ORIGINAL RESEARCH

Effect of Triazine Comonomer Substitution on the Structure and Glass Transition Temperature of Monomethacrylate-based Resin Polymer: An *In Vitro* Study

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Abstract

Aim and objectives: The present research aimed to characterize and deduce the structure of a novel denture base copolymer containing antimicrobial triazine comonomer by nuclear magnetic resonance (NMR) and energy-dispersive X-ray (EDX) spectroscopies. Also, it aimed to evaluate the glass transition temperature (T_g) with the addition of TATA at different concentrations.

Materials and methods: The trial groups G10 and G20 were thermo-polymerized with triazine comonomer, whereas the control group G0 was polymerized without the triazine. NMR and EDX spectroscopies assessed copolymerization along with deducing elemental composition in mass %. The surface topographies were observed through field-emission scanning electron microscopy (FESEM). The T_g of the resultant copolymer was examined by differential scanning calorimetry. Pertinent statistical tests with relevant multiple comparison tests were exercised to compare the mean T_g of the groups.

Results: The configuration of a new copolymer containing triazine comonomer was manifested with additional protons and carbon atoms. Nitrogen was detected in the EDX spectroscopy of the trial groups. The T_g of the new copolymer was higher than the G0. The triazine comonomer in the copolymer at 20% concentration exhibited the highest T_g.

Conclusion: The triazine comonomer substitution produced a novel denture base copolymer with enhanced T_g.

Clinical significance: The novel denture base copolymer may possess enhanced biomechanical properties due to the TATA's cross-linking capability. Nevertheless, the antimicrobial property of the triazine comonomer incorporated in the denture base composition might be beneficial in inhibiting the microbial colonization on the denture's surface.

Keywords: Antimicrobial, Copolymer, Denture base, Glass transition temperature, Triazine.

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Introduction

Mastering polymer composition is mandatory in understanding the chemistry and application of a dental biomaterial in clinical practice. Poly(methyl methacrylate) [P(MMA)] is an extensively employed denture base polymer (DBP). Despite its popularity, dominance, and advantages, several untoward tissue reactions were reported owing to its improper monomeric conversions.[1,2] Incomplete degree of conversion (DC) ruins polymer's glass transition temperature (T_g). Therefore, jeopardized T_g renders the polymer to be unsteady at mouth temperatures when compared to highly converted polymers.[3,4]

The credibility of T_g is considered important from a clinical frame of reference. This is because the temperature fluctuations in the oral environment exhibit repercussions on the polymer properties. The amorphous DBP is rigid/brittle at a low temperature which can flex as flexible as rubber with high viscosity at an elevated finite temperature and the transformation process is designated by T_g.[5] Normally, the DBP would not encounter temperatures beyond the T_g during its clinical service. However, finishing by abrasives, dry buffing in the laboratory, or cleaning/storing in extremely hot water by the patients are certain circumstances when the DBP might approach or cross beyond the T_g.[6] Under these critical circumstances, the DBP might experience peremptory deformation since thereafter minor pressure or impact can cause distortion or dimensional aberrations. T_g is associated with the DC of DBP since

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the T_g is straightaway associated with the polymeric molecular weight (Mw)[7] and gets diminished due to the residual monomers' plasticizing effect.[8]

Multitude compositional adjustments were executed to develop a DBP with superior properties. Monomeric alterations or substitutions are not uncommon.[9] Any substituted or added comonomer has to react through the reactive functional group with the propriety conventional methyl methacrylate (MMA) liquid monomer to form a novel copolymer with desired properties. Copolymerization (CP) is a pivotal process that dictates the essential properties of the DBPs.[10] A favorable reaction between the propriety and a substituted comonomer is obligatory to enhance the DBP's properties. This interaction can be determined and ascertained by using field-emission scanning electron microscopy (FESEM) with energy-dispersive X-ray (EDX) spectroscopy.[11] Nevertheless, copolymeric functional phenotypes can be identified through nuclear magnetic resonance (NMR) spectroscopy to delineate the structure of a compound.[12]

