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Determination of neutralization capacity and stability of a basic methacrylate monomer using NMR

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Abstract

The durability of dental resin depends on the stability of the polymer. The neutralizing capacity of a basic methacrylate monomer and its chemical stability were measured using nuclear magnetic resonance (NMR) spectroscopy. Lactic acid solution was titrated with 2-(dimethylamino)ethylmethacrylate (DMAEMA) or 2-hydroxyethylmethacrylate (HEMA) and its chemical shifts monitored. Addition of DMAEMA alters the chemical shift proportionally to pH neutralization, whereas HEMA has no impact. Chemical shifts were used to quantify both the change in pH and monomer stability. The results demonstrate that neutralization by basic monomer can be achieved and that this can be measured using an NMR assay.

Keywords

neutralization; stability; basic monomer; lactic acid; nuclear magnetic resonance spectroscopy

INTRODUCTION

Interest in dental composites as an alternative to amalgam has been prompted by the public's concern about mercury release from dental amalgam [1–2]. Despite their extensive use, the short clinical lifetime of composites is a significant limitation [3–5]. The primary factor in the premature failure of composite restorations is recurrent caries at the margins of these restorations [6]. The composite is too viscous to bond directly to the tooth and thus, a low viscosity adhesive must be used to form a bond between the tooth and composite. Acid-etching provides effective mechanical bonding between the enamel and adhesive, but bonding to dentin has been fraught with problems. Class II composite restorations i.e., restorations that involve the biting surface plus one or both of the proximal surfaces, are particularly vulnerable to early clinical failure because of secondary decay at the gingival margin.

Clinicians frequently find very little enamel available for bonding at the gingival margin of class II composite restorations and thus, the bond at this margin depends on the integrity of the adhesive seal formed with dentin. At the vulnerable gingival margin, the dentin adhesive can be the primary barrier between the prepared tooth and the surrounding environment. A failed adhesive means that there are gaps between the tooth and composite. In moderate to large posterior class II composite restorations, secondary decay at the gingival margin is

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linked to failure of the bond between the tooth and composite and increased levels of the cariogenic bacterium, *Streptococcus mutans*, at the perimeter of these materials [7–9].

Streptococcus mutans is a Gram-positive, facultatively anaerobic microorganism and a major causative agent of dental decay. Adhesion of *S. mutans* to the tooth surface creates an environment that supports the subsequent attachment and growth of other bacterial species, ultimately forming a micro-ecosystem known as a biofilm. In addition to its role as a "pioneer" organism in biofilm formation, *S. mutans* produces lactic acid; the lactic acid damages the adjacent tooth surface by demineralization. Dental plaque biofilm cannot be eliminated [10], but the pathogenic impact of the biofilm at the margin of composite restorations could be reduced by engineering novel anti-cariogenic dentin adhesives.

Although numerous monomers have been investigated [11–17] the lack of dentin adhesives that are both effective and durable continues to be a major problem with the use of composites in direct restorative dentistry. Here, we measure the neutralization capacity and chemical stability of 2-(dimethylamino) ethylmethacrylate (DMAEMA), a basic functional monomer because it may be used as a *co*-monomer in dentin adhesives for reducing lactic acid induced decay of the tooth structure. While the neutralization of acidic polymers for drug release has been investigated [18], to our knowledge this is the first study to examine the neutralization capacity of a basic methacrylate monomer that has the potential to be incorporated into dentin adhesives. In the present study, DMAEMA was chosen as a neutralizing *co*-monomer, because it is the most commonly used photo *co*-initiator in dental materials [13, 19]. The present study tests the hypothesis that the amino group from the basic methacrylate monomer will act as a buffer and neutralize lactic acid, which is produced and acidifies the oral microenvironment. A straightforward NMR-based assay was used to measure the pH and buffering capacity of DMAEMA in lactic acid solutions.

