RESEARCH PAPER

Synthesis and Characterization of a Urethane Dimethacrylate Monomer Containing a Quaternary Ammonium Salt for Use as a Component of Orthodontic Adhesive Primer

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Received: May2022 Revised: July2022 Accepted: July2022

DOI: 10.22068/ijmse.2810

Abstract: *Quaternary ammonium compounds (QACs) are among the most commonly used antibacterial agents. The aim of this study was to synthesize a dimethacrylate monomer functionalized with a QAC and to study its effect on the properties of an orthodontic adhesive primer. Urethane dimethacrylate monomer functionalized with a QAC (UDMAQAC) was synthesized and then characterized by nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FTIR). 5, 10, 15 and 20 wt% of UDMAQAC was added to an orthodontic adhesive primer (control group). FTIR analysis was used to measure the degree of conversion (DC). The bond strength of dental brackets was measured by shear bond strength (SBS) test and adhesive remaining index (ARI) was evaluated by stereomicroscope. Agar diffusion test and MTT assay were used to evaluate the antibacterial property and cell viability, respectively. Statistical analysis included one-way ANOVA with Tukey's post hoc test and Kruskal-Wallis nonparametric test ($P<0.05$). Although the obtained data did not show significant differences between the SBS and DC of different groups, but the highest values were obtained by adding 10 wt% monomer. Adding more than 10 wt% UDMAQAC resulted in significant increase in antibacterial property. The 15 and 20 wt% groups showed significantly lower cell viability.*

Keywords: *Quaternary Ammonium Compound; Antibacterial; Bond Strength.*

1. INTRODUCTION

Use of adhesive systems containing antibacterial agents is on the rise [1]. Orthodontic primers and adhesives are no exception to this rule, especially because plaque accumulation and development of white spot lesions around orthodontic brackets are among the main complications of fixed orthodontic treatment. It has been reported that placement of fixed orthodontic appliances increases the oral Streptococcus mutans count [2, 3]. These anaerobic microorganisms produce organic acids at a pH below 5.5, and demineralize the tooth structure as such [4]. Also, resin adhesives remaining around orthodontic brackets can create a suitable environment for bacterial accumulation [5]. Several disinfecting agents have been incorporated in the composition of composite resins and dental adhesives to confer antibacterial activity. Quaternary ammonium compounds (QACs) are among the most commonly used antibacterial agents. The optimal antibacterial properties of QACs have been well documented in the literature [1, 6]. QACs can react with monomers, form covalent bonds, and confer antibacterial properties. QACs have a positive charge while the cell wall of the majority of bacteria has a negative charge due to the presence of phosphatidylethanolamine which accounts for 70% of the cell wall structure. The interactions of the bacterial cell wall with QACs impair the electrical balance of the cell membrane and result in its disruption under osmotic pressure. Accordingly, the long lipophilic alkyl chain binds to the bacterial cell wall and penetrates into the cytoplasm, enhancing the bacterial cell elimination by the contact killing mechanism [1, 7]. QACs are used in different forms in dentistry such as monomers and fillers [1]. In this regard, 12-methacryloyloxy-dodecyl-

pyridinium-bromide (MDPB) is among the most well-known monomers that has been incorporated in the composition of Clearfil SE Protect dental adhesive [8]. The monomers used in dental adhesives are dimethacrylate monomers, while many of the synthesized QAC monomers only have one methacrylate group and it may not work well as a cross-linker [9]. This study aimed to describe the synthesis of a dimethacrylate monomer functionalized with a QAC and its characterization. The effect of addition of this monomer on the properties of an orthodontic adhesive primer was also evaluated.

2. EXPERIMENTAL PROCEDURES

2.1. Synthesis of urethane dimethacrylate monomer functionalized with a QAC

In this study, a new compound was synthesized which included a urethane dimethacrylate monomer functionalized with a QAC. For this purpose, the urethane acrylate monomer containing a QAC was synthesized in three chemical phases, and added to a commercial orthodontic adhesive primer. All chemical ingredients were purchased from Merck (Merck KGaA, Darmstadt, Germany) or Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA) with high purity.

2.1.1. Synthesis of QAC [BIS (2-hydroxyethyl) hexadecyl methyl ammonium bromide]

For the synthesis of QAC, 0.01 M 1-bromohexane decane (equal to 30.54 g) along with 0.01 M N-methyl di-ethanol amine (equal to 11.91 g) were mixed in a 3-outlet reactor equipped with a condenser, thermometer, and nitrogen gas inlet; 80 mL of isopropyl alcohol was added to the mixture and stirred at 80°C for 2 hours. The obtained product was cooled and deposited by adding 200 mL of diethyl ether as an anti-solvent. The deposits were filtered using a filter paper (0.22 µm, Whatman, USA) and completely dried in a vacuum oven (VWR, USA) at 45°C for 4 hours. A total of 39 g of the product was obtained, and the reaction efficiency was 92% (Fig. 1).

2.1.2. Synthesis of urethane dimethacrylate monomer containing QAC (UDMAQAC) in two phases

For this purpose, 0.05 M QAC (equal to 21.22 g) was dissolved in 80 mL of anhydrous acetone, and in the first phase, 0.1 M isophorone diisocyanate (equal to 22.2 g) was added to the contents of the reactor; 0.06 g stannous octoate catalyst was also added, and the mixture was stirred for 4 hours in the reactor. Next, 0.1 M hydroxyethyl methacrylate monomer (equal to 13 g) was added to the reactor contents plus 0.06 g stannous octoate. To prevent the polymerization of acrylate units of hydroxyethyl methacrylate monomer, 0.1 g 4-methoxy phenol inhibitor was added, and the reactor contents were stirred at 55°C for 4 hours. The temperature of the reactor contents was reached to room temperature, 200 mL of diethyl ether anti-solvent was added, the deposits were filtered by a filter paper, rinsed three times with 50 mL of diethyl ether, and completely dried in an oven at 40°C for 4 hours. Accordingly, 49 g deposit was obtained, and the reaction efficiency of the synthesis of urethane acrylate containing QAC was approximately 87% (Figs. 2a, 2b).

