

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF NOVEL, ANTI-BACTERIAL MONOMERS FOR DENTAL AND BIOMEDICAL APPLICATIONS

Joseph M. Antonucci, Bruce O. Fowler, Diana N. Zeiger,
Nancy J. Lin and Sheng Lin-Gibson

Polymers Division, National Institute of Standards and Technology,
Gaithersburg, MD 20899-8543

Official contribution of the National Institute of Standards and Technology;
not subject to copyright in the United States

Introduction

Since its introduction in the 1960s, thermosetting (meth)acrylic resins, including triethylene glycol dimethacrylate (TEGDMA), 2,2-bis[*p*-(2'-hydroxy-3'methacryloxypropoxy)phenylene]propane (bis-GMA) and their blends have been increasingly used as the matrix phases of polymeric dental composites.¹ Over the past several decades, considerable efforts have been expended to improve the quality and durability of these restorative materials.² Coupled with improvements in dental adhesive systems for bonding composites to tooth structure, the clinical utility of these materials has steadily grown, and now they are the material of choice for direct, esthetic restorations. However, major failings of all acrylic resin-based composites are the significant volumetric shrinkage and stress development that accompany the polymerization process, especially in the case of photopolymerization.³ Polymerization shrinkage and stress challenge the composite-tooth bonds, making the interphase vulnerable to marginal gap formation, microleakage, bacterial infiltration, and eventual development of secondary (recurrent) caries, or tooth decay, at the tooth-composite interface.⁴

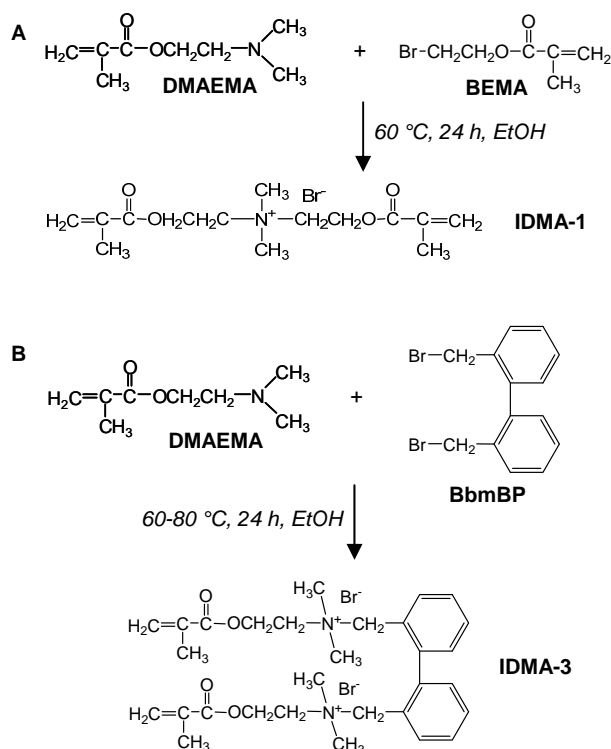
Although progress has been made in reducing polymerization shrinkage through resin and filler modifications, and in designing improved dental adhesive systems, composite fillings still fail clinically because of bacterial infiltration and secondary caries development. One approach to prevent the occurrence of recurrent caries is to use polymeric dental materials with biocidal properties. The objective of this study was to adapt the classical, facile, and versatile Menshutkin reaction⁵ (the addition reaction of tertiary amines with organo-halides) for the synthesis of free radical, thermosetting monomers that have quaternary ammonium groups in their chemical structures (Scheme 1). These reactive monomers would also have solubility parameters similar to common dental resins. Thus, these monomers are expected to be easily miscible with current dental resin systems, to have low leachability due to their multiple vinyl groups, and most importantly, by virtue of their quaternary ammonium functionalities, to yield copolymers and composites that are anti-bacterial by contact.



Scheme 1. General synthesis of quaternary ammonium monomers.

Experimental*

Synthesis of bis(2-methacryloyloxy-ethyl) dimethyl-ammonium bromide [IDMA-1] and 1,1'-bis[o-(2-methacryloyloxyethyl) 2-methylphenylene]dimethylammonium bromide [IDMA-3]. The synthesis of IDMA-1 is shown in Scheme 2A. In a tared vial equipped with a magnetic stir bar was placed 1.57 g (10 mmol) of 2-(*N,N*-dimethylamino)ethyl methacrylate (DMAEMA), 1.93 g (10 mmol) of 2-bromoethyl methacrylate (BEMA), and 3 g ethanol. The vial was capped, and the mixture was heated at 60 °C and stirred for 24 h. After removal of the solvent and residual reagents, a clear, colorless, viscous product (IDMA-1) was isolated in near quantitative yield. IDMA-3 was synthesized in a similar manner from



Scheme 2. Reaction scheme for IDMA-1 (A) and IDMA-3 (B).