MATERIALS & METHODS

Materials

L(+)-lactic acid (LA, 98%), 2-(dimethylamino) ethyl methacrylate (DMAEMA, 98%), 2-hydroxyethylmethacrylate (HEMA, 99%), and deuterium oxide (99.8 atom % D) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were reagent grade and used without further purification. The 0.1 M lactic acid in D_2O was prepared and divided into six groups of amber vials in which each group contained 3 mL of 0.1 M LA. Each group was treated with the different molar concentration of 2-dimethylaminoethyl methacrylate and vigorously stirred using a vortex mixer for 30 seconds. The pH was measured using a standard pH meter and NMR analysis of the solutions was performed, using the chemical shifts of LA as a probe for pH.

Methods

Nuclear Magnetic Resonance (NMR) Spectroscopy—^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance DRX 500 spectrometer equipped with a broadband probe. Samples of 0.1 M LA, with varied amounts of monomers, were prepared in 99.8% D_2O. Chemical shifts (δ) are reported in parts per million (ppm) and referenced relative to H_2O (4.7036 ppm) at 25 °C. ^{1}H-detected spectra were collected using 16 scans, and ^{13}C-detected spectra were acquired using 50 scans.

pH measurements—The pH measurements were carried out using an Accument portable AP 110 pH meter equipped with an Accumet combination microelectrode with a calomel reference. Calibration was done using commercial buffers (Fisher Scientific, pH 4.01, 7.00, and 10.01).

RESULTS

The ^{1}H and ^{13}C NMR spectra of lactic acid in D_2O are shown in Fig. 1. The peaks around 1.32 and 4.30 ppm in Fig. 1(a) are expanded to show the splitting of the multiplets in the spectrum. As can be seen, a doublet at 1.3411 and 1.3271 ppm (peak “a”), a singlet at 2.14 ppm (peak “b”) and a quartet at 4.3173, 4.3033, 4.2893, and 4.2753 ppm (peak “c”) correspond to the three methyl protons, hydroxyl proton, and methine proton of lactic acid, respectively. In the ^{13}C NMR spectrum (Fig. 2b), peaks “1, 2, and 3” at 19.22 ppm, 66.38 ppm, and 178.59 ppm represent the methyl carbon, methine carbon, and carbonyl carbon, respectively. The peak at 178.59 ppm (marked as C* in Fig. 1b) was used to monitor chemical shift changes during the titrations performed with DMAEMA or HEMA.

Fig. 2 (a–d) shows ^{1}H and ^{13}C NMR spectra of the methine proton and carbonyl carbon of 0.1 M of lactic acid in D_2O treated by different concentration of DMAEMA (0.02, 0.04, 0.06, 0.08, and 0.10 M) and HEMA (0.02, 0.06, and 0.10 M). In Figs. 2a–b, the bottom, second-, third-, fourth-, fifth-, and sixth-row spectra correspond to the molar concentration of DMAEMA of 0 M and 0.02, 0.04, 0.06, 0.08, and 0.10 M, respectively. In Fig. 2 (c–d), the bottom, second-, third-, and fourth-row spectra correspond to the molar concentration of HEMA of 0, 0.02, 0.06, and 0.10 M, respectively. As seen in Fig. 2a, the resonances of the methine proton on the carbon adjacent to the carbonyl in lactic acid are shifted gradually to lower chemical shift with increasing the molar concentration of DMAEMA, for example in ^{1}H, from 4.317, 4.303, 4.289, and 4.275 ppm at 0.0 M of DMAEMA to 4.032, 4.018, 4.004, and 3.990 ppm at 0.10 M of DMAEMA. Similarly, the carbonyl carbon of lactic acid in the ^{13}C NMR spectra (Fig. 2b) shifted to higher chemical shift with increasing the DMAEMA concentration, from 178.59 ppm at 0.0 M of DMAEMA to 182.38 ppm at 0.10 M. However, inclusion of HEMA even at high concentration in the lactic acid solution has no impact on the chemical shift both ^{1}H- and ^{13}C NMR spectra (Figs 2c–d). Fig. 3 shows three-dimensional plot of pH versus ^{13}C NMR chemical shift (ppm) of the carbonyl group (C=O) from lactic acid in solutions titrated with increasing concentrations of HEMA or DMAEMA monomers. The pH of 0.1M solution of lactic acid in water was increased from pH 2.4 (at 0 M of DMAEMA) to pH 9.3 (at 1.0 M of DMAEMA) as the DMAEMA concentration increased. On the contrary, lactic acid solution treated by HEMA showed no change in pH (around pH 2.4).

