



Effect of processing and curing procedures on residual monomer levels of denture base materials

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Abstract

Objectives: To investigate whether processing and curing procedures have an effect on the residual monomer levels of denture base polymers.

Materials and methods: Three denture base polymers, polymerized by two different processing techniques; injection molding technique (SR Ivocap High Impact), compression molding technique (SR Triplex Hot and Probase Hot) were selected. Six disc-shaped specimens of each brand, with diameter of 50 mm and 3 mm thick, were prepared from separate mixes. The mixing ratios and processing methods were achieved according to the manufacturer's instruction. Each brand was polymerized by both long and short curing procedures. Residual monomer was extracted from the ground specimen and determined by gas chromatography following ISO 20795-1:2013[E]. Two-way ANOVA was used to analyze the data at $\alpha=0.05$.

Results: There was a significant difference among the three brands. SR Ivocap showed the significantly highest residual monomer level, followed by SR Triplex Hot, and Probase Hot, respectively. In terms of curing procedure, the residual monomer levels of SR Ivocap and SR Triplex Hot was significantly greater in short cured group than long cured group.

Conclusions: The injection molding material had a higher residual monomer level than the compression molding material. Longer curing procedure was able to reduce the residual monomer level.

Keywords: compression molding technique, denture base material, gas chromatography, Injection molding technique, PMMA, residual monomer

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Introduction

Poly (methyl methacrylate) or PMMA is usually used to fabricate denture bases for removable prostheses, artificial teeth, custom impression trays, provisional restorations, maxillofacial appliances, and also used as esthetic tissue replacement for severe gingival recession.^{1,2} From the polymerization reaction of PMMA, not all monomers are able to convert to polymer. Some monomer remain unreacted and become trapped within the polymer mass that is called "residual monomer". The unreacted residual monomer may alter the properties and stability of the final polymer and also result in many adverse effects with poor biocompatibility that is allergic reactions, or chemical burns.^{3,4,5} The unreacted residual monomer acts as a plasticizer in the denture base. After polymerization, the residual monomer initially adversely affects the physical and mechanical properties of the materials^{6,7} until the monomer leaches out over several days or weeks.² As the monomer is leached out from the denture, the modulus of elasticity of the denture increases.⁵ Many other mechanical properties of denture base are also affected by the amount of residual monomer, for example, residual monomer can greatly reduce the glass transition temperature⁸, increase the flexibility of the material, minimize the tensile strength, increase water sorption^{7,9,10}, and also spoil the appearance of the denture base polymer¹¹ as the porosity is prone to collecting stains, debris and microorganisms.²

Unreacted MMA is toxic to cell culture. Sheridan et al.¹² found that the greatest cytotoxic effect occurred at first 24 hours, and less impaction was observed as specimen immersion time increased. Residual monomer also triggers allergic reactions in individual hypersensitivity to methyl methacrylates, usually a delayed-type reaction^{4,5}, irritates the mucosa and causes inflammation, or burning mouth syndrome^{2,4,11,13}

by leaking into surrounding tissues.¹⁴ Clinical signs and symptoms of irritated mucosa are always reported as erythema, erosion, or even a burning sensation on the oral mucosa.¹³ The release of MMA into the oral cavity may be affected by the oxidation reaction that converts MMA into formaldehyde, and hydrolysis reaction that converts MMA into methacrylic acid.¹⁵ These toxic substances may alter their cytotoxic potential.¹²

At the present time, there are many modifications of the polymerization method to achieve minimal residual monomer contents. Furthermore, the curing process should be carefully regulated to avoid the effects of uncontrolled temperature.¹⁶ Different polymerization methods and curing conditions have an effect on residual monomer contents.¹⁷ The level of residual monomer can be minimized by heating the resin more gently¹⁶, extending curing time, and applying higher temperature or terminal boiling.^{9,14,18,19,20} One of those methods to minimize porosity is pressure and temperature control.²¹

Poly (methyl methacrylate) used as denture base materials in this investigation can be processed by more than one technique, as recommended by the manufacturer. Hypothesized that different processing techniques and curing procedures may have an effect on the amount of residual monomer, the aim of this study was to investigate the effect of processing and curing procedures on the residual monomer levels in denture base materials.