DMAEMA and 2,2'-bis(Bromomethyl)-1,1'-biphenyl (BbmBP) as shown in Scheme 2B.

Characterization. Fourier transform infrared spectroscopy (FTIR) and proton-nuclear magnetic resonance spectroscopy (¹H-NMR) analyses were used, following standard protocols, to evaluate the structure of the resulting ionic liquids IDMA-1 and IDMA-3. FTIR spectra of the products and starting materials were recorded from the samples between ThBr.I plates in the 4000 cm⁻¹ to 400 cm⁻¹ region with a wave number expanded uncertainty of 0.5 cm⁻¹. ¹H-NMR was used to qualitatively confirm the FTIR analysis.

Resin Formulation. Resins containing IDMA-1 were prepared in order to assess the bacteria response to the polymers. IDMA-1 was mixed with bis-GMA/TEGDMA (50:50 mass fraction) for a final IDMA-1 concentration of 10 %, 20 %, and 30 % (by mass). Control resins contained no IDMA-1. Resins were activated for visible light (470 nm) photopolymerization with camphorquinone and ethyl 4-N,N-dimethylaminobenzoate. Polymer disks of 4 mm in diameter x 1 mm in height were prepared by irradiating (Dentsply Triad 2000, 250 W, 120 V) the activated resin blends between two glass slides (1 min per side).

Bacteria culture and imaging. *Streptococcus mutans* (*S. mutans*) were cultured in brain heart infusion (BHI) broth with 0.5 µg/mL bacitracin. Polymers were sterilized with 70 % (volume fraction) ethanol for 20 min, soaked in phosphate buffered saline (PBS) overnight, and inoculated with *S. mutans* prepared at an optical density (OD₆₀₀) of 0.06 in PBS. After 4 h incubation at 37 °C, 5 % CO₂ (by volume), samples were washed 3X to remove nonadherent bacteria, fixed with 37 mg/mL formaldehyde in PBS, and stained for 1 h with 1 µmol/L SYTOX green. Samples were imaged using a Zeiss laser scanning confocal microscope with a 40x water immersion objective. Three samples were evaluated for each IDMA-1 concentration (*n* = 3). Five image stacks were collected on each sample, and projection images were prepared using the manufacturer's software.

Results and Discussion

Because of the widespread incidence of recurrent caries, there is a need for effective anti-bacterial, polymeric dental materials. The presence of multiple quaternary ammonium functional groups in ionenes (synthesized by step-growth polymerization based on the Menshutkin reaction) imparts cariostatic properties to these linear polymers.⁶⁻⁷ As a way to impart anti-cariogenic properties to resin-based dental materials (sealants, adhesives, composites, etc), we have successfully adapted the classical Menshutkin reaction to the synthesis of resin-compatible, thermosetting methacrylic monomers and, recently, to the synthesis of silane coupling agents with quaternary ammonium groups. Interestingly, our synthetic approach also seems to offer a facile route to ionic liquid monomers.

All reactions were indicated to be successful, based on the characterization results from FTIR and ¹H-NMR. For IDMA-1, FTIR showed the disappearance of the C-Br absorption bands from BEMA (575 cm⁻¹, 512 cm⁻¹) as well as the N(CH₃)₂ bands (2822 cm⁻¹, 2771 cm⁻¹) from DMAEMA (Figure 1A). For IDMA-3, the disappearance of these bands was also shown (Figure 1B), and new bands, at N = 1048 cm⁻¹, 981 cm⁻¹, 886 cm⁻¹, and 712 cm⁻¹, are probable bands of the quaternary NR₄⁺ complex. The new bands at 3412 cm⁻¹ and ≈ 550 cm⁻¹ are from hydrogen-bonded water. ¹H-NMR confirmed the assigned structures of IDMA-1 and IDMA-3.

