# Synthesis and Photopolymerizations of First Monomers with Phosphonate and Bisphosphonate or Phosphonic and Bisphosphonic Acid Functionalities for Potential Dental Applications

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**ABSTRACT**: The first monomers containing both phosphonate and bisphosphonate (M1) or phosphonic and bisphosphonic acid (M2) functionalities are synthesized, aiming to improve binding abilities of self-etching adhesive systems and composites: An amine having both phosphonate and bisphosphonate functionalities is prepared via Michael addition reaction between diethyl (6-aminohexyl)phosphonate and tetraethyl vinylidene bisphosphonate, its reaction with 2-isocyanatoethyl methacrylate gives M1 which is converted to M2 by selective dealkylation of the phosphonate/bisphosphonate ester groups. Their copolymerization with commercial dental monomers (bisphenol A glycidyl methacrylate, triethylene glycol dimethacrylate, and 2-hydroxyethyl methacrylate) investigated by photo-differential scanning calorimetry shows adequate photopolymerization rate and conversion. X-ray diffraction, Fourier transform infrared, and X-ray photoelectron spectroscopy analyses of M2-treated hydroxyapatite particles show formation of stable M2-calcium salts. These monomers are assessed to be not toxic according to MTT standards by *in vitro* cytotoxicity studies with NIH 3T3, U2OS, and Saos-2 cells. All these properties make these monomers potential candidates as biocompatible components for dental adhesives and composites. © 2018 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2018**, *56*, 2739–2751

**KEYWORDS**: bisphosphonic acid; dental adhesive; hydroxyapatite; photopolymerization; phosphonate

**INTRODUCTION** Dental composites are biomaterials which consist of a resin matrix, fillers, coupling agents, initiators, and inhibitors.[1] None of these ingredients have strong interaction with the tooth tissues (enamel and dentin), enough to ensure an acceptable lifetime for fillings. To provide said interaction, dental adhesives which follow an etch-and-rinse or a self-etch approach are used.[2–9] Both kinds of adhesives remove the smear layer which is formed on top of the dentin during cavity preparation using dental burs, generate an etch pattern on the enamel and lead diffusion of comonomers into the created porosities. After polymerization, they are micromechanically interlocked to form a hybrid layer.[10] Self-etch adhesives do not require a separate etching step, thanks to the acidic monomers in their mixtures. The acidic monomers contain functional groups such as carboxylic acids, dihydrogen phosphate or phosphonic acids, and simultaneously condition and prime the dental substrate.[9] Self-etch adhesive systems can be classified according to pH of their solutions as strong (pH < 1), mild (1 < pH < 2.0), and ultra-mild (pH > 2.5) which determine the interaction/infiltration depth of adhesives in dentin.[8]

In addition to micromechanical interlocking, chemical interaction of functional monomers with hydroxyapatite (HAP) (the inorganic component of teeth, $Ca_{10}(PO_4)_6(OH)_2$) is important to achieve good bonding and durability. The interaction of acidic monomers with dental tissues was described by a mechanism called the adhesion–decalcification concept (AD-concept).[11] According to this mechanism, the acidic monomer forms an ionic bond with calcium, and $OH^-$ and $PO_4^{3-}$ ions are released from HAP. If the ionic bond is stable, the monomer will remain bonded to HAP. If the ionic bond is not stable, decalcification with the release of $Ca^{2+}$ and phosphonate ions form HAP will occur.

The bonding performance of the adhesives depends on the structure of the acid monomer and small differences, such as in hydrophobicity, may result in significant changes in adhesive performance.[12–17] For example, 10-methacryloyloxydecyl diphosphate (MDP) forms stable calcium salts while 2-methacryloxyethyl phenyl hydrogen phosphate (Phenyl-P) deposits unstable calcium salts, and shows lower chemical interaction and dentin bond strength.[15] The presence of ester or ether groups in spacer chains may also affect the

hydrophilicity and influence the interaction of functional monomers with dental tissues.[12] Therefore, there seems to be room for improvement in the field of acidic dental monomers, and synthesis of functional monomers with better binding potential to HAP has been the subject of extensive research effort.

In addition to phosphonic[18–27] and phosphoric acid[28]-functionalized dental monomers, bisphosphonic acid-containing ones have been synthesized to be used in self-etching adhesives recently.[29–33] Bisphosphonic acids are synthetic analogues of inorganic pyrophosphate, are resistant to enzymatic hydrolysis and have ability to bind strongly to bone surface.[34–36] The two phosphonate groups are responsible for binding to bone mineral.

Besides good interaction with dental tissues, dental monomers should have a high rate of homopolymerization or copolymerization under light exposure with the comonomers of the dental formulation. Hydrogen bonding monomers are an important class of dental monomers, since the hydrogen bonds can bring the monomers closer, hence enhance reactivity. A set of such monomers were investigated by Berchtold and Jansen, who found monomers containing urea to be the most reactive.[37,38] The significantly enhanced polymerization rates and conversions of these monomers were explained by a combination of hydrogen abstraction/chain-transfer reactions due to labile hydrogens and hydrogen bonding imparted by the urea linkages. Recently, we described the synthesis of two monofunctional phosphonated urea methacrylates for dental materials.[39] The photopolymerization reactivities of these monovinyl methacrylates were found to surpass the reactivity of triethylene glycol dimethacrylate (TEGDMA), the most common crosslinker in dental formulations. These monofunctional methacrylates with enhanced rates and conversions were found to have potential to be used as diluent monomers in dental formulations. Then, phosphonic acids bearing urea groups were synthesized to improve the performance of self-etching adhesives by Moszner and coworkers and the presence of a urea group was shown to significantly increase both the rate of polymerization and the shear bond strength to both dentin and enamel.[40]

In this work, the first methacrylate monomers that contain both phosphonate and bisphosphonate or phosphonic and bisphosphonic acid functionalities were synthesized to improve self-etching dental adhesives' and/or dental composites' performances via enhanced HAP interaction. Their photopolymerization behaviors and interactions with HAP were investigated.

## EXPERIMENTAL

### Materials

Diethyl (6-aminohexyl)phosphonate was synthesized in three steps according to the literature procedures.[41–43] Tetraethyl vinylidene bisphosphonate was prepared from tetraethyl methylene bisphosphonate by the method of Degenhardt and Burdsall.[44] MDP was supplied by Ivoclar Vivadent AG Schaan, Lichtenstein. Roswell Park Memorial Institute (RPMI) 1640 medium (with L-glutamine and 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)), penicillin/streptomycin and trypsin–ethylenediaminetetraacetic acid (EDTA) were purchased from Multicell, Wisent (Quebec Canada). Fetal bovine serum (FBS) was obtained from Capricorn Scientific GmbH (Ebsdorfergrund Germany). Thiazolyl blue tetrazolium bromide (MTT) and phosphate buffered saline (PBS) tablets were provided by Biomatik (Cambridge, Ontario, Canada). All other chemicals and solvents, including diethyl amine, 2-isocyanatoethyl methacrylate (IEM), 2-hydroxyethyl methacrylate (HEMA), 1,6-dibromo hexane, trimethylsilyl bromide (TMSBr), triethyl phosphite, potassium phthalimide, hydrazine monohydrate, paraformaldehyde, tetraethyl methylene bisphosphonate, bisphenol A glycidyl methacrylate (bis-GMA), triethylene glycol dimethacrylate (TEGDMA), *para*-toluene sulfonic acid, 2,2-dimethoxy-2-phenylacetophenone (DMPA), phenylbis (2,4,6-trimethylbenzoyl)-phosphine oxide (BAPO) were obtained from Aldrich Chemical Co. (St. Louis, MO) and used without purification. Then, 96-well plates were purchased from Nest Biotechnology (Wuxi, Jiangsu China). NIH 3T3 mouse embryonic fibroblast cells, Saos-2 human osteosarcoma cells, and human bone osteosarcoma epithelial cells (U2OS) were a kind gift of Halil Kavakli (Department of Molecular Biology and Genetics, Koc University, Istanbul, Turkey).

### Characterization

Proton nuclear magnetic resonance ($^1$H-, $^{13}$C-NMR) spectra were recorded on a Varian Gemini (400 MHz) spectrometer with deuterated chloroform ($CDCl_3$) or methanol (MeOD) as solvent, and tetramethylsilane as an internal standard. Fourier transform infrared (FTIR) spectra of monomers were recorded with a Nicolet 6700, Thermo spectrometer. Combi Flash Companion Teledyne ISCO Flash Chromatography with C18 reverse phase column was used for purification of monomers. X-ray diffraction (XRD) patterns of HAP particles treated with the monomers were recorded using powders by coupled θ-2θ XRD (D8 Advance; Bruker, Karlsruhe), with a Cu Kα source (λ = 1.5418 Å) at operating parameters of 40 kV and 40 mA and with a step size of 0.01°. X-ray photoelectron spectroscopy (XPS) studies were carried on a Thermo Scientific K-Alpha X-ray Photoelectron Spectrometer. Scanning electron microscopy (SEM) images of monomer treated enamel and dentin samples were recorded by SEM (FEI-Philips XL30). Mass spectrometry (MS) experiments were carried out by using MS Q-time-of-flight (TOF) (model:G6530B) mass spectrometer equipped with an ion source dual electrospray ionization.

