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Modified Heat Cured Acrylic Resin Denture Base Material: Residual Monomer

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ABSTRACT

Aims: to estimate the amounts of residual methyl methacrylate (MMA) of heat cured acrylic resin denture base material modified with Acrylic Acid (AA), Zinc Oxide (ZnO) and Zinc Diacrylate (ZDA) additives.

Methods: thermally activated ZnO and ZDA powders were synthesized. Heat cured acrylic resin denture base material had modified by adding 5% or 10% by weight from each of AA, ZnO and ZDA. High Performance Liquid Chromatography (HPLC) was used as the analytical monitoring method to quantify the residual MMA in the cured samples. One-way ANOVA test was used to compare the results.

Results: minimum amounts of residual MMA were estimated in heat cured acrylic resin modified by AA, while the addition of each of ZnO and ZDA to heat cured resin results in statistically significant larger amounts ($p \leq 0.05$) of residual MMA than the control sample. The analyzed residual MMA quantities of all polymerized samples were lesser than ADA standardization (2.2% mass fraction).

Conclusion: modifying heat cured acrylic resin with ZnO or ZDA additives result in larger residual MMA than modifying it with AA additive.

Keywords: Acrylic acid; Acrylic resin; Denture base; HPLC; Methyl methacrylate; Residual monomer; ZnO; Zinc diacrylate.

INTRODUCTION

During the polymerization reaction of the acrylic resins, the conversion of monomer into polymer is not complete and varying amounts of free or nonreacted monomer remain in the polymerized resin [1-3]. The presence of the nonreacted residual monomer has an adverse effect on physical and mechanical properties [4-7] as well as on the biocompatibility of the cured denture base resins [8-16].

Several studies have been estimated different amounts of residual monomers released from acrylic denture base materials [2-3]. The type of denture base acrylic resin is a decisive factor affecting the amounts of residual monomer. It was found that heat cured acrylic resins have residual monomer contents greater than microwave polymerized acrylic resins' types and lesser than both autopolymerized [17-20] and light polymerized types ,

Residual monomers released from heat cured acrylic resins could exhibit variable amounts depending on the mixing polymer to monomer ratio [4,20], time [1,5,6,13,20,21] and temperature [1,5,12,22-24] of polymerization cycle. The ISO-1567 standards [25] and ADA Specification No. 12 [26] standardized the upper limit (maximum) for the residual monomer level to be 35.37mg g^{-1} (2.2 % mass fraction) of the quantity of the specimen for the denture base polymers. Levels of residual monomer < 1% can be obtained using conventional laboratory polymerizing procedures [27].

Miettinen and Vallittu [17] assessed if there is any effect of reinforcing acrylic resins with glass fibers on the residual monomer contents. Their study revealed that release of residual MMA from heat-cured test specimens with glass fiber reinforcement was significantly higher than that from unreinforced test specimens (P = 0.003), while in chemical-cured test

specimens, with and without glass fiber reinforcement, the amount of MMA released did not differ (P = 0.50). Santos et al. [28] modified the PMMA bone cement by adding acrylic acid to enhance its mechanical performance. Although this addition was beneficial, it resulted in higher residual monomer values.

According to ISO-1567 standards [25], the residual monomers in most dental polymers are analyzed with a semi-quantitative method of solubility. A number of methods have been developed to determine the levels of residual MMA monomer. High performance liquid chromatography (HPLC) has expanded applications in analytical chemistry and it is suitable for determining the amount of residual monomer in denture-base acrylic resin [23].

MATERIAL AND METHOD

Six groups of modified heat cured acrylic resin samples and one control sample group were prepared and termed as follow: G1: Unmodified acrylic resin sample without any additive (control sample), G2: Modified acrylic resin sample contain 5% (AA), G3: Modified acrylic resin sample contain 10% (AA), G4: Modified acrylic resin sample contain 5% thermally activated (ZnO), G5: Modified acrylic resin samples contain 10% thermally activated ZnO, G6: Modified acrylic resin samples contain 5% (ZDA) and G7: Modified acrylic resin samples contain 10% (ZDA).

The AA was added as received without any preparation. ZnO was thermally activated by heating it in a furnace at 950°C for 3 hours. After cooling, the powder was weighed. This procedure was repeated until a constant weight was obtained. Then ZnO powder was milled with aVibratory Disc Mill and the milled powder was sieved with a 25µm sieve to get a very fine powder. This ZnO powder was carefully collected and stored in a perfectly sealed container until use.