In this present study, the residual monomer levels among the three brands of denture base materials which were processed differently (i.e. compression molding vs injection molding techniques) were compared. In addition, the comparison of the residual monomer levels between long curing and short curing procedures of denture base materials were investigated.

Materials and methods

Three commercial denture base polymer products were used in this investigation. The batch number, manufacturers, processing procedures, mixing proportions of powder to liquid, mixing and working time, and curing procedures of these commercial denture base products are listed in Table 1.

Preparation of test specimen disc

Stainless steel molds were mounted with dental stone into the denture flasks of both conventional compression and injection molding techniques following the manufacturer instruction manuals. For each group, six specimens (a diameter of 50 mm and a thickness of 3 mm) were prepared from separate mixes. Powder to liquid ratio is presented in Table 1. Then, they were properly polymerized by long curing procedure and short curing procedure as shown in Table 1. The specimens were kept in the dark chamber in a laboratory environment for 24 hours. The specimen discs were wet-grinded under running water for cooling and lubricating

at both sides with the metallographic grinding papers [with a grain size of approximately 30 μm (P500) and 15 μm (P1200)] until the 2- mm thick specimens were obtained. They were stored in the dark chamber in a laboratory environment for 24 hours prior to monomer extraction.

Extraction of monomer

Three sample solutions were taken from each test specimen disc. Each sample solution was prepared by weighing approximately 650 mg from the specimen disc, which was broken into small grains by cutting with the nipper, into a closable 10 ml volumetric glass flask. Acetone diluting solution was added, then agitated for 72 hours at room temperature. A 2 ml aliquot of each previously prepared sample solution was transferred to each separate one-mark closable 10 ml volumetric glass flask, then 100 μl of the Internal Standard (I.S.) solution was added, and the flask was filled up by methanol solution. After that, 5 ml of the polymer and monomer containing slurry was transferred to centrifuge (Centrifuge, Beckman

Table 1 Materials used in this investigation

Material	Manufacturer	Processing procedure	Powder-liquid ratio	Mixing time	Working time	Curing procedure
SR Triplex Hot® (Batch No. R32692)	Ivoclar Vivadent Schaan, Lichtenstein	Heat-cured compression molding	2.34g : 1ml	10 min. (including time for leaving mixture to become dough stage)	20 min.	<i>Long curing procedure</i> Started at room temp., heated up to 100°C (within 1 h.), and boiled for 45 min. <i>Short curing procedure</i> Started at 100°C, and boiled for 20 min.
ProBase Hot® (Batch No. R48140)	Ivoclar Vivadent, Schaan, Lichtenstein	Heat-cured compression molding	2.25g : 1ml	10 min. (including time for leaving mixture to become dough stage)	20 min.	<i>Long curing procedure</i> Started at room temp., heated up to 100°C (within 1 h.), and boiled for 45 min. <i>Short curing procedure</i> Started at 100°C, and boiled for 40 min.
SR Ivocap High I mpact® (Batch No. R35688)	Ivoclar Vivadent Schaan, Lichtenstein	Heat-cured Injection-molding	0.67g : 1ml	5 min.	10 min.	<i>Long curing procedure</i> Started at 100°C and boiled for 90 min. <i>Short curing procedure</i> Started at 100°C, and boiled for 35 min.

J2-21, Beckman, California, USA) at $3000 \times g_n$ m/s² for 15 minutes. Then, a 3 ml aliquot of each centrifuged solution was ready for injection to the tube of gas chromatography machine.

Preparation of standard solution

Five standard solutions with concentrations 120, 1200, 3000, 6000, and 8000 ppm of MMA monomer were prepared with 100 µl of the I.S. solution, and injected to the tube of gas chromatography machine.

