Titration and pH Measurement

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University of California, San Diego
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University of California, San Diego
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Titration and pH Measurement

Patricia A Jennings, *University of California, San Diego, California, USA*

Christine A Mullen, *Texas A&M University, College Station, Texas, USA*

Melinda Roy, *University of California, San Diego, California, USA*

Based in part on the previous version of this Encyclopedia of Life Sciences (ELS) article, Titration and pH Measurement by Patricia A Jennings and Christine A Mullen.

Titration is the quantitative addition of a solution of known concentration to a solution of unknown concentration until the reaction between them is complete to determine the concentration of the second solution. An acid–base titration is the quantitative determination of the concentration of an acid or a base. Titration of an acid with a base requires that the pH, or relative concentrations of the two reactants, be monitored. pH can be assessed by litmus paper or by indicators, for example, phenolphthalein, but these methods lack precision. Typically, pH measurement in the laboratory is done by measuring the cell potential of that sample in reference to a standard hydrogen electrode. A plot of the pH of an acidic (or basic) solution as a function of the amount of added base (or acid) is a titration curve. From this, the endpoint or equivalent points can be determined.

Introduction

Acid–base titration is one of the oldest tools of analytical chemistry. It is the determination of the concentration of an acid or base by neutralising the analyte with an acid or base of known concentration. The method was first described by Glauber (1658). It called for potash – potassium carbonate from wood ashes – to be added to nitric acid until gas no longer evolved. We now know that this simple method was a reliable way to produce a solution with a hydronium (H_3O^+) ion concentration of $5.0 \times 10^{-5}\,mol\,L^{-1}$. Since that time, much effort has been directed at expanding the usefulness of acid–base titrations as well as the methods for H_3O^+ ion measurement.

A convenient scale, the pH scale, is now used to report the relative acidity and basicity of aqueous solutions. Briefly, pH is defined as $pH = \log_{10}\frac{1}{[H_3O^+]} = -\log_{10}[H_3O^+]$. **See also**: pH and Buffers

For a neutral solution at the standard state temperature of 25°C, the H_3O^+ ion concentration is 1.0×10^{-7} and the pH equals 7.0. Solutions with a pH less than 7 are acidic and solutions with a pH greater than 7 are basic or alkaline.

Many biochemical processes are pH-dependent and many enzymes perform proton transfers, that is, acid–base catalysis. Hence, accurate and precise measurement and control of pH is important. Many of the chemical reactions that occur in aqueous solutions either release or use protons, which then alter the pH value. For example, protein degradation increases basicity and carbohydrate metabolism increases acidity. With few exceptions, however, chemical reactions of living organisms must occur within a pH range of 6.9–7.5. Measurement of pH is therefore one of the most crucial and frequently used procedures in the life sciences. **See also**: Acid–Base Catalysis by Enzymes; Cell Biophysics; Organic Reaction Mechanisms

Outline of Methods

The pH value of a solution can be measured with a strip of litmus paper, or a chemical called an indicator or an instrument called a pH meter. Litmus paper indicates the presence of acids or bases by changing its colour to red or blue, respectively (**Figure 1**). Unfortunately, the red and blue coloration does not give any indication of the strength of the acid or base. Other, more sensitive types of test strips are impregnated with combinations of acid–base indicators that turn various colours as the pH changes. Although these 'universal' test strips give only approximate values, they also provide fast, convenient, portable and inexpensive indications of pH.