### Synthesis of Phosphonate- and Bisphosphonate-Functionalized Amine, Tetraethyl (2-((6-(diethoxyphosphoryl)hexyl)amino)ethane-1,1-diyl) bis(phosphonate) (A1)

Diethyl (6-aminohexyl)phosphonate (0.24 g, 1 mmol) and tetraethyl vinylidene bisphosphonate (0.3 g, 1 mmol) were mixed with a magnetic stir bar on a hotplate stirrer at room temperature for 2 days under inert atmosphere. The reaction mixture was washed with petroleum ether (3 × 10 mL) to

remove excess tetraethyl vinylidene bisphosphonate and dried under reduced pressure for 12 h. The product was obtained as yellow liquid in 63% yield.

$^1$H NMR (400 MHz, $CDCl_3$, δ): 1.34 (m, 18H, $OCH_2CH_3$), 1.52 (m, 8H, $PCH_2CH_2$), 1.70 (m, 2H, $CH_2$-P), 2.56 (t, $^3J_{HH}$ = 7.2 Hz, 2H, $CH_2CH_2NH$), 2.66 (dt, $^3J_{HH}$ = 6 Hz, $J_{HP}$ = 23.6 Hz, 1H, P-CH-P), 3.12 (td, $^3J_{HH}$ = 6 Hz, $J_{HP}$ = 16.6 Hz, 2H, $NHCH_2CH$-P), 4.12 (m, 12H, $OCH_2CH_3$) ppm. FTIR (attenuated total reflection [ATR]): 954, 1016 (P-O), 1239 (P=O), 2932 (C-H), 3476 (NH) cm$^{-1}$.

## Synthesis of Monomers

### *Synthesis of 2-(3-(2,2-Bis(diethoxyphosphoryl)ethyl)-3-(6-(diethoxyphosphoryl)hexyl)ureido)ethyl methacrylate (M1)*

To an ice-cold solution of A1 (0.22 g, 0.415 mmol) in 1.5 mL of dry chloroform under stream of nitrogen, IEM (0.066 g, 0.430 mmol) was added dropwise. The solution was stirred at room temperature overnight under a stream of nitrogen and then extracted with 1 wt % HCl (2 × 6.2 mL) and brine (2 × 6.2 mL). The organic layer was dried over $Na_2SO_4$, filtered, and evaporated under reduced pressure to leave the crude product which was purified by reversed-phase flash chromatography on C18, eluting with water/methanol (50/50). The pure product was obtained as pale yellow viscous liquid in about 30–40% yield.

$^1$H NMR (400 MHz, $CDCl_3$, δ): 1.30 (m, 18H, $OCH_2CH_3$), 1.52 (m, 8H, $PCH_2CH_2$), 1.68 (m, 2H, $CH_2$P), 1.91 (s, 3H, $CH_3C=CH_2$), 2.87 (tt, $^3J_{HH}$ = 5.8 Hz, $J_{HP}$ = 23.6 Hz, 1H, PCHP), 3.25 (t, $^3J_{HH}$ = 7.6 Hz, 2H, $CH_2CH_2N$), 3.49 (q, $J_{HH}$ = 5.6 Hz, 2H, $OCH_2CH_2NH$), 3.75 (td, $^3J_{HH}$ = 6 Hz, $J_{HP}$ = 14.2 Hz, 2H, $CH_2CHP$), 4.11 (m, 14H, $OCH_2CH_3$, $NHCH_2CH_2O$), 5.55, 6.14 (s, 2H, $C=CH_2$) ppm. $^{13}$C NMR (101 MHz, $CDCl_3$, δ): 16.35 ($OCH_2CH_3$), 18.27 ($CH_3C=CH_2$), 22.29, 24.89, 26.29, 27.67, 30.42 ($[CH_2]_5$-P), 36.85 (PCHP), 40.01 ($NCH_2CH_2$), 44.64 ($OCH_2CH_2NH$), 47.75 ($NCH_2CH$-P), 61.34, 62.55, 63.06, 64.07 ($OCH_2CH_3$, $NHCH_2CH_2O$), 125.65, 136.15 ($C=CH_2$), 157.74 (O=C-NH), 167.42 (O=C-$OCH_2$) ppm. FTIR (ATR): 958, 1020 (P-O), 1239 (P=O), 1560 (N-H), 1632, 1716 (C=O), 2984 (C-H), 3310 (NH) cm$^{-1}$. Q-TOF-MS (m/z): Calcd for $C_{27}H_{55}O_{12}P_3$, 692.2965. Found: 692.3073 $[M + H]^+$.

### *Synthesis of 2-(3-(2-(Methacryloyloxy)ethyl)-1-(6-phosphonohexyl)ureido)ethane-1,1-diyl)bis (phosphonic acid) (M2)*

To an ice-cold solution of M1 (0.24 g, 0.346 mmol) in dry dichloromethane (DCM) (0.7 mL), TMSBr (0.238 g, 1.56 mmol) was added dropwise under nitrogen stream. The mixture was stirred at 30 °C for 5 h. DCM and excess TMSBr were removed under reduced pressure. Methanol (0.7 mL) was added and the mixture was stirred for 15 min. The solvent was removed under reduced pressure and the product was obtained as yellow viscous liquid in 95% yield.

$^1$H NMR (400 MHz, MeOD, δ): 1.60 (m, 8H, $PCH_2CH_2$), 1.73 (m, 2H, $PCH_2CH_2$), 1.93 (s, 3H, $CH_3C=CH_2$), 2.67 (tt, $^3J_{HH}$ = 5.6 Hz, $J_{HP}$ = 23.2 Hz, 1H, PCHP), 3.31 (m, 2H, $NCH_2CH_2$), 3.46 (t, $^3J_{HH}$ = 5.6 Hz, 2H, $NCH_2CH_2P$), 3.82 (td, $^3J_{HH}$ = 6 Hz, $J_{HP}$ = 14.4 Hz, 2H, $NCH_2CH$-P), 4.20 (t, $^3J_{HH}$ = 5.6 Hz, 2H, $NHCH_2CH_2O$), 5.63, 6.13 (s, 2H, $C=CH_2$) ppm. FTIR (ATR): 914, 983 (P-O), 1250 (P=O), 1627 (C=C), 1627, 1693 (C=O), 2931 (C-H), 3500–2500 (NH, OH) cm$^{-1}$. Q-TOF-MS (m/z): Calcd for $C_{15}H_{31}N_2O_{12}P_3$, 524.1089. Found: 524.1162 $[M + H]^+$.

## Etching Performance

Three human caries-free permanent premolar teeth were used for analysis. They were horizontally cut with the aid of a carbon separating disk under water cooling, leaving the dentin open at the coronal portion. In the same way, a horizontal section with a thickness of 4 mm was obtained from the tooth crown with a second incision from the enamel-cement junction. Dentin and enamel surfaces of the tooth sections were ground under water cooling with 350, 600, 800, and 1200 grit silicon carbide abrasive papers, respectively, to provide smear layer removal and homogenization of surface roughness. Each grinding operation was applied for 1 min to ensure standardization. M2 (20 wt %) containing ethanol/water (1:1, v/v) solution was applied continuously with a brush to the enamel and dentin surfaces for 15 s. The surfaces were then washed with 2.5 mL of distilled water and then with 1 mL of ethanol and dried with strong air for 5 s. The surface of the samples was examined with the SEM.

## HAP Interaction

HAP (0.1 g) was dispersed in a M2/ethanol/water (0.5 g, 15:45:40 wt %) mixture by stirring for 1 day at room temperature. M2-coated HAP particles were separated from the mixture by centrifugation. The particles were washed with ethanol (2 × 10 mL) and water (2 × 10 mL), dried at room temperature.[13] The crystal phases on M2-coated HAP particles were identified by a powder XRD. M2-coated HAP particles and their solution phase were also analyzed by FTIR. The samples were additionally examined by XPS (wide and narrow scans).