Developing a novel DBP possessing the antimicrobial property is necessary to prevent microbial colonization and denture plaque formation that would debilitate the geriatric oral and general health. 1,3,5-triazine derivatives were extensively studied for their antimicrobial properties,[13,14] terminating primarily gram-positive bacteria.[15] 1,3,5-triacryloylhexahydro-1,3,5-triazine (TATA) was used in orthodontic adhesives which notably diminished bacterial growth.[16] TATA is an antibacterial acrylate cross-linking monomer containing three reactive polymerizable aliphatic carbon-carbon double bonds (C=C). However, there is no literature found on copolymerizing TATA with DBP P(MMA) and the effects on its T_g. Hence, the present research aimed to characterize a novel DBP containing TATA antimicrobial comonomer and also to evaluate the T_g with the addition of TATA at different concentrations. The null hypothesis was that the TATA incorporation in the DBP does not influence T_g upon evident CP.

Materials and Methods

The research was conducted in the Annamalai University, Chidambaram, and Central Electrochemical Research Institute, Karaikudi. Poly (methyl methacrylate) [P(MMA)] powder (Mw ~ 350×10^3 g/mol), dibenzoyl peroxide (DBPO), methyl methacrylate (MMA) with ≤30 ppm mequinol as an inhibitor, *n*-butyl methacrylate (BMA), tricyclodecanedimethanol diacrylate (TCDDMDA), and 1,3,5-triacryloylhexahydro-1,3,5-triazine (TATA) were purchased from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis, USA) and used as received. TATA and 2% DBPO were mixed in P(MMA) powder in a ball miller for an hour at 25 (±1)°C. The monomer liquid was prepared by adding 10% BMA and 10% TCDDMDA in MMA contained in an Erlenmeyer flask with a magnetic stirrer and stirred for 30 minutes at 25 (±1)°C when the flask was sealed airtight to prevent the volatilization of the monomeric integrants. The groups of the research differed only in the powder component. A control group G0 consisted of P(MMA) powder and 2% dibenzoyl peroxide. Trial groups G10 and G20 additionally consisted of TATA comonomer at 10 and 20% concentration, respectively. The liquid composition was uniform for all the groups. The study groups and their composition were tabulated in Table 1. Thermo-polymerization was carried out at 74°C for 8 h with terminal boiling at 100°C for 30 minutes in an acrylizer.

^{1}H-/proton- and ^{13}C-/carbon-NMR spectra of the groups were recorded and analyzed digitally in an NMR spectrometer (Ascend™ 500; Bruker, GmbH, Germany). The polymerized specimens were finely ground to powder. For ^{1}H- ($n = 1$ per group) and ^{13}C-NMR ($n = 1$ per group), polymerized resin specimens were ground to a fine powder. Twenty and 30 mg powder were dissolved in 1 mL of deuterated chloroform ($CDCl_3$) for ^{1}H- and ^{13}C-NMR, respectively. For internal standards, tetramethylsilane was employed. A polymerized cuboidal specimen ($n = 1$) for each group measuring $5 \times 5 \times 3$ mm^3 was assessed for FESEM-EDX. The surface features were scanned with FESEM (Carl Zeiss, Supra 55VP, Germany). The freshly forged specimens were positioned on the mount and adjusted to 10 mm from the beam source. The elemental integrants in mass percent were deduced through EDX spectroscopy (JEOL-JSM-IT 200; Tokyo, Japan). The T_g was evaluated by differential scanning calorimetry (DSC) (Netzsch, STA 449 F3 Jupiter®, Selb, Germany) under a nitrogen atmosphere. Polymerized specimens were finely crushed to ~200 mg powder ($n = 10$) for each group. Twenty milligrams of powder from each group was heated on an aluminum mount (from 50 to 200°C) at a rate of 20°C/minute. An in-built software program (Proteus®) was used to determine the T_g by the method explained by Ajay et al.[11]

The data analysis for the T_g was done employing Statistical Package for the Social Sciences software (SPSS Inc., Chicago, Illinois, USA; version 21.0). The obtained data were normally distributed according to the Shapiro-Wilks test ($p > 0.05$). To analyze the difference among the groups, one-way ANOVA was employed, and to analyze differences between the groups, a *post-hoc* Tukey HSD test ($\alpha = 0.05$) was exercised.

