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Freezing for Better Biocompatibility: Residual Monomer Reduction in High-Impact vs. Conventional PMMA Acrylic Resins

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Abstract

Introduction: Residual monomer content (RMC) in denture base acrylic resins is a recognized factor contributing to oral mucosal irritation and allergic reactions.

Objective: This *in vitro* study aimed to evaluate and compare the RMC in conventional and deep-frozen high-impact, as well as conventional heat-cure polymethylmethacrylate (PMMA) acrylic resins, following immersion in water for 24 and 48 hours.

Materials and Methods: A total of 120 disc-shaped specimens were fabricated from two heat-cure acrylic resins – a conventional PMMA (DPI) and a high-impact PMMA (Trevalon HI) – using the compression molding technique. Group I (n=60) specimens were prepared using conventional resin mixing, packing, and a long curing cycle. For Group II (n=60), specimens were prepared by deep-freezing the mixed acrylic, followed by thawing, packing, and a long curing cycle. Each group was further subdivided based on storage duration in distilled water (24 hours and 48 hours). The pulverized form of each specimen was subsequently analyzed for RMC using High-Performance Liquid Chromatography (HPLC). The acquired data were subjected to appropriate statistical analysis.

Results: The mean RMC was highest in Group I conventional heat-cure PMMA (DPI) specimens stored for 24 hours. Conversely, the lowest mean RMC was observed in Group II high-impact PMMA (Trevalon HI) specimens after 48 hours of water storage. A paired t-test

revealed a statistically significant difference among all comparison groups ($p < 0.001$).

Conclusion: Deep-freezing the mixed high-impact Trevalon HI PMMA acrylic resin, followed by storage in water for 48 hours, significantly reduced the residual monomer content. This technique could potentially enhance the biocompatibility of denture base resins.

Keywords: Acrylic Resins; Denture Bases; High-Performance Liquid Chromatography; Polymethyl Methacrylate; Residual Monomers.

Introduction

Denture base materials have remained a challenge in dentistry and with the introduction of heat cure acrylic resin in 1937 by Dr. W.H Wright, the discipline of dental prosthesis revolutionized. Acrylic resin is still the most widely accepted material and is the principal choice when it comes to denture bases. [1] The polymerization reaction in denture base resins is an addition reaction that involves the activation of the initiator and results in the conversion of methyl-methacrylate (MMA) into Poly-methyl methacrylate (PMMA) during which the monomer molecules are converted into polymers. It usually activates through heat polymerization, chemical or auto-polymerization, or light polymerization.[2] During this process, not all the monomer molecules are converted and thus, some unreacted residual monomers remain un-polymerized.[3] Generally, 3% to 5% free monomer is displayed in resins polymerized via chemical activation, whereas heat-activated resins exhibit 0.2% to 0.5% residual monomer.[4] The unreacted monomer may leach out into the saliva which actually causes the cytotoxic effects in the oral cavity.[5] Residual monomer content has the potential to cause irritation, inflammation, and an allergic response of the oral mucosa. Clinical signs and symptoms most frequently reported include erythema, erosion of oral mucosa, and a burning sensation on the mucosa and tongue.[6] In addition, the mechanical properties of the polymer which are affected are tensile strength, modulus of elasticity and surface hardness which have been found to be lower with greater residual MMA concentration.[7] The explanation is the plasticizing effect of the MMA. Therefore, it is desirable on both accounts to reduce the value of residual MMA concentration in the processed denture to the point where both biological and mechanical effects are negligible, irrespective of the denture base material used. Evidences from previous studies have proved that residual monomer concentration varies with the methods and the conditions of polymerization also. On the basis of the review of literature, it may be concluded that the cytotoxic effect of denture base acrylic can be reduced by storage time in water, powder to liquid ratio, polymerization method (conventional or microwave), and cycle, 3D printing of dentures, subtractive manufacturing of Polident Pink by CAD-CAM for denture base fabrication and by deep freezing of heat cure acrylic resin.[4,8-13] Isik and Harrison in year 2005 introduced deep freezing of heat cure acrylic resin as a method for fabrication of denture bases.[12] Freezing the mixed dough prior to packing extends the working time which allows the laboratory to use portions as and when necessary for economy of both material and time; supposedly without adversely affecting properties of the polymerized material. Deep freezing the heat cure acrylic material up to 1 month in the dough stage did not alter the impact strength, hardness, flexural modulus, and flash thickness. Recent evidence published in 2025 has further reinforced the relevance of deep-freeze techniques, demonstrating that controlled sub-zero conditioning combined with modern polymerization cycles can enhance monomer conversion efficiency and reduce cytotoxic potential. Studies in 2025 also highlight that integrating deep freezing protocols with emerging