## Photopolymerization

Photopolymerization kinetics of the monomers was monitored using photo-differential scanning calorimetry (DSC) (Q250; TA Instruments Philadelphia, PA). The monomers M1, M2, and their mixtures with dental monomers (3–4 mg) containing 2.0 mol % initiator were placed on an aluminum DSC pan and equilibrated for 5 min under nitrogen flow to remove oxygen. Then, they were exposed to a light source (OmniCure 2000) with light (intensity of 20 mW cm$^{-2}$, λ = 320–500 nm) on the sample under pure nitrogen flow. All polymerizations were performed at 37 °C for 5 min in duplicate. The heat flow was monitored as a function of time with DSC. The double-bond conversion (DBC) was calculated[45] using:

$$\mathrm{DBC} = \frac{\Delta H_p}{\Delta H_{0p}} \quad (1)$$

where $\Delta H_p$ (J g$^{-1}$) is the overall enthalpy and $\Delta H_{0p}$ (J g$^{-1}$) is the theoretical enthalpy obtained for 100% conversion of the mixtures. $\Delta H_{0p}$ can be obtained.[45]

$$\Delta H_{0p} = \sum \Delta H_{0i} P_i / M_i \quad (2)$$

where $i$ runs over polymerizable double bonds, $\Delta H_{0i}$ is the theoretical enthalpy of polymerization, $M_i$ is the molar mass of the monomer, and $P_i$ is the amount used in the formulation (wt %). As all our monomers are methacrylates, $\Delta H_{0i}$ = 54.8 kJ mol$^{-1}$ = $\Delta H_0$ per double bond,[46,47] we obtain

$$\Delta H_{0p} = \Delta H_0 \sum n_j P_j / M_j \quad (3)$$

where now $j$ runs over monomers, $n_j$ is the number of methacrylate units, and $M_j$ and $P_j$ are as above. The rate of polymerization is calculated by

$$\text{Rate} = \frac{Q}{\Delta H_{0p} m} \quad (4)$$

where $Q$ is the heat flow per second and $m$ is the mass of monomer in the sample.

### *In vitro* Cytotoxicity Assay

Cytotoxicity of the monomers on mouse embryonic fibroblast cells (NIH3T3), human bone osteosarcoma epithelial cells (U2OS), and human osteoblastic osteosarcoma cells (Saos-2) was determined with MTT cell viability assay. Cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin antibiotic solution and grown in a 5% $CO_2$-humidified incubator at 37 °C. Trypsin–EDTA was used for the detachment of cells from culture plates. For the MTT assay, cells were seeded at a density of $1 \times 10^4$ cells/well in 96-well plates. The next day, the medium was removed and the monomers were added in the 25–200 μg mL$^{-1}$ concentration range with fresh culture medium. After 24 h incubation, the medium in each well was replaced with 150 μL of culture medium and 50 μL of MTT solution (5 mg mL$^{-1}$ in PBS) which forms purple formazan crystals as a result of mitochondrial activity of viable cells. The cells were further incubated for 4 h and subjected to a gentle shaking for 15 min following addition of ethanol: dimethyl sulfoxide (DMSO) (1:1 v/v) solution to dissolve the formazan crystals. Absorbance intensity at 600 nm was measured with a reference wavelength of 630 nm using ELx800 Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT). Cells that are not treated with monomers were used as controls by assuming 100% viability. Relative cell viability was calculated according to the following formula:

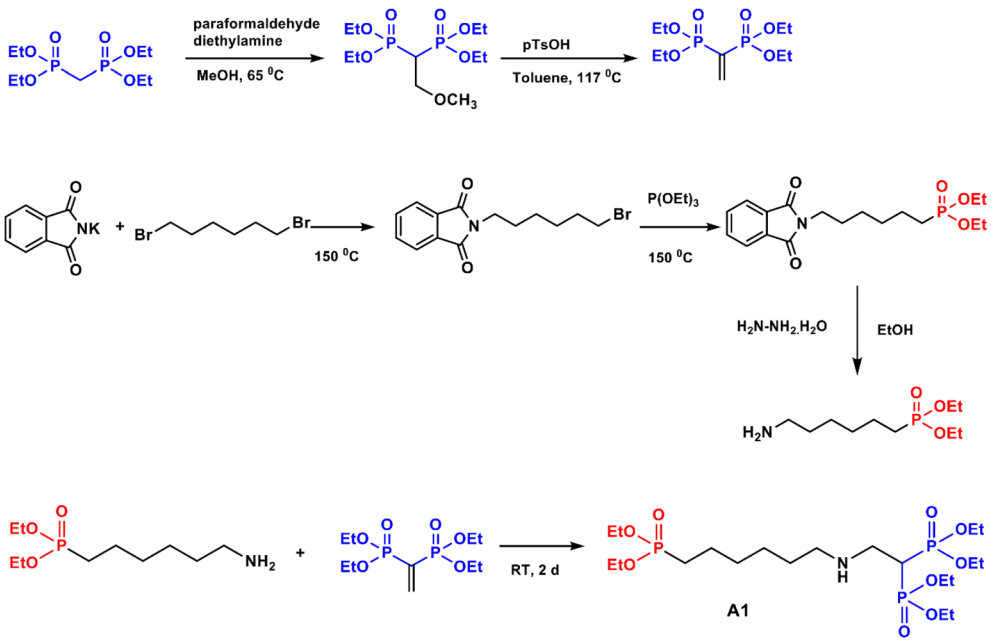
$$\text{Cell viability } (\%) = \left[\frac{\text{sample absorbance}}{\text{control absorbance}}\right] \times 100 \quad (n = 5) \quad (5)$$

Controls were untreated cells. Statistical analysis of the results was conducted by using the nonparametric Kruskal–Wallis test with Dunn's multiple comparison post-test or Mann–Whitney test of GraphPad Prism 6 software package (GraphPad Software, Inc., San Diego, CA). Data were expressed as mean values ± standard deviation (SD). All tests were two-tailed. Statistical significance was determined at $p < 0.05$.

## RESULTS AND DISCUSSION

### Synthesis and Characterizations of Monomers

A novel phosphonate- and bisphosphonate-functionalized secondary amine (A1) was synthesized and used as a starting material for dental monomers (Scheme 1). A1 was obtained as a viscous liquid in 63% yield; it is soluble in polar (e.g., water, methanol, and ethanol) and weakly polar (e.g., DCM and




**SCHEME 1** Synthesis of the phosphonate and bisphosphonate-functionalized amine A1. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 1** Solubilities of A1, M1, and M2 in Selected Solvents

| Amine/ Monomer | Petroleum Ether | Diethyl Ether | DCM | THF | MeOH | $H_2O$ |
|---|---|---|---|---|---|---|
| A1 | – | + | + | + | + | + |
| M1 | – | + | + | + | + | + |
| M2 | – | – | – | – | + | + |

tetrahydrofuran [THF]) organic solvents but insoluble in very nonpolar organic solvents (e.g., petroleum ether and hexanes) (Table 1). The structure of the amine was confirmed by $^1$H NMR and FTIR. The $^1$H NMR spectrum of A1 showed methine proton of bisphosphonate at 2.66 ppm (doublet of triplets); methylene protons adjacent to nitrogen at 2.56 (triplet) and 3.12 (triplet of doublets) ppm (Fig. 1).

A1 was reacted with IEM to synthesize a novel methacrylate monomer (M1) containing both phosphonate and bisphosphonate groups and a six-carbon aliphatic chain as spacer between them to avoid steric hindrance during polymerization and also obtain suitable hydrophilic–hydrophobic balance, especially for acidic monomers (Scheme 2). This monomer is soluble in water (Table 1).

The structure of the monomer was verified with $^1$H, $^{13}$C NMR, and FTIR. In the $^1$H NMR spectrum, peaks at 1.68 (multiplet), 2.87 (triplet of triplet), and 3.75 (triplet of doublets) ppm are due to methylene protons adjacent to phosphorus, methine proton, and methylene protons next to bisphosphonate group, respectively (Fig. 2). The broad peak at around 6.0 ppm is typical for the NH peak of urea group. In the $^{13}$C NMR spectrum of this monomer, the triplet seen at 36.85 ppm is due to methine carbon of the bisphosphonate group (Fig. 3). FTIR spectrum of M1 shows peaks at 1632, 1716, and 1560 cm$^{-1}$ belonging to two C=O and NH stretchings, respectively (Fig. 4). The silylation of monomer M1 with TMSBr followed by methanolysis of the silyl ester groups subsequently gave the new phosphonic and bisphosphonic acid-functionalized methacrylate (M2) in 95% yield (Scheme 2). This monomer is also soluble in water and alcohols (methanol and ethanol) but not in other selected organic solvents (Table 1). $^1$H NMR spectrum of M2 shows the disappearance of almost all of the phosphonic and bisphosphonic ester peaks at 1.30 and 4.11 ppm (Fig. 2). In the FTIR spectrum of M2, broad NH and OH (2500–3500 cm$^{-1}$), two different C=O (1627 and 1693 cm$^{-1}$), P=O (1250 cm$^{-1}$), and P-O (914 and 983 cm$^{-1}$) peaks were observed (Fig. 4).