The (ZDA) powder was synthesized as follow; ZnO (8.14 g, 0.1 mol) and 33 ml deionized water were introduced in a round bottom flask. After strong stirring, the mixture turned into suspension. Then (14.4 g, 0.2 mol) acrylic acid was added drop wise within 10 min at 5° C. The reaction was carried out at room temperature for 24 hours. Then the insoluble precipitate was filtered off using suppression Buchner and the filtrate was collected. Finally, the filtrate was dried under reduced pressure at room temperature to obtain zinc diacrylate, and further purification wash with methanol absolute was done. ZDA was dried on air at room temperature and milled using porcelain mortar and pestle.

Heat cured denture base acrylic resin prothyl press EVO 162 (Zhermack® technical Italy/ Complies with UNI EN ISO 20795-1) was used to prepare the tested sample. Flasking, mould separation, packing and clamping procedures followed the daily routine work for sample preparation in dental laboratory.

The manufacturer's recommended powder to liquid mixing ratio for Zhermack heat-cured acrylic resin (10 g powder to 4.5 ml liquid) was followed. Composites with varying additives were prepared by replacing a weight fraction of the pre-mixed PMMA powder and MMA liquid with an equal weight of additive. In other words, in samples with additives, each of ZnO, AA or ZDA was added either in (5%) or in (10%) by weight of the mixture.

The curing cycle was achieved at 73°C for 90 min, then after; the water bath temperature was raised to 100°C and left boiled for 60 min. It was then switched off and let it to cool down slowly while the flasks still there. The cured specimens were immersed in distilled water for 24 hours at 23°C. Then, they were examined visually without magnification to ensure clearness from porosity. Outer parts of the specimens were removed and each specimen was then fragmented by a cutter into suitable small pieces and 50 mg of these fragments were weighed into a glass vial using an electronic balance.

Solvent extraction of the MMA monomer from polymerized denture base material was carried out followed by chromatographic analysis. High Performance Liquid Chromatography (HPLC) was chosen as the analytical monitoring method to quantify the residual methyl methacrylate (MMA) content in the sample. The apparatus used was HPLC Liquid Chromatography LC-20AD Shimadzu/ Japan .

A reserved-phase partition chromatographic column CAPCELL PAK C18 (Dos-3) 250 mm length and 4.6 mm bore (internal diameter) was used coupled to a pre-column of reverse phase. The detection was achieved at UV wavelength 205 nm at a constant room temperature. The separation was made satisfactory with a mobile phase system composed of acetonitrile / water ($CH3CN/H_2O$) 50:50 (%v/v) with a flow rate of 1.0 mL.min-1 .

Basically, preparation involved extracting all the residual monomer from the samples by dissolving them in 1 ml of acetone in sealed test tube separately and then 10 ml of methanol was added to each solution to precipitate the polymer. The supernatant of solution was filtered through a 0.45 µm pore Millipore filter and 10 ml of the sample solution was injected. To ensure that a constant volume of the sample solutions and the calibration solutions are injected, a loop with a fixed

volume (20 µl) was used. The solutions were then analyzed by HPLC. This preparing and measuring procedure was previously cited [18,26,29].

Firstly was performed a qualitative analysis by comparing the chromatograms of the samples with that of the standard. External standard calibration procedure was used for the quantitative determinations. The peak-areas of the chromatograms obtained from the leaching monomer were compared to a standard linear calibration curve obtained by plotting the peak areas of MMA in the calibration solutions against the respective concentrations of the monomer-standard expressed in micrograms per milliliter (µg/mL). The peak areas of MMA were determined electronically by a computing registration and integration. The concentration of MMA monomers (RM_{ch}) in the sample solutions was calculated (in µg/mL) using a linear regression equation obtained from a calibration graph by referring the peak areas from the HPLC analysis of the samples, to the standard calibration curve. This calculating procedure was previously described [23,30]. The following equation was used to calculate total amount of MMA monomer in the sample solution, RM_s (µg):

$RM_s = RM_{ch} \times V$

Where;

RM_s is the total mass of residual monomer extracted from the dissolved sample mass in µg

RM_{ch} is the concentration of the residual monomer as it was measured by the calibration curve in µg/mL

V is the total volume of the sample solution in mL

This value was used to calculate the weight percentage of extracted MMA with respect to the weight of the polymerized specimen (w/w %) by the following equation;

$$RM\ (\%\ \text{mass fraction}) = \frac{RM\ s}{\text{specimen}\quad \text{mass}} \times 100$$

One Way Analysis of Variance (ANOVA) was used. The statistical results were considered significant at $p \leq 0.05$.