Gas chromatography

An Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Delaware, USA) equipped with a flame ionization detector (FID) system was used. The injection port for samples was in the split mode (1:20) with the temperature maintained at 200°C. The carrier gas (Helium) had a flow rate of approximately 1 ml/min. The detection was carried out by a flame ionization detector with the temperature of 200°C and the ratio of H₂/air flow at 45/450 ml/min.

Gas chromatogram of standard solution

The peaks of gas chromatogram were evaluated, and the retention time of MMA and I.S. was determined. A scatter plot was made by plotting the concentrations of the standard solutions and the ratios of the peak area of A'_{MMA} to A'_{I.S.} of five standard solutions (Figure 1).

From a scatter plot, a regression line between $\frac{A'_{MMA}}{A'_{I.S.}}$ and concentration of MMA was drawn.

The equation of this regression line is $y = 0.0015x$**Equation (1)**

Determination of residual monomer in sample solutions

The peak areas of MMA and I.S. of each sample solution were recorded when the sample solutions were injected into the column of gas chromatography machine. The concentration of MMA (µg/ml) or c_{MMA} in the sample solution was calculated using equation of regression line (Equation 1).

c_{MMA} was used to calculate the total amount of MMA in the sample solution (m_{MMA}) in micrograms (µg) using Equation (2).

$$m_{MMA} = \left[c_{MMA} \times \left[\frac{10}{2} \right] \times 10 \right] \dots\dots\dots \text{Equation (2)}$$

Subsequently, m_{MMA} was used to compute the percentage of residual monomer by Equation (3).

$$\text{Residual monomer (\%)} = \frac{m_{MMA}}{m_{SAMPLE}} \times 100 \dots\dots\dots \text{Equation (3)}$$

Where m_{SAMPLE} was the mass of sample, in micrograms (µg). The determination of the percentage residual monomer was performed as a dependent variable, while the processing

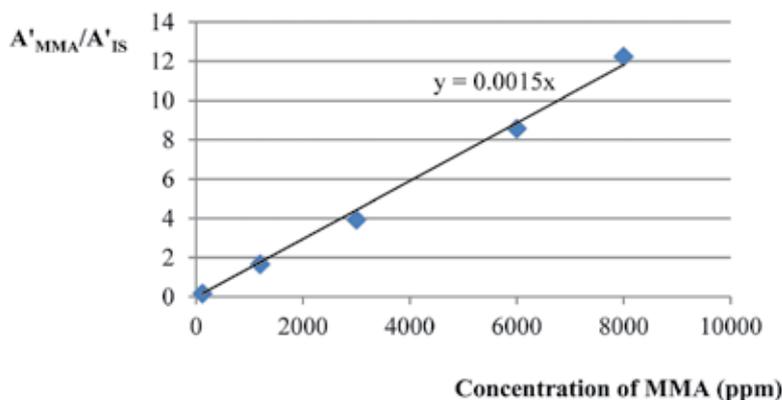


Figure 1 Concentration of MMA vs. $\frac{A'_{MMA}}{A'_{I.S.}}$ ratios of five standard solutions

techniques and curing procedures acted as independent variables. Mean residual monomer value of each specimen disc was then calculated from the three sample solutions.

After the determination of the levels of residual monomer was complete, normality of the data was determined by the Shapiro-Wilk test and the homogeneity of variances was carried out using Levene's test. After that, two-way analysis of variances (ANOVA) was done at $\alpha = 0.05$ to compare the effect of the two factors. The statistical analysis was done by SPSS for windows version 16.0.²²

Results

The mean and standard deviation (S.D.) of residual monomer levels are presented in Table 2.

Data distribution was normal ($p > 0.05$). Two-way ANOVA (at $\alpha = 0.05$) revealed that there was statistically significant interaction ($p < 0.05$) between the two factors in affecting the residual monomer concentration as shown in Table 3. Then, simple effect analysis was done. It was found that in terms of long curing

and short curing procedures, there was a statistical significant mean effect difference among the three brands. From the view of brands of material, there was a significant mean effect difference between short and long curing procedures in SR Triplex Hot and SR Ivocap, but not in ProBase Hot ($p = 0.057$). This result indicated that the curing procedure did not affect the residual monomer level of ProBase Hot.