## HAP Interaction

The pH value of aqueous solution of M2 (1 wt %) was found to be 1.66, which is in the range of mild self-etching monomers (pH ~ 2) according to Van Meerbeek and coworkers[13] To determine the etching performance of M2 on tooth tissues, M2/ethanol/water (15:45:40 wt %) solutions were prepared and applied to tooth surfaces. Since MDP is the most commonly used and intensively studied monomer in dental adhesives, we used the same procedure to prepare MDP-treated tooth surfaces as control. SEM photomicrographs of enamel and dentin treated with M2 and MDP are shown in Figure 5. The results indicate the importance of structural differences on etching behavior of the monomers with dental tissues. After application of MDP, the smear layer was completely removed, the dentinal tubules were open, and MDP-Ca salts were formed (5b). In contrast, M2 left some smear debris, most tubules were patent while some of them remained partially occluded (5e). Similar to MDP, formation of some M2-Ca salts were observed on the dentin surface (5e). SEM images showed HAP crystals on enamel for both monomers (5c and 5f). As a result, partial disappearance of smear layer and demineralization on dentin indicates that M2 can be used as an etching primer.

Interaction between HAP and M2 was investigated with FTIR, XPS, and XRD analysis. Monomer-treated HAP particles were prepared as described in the literature.[13] FTIR spectrum of

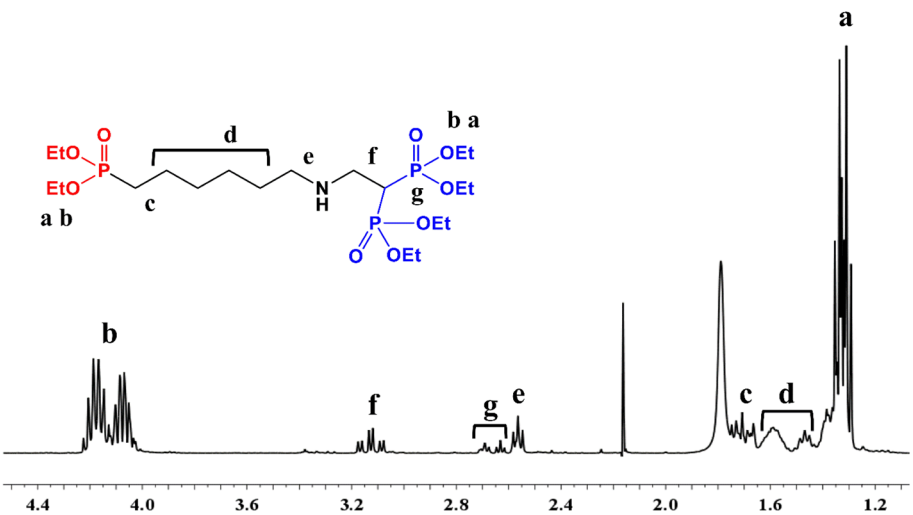
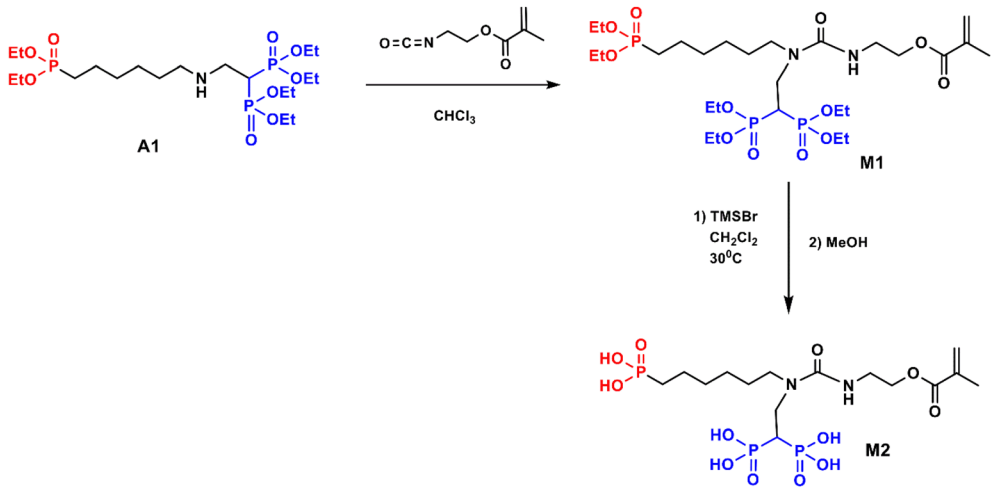



**FIGURE 1** $^1$H NMR spectrum of A1. [Color figure can be viewed at wileyonlinelibrary.com]




**SCHEME 2** Synthesis of M1 and M2. [Color figure can be viewed at wileyonlinelibrary.com]

HAP particles features the characteristic $PO_4$ peaks at ~ 960 and 700 $cm^{-1}$ [Fig. 6(c)]. The spectrum of monomer-treated HAP particles shows the characteristic peaks of monomers, two different C=O and C=C around 1700 and 1630 $cm^{-1}$, which indicates that M2 can bind to HAP and form a stable salt with it [Fig. 6(c)]. XPS spectrum of M2-treated HAP particles shows an increase in C 1s peak compared to untreated HAP [Fig. 6(a)]. This peak is the combination of three peaks: C-C, C-H, C=C binding peak (284.6 eV), C-O binding peak (286 eV), and COO (ester) peak (288 eV). These were also compared to MDP, the most common dental adhesive monomer. XRD spectrum of MDP-treated HAP particles shows that MDP forms a MDP-calcium salt ($CaMHP_2$) structure (peaks at 2θ = 2.24°, 4.56°, 6.86°, and 20°) and $CaHPO_4{\cdot}2H_2O$ (DCPD) (2θ = 11°).[13] The peak at 2θ = 5° in the XRD spectrum of M2-treated HAP particles [Fig. 6(b)] is probably due to the monomer-Ca salt ($CaMHP_2$) adsorbed onto HAP surface [Fig. 6 (d)]. This result can be explained by the presence of six-carbon alkyl group in the molecule, which decreases solubility of the M2-calcium salt in the aqueous environment according to the "AD-concept" and leads to an improvement in the chemical adhesion of M2 to HAP.

