

High Resolution Acid-Base Titration in Acoustic Dispensing

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Hypothesis

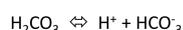
Distilled water contains dissolved CO₂ that is in equilibrium with CO₂ in the atmosphere. And it is well known that the CO₂ is in equilibrium with carbonic acid[1]. Adding phenol red to this distilled water indicates by color what the pH of the water is. This is because phenol red absorbs light in the wavelengths peaked at 443nm and 570nm based on a preliminary survey of the absorbance spectrum for phenol red. Although the change in absorption is not linear with respect to pH for the entire pH there is a range where the pH may be accurately determined.

The fundamental reactions in the experiment are understood[1,2]. The main question is: What is the best way to acquire accurate results from titration with phenol red indicator? Of course this experiment could be expanded to using other titrates and indicators depending on the reactions to be studied. However, as far as this reaction is concerned the pH range is approximately from 5.5 to 8.5 which are within the indicator range.

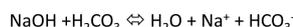
Therefore, the first reaction of concern is:



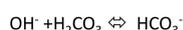
And the carbonic acid dissociates by:



And when adding NaOH, a strong base, to this solution it dissociates completely and the OH⁻ ions react with the H⁺ ions dissociated from the carbonic acid. Consider the following reaction:



The Na⁺ ions have the same concentration on both sides of the arrow because the sodium hydroxide is a strong base and dissociates completely in the aqueous solution we can write:



Where the equilibrium equation gives the OH⁻ ion concentration:

$$[\text{OH}^-] = \frac{K_b[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]}$$

By taking the log base 10 of both sides of the equation :

$$\text{pOH} = -\log\left(\frac{K_b[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-]}\right)$$

By realizing that the number of moles of HCO₃⁻ are a function of the number of OH⁻ ions introduced into the solution and the volume of solvent added we have:

$$[\text{HCO}_3^-] = \frac{(C_i V_i + C_{\text{NaOH}} V_{\text{NaOH}})}{(V_i + V_{\text{NaOH}})}$$

$$[\text{H}_2\text{CO}_3] = \frac{(C_a V_i - C_{\text{NaOH}} V_{\text{NaOH}})}{(V_i + V_{\text{NaOH}})}$$

By using pH = 14 - pOH and the initial concentration and volumes of the acid and ions in the distilled water we can calculate the pH of the water in the well. The initial concentration of both the carbonic acid and the dissociated ion form are known to be 15.6nM and 78nM respectively from the equilibrium condition of CO₂ in equilibrium with distilled water [2]. Therefore, the expected pH of the distilled water as a function of NaOH added may be seen in figure 1.

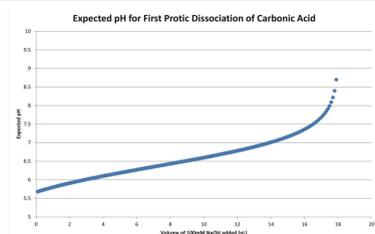


Figure 1 By using the known equilibrium constant for carbonic acid and the current concentration of CO₂ in the atmosphere the expected pH as a function of NaOH added is calculated.

Abstract

Acoustic dispensing is a tool for any chemist with the desire to accurately and precisely transfer liquids in the volume range between 1nL and 1μL. This experiment demonstrates the utility of the previous statement by performing a strong base-weak acid titration in the transfer volume range from one nanoliter to several microliters. We have chosen NaOH as our strong base. And we are using distilled water which contains CO₂ dissolved from the ambient atmosphere to become carbonic acid as the weak acid. The challenge faced is the rate at which CO₂ continues to be dissolved into solution during this measurement and converted to carbonic acid. This titration is performed with three concentrations of base in order to highlight the advantages and drawbacks of each choice available. Lower concentration requires higher volume titrate in order to complete the reaction which requires more time. While higher concentration depends on higher resolution in volume precision. This work demonstrates a new method of acoustic dispensing that incorporates the speed of dispensing large drops with a resolution of less than a nanoliter. By using the indicator phenol red, which has two wavelength absorption frequencies as a function of pH, we are able to resolve the acid-base reaction midpoint and endpoint for the first hydrogen ion dissociation.

Results

Figure 2 shows a titration curve obtained by dispensing 100mM NaOH into distilled water with phenol red indicator and the absorption of the 443nm and 570nm wavelengths were read. From this data the 570nm absorption line seems to have more dynamic range than the 443nm line. This curve tends to follow the expected pH curve shown in figure 1. However, the curve deviates from the expected curve after the 17nL volume added indicating another effect. The assumption for figure 1 is that the NaOH would neutralize the acid and then the pH would be a function of the OH⁻ ions remaining in the solution. There are two problems with this assumption. First, the acid is diprotic; therefore the second proton will begin to be dissociated after the first is consumed. Second, more CO₂ dissolved in the solution will convert to carbonic acid when the equilibrium is disturbed.