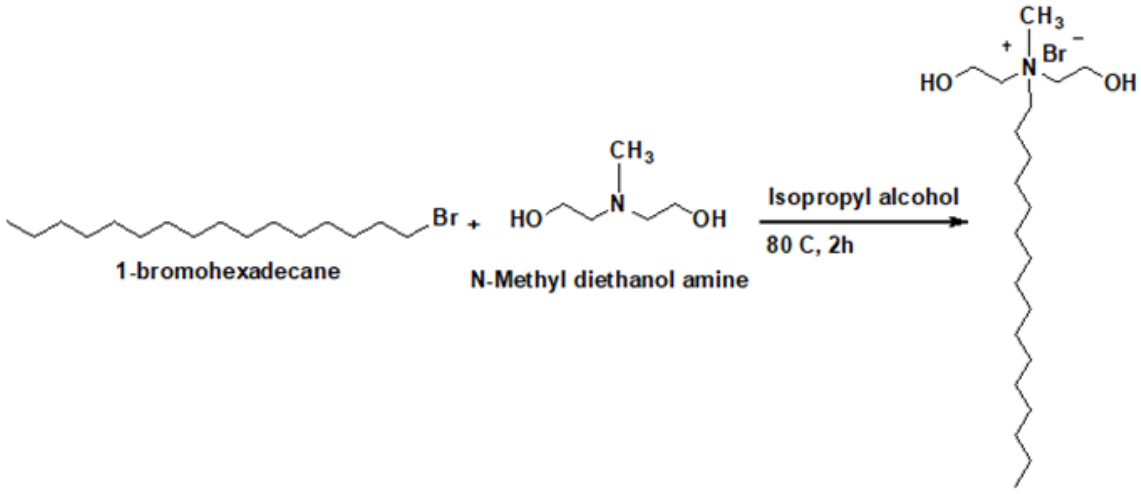

Fig. 1. Synthesis process and chemical formula of QAC

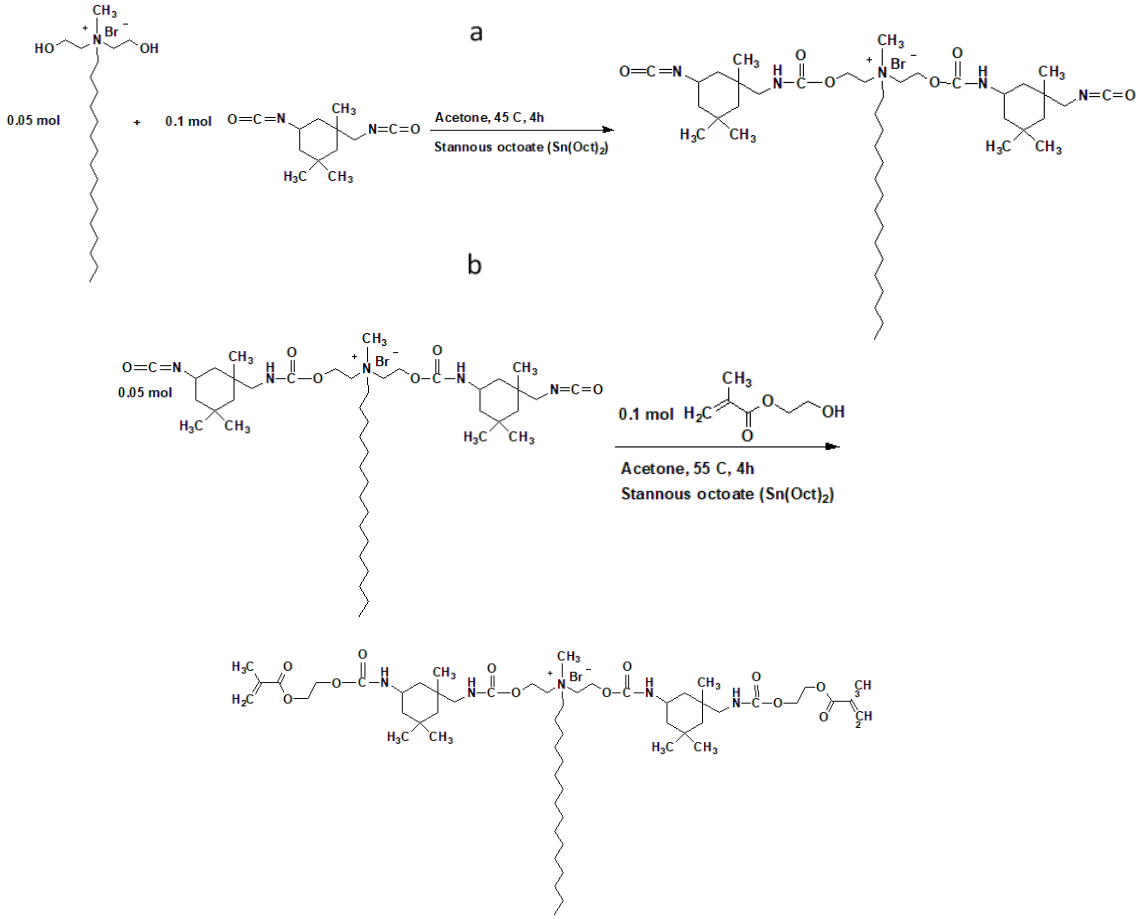

Fig. 2. Synthesis of urethane dimethacrylate monomer containing QAC (UDMAQAC) in two phases a (phase I) and b (phase II).

2.2. Characterization

In the present study, the following analyses were carried out to confirm the molecular structure of QAC and the final product (UDMAQAC):

2.2.1. Nuclear magnetic resonance (NMR) analysis

The ^{13}C NMR and ^{1}H NMR analyses were conducted on the synthesized material (DRX-500 AVANCE instrument; 500.1 MHz for ^{1}H, 125.0 MHz for ^{13}C, solvent: CDCl3).

