

ORIGINAL ARTICLE

Ultrasonic cleaning reduces the residual monomer in acrylic resins



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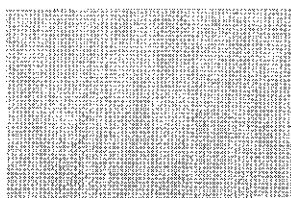
Abstract *Background/purpose:* The residual monomer remaining in acrylic resin can cause an allergic reaction and is toxic to oral soft tissue. This study determined the effect of the duration of ultrasonic cleaning on the amount of residual methyl methacrylate monomer in one heat-polymerized acrylic resin, Meliodent, and three autopolymerized acrylic resins, Unifast Trad Ivory, Unifast Trad Pink, and Unifast III.

Materials and methods: Thirty-six disc-shaped specimens of each brand were prepared and randomly divided into six groups: control (no treatment), positive control, and ultrasonic treatment in 50°C water for 3 minutes, 5 minutes, 10 minutes, or 15 minutes. The residual monomer was extracted and analyzed using high performance liquid chromatography.

Results: There were no significant differences in the residual monomer amount in the Meliodent groups. The amounts of residual monomer in the autopolymerized acrylic resin positive control group and ultrasonic treatment groups were significantly lower than those of the control group for the Unifast Trad Ivory, Unifast Trad Pink, and Unifast III groups ($P < 0.05$). The amount of residual monomer was not significantly different between the ultrasonic treatment in 50°C water (3 minutes for Unifast Trad Pink and 5 minutes for Unifast Trad Ivory and Unifast III) groups and the positive control group ($P > 0.05$).

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Conclusion: Ultrasonic treatment with 50°C water for 3–5 minutes for autopolymerized resin and 3 minutes for heat-polymerized acrylic resin reduced the amount of residual monomer similarly to previously recommended methods, using shorter treatment times. Copyright © 2016, Association for Dental Sciences of the Republic of China. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Acrylic resin is used to fabricate denture bases and provisional restorations. One disadvantage of acrylic resin is residual monomer, the unreacted monomer remaining after polymerization is completed.¹ Several studies have shown the adverse effects of residual monomer on the mechanical and physical properties of acrylic resins, such as decreased impact strength and color instability.^{2–4} Moreover, residual monomer has been demonstrated to be toxic, irritating to oral mucosa, and can cause allergic reactions in oral tissue.^{5–8} According to ISO 20795-1:2013, the maximum residual monomer content should not exceed 2.2% for heat-polymerized acrylic resin and 4.5% for autopolymerized acrylic resin.⁹

Previous studies have suggested methods to reduce the residual monomer content in acrylic resin by changing the polymerization conditions (e.g., increased curing time and temperature, increased power during microwave curing) or using techniques to dry the plaster in the processing flask.^{10–13} Other studies have suggested treating acrylic resin subsequent to polymerization to reduce the residual monomer, or its release from the resin. Immersing the resin in water at 37°C for 24 hours was recommended by Vallittu et al.¹⁴ It has also been shown that immersing acrylic resin in 50°C water for 1 hour reduced residual monomer.¹⁵ These methods have been widely used for heat-polymerized and autopolymerized resins. However, many of the previously recommended methods require increased chair-time and are not practical in clinical or laboratory situations.^{16,17} Thus, a different method of reducing residual monomer would be advantageous.