Results

NMR Spectroscopy

The proton-NMR signals at δ 3.951 and 3.920 corresponding to >N-C***H***$_2$-N< indicate the TATA's presence in the trial group specimens. Conspicuous signals at δ 2.093–2.097 (G10) and δ 2.106–2.110 (G20) concerning -CH_2-Ć***H***(CO)-CH_2- in the trial

Table 1: Groups and their chemical composition

	Composition	
Group	*Powder*	*Liquid**
Control G0	P(MMA) powder with Mw 350×10^3 g/mol, 2% dibenzoyl peroxide as initiator.	MMA with ≤30 ppm mequinol as an inhibitor, 10% *n*-butyl methacrylate as a plasticizer, and 10% tricyclodecanedimethanol diacrylate as cross-linker.
Trial G10	P(MMA) powder with Mw 350×10^3 g/mol, 2% dibenzoyl peroxide as initiator, and 10% TATA.	
G20	P(MMA) powder with Mw 350×10^3 g/mol, 2% dibenzoyl peroxide as initiator, and 20% TATA.	

The powder-liquid ratio was 3:1 and kept constant for all the groups. *For NMR, the liquid consists of plain MMA with no plasticizer and cross-linker

groups confirmed the CP of TATA with the MMA and affirmed the formation of novel P(MMA-*Co*-TATA) denture base copolymer. These characteristic signals were absent in the G0 (Fig. 1). The carbon-NMR signals of trial groups at δ 20.18 (G10); 20.19 (G20) attributed to –**C**H$_2$–, δ 56.89 (G10); δ 56.91 (G20) for >N-**C**H$_2$-N<, and δ 165.03 (G10); δ 165.06 (G20) for amide **C**=O indicate the presence of TATA. Peculiar peak signal at δ 40.05 (G10); δ 40.08 (G20) attributed to α–**C**H tertiary carbon confirmed the CP and ascertained novel P(MMA-*Co*-TATA) denture base copolymer formation. The above ^{13}C-NMR signals were not observed in the G0 (Fig. 2). The appearance of weak signals at δ 5.484, 5.731, 6.026, and 6.302 in proton-NMR; signals at δ 126.11, 130.90 in carbon-NMR of G0 endorse the presence of some unreacted monomer that was not observed in the G10 and G20 groups. Table 2 tabulates the peak signal values of ^{1}H- and ^{13}C-NMR spectroscopies for the study groups.

FESEM-EDX Spectroscopy

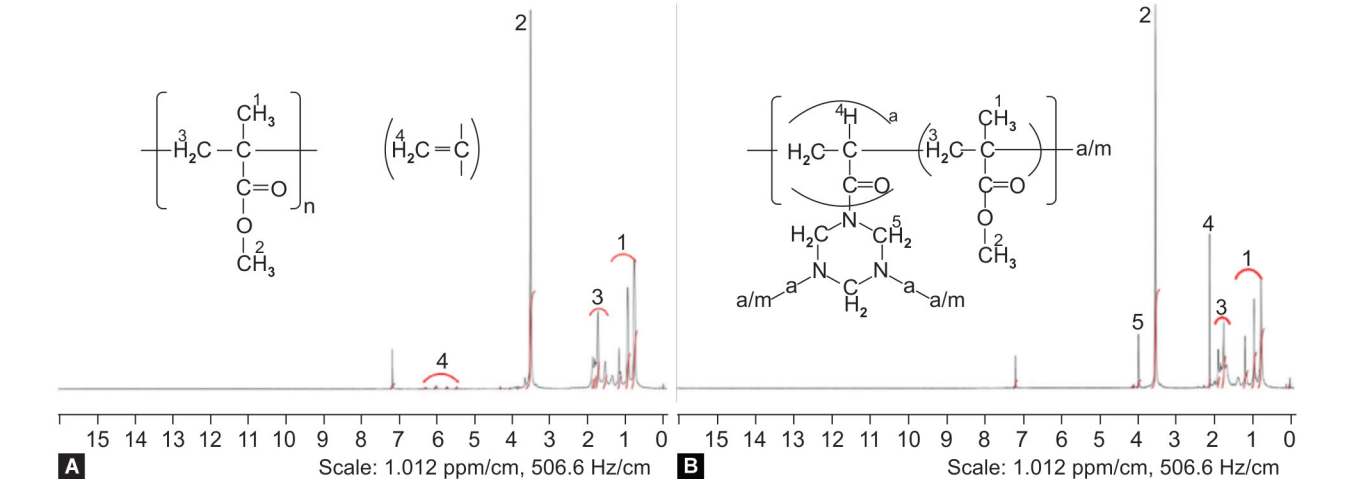
FESEM analysis elicited surface topographical variations among the groups (Fig. 3). The G20 surface appeared smooth, regular, and uniform (Fig. 3C). However, the G0 had a coarse, jaggy, and scabrous surface with a pronounced "rock-strewn" appearance (Fig. 3A). G10 presented a comparatively more crinkled surface exhibiting an "undulated" appearance than the G20 (Fig. 3B). At the same time, the G10 surface was far smoother than the G0. Hence, incorporating TATA in DBR augmented the surface topography of the P(MMA-*Co*-TATA) copolymer. The elemental integrants of the control and trial groups were detected and tabulated in Table 3. The presence of nitrogen (N) in the trial groups ascertained the TATA's incorporation that was absent in the G0 (Fig. 4). Carbon and oxygen were the other two elements detected in all the groups.