How do these effects reveal themselves in the data?

First, the shape of the endpoint of this curve suggests that H⁺ ions are not consumed as quickly as one might expect.

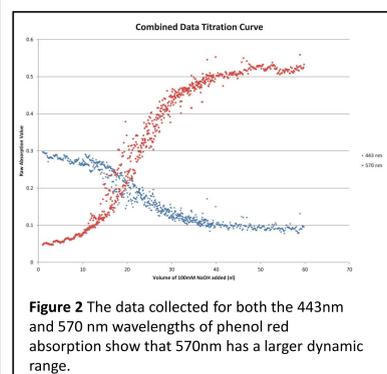


Figure 2 The data collected for both the 443nm and 570 nm wavelengths of phenol red absorption show that 570nm has a larger dynamic range.

Another way to ask this question is to consider doing the same experiment with three different concentrations of NaOH. In a practical sense this is exactly how chemists get around the difficulty in being able to measure small quantities of liquid. By using a lower concentration one is able to transfer the same amount of solute by measuring a larger volume of liquid. Therefore, by considering the concentration of NaOH initially introduced in the well the reaction should be the same and the phenol red absorption should be the same. Figure 3 shows this data.

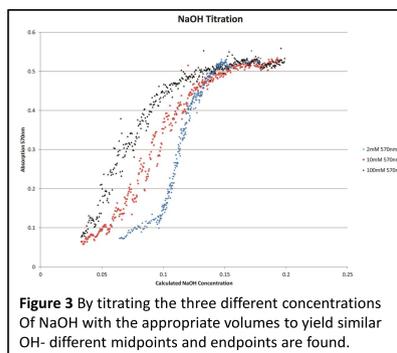


Figure 3 By titrating the three different concentrations of NaOH with the appropriate volumes to yield similar OH⁻ different midpoints and endpoints are found.

The data clearly shows that the reaction does not behave the same under the introduction of the same amount of NaOH. So, what is different? Besides using a different concentration of NaOH and a different volume of that solution the other factor different in each of these processes is the time required to dispense the given volume. The 2mM solution took over 27 minutes to dispense and 33:26 overall dispensing and reading. The 10mM solution took 4 minutes to dispense and 10:37 overall to dispense and read. And, the 100mM solution took 1:20 to dispense and 5:17 overall to dispense and read. Therefore it seems clear that the introduction of more H₂CO₃ into the solution clearly effects the ability to accurately measure the endpoint of this reaction. But how quickly is this reaction happening? Figure 4 shows the same titrated plate being read multiple times at approximately 3 minute intervals.

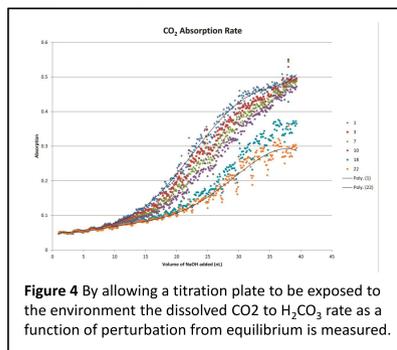


Figure 4 By allowing a titration plate to be exposed to the environment the dissolved CO₂ to H₂CO₃ rate as a function of perturbation from equilibrium is measured.

Analysis

Figure 4 shows that conversion of H₂CO₃ in the water is faster when the equilibrium is further from the equilibrium value. The effect yields a lower pH value reading than the initial value if it were read at exactly the dispense time. We should consider the conversion of CO₂ to cause a drift in the value of the absorption in the 570nm wavelength. If we know how strong this effect is, then can it be predicted? If it can be predicted then can an initial value be derived?

The interval between dispensing into each well and reading the data is derived from the dispense log and the plate reader logs. In the case of the 100mM NaOH dispense the total time from beginning of dispensing to end of reading took 5 minutes and 17 seconds. The dispense log records 80 seconds total dispense time. The plate reader recorded 86 seconds of read time leaving three minutes to collect the plate, spin and shake the solution. The total time for each well varied only slightly between 291 seconds and 298 seconds.

By using the data from the data sets labeled 1 minute and 7 minutes for the time difference the drift of the absorption line at 570nm is shown in figure 5.