2.2.2. Fourier transform infrared spectroscopy (FTIR):

The IR spectra of the synthesized materials were recorded by a spectrophotometer (Nicolet IS10.USA) using a KBr disc at 400-4000 cm^{-1} wavelength range, and 4 cm^{-1} resolution.

2.3. Addition of synthesized monomer to a commercial orthodontic adhesive primer:

Different weight percentages of the UDMAQAC were added to a commercial primer (Transbond XT primer; 3M Unitek, Monrovia, California, USA). To ensure complete mixing of the primer liquid with urethane acrylate monomer, mixing was performed in the dark in an oven (Memmert GmbH, Schwabach, Germany) at 60°C for 5 minutes. The study groups were as follows:

(I) Control: Transbond XT light cure adhesive primer
(II) 5-UDMAQAC: 95 wt% Transbond XT adhesive primer + 5 wt% UDMAQAC
(III) 10-UDMAQAC: 90 wt% Transbond XT adhesive primer + 10 wt% UDMAQAC
(IV) 15-UDMAQAC: 85 wt% Transbond XT adhesive primer + 15 wt% UDMAQAC
(V) 20-UDMAQAC: 80 wt% Transbond XT adhesive primer + 20 wt% UDMAQAC

The following analyses were then performed on the abovementioned four groups:

2.4. Assessment of degree of conversion (DC) of monomer to polymer:

Attenuated total reflectance (ATR)-FTIR analysis

was performed to calculate the DC. The specimens (n= 8) were selected from each group, and their ATR spectra were recorded before and immediately after curing. Curing was carried out using a light curing unit (Woodpecker Med. Instrument, Guilin, China) with 470 nm wavelength and light intensity of 1200 mW/cm^2 for 20 seconds. Two absorbance peaks are important in calculation of DC: An aliphatic peak at around 1637 cm^{-1} (C=C) that changes after curing, and an aromatic peak at around 1608 cm^{-1} (C=C) which is constant, and serves as an internal standard. DC was calculated using the following formula:

$$DC\% = \left[1 - \frac{(1637\ cm^{-1}\ /\ 1608\ cm^{-1})\ peak\ area\ after\ curing}{(1637\ cm^{-1}\ /\ 1608\ cm^{-1})\ peak\ area\ before\ curing}\right] \times 100$$

2.5. Assessment of cell viability (following exposed to cured adhesive primers)

To prepare disc-shaped specimens (n= 8), the materials were applied in molds with an internal diameter of 6 mm and thickness of 1 mm, and cured for 20 seconds. After removing from the molds, the specimens were cured again for another 20 seconds from all directions. The specimens were then sterilized with ethylene oxide gas. To assess the cytotoxicity of specimens and their effect on cell growth and proliferation, extraction was performed according to ISO 10993-5. In this process, 1 mL of culture medium was added per each 6 cm^2 surface area of each sterile specimen. After 3 days, the culture medium was extracted.

To assess the cytotoxicity of primers, the methyl thiazolyl tetrazolium (MTT) assay was performed, which is based on conversion of yellow tetrazolium powder to water insoluble purple formazan crystals. L929 cells (NCBI C161, Pasteur Institute Cell Bank, Tehran, Iran) were cultured at 37°C (5% CO_2, 95% humidity) in RPMI (Roswell Park Memorial Institute) 1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (pen-strep).

To assess cell proliferation, 1 x 10^4 cells along with 100 µL of culture medium were added to each well of a 96-well plate, and incubated at 37°C for 24 hours in order for the cells to adhere to the bottom of the plate. After ensuring cell adhesion, the overlaying culture medium was removed, and 90 µL of the extract of the samples and twice-diluted samples along with 10 µL of fetal bovine serum were added to each well. The cells were then exposed to the extracts for 24 and 72 hours. Next, the culture medium was removed and replaced with 100 µL of 0.5 mg/mL MTT in each well, followed by 4 hours of incubation. After 4 hours, the overlaying medium was removed, and isopropanol was added to the cells to dissolve the formed purple crystals. The concentration of the dissolved crystals in isopropanol was measured by an ELISA Reader (STAT FAX 2100, USA) at 570 nm wavelength. The mean optical density (OD) of the complete culture medium was considered as 100% viability. The relative viability of the treated cells was calculated as a percentage relative to the untreated control cells.

2.6. Assessment of antimicrobial activity of cured and uncured adhesive primers

The agar diffusion test was used to assess the antibacterial activity of cured and uncured specimens. Eight discs with 6 mm diameter and 1 mm thickness were fabricated in each group as explained earlier to serve as cured specimens. After curing, the specimens were sterilized by ethylene oxide gas. Sterile paper discs with 6 mm diameter and 1.5 mm thickness were used for uncured specimens. The paper discs were dipped in uncured primers (20 µL) in the five groups.

For the agar test, Streptococcus mutans (IBRC-M 10682) was used to prepare a microbial suspension with a cell density of 1.5 × 10^8 CFU/mL (0.5 McFarland standard). The suspension was densely cultured on tryptic soy agar (TSA). Next, the cured and uncured specimens were placed on the respective plates (15 mm × 100 mm) containing the bacteria. The plates were incubated at 37°C and 5% CO_2 for 48 hours, and then the diameter of the bacterial growth inhibition zones was measured.

2.7. Measurement of shear bond strength (SBS) by a loop wire

To assess the effect of new experimental primers SBS, 60 extracted sound first premolars (n= 12 in each group) with no caries, restorations, or cracks were used. Soft tissue residues were removed, and the teeth were disinfected by immersion in 0.5% chloramine T solution at 4°C for 7 days. The teeth were then etched with 32% etchant (Scotchbond Universal; 3M ESPE, USA) for 20 seconds. The five primer groups were used for bracket bonding. Next, Transbond™ XT Light Cure Paste Adhesive

(3M Unitek, Monrovia, California, USA) was applied on stainless steel standard edgewise (American orthodontics, Sheboygan, USA) premolar bracket bases with 0.018-inch slot size and 12.62 mm^2 bracket base area according to the manufacturer's instructions. The compositions of main primer and adhesive are shown in Table 1.

