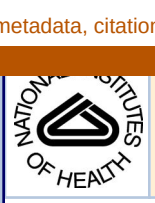

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Characterization of Acid-neutralizing Basic Monomers in Co-solvent Systems by NMR

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Abstract

Metabolic activity of the oral microbiota leads to acidification of the microenvironment and promotes demineralization of tooth structure at the margin of composite restorations. The pathogenic impact of the biofilm at the margin of the composite restoration could be reduced by engineering novel dentin adhesives that neutralize the acidic micro-environment. Integrating basic moieties into methacrylate derivatives has the potential to buffer against acid-induced degradation, and we are investigating basic monomers for this purpose. These monomers must be compatible with existing formulations, which are hydrophobic and marginally miscible with water. As such, cosolvent systems may be required to enable analysis of monomer function and chemical properties. Here we present an approach for examining the neutralizing capacity of basic methacrylate monomers in a water/ethanol co-solvent system using NMR spectroscopy. NMR is an excellent tool for monitoring the impact of co-solvent effects on pKa and buffering capacity of basic monomers because chemical shift is extremely sensitive to small changes that most other methods cannot detect. Because lactic acid (LA) is produced by oral bacteria and is prevalent in this microenvironment, LA was used to analyze the effectiveness of basic monomers to neutralize acid. The ^{13}C chemical shift of the carbonyl in lactic acid was monitored as a function of ethanol and monomer concentration and each was correlated with pH to determine the functional buffering range. This study shows that the buffering capacity of even very poorly water-soluble monomers can be analyzed using NMR.

Keywords

basic monomer; co-solvent; lactic acid; neutralization; nuclear magnetic resonance spectroscopy

Global concerns about mercury in the environment are driving the discontinuation of dental amalgam and thus, polymer-based composites are rapidly replacing dental amalgams in the reconstruction of posterior teeth. Composite restorations have higher failure rates, more recurrent decay and substantially shorter clinical life spans as compared to dental

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amalgam [1–7]. The longevity of the composite restoration depends heavily on the integrity of the adhesive that bonds the composite material to the tooth surface. Methacrylate monomers are used ubiquitously in adhesive formulations because of their biocompatibility, rapid polymerization, straightforward photochemistry and compatibility with composite resin. The methacrylate-based adhesives are, however, vulnerable to hydrolytic attack via enzymatic hydrolysis catalyzed by salivary enzymes, particularly esterases [8–17] and chemical hydrolysis catalyzed by acids or bases.

The pioneer organism, *Streptococcus mutans*, binds to composite restorations at the adhesive/tooth interface, and its attachment facilitates degradation of the tooth and restoration by acidifying the microenvironment. We have hypothesized that the longevity of a composite restoration may be extended by combating acid-induced degradation. As such, methacrylate monomers are being designed to incorporate moieties capable of acting as proton sponges that buffer the microenvironment and resist acidification.

Buffers are used in numerous biological and chemical applications to control the pH of solutions. Monomers that have basic functional groups have the potential to mitigate acidic excursions. Determining the pKa of these moieties is important to engineering polymers that are compatible with the current hydrophobic formulations and also are able to soak up protons in the pH range required to effectively protect the composite reconstruction. The pKa of an individual chemical group is affected by the network of molecules surrounding the ionizable moiety, including the solvent and adjacent groups within the molecular structure. It is most common to analyze compounds and measure pKa values in a single solvent, e.g. water.

In adhesive formulations it may be necessary to utilize a co-solvent system to achieve solubility of the components and accommodate the aqueous environment at the tooth surface. The dielectric constant of the solvent will impact the pKa measurement. Assays that permit quantitative analysis of the pKa of poorly water-soluble compounds are lacking, but such assays are required to characterize the behavior of basic monomers that are miscible with hydrophobic formulations. In this study, lactic acid (LA) was mixed with a range of concentrations of each of a series of basic monomers, e.g. 2-dimethylaminoethyl methacrylate (DMAEMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), 2-N-morpholinoethyl methacrylate (MEMA), and 2-(tert-butylamino)ethyl methacrylate (TBAEMA), to assess their ability to neutralize acid and act as a “proton sponge.” Because the solubility of these monomers in water varies, the dielectric of the solvent was altered by titrating the ratio of water to ethanol up to 60% ethanol for some monomers. These results were used to determine the relationship between solvent dielectric constant and the pH range over which buffering occurs. The pH and NMR spectra of each sample were monitored as a function of monomer and/or ethanol concentration, and the chemical shift of the carbonyl ^{13}C in LA was correlated with pH in this co-solvent system.

Materials and Methods

Materials

L(+)-lactic acid (LA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), 2-N-morpholinoethyl methacrylate (MEMA), and 2-(tert-butylamino)ethyl methacrylate (TBAEMA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The ethanol used was 200 proof and reagent grade. The deuterium oxide (D_2O) used had 99% ^2H atoms and was filtered through a 0.22 um filter.