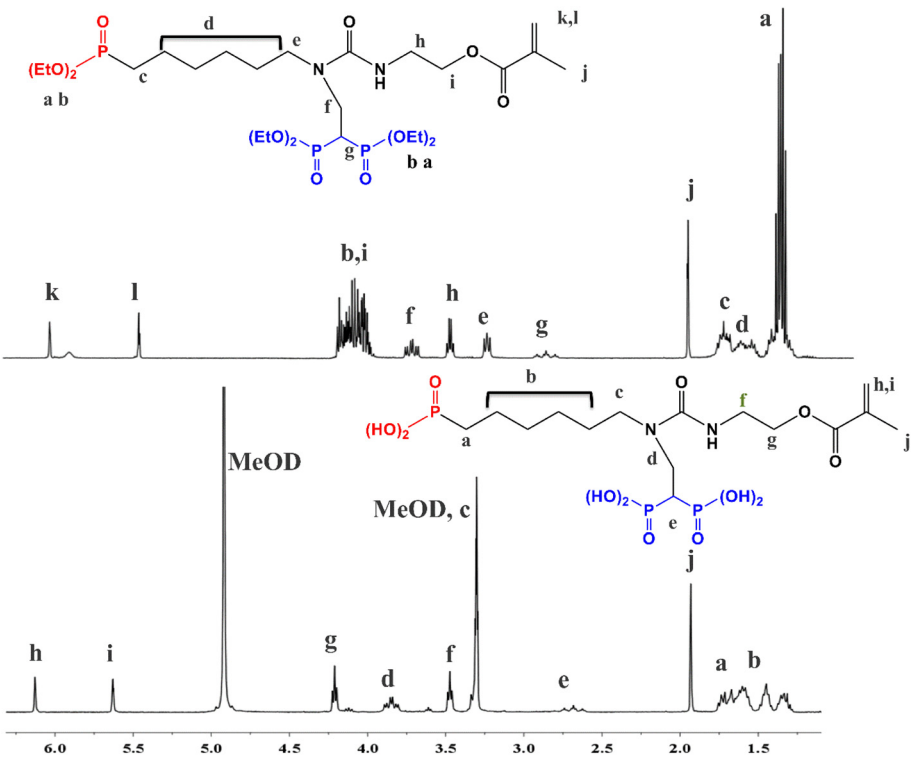



**FIGURE 2** $^1$H NMR spectra of M1 and M2. [Color figure can be viewed at wileyonlinelibrary.com]

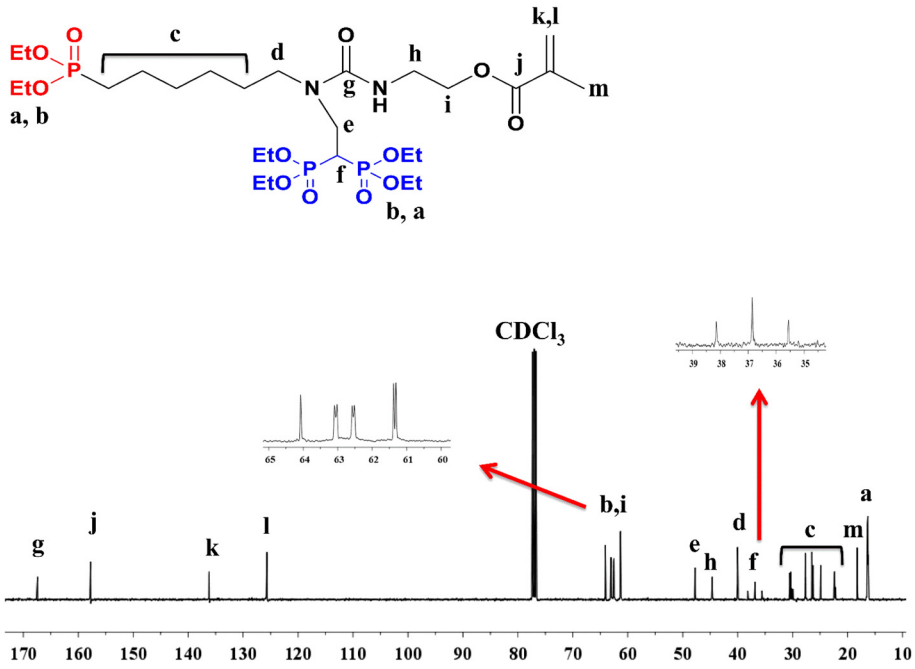



**FIGURE 3** $^{13}C$ NMR spectrum of M1. [Color figure can be viewed at wileyonlinelibrary.com]

### Photopolymerization

We first studied the homopolymerization behaviors of M1 and M2, and compared with that of HEMA, a common monomer used in dental adhesives and composites; and then performed copolymerizations of relevant HEMA:M1, and HEMA:M2 mixtures. We also investigated similar bis-GMA:TEGDMA:M1 and bis-GMA:TEGDMA:M2 mixtures, since the 50:50 bis-GMA:TEGDMA mixture is also a similarly common base material. Finally, we studied a HEMA:water:M2 mixture since dental adhesives usually contain water in their formulations. The polymerizations were performed/studied with photo-DSC at 37 °C, using BAPO or DMPA (2 mol %) as photoinitiator, usually depending on the degree of polarity of the formulation. Whenever appropriate, we also try to get an idea on the possible effect of the choice of the photoinitiator.

### Homopolymerizations

Figure 7 shows the rates of polymerization and conversions of HEMA, M1, and M2 (using BAPO) as functions of time; and from the plots, the maximum rates of polymerization ($R_{p\max}$), times ($t_{\max}$) of the maximum polymerization rate and conversions can be obtained. It can be seen that M1 and M2 have much lower $t_{\max}$ values compared to HEMA (2.4, 4.8, and 7.8 s for M1, M2, and HEMA). This may indicate higher reactivity of the synthesized monomers. However, $R_{p\max}$ values are found to be lower than HEMA, which is probably due to very high viscosity of M1 and M2 compared to HEMA, M2 being even more viscous than M1 resulting from additional hydrogen bonding due to (bis)phosphonic acid groups. Therefore, the conversions reached were low (around 77 and 66% for M1 and M2 after 120 s) compared to HEMA (99%), showing a decrease with a decrease in flexibility.

### Copolymerizations with HEMA

In dentistry, composites and self-etching adhesive resin formulations contain about 15 wt % of adhesive monomers, and similar formulations are investigated in the literature.[48] Therefore, we also investigated copolymerizations of HEMA:M1 (90:10 and 80:20 mol %) and HEMA:M2 (90:10 and 70:30 mol %) mixtures (Figs. 7 and 8).

It was observed that incorporation of M1 or M2 into HEMA reduced $t_{\max}$ values compared to HEMA depending on their concentrations in the formulations, indicating higher reactivity of these monomers (Figs. 7 and 8). The $t_{\max}$ values of HEMA, HEMA:M2 (90:10 mol %), and HEMA:M2 (70:30 mol %) were found to be 7.8, 4.2, and 3.0 s, respectively, with BAPO; those of HEMA, HEMA:M1 (90:10 mol %), and HEMA:M1 (80:20 mol %), 20.4, 15, and 13 s with DMPA.

The conversion values of HEMA:M1 systems were found to be around 76–87% (DMPA) (120 s) and 96% (BAPO), while 74–86% (BAPO) for HEMA:M2 systems, respectively (Figs. 7 and 8). The similar or lower conversion values of the mixtures can be explained again by the higher viscosities of M1 and M2 compared to HEMA, leading to a decrease in the mobilities of reactive species which result in fast gelation of the mixture. Actually, the shoulders at the onset of autoacceleration were observed earlier when the M1 amount is higher in the mixture (Fig. 8). The shoulders were not that obvious for fast polymerizing systems using BAPO (Fig. 7).

It was observed that the initial rates of polymerization of M1 and its mixtures were significantly higher than HEMA (Figs. 7 and 8); however, the $R_{p\max}$ values are similar or lower. For example, the $R_p$ values were found to be 0.01,

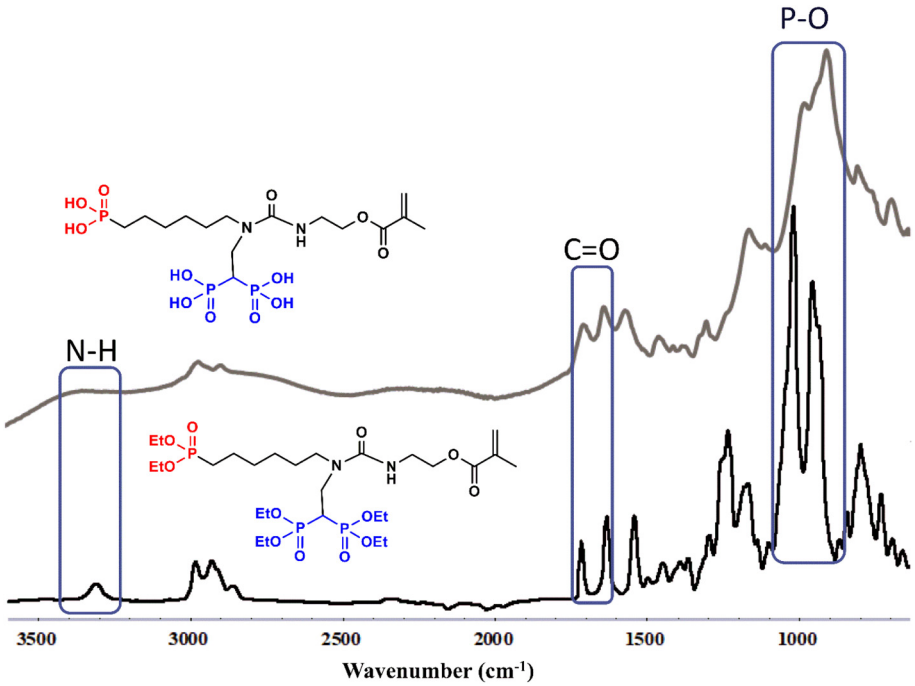



**FIGURE 4** FTIR spectra of M1 and M2. [Color figure can be viewed at wileyonlinelibrary.com]

0.025, and 0.026 for HEMA, HEMA:M1 (90:10 mol %), and HEMA:M1 (80:20 mol %) at 6 s of polymerization (using DMPA), indicating that autoacceleration occurred earlier with the mixtures. This behavior is generally observed for multifunctional monomers; crosslinking leads to an increase in viscosity which causes a reduced termination rate and increase in $R_p$. Although the synthesized monomers are monofunctional, they increase viscosity of the formulations, leading to the same behavior.