The brackets were positioned at the center of the buccal surface of tooth crowns (both mesiodistally and occluso-gingivally) by applying equal load. After aligning the longitudinal axis of brackets with the longitudinal axis of tooth crown, excess adhesive was carefully removed by a scaler, and each tooth was light-cured from the mesial, distal, occlusal, and gingival surfaces for a total of 40 seconds. To ensure complete curing, the specimens were incubated in distilled water at 37°C for 24 hours. Next, they underwent thermocycling (Vafaei Industrial, Iran) between 5-55°C with a dwell time of 20 seconds in each bath, and a transfer time of 10 seconds.

The specimens were mounted in self-cure acrylic resin. To ensured that all brackets on all teeth were mounted in the same orientation, the teeth were fixed to a rectangular stainless-steel wire with a ligature wire. Each tooth was positioned right at the center of each mold, and then the rectangular wire was fixed to the mold with sticky wax to ensure no movement of the teeth when applying acrylic resin. The teeth were embedded in acrylic resin to the level of their cementoenamel junction [10]. After polymerization of acrylic resin and detaching the teeth from the rectangular wire, the SBS of bracket to tooth surface was measured by a universal testing machine (Zwick/Roell Z050, Ulm, Germany). The specimens were positioned in the machine such that the bracket base was parallel to the load application vector. The loop wire was placed below the bracket gingival wings and pulled upward applying a shear stress to the adhesive interface in gingivo-occlusal direction. The cross-head speed was 1 mm/minute, and 1 kN load cell applied load until failure. The value at the time of debonding was recorded in Newtons for each specimen. The values in Newtons were then divided by the bracket base area in square millimeters to calculate the SBS in megapascals (MPa). After debonding of specimens, the debonded tooth and bracket surfaces were inspected under a stereomicroscope (SMZ800; Nikon, Tokyo, Japan) at x10 magnification, and the adhesive remnant index (ARI) score was calculated based on the amount of residual adhesive on the enamel surface as follows:

Score 1: No adhesive remaining on the enamel

Score 2: Less than 50% of adhesive remaining on the enamel

Score 3: ≥ 50% of adhesive remaining on the enamel

Score 4: Entire adhesive remaining on the enamel, and the impression of bracket mesh on the adhesive is visible [11].

2.8. Statistical Analysis

The bond strength, MTT assay and antibacterial test were assessed by one-way ANOVA and Tukey's post hoc test. ARI evaluation was performed by Kruskal-Wallis nonparametric test. A p-value lower than 0.05 was considered statistically significant ($P< 0.05$). The data were analyzed using IBM® SPSS® 26 Statistics software.

3. RESULTS AND DISCUSSION

An antibacterial agent can be acceptably incorporated in the composition of an adhesive only if it confers strong antimicrobial activity and shows no or minimal adverse effects on the properties of adhesive such as its SBS and biocompatibility.

Table 1. The compositions of the main adhesive primer and paste

Material	Manufacturer	LOT No	Composition
Transbond™ XT Light Cure Adhesive Primer	3M Unitek, Monrovia, California, USA	N701939	Bisphenol-a diglycidyl ether dimethacrylate, Triethylene glycol dimethacrylate (BISGMA) (TEGDMA), 4-(Dimethylamino)-benzeneethanol
Transbond™ XT Light Cure Adhesive Paste	3M Unitek, Monrovia, California, USA	N689386	Silane-treated quartz, Bisphenol A diglycidyl ether dimethacrylate (BISGMA), Bisphenol A dimethacrylate, Silane-treated silica, Diphenyliodonium hexafluorophosphate, Triphenylantimony

3.1. Characterization of the synthesized monomer by NMR and FTIR

3.1.1. NMR

^{1}HNMR (QAC): Figure 3a shows the location of protons of the QAC. Additional peaks (marked with letter i) are related to the stannous octoate catalyst, that despite purification, were not completely separated from the product.

^{13}CNMR (QAC): The starred carbon peak in the following formula appeared at around 58.7 ppm, which was deshielded due to approximation of this carbon to nitrogen compared with other carbons. It indicates the binding of this carbon to nitrogen, and confirms the synthesis of the claimed product (marked with letter g) (Fig. 3b).

^{1}HNMR (UDMAQAC): The two terminals $=CH_2$ functional groups with a vinyl H are located in the structure of final product in cis and trans forms relative to each other. The hydrogen in cis form appeared at 5.5 ppm, and the hydrogen in trans form appeared at 6.1 ppm, confirming the presence of methacrylate groups at the two ends of the molecule. These two peaks are marked with letter q in HNMR spectra (Fig. 4a).

^{13}CNMR (UDMAQAC): The carbon peak related to the two terminal methacrylate groups ($=CH_2$) appeared at 126 ppm, marked with letter m.

The peak related to C=O (carbonyl) of polyurethane group (-NH-(C=O)-O-) appeared at 155 ppm, and marked with letter O. These two peaks indicate the final product structure (Fig. 4b).

3.1.2. FTIR

FTIR (QAC): The FTIR spectrum of the intermediate product confirmed the synthesis of QAC monomer (Fig. 5a). The stretching vibration of the QAC appeared as a distinct and relatively strong peak at 3293 cm^{-1}.

The stretching vibration of C=C related to CH2 and CH_3 units of the synthesized monomer appeared at 2919 and 2851 cm^{-1}, respectively, and small narrow peaks adjacent to each other at 1420 to 1483 cm^{-1} indicated the bending vibrations of CH_2 in the alkyl and methylol groups, respectively. The bending vibration of CH_3 in methyl groups of methylol was noted at 1380 and 1396 cm^{-1}. The stretching vibration of C-N of the synthesized product appeared at 1258 cm^{-1}. A wide peak at 1050 cm^{-1} was related to the stretching vibration of C-O of alkyl group at the terminal end of the synthesized monomer. The QAC was used to confer antibacterial activity and the methacrylate groups were used to form covalent bonds to the polymer network.