Ultrasonic waves have been used in many applications, such as cleaning, sonochemistry, medical imaging (ultrasound), underwater acoustics (sonar), and spot welding of metals. In addition, ultrasonic cleaners are widely used in dental clinics for cleaning instruments. Ultrasonic cleaning is the application of high frequency electric energy, which is converted by an ultrasonic transducer into ultrasonic energy. The ultrasonic energy enters the liquid in the ultrasonic cleaner tank, causing the formation, growth, and collapse of microscopic vacuum bubbles, a process known as cavitation. When the bubbles form at a liquid-solid interface collapse, they release energy that removes contamination from a material's surface.¹⁸

Ultrasound treatment has been used to enhance extraction rates and yields since the 1950s.¹⁹ Cravotto et al.²⁰ used ultrasonic treatment to improve the extraction of oils from plants, and found that ultrasound treatment enhanced the release of soluble compounds from plant material, enhanced mass transfer, and facilitated solvent access into the cells. Charasseangpaisarn and

Wiwatwarrapan²¹ found that using an ultrasonic cleaner with different frequencies reduced the residual monomer in acrylic resin to the same extent as previously recommended methods (immersion in room temperature water for heat-polymerized resins or immersion in 50°C water for 1 hour for autopolymerized resins).

The purpose of the present study was to investigate the effect of different ultrasonic treatment times on the amount of residual monomer in heat-polymerized and autopolymerized acrylic resin, compared to a standard method and an untreated control.

Materials and methods

Sample preparation

One brand of heat-polymerized acrylic resin (Meliodent, Heraeus Kulzer, Hanau, Germany) and three different brands of autopolymerized acrylic resin (Unifast Trad Ivory, Unifast Trad Pink, and Unifast III, GC Corporation, Tokyo, Japan) were used in this study (Table 1). Each sample was prepared in a circular stainless steel mold (50 mm in diameter and 3.0 ± 0.1 mm deep) and invested in dental stone in a dental flask. The samples were processed followed the manufacturer's instructions. After processing, the samples were kept in the dark for 24 ± 5 hours prior to grinding and subsequently kept at -28°C to prevent residual monomer evaporation.

The samples were wet ground on both sides with P500 metallographic grinding paper to 2.0 ± 0.1 mm in thickness, and wet-ground with P1200 paper on the edge until smooth (Figure 1A). Thirty-six samples of each brand were prepared and randomly divided into six experimental group. ($n = 6$) treated as follows: Group 1, left untreated (C); Group 2, treated using the previously recommended method of immersion in room temperature water for 24 hours for heat-polymerized acrylic resin or 50°C water for 1 hour for autopolymerized acrylic resin; Groups 3, 4, 5, and 6, treated by immersion in an ultrasonic bath at 50°C for 3 minutes, 5 minutes, 10 minutes, and 15 minutes, respectively. The ultrasonic cleaner (VGT 1990 QTD, Guangdong GT Ultrasonic Industrial Co., Shenzhen, China) was set at 40 kHz and 100 W. The sample discs were stored in the dark for 24 ± 1 hours followed by monomer extraction according to ISO 20795-1:2013.

Residual monomer extraction procedure

Each specimen disc was broken into small pieces, and pieces totaling approximately 650 mg were weighed to

Brand	Powder:Liquid ratio (g/mL)	Manufacturer	Lot No.	Details
Meliodont	2.2:1	Hereaus Kulzer, Hanau, Germany.	Powder: 33May105 Liquid: 140411	Heat-polymerized acrylic resin for denture bases
Unifast Trad (Ivory)	2:1	GC Corporation, Tokyo, Japan.	Powder: 1204133 Liquid: 1202011	Autopolymerized acrylic resin for provisional crowns
Unifast Trad (Pink)	2:1	GC Corporation.	Powder: 1205102 Liquid: 1202011	Autopolymerized acrylic resin for relines or repair of denture bases
Unifast III	2:1	GC Corporation.	Powder: 1303113 Liquid: 1303191	Autopolymerized acrylic resin for provisional crowns