RESULTS

The results of the residual MMA quantities in the cured acrylic resin specimens expressed as percentage of the residual monomer (RM) mass fraction are shown in the figure.

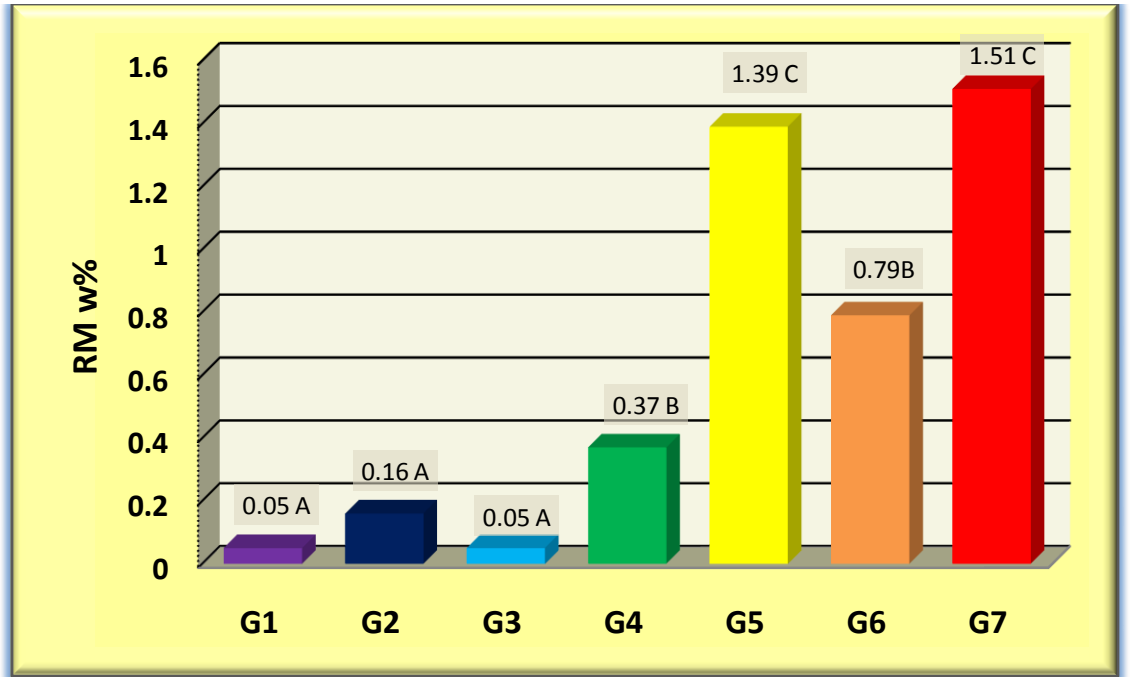

Figure: Means and ANOVA Test of the Residual Monomer's (RM) Percentage for the cured acrylic resin specimens. Different letters indicate significant differences at p value ≤ 0.05

All acrylic resin specimens were cured under identical conditions, their analyzed fragments were prepared according to the same procedure and the residual monomer extraction method was equal for all.

Statistical calculations reveal a wider assortment of the RM means ranged between the smallest percent for G1 and G3 (0.05%) to the greatest values for G5 and G7 (1.39% and 1.42 % respectively). RM concentration of the G2 is closest to G1 and G3 (there is no significant difference at $P \leq 0.05$), while other sample groups (G4, G5, G6 and G7) exhibit significant higher RM contents at $P \leq 0.05$. In addition the latter groups show significant differences in their RM among themselves.

DISCUSSION

Lung and Darvell [13] proved that the residual monomer is inevitable for all PMMA-based products no matter what the curing conditions are. The polymerization reaction never reaches 100% conversion and monomers remain free within the material, which can be released to the oral cavity [7].An important consideration is the impact the residual monomer has on the physical, mechanical and biological properties of the denture base resins. It was proved that resins used for the manufacture of denture bases have displayed various degrees of in vitro cytotoxicity [10,11,14] and in vivo allergic responses [8,9,10] caused by nonreacted components remaining after the polymerization process.

In addition, high levels of residual monomer have a deleterious effect on the physical and mechanical properties of acrylic denture base materials [4-7]. Therefore, it is very important to estimate if there is any change in the amounts of residual monomer released from heat cured acrylic resin after modifying it with AA, ZnO and ZDA addition in their proposed proportions.