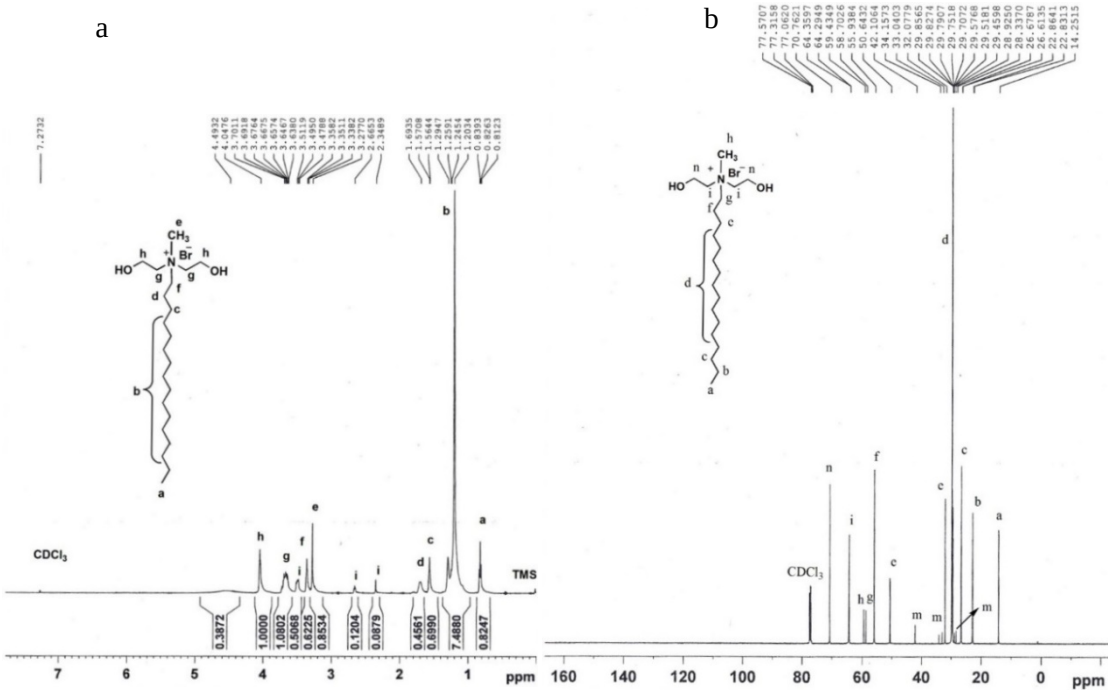

Fig. 3. ^{1}H NMR (a) and ^{13}C NMR spectra (b) of QAC

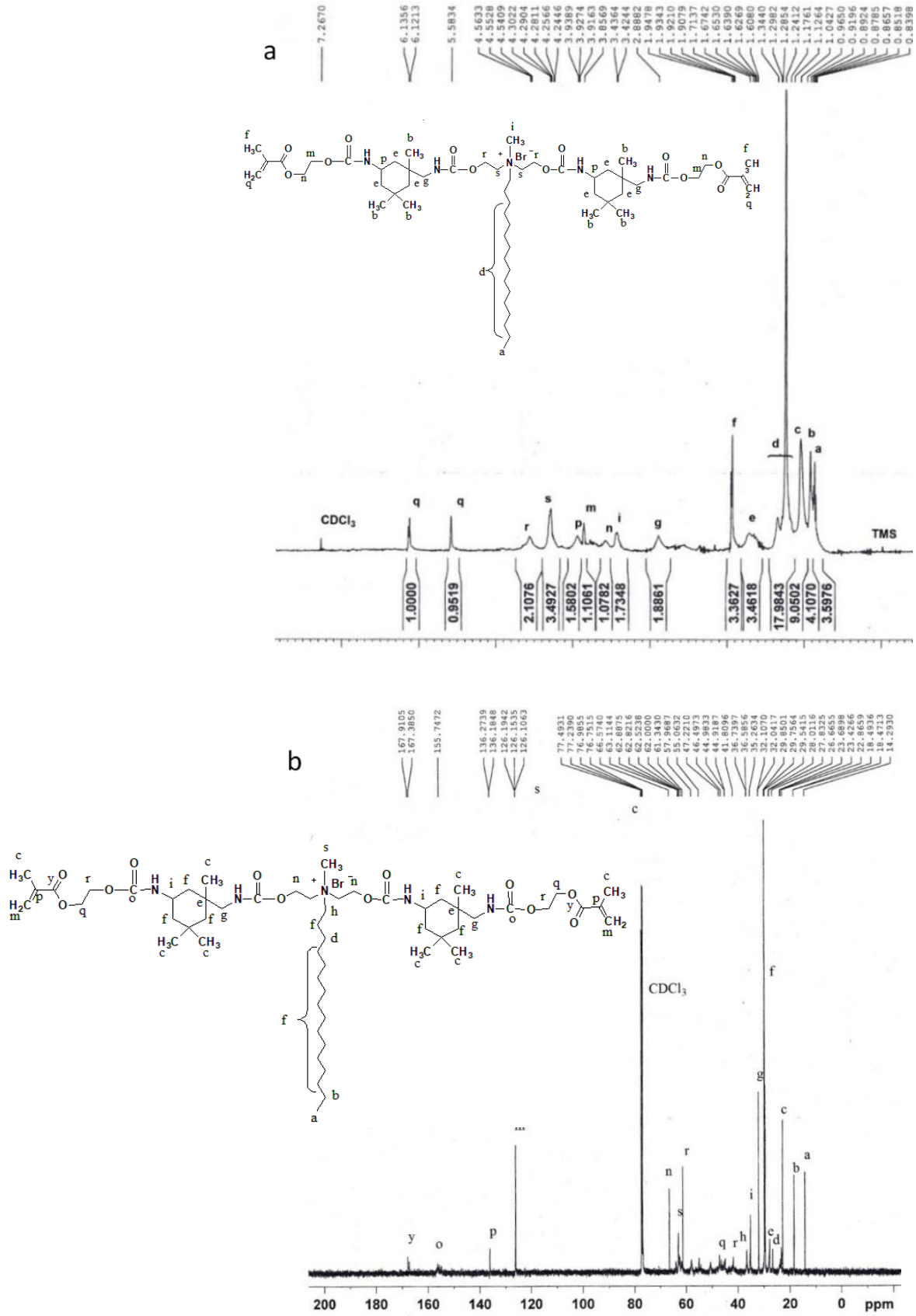

Fig. 4. ^{1}H NMR (a) and ^{13}C NMR spectra (b) of UDMAQAC

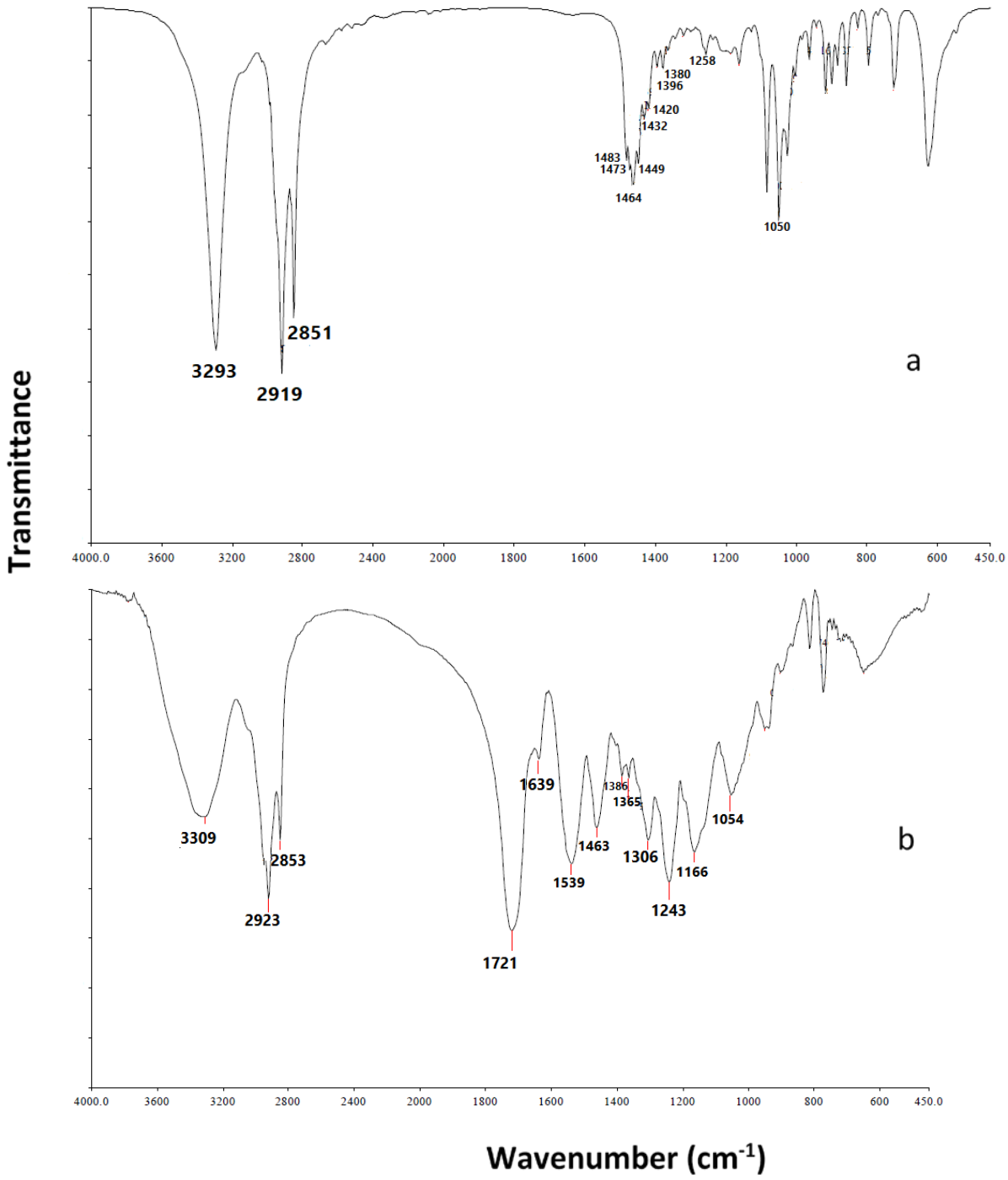

Fig. 5. FTIR spectra for QAC (a) and UDMAQAC (b)

FTIR (UDMAQAC): The FTIR spectra of the final product confirmed the synthesis of a urethane dimethacrylate containing QAC by using isophorone diisocyanate. The stretching vibration of QAC was seen as a distinct relatively strong peak at 3309 cm^{-1}. The stretching vibration related to CH_2 and CH_3 units of the synthesized QAC, isophorone diisocyanate, and hydroxyethyl methacrylate appeared at 2923 and 2853 cm^{-1}, respectively. The stretching vibration of C=O of carbonyl group of acrylate monomers and urethane bonds appeared as a wide peak at 1721 cm^{-1}. A small peak at 1639 cm^{-1} indicated the stretching vibration of C=C of acrylate unit. A wide peak at 1243 cm^{-1} was related to the bending vibration of C-N of urethane bonds, and the bonds

related to the attachment of QAC to urethane acrylate structure. The frequency of bending vibration of CH_2 in the structure of synthesized monomer appeared at 1463 cm^{-1}. The bending vibration of CH_3 in methyl groups of methylol units, diisocyanate, and methyl methacrylate monomer appeared at 1386, 1365, and 1306 cm^{-1}, respectively. The peak at 1243 cm^{-1} belonged to the stretching vibration of C-N in the synthesized product. The stretching vibration of C-O in urethane bonds appeared in the form of two wide peaks at 1166 and 1054 cm^{-1}[12] (Fig. 5b).