Methods

Sample Preparation—Triplicate samples with monomer concentrations of 0, 0.020, 0.040, 0.060, 0.080, 0.10, 0.50 and 1.0 M were analyzed. Each was prepared by mixing pre-determined amounts of solvent, lactic acid, and monomer stock solutions to achieve the desired concentration of each component. The solvent stock solutions of 0, 5, 15, 30, 40, 50 and 60% ethanol were prepared by mixing D_2O and ethanol by volume. 1.0 M lactic acid (LA) stock was prepared, and each sample was made by adding an equivalent volume of this stock to the various co-solvent solutions. Monomers were diluted into the stock solvents to 1.5 M. The three component mixtures were pipetted into 1.5 mL conical centrifuge tubes, mixed by vortex for 10 seconds and allowed to equilibrate for 30 minutes to ensure samples were miscible and no phase separation had occurred. Based on visual inspection of the samples for clarity and miscibility, DMAEMA and MEMA were soluble up to 1.0 M monomer concentration in 0–60% ethanol in water. At the same monomer concentration TBAEMA was soluble in 50% ethanol and DEAEMA was soluble in 60% ethanol.

pH and pKa Measurements—The pH measurements were performed with a Fisher Scientific (Waltham, MA, USA) Accumet Research AR25 pH meter equipped with a micro-probe. Calibration was done using commercial buffers (Fisher Scientific, pH 4.01, 7.00, and 10.01). Measurements were taken with application of gentle agitation. Average values and standard deviations were calculated from measurements of triplicate samples. Each pKa determination was performed by titrating the monomer with hydrochloric acid (HCl) and titrating lactic acid with sodium hydroxide (NaOH). In each case the pH was measured after the addition of every aliquot. The pKa was determined to be the pH at the inflection point of graphs of the titration data; this value was obtained by taking the derivative of the sigmoidal curve fit to the data. Titrations were performed in triplicate and the average values and standard deviations were calculated from these measurements.

Nuclear Magnetic Resonance (NMR) Spectroscopy—After the pH was measured, 600 μL of each sample was loaded into individual standard 5 mm NMR tubes. 5% D_2O was present in each sample to maintain lock. ^{13}C NMR spectra were collected at 25 °C on a Bruker Avance DRX 500 spectrometer equipped with a broadband probe. 1024 transient scans were acquired for signal averaging. ^1H decoupling was performed to minimize broadening of the ^{13}C signals by protons. Spectra are referenced to ^1H in H_2O at 4.7036 ppm, and ^{13}C was referenced indirectly, using a $^{13}C/^1H$ ratio of 0.251449530.

Results

The ^{13}C NMR spectrum of 0.1 M lactic acid (LA) in water at pH 2 yields peaks at 19.22 ppm, 66.38 ppm, and 178.59 ppm [5]. The peak at 178.59 corresponds to the carboxyl carbon in its fully protonated state, and the position of this peak is sensitive to changes in pH. Replicates indicate the chemical shift is 178.57 ± 0.02 ppm (data not shown). The ^{13}C NMR spectrum of ethanol (EtOH) has peaks for the methyl and methylene carbons, at approximately 18 and 58–66 ppm (depending on solution conditions), respectively. There is complete separation between the chemical shift of the carboxyl carbon in LA and the carbon signals from ethanol, and as such, the co-solvent does not interfere with the ability to track changes in pH using LA as a probe.

The relationship between the amount of ethanol and the pH of the solutions containing 0.1 M lactic acid was examined to establish the baseline for changes resulting from differences in dielectric of the co-solvent system. The dielectric constants (ε) of pure water and ethanol at 25 °C are 80.4 and 24.3, respectively (CRC). Increasing the percentage of ethanol in the co-solvent system decreases the dielectric. As a reasonable first approximation, the dielectric of the binary mixture is proportional to the sum of the individual ε weighted by mole fraction. The change in pH was determined to be linear over the 0–60% (v/v) ethanol range tested (Figure 1A). The data were plotted and fit to a linear equation, which indicates the pH of the 0.1 M LA solution increases 0.135 pH units for each 10% ethanol increment added. This equates to an increase in the pH of 0.675 when comparing 50% ethanol to the water only sample. The linear fit yields an R^2 value of 0.9623, and the theoretical y intercept, which reflects the pH of the 0% ethanol solution, is 2.09, which agrees reasonably well with the measured value of 2.17 ± 0.06.

A plot of ethanol concentration versus chemical shift was generated to assess linearity of the NMR measurement in the co-solvent system. It was determined from our previous study that the carbon within the carboxylic acid moiety of LA is an excellent probe for tracking the pH of the solution, because its chemical shift position reflects the protonation state of this group [5]. ^{13}C NMR spectra of LA were collected in the presence of various amounts of ethanol and the chemical shift position of the carboxyl carbon monitored (Figure 1B). The plot of this data shows a linear relationship (R^2=0.9989) between ethanol concentration and the chemical shift position up to 50% ethanol. Over this concentration range, the chemical shift position decreases by 0.148 ppm for each 10% increase in ethanol in solution, and an offset of 0.0148 ppm/1% ethanol can be applied to relate the value measured in co-solvent to that of water. At 60% ethanol, however, a large deviation from the linear-extrapolation value is observed. Based on the equation derived from the fit of the data from 0–50% ethanol, the expected chemical shift value in 60% ethanol is 177.66 ppm, but the measured value is 177.34 ppm. In this case, the chemical shift value overestimates the pH by 0.16 units, a value expected for a solution containing 72% ethanol. Consequently, data collected in greater than 50% ethanol cannot be adjusted accurately using the linear correction factor applicable to lower ethanol concentrations. As such, quantitative analysis is restricted to samples containing 0–50% ethanol.