We are concerned with acid–base titrations, in which the concentration of an acidic (or basic) solution (the analyte) is determined by the delivery of a measured volume of base (or acid) of known concentration (the titrant; see **Figure 1**). The titrant must react rapidly and completely with the analyte and therefore is usually a strong acid or base. In
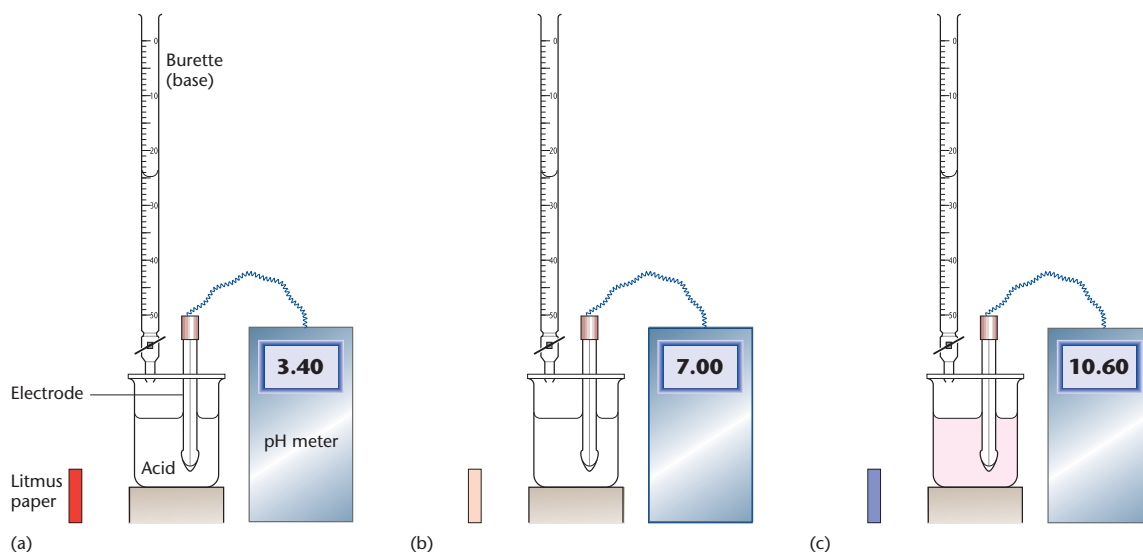
Figure 1 Comparisons of the pH measurement capability of litmus paper, the indicator phenolphthalein and a pH meter at different stages of a strong acid–strong base titration. (a) Just before the equivalence point, the litmus paper indicates that the solution in the beaker is acidic by its bright red colour. This conclusion is confirmed by the fact that the phenolphthalein added to the solution in the beaker is colourless. The pH meter indicates that the precise solution pH is 3.40, which is equal to a H_3O^+ concentration of 4.0×10^{-4} mol L^{-1}. (b) At the equivalence point, the litmus paper fails to display any appreciable colour change, suggesting a neutral solution; however, quantitation of the H_3O^+ concentration is problematic. As the phenolphthalein has not yet reached its colour transition range, and is therefore still colourless, it indicates that the solution is either acidic or neutral. Only the pH meter is capable of indicating that the solution is neutral (pH 7.0). (c) Just beyond the equivalence point, the litmus paper indicates that the solution in the beaker is basic by its bright blue colour. The phenolphthalein verifies the pH increase with a pink colour change. In theory, this is the titration endpoint. In practice, however, it is very difficult for the experimenter not to overshoot this point and add too much base. Thus, depending on the experimenter's dexterity, the pink colour may or may not denote the true end point. Finally, as it has done at each of the three titration points, the pH meter again measures and indicates the precise solution pH of 10.60, which is equal to a H_3O^+ concentration of 2.5×10^{-11} mol L^{-1}.

principle, the titrant is dispensed from a burette (a device for accurately delivering known volumes of solutions) until the amount added is chemically equivalent to the amount of analyte. This point is called the equivalence point. In practice, titration continues until the reaction between the acid and the base is judged to be complete by an indicator, a substance that changes colour near the equivalence point. The point at which the indicator actually changes colour is called the endpoint of the titration. The most common acid–base indicators are compounds that are themselves weak acids. They exhibit one colour as the weak acid and a different colour as the conjugate base. For example, phenolphthalein, a commonly used indicator, is colourless in its acidic form and pink in its basic form. The goal is to choose an indicator such that the endpoint occurs at the equivalence point. For a typical acid–base indicator, the colour transition occurs over a range of pH values given by $pK_a \pm 1$. pK_a is defined as $-\log K_a$, where K_a is the acid dissociation constant.

For more precise measurements, the pH meter is the instrument of choice. Most common pH meters provide values accurate to 0.01 pH unit. In addition, this method of pH measurement is not dependent on the ability to see colour. Measurement of pH with a pH meter is a simple process, but care must be taken to avoid errors and maintain reproducibility (Spitzer and Werner, 2002). A pH meter is a potentiometer used to measure the H_3O^+ concentration in solution. An electric potential is measured