3.2. DC

The DC values are listed in Table 2. DC has a prominent role in durability of bonds to tooth structure. High DC not only improves the mechanical properties of the bonding layer, but also decreases the permeability of the bonding interface, and minimizes degradability [13]. On the other hand, given that the degree of cross-linking of antibacterial compounds in polymer network is low, there is a concern that safety will decline due to the release of antibacterial agents [14]. The obtained data showed that inclusion of the antibacterial monomer did not affect the DC of UDMA-based monomer adversely. This phenomenon is ascribed to the chain transfer reactions caused by UDMA's –NH– groups [15, 16]. UDMA has a higher DC than bis-GMA. It is due to the absence of a phenol ring in the UDMA monomer chain which results in high flexibility. The stiff molecular structure of bis-GMA (as the main component of orthodontic primer) and its strong hydrogen bonds between hydroxyl groups make it more rigid [9, 17]. On the other hand, use of dimethacrylate monomers containing QAC with cross-linking polymerization can increase the DC, compared with mono-methacrylate monomers with linear polymerization [1]. The obtained results indicated that addition of urethane monomer had no adverse effect on DC. This result was consistent with the finding of some other studies which showed no effect or even slight increase in DC values by adding methacrylate monomers containing quaternary ammonium salt [18, 19]. Although no significant difference was observed between the groups, it is clear that up to 10 percent increase in monomer content, the amount of DC values increased and then a decrease in DC was observed. Further increase in monomer concentration may increase the viscosity, and results in lower mobility of monomers, which can adversely affect the DC and the descending trend would probably be more noticeable in higher concentrations [7]. ATR spectra of one of the groups are shown in Fig. 6.

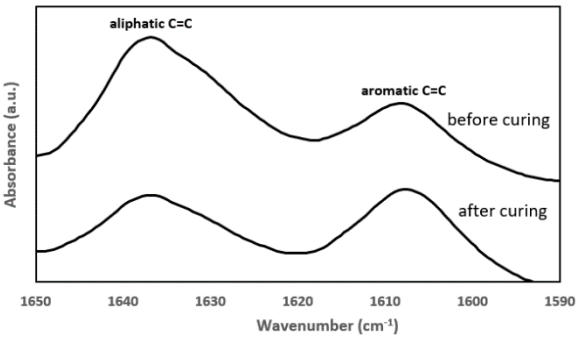

Fig. 6. ATR spectra of uncured and cured adhesive primers were used for measuring degree of conversion.

3.3. SBS test

The current results demonstrated that addition of QAC did not cause a significant difference in SBS of the synthesized products compared with the control group, and the resultant SBS remained above the acceptable threshold of 6-8 MPa [20] (Table 2). Assessment of ARI scores also revealed no significant difference among the groups (Table 3). This result was in agreement with a previous study by Assad-Loss et al which showed no adverse effect of addition of QAC to adhesive on SBS of orthodontic brackets [21]. However, similar to DC values, the obtained SBS values decreased in the groups with more than 10% UDMAQAC content. Besides the adverse effect of the higher concentration of QAC on DC values which decreases the bond strength, it may also lead to the formation of a condensed layer which acts as a stress-riser causing microcracks in adhesive layer [22].

3.4. Antibacterial test

Among the uncured specimens, the control group showed no growth inhibition zone (Fig. 7). However, all groups containing QAC showed growth inhibition zones. By an increase in UDMAQAC concentration, the antibacterial properties increased, such that the 5% specimens had no significant difference with the control group but 10%, 15% and 20% groups showed antibacterial activity significantly higher than the control group.

Table 2. The mean values for DC (degree of conversion) and SBS (shear bond strength), SD: standard deviation

group	DC		SBS	
	Mean (%)	SD(±)	Mean (MPa)	SD(±)
control	77	8.4	17.4	4.0
5-UDMAQAC	79.3	8	18.3	5.8
10-UDMAQAC	81.3	6.3	20.6	5.2
15-UDMAQAC	74.3	6.7	16.5	5.6
20-UDMAQAC	72.8	9	15.6	4.4

Table 3. adhesive remnant index (ARI) based on the numbers of specimens for each score

group	ARI Score			
	0	1	2	3
control	1	5	5	1
5-UDMAQAC	1	3	6	2
10-UDMAQAC	0	4	6	2
15-UDMAQAC	2	4	6	0
20-UDMAQAC	1	6	4	1

It is in consistent with the study of Liang et al. which showed that quaternary ammonium dimethacrylate monomer has antibacterial effect when its concentration reaches 10% or more [23]. The antibacterial test results indicated no antibacterial activity for any of the cured specimens (inhibition zone diameter= 0). As mentioned earlier, QACs form covalent bonds to the molecular structure of adhesive and resin. Resultantly, the QAC shows insignificant diffusion when bonds with cured primer, and its antibacterial effect would be limited to the surface of material (contact killing mechanism) [24, 25]. For a more detailed study of antibacterial properties in the cured state, it is recommended to use direct contact method instead of extraction method [26]. The antibacterial mechanism of QACs is based on the electric imbalance produced between negatively charged bacterial cells and positively charged nitrogen that leads to increased osmotic pressure [27]. The product synthesized in the present study had a hydrocarbon chain with 16 carbon atoms. Previous studies have demonstrated that QACs have maximum antibacterial activity when the number of carbon atoms in their alkyl chain is 12 to 16 [28-30]. This finding suggests another mechanism for the antibacterial properties of QACs, which is based on the penetration of ammonium-bound hydrocarbon chains into cell membranes followed by its disruption [27]. Despite the conflicting results, Chen et al showed that the presence of counter-ion such as bromide further adds to the antibacterial properties of the material [31].