In addition, a pH titration was performed to determine the pKa of the carboxylate moiety of lactic acid in each co-solvent condition (Table 1). The pKa was plotted as a function of ethanol concentration to characterize the ionization behavior of lactic acid in the cosolvent system (Figure 2). To assess the behavior of a basic moiety in the co-solvent system, the basic monomer DMAEMA was examined using the same approach. A complete assessment of DMAEMA was performed, and in addition, other basic monomers were examined under select conditions. The measured pKa values for these compounds are reported in Table 1. Figure 2 shows a linear increase in pKa of the carboxyl group in LA with increasing amounts of ethanol. The R^2 value of 0.9993 indicates an excellent linear fit to the data. The pKa increases by approximately 0.2 units for each 10% increase in ethanol concentration, resulting in the pKa of LA being raised to 4.6 in 50% ethanol. The pKa of DMAEMA in water was determined to be 8.62. A linear fit of % ethanol versus pKa yields an excellent fit ($R^2 = 0.9811$) and indicates addition of each 10% increment of ethanol depresses the pKa of DMAEMA by 0.12. Table 1 compiles the pKa values measured in this study.

A lowering of the dielectric constant depresses the pKa of bases and elevates the pKa of acids; thus, in a system containing both an acid and base the buffering ranges approach one another and overlap to a greater extent than in a purely aqueous system. This property is manifest in the plot of the pH profiles of DMAEMA and LA as a function of the percentage of EtOH in the co-solvent system (Figure 3). The basic pKa of the DMAEMA monomer decreases linearly with increasing EtOH in the solvent. Conversely, the pKa of LA increases linearly with increasing EtOH.

Figure 3 shows the effect of DMAEMA concentration on the chemical shift of the LA carboxyl carbon and the pH in the co-solvent systems with varied ethanol content. Inclusion of increasing amounts of DMAEMA raises the pH of the solution as lactic acid is neutralized by the basic moiety in the monomer. Neutralization of LA is observed when DMAEMA is added to all of the co-solvents. Regardless of the percent ethanol in the co-solvent system, the midpoint of the transition occurs at pH 6.2 ± 0.1 and 0.16 M DMAEMA. Because the pKa of the amine moiety in DMAEMA is much higher than neutral, the pH of the solution continues to elevate, resulting an alkaline pH of the solution at the end point when the monomer is in great excess. Because the ^{13}C carboxyl chemical shift of LA is directly related to the pH of the solution, the chemical shift can be adjusted to an effective 0%-ethanol condition by subtracting the product of the correction factor (0.0148 ppm/1% EtOH) times the percent ethanol. Adjusting the data using this correction factor, leads to the curves collected in 0–50% ethanol overlaying each other (Figure 3B). The pKa values were adjusted as a function of ethanol concentration to calculate an effective pKa (pKa^0). A comparison of the measured and corrected values for LA and DMAEMA is presented in Table 2. The table shows the calculated values correspond well to the measured values; small, random deviations within error of the measurement are observed for both compounds.

The commercially available monomers DEAEMA, MEMA and TBAEMA were also examined for their ability to control pH in the presence of LA. The pKa of these basic monomers differs. The pKa value of each was determined in the appropriate co-solvent system for analysis (Table 1). DEAEMA required 60% ethanol to be solubilized and the pKa in this solution is 8.2. MEMA is soluble in water and has a pKa of 6.2. TBAEMA is soluble

in 50% ethanol and has a pKa of 9.0 in this co-solvent. Each monomer was titrated into the corresponding solvent containing LA and the pH and chemical shift values measured (Figure 4). The data show MEMA is able to neutralize LA more effectively than DMAEMA and the end point of the titration is near neutral even when the monomer is present in large excess. Because the pKa of MEMA is lower than DMAEMA, it begins buffering and raising the pH of the solution under more acidic conditions and when added at lower concentration than DMAEMA. As was observed with DMAEMA in the co-solvent system, DEAEMA and TBAEMA have elevated pH at the beginning of the titration, but the results show the monomers differ in their titration end point, which corresponds to the pKa of the base.

Discussion

Production of lactic acid (LA) by *Streptococcus mutans* is a primary factor in the deterioration of composite dental restorations [18]. To protect against acid-induced degradation, incorporation of basic monomers capable of neutralization is being investigated for next generation dental polymers. We previously demonstrated NMR may be used to track lactic acid neutralization in aqueous solution by monitoring the chemical shift of the carboxyl carbon in LA [5]. Because dental adhesive is hydrophobic and the oral environment is aqueous, miscibility of the basic monomer with both resin and water is an important aspect of compatibility with the formulation. As such, analysis of monomers may require the use of co-solvent to achieve sufficient solubility.

In this study, we sought to demonstrate the NMR approach may be applied to examine monomers of various composition and solubility in a co-solvent system. Here, we employed ^{13}C NMR to assess the ability of several monomers to neutralize acid, and we varied the dielectric (ε) properties using an ethanol/water co-solvent system to establish comparison of neutralization capacity can be made over a range of ε values. Ethanol was selected because it is used in existing formulations and does not interfere with ^{13}C NMR measurement of the LA carboxyl chemical shift.

Comparison of four different monomers shows their neutralization capacity in co-solvent parallels the pKa of the base. The pKa of MEMA (6.2) results in the most effective buffering in the relevant pH range and a less alkaline end point. The other monomers have much higher pKa values and provide less neutralizing capacity in the acidic range per monomer by comparison. The more basic pKa value also results in a more alkaline pH at the end point of the titration for these monomers.