digital denture workflows improves dimensional stability and reduces internal polymerization stresses, supporting its continued evaluation as an effective adjunct in denture base processing.[14-17] Thus, the aim of this in vitro study was to evaluate and compare the amount of residual monomer content between conventional and the deep frozen heat cure acrylic resins of two different types when polymerized at 74°C for 8 hours.

Materials and Methods

The present in vitro study was done to evaluate the residual monomer content of 120 specimens made by using two different heat cure polymethylmethacrylate resins (60 DPI and 60 Trevalon HI) and divided into two groups as follow:

Group I (Conventional) PMMA specimens: 60 specimens of Heat Cure Polymethylmethacrylate acrylic resins (30 DPI & 30 Trevalon HI) were fabricated by conventional mixing, packing and curing. (Fig. 1)

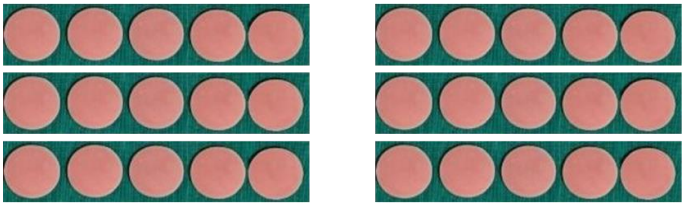


Figure 1: Group I (Conventional) PMMA Specimens (DPI & Trevalon Hi)

Group II (Deep frozen) PMMA specimens: 60 specimens of Heat Cure Polymethylmethacrylate acrylic resins (30 DPI & 30 Trevalon HI) were fabricated by deep freezing the mixed powder and liquid for 1 month. At the end of the storage time, sufficient dough was removed, thawed, packed, and polymerized, and specimens were prepared. (Fig. 2)

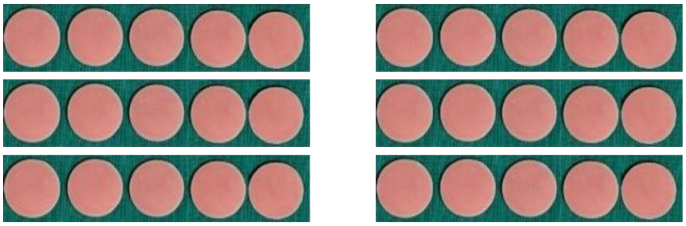


Figure 2: Group II (Deep Frozen) PMMA Specimens (DPI & Trevalon Hi)

To prepare each sample, highly polished customized acrylic disc measuring 50mm diameter x 2mm thickness was invested into Type III Dental stone. (Fig. 3,4) After setting of investment, flask was opened, acrylic disc was removed. Heat cure resin (DPI/Trevalon HI) was mixed with monomer 2:1 by weight and packed into mould. After 1 hour bench curing, long curing was done at 74°C for 8 hours. After overnight bench cooling, sample was retrieved and flash was removed with BP knife.

To fabricate Group II PMMA specimen polymer and monomer were weighed and mixed in a porcelain jar as described in the conventional method and it was transferred into an airtight container immediately. The container was covered with cling film and aluminium foil was wrapped around it. The container was transferred to Skadi Upright Deep Freezer and stored at -18°C for a period of 1 month. (Fig.5) After 1 month, the mix was removed, allowed to thaw, and packed in the invested mould. The specimens were cured in a similar manner as in the conventional heat-cure resin group. In this way 60 conventional PMMA specimens (30 DPI & 30 Trevalon HI) were made.

