MEASUREMENT OF RESIDUAL MONOMER IN AUTOPOLYMERIZED ACRYLIC RESINS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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ABSTRACT

Background & Objective: Autopolymerized acrylic denture base resins are widely used in dentistry due to many desirable properties. However, it is possible to have residual monomer leakage from these resins even after setting is completed. Therefore, the objective of present study was to measure the amount of residual monomer leached from two commercial acrylic polymers by High Pressure Liquid Chromatography (HPLC) at different time intervals.

Methods: Two Autopolymerized denture base polymers, pink-veined (Universal Dental, Meadway, England) and clear acrylic (Universal Dental, Meadway, England) were selected to measure the residual monomer concentration by HPLC (Waters System, Woldbronn, USA). Twelve disc-shaped specimens (5 mm × 25 mm) were prepared for each group (pink) and clear acrylic) and stored in distilled water sealed glass container at 37˚C at time intervals of 1, 2, 6, 12, 24 hours and 1 week. Mean concentration was recorded in parts per million (ppm) as final monomer concentration of the specimen. The data was analyzed by ANOVA and t-test to determine the differences in monomer released at different time intervals and p value < 0.05 was considered significant.

Results: HPLC showed statistically significant differences among two groups (P < 0.05) in the amount of eluted monomer There were significant differences at one hour (T 1) (p = 0.001), at two hours (T2) (p = 0.011) and insignificant difference (p = 0.606) was observed at the six hours (T3) interval in the residual monomer release in two groups. However, at 12 hours (T4) and one week (T6) intervals, there was a gradual decrease of residual monomer release in both groups.

Conclusion: There was significant (P < 0.05) amount of residual monomer released from both pink-veined and clear Autopolymerized resins at different time intervals.

Key words: Residual monomer, Autopolymerized denture base resin, HPLC.

INTRODUCTION

Acrylic resin or polymethyl methacrylate (PMMA) are frequently used in dentistry for the construction of maxillofacial prosthesis, dentures, surgical splints, and orthodontics appliances.[1] The biocompatibility, low cost, good physical and chemical characteristics of PMMA have led to their multiple use in the oral prosthesis.[2]

PMMA are polymerized by different methods such as autopolymerization, photopolymerization, microwave polymerization and thermopolymerization but autopolymerization technique is commonly used in dentistry due to clinical applications and fast setting.[3]

Auto-polymerizing or cold-cure acrylic resins are manipulated by various methods. The dough technique involves mixing powder and liquid, whereas in sprinkle-on (addition) technique the polymer is saturated by its monomer.[2] In the polymerization process of acrylic resins, the powder and liquid (monomer) are mixed to obtain a homogenous mass. However, not all monomer is involved in the saturation process and some remains unreacted. Therefore, residual monomer concentration varies depending on the methods and conditions of polymerization.[4,5] The residual monomer is either trapped in the polymer or leached into the surrounding environment.[6] Leaching of monomer may produce a local or systemic reaction to cause irritation, pain, inflammatory and allergic reactions to oral mucosa.[7-10]

Several studies have reported a high amount of residual monomer release[11-13] from the chemical-cured acrylic resins, especially during the first 24 hours and the cytotoxic effects of the residual monomer has also been reported.[14-16]

Different methods such as gas chromatography, infrared spectrometry and chemical detection have been described to evaluate the levels of residual MMA monomer.[2-4] HPLC is popular in analytical chemistry

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as it is a nondestructive method that enables simultaneous analysis of various substances and provides correct estimates of the degree of residual monomer in acrylic resins. 12 As far as the scientific data on the amount of unreacted residual monomer released from denture based polymers is concerned, there is limited date available on leaching of residual monomer after one hour time interval.

Therefore, this study was carried out to determine the amount of residual monomer leached from two polymers by high pressure liquid chromatography (HPLC).

METHODS

The study was conducted in Government College University, Lahore. The duration of the study was one month. Two commercial brands of autopolymerized denture base polymers, super-cure deep pink-veined (Universal Dental, Meadway, England) and super-cure shade clear (Universal Dental, Meadway, England) were selected. Test samples were prepared following manufacturer's recommendations for powder to liquid ratios by volume and polymerization time according to EN ISO standard 10993-12:2012 17 under aseptic conditions using sterile Teflon (PTFE) standardized mould measuring 25 mm in diameter and 5 mm in thickness (Figures 1a, 1b, 1c).