Based on the relationship determined for DMAEMA's pKa and the pH of the solution in increasing amounts of ethanol, a correction factor can be applied to account for the effect of the co-solvent on pH and the pKa of the amine moiety. If the pH data for the 50% ethanol-containing TBAEMA solution is adjusted by 0.675 (0.0135/1%×50% ethanol) to emulate an ethanol-free environment, TBAEMA would have an effective pKa (pKa^0) of 9.71 in water. Based on the raw data, TBAEMA appears to neutralize LA better than DMAEMA, but applying the correction factor to remove the effect of the co-solvent reveals that in fact DMAEMA is more effective than TBAEMA. Applying the pH correction factor to DEAEMA in 60% ethanol yields a pKa^0 of 8.96. By extrapolating the pKa of the basic

monomers to an effective 0%-ethanol condition a comparison of their ability to neutralize LA can be made. This study indicates monomers with a pKa^0 value close to neutral have the best properties for neutralizing LA in aqueous solution and that MEMA > DMAEMA > DEAEMA > TBAEMA when extrapolated to pure water.

As can be seen in the plots, pH and pKa values for LA and basic monomers increase linearly over the full range of co-solvent conditions examined. In the NMR assay, as the dielectric decreases, the chemical shift position of LA increases and the pKa of the base decreases. Our data show this relationship is linear up to 50% ethanol, which has a dielectric of 52.4 at 25 °C. The addition of monomer, like ethanol, also affects the dielectric of the solution. These values for pure monomer are rarely reported, preventing calculation of the co-solvent's dielectric. Here, only a small proportion of monomer is added to the co-solvent system, and its effect on ε is negligible in our experiments. At high concentrations (~M) of monomer, its influence would be relevant, and the effects could be analyzed using the NMR assay up to an equivalent mole fraction by modulating the proportion of monomer and ethanol in the solution.

One additional consideration to make in evaluating buffering is how incorporation of the basic monomer into the methacrylate polymer may affect its pKa and performance in the oral environment. The pKa of ionizable moieties can be altered, in some cases dramatically, by local environment. For example, carboxyl moieties in folded proteins have been shown to deviate by several orders of magnitude (ranging from below 2 to above 9) from the value obtained for the soluble amino acid in aqueous solution [19]. Such large deviations result largely from well-structured hydrogen bonding and electrostatic interactions with neighboring residues, but perturbation may also result from proximal charges and hydrophobic surfaces [20] [21] [22]. As such, the pKa of the basic moiety in dental monomers may be altered to some extent by incorporation into a hydrophobic resin and proximity to neighboring basic monomers and hydrogen bonding partners. The LA-based NMR assay also may be applied to determine the effective pKa and buffering capacity of polymers containing basic moieties. Correlating monomer properties with the buffering capability of the basic moiety in the solid resin is expected to enable design and selection of monomers with optimal neutralization performance characteristics.

Conclusion

Dental adhesive formulations are hydrophobic, and to avoid phase separation when incorporating basic moieties, the monomers need to be miscible in these hydrophobic formulations. The NMR assay, originally developed to examine the neutralization capacity of monomers in aqueous solution, was modified for use in characterizing the behavior of less water-soluble species that require analysis in a co-solvent system. This study shows a direct linear correlation between solution pH and chemical shift of the ^{13}C carboxyl in lactic acid up to 50% ethanol. Because of the straightforward relationship between pH, chemical shift and dielectric, the buffering properties of a basic monomer examined in the co-solvent system can be adjusted using a correction factor to enable comparison of samples collected using different co-solvent conditions.