When comparing the percentage of residual monomer levels of denture base materials in long and short curing procedures, SR Ivocap had the significantly highest residual monomer level, followed by SR Triplex Hot, and ProBase Hot, respectively (Figure 2).

Discussion

Polymerization of conventional denture base polymers requires an initial low temperature and sufficient time to prevent boiling of the MMA monomer. This may be time-consuming especially for the laboratory. There are many commercial denture base materials available in dental market, which are claimed to have superior physical and mechanical properties.

Table 2 Percentages by mass of residual monomer level of MMA (S.D.) (n=6)

Brand of material	Curing procedure	
	Long curing procedure	Short curing procedure
SR Triplex Hot	0.8769 (0.0560) ^{a,A}	1.4755 (0.1000) ^{b,B}
ProBase Hot	0.2596 (0.0284) ^{c,C}	0.3284 (0.0165) ^{c,D}
SR Ivocap	1.3293 (0.0431) ^{d,E}	2.0616 (0.0752) ^{e,F}

*Within the same material (horizontal), same lower-case letter indicates no significant difference. Different capital superscript letters (vertical) indicate significant difference among all three brands (simple effect analysis, $\alpha = 0.05$), S.D.: standard deviation.

Table 3 Results of a two-way ANOVA for analyzing two factors: brands of material and curing procedures ($\alpha = 0.05$)

Source	Sum of Squares	Df	Mean Square	F	P-value
Brand	12.048	2	6.024	1,663	<0.05*
Curing Procedure	1.959	1	1.959	541.048	<0.05*
Brand*Curing Procedure	0.739	2	0.369	102.013	<0.05*
Error	0.109	30	0.004		
Total	54.942	36			

*Statistical significance at $\alpha = 0.05$

Most of them were designed to simplify the processing technique, to reduce production time, or to minimize residual monomer levels that may cause mucosal irritation¹⁴ or have an effect on mechanical properties.^{6,7,9,14,23} Many products with different processing procedures were introduced to minimize residual monomer and processing time. Undurwade and Sidhaye²⁴ mentioned that pressure played a role in accelerating initial polymerization. Thirty minutes curing time of denture base polymer under pressure resulted in a minimal residual monomer content which was not more than that of other conventional rapid curing cycle, since sufficient pressure helped elevate the boiling point of MMA monomer and prevent porous formation in the materials during exothermic reaction.²⁵ SR Ivocap High Impact chosen in this study represented an injection molding denture base polymer²⁶ due to its continual applying pressure during processing. It was used to compare residual monomer level with other two brands of denture base materials which were polymerized by compression molding technique. (Type 1: Heat-polymerizable materials classified by ISO 20795).²⁷

From the manipulation aspect, the compression molding technique is easier to fabricate and has lower capital costs than the injection molding technique. The injection molding technique requires highly trained lab technician, has difficulty in mold design, and uses complicated and expensive equipment. In addition, inadequate spruing might lead to underfilled molds. However, it has advantages of the availability in prepacked-cartridge form, rapid mechanical mixing without steps of direct handling, and producing a homogenous mixture.

The residual monomer level from the previous study²⁸ was various due to the difference in polymerization procedures and ambient conditions. Some residual MMA was lost due to diffusion to the surroundings during polymerization or during the monomer extraction process since it was an experiment under open system.¹⁹ This experiment was conducted under control in the step of specimen preparation, monomer extraction, and determination of residual monomer content according to ISO 20795-1:2013[E] Part 1: Denture base polymers²⁷ in order to minimize monomer loss.