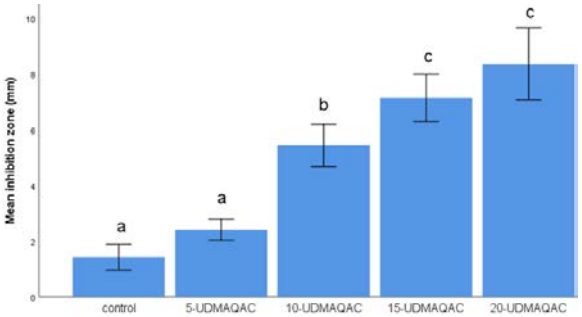

Fig. 7. Mean inhibition zone (mm) obtained from agar diffusion test

3.5. Cytotoxicity testing of uncured primers

The 100% concentration of all groups evaluated in this study showed low cell viability at 24 and 72 hours (Fig. 8a, 8b). In use of diluted samples, cell viability was significantly higher in all groups at both time points. It is worth mentioning that dilution of samples resembles the process which happens in vivo due to volume of saliva [32]. The results revealed a significant increase in cytotoxicity by addition of QAC compared with the control group; this increase was concentration-dependent, such that 20% group showed the lowest cell viability. The cytotoxicity of 3M primer is due to the presence of unreacted monomers, and solvents added to decrease its viscosity [33]. Although the DC of different groups was not significantly different, the amounts of unreacted monomers is higher in groups with higher monomer concentration.

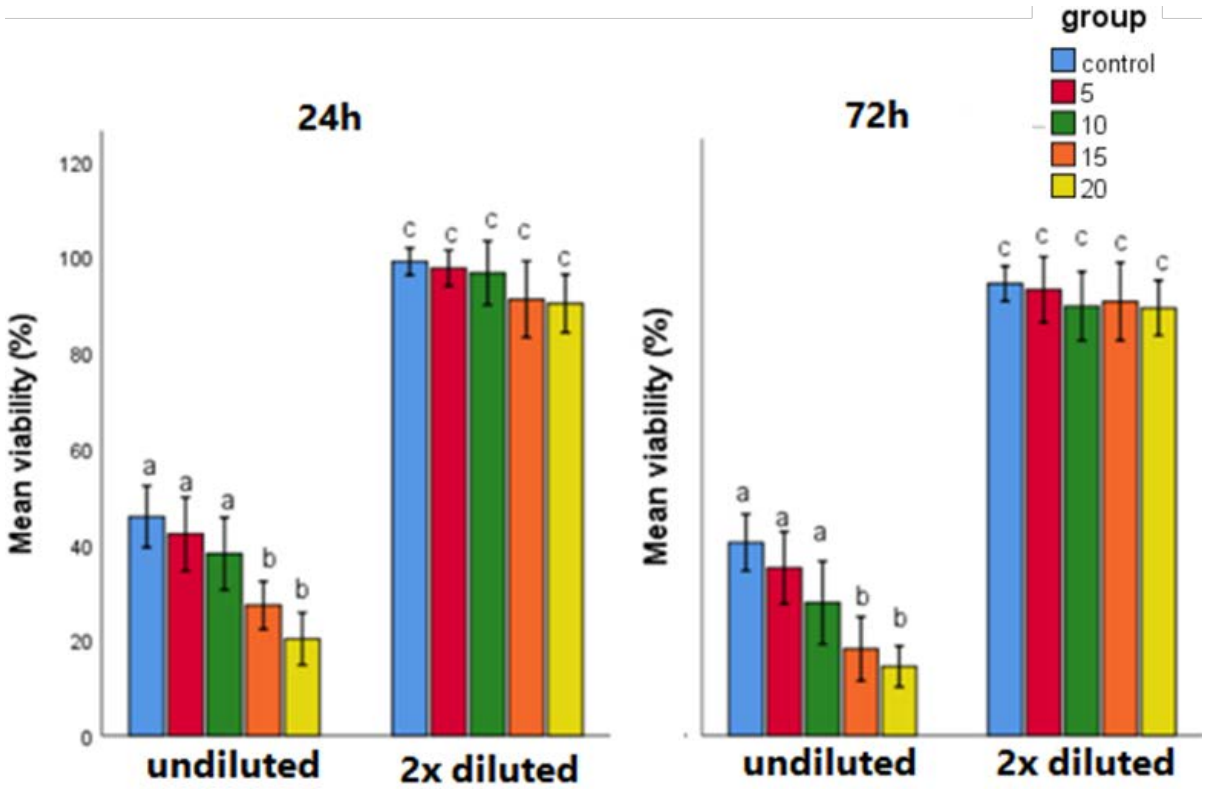

Fig. 8. Mean viability for each time period (24 h and 72 h), at 2 concentrations of extracted medium. Different lowercase letters indicate significant differences between groups.

The antibacterial agent is co-polymerized by forming covalent bonds with the polymer network, and is therefore completely immobilized [32]. Thus, the QAC cannot be released over time, or has a small release. Therefore, the material preserves its antibacterial activity at the surface while becoming more biocompatible. The dimethacrylate monomer containing QAC binds to the main polymer chain through its both terminal ends; while in compounds with one methacrylate group, this bond is mediated only through one terminal end of the monomer, and the other end of the monomer remains free. The free terminal end of the monomer has a plasticizing effect, which can compromise the polymer network. Moreover, it can increase the risk of leaching, and subsequent cytotoxicity of mono-methacrylates compared with dimethacrylates. Therefore, mono-methacrylate compounds containing QACs are used in lower concentrations compared with dimethacrylates, which can result in lower antibacterial properties [14, 34-36]. The miscibility of quaternary ammonium dimethacrylate which is caused by its low viscosity is thought to reduce leaching [19, 37].

4. CONCLUSIONS

DC and SBS values of orthodontic primer increased by adding urethane methacrylate monomer functionalized with a QAC up to 10%, and then decreased at higher concentrations. However, no significant differences were observed between groups. 15 and 20% groups showed the highest antibacterial effect with the lowest cell viability. According to the obtained results, 10 wt%. UDMA containing QAC seems to be the most proper percent for use in orthodontic adhesive primer.

REFERENCE

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