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References

1. Bernardo M, Luis H, Martin MD, Leroux BG, Rue T, Leitao J, DeRouen TA. Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial. Journal of the American Dental Association. 2007; 138(6):775–783. [PubMed: 17545266]
2. Levin L, Coval M, Geiger SB. Cross-sectional radiographic survey of amalgam and resin-based composite posterior restorations. Quintessence International. 2007; 38(6):511–514. [PubMed: 17625635]
3. Mjor IA, Dahl JE, Moorhead JE. Age of restorations at replacement in permanent teeth in general dental practice. Acta Odontologica Scandinavica. 2000; 58(3):97–101. [PubMed: 10933556]
4. Opdam NJM, Bronkhorst EM, Loomans BAC, Huysmans M. 12-year Survival of Composite vs Amalgam Restorations. Journal of Dental Research. 2010; 89(10):1063–1067. [PubMed: 20660797]
5. Park J, Ye Q, Spencer P, Laurence JS. Determination of neutralization capacity and stability of a basic methacrylate monomer using NMR. Int J Polym Mater. 2012; 61:144–153. [PubMed: 22544985]
6. Simecek JW, Diefenderfer KE, Cohen ME. An evaluation of replacement rates for posterior resin-based composite and amalgam restorations in US Navy and Marine Corps recruits. Journal of the American Dental Association. 2009; 140(2):200–209. [PubMed: 19188417]
7. Soncini JA, Maserejian NN, Trachtenberg F, Tavares M, Hayes C. The longevity of amalgam versus compomer/composite restorations in posterior primary and permanent teeth: findings From the New England Children's Amalgam Trial. J Am Dent Assoc. 2007; 138(6):763–772. [PubMed: 17545265]
8. Donmez N, Belli S, Pashley DH, Tay FR. Ultrastructural correlates of in vivo/in vitro bond degradation in self-etch adhesives. Journal of Dental Research. 2005; 84(4):355–359. [PubMed: 15790743]
9. Finer Y, Jaffer F, Santerre JP. Mutual influence of cholesterol esterase and pseudocholinesterase on the biodegradation of dental composites. Biomaterials. 2004; 25:1787–1793. [PubMed: 14738842]
10. Finer Y, Santerre JP. The influence of resin chemistry on a dental composite's biodegradation. Journal of Biomedical Materials Research. 2004; 69A:233–246. [PubMed: 15057996]
11. Finer Y, Santerre JP. Salivary esterase activity and its association with the biodegradation of dental composites. Journal of Dental Research. 2004; 83:22–26. [PubMed: 14691108]
12. Kostoryz EL, Dharmala K, Ye Q, Wang Y, Huber J, Park JG, Snider G, Katz JL, Spencer P. Enzymatic biodegradation of HEMA/bisGMA adhesives formulated with different water content. J Biomed Mater Res B Appl Biomater. 2009; 88(2):394–401. [PubMed: 18395826]
13. Munksgaard EC, Freund M. Enzymatic hydrolysis of (di)methacrylates and their polymers. Scandinavian Journal of Dental Research. 1990; 98:261–267. [PubMed: 2349453]
14. Park JG, Ye Q, Topp EM, Kostoryz EL, Wang Y, Kieweg SL, Spencer P. Preparation and properties of novel dentin adhesives with esterase resistance. Journal of Applied Polymer Science. 2008; 107(6):3588–3597. [PubMed: 22919119]
15. Park JG, Ye Q, Topp EM, Lee CH, Kostoryz EL, Misra A, Spencer P. Dynamic mechanical analysis and esterase degradation of dentin adhesives containing a branched methacrylate. J Biomed Mater Res B Appl Biomater. 2009; 91(1):61–70. [PubMed: 19358261]
16. Park JG, Ye Q, Topp EM, Spencer P. Enzyme-catalyzed hydrolysis of dentin adhesives containing a new urethane-based trimethacrylate monomer. J Biomed Mater Res B Appl Biomater. 2009; 91(2):562–571. [PubMed: 19582843]
17. Yourtee DM, Smith RE, Russo KA, Burmaster S, Cannon JM, Eick JD, Kostoryz EL. The stability of methacrylate biomaterials when enzyme challenged: Kinetic and systematic evaluations. Journal of Biomedical Materials Research. 2001; 57(4):523–531.

18. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res. 2011; 90(3):294–303. [PubMed: 20924061]

19. Thurlkill RL, Grimsley GR, Scholtz JM, Pace CN. Hydrogen bonding markedly reduces the pK of buried carboxyl groups in proteins. J Mol Biol. 2006; 362(3):594–604. [PubMed: 16934292]

20. Petong P, Pottel R, Kaatze U. Water-ethanol mixtures at different compositions and temperatures. A dieletric relaxation study. Journal of Physical Chemistry A. 2000; 104(32):7420–7428.

21. Mohsen-Nia M, Amiri H, Jazi B. Dielectric Constants of Water, Methanol, Ethanol, Butanol and Acetone: Measurement and Computational Study. Journal of Solution Chemistry. 2010; 39(5): 701–708.

22. Wang PM, Anderko A. Computation of dielectric constants of solvent mixtures and electrolyte solutions. Fluid Phase Equilibria. 2001; 186(1–2):103–122.