that depends on the voltage difference between a reference electrode and a glass electrode that is sensitive to H_3O^+ concentration. A combination electrode, in which both the reference electrode and the glass electrode are incorporated into one slim tube, is now standard in research laboratories (see **Figure 1**). The glass electrode's membrane acts as if it is selectively permeable to H_3O^+, whereas other cations and anions are excluded. This permeability results in a potential across the membrane that is a linear function of the pH. The magnitude of the potential difference is measured with a voltmeter. Because of variations among individual electrodes, one must standardise the electrode and meter with solutions of known H_3O^+ concentration, as described, for example, in Naumann *et al.* (2002). For routine calibration, it is sufficient to use two buffer solutions, one with a pH above that of the sample solution and the other with a pH below that of the sample solution. In cases where high accuracy is required, multiple-point calibration with three or four calibration solutions is best. The most widely used pH calibration buffers for the biological sciences have pH values of 4, 7 and 10. The measured potential is also a function of temperature. For accurate pH measurement, the temperature compensation control on the meter must be adjusted to the temperature of the solution being measured. Once the meter has been standardised and readings are stable, the pH of a test solution can be measured. Errors in pH measurement may arise in the presence of high sodium ion concentration, at high ionic strength,
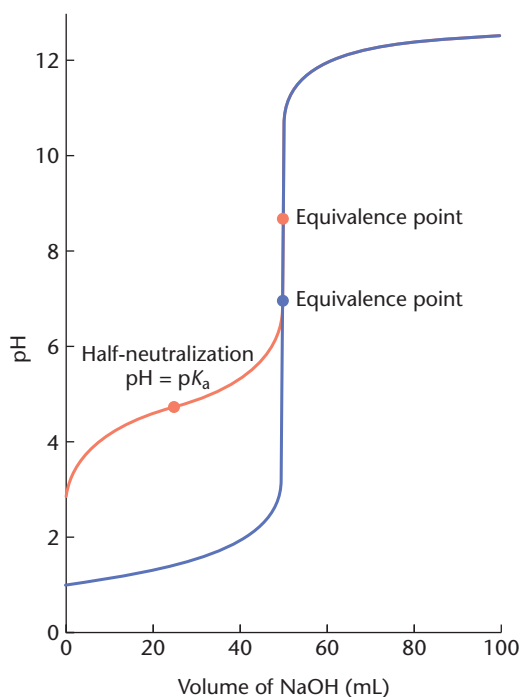
Figure 2 Curves for the titration of 100 mL of 0.1 mol L⁻¹ sodium hydroxide with a strong acid (100 mL of 0.1 mL⁻¹ hydrochloric acid, blue curve) and with a weak acid (100 mL of 0.1 mol L⁻¹ acetic acid, red curve). Note that the weak acid's pH changes least rapidly near the half-neutralisation point. Here, the concentration of weak acid approximates the concentration of its conjugate base, and the ability of the solution to resist pH changes, known as buffering, is optimal.

such as with ammonium sulphate solutions and with electrodes fouled by proteins or other biopolymers.

The progress of an acid–base titration is often monitored by plotting the pH of the solution being analysed as a function of the amount of titrant added. Such a plot is called a titration curve. The centre of the vertical region (inflection point) of the curve indicates the equivalence point (**Figure 2**). Multiple equivalence points will produce multiple inflection points in the titration curve. Titrations may also be monitored with instruments that respond to certain properties of the solution that change in a characteristic way during the titration. Spectroscopic methods are frequently employed to determine the global or local titration behaviour of biological molecules. Titrators that produce titration curves and measure the equivalence point automatically are commercially available.

Applications

Titration of strong acids and bases

The calculation of a titration curve requires the decoupling of the effects of reaction and dilution as in Macca and Solda (2002a, b). This is achieved by considering separately the change in the number of moles of acid or base and the change in volume. The concentrations of acid and base change because of both of these factors and cannot be used directly. In the region of excess acid, first the number of moles of acid present before neutralisation is determined. Then the number of moles of added base needs to be calculated. Finally, the pH is determined and the acid concentration as a function of added base volume is calculated. The calculation is repeated for a sufficiently large number of points to permit a smooth curve to be drawn.

As shown in **Figure 2**, the pH rises slowly with the amount of added base until the vicinity of the equivalence point is reached, where it rises sharply. As both acid and base are strong, the pH at the equivalence point is 7.0 and the curve is symmetric about this point. The curve for the titration of a strong base with a strong acid is just the reverse of the one obtained for the titration of a strong acid: the pH decreases with added acid and shows the same behaviour near pH 7.

Titration of weak acids and bases

The titration of a weak acid by a strong base requires a considerably more complicated analysis than the titration of a strong acid by a strong base, as in Macca and Solda (2002a, b). Understanding this type of titration is crucial, however, as many biologically important compounds contain weakly acidic and basic groups, and the response of these groups to pH changes is often of considerable importance to their function, for example, protein–ligand interactions and enzyme activity. The calculation of the titration curve is divided into four parts. Before the addition of base, the pH is that of the weak acid. In the region of acid excess, the equilibrium is that of a weak acid and its conjugate base. At the equivalence point, the equilibrium is that of the conjugate base. Beyond the equivalence point, the acid equilibrium no longer plays a role and the pH in that region is determined by the concentration of excess base. **See also**: Enzymes: The Active Site; Protein–Ligand Interactions: General Description

An acid–base buffer typically consists of a weak acid and its conjugate base. Acid–base buffers confer resistance to a change in the pH of a solution when hydrogen or hydroxide ions are added. For example, blood pH is largely maintained by the carbonic acid (HCO_3^-)/bicarbonate (H_2CO_3) balance. **See also**: Acid–Base Balance Disorders