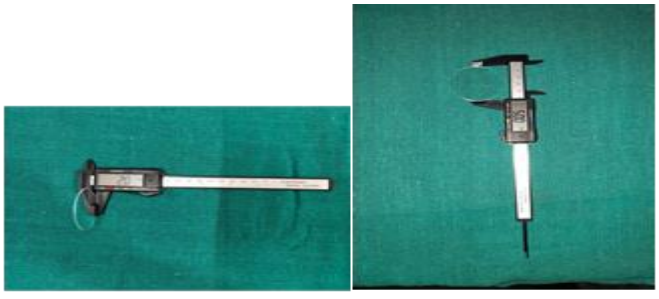


Figure 3: Acrylic disc

Figure 4: Mould of acrylic disc invested in dental stone

Figure 5: Storage of mixed Polymethylmethacrylate resins in airtight plastic containers covered with aluminium foil kept in deep freezer

Determination of residual monomer in the Heat Cure Resins using High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) was used to evaluate the quantity of residual MMA content in all the 120 specimens of heat-cure resin. The powdered form of specimens was obtained using an acrylic trimming bur with slow speed on micromotor (Fig. 6). 50 mg Powdered sample from each specimen was dissolved in 1 ml of acetone, and then 10 ml of methanol was added to the solution to precipitate the polymer. The supernatant of the solution was filtered through a 0.22 µm pore Millipore filter. HPLC analysis was performed using Agilant technology equipped with C18 column. 20µl of the sample solution was injected and analysed at 40°C at a flow rate of 0.12 ml per minute with acetonitrile water (50/50). (Fig. 7)

Residual methyl methacrylate calibration curve (Area mAU) was recorded for each specimen of both the groups with HPLC. RMC in weight % for each sample was determined by measuring peak area with the help of software and comparing it with those obtained from known standard monomer amounts. The data so obtained was subjected to statistical analysis.

Group I DPI(24 hours) Group I Tre HI(48 hours) Group II DPI(24 hours) Group II Tre HI(48 hours)

Figure 6: Powdered Samples of Group I and Group II Specimens

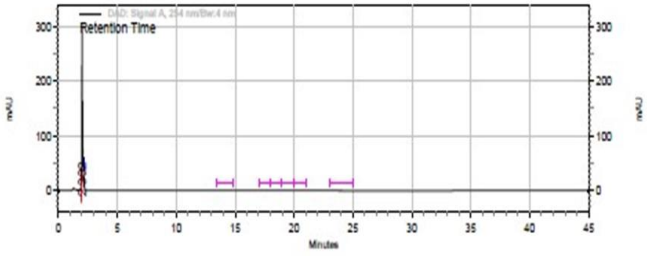
Figure 7: High-Performance Liquid Chromatography (HPLC) system (Agilant Technologies 1260 Infinity)

Observations

Residual monomer content was evaluated in 120 heat-cure PMMA specimens. Group 1 included 60 conventionally processed samples (30 DPI, 30 Trevalon Hi), and Group 2 included 60 samples prepared after deep-freezing the polymer–monomer mix for one month. All specimens were polymerized using a long-curing cycle at 74 °C for 8 hours and subdivided based on water storage duration (24 h and 48 h). Each specimen was ground into powder and analysed for residual monomer using HPLC. Peak areas obtained from chromatograms were compared with standard monomer values to

calculate residual monomer content. (Graph 1) The compiled data were statistically analysed using mean, standard deviation, standard error, and t-test to compare both groups.

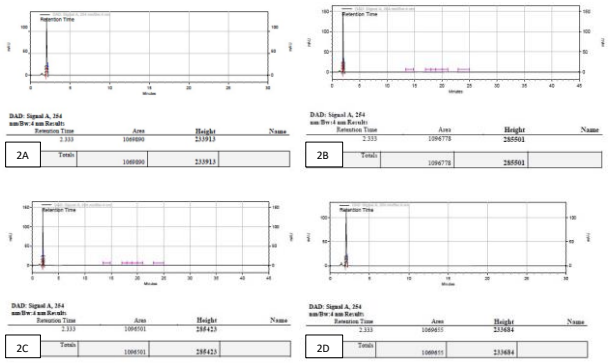
Graph 1: Standard Calibration Curve for Methyl Methacrylate (DPI)



DAD: Signal A, 254 nm/Bw:4 nm Results

Retention Time	Area	Height	Name
2.332	2394277	599899	
Totals	2394277	599899	

Graphs 2: (A, B, C, D)



Graph 2(A) Residual Methylmethacrylate Calibration Curve obtained from 1gm of Group I Conventional PMMA (DPI) stored in water for 24 Hours