### A Case with Water

To see an example of the possible effect of water in the formulation, a mixture of HEMA:water:M2 (50:40:10 mol %) was photopolymerized with BAPO (DMPA is not soluble in water)

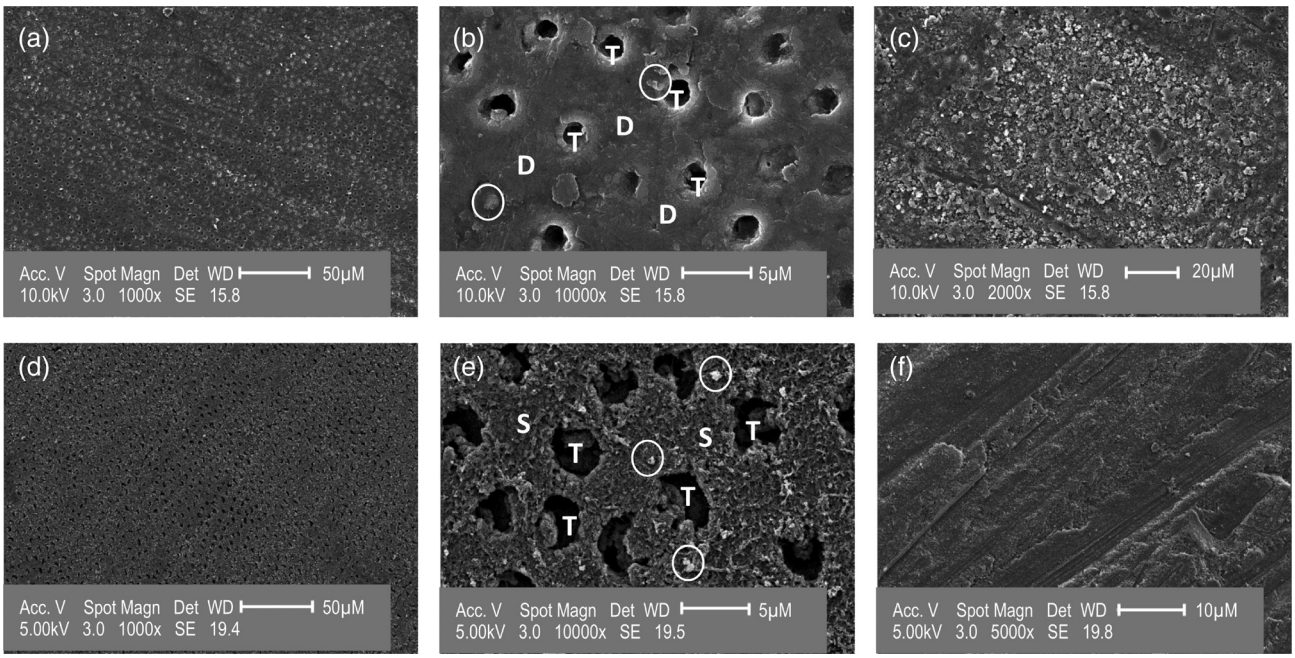



**FIGURE 5** SEM images of dentin ([a, d] 1000× and [b, e] 10,000×) and enamel ([c] 2000× and [f] 5000×) etched with 20 wt % MDP (a–c) or M2 (d–f ); containing EtOH/$H_2O$ (1:1, v/v) solution. Dentin surface (D) and dentin tubules (T) and smear layer (S). Circle indicates monomer-$Ca^{2+}$ salts.

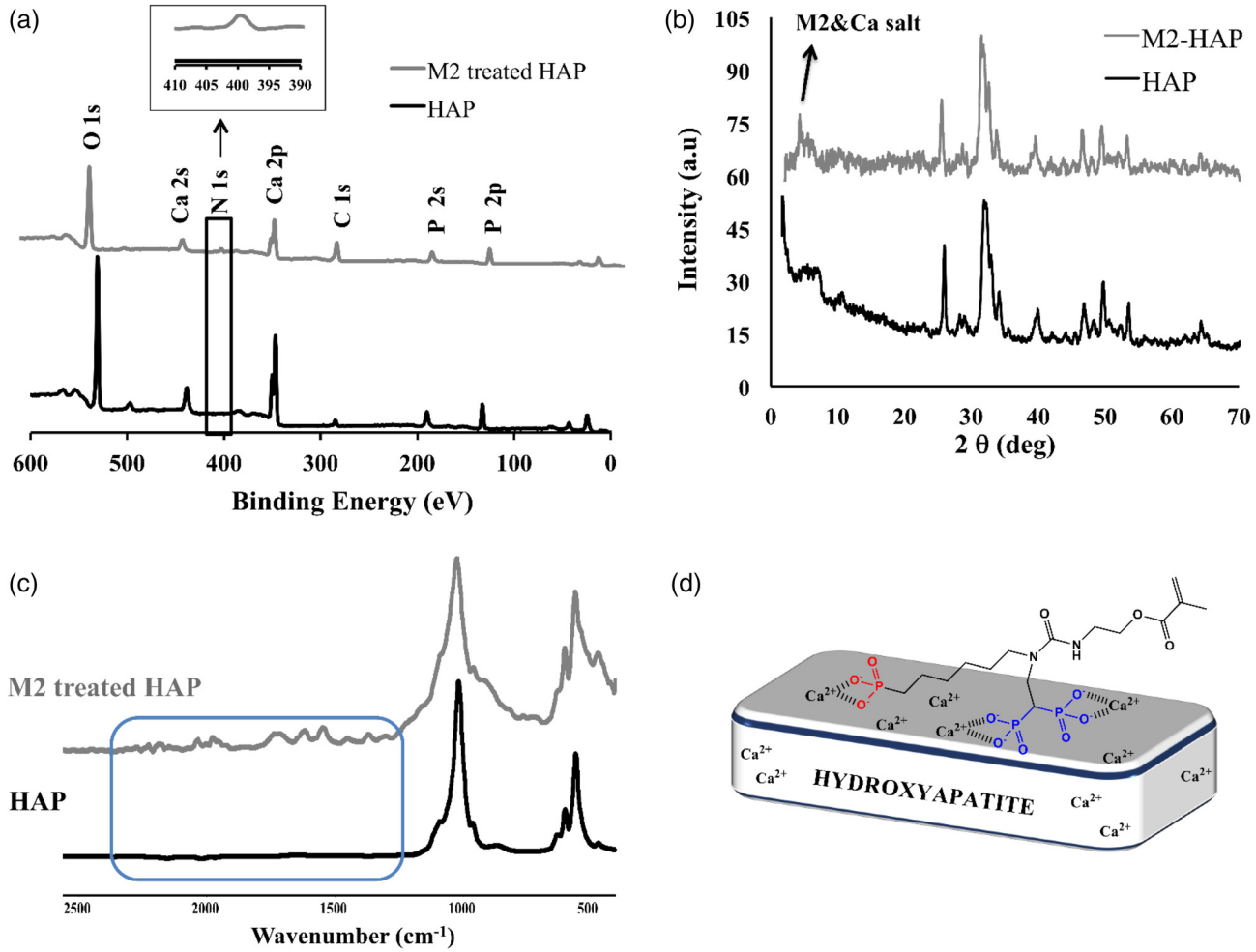



**FIGURE 6** (a) Narrow scan XPS spectra of HAP (bottom spectrum) and M2-treated HAP (top spectrum). (b) XRD spectrum of HAP (bottom spectrum) and M2-treated HAP (top spectrum). (c) FTIR spectrum of HAP (bottom spectrum) and M2-treated HAP (top spectrum). (d) Schematic diagram of the formation of M2-Ca salt. [Color figure can be viewed at wileyonlinelibrary.com]

(Fig. 9). The addition of M2 was found to actually decrease $t_{max}$ values while $R_{pmax}$ and conversion values did not change significantly compared to the HEMA:water systems. $R_{pmax}$, $t_{max}$, and conversions were found to be 0.0501 $s^{-1}$, 6 s, and about 81% (after 120 s) for HEMA:water:M2 (50:40:10 mol %). In the literature kinetics of water soluble acid monomers, such as acrylic and methacrylic acid in water were investigated by pulsed-laser polymerization technique and the $k_p$ values in polymerization experiments in water were found to be significantly larger than the corresponding values obtained in methanol or DMSO.[49]

### Copolymerizations with bis-GMA-TEGDMA

Bis-GMA has been used as the base monomer in dental resins because of its low polymerization shrinkage and outstanding mechanical properties. However, its high viscosity due to hydrogen bonding leads to low polymerization conversion and makes it difficult to handle. Therefore, reactive diluents such as TEGDMA are used to increase the polymerization yield. Hence, we also investigated the potential of the synthesized monomers to partially replace TEGDMA in such composite resins.