Figure 2 also displays a titration curve resulting from such an analysis. The starting pH of the weak acid titration is higher (less acidic) than that of the strong acid titration, because of incomplete dissociation of the weak acid. Furthermore, the weak acid solution exhibits a much faster initial rise in pH than does the strong acid solution. After the initial rise, the curve features a long plateau, reflecting the formation of the weak acid–conjugate base buffer. Here, the concentrations of the weak acid and its conjugate base are both relatively large and their ratio changes little with addition of base. The pH in the middle of this region, the point halfway to the equivalence point, is equal to the pK_a of the weak acid. This fact is clearly demonstrated by the Henderson–Hasselbalch equation (eqn [1]), which

relates the ionisation constant to the pH of a solution of a weak acid.

$$pH = pK_\alpha + \log_{10} \frac{[A^-]}{[HA]} \qquad [1]$$

$[A^-]$ is the concentration of the conjugate base and $[HA]$ is the concentration of the weak acid. When $[A^-] = [HA]$, pH equals pK_a, because $\log_{10}([A^-]/[HA]) = \log_{10} 1 = 0$.

As the equivalence point is approached, the pH increases rapidly. The pH change near the equivalence point, however, is much smaller than in the case of the strong acid. Indeed, the weaker the acid being titrated, the smaller is the vertical region around the equivalence point. This allows much less flexibility in choosing the method of measurement. Furthermore, the pH at the equivalence point is greater than 7. This is because the conjugate base of the weak acid, which remains in solution at the equivalence point, abstracts hydrogen ions from water molecules to produce hydroxide ions. Beyond equivalence, the excess base represses that reaction, so that the titration curves for the weak and the strong acids become identical. The curve for the titration of a weak base with a strong acid has the same four regions as the curve for the titration of a weak acid with a strong base. The shape of the curve is the reverse of the one obtained with a weak acid, with the pH decreasing with the amount of added acid.

Future Developments

Different pH measuring problems require differing sensors (Vonau, 2010). Glass is a versatile structural material because of its chemical resistance and the ease with which it can be formed into complex shapes. The glass electrode can therefore be used without interference in solutions containing strong oxidants, strong reductants, proteins and gases. Consequently, glass electrodes have been prepared in almost every conceivable geometrical form and size for special applications. The current trend is towards smaller electrodes, capable of measurement in diverse sites such as tooth cavities, the stomach or single drops of sweat. Stomach probes, microcombination electrodes with diameters less than 3 mm, can be swallowed to indicate the acidity of the digestive tract. Short glass electrodes with electrodes less than 8 mm in length are used in medicine to continuously monitor patient carbon dioxide levels. It is also possible to draw membrane glasses into capillaries. These electrodes have approximate internal diameters of 1 mm and are suitable for use with samples of only a few microlitres. Ultramicro-glass electrodes are prepared as spheres 100–500 μm in diameter and reduce the required sample volume to 2 nL.

Ultramicroelectrodes with diameters down to 1 μm are used for the measurement of the pH inside a living cell. These electrodes cannot be manoeuvred manually and require the use of a micromanipulator. **See also**: Intracellular pH Measurement

The inherent disadvantage of the glass electrode is that it is breakable. Thus, future developments in pH measurement are directed at the development of more rugged materials that share the malleability, sensitivity and stability of glass. In addition, there is great need for even smaller pH electrodes, capable of measuring the solution pH of cellular compartments such as the nucleus, mitochondria and ribosomes in a dynamic fashion. The ideal pH electrode would be targetable to the lumen of an organelle and allow direct measurement of intraorganellar pH and analysis of pH regulatory mechanisms.

The invasiveness of the microelectrodes limits their application to larger cells. Other techniques are being developed to determine intracellular pH and study its regulation, including nuclear magnetic resonance (NMR) and fluorescent spectroscopic methods. **See also**: Intracellular pH Measurement

Summary

pH titration is an old analytical method that has expanded its usefulness to the life sciences. The goal of an acid–base titration is to determine the volume of a liquid acid (or base) of known concentration required to react completely with a base (or acid). The pH of a solution can be measured using indicators, litmus paper or, most accurately, a glass electrode and pH meter. A plot of the pH of an acidic (or basic) solution as a function of the amount of added base (or acid) is a titration curve. Titration curves are of two varieties: those for strong acids and strong bases, and those for weak acids and weak bases. Life scientists are more concerned with the behaviour of weak acids and bases as they are common in biological systems and play important roles in metabolism and regulation. Future developments in titration and pH measurement concern the construction of more durable electrodes capable of measuring dynamic pH changes at the subcellular level.

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