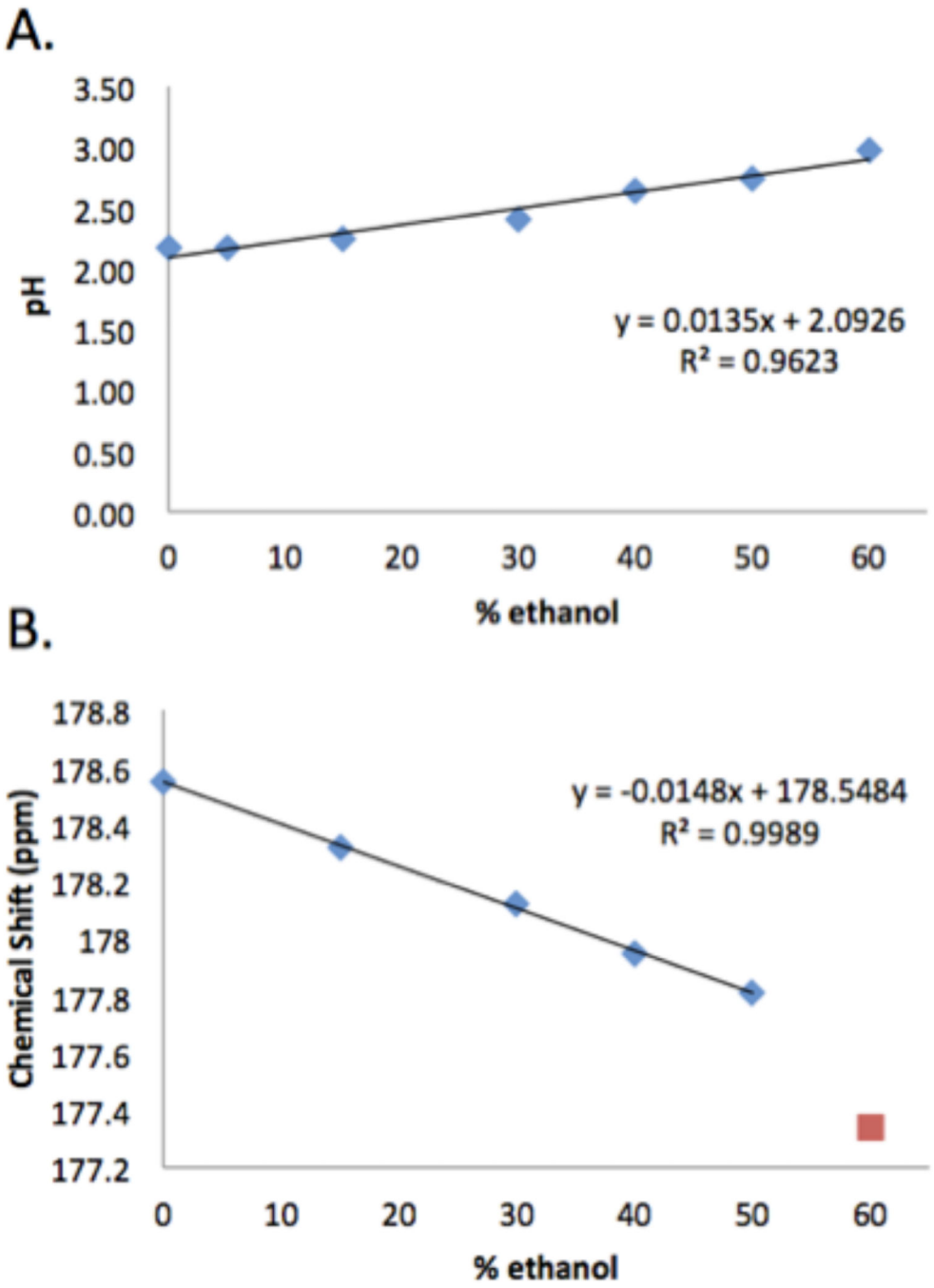

Figure 1.

Plots of ethanol concentration versus pH and chemical shift. A) Plot of pH vs % ethanol. The pH of solutions containing 0.1 M lactic acid in 0, 5, 15, 30, 40, 50 and 60% ethanol (v/v in water) were measured at 25°C. All data points were fit to a linear function, yielding an R^2 value of 0.9623, as shown on the plot. B) Plot of chemical shift position of the ^{13}C carbonyl from lactic acid as a function of ethanol concentration. Data for samples containing 50% ethanol or less were fit to a linear function, yielding an R^2 value of 0.9989. The chemical shift is not linear with respect to ethanol concentration at 60%.