Glass Transition Temperature

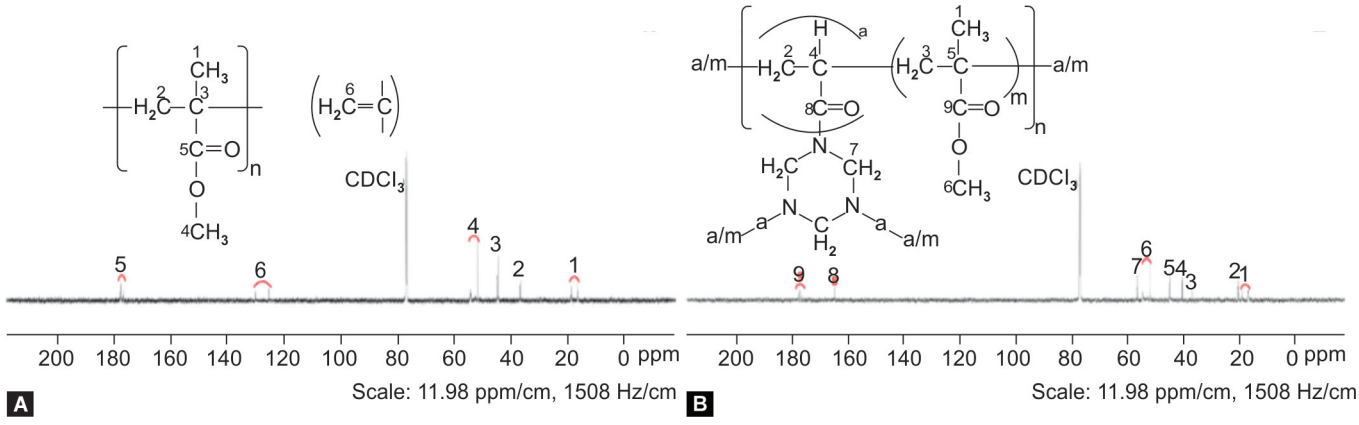
The mean (± standard deviation [SD]) T_g (°C) of G0, G10, and G20 groups was 105.112 ± 0.641, 115.741 ± 0.559, and 119.126 ± 0.487, respectively (Table 4). Statistically, a significant difference was observed among and between the groups (p = 0.000) (Table 5). The P(MMA-*Co*-TATA) copolymer with 20% TATA had the highest T_g. Figure 5 depicts the DSC curves of the groups. A characteristic exothermic post/cold crystallization curve was observed exceptionally in the G20 at approximately 157°C (T_c), absent in the G10 and G0 groups.