When the polymerization rates and conversions (using DMPA) of formulations consisting of bis-GMA:TEGDMA (50:50 mol %) and bis-GMA:TEGDMA:M1 (50:40:10 mol %) were compared (Fig. 10), it was observed that replacement of 10 mol % of TEGDMA with M1 slightly increased both the rate and conversion. Similar behavior was observed for bis-GMA:TEGDMA:M2 (50:40:10 mol %) formulation compared to bis-GMA:TEGDMA (50:50 mol %) using BAPO.

This enhancement can be explained with the higher reactivity and/or lower double bond count of M1 and M2 with respect to TEGDMA. M1 and M2 have the capability of hydrogen bonding due to the urea linkage in their structure, which is an important factor of rate enhancement. It is known that that higher functionality leads to higher reaction rates and more rapid onset of gelation and vitrification due to formation of networks, which reduces the conversion.[50] Thus, replacement of TEGDMA with the synthesized monofunctional monomers increases both the rates and conversions.

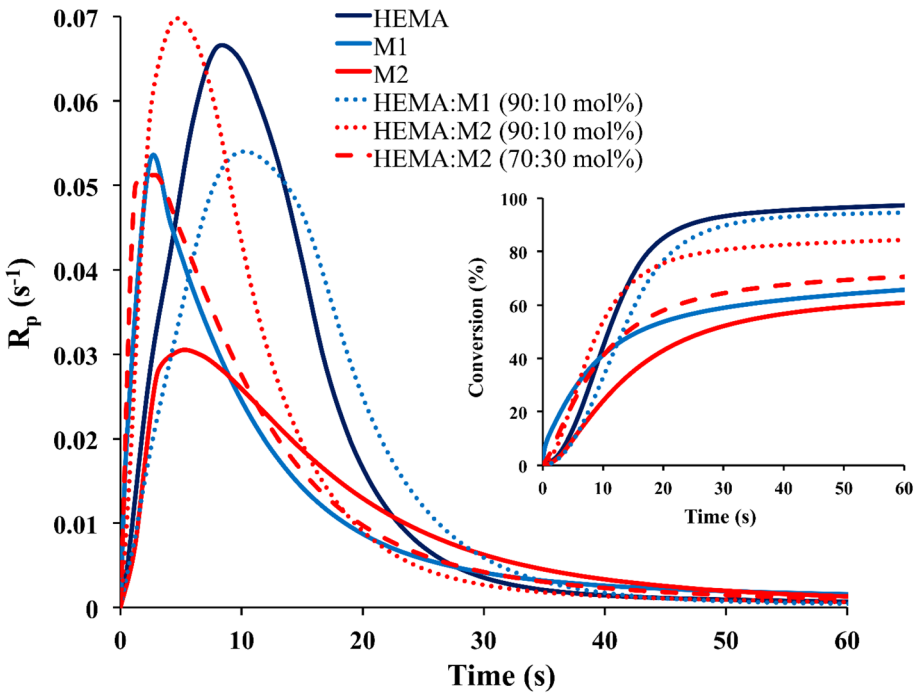



**FIGURE 7** $R_p$ versus time and conversion versus time plots for M1, M2, HEMA, HEMA:M1 (90:10 mol %), HEMA:M2 (90:10 mol %), and HEMA:M2 (70:30 mol %) mixtures at 37 °C with BAPO (2 mol %). [Color figure can be viewed at wileyonlinelibrary.com]

### Comparisons of Photoinitiators

As stated above, two different photoinitiators, BAPO and DMPA were used in this work. BAPO is a polar molecule, hence soluble in water, whereas DMPA is not. Therefore, not all formulations were suitable for use of DMPA.

It was observed that BAPO led to faster photopolymerization in HEMA systems when the comparison was made; with similar or higher $R_{p\max}$ and shorter time $t_{\max}$ to reach it; and somewhat higher conversion. For example, $R_{p\max}$ values of HEMA were found to be 0.045 and 0.066 $s^{-1}$ for DMPA and

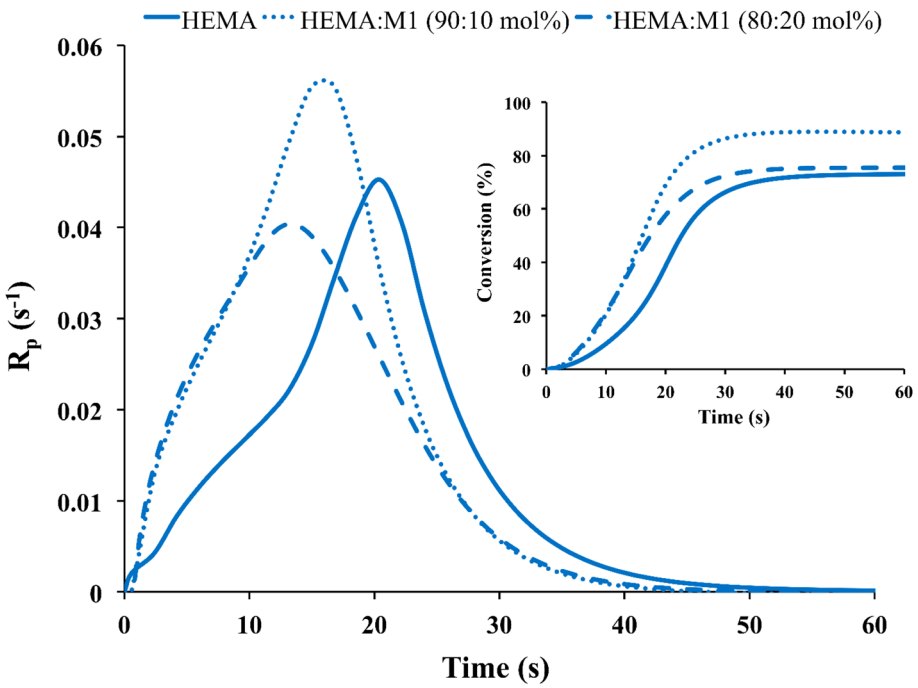



**FIGURE 8** $R_p$ versus time and conversion versus time plots for HEMA, HEMA:M1 mixtures at 37 °C with DMPA (2 mol %). [Color figure can be viewed at wileyonlinelibrary.com]

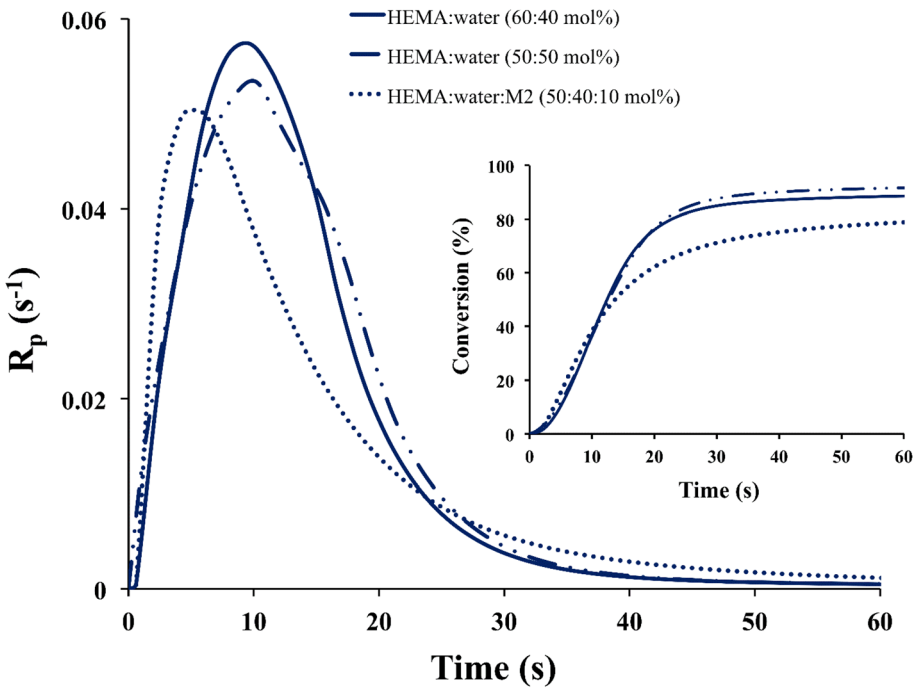



**FIGURE 9** $R_p$ versus time and conversion versus time plots for HEMA:water (60:40 mol %), HEMA:water (50:50 mol %), HEMA:water: M2 (50:40:10 mol %) mixtures at 37 °C with BAPO (2 mol %). [Color figure can be viewed at wileyonlinelibrary.com]