Graph 2(B) Residual Methylmethacrylate Calibration Curve obtained from 1gm of Group I Conventional PMMA (Trevalon Hi) stored in water for 24 Hours

Graph 2(C) Residual Methylmethacrylate Calibration Curve obtained from 1gm Of Group II Deep Frozen PMMA (DPI) stored in water for 24 Hours

Graph 2(D) Residual Methylmethacrylate Calibration Curve obtained from 1gm Of Group II Deep Frozen PMMA (Trevalon Hi) stored in water for 24 Hours

Graph 3: Bar Graph of Mean Residual Monomer Content (Wt%) of All Groups

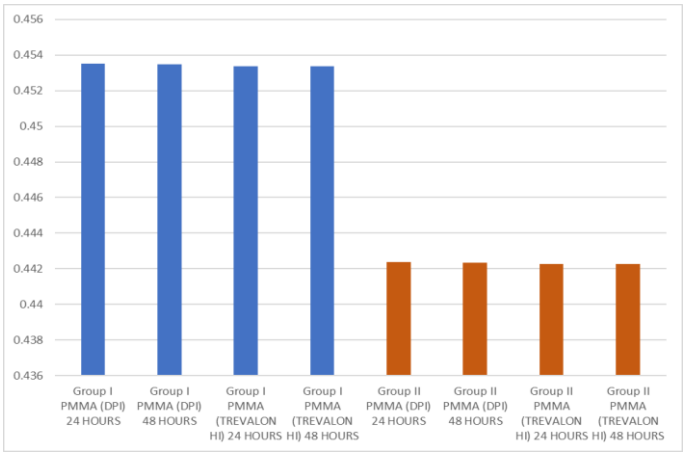


Table 1: T-Test for Equality of Means between Group I Pmma (Dpi) 24 Hours & Group Ii Pmma (DPI) 24 Hours

t-test for Equality of Means								
t	df	Mean Difference	Std. Error Difference	95% CI		99% CI		p-value
				Lower	Upper	Lower	Upper	
16815.389	28	0.0111191	0.0000007	0.0111178	0.0111205	0.0111173	0.0111210	0.001

Table 2: T-Test for Equality of Means between Group I Pmma (Dpi) 48 Hours & Group Ii Pmma (Dpi) 48 Hours

t-test for Equality of Means								
t	df	Mean Difference	Std. Error Difference	95% CI		99% CI		p-value
				Lower	Upper	Lower	Upper	

15517.161	28	0.0111389	0.0000007	0.0111375	0.0111404	0.0111369	0.0111409	0.001

Table 3: T-Test for Equality of Means Between Group I Pmma (Trevalon Hi) 24 Hours & Group Ii Pmma (Trevalon Hi) 24 Hours

t-test for Equality of Means								
t	df	Mean Difference	Std. Error Difference	95% CI		99% CI		p-value
				Lower	Upper	Lower	Upper	
10578.816	28	0.0111014	0.0000010	0.0110993	0.0111036	0.0110985	0.0111043	0.001

Discussion

This in vitro study aimed to evaluate and compare the RMC of two widely used heat-cure PMMA denture base resins, a conventional one and the other a high impact resin, processed via conventional and deep-freezing techniques. The RMC of each specimen was determined using High-Performance Liquid Chromatography (HPLC), a highly sensitive and reliable analytical technique. HPLC is commonly used to measure RMC in PMMA denture base resins due to its high sensitivity, specificity, and accuracy. It enables precise quantification of trace amounts of unreacted MMA monomer, which is critical for evaluating the biocompatibility and mechanical properties of the resin.[20] The ability of HPLC to selectively detect MMA even in complex mixtures makes it superior to less sensitive techniques such as UV spectrophotometry or gas chromatography in certain applications.[21] Furthermore, HPLC provides reproducible and reliable results, making it suitable for comparative studies assessing the impact of different polymerization techniques, storage conditions, or material modifications on RMC.[22] Rashid et al., reviewed and emphasized HPLC as an effective method for detecting residual MMA in dental polymers.[6] Several other researchers have similarly employed HPLC for quantifying RMC in various types of acrylic resins, supporting its role as a preferred analytical technique in dental material research.[5]