Discussion

The CP of TATA with MMA and the formation of novel copolymer P(MMA-*Co*-TATA) were confirmed using NMR and EDX spectroscopies. The effect of CP of TATA commoner with the DBR on the T_g was demonstrated in the present research. The chemical structure of the thermo-polymerized DBR was successfully altered by the substitution of antimicrobial triazine-based TATA



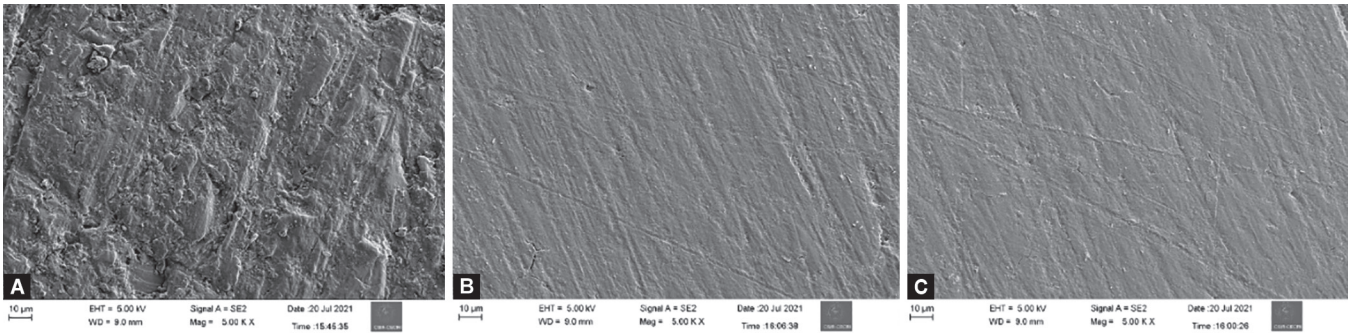
Figs 1A and B: ^{1}H-NMR spectra of (A) G0 group; (B) G10 and G20 groups



Figs 2A and B: ^{13}C-NMR spectra of (A) G0 group; (B) G10 and G20 groups

Table 2: NMR peak intensities of the groups

	Group (δ/ppm)		
^{1}H-NMR: protons of the ingredients	*G0*	*G10*	*G20*
┤C-C***H***$_3$	0.772–1.361	0.774–1.361	0.759–1.172
┤C-C***H***$_2$-C├	1.543–1.871	1.556–1.872	1.733–1.873
-CH_2-Ć***H***(CO)-CH_2-	**Absent**	**2.093–2.097**	**2.106–2.110**
-O-C***H***$_3$	3.524	3.523	3.524
>N-C***H***$_2$-N<	**Absent**	**3.951**	**3.920**
=C***H***$_2$	**5.484, 5.731, 6.026, 6.302**	**Absent**	**Absent**
^{13}C-NMR: carbons of the ingredients			
α–***C***H_3	16.54, 18.74	16.47, 18.74	16.42, 18.74
-***C***H_2- (TATA)	**Absent**	**20.18**	**20.19**
β–CH_2– (MMA)	37.23	37.34	37.30
α–***C***H (tertiary carbon)	**Absent**	**40.05**	**40.08**
┼ ***C*** (quaternary carbon)	44.57, 44.91	44.57, 44.90	44.54, 44.89
-O-***C***H_3	51.80, 54.20, 54.42	51.78, 54.18, 54.42	51.84, 54.22, 54.41
>N-***C***H_2-N<	**Absent**	**56.89**	**56.91**
=***C***H_2	**126.11, 130.90**	**Absent**	**Absent**
C=O (amide)	**Absent**	**165.03**	**165.06**
C=O (ester)	176.98, 177.80, 178.09	176.95, 177.82, 178.10	177.86, 178.15

