

CHAPTER 4

MEASUREMENT OF pH AND ACIDITY

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CHAPTER 4

MEASUREMENT OF pH AND ACIDITY

4.1 INTRODUCTION

Assuring that the pH is 4.6 or lower in every part of a food within each container is a critical requirement in the production of safe acidified foods. An essential part of assuring pH control is the accurate measurement of the finished **equilibrium pH** of products. In addition, it may be useful to determine the pH of ingredients because use of ingredients that are in the correct pH range may be necessary to get the correct finished equilibrium pH. The measurement of pH is, in most cases, a simple process. It consists of putting a pH electrode into a sample and then reading the pH displayed on the pH meter. However, specific steps must be taken to be sure the pH meter is operating correctly, that a proper sampling procedure is used, and that appropriate records are maintained.

Methods for determinations of pH and acidity for acidified foods are described in 21 CFR Section 114.90.

4.2 SAMPLE PREPARATION

For any analytical measurement taking a representative sample for analysis is a critical step. If a non-representative sample is used or a sample is not properly prepared, results of testing may be meaningless. Remember the following key points:

- (1) Most foods are mixtures of solids and liquid. In an acidified food it is essential that all water-containing parts of a food, both solids and liquids, have a pH of 4.6 or lower. Therefore, in some cases the liquid and solid parts of a food must be separated, weighed, and the pH determined separately. The final equilibrated pH can be determined by measuring the pH of a mixture of liquid and slurry of the solid part of the food mixed in the same proportions as was present in the product.
- (2) For acidified products, like cucumber pickles, where pH equilibration occurs readily, the equilibrated pH can be determined simply by blending the entire contents of a jar and then measuring the pH of the slurry.

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- (3) In marinated products that contain oil, the oil can interfere with pH and acidity determinations. For products of this type the oil should be separated and removed before measuring pH on the water phase of the product.
- (4) The pH meter should be standardized when the electrodes and standardization buffers are between 68 and 86°F (20-30°C). Ideally, the temperature of samples should be the same as the temperature of the buffer solutions used to standardize the pH meter. Therefore, samples that are hotter or colder than the standardization temperature should be allowed to equilibrate to that temperature before the pH is measured.
- (5) If a food sample is difficult to blend, distilled water up to 20 percent of the weight of the sample can be added to make blending easier. Addition of small amounts of distilled water to food samples does not cause significant changes in pH.

4.3 pH DETERMINATION USING A POTENTIOMETRIC METHOD

Potentiometric pH determinations are done with a pH meter. This is the preferred way to do pH measurements of foods. A variety of pH meters is available, from a small hand unit not much larger than a pen to a complex bench-top unit suitable for research purposes (Fig. 4.1). pH meters may have an analog (pointer dial) or, more commonly today, a digital readout. The cost can vary from less than \$50 to \$4,000. However, all pH meters function in a similar way and require similar care and attention to assure that they function properly. The first rule for maintaining a pH meter in good working condition is to follow the manufacturer's instructions for maintenance and storage of both the instruments and electrodes.

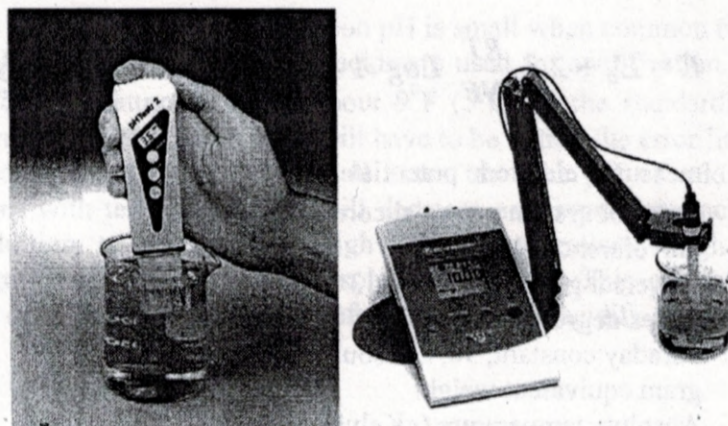


Fig. 4.1 Hand-held and bench-top digital pH meters.

pH meters measure an electrical potential difference in millivolts between a reference electrode and a measuring electrode (Fig. 4.2). This millivolt reading is automatically converted to a pH value on the instrument display. As expected from the discussion of the pH scale in the previous chapter, the pH scale goes from 0 to 14 pH units. The reason pH meters work is that there is a linear relationship between pH and the potential difference measured by the pH electrodes.

