

Short communication

Explanation for the enhanced dissolution of silica column packing in high pH phosphate and carbonate buffers

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Abstract

It has been reported that at high pH, the rate of bonded phase packing degradation in methanol/water mobile phases is greater for carbonate and phosphate buffers than for amine buffers. This conclusion was based on buffer pH determined in the aqueous buffer before dilution with methanol. Changes in buffer species pK_a , and therefore buffer pH, upon methanol dilution are consistent with the observed degradation results. Measurements of pH in the methanol/water solutions confirm that the carbonate and phosphate buffers were considerably more basic than the amine buffer, even though all the buffers were pH 10 before dilution with methanol. These results demonstrate that it can be misleading to extrapolate aqueous pH data to partially aqueous solutions. Measurements of pH in the mixed solvent provide more reliable predictions of column and sample stability.

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1. Introduction

In a paper on silica-based liquid chromatography (LC) column stability, the authors prepared pH 10 buffers in water, diluted the buffers 1:1 with methanol and measured silica dissolution rate under chromatographic conditions [1]. They were surprised to find very large differences in the dissolution rates for buffers prepared with different anions in spite of the fact that all the buffers were “pH 10”. The silica dissolution rate for phosphate was 10 times greater than the rate for borate or glycine, while the carbonate rate was at least 10 times higher than the rate for

phosphate. In a later paper, similar differences between phosphate and amine buffers were observed [2]. The authors state that “the mechanism for this effect needs clarification”. As a result of this work, it has become part of LC lore that phosphate buffers should not be used for high pH LC buffers because of their aggressive attack on silica. Instead, amine buffers should be used.

The pioneering work on pH and buffers by Bates [3] and de Ligny et al. [4] in partially aqueous solutions provides an explanation for these unexpected results. When water is diluted with methanol, the main effect, up to about 50% methanol, is a lowering of the solution dielectric constant. With a smaller dielectric constant, the dissociation of neutral species to form charged species, or charged species

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to make more highly charged species, becomes less favorable. Therefore, species such as H_3PO_4 and HPO_4^- become weaker acids in methanol/water mixtures. As a rule, any neutral or negatively charged acid becomes weaker. On the other hand when cationic acids such as ammonium ion and protonated amines dissociate, a more highly charged species is not formed. Therefore, as a rule, cationic acids become stronger in methanol/water solutions. These rules apply for many acids used to prepare buffers at least up to 50% methanol. These rules also apply when other lower dielectric constant solvents are mixed with water, for example ethanol and acetonitrile [5]. To illustrate these rules examples of water, and water/methanol, $\text{p}K_a$ values for some buffer acids are shown in Table 1. From these data, as well as the rules for the behavior of buffers in methanol/water, it would be expected that the pH of the phosphate and carbonate buffer used in the stability study would increase when methanol was added, while the pH of the glycine buffer would be expected to decrease. Therefore, the trends in the observed dissolution rates are in fact in the expected order.

It is possible to confirm the hypothesis that $\text{p}K_a$ changes resulted in the observed differences in silica dissolution by measuring the pH values of the methanol/water buffers. It is well established that the glass electrode responds nearly ideally to proton activity in methanol/water mixtures [6]. Therefore, a pH measurement of the buffers after methanol dilution will provide a reliable relative measure of the acidity, or basicity, of these solutions. Absolute hydrogen ion activities in 50:50 methanol/water can be measured by calibrating the pH electrode in a pH standard prepared in 50:50 methanol/water [6].

Table 1
 $\text{p}K_a$ values in water (first entry) and 50:50 methanol water (second entry)

Acid	$\text{p}K_a$
Acetic	4.8/5.7
Dihydrogen phosphate	7.2/8.4
Phosphoric	2.2/3.2
Ammonium	9.2/8.8

Data are from Refs. [4,9].

However, only a relative measure of hydrogen ion activity is necessary to confirm the hypothesis, and this was the approach taken in our study. If the glass electrode is calibrated in an aqueous solution and then used to measure a methanol/water solution, a correction will have to be applied to the measurement to calculate hydrogen ion activity. The correction accounts for the difference in standard state and junction potential between water and water/methanol. For the solutions under consideration, this correction is small [7]. The measurements will be 0.1–0.2 pH units higher than if the electrode was calibrated with a 50:50 methanol/water buffer.