For the stability study of DMAEMA at different pH condition, the pH of solution of DMAEMA in water was adjusted to pH 2 and 7 by adding dropwise HCl. The degree of hydrolysis of DMAEMA in D_2O at 25 °C with different pH as a function of time was determined by comparing the integrated intensity of the oxymethylene protons (peak “d”) of DMAEMA at 4.344, 4.333, and 4.323 ppm to the corresponding protons of dimethylaminoethanol (peak “d`”) hydrolyzed at 3.811, 3.800, and 3.789 ppm (Figs. 4–5). As shown in the third row from the bottom of Fig. 4, all the peaks were clearly assigned to DMAEMA and its hydrolysis products, methacrylate and dimethylaminoethanol (DMAE). DMAEMA after storage in aqueous solution at 25 °C for 30 days was equally stable (degraded 1–4%) at pH 2 and 7, but the monomer was much less stable at pH 10 (82% degraded at 24 hrs and 100% at 30 days). However, no degradation in HEMA occurred at 30 days (data not shown).

DISCUSSION

Lactic acid (LA) is the primary compound produced during acidification of the oral cavity by microbes. In this study, we have developed an NMR-based assay for detecting the solution pH and changes in pH of samples containing lactic acid. Because LA is an acid, the addition of basic material that contains buffering moieties neutralizes the acidic

microenvironment, which is relevant to dental decay processes in the mouth. The degree of change in pH can be tracked by NMR assay. DMAEMA, which is commonly used as a *co*-initiator in dentistry, was used to neutralize the acidic solution containing LA in water because it has a basic amino group, and HEMA, which lacks this moiety, was used as a reference.

With increasing the concentration of DMAEMA, the chemical shifts in ^{1}H NMR spectra of both methine proton and methyl protons in lactic acid gradually decreased (Fig. 2a). This shift could be explained by the fact that LA reacted with DMAEMA as an acid-base reaction, resulting in carboxylate anion, which caused methine proton and methyl protons to be more shielded than carboxylic acid of neat LA. This change in chemical shift position is larger for the methine proton compared to the methyl protons, because the methine proton is closer to the titratable carboxylate group. In general, the influence of a substituent on chemical shift decreases with increasing distance [20]. The chemical shift of the carbonyl carbon in the ^{13}C NMR spectra (Fig. 2b and Fig. 3) shifted downfield with increasing pH. The carbonyl carbon in LA is strongly influenced by the protonation state of the adjacent oxygen atom, which is influenced by the bulk buffering effect of the amino group in DMAEMA. In addition, the carbonyl carbon in the ^{13}C NMR appears at very high chemical shift due to the strong electron withdrawing effect of the oxygen. Thus, this carbonyl carbon peak in ^{13}C NMR spectrum is very diagnostic as there are very few other functional groups that would give a peak at this position.

HEMA does not alter the pH and chemical shift of the 0.1 M LA solution, whereas increasing amounts of DMAEMA demonstrates obvious buffering and neutralization. As can be seen in Fig. 2c–d and Fig. 3, inclusion of HEMA, the monomer currently used in the methacrylate dentin adhesive, even at high concentration in the LA solution, has no impact on the pH of the solution, and it cannot buffer or neutralize LA. The addition of increasing amounts of DMAEMA, which contains a basic amine, does, however, shift the pH and ^{13}C NMR carbonyl carbon peak of the acidic LA solution making it more neutral. These data show that neutralization can be achieved by monomers such as DMAEMA and that the buffering capacity can be measured in a straightforward NMR experiment using LA as a probe.