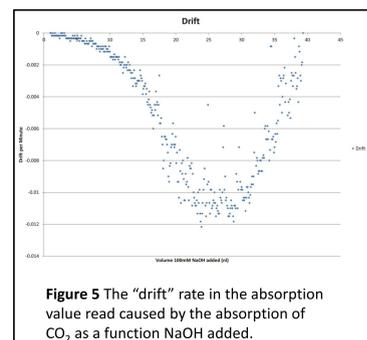


Figure 5 The "drift" rate in the absorption value read caused by the absorption of CO₂ as a function NaOH added.

The corrected titration curve in figure 6 is created by adding the drift to each point. One is able to determine the midpoint and the endpoint of the titration. The curve begins when no NaOH has been introduced and the value for the 570nm absorption is 0.05. The endpoint value for the 570nm absorption is 0.51. Therefore, the midpoint corresponds to the average of these two values or 0.28. The amount of 100mM NaOH required to reach the midpoint is 20nL and the amount required to reach the end point is 30nL.

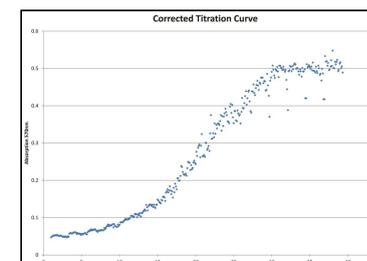


Figure 6 By using the rate of drift and the time between dispense and read the points are replotted as the initial value.

Materials:

- EDC BioSystems Gen5 ATS with *Volume on Demand*
- Thermo Multi Drop Combi bulk liquid dispenser
- BMG Labtech CLARIOstar plate reader
- Centrifuge
- Greiner 781 865 UV star plasma treated 384 microtiter plates
- Corning 3762 Microtiter Plates
- Four 50mL Falcon Tubes
- Phenol Red Indicator Solution
- 1 M NaOH Solution
- Distilled Water

Procedure:

- 1) Prepare three NaOH solutions (100mM, 10mM and 2mM) from the 1M solution by pipetting 36mL of distilled water into each of two 50mL Falcon Tubes, and 38mL into the third.
- 2) Pipette 4mL of 1M NaOH into the first tube, cap it and label it 100mM NaOH
- 3) Agitate the 100mM tube, replace into the rack and remove the cap. Pipette 4mL of 100mM solution into the next tube and label it 10mM.
- 4) Agitate the 10mM tube and pipette 2mL into the third tube. Label the third tube 2mM NaOH.
- 5) Prepare a fourth Falcon tube by filling it with 50mL of distilled water and adding 25 drops of phenol red to the solution. Agitate.
- 6) Create plate maps to dispense enough OH⁻ ions from minimal to enough to neutralize the acid in the plate plus more. This amounts to at least 20nL of the 100mM NaOH max, but we used 40 or 60nL as insurance. It would be 600nL for the 10mM and 3uL for the 2mM. By using the *Volume on Demand* method, volumes may be specified without regard to drop volume.
- 7) Prepare a Corning 3762 plate as the destination plate. Use the multi drop dispenser to fill the plate with the phenol red solution. Spin in the centrifuge for 30 seconds to remove bubbles.
- 8) Prepare a Greiner 781 865 plate as the source by pipetting 60uL into the well defined in the plate map as the source well.
- 9) Run the prepared destination plate in the reader set to read absorbance for the 443nm and 570nm wavelengths to establish a baseline.
- 10) Dispense NaOH into destination plate and read after 10 second centrifuge and 10 second shaking.
- 11) Collect data and record concentration associated with this run.
- 12) Repeat with next concentration.

Conclusion

Titration of high concentration titrates increases the speed of acoustic dispense titration and limits the effect of the environment on the product. *Volume on Demand* enables a resolution of 0.1nL and to dispense 384 unique individual volumes from 1 nanoliter to 39.4 nanoliters in 80 seconds. This dispense speed is actually faster than the reader. However, even with this speed, the total time between reading and dispensing is 4.85 minutes. This delay does distort the titration curve. By collecting additional reader data it is possible to reconstruct what the actual absorption reading would have been at the time of dispense.

References

- 1) Keenan, C.W.; Wood, J.H.; Kleinfelter, D.C. *General College Chemistry*, 5th ed.; Harper and Row: New York, NY, 1957; pp 353-358.
- 2) Brinkman, R.; Margaria, R.; Roughton, F.J.W. *The Kinetics of the Carbon Dioxide - Carbonic Acid Reaction*; The Philosophical Transactions of the Royal Society of London Series A, Vol. 232.; Harrison and Sons, Ltd. London 1933; pp 65-97.