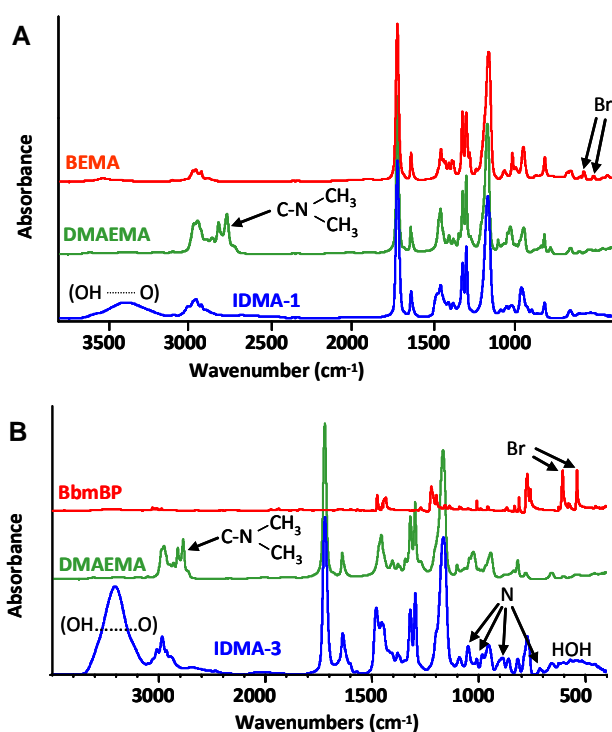


Figure 1. FTIR analysis of IDMA-1 (A) and IDMA-3 (B).

Preliminary qualitative evaluation for anti-bacterial activity indicates that IDMA-1 in Bis-GMA/TEGDMA terpolymers affects bacteria colonization on the polymer surface at 4 h (Figure 2). Initial colonization of *S. mutans* on polymers was greatly affected as the mass fraction of IDMA-1 increased up to 30 %. With 10 % IDMA-1, there appeared to be a slight reduction in bacteria number. As the mass fraction increased to 20 % and 30 %, the number of bacteria decreased, and the morphology of the microcolonies changed from individual bacteria to irregularly shaped groups of bacteria.

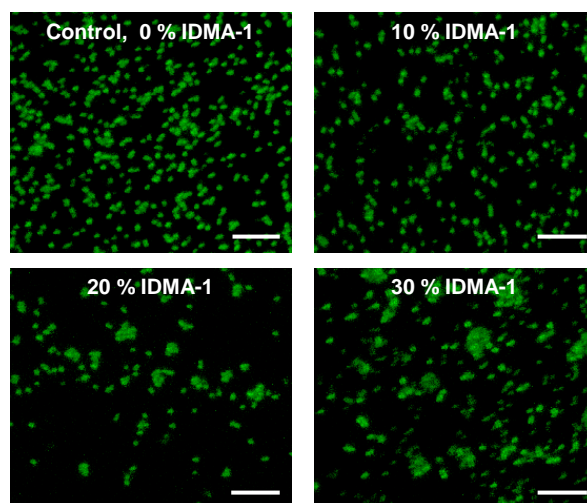


Figure 2. *S. mutans* colonization of polymers with varying amounts of IDMA-1. Scale bars = 10 μm.

Custom image analysis macros in ImagePro Plus are being used to quantify the fraction of the surface covered by *S. mutans*. The macros also count the object density and the object area. Using these parameters, differences in *S. mutans* colonization at 4 h will be quantified and compared using statistical methods. Future studies will include longer culture times, viability staining, and the testing of IDMA-3.

Conclusions

The facile, versatile Menshutkin reaction can be utilized to produce a wide variety of potentially anti-bacterial monomers, oligomers, and polymers that are expected to have wide application in dental and other biomedical materials. In addition, the Menshutkin reaction can provide a facile synthetic route to ionic liquid monomers. Moreover, preliminary bacteria colonization studies have indicated that polymers containing IDMA-1 have reduced bacteria growth on their surfaces.

Acknowledgements

Reported work was supported by Interagency Agreement YI-DE-7005-01 between the National Institute of Dental and Craniofacial Research (NIDCR) and the National Institute of Standards and Technology (NIST). Contribution of bis-GMA and TEGDMA from Esstech, Inc. is gratefully acknowledged.

* Disclaimer

Certain commercial materials and equipment are identified in this article to specify the experimental procedure. In no instance does such identification imply recommendation or endorsement by NIST or that the material or equipment identified is necessarily the best available for the purpose.

References

- (1) Bowen, R.L. *J. Am. Dent. Assoc.* **1963**, *66*, 57.
- (2) Antonucci, J.M. and Stansbury, J.W. In *Desk Reference of Functional Polymers: Synthesis Applications*; R. Arshady, Ed. ACS **1997**, 719.
- (3) Braga, R.R. and Ferracane, J.L. *Crit. Rev. Oral Biol. Med.* **2004**, *15*(3), 176.
- (4) Imazato, S. *Dental Materials* **2003**, *19*(6), 449.
- (5) Menshutkin, N. *Z. Physik. Chemie* **1890**, *5*, 589.
- (6) Noguchi, H. and Rembaum, A. *Macromolecules* **1972**, *5*(3), 253.
- (7) Rembaum, A. U.S. Patent 4,013,507, **1997**.