In the local microenvironment in the mouth, the pH often becomes acidic as a result of LA production by microbes. Therefore, the hydrolytic stability of materials exposed to different pH conditions was investigated in this study. The results presented here indicate that in acidic or neutral aqueous solution DMAEMA was quite stable, as after 30 days only 3–4 % of DMAEMA was hydrolyzed at pH 2 and pH 7. At pH 10, however, the monomer is much less stable, and it becomes completely hydrolyzed into methacrylic acid and dimethylaminoethanol within 30 days. Instability at pH 10 is likely due to ester hydrolysis involving hydroxide anion [21].

The concept demonstrated in the present study suggests that basic monomer may be used to buffer acidic microenvironments. Thus, inclusion of basic monomers such as DMAEMA as a component of methacrylate dentin adhesives may offer enhanced durability in the environment of the mouth. However, it should be recognized that the buffering capacity of the polymer may be lower than that of the monomer, but the approach presented here may be used to characterize this difference. Future studies will include the preparation and evaluation of dentin adhesives containing DMAEMA as a *co*-monomer with HEMA and also development of new basic monomers having neutralizing capability and hydrolytic stability in aqueous, acidic environments.

CONCLUSION

In this study, neutralization capacity of the basic functional monomer DMAEMA that contains a buffering moiety has been studied with lactic acid solution using an NMR assay. The NMR chemical shift is extremely sensitive to small changes that most other methods cannot detect, making it an excellent probe for monitoring perturbations to the nucleus of interest. The results show that neutralization can be achieved by monomers such as DMAEMA and that the buffering capacity can be measured in a straightforward NMR experiment using lactic acid as a probe. Thus, basic monomer, when included as a component of methacrylate dentin adhesives, may offer the ability to reduce lactic acid induced dental decay at the margins of composite restorations. The degree of hydrolysis of DMAEMA in water at 25 °C in different pH solutions as a function of time was also determined by NMR. ^{1}H NMR studies indicated that DMAEMA after 30-day storage at 25 °C was quite stable in acidic or neutral aqueous solution. However, the most pronounced extent of hydrolysis is observed at pH 10.

Acknowledgments

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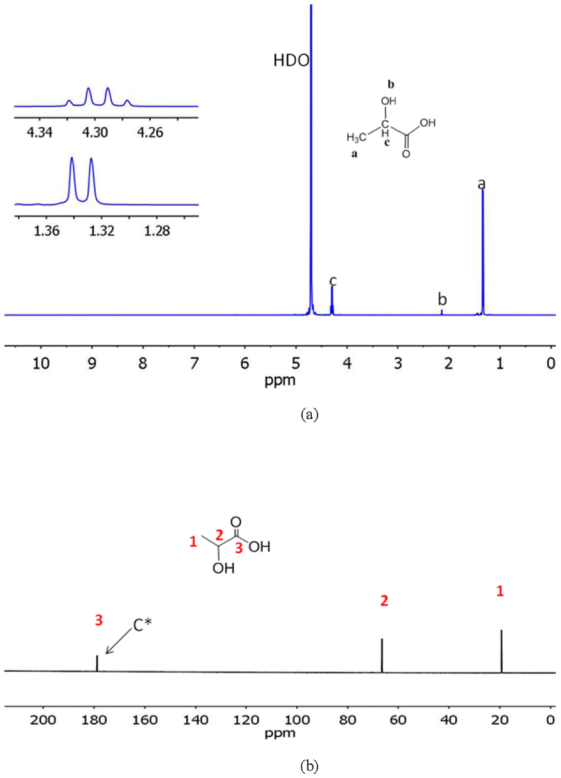

FIGURE 1.

