recovery %) was in a range of 96.1–101.2% through the analysis of quality control samples. The DI-SPME method could appropriately measure the monomers eluted from dentin resin composites in both saliva and ethanol matrices. The concentration ranges might be extended to three orders of magnitude with good precision and accuracy, as shown in Table 1.

Table 1
Precision and accuracy of DI-SPME method for monomers.

	TEGDMA	UDMA	Bis-GMA
Intra-assay precision (CV%)			
QC1	5.42	4.25	8.15
QC2	2.81	3.06	3.68
Inter-assay precision (CV%)			
QC1	5.57	4.80	5.13
QC2	2.84	2.30	2.65
Overall recovery (%)			
QC1	99.0	99.5	101.2
QC2	99.3	98.9	96.1
LoD (μg/mL)	0.03	0.27	0.06

Method validation was performed on standards dissolved in blank saliva, and a correlation was made between this DI-SPME method and the liquid injection method with no significant differences ($p > 0.2$). Both the DI-SPME and direct injection methods showed fairly good precision and accuracy. However, the DI-SPME method provided a lower concentration for sample analyses (about tenfold lower) in comparison with the liquid injection method. The method detection limit of the DI-SPME method was 0.03, 0.27 and 0.06 μg/mL for TEGDMA, UDMA and Bis-GMA, respectively. It has been reported that monomers of TEGDMA, UDMA and Bis-GMA leach out from sealants in minor quantities [8,9]. Therefore, the DI-SPME method developed herein can be a more sensitive method with less sample pretreatment procedures for the dentin monomer analysis.

One of the important concerns pertaining to the DI-SPME technique is attributed to matrix interferences through direct contact of fiber with the biological sample. The fiber surface should suffer contamination by complex endogenous substances and from the formation of a diffusion barrier due to clotted proteins. In the present study, each fiber was repeatedly used more than ninety times by following normal operation procedures and scrupulous fiber condition after each analysis. The effect of matrix interferences on SPME fiber can be neglected herein.

3.5. Leaching test on commercial dentin composites

In order to attest to the applicability of the DI-SPME method, five commercial dentin resins in different combinations of monomers were employed for the leaching test where terpolymer product 1 contained TEGDMA, UDMA and Bis-GMA; copolymer product 2 and product 3 contained TEGDMA and Bis-GMA from two different brands; copolymer product 4 contained TEGDMA and UDMA; and product 5 contained only UDMA monomer. The US FDA recommends a 75% (v/v) ethanol–water solution for the clinically relevant food/oral simulating liquid that several researchers used as an extraction solvent [14]. Atkinson et al. suggested that an oral fluid should be included for *in vitro* testing of the dental materials [23]. Therefore, leaching tests were conducted herein using two different matrices, *i.e.*, saliva and a 75% ethanol solution, respectively.

The results on leaching characteristics of composites in matrices of saliva and 75% ethanol solution are shown in Table 2. Most of the eluted monomers were detected in both the saliva and ethanol solution on days 1 and 3, and they could not be detected in saliva after the 5-day incubation period. TEGDMA in product 2 was detected only for 1-day incubation in saliva. UDMA in product 5 was not detected in saliva even for 1-day immersion. It is speculated that the dental materials interacted with the oral fluid, which may contain various salivary enzyme, bacterial protease and other components. Therefore, the breakdown interactions of the resin composites would proceed in the saliva matrix [23]. This result was

Table 2
Amount of different monomers eluted from commercial resin products.