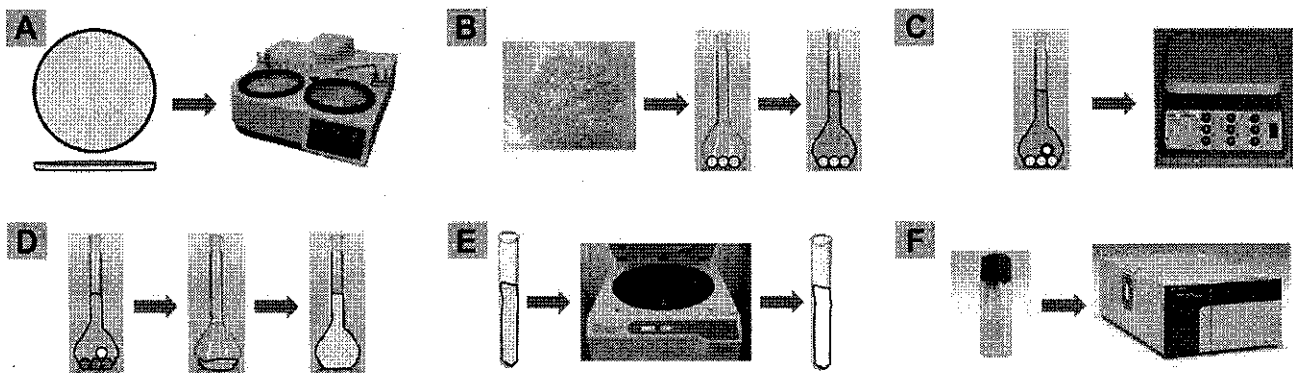


Figure 1 The residual monomer extraction procedures according to ISO 20795-1:2013. (A) The specimens were polished with a polishing machine; (B) the specimens were fragmented, approximately 650 mg were placed into a 10 mL volumetric flask, and tetrahydrofuran diluting solution was added to 10 mL (C) a polytetrafluoroethylene (PTFE) magnetic stirring bar was added and stirred with a magnetic stirring machine for 72 ± 2 hours; (D) supernatant (2 mL) was transferred to another volumetric flask and methanol diluting solution was added to 10 mL; (E) solution (5 mL) was transferred into a glass tube and centrifuged at 1085.7 g at 25°C for 15 minutes; and (F) $1 \mu\text{L}$ of supernatant was injected to the high performance liquid chromatography (HPLC) system.

four decimal places using a digital scale (Sartorius BP110s, Sartorius, Göttingen, Germany), and placed into a 10 mL volumetric flask (Duran, Wertheim/Main, Germany). Tetrahydrofuran diluting solution (Merck KGaA, Darmstadt, Germany) was added to a 10 mL final volume (Figure 1B). Each flask was stirred using a clean 3-mm polytetrafluoroethylene coated magnetic stirring bar (Cowie Technology, Middlesbrough, UK) on a magnetic stirrer (Barnstead PMC 509C, Thermo Fisher Scientific Inc., Waltham, MA, USA) for 72 ± 2 hours at room temperature (Figure 1C). Some 2 mL of the resultant slurry was transferred to another 10 mL volumetric flask, and methanol diluting solution (Merck KGaA) was added to a 10 mL final volume (Figure 1D). Some 5 mL of slurry was transferred using a volumetric pipette into a glass centrifugation tube and centrifuged (Avanti J-E, Beckman Coulter, Indianapolis, IN, USA) at 1085.7 g for 15 minutes at 25°C (Figure 1E). One μL of the supernatant of each sample was analyzed by a high performance liquid chromatography (HPLC) system (Shimadzu 20A Prominence HPLC, Shimadzu Corporation, Kyoto, Japan) using a reverse-phase LC-18 ($5 \mu\text{m}$ particle diameter, 4.6 cm internal diameter \times 15 cm length) analytical column, maintained at 40°C , with a 66% methanol and 34% water isocratic elution. The flow rate

was 1.5 mL/min and the UV wavelength was detected at 205 nm (Figure 1F).