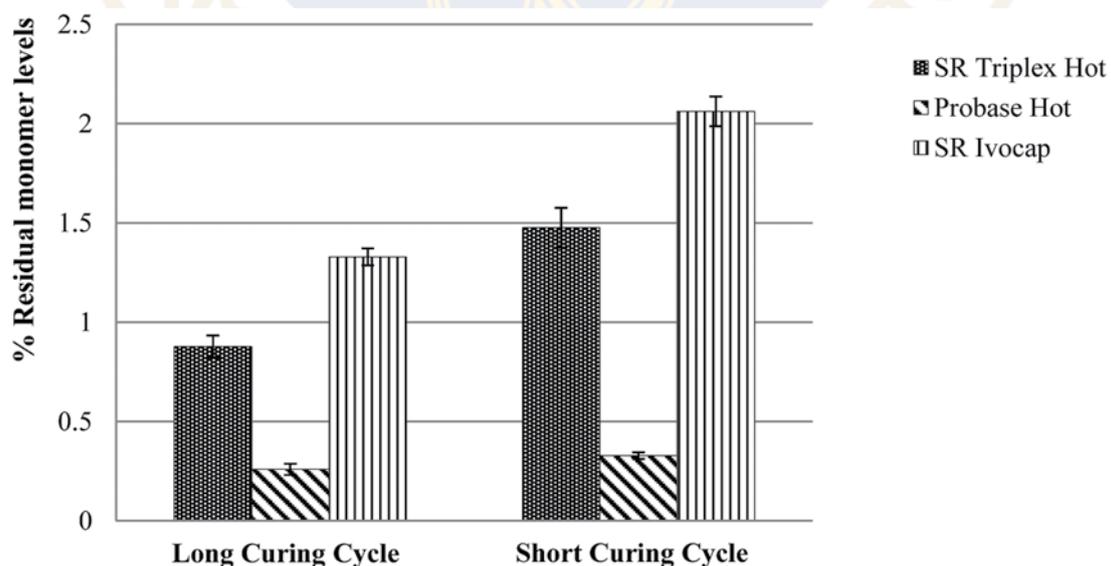


Figure 2 Comparisons of % residual monomer levels of denture base materials in long and short curing procedures

Preparation of the specimens involved grinding and polishing the specimen discs from the thickness of 3 mm to 2 mm as mentioned in the ISO 20795-1:2013 [E].²⁷ The surface treatment of denture base after polymerization such as polishing may reduce the residual monomer amount compared to the untreated specimens. It was due to the increase of temperature of the specimens during polishing.¹⁵ Therefore, careful polishing the specimen discs with sufficient lubricant to avoid forming frictional heat until the desired thickness was achieved. Each disc specimen was broken into small fragments to the size that was able to pass through the neck of one-mark 10 ml volumetric glass flask. In many studies, the specimens were ground using a drilling bur that may cause loss of monomer or depolymerization by the frictional heat.²⁷ In this study, the specimen disc was broken into small pieces by cutting with the nipper.¹⁹

There are many effective techniques for residual MMA monomer determination. Gas chromatography is widely used for investigating the residual monomer due to the high volatility of the monomer, and also the simplicity, rapidity, and high reproducibility of this technique.²⁹ It can also be served as qualitative and quantitative analysis even small injected amount of the sample.³⁰

This study investigated whether the amount of residual monomer was influenced by the processing techniques or curing procedures. The result from our study showed that the residual monomer level of SR Ivocap > SR Triplex Hot > ProBase Hot. In case of the effect from processing technique on the residual monomer level, the continuous pressure application in injection molding technique actually helps accelerate the polymerization reaction to be much more complete because the sufficient pressure applied during polymerization will not boil the residual monomer as the boiling point of MMA monomer is raised up. This might

be a result of reduction of residual monomer level.^{24,25} In contrast, this investigation found that the denture base that was processed by injection molding technique (SR Ivocap) had higher residual monomer levels than that of compression molding technique (SR Triplex Hot and ProBase Hot). This might be because the concentration of cross-linking agent has an effect on the residual monomer level more than processing technique. The material safety data revealed that there is higher amount of cross-linking agent in SR Ivocap and SR Triplex Hot (3-10%)^{31,32} than in ProBase Hot (1-10%).³³ High cross-linking agent contents will form a rigid polymer structure¹ that obstructs the conversion of MMA monomer, especially at the curing temperature lower than the glass transition temperature or T_g .³⁴ In contrast, Lee et al.³⁵ who also investigated the residual monomer content of SR Ivocap denture base on the 7th day after polymerization concluded that SR Ivocap, representing the injection molding denture base materials, had higher residual monomer levels than that of compression molding denture base materials but not significant difference. It was also mentioned that SR Ivocap contained higher cross-linking agent (5.6%), which hindered the monomer conversion.