Sample	Constituent	1 d		3 d		5 d	7 d
		Saliva	EtOH	Saliva	EtOH	EtOH	EtOH
Product 1	TEGDMA	0.006 ± 0.001	0.006 ± 0.001	0.015 ± 0.004	0.048 ± 0.003	0.097 ± 0.006	0.140 ± 0.007
	UDMA	0.041 ± 0.005	0.041 ± 0.001	0.041 ± 0.005	0.088 ± 0.007	0.285 ± 0.009	0.347 ± 0.008
	Bis-GMA	0.036 ± 0.011	0.036 ± 0.001	0.068 ± 0.011	0.182 ± 0.009	0.442 ± 0.010	0.543 ± 0.005
Product 2	TEGDMA	0.186 ± 0.033	0.246 ± 0.022	ND	0.493 ± 0.027	0.744 ± 0.023	0.996 ± 0.026
	Bis-GMA	0.081 ± 0.018	0.101 ± 0.013	0.171 ± 0.009	0.319 ± 0.011	0.747 ± 0.073	0.765 ± 0.083
Product 3	TEGDMA	0.028 ± 0.006	0.031 ± 0.002	0.063 ± 0.004	0.103 ± 0.004	0.251 ± 0.003	0.354 ± 0.005
	Bis-GMA	0.010 ± 0.001	0.011 ± 0.001	0.030 ± 0.003	0.027 ± 0.003	0.204 ± 0.007	0.331 ± 0.010
Product 4	TEGDMA	0.014 ± 0.005	0.010 ± 0.001	0.013 ± 0.002	0.036 ± 0.002	0.058 ± 0.002	0.078 ± 0.003
	UDMA	0.061 ± 0.014	0.073 ± 0.005	0.046 ± 0.005	0.103 ± 0.009	0.159 ± 0.012	0.615 ± 0.040
Product 5	UDMA	ND	0.033 ± 0.001	ND	0.070 ± 0.003	0.124 ± 0.008	1.341 ± 0.029

Amount eluted (wt%): mean ± standard deviation of triplicate analyses.
ND: not detected.
Product 1: Tetric Ceram (Ivoclar Vivadent AG, Schaan, Liechtenstein); Product 2: Palfique Estelite (Tokuyama dental Corp., Tokyo, Japan); Product 3: Spectrum (Dentsply DeTrey GmbH, Konstanz, Germany); Product 4: Compoglass flow (Ivoclar Vivadent AG, Schaan, Liechtenstein); Product 5: Dyract (Dentsply DeTrey GmbH, Konstanz, Germany).

in agreement with the study of Spahl et al. who found that UDMA was identified in minor concentrations in aqueous extracts [8]. In addition, Atkinson et al. indicated that hydrolytic salivary enzymes promoted increases in methacrylate-based resin wear [23].

As shown in Table 2, the weight percent leached for all target products was larger in the ethanol solution than those in the saliva matrix. Several researchers have indicated that 75% ethanol solution has a good ability to swell the polymer network consisting of TEGDMA, UDMA and Bis-GMA monomer units, and could promote the eluting of unreacted monomers from bulk resins [8,23].

Several studies reported that unbound monomers and additives are easily extracted by saliva and/or dietary solvents during the first 24 h after polymerization [5,14]. As shown in Table 2, different brands of composites leached at different amount percentages for individual resin components. The longer the time incubated, the larger the amount was eluted.

3.6. Sorption kinetic of DI-SPME

For the DI-SPME method, the analyte is transferred by diffusion through the bulk solution and then into the fiber coating [24]. Under ideal agitation conditions, the effect of diffusion of analytes in the bulk solution can be negligible. Mass transfer of the analyte into the fiber coating of SPME can be described by the solution-diffusion model. The analyte is first adsorbed onto the surface of the fiber and then diffused into the fiber [25]. The polymer fiber is coated onto the surface of the silica fiber of SPME as a hollow cylinder. Because the thickness of the fiber coating L is very thin, it can be assumed as a plate. Hence, the diffusion of the analyte into the fiber coating can be expressed by Fick's law as follows:

$$\frac{\partial C_f}{\partial t} = D_f \frac{\partial^2 C_f}{\partial x_f^2} \tag{1}$$

where D_f is the diffusion coefficient ($L^2\,T^{-1}$) of the analyte in the fiber coating; C_f is the analyte concentration in the fiber ($M\,L^{-3}$); and x_f is the distance along the direction of diffusion (L).

Prior to the extraction of the sample, the concentration of the analyte in the fiber coating is zero. The analyte molecules diffuse into the fiber, but they cannot enter into the center of SPME. For a fiber coating of thickness L, the initial and boundary conditions of Eq. (1) are as follows:

$$C_f = 0, \quad 0 \le x_f \le L, \quad t = 0$$

$$\frac{\partial C_f}{\partial t} = 0, \quad x_f = L, \quad t > 0$$

As the fiber coating is far smaller than the sample matrix in terms of volume, the analyte concentration in the fiber, C_f ($M\,L^{-3}$), can be represented as follows:

$$\frac{C_f}{C_\infty} = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left[-D_f(2n+1)^2\pi^2\frac{t}{L^2}\right]\cos\frac{(2n+1)\pi x_f}{L} \tag{2}$$

where C_∞ is the analyte concentration in the fiber coating when the sorption reaches equilibrium ($M\,L^{-3}$).