^{1}H- (a) and ^{13}C- (b) NMR spectra of lactic acid in D_2O. The chemical structure of lactic acid is labeled and shows the corresponding peak assignments in each spectrum.

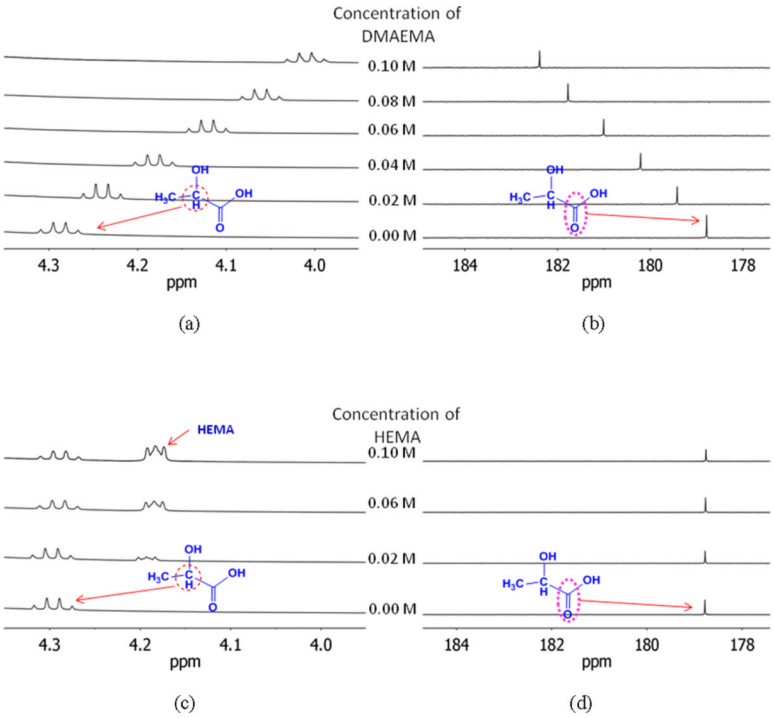

FIGURE 2.

^{1}H and ^{13}C NMR spectra of the methine and carbonyl moieties of lactic acid in D_2O when treated with different concentrations of DMAEMA and HEMA. ^{1}H spectra for the DMAEMA (a) and HEMA (c) titrations. ^{13}C spectra for the DMAEMA (b) and HEMA (d) titrations.

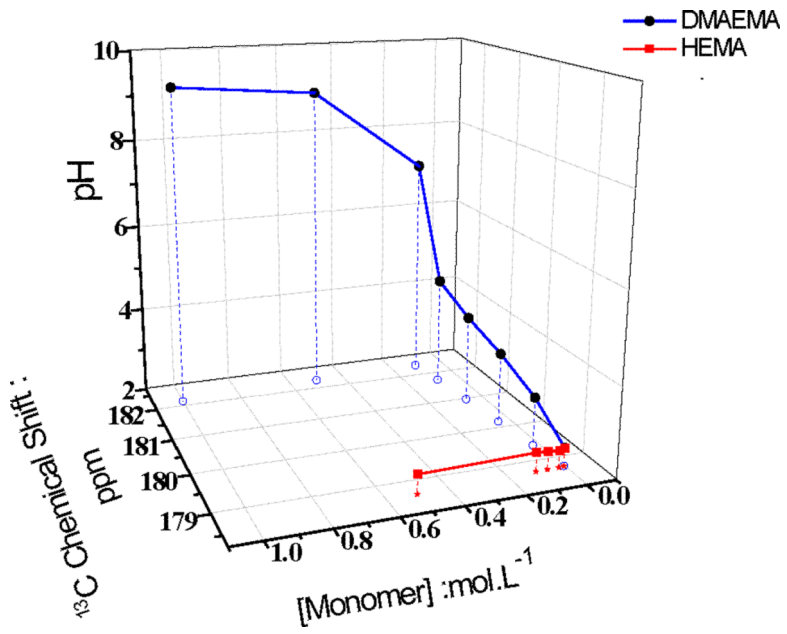

FIGURE 3.