Between the two compression molding denture base polymers, SR Triplex Hot had significantly higher residual monomer concentration. It was noted that higher chemical activator concentration of ProBase Hot (Dibenzoyl peroxide \leq 2.5%) than that of SR Triplex Hot (Benzoyl peroxide 0.5-1.5%) caused higher rate of free radicals formation in the stage of initiation, hence the more completion of polymerization could occur.¹⁸

Manufacturers provide various choices of curing procedure for their products. Laboratory technicians mostly choose the method that minimizes their laboratory time. In this investigation, two curing procedures were

chosen to compare the residual monomer level after polymerization. For SR Triplex Hot and ProBase Hot, one was "short curing procedure" representing the shortest polymerization time with the initial temperature of boiling water (100 °C). The other was "long curing procedure" which was the recommended procedure, having longer boiling time by starting at room temperature and gradually heated up to 100 °C within 1 hour, and then cured at 100 °C for 45 minutes. Both procedures were mentioned in the manufacturer's instruction manual. It was found that the residual monomer contents of Triplex Hot when polymerized by long curing procedure were considerably lower than when polymerized by short curing procedure. This may be due to the effect of initial temperature since there was no initial peak heating in the long curing cycle that might cause exothermal boiling of the MMA monomer to form porosity in the material.^{7,36} Also, the conversion of monomer was time-dependent.²⁰ The materials polymerized by long term boiling showed lower residual monomer level than that of the short term group. Lung and Darvell¹⁹ found that the amount of residual monomer decreased steadily from 0.25% to as low as 0.07% by extending the processing time. Harrison and Huggett³⁷, Kedjarune et al.,¹⁴ and Dogan et al.⁹ also found that the extension of polymerization time reduced the residual monomer level due to more completion of polymerization reaction. However, the curing temperature should be high enough to optimize the complete polymerization.

Although the residual monomer concentration of ProBase Hot when polymerized by short curing procedure was higher than that of the long curing group, there was no statistically significant difference. It might result from the improvement of formula of the products, such as adding the chemical activator to start the polymerization at once after mixing.³⁸ Due to the limitation of commercial products, the

researchers do not exactly know the composition of these materials, that is not mentioned in the commercial document, that might have an effect on the amounts of residual monomer.

All the three brands of denture bases used in this study had passed the biosafety according to ISO 20795-1:2013[E] specification²⁷ which quoted a standard acceptable maximum level of residual methyl methacrylate of denture base polymer type 1 not to exceed 2.2% mass fraction, therefore all of them can be cured using short curing procedure as mentioned in the instruction manuals for the main advantage of reducing laboratory time.

There was a limitation of different mixing ratios of polymer to monomer in each brand. As mentioned by Jerolimov et.²³ that the curing procedures had much greater influence to the residual monomer level than the mixing powder to liquid ratio, so the different mixing ratio of each brand was not taken into consideration in this investigation.

This study was also limited by the brands of denture base materials. Future research will be designed to investigate the residual monomer levels of the other type of injection molding denture base material and other commercially compression molding denture base materials from different manufacturers.

In conclusion, within the limitations of this study, it can be concluded that;

Denture base that was polymerized by injection molding technique had higher residual monomer concentration than compression molding technique.

Long curing procedure had an effect on reducing residual MMA monomer content except Probase Hot of which there was no significant difference between long curing and short curing procedure.

Though SR Ivocap polymerized by short curing procedure had the highest residual methyl methacrylate monomer level, it did not exceed

the limit (2.2% mass fraction for denture base polymers Type 1) as mentioned in ISO 20795-1:2013[E] specification.²⁷ It can be concluded that the residual monomer levels of all three brands of denture base materials polymerized by long curing and short curing cycle were clinically acceptable.

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