From Eq. (2), the mass of the analyte sorbed in the fiber coating, M_t (M), can be written as follows:

$$C_t = \frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-D(2n+1)^2\pi^2\frac{t}{L^2}\right] \tag{3}$$

where M_∞ is the mass of the analyte sorbed in the fiber at equilibrium.

The sorption curve of the analyte in the fiber coating is determined by plotting C_t against (Time)$^{0.5}$. According to Eq. (3), the

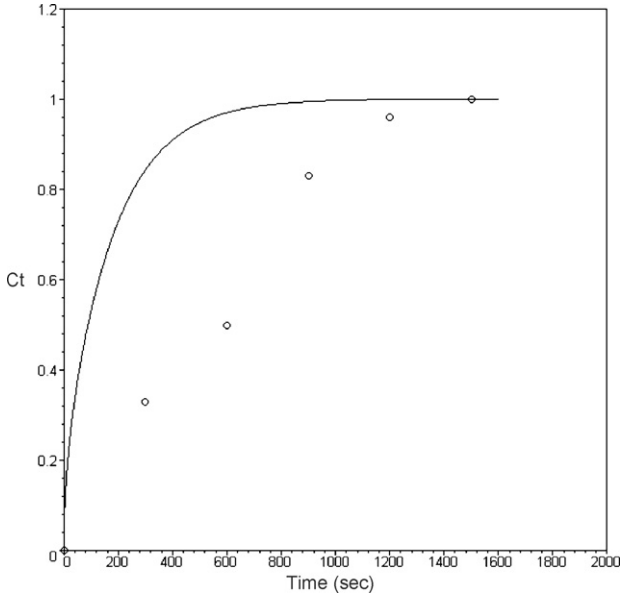

Fig. 7. Simulations of UDMA for HPLC analysis.

diffusion coefficient of the analyte in the fiber coating can be determined by the sorption curve and the following equation [26]:

$$D_f = \pi \left(\frac{L\theta}{4} \right)^2 \quad (4)$$

where θ is the slope of the initial linear line of the sorption curve.

Using the experimental results of Fig. 6, the diffusion coefficient of UDMA in the PD coating was 2.6×10^{-9} cm^2/s as determined by Eq. (4). The sorption concentrations of UDMA in PD/SPME were further obtained by substituting D_f into Eq. (3). As shown in Fig. 7, D_f obtained by Eq. (4) was unable to model the UDMA concentrations of DI-SPME except for the equilibrium concentrations. Fig. 7 indicates that the experimental concentrations of UDMA were obviously lower than the modeling results. This discrepancy implied that the outside surface of PD coating may not be in equilibration with UDMA in the sample matrix within an extraction time of 20 min. In addition, plasticization of the PD coating can enhance the swelling of the SMPE fiber. As shown in Fig. 7, the observed deviation may be due to neglecting the effects of increase in thickness of the SPME fiber in the modeling equations. For a practical purpose, the extraction time for SPME can be determined by solving Eq. (3) as setting $C_t = 0.95$ [21]. However, the values of D_f should be obtained first.

4. Conclusions

In this study, a DI-SPME-HPLC method with PDMS/DVB fiber coating was developed to analyze the leaching of dental composite resins in saliva. The sample vial was agitated at a rate of 200 rpm and at 40 °C during the period of extraction. The operation conditions were optimized to be SPME extraction time of 20 min and desorption time of 15 min. The calibration curve was extended over the range of 0.30–300 μg/mL, and the limits of detection showed more than a 10-fold greater sensitivity compared to the direct injection method. The DI-SPME method provided reproducible and quantifiable results for measuring multiple components of dentin resins in the saliva matrix. The DI-SPME-HPLC method established herein would be a simple, efficient and practical approach to analyze the resins elution of denture wearers.

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