Residual monomer determination

The amount of residual methyl methacrylate (MMA) was determined from a standard calibration curve ($R^2 > 0.99$) that was prepared by plotting the HPLC peaks of known amounts (approximately 6 mg, 60 mg, 150 mg, 300 mg, and 400 mg) of MMA and represented as percent by mass of the specimen. The standard curve was used to determine the concentration of MMA in micrograms, C_{MMA} , per milliliter of analyzed sample solution.

The standard calibration curve was calculated from known concentrations of methyl methacrylate solution (Figure 2), which had the following equation:

$$(R^2 > 0.995): f(x) = (1.38539 \cdot 10^7)x - 372563 \quad (1)$$

where $f(x)$ = absorbance area of MMA by UV detector and x = MMA concentration.

The MMA peak of the sample solutions was identified using the same elution time of a known MMA solution (Figure 3). The total quantity of MMA in the sample

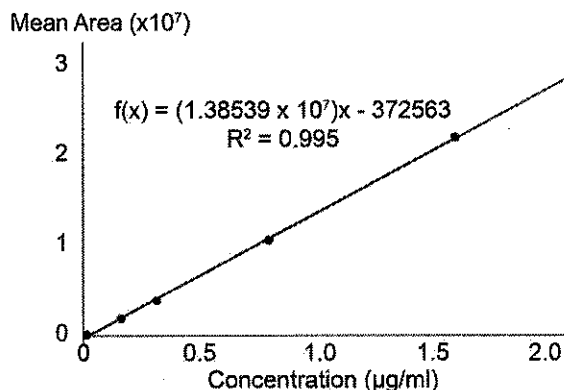


Figure 2 The standard calibration curve of known concentrations of MMA monomer.

solution, m_{MMA} , in micrograms, was calculated according to the following equation:

$$m_{\text{MMA}} = \left[c_{\text{MMA}} \times \left(\frac{10}{2} \right)^* \times 10^{**} \right] \quad (2)$$

where * was the tetrahydrofuran amount (milliliter) and ** the methanol amount (milliliter) used for extraction.

The residual monomer (% mg) was calculated using the following equation:

$$\text{Residual monomer (\%mg)} = \frac{m_{\text{MMA}}}{c_{\text{MMA}}} \times 100 \quad (3)$$

Each specimen was divided into three solutions (9 solutions in total) and the solutions were tested for pass/fail determination of residual monomer.⁹ The three solutions per sample were averaged to generate the representative value of each specimen. Therefore, six values were obtained for each experimental group. The data from the heat-polymerized and autopolymerized acrylic resins were analyzed separately. The heat-polymerized acrylic resin data were analyzed with one-way analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test at a 95% confidence level (SPSS version 17, IBM, Armonk, NY, USA). The autopolymerized acrylic resin data were analyzed with two-way ANOVA followed by Tukey's HSD test at a 95% confidence level. The mean amount of residual monomer was compared between products.

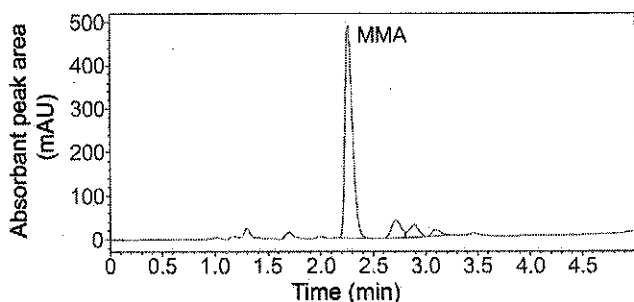


Figure 3 Representative MMA high performance liquid chromatography (HPLC) chromatogram.

Results

Table 2 shows the two-way ANOVA analysis of the amount of residual monomer in the autopolymerized acrylic resin groups. The amount of residual monomer was significantly affected by the type of treatment, brand of acrylic, and interaction between types of treatment and brand ($P < 0.05$).