HPLC was used for determination of residual MMA without the need of a preliminary extraction from the media, revealing to be a good method for MMA quantification [31]. HPLC is a common, versatile and extremely precise technique used to separate, identify and quantify different chemical components; pharmaceuticals, biomolecules, polymers and many organic and ionic compounds. It is used in various environments, including medical, forensic, environmental and manufacturing. Generally, it is quick, automated and highly accurate. HPLC has a number of advantages over other techniques. It has rapid and precise quantitative analysis, highly automated operation, high sensitivity detection, quantitative sample recovery, and amenable to diverse samples [32]. It has distinct advantages over GC for the analysis of organic compounds. It is more amenable to polar, non-volatile and thermally labile compounds, like most biochemicals, drugs and metabolites. Compounds can be analyzed at room temp without decomposition or the necessity of making volatile derivatives. GC vaporizes the sample while HPLC by having the sample remain in its liquid state increases the stability of the compound [33].

In comparison with UV spectroscopy, although UV method does not require the elaborate treatment and time consuming procedures usually associated with chromatographic method, HPLC method is more accurate and precise than UV method. It has better absorptivity onto either the mobile or stationary phase, better overall separation, eliminates dilution errors, less prone to interferences, and work well with trace quantities [34].

In studies used UV spectroscopy for measuring residual MMA, a specimen of definitive linear measurements is immersed in a proper volume of distilled water or other media (like natural or artificial saliva) for specific time intervals. The authors notice that UV spectroscopy measures only the residual monomer amount that leached from the specimen to the surrounding media during the proposed limited time interval while monomers not released to the aqueous media can stay in the matrix resin as pending chains [35]. Hence, we can conclude the following;

- Releasing of the residual MMA is a time dependant factor. It is leached slowly from the acrylic resin with high leaching rate during the first 1-2 days of immersions [24,36-38].
- This leaching rate depends on the thickness of the specimens. Slower leaching occurs in thicker sections [23].
- The sample should be incubated during overall immersion in 37°C to simulate the oral cavity temperature. This is because the leaching of MMA could be affected by the temperature. High amounts of leaching had been noticed to occur in elevated temperatures [4,39].
- Finally, the released MMA amounts and rates differ according to the immersion medium weather it is distilled water, natural or artificial saliva.
  All these are basic contributing factors that have essential effects on the quantities of the released MMA that should be measured, regardless of other changeable variables like acrylic resin type, polymerization process and manipulative procedures.

For all the former reasons, and from the point of our view, HPLC analysis method is superior. Therefore, HPLC had been chosen to estimate the concentration of the nonpolymerized MMA presents in a definitive mass, regardless of its dimensions, by dissolving it in a proper solvent.In our study, the UV detection was achieved with a wavelength of 205 nm as it is suitable for low concentration of MMA in the sample solution. Acetonitrile / water (CH_3CN/H_2O) 50:50 (%v/v) was

chosen as it is an appropriate mobile phase composition that secured a satisfactory separation of all substances to ensure correct quantification of the MMA content in the sample solution [26].

Without doubt, a limited comparison of our RM results with other studies was achieved. Factors to be considered in this respect are the acrylic resin type, liquid/powder mixing ratio, curing duration and technique and post polymerization treatment. Discrepancies may also arise from the various analytical techniques used, sample treatments such as drilling or cutting of the sample for extraction, and the temperature of extraction, as well storage conditions (in water or air) and duration. It is also not possible to take into account the effect of the variation of temperature during the initial exotherm [13] and the variety of additives. Since the RM content depends on all these previously mentioned variables, specific comparisons cannot be made.

Our study showed that the control sample (G1) has the smallest RM percent (0.05%). This disagrees with the results of Mohamed et al. [18] which showed rather higher RM contents of the heat cured acrylic resin (1.44%). Although the same HPLC analyzing procedure was followed in both studies but there are focal differences in the acrylic resin brand, curing conditions and the post curing water immersion which might responsible for such discrepancy .

In our results, both G2 and G4 exhibit the lowest RM values among the additive sample groups. The RM of G2 is 0.16% with no significant difference from that of control group (0.05%). Moreover, G3 has RM (0.05%) as same as G1. The acrylic acid polymerized with the MMA to produce a random copolymer Poly (methyl methacrylate-co-acrylic acid). Therefore, when AA was used as an additive, a more complete polymerization and conversion could be expected, thus resulting in lower level of RM. This is particularly clear with the 10% of AA. Adversely, Santos et al. [28] made a note of an important increase of residual monomer of autopolymerized acrylic resins after an addition of AA. However, it is possible that the presence of tertiary amines as a chemical activator in autopolymerized products could have an effect on the degree of polymerization and consequence high RM amounts [5].