If the electrode is calibrated in water and used to measure several dilute buffer solutions, all with the same methanol/water composition, the junction potential and standard state correction will be nearly the same for each methanol/water buffer. This assumption is particularly reliable in this case because these junction potentials and standard state corrections are small. The differences in the measured pH of the methanol/water buffers provide an accurate measure of the differences in hydrogen ion activity between these solutions. These differences can then be correlated with observed dissolution rates.

2. Experimental

2.1. Apparatus and reagents

ACS reagent-grade sodium carbonate, sodium bicarbonate, anhydrous dibasic sodium phosphate and sodium borate were from Mallinckrodt (Paris, KY, USA). ACS reagent-grade sodium hydroxide and ultrapure glycine were from J.T. Baker (Phillipsburg, NJ, USA). ACS HPLC-grade methanol was from Aldrich (Milwaukee, WI, USA). Water was obtained from a Milli-Q RG Ultra-pure Water System from Millipore (Bedford, MA, USA). pH measurements were made using an Orion model 420A pH meter (Thermo Orion, Beverly, MA, USA) with an Orion model 9157BN Triode pH electrode. The pH electrode was calibrated using certified Orion pH 7.00 and pH 10.01 buffers.

Table 2
0.1 M Buffer pH before and after methanol addition

0.1 M Buffer	pH Before	pH After	Difference
Glycine NH ₂ CH ₂ COOH	9.94	9.83	-0.11
Sodium borate Na ₂ B ₄ O ₇	9.93	10.17	+0.24
Sodium phosphate Na ₂ HPO ₄	10.00	10.65	+0.65
Sodium bicarbonate NaHCO ₃	10.00	11.40	+1.40

2.2. Procedure

The 0.10 M pH 10 carbonate buffer was prepared by adding 0.10 M sodium carbonate to 0.10 M sodium bicarbonate until pH 10.0 was reached. The 0.10 M pH 10 phosphate, borate, and glycine buffers were prepared by adding 1.0 M sodium hydroxide to solutions of the corresponding acids until pH 10.0 was reached and then the solution was diluted to volume to make the solution 0.10 M. The pH after dilution to volume was measured and this value tabulated in Table 2. The 50:50 methanol/water buffers were prepared by adding 10 ml of methanol to 10 ml of the 0.1 M pH 10 buffer. The vial was capped, mixed and allowed to set until it reached room temperature. Once at room temperature, the pH of the solution was measured and tabulated in Table 2.

3. Results and discussion

Table 2 shows the measured pH of the various buffers used in the dissolution study [1] before and after dilution with methanol. After adding methanol, the pH of the carbonate solution is considerably higher than the other buffers which is consistent with the very high rate of silica dissolution observed with this buffer. This solution is nearly 40 times more basic than the glycine buffer. The pH of the phosphate buffer is higher than the glycine and borate buffers, again in agreement with the observed relative dissolution rates. While the pH of the borate buffer is higher than that of the glycine buffer, the silica dissolution rate in these buffers is about the same. This result is not necessarily inconsistent with

the pH data. Below some pH, the silica will not dissolve at an appreciable rate and the observed dissolution rate will become insensitive to changes in buffer pH. We believe this is the case for the glycine and borate buffers.

These results do not rule out the possibility that the structure of the buffer anion has some effect on rate as proposed by the authors of the dissolution work [1]. Complexation between phosphate and silica was proposed but this hypothesis would not explain the even greater rate observed for the carbonate buffer. Rate studies where the buffers are at the same pH in methanol/water would need to be done to investigate potential anion structure effects. Until that data are available, it can be concluded that large changes in pK_a upon dilution with methanol explain part, if not all, of the differences in dissolution rate. Furthermore, the data suggest that phosphate may be no worse than amine buffers when the mobile phase pH is the same.

Phosphoric acid is often used to acidify mobile phases for the separation of acid compounds [8]. In the silica dissolution paper, the authors noted that phosphoric acid did not enhance the solubility of silica. When methanol, or acetonitrile, is added to an aqueous phosphoric acid solution, the phosphoric acid becomes a weaker acid and the hydrogen ion activity of the mobile phase will become smaller [5]. Therefore, if a column shows an acceptable acid stability in water at a given phosphoric acid concentration, it will be even more stable toward acid hydrolysis upon dilution with methanol or acetonitrile in agreement with the silica stability data [1].

These experiments show that it can be misleading to extrapolate aqueous pH data to partially aqueous solvents. The pH of the mixed solvent must be determined to reliably interpret column, or sample, stability studies in partially aqueous solvents.

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