One-way ANOVA and Tukey's HSD were used to analyze the differences between the groups (Table 3). For the autopolymerized acrylic resins, the amounts of residual monomer in the positive control group of each brand were significantly lower ($P < 0.05$) than that of its control group. The amount of residual monomer in the respective positive control group was not significantly different ($P < 0.05$) from that of the ultrasonic treatment for 5 minutes or 10 minutes Unifast Trad Ivory groups, all ultrasonic treatment Unifast Trad Pink groups, or the ultrasonic treatment at 5 minutes, 10 minutes, or 15 minutes Unifast III groups. There were no significant differences between the amount of residual monomer in the control groups of each brand ($P > 0.05$). However, the Unifast III positive control group demonstrated significantly less residual monomer than that of the Unifast Trad Ivory and Pink positive control groups ($P < 0.05$).

Table 3 also shows the one-way ANOVA analysis and Tukey's HSD of the amount of residual monomer in the heat-polymerized acrylic resin groups. The amount of residual monomer was not significantly different between the groups ($P > 0.05$).

Discussion

Residual MMA monomer in acrylic resin is toxic to oral tissues and acts as a plasticizer, affecting the mechanical and physical properties of the resin.^{2-4,22,23} The International Organization for Standardization (ISO) has defined the maximum allowable amount of residual monomer in acrylic resin in ISO 20795-1:2013, classified by type of resin. The materials in the present study were MMA-based heat-polymerized acrylic resin (Meliodent) and

Table 2 Two-way ANOVA analysis of the amount of residual monomer in autopolymerized acrylic resin.

Source	Type III sum of Squares	df	Mean Square	F	P
Corrected model	29.449 ^a	17	1.732	26.977	< 0.001
Intercept	345.120	1	345.120	5.375E3	< 0.001
Brand	8.220	2	4.110	64.007	< 0.001
Treatment	17.870	5	3.574	55.656	< 0.001
Brand* treatment	3.359	10	0.336	5.231	< 0.001
Error	5.779	90	0.064		
Total	380.348	108			
Corrected total	35.228	107			

ANOVA = analysis of variance.

^a $R^2 = 0.936$ (adjusted $R^2 = 0.805$).

Table 3 The mean amount of residual monomer (mg%) with standard deviation and Tukey's HSD analysis.^{a,b}

	Autopolymerized			Heat-polymerized
	Unifast Ivory	Unifast Pink	Unifast III	Meliodent
Control	2.87 (0.44) ^G	2.45 (0.20) ^{F,G}	2.43 (0.28) ^{F,G}	1.62 (0.14) ^a
Positive control	1.65 (0.17) ^{C,D}	1.54 (0.14) ^{C,D}	0.96 (0.13) ^A	1.54 (0.29) ^a
Ultrasonic 3 minutes	2.58 (0.49) ^{F,G}	1.76 (0.36) ^{C,D}	1.51 (0.90) ^{B,C,D}	1.66 (0.22) ^a
Ultrasonic 5 minutes	1.40 (0.27) ^{A,B,C,D}	1.59 (0.20) ^{C,D}	1.36 (0.16) ^{A,B,C}	1.72 (0.06) ^a
Ultrasonic 10 minutes	1.90 (0.18) ^{D,E}	1.71 (0.26) ^{C,D}	1.00 (0.10) ^{A,B}	1.59 (0.09) ^a
Ultrasonic 15 minutes	2.32 (0.24) ^{E,F}	1.74 (0.30) ^{C,D}	1.39 (0.16) ^{A,B,C,D}	1.55 (0.07) ^a

^a The groups with identical letters were not significantly different ($P > 0.05$).