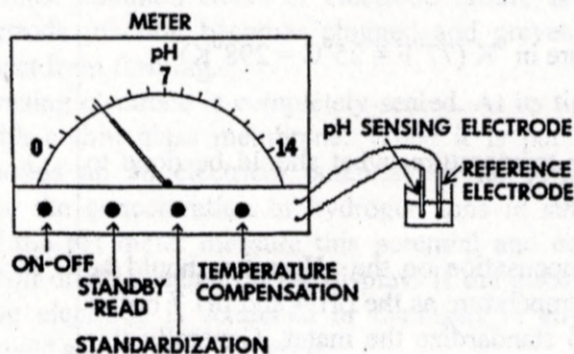


Fig. 4.2 Schematic drawing of an analog pH meter with reference and sensing electrodes.

The Nernst equation is the fundamental equation for the potentiometric measurement of any ion. This is *not* to be memorized. It is here for reference and to notice the **T** in the equation. The **T** means that pH measurements will be affected by temperature and that you need to compensate appropriately for temperature as you make pH measurements

Nernst equation $E = E_o + 2.3 \frac{RT}{NF} \text{Log } A$

Notes

Where:	E	=	measured electrode potential
	E _o	=	constant system potential corrected for by the reference electrode
	R	=	universal gas constant, 8.313 joules/degree/g-mole weight
	F	=	Faraday constant, 96,490 coulombs per gram equivalent weight
	T	=	Absolute temperature (° Kelvin)
	N	=	Charge of the ion
	A	=	Activity of the ion being measured

Most pH meters are designed so that a pH 7.00 buffer gives a 0 millivolt reading. pH values below 7 will give positive millivolt readings, while pH values above 7 will have negative millivolt readings. On the pH scale of the pH meter the millivolt reading that a sample gives is automatically converted to a pH value using the following equation.

$$pH = 7.00 - \frac{(mV)}{(0.1982 XT)}$$

mV = millivolts

T = temperature in °K (77°F = 25°C = 298°K)

Since pH is affected by temperature, what should be done to properly account for temperature?

- (1) Temperature compensation on the pH meter should be set at the same temperature as the pH 4 and pH 7 buffer solutions used to standardize the meter. Generally this should be done near 77°F or 25°C.
- (2) The temperature of the sample should be equilibrated to the same temperature as the standardizing buffers before measuring pH.

The effect of temperature on pH is small when common organic acids like acetic, lactic or citric acids are used for acidification. If the sample temperature is within about 9°F (5°C) of the standardization temperature, pH measurements will have to be within the error limits of the standard buffers. To the small extent that the pH of acid foods changes with temperature, pH will decrease as temperature increases. In addition to temperature, high salt concentrations (high ionic strength) can lower the pH of solutions slightly. This pH lowering effect of salt is too small to be useful as a means to acidify products.

4.4 pH ELECTRODES

Historically it was necessary to use two different electrodes to make a pH measurement. One electrode is the pH sensing electrode. The other is the reference electrode. Both electrodes must be placed in a sample at the same time to do a measurement. Today combination electrodes are usually used. The two separate electrodes are still there, but they are incorporated into a single glass or plastic electrode body.

The reference electrode contains a specially prepared metal wire immersed in a concentrated solution of potassium chloride. There is a porous ceramic or fiber junction which looks like a rough spot on the side of a combination electrode. This junction must be immersed in the sample and not clogged so a very slow flow of potassium chloride solution goes into the sample as a measurement is made. This establishes an electrical contact between the reference electrode and sample. The most common cause of electrode failure is when this reference electrode junction becomes clogged and prevents a stable electrical contact from forming.

The sensing electrode is completely sealed. At its tip there is a glass bulb with a thin glass membrane. When it is put in a water solution, it builds up an electrical potential on its surface that is proportional to the concentration of hydrogen ions in solution. The electronics of the pH meter measure this potential and convert it to either a millivolt or pH reading on the display. If the glass membrane on the sensing electrode is scratched or damaged in any way, the electrode is ruined and must be replaced.

Manufacturers of both pH meters and pH electrodes provide specific instructions for the care and maintenance of their products. These specific instructions should be followed. Since pH meters are electronic devices, they are subject to corrosion, especially in humid, acid atmospheres, as is the usual situation in a plant processing acidified foods. Therefore, an effort should be made to keep them in a room with low humidity, off the processing floor. Should on-line measurements be required, unbreakable epoxy, plastic, or ceramic electrodes should be used. Electrodes are kept immersed in a solution when they are not being

used. Some electrode manufacturers recommend storage in pH 7 standard buffer, others in pH 4 buffer, and still others in dilute hydrochloric acid solution. Use the storage solution suggested by the electrode manufacturer. Electrodes should not be left to dry out in the air for more than a few minutes.

Notes

4.5 STANDARDIZATION

It is necessary to standardize a pH meter to get an accurate pH measurement. A pH meter should be standardized at least once a day. The pH of pH 4.0 standard buffer can be checked as often as necessary to ensure accurate readings. If the pH measurement on the standard buffer deviates significantly from pH 4.0, the meter should be re-standardized. When samples contain oil or grease which can coat the electrodes, standardization should be done every two or three samples. Finally, in a situation where the objective is to have an equilibrated pH near the critical pH of 4.6, the pH meter should be standardized after each sample.