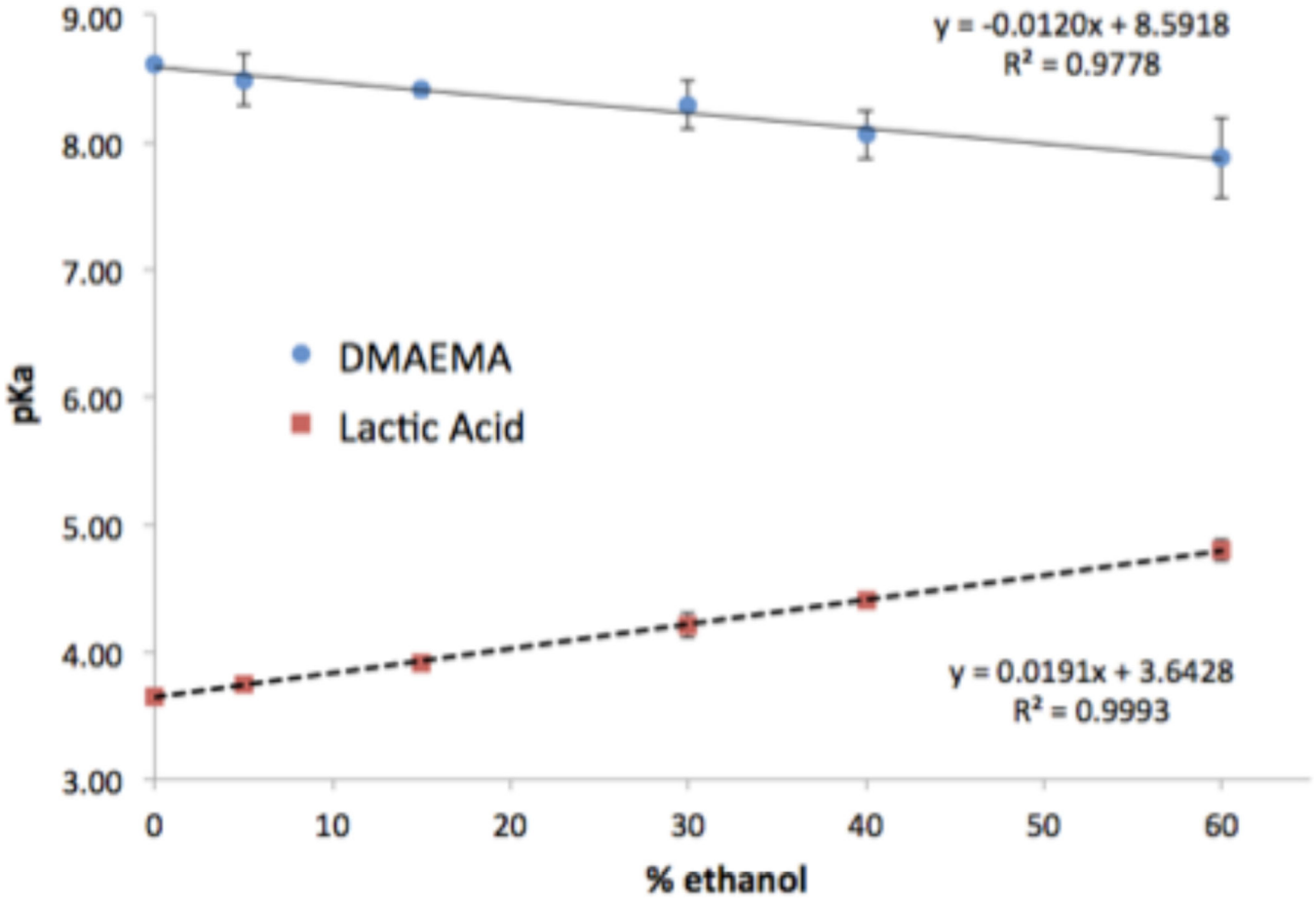

Figure 2.

Plot of lactic acid and DMAEMA pKa values as a function of ethanol concentration in the co-solvent system. Data for DMAEMA is shown in blue circles and lactic acid is shown in red squares. Linear regression lines are shown with their respective equations adjacent to the data.

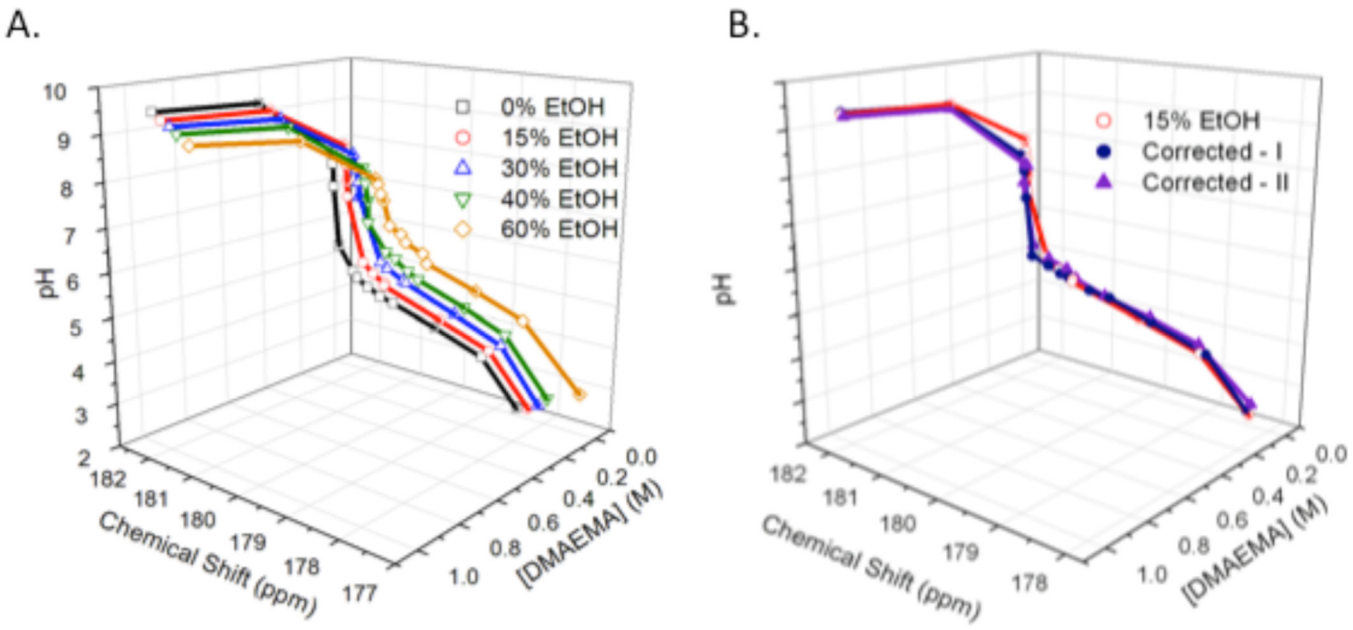

Figure 3.

Plot of ^{13}C NMR chemical shift and pH vs DMAEMA concentration as a function of increasing amounts of ethanol. A) The pH of each LA solution containing various amounts of ethanol was measured directly and correlated with the chemical shift of the lactic acid carboxyl carbon peak during titration with DMAEMA. B) Two different approaches were used to compare data sets collected at different ethanol concentrations to the measured values at 15% EtOH. The plot labeled Correction I was derived from adding the difference of the 30%-15% data sets to the 0% data to emulate 15% EtOH. The plot of Correction II was derived from subtracting 40%–30% and then multiplying this 10% difference by 1.5 to emulate 15% EtOH. These difference plots compare well with the measured data, indicating a consistent offset can be applied on a percent ethanol basis to effectively emulate another co-solvent condition.