The powder (2.5 g) was measured on an electronic balance and liquid (1 ml) was measured in calibrated glass cylinder. At the dough stage, the samples were packed into the Teflon mould, covered with cellophane paper, closed with a lid and placed in dental pressure pot for 10 minutes under 20 pounds psi pressure. Following removal from the mould, each sample was polished with pumice slurry and immediately immersed in a glass flask containing 20 ml of distilled water and sealed with bees wax to avoid leakage and evaporation of the monomer.[11]

Twelve samples were prepared for each group. Group (pink acrylic) and group (clear acrylic) was subdivided in six subgroups according to different storage time. The distilled water storage time interval for residual monomer detection was 1 hour (T1), 2 hours (T2), 6 hours (T3), 12 hours (T4), 24 hours (T5) and 1 week (T6).

HPLC (Waters System, Woldbronn, USA) was used to identify the residual monomer (MMA) content at the time intervals of T1, T2, T3, T4, T5 and T6 of immersion in distilled water after the sample preparation. The analysis used an Agilent UV detector at 230 nm, an Ace 5 C18 column of 25 cm length, particle size of 1 µm as stationary phase, a binary pump and manual injector systems. For the mobile phase, a mixture (50:50 volume ratios) of acetonitrile (HPLC grade) and distilled water was used and flow rate was 1 ml/min. The residual monomer concentration was determined by measuring the absorbance at 230 nm using the calibrated standard solution at 1600 ppm. To quantify the amount of residual monomer release from acrylic resin, 20 µl extracted solution of each sample was injected three times into the chromatographer and mean concentration was recorded in ppm as the final monomer concentration.[12]

The SPSS 20.0 software program (SPSS Inc, Chicago, IL, USA) was used for statistical analysis to determine the differences within each group over different periods and ANOVA test and t-test was used to determine the differences in monomer released at different time intervals. The data is presented as mean and standard deviation and a value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Figures 2 and 3 demonstrate chromatogram of two different samples which were prepared in this study. Mean and standard deviation of residual monomer concentration between two different groups at different time intervals are presented in Table 1. Leaching profile of monomer is shown in Figure 4.

Table 1: *Mean residual monomer concentration in ppm at various time intervals between two groups (Pink and Clear acrylic).*

Time	*Groups*	*Mean (ppm)*	*Standard Deviation*	*P-value (ANOVA)*
T1	Pink	86.80	48.47	.001
	Clear	233.40	42.60	
T2	Pink	27.40	12.82	.011
	Clear	82.80	35.10	
T3	Pink	126.20	9.34	.606
	Clear	116.40	39.76	
T4	Pink	94.40	8.96	.001
	Clear	143.40	20.23	
T5	Pink	82.60	2.41	.012
	Clear	101.60	12.93	
T6	Pink	62.80	9.42	.008
	Clear	78.80	4.15	

The residual monomer released from two Autopolymerized acrylic resins at six different time intervals (mean ±standard deviation [SD]).
P-value < 0.05 is significant according to ANOVA.

The results revealed statistically significant difference in amount of residual monomer release within groups at different time intervals except at the T2 interval (Table 1). ANOVA showed that the maximum