The reduced RMC observed in the deep-frozen groups may be attributed to more favorable polymerization kinetics. The deep-freezing method, which involved storing the monomer-polymer mix at -18°C for one month, likely improved the stability and homogeneity of the dough stage. This stabilization could allow for more complete conversion of monomer to polymer upon curing.[12] The process of thawing and subsequent polymerization also possibly facilitate a slower and more uniform curing process, enhancing the degree of polymerization. Similar findings were reported by Jadhav et al., who demonstrated significantly lower RMC in deep-frozen PMMA samples.[19]

Trevalon HI consistently exhibited lower RMC values compared to DPI across all groups. This may be attributed to differences in material formulation, including powder-to-liquid ratio, particle size distribution, and the presence of polymerization modifiers, which can impact the degree of monomer conversion.[28] It incorporates a higher concentration of cross-linking agents, such as butanediol dimethacrylate, which enhances the polymerization process by forming a more extensive cross-linked network, effectively reducing the amount of unreacted MMA.[23] Additionally, Trevalon HI is engineered for greater polymerization efficiency through the use of optimized initiators and accelerators, which promote more complete monomer-to-polymer conversion. The resin also typically features a higher polymer-to-monomer ratio, meaning less MMA is

present initially, further contributing to the reduced RMC.[24] Moreover, the use of pre-polymerized PMMA beads with controlled particle size during manufacturing facilitates better monomer diffusion and more complete polymerization.[25] Owing to these properties Chokattu et al. also reported lower RMC in high impact resin (Trevalon Hi).[27]

Extended water storage also plays a critical role. Even in conventionally processed resins storage for 24 hours yielded higher RMC values compared to 48 hours immersed in water. This further reduced when deep frozen material was used. Here also storage in water for 48 hours resulted lower RMC than with 24 hours immersion. This is because storage of specimens in distilled water for 48 hours allowed for further leaching of unreacted MMA monomer, aligning with the observations of Bayraktar et al. and Huda et al., who emphasized that prolonged immersion helps reduce monomer levels due to diffusion at body temperature analogues.[4,18]

A consistent trend was observed across both materials and processing methods i.e. deep freezing the mixed acrylic resin for one month prior to polymerization, followed by 48-hour water storage, significantly reduced the RMC. These differences were statistically significant ($p < 0.001$), affirming that both the processing technique and post-polymerization water storage duration influence the final residual monomer levels.

All RMC values in the study were within the permissible limits outlined by ISO 1567 for denture base resins, supporting the clinical acceptability of both materials and processing techniques.[26] However, the notably lower RMC in the deep-frozen Trevalon HI specimens indicates a potentially superior alternative for patients with increased sensitivity or allergic predispositions to residual MMA.

A limitation of the study is the logistical requirement of the deep-freezing protocol, which necessitated the use of an ultra-low temperature freezer (–18°C) and a one-month storage duration. Despite this, the significant reduction in RMC and its implications for improved biocompatibility suggest that this technique warrants consideration in clinical situations demanding low monomer content. Incorporating this method could be especially beneficial in fabricating prostheses for immunocompromised individuals or those with prior allergic responses to acrylic resins.

Future investigations should explore the impact of deep freezing not only on RMC but also on other critical properties such as flexural strength, surface hardness, color stability, and cytotoxicity, particularly under *in vivo* conditions. Further comparative analyses involving additional commercially available PMMA resins and emerging technologies such as CAD-CAM milled and 3D-printed denture base materials would provide a broader perspective on the clinical relevance and feasibility of deep-freezing techniques.

Conclusion

Least amount of the residual monomer content was found in deep frozen (Trevalon HI) specimens stored in water for 48 hours. Thus, it was concluded that type of denture base resin, deep freezing the mixed acrylic for one month and storing in water for 48 hours can reduce the residual monomer content.

Clinical relevance of the present study is that deep freezing the mixed heat cure PMMA for one month may be considered as an economical and feasible method to be used for the fabrication of prosthesis. Low residual monomer concentration in deep frozen acrylic than conventional PMMA has an added advantage of less allergic susceptibility.

The limitation of present study is that it needs special deep freezer and prior storage of mixed acrylic for one month in deep freezer. However, after considering the less residual monomer content, this limitation can be easily managed. Further in vitro and in vivo studies along with other parameters may be conducted.

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