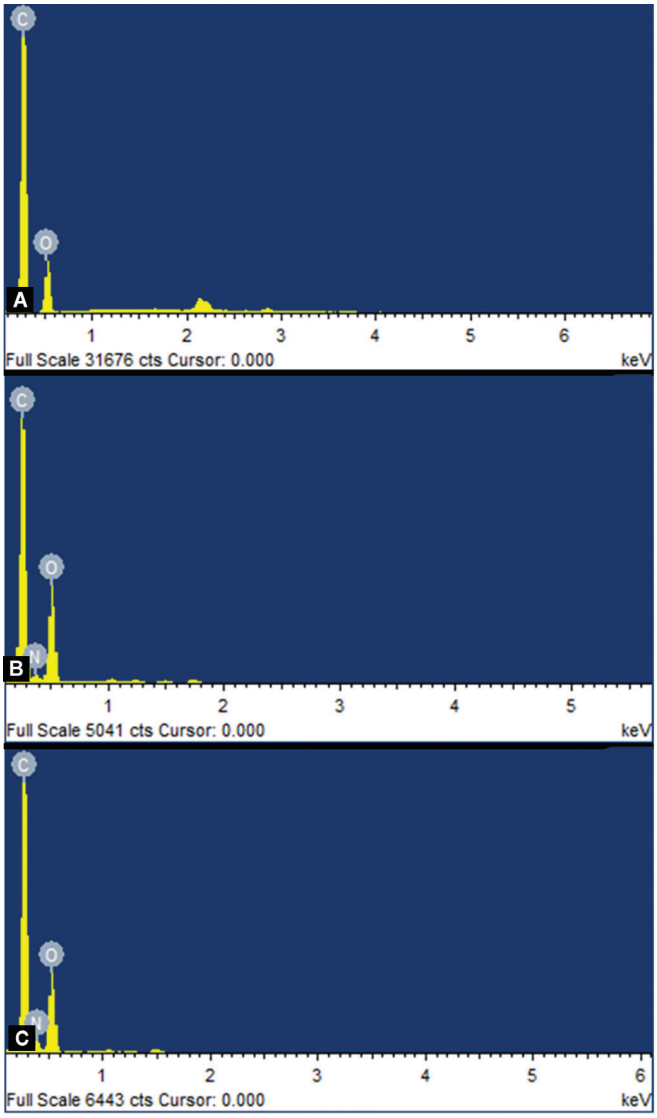


Figs 3A to C: FESEM surface topographies of (A) G0; (B) G10; (C) G20

comonomer yielding in the novel P(MMA-*Co*-TATA) copolymer. The CP in the G10 and G20 was ascertained using NMR which detected additional protons and carbons of the TATA in the spectra. This was indeed confirmed and attested by the EDX spectroscopy which detected the "N" atoms in the trial groups. Ajay et al.[11] copolymerized a cycloaliphatic cross-linking comonomer TCDDMDA with the DBR and confirmed the CP through NMR and EDX spectroscopies. Substitution of this comonomer resulted in better physicomechanical and biological properties of the DBR.[17,18] Therefore, in the present research, the conventional cross-linker ethylene glycol dimethacrylate was deliberately replaced completely by TCDDMDA cross-linker. He et al.[19] synthesized and characterized various quaternized N-containing antibacterial comonomers by NMR spectroscopy which were substituted in the dental composite resins to yield antibacterial properties. Ayaz et al.[20] confirmed the CP of N-based acrylamide comonomer with the DBR by NMR spectroscopy where the formed copolymer showed a new peak signal denoting –NH_2 functional group.

In the present research, the substitution of antimicrobial TATA cross-linking commoner in the DBR increased the T_g in the trial groups. This is congruent with a previous study that postulated that the higher the cross-linking density and degree of cure, the higher the T_g.[21] However, the plausible association between T_g and the cross-linker has not been found yet. In the literature, divinyl cross-linker in P(MMA) significantly modified the T_g.[22] Nevertheless, the association between T_g and cross-linker's chain length and concentration is oddly a convoluted theory. Various polymerization cycles can bring aberrations in the T_g till 20°C.[23] Besides TATA, there were myriad other comonomers that had been experimented with DBR and evaluated their impact on the T_g.[10] In particular, fluoro-monomers with long C-chain and cycloaliphatic acrylate cross-linker elevated the T_g.[11,24,25] On the contrary, itaconate[26,27] and nitro-monomers[28] lowered the T_g. Therefore, from the above context, it is discernable that the type of substituted comonomer also has a strong influence on the T_g.