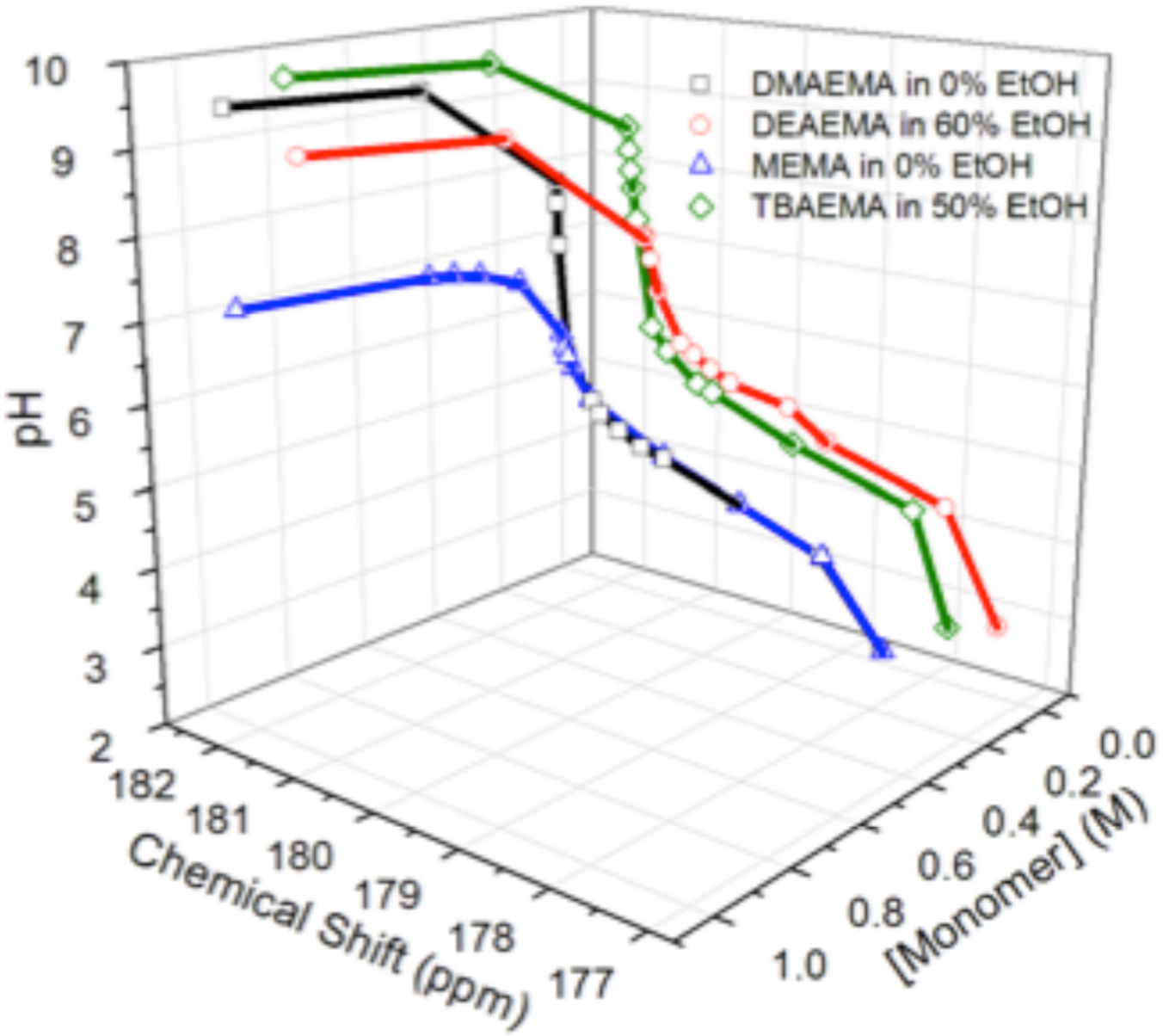

Figure 4.

Plot shows a set of basic monomer's ability to neutralize LA. The ^{13}C carboxyl chemical shift of lactic acid and the pH of the solution were monitored as a function of monomer concentration in the co-solvent system. The measured values are plotted and have not been adjusted to account for solvent effects.

Table 1

Experimentally determined pKa values in co-solvents

Species	EtOH (%)	pKa	Std. Dev.	Monomer Species	EtOH (%)	pKa	Std. Dev.
Lactic Acid	0	3.65	0.05	DMAEMA	0	8.62	0.04
Lactic Acid	5	3.75	0.01	DMAEMA	5	8.49	0.20
Lactic Acid	15	3.91	0.03	DMAEMA	15	8.41	0.05
Lactic Acid	30	4.21	0.10	DMAEMA	30	8.29	0.19
Lactic Acid	40	4.41	0.01	DMAEMA	40	8.06	0.19
Lactic Acid	60	4.80	0.09	DMAEMA	60	7.88	0.31
				TBAEMA	50	9.03	0.05
				DEAEMA	60	8.15	0.03
				MEMA	0	6.22	0.05

Table 2

Effective pKa (pKa^0)

Lactic Acid			DMAEMA		
% EtOH	**pKa (measured)**	**pKa^0**	**% EtOH**	**pKa (measured)**	**pKa^0**
0	3.65	-	0	8.62	-
5	3.75	3.65	5	8.49	8.56
15	3.91	3.61	15	8.41	8.61
30	4.21	3.61	30	8.29	8.69
40	4.41	3.61	40	8.06	8.60
60	4.8	3.60	60	7.88	8.69

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