^b The upper and lower case letters were separate analyses.

autopolymerized acrylic resins (Unifast Trad Ivory, Unifast Trad Pink, and Unifast III). The mean amount of residual monomer of Meliodent was lower than the 2.2 mg% for heat-polymerized acrylic resin and the residual monomer in the other groups was lower than the 4.5 mg% for autopolymerized acrylic resin as required by ISO. In the control group, heat-polymerized acrylic resin showed less residual monomer compared with autopolymerized acrylic resin as reported in previous studies.^{14,15,24} In the present study, when the acrylic resin was treated by the ultrasonic method with suitable timing, the amount of residual monomer in the heat-polymerized and autopolymerized acrylic resins was not different. In the autopolymerized resin groups, the Unifast III groups demonstrated the lowest residual monomer, most likely because the Unifast III liquid is lower in MMA (91%) compared with the Unifast Trad liquid (> 95%).

Previous studies have investigated the release of residual monomer from acrylic resin into the environment and that remaining in the resin.^{8,10,14,15,25} The present study focused on the residual monomer, using the method for residual monomer determination described by ISO. The amount of residual monomer in autopolymerized acrylic resin was significantly higher in the control group of each brand compared with its positive control group (immersion in 50°C water for 1 hour), as reported by Tsuchiya et al,¹⁵ who recommended this method to reduce the risk of adverse reaction of oral tissue to residual monomer. The amount of residual monomer in the ultrasonic treatment for various times groups was also significantly lower than that of their respective control groups. The appropriate time for ultrasonic treatment was determined by the ultrasonic treatment group that required the least time that resulted in an amount of residual monomer that was not significantly different from that of the positive control group. These findings indicate that ultrasonic treatment of autopolymerized acrylic resin for 3 minutes for the denture base repair material, Unifast Trad Pink, and 5 minutes for the provisional crown materials, Unifast Trad Ivory and Unifast III, was sufficient to reduce residual monomer to the same level as immersion in 50°C water for 1 hour. The results showed that the amount of residual monomer tended to increase as the time of ultrasonic treatment increased. This may be because longer ultrasonic treatment time resulted in greater cavitation energy and caused the depolymerization of the polymer chains, as was reported in previous studies.^{26,27}

In contrast with autopolymerized acrylic resin, the mean amount of residual monomer in the heat-polymerized acrylic resin control group was not significantly different from immersion in room temperature water for 24 hours, as found by Vallittu et al.¹⁴ This residual monomer reduction method was recommended because the leaching of residual monomer from acrylic resin was significantly higher in the first 24 hours. The reduction method of residual monomer by ultrasonic treatment was also not significantly different from the control group, as found by Charasseangpaisarn and Wiwatwarrapan.²¹ The remaining residual monomer in heat-polymerized acrylic resin that could not be extracted can be explained by the non-extractable monomer theory that residual monomer is still trapped in long polymer chain molecules after various monomer reduction treatments.²⁸

Ultrasonic treatment may affect the amount of residual monomer in acrylic resin in two ways. First, ultrasonic treatment may enhance the extraction rate of the residual monomer from the resin. Second, ultrasonic treatment may cause postpolymerization of the residual monomer. A limitation of our study is that the precise mechanism by which ultrasonic treatment affected the residual monomer was not identified. Further studies should investigate the amount of residual monomer that was released from the acrylic resin by ultrasonic treatment compared with previously recommended methods. Moreover, the physical properties of acrylic resin, such as the mechanical or optical properties after ultrasonic treatment, should be determined due to the negative correlation between the amount of residual monomer and the properties of acrylic resin. Future studies should also investigate the degree of conversion, surface hardness, and fracture toughness of the resin before and after ultrasonic treatment to confirm that depolymerization did occur at the surface of the resin.

In conclusion, the minimum time required for ultrasonic treatment at 40 kHz in 50°C water to reduce the amount of residual monomer in heat-polymerized acrylic resin (Meliodent), relin or repair autopolymerized acrylic resin (Unifast Trad Pink), and autopolymerized provisional crown resin (Unifast Trad Ivory and Unifast III) is 10 minutes, 3 minutes, and 5 minutes respectively. Ultrasonic treatment is an innovative method for reducing the residual monomer remaining in acrylic resin with the advantage of being practical and achievable, requiring less chair time and laboratory time.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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