In heat polymerization, it seems that the high curing temperature enhanced copolymerization of methyl methacrylate with a more reactive monomer (AA). Thus the AA consumed the MMA and reduced the RM. On the other hand, AA is a liquid; therefore its addition lowered the viscosity of the resin mixture and permitted the allowable mobility of the free radicals. This let the polymerization reaction to extend further, resulting in fewer residual monomers. Significant higher RM contents were estimated in both G4 and G5. A study of Miettinen and Vallittu [17] suggested that the use of glass fiber reinforcement as an additive to the heat-cured denture PMMA statistically increases the release of residual MMA from the material. In fact, these raised RM values were partly expected in our study. In both sample groups, G4 and G5, the viscosity of the resin mixtures greatly increased after the addition of ZnO powder during sample preparation. This increased viscosity may interfere with the molecular mobility making the conversion incomplete. This reason could also explain why the RM concentration of G5 (ZnO10%) is significant higher than G4 (ZnO5%) as the mixture viscosity correlates with the quantity of the added ZnO powder.

A significant higher RM content had been recorded for both G6 and G7. It was predictable that the addition of ZDA caused copolymerization and cross linking of the methyl methacrylate, but actually it did not decrease the total residual monomer content. This may be due to the limited conversion occur as a result of verification of the cross linking and the subsequent restricted mobilization of the free radicals [40]. This may also explain why the RM of G7 was significantly higher than that of G6 as the cross linking comes about in proportion with the ZDA percent in the sample group.

Since the polymerization process is rather more complicated than an elementary stepwise mechanism, with chain-transfers, mutual annihilation and other complications [13], it might be that other experimental work, including rather different additive percents and polymerization methods, needs to be reevaluated. Having mapped an equilibration between rather different additives percents on one hand and the polymerization time and method on the other hand, it is feasible to make direct and unambiguous predictions of the residual monomer to be expected in processed denture base acrylic materials. This may enable less-irritant dentures to be fabricated by identifying more appropriate conditions. This is worthy of further and deeper study.

Evidently, the analyzed RM quantities of all polymerized sample groups did not show any obvious sign of approaching the upper limit of RM of the heat-cured denture base polymers standardized by ADA specification no.12 (2.2% mass fraction). These favorable results obtained in our study suggest that the 24 hours immersion of the cured sample in distilled water at 23°C before their preparation for HPLC analysis may be responsible for RM reduction. This step was done to simulate what is the operator really do i.e. water immersion of the acrylic denture for at least 24 hours. before its intraoral insertion. The lowest residual monomer content could be obtained by a terminal boiling and then storing in distilled water for at least one day [6]. Therefore, most of the nonpolymerized monomer was leached out the specimens during this immersion. Another

reason may be the use of external standard for RM calibration. Therefore, the reliability of the results was dependent on the reproducibility of pipetting in the sample preparation, which may adversely affect the precision of measurement and increase the possibility of error because of operator technique variability [5,40].

The third expected reason for this great reduction of the RM is the submitting of the cured samples to the post-polymerization immersion in hot water by keeping the flasks soaked in water after the water bath had been switched off until the water cooled. Many investigators have determined the effect of post curing immersion in hot water on the levels of RM in conventional heat-polymerizing acrylic resins. They reported that significant reductions in monomer concentration were seen as a result of this procedure [4,39]. Different mechanisms might help explain this reduction. It has been observed that the concentration of the RM in the polymerized resins can be diminished by diffusion into water and that the release of RM is a temperature-dependent process, thus increasing the temperature enhances the diffusion.

It has also been demonstrated that the fall in RM levels that takes place after polymerization is due to further polymerization at the sites of active radicals, and at higher temperatures, monomer molecules should diffuse more rapidly to these active sites and the rate of fall in monomer levels should increase. Another explanation that has been pointed out as a contributing factor to the reduction of the free RM is its hydrolysis to methacrylic acid [4,41,42]. Hence, the mechanisms of diffusion, post-polymerization reaction and hydrolysis could have been responsible for the decrease in the concentration of RM promoted by the water-bath post-polymerization soaking and this may account for part of the observed discrepancy towards lower than predicted values in this study.

CONCLUSION

Modifying heat cured acrylic resin denture base material by the addition of 5% and 10% weight fraction from each of ZnO and ZDA significantly increases the amounts of residual MMA ($p \leq 0.05$), while modifying it by addition of AA has no effect on residual MMA amounts. The analyzed residual MMA quantities of all polymerized samples were lesser than ADA standardization (2.2% mass fraction).

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