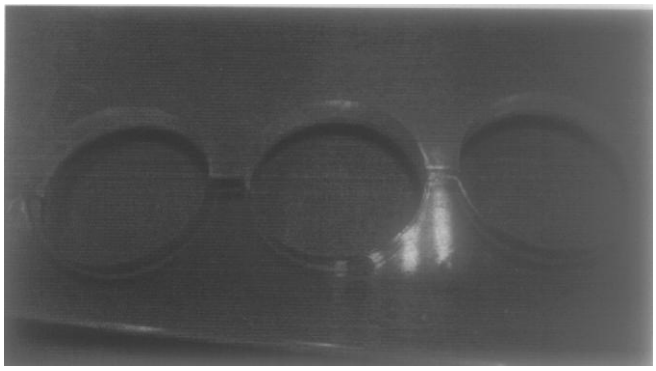

Fig. 1 (a): *Teflon mold used for specimen preparation.*

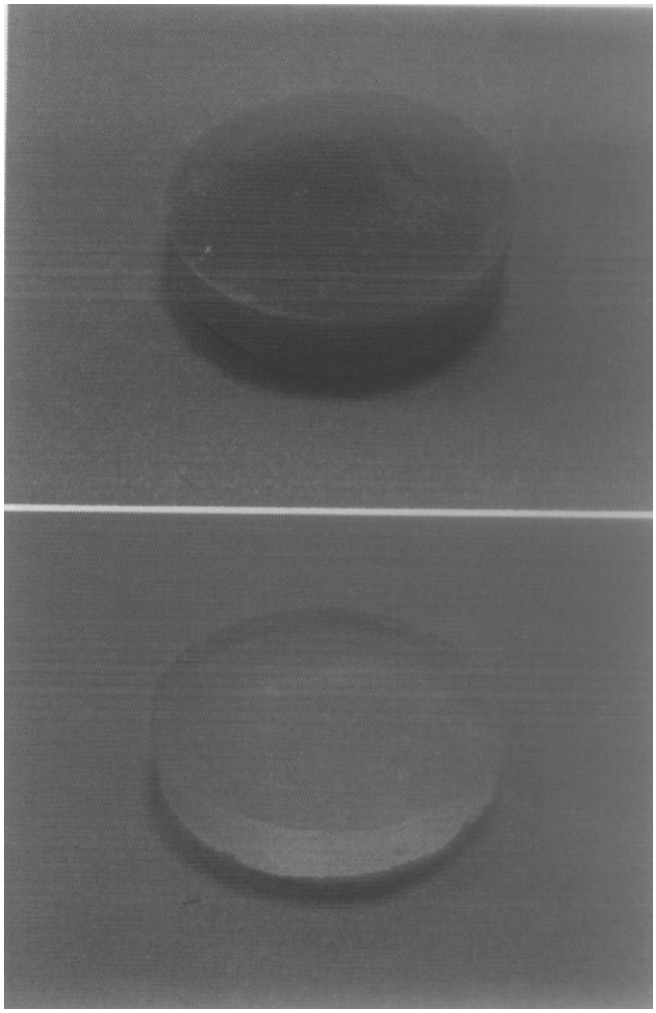

Fig. 1 (b): *Specimen of pink acrylic Figure 1 (c): Specimen of clear acrylic.*

amount (233 ± 42.60 ppm) of monomer release was from clear acrylic after one hour (T1) of immersion in distilled water, whereas the lowest amount of monomer release (27 ± 12.82 ppm) was from pink veined acrylic after two hours (T2).

According to HPLC method, the amount of residual monomer ranged from 27.40 ppm to 233.40 ppm. There were significant variations in amount of residual

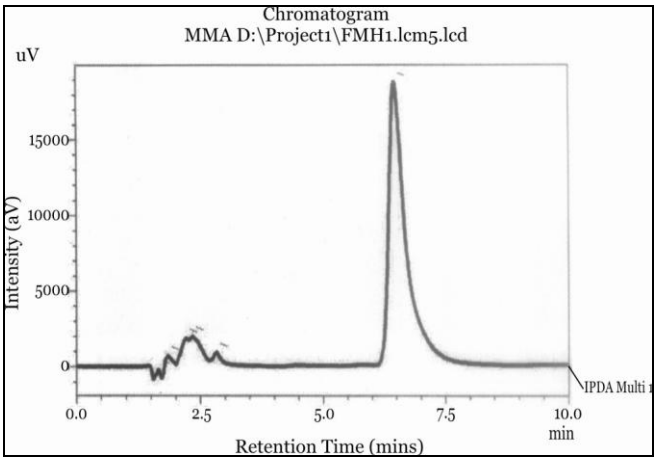

Fig. 2: *Typical chromatogram of standard monomer concentration (1600ppm).*

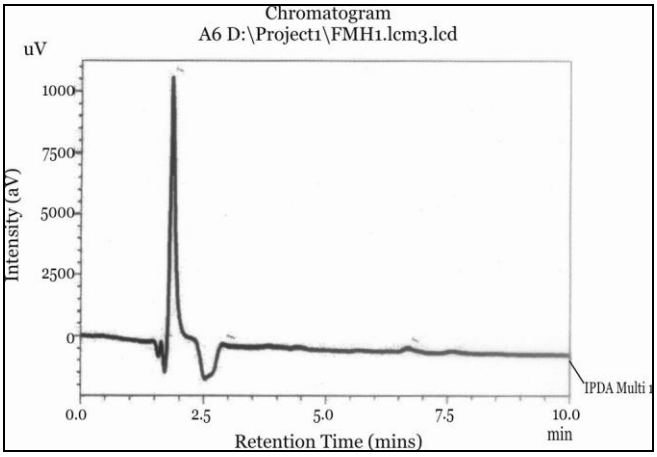

Fig. 3: *A sample chromatogram of specimen after soaking in distilled water for 6 hours' time interval.*

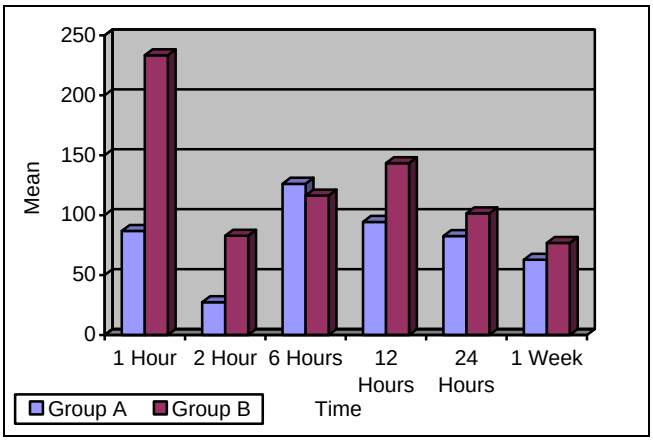

Fig. 4: *Mean monomer release (ppm) of pink acrylic (group A) and clear acrylic (group B) at different time intervals.*

monomer release between the two groups at T 1 (p = 0.001) and T2 time intervals (p = 0.011), whereas insignificant difference (p = 0.606) was observed at the T3 interval. There was gradual decrease of residual monomer release at T3 and T5 time intervals in both groups (Table 1, Figure 4).