^{13}C NMR chemical shift of carbonyl group (C=O) and pH change of 0.1 M lactic acid solution titrated with increasing concentration of DMAEMA. There is a corresponding increase in pH and chemical shift when the titration is performed with DMAEMA, whereas no change in pH or chemical shift position is observed when HEMA is added.

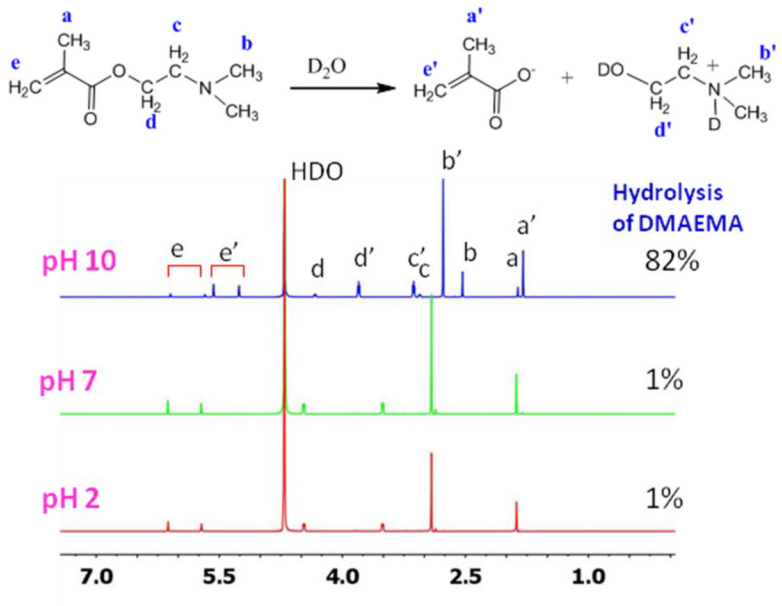

FIGURE 4.

Representative ^{1}H NMR spectra of 0.1 M of DMAEMA stored for 1 day in D_2O at 25 °C as a function of pH change.

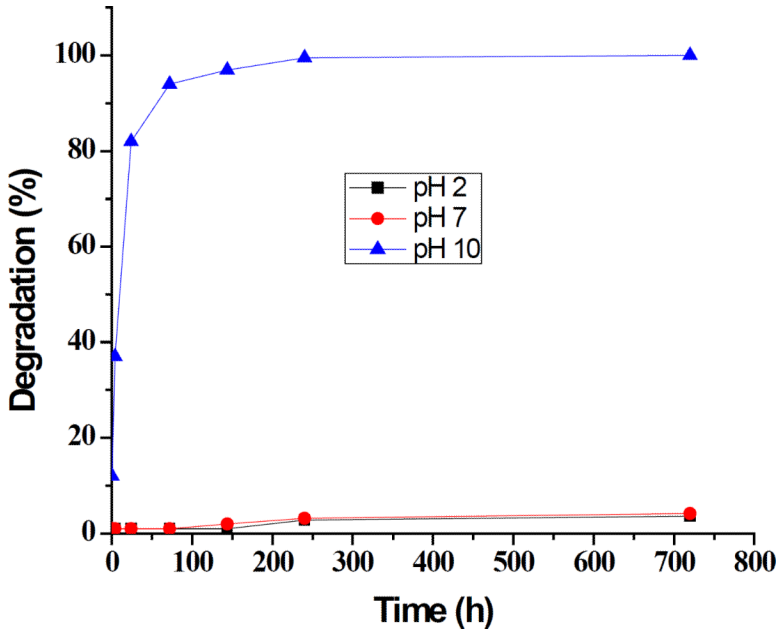

FIGURE 5.
Hydrolysis of DMAEMA at pH 2 (black), 7 (red), and 10 (blue) as a functionof time.