BAPO systems; and HEMA:M1 (90:10 mol %) formulations gave $t_{max}$ values of 15 and 10 s using DMPA and BAPO photoinitiators, respectively. The conversion values of HEMA:M1 systems were found to be around 76–87% (DMPA) and 96% (BAPO). The shoulders at the onset of autoacceleration were observed earlier in DMPA polymerized systems when M1 amount is higher in the mixture (Fig. 8). The shoulders were not that obvious for fast polymerizing systems using BAPO (Fig. 7). In fast polymerizing bis-GMA: TEGDMA systems no significant difference was observed in

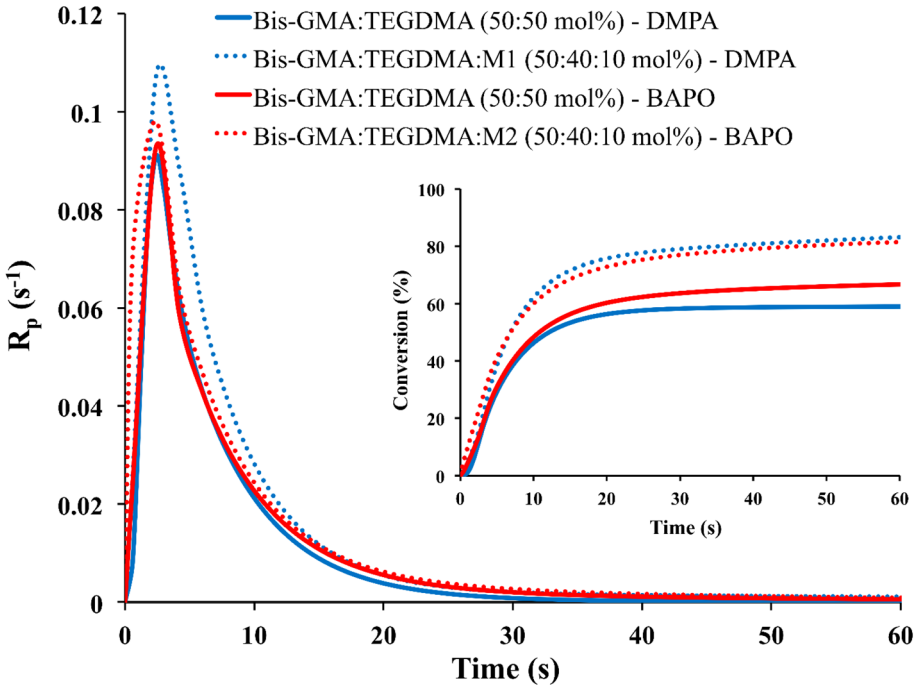



**FIGURE 10** $R_p$ versus time and conversion versus time plots for bis-GMA:TEGDMA (50:50 mol %) and bis-GMA:TEGDMA:M1 (50:40:10 mol %) at 37 °C with DMPA (2 mol %); bis-GMA:TEGDMA (50:50 mol %), and bis-GMA:TEGDMA:M2 (50:40:10 mol %) at 37 °C with BAPO (2 mol %). [Color figure can be viewed at wileyonlinelibrary.com]

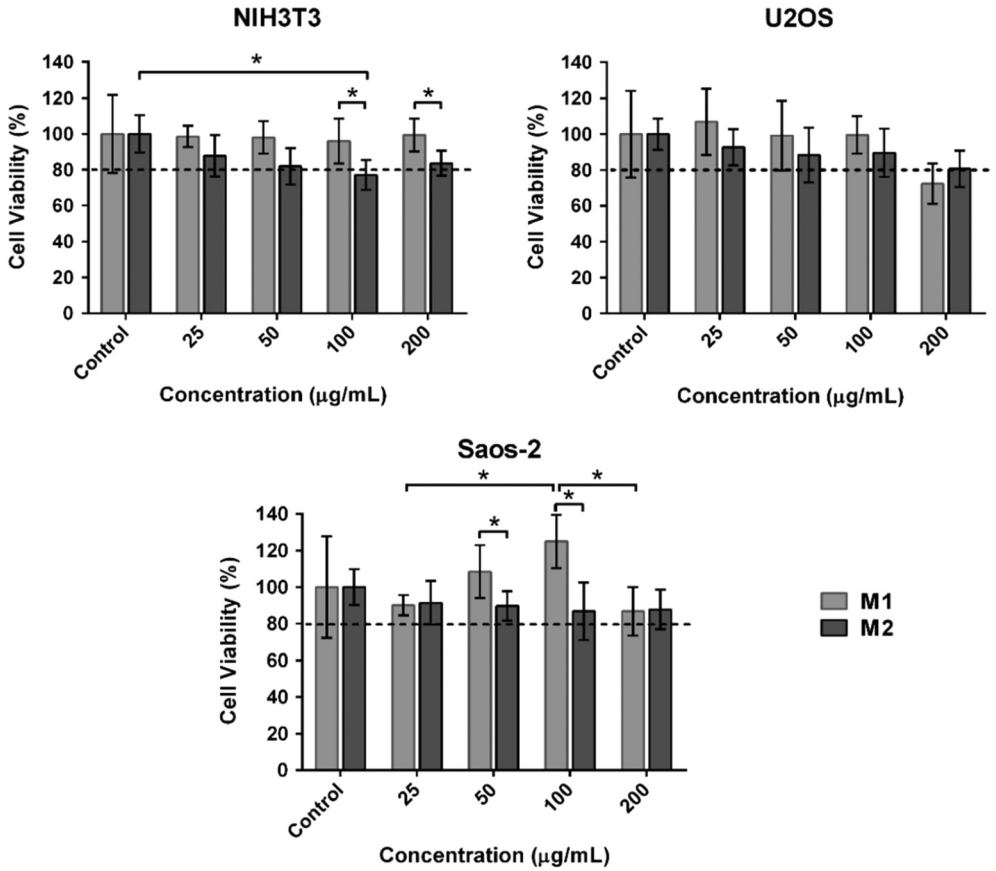



**FIGURE 11** Viability of NIH 3T3, U2OS, and Saos-2 cells treated with M1 and M2 after 24 h incubation measured with MTT. The data are expressed as mean ± SD ($n = 5$), (*$p < 0.05$, **$p < 0.01$).

$R_{p\max}$, conversion and $t_{\max}$ values using BAPO or DMPA as photoinitiators.

### *In vitro* Cytotoxicity Assay

The release of methacrylate monomers used in dentistry and orthodontics into the oral environment is of special concern because they can migrate into many organs with the bloodstream and induce adverse biological effects.[51] Monomer release may be due to unreacted monomers during the curing process and/or form as a consequence of mechanical stress experienced by the cured polymer during the chewing process. Also, degradation of monomers and polymers, especially ester functionalized ones by esterase enzymes present in the saliva result in release of new products. The monomers developed here are also methacrylates and their toxicity needs to be determined to evaluate their suitability for medical purposes. The cytotoxicities of M1 and M2 were tested on NIH3T3, U2OS, and Saos-2 cells using the standard MTT assay. Materials resulting in more than 80% cell viability are accepted as noncytotoxic according to ISO 10993-5.[52] Figure 11 shows that no significant cytotoxicity was detected between 25 and 100 μg $mL^{-1}$ in 24 h. For NIH3T3 and Saos-2, the dose can be pushed toward 200 μg $mL^{-1}$ as well, at which concentration some differences start to appear between M1 and M2 in favor of M1 in NIH3T3 data. Otherwise, there seems to be no significant difference between the phosphonate/bisphosphonate and phosphonic/bisphosphonic acid pendant groups on the cytotoxicity of the monomers on these cell lines.

## CONCLUSIONS

A phosphonated primary amine where the amine group serves as a useful handle for attaching a bisphosphonate group was synthesized. This method can be used for the synthesis of different phosphonate and bisphosphonate-functionalized secondary amines with tunable hydrophilicities. The synthesized amine was used for the preparation of novel phosphonate and bisphosphonate (M1) or phosphonic and bisphosphonic acid-functionalized (M2) methacrylates. These monomers were evaluated for possible applications in dental composites and adhesives. Photopolymerization results indicated that partial replacement of TEGDMA in bis-GMA-TEGDMA mixtures with these monomers enhances both the rates and conversions, indicating their potential as reactive diluents in dental composites with improved biocompatibility and binding properties. The copolymerization behavior of M2 with HEMA in aqueous solution indicated its potential as a biocompatible adhesive monomer in dental adhesives. The pH values of the acid monomers were found to be in the range of mild self-etching adhesives. SEM analysis revealed that the acid monomer can partially remove the smear layer on dentin and cause demineralization. The chemical bonding efficiency of M2 to HAP was confirmed using XRD by the formation of hydrolytically stable

monomer Ca salts and FTIR indicating characteristic peaks of monomers. The monomers are biocompatible with NIH 3T3, Saos-2, and U2OS cells.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