DISCUSSION

Poly methyl methacrylate is commonly used for fabrication of acrylic resin based appliances. The most unfavorable characteristic of denture based polymers is the presence of unreacted monomer which acts as a plasticizer and decreases their strength. The biological effects of methyl methacrylate must be kept in mind during fabrication acrylic appliances. MMA is a well-known allergen and if remained in contact with oral mucosa it may cause local adverse reactions, such as burning sensation, necrosis, fissures, edema and pain to the mucosa as well as systemic reactions.[12,23-25]

Goncalves et al. reported that acrylic resins exert cytotoxic effects on epithelial cells. Therefore, it was recommended to reduce the residual monomer content as low as possible before dental prostheses are inserted. To reduce the residual monomer content, post-polymerization treatments are reported in literature, such as microwave irradiation and immersion in hot water.[14] Nonetheless, the risk of adverse effects of polymer based materials is higher for personnel in dental clinics and laboratories during handling of this materials.[13]

The results of our study showed that the residual monomer rate was significantly higher (233.40 ppm) in clear acrylic as compared to pink veined acrylic after one hour of storage in distilled water (Fig. 4). There was variation in amount of residual monomer release from one hour to six hours, but after 12 hours (T4) the monomer release was decreased gradually (Table 1, Fig. 4). The variation in amount of monomer release from one to 12 hours may be due to great difference in standard deviations during these study hours (Table 1). Significant ($p < 0.05$) monomer elusion was observed when one hour (T1) immersion time was compared against two (T2), 12 (T4), 24 (T5) hours and one week (T6). This result is in accordance with Meister et al.[21] who reported that significant residual methyl methacrylate was released from tested acrylic samples within first 24 hours of immersion in water. It suggested that two mechanisms would be responsible for 24-hour reduction in monomer release; late conversion of residual monomer into polymer once there are free radicals that remain on the surface after curing; and diffusion of the monomer out of acrylic specimen.

Ica et al.[12] reported that the maximum amount of residual monomer release from cold cure orthodontic acrylic resins was till 24 hours and then gradual reduction till one week. Our study showed that there was significant difference ($p < 0.05$) in monomer elusion from both pink and clear acrylic at one week (T6). Conversely, Nik et al. observed no difference in amount of monomer release from different study groups after one week.[11] However; they concluded that this difference may be due to different polymerization techniques and different brand of orthodontic cold cure resins used in the study. Significant amount of residual monomer was reported by researcher after 48 hours of immersion of samples in water followed by long time release of monomer till 15 days.[22]

Several factors such as temperature, time, polymerization technique, method of specimen preparation, thickness of the acrylic resin and polymer-monomer ratio influence the release of residual monomer. Pithon et al. showed that the amount of leached residual monomer is generally around 1.5% to 4.5% in self-curing acrylic resins.[19] Yilmaz et al. used ISO 1567 as a reference for the release of residual monomer as it limits the level of residual monomer to 4.5% for self-curing acrylic resins.[20]

Furthermore, biocompatibility of Autopolymerized acrylic resin has been studied by different researchers who suggested that this material is cytotoxic.[23-25] But water storage of denture base acrylic resins can lead to reduction of residual methyl methacrylate resins by diffusion into water.[24] Therefore, it is suggested that one of the methods for reducing the monomer release is storing the denture and acrylic appliance in water for 24 hours before insertion in patients' mouth. The water storage for one week may be recommended to minimize the adverse effects of residual monomer leaching from Autopolymerized acrylic resins since monomer elusion continues for one week. However, for feasibility purposes and high patient demands, at least 24 hours of water storage time must be undertaken before delivering such prostheses to the patients. In vitro investigations for longer intervals of water storage is recommended using artificial saliva instead of water.

In ***conclusion*** within limitation of this study there was significant amount of residual monomer released from both pink-veined and clear Autopolymerized resin. The eluting residual monomer leached from both types of acrylic gradually reduced from 12 hours (T4) till one week (T6) of water storage.

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Authors' Contribution

AQ: She is the main author of the study who wrote the article and bore all the finances for the study. UWJ: Conducted the research project, contributed in manuscript writing, and reviewed the article. MAF: Contri-

buted in article write-up, statistical analysis and its review. NY: Contributed in article write-up and its review.

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