The tacticity of the polymer also influences the T_g. The T_gs for a few atactic P(MMA) powders were more than the conventionally accepted value of 105°C. Thermo-polymerization through free radical initiation at 60°C resulted predominantly (76%) syndiotactic P(MMA) with a T_g of 119°C.[29] P(MMA) of the G0 group in the present research exhibited a T_g of 105.11°C which is less than 119°C. This is attributed to the presence of *n*-BMA plasticizer which decreased



Figs 4A to C: EDX spectra of (A) G0; (B) G10; (C) G20

Table 3: Elemental composition by EDX spectroscopy

	Elemental mass %		
Group	*C*	*O*	*N*
G0	71.03	28.97	Absent
G10	51.40	40.69	7.91
G20	51.59	36.64	11.77

Table 4: One-way ANOVA—comparison of mean T_g among the groups

Group	*Mean ± SD (°C)*	*f value*	*p value*
G0	105.112 ± 0.641	1668.43987	0.000
G10	115.741 ± 0.559		
G20	119.126 ± 0.487		

the T_g. However, with an increase in the curing temperature, the resultant polymer turns into increasingly atactic. In the current research, thermo-polymerization was executed at 74°C for 8 hours followed by terminal boiling at 100°C for 30 minutes. Hence, the resultant P(MMA-*Co*-TATA) copolymer was atactic

Table 5: *Post-hoc* Tukey HSD test for T_g

Group (i)	*Compared group (j)*	*Mean difference (i–j)*	*Q value*	*p value*
G0	G10	−10.629*	−59.3729	0.000
	G20	−14.014*	−78.2813	0.000
G10	G20	−3.385*	−18.9084	0.000

*Mean difference is significant at 0.05 level

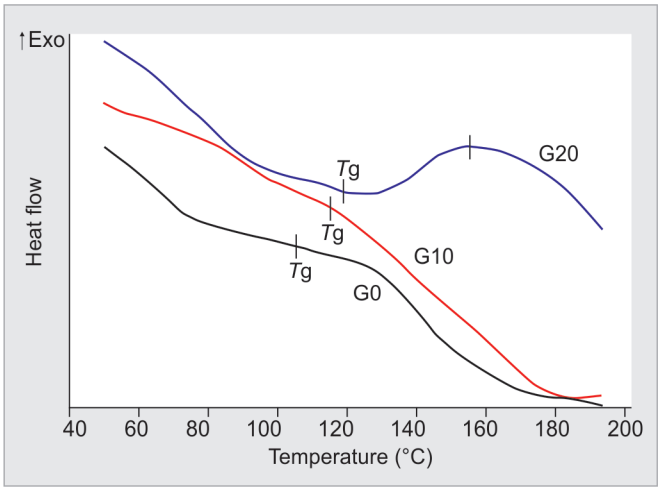


Fig. 5: DSC curves of the groups for T_g

which is amorphic rather than crystalline. The entropy value for the amorphous polymer is high due to high disordering leading to high T_g. Hence, this can be the possible explanation for high T_g for the P(MMA-*Co*-TATA) copolymer. A raise in the Mw reduces the chain end concentration and free volume and in turn increases the T_g in straight chain polymers. In this research, P(MMA) powder with Mw 350 × 10^3 g/mol was utilized in all groups. Therefore, the increased T_g in the trial groups was purely attributed to the TATA cross-linking comonomer. Fox and Flory[30] found a direct proportionality between the T_g and Mw concerning polystyrene. Another possible reason for high T_g may be attributable to the molecular structure of the TATA which is bulky with a rigid side group decreasing the chain mobility during heating. Other factors such as branching, length of the polymeric chain, the interaction of bonds, and polymer chain rigidity influencing the T_g are beyond the discussion scope.

In this research, only two concentrations of TATA were incorporated in DBR. The copolymerizability and its effect on T_g more than 20% concentration have not been tested so far. This research is the first of its kind to employ an antimicrobial triazine-based monomer in DBR. Hence, the results ought to be deciphered with prudence. Future research should be mandatorily conducted by evaluating the anti-microbial efficacy, other physico-mechanical properties, and biocompatibility by increasing the TATA's concentration.

Conclusion

Within the research constraints, the copolymerization of the antimicrobial cross-linker TATA was established successfully resulting in a novel denture base copolymer P(MMA-*Co*-TATA) possessing high T_g and smooth surface topography when compared to P(MMA).

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