Standardization is normally done for acidified foods with pH 7.0 and pH 4.0 buffers. This gives an accurate reading within the pH region of interest, pH 4.6. Then the pH of 9.18 standard buffer should be measured. If the electrodes and pH meter are working properly, the pH reading on the pH 9.18 buffer should be between pH 8.88 and pH 9.48. The electrodes and meter should be checked out according to the manufacturer's instructions if the pH reading is outside this range.

Due to the effect of temperature on pH discussed earlier, the temperature of the standard buffer solutions should be the same as the temperature of the samples that are to be measured. If a magnetic stirrer or other type of stirring device is used to mix samples while pH measurements are done, the standard buffers should be stirred in the same way during standardization. Continuous monitoring of pH is required by FDA regulation; therefore, a backup electrode and sufficient standard buffers should always be available.

4.6 pH MEASUREMENT

To make a pH measurement, thoroughly rinse the electrode with distilled water using a squirt bottle, or with some of the next sample. Excess water on the electrode can be removed by blotting, *not wiping*, with a soft tissue paper. Wiping the tip of the sensing electrode even with a soft tissue can damage the hydrogen ion sensitive glass membrane. If sufficient sample is available, the electrode may be rinsed in the next sample instead of rinsing with water. The rinsed electrode is placed in the sample deep enough so the reference electrode junction is immersed in the sample. It should take less than one minute (usually 20 to 30 seconds)

for the pH reading to stabilize. If it takes much longer for stabilization to occur, the electrode may have become fouled with fat or protein from previous samples or the electrode may be permanently damaged. If it is fouled, the electrode response may be restored by *gently* wiping the tip with a tissue saturated with 75 percent methanol solution or soak the electrode for about five minutes in 0.1N HCl, rinse with water, and then let it soak overnight in buffer solution. Again, manufacturer's instructions should be followed for the specific electrode you are using. Once the pH meter reading stabilizes, record the pH. Remove the electrode from the sample, rinse, and continue on to the next sample.

Notes

4.7 COLORIMETRIC ESTIMATION OF pH

Section 114.90 (b) of the Good Manufacturing Practice (GMP) states that a colorimetric method may be used for pH determination if the pH is below 4.0. This means that a processor is not required by the regulation to measure pH with a pH meter if high acid products with an equilibrated pH lower than 4.0 are being produced.

Today, inexpensive, pocket-size pH meters are available in the range of \$50 to \$100. Even the least sophisticated of these meters, which only allow pH readings to be made to the nearest 0.1 pH unit, are preferable to pH paper or indicator dyes. So, even though the regulations allow colorimetric techniques to be used, a pH meter is strongly recommended.

4.7.1 Indicator Paper

Indicator paper (or pH paper) is a colorimetric technique that may be used. Rolls or strips of pH paper for use in the range of pH 3.0 to 5.0 are available from scientific supply companies. The strip or paper is dipped into the sample. Color develops immediately. The color is compared to color standards which come with the pH paper. Care must be taken to be sure the pH paper is stored so it is not exposed to acid vapors or constant light. If highly colored samples are used, it will be necessary to show that the color of the sample does not affect the color comparison.

4.7.2 Indicator Solutions

In addition to pH paper, solutions containing pH sensitive dyes are available commercially. Sample liquid is mixed with the indicator solution in test tubes or on spot plates. The color changes immediately in response to the pH of the sample. The pH is estimated by comparison with color standards. Bromphenol blue is recommended as a suitable dye for the pH 3.0 to 4.6 range. Again, it must be emphasized that use of this

colorimetric method to control pH is only allowed for products with an equilibrium pH below 4.0.

Notes

4.8 TITRATABLE ACIDITY

The discussion in the chapter on pH and acidity emphasizes that *pH and titratable acidity are not the same*. pH is a measure of the amount of free hydrogen ions in a solution. Titratable acidity is a measure of both bound and free hydrogen ions in a solution. It is measured by titration of acid in the food with standardized NaOH solution. There is no fixed relationship between pH and titratable acidity in a food. However, experience has shown that titratable acidity can be relied upon as an indicator that pH is no higher than some maximum value for a particular product formulation. This relationship must be established by experience for the particular ingredients and the way in which they are used. For this reason, the regulation allows use of titratable acidity measurements to control a process and for documentation of a final maximum pH if the equilibrium pH of a product is below 4.0.

Even if pH measurements with a pH meter are routinely done, titratable acidity is an important analytical method to assure that ingredients contain the expected amount of acid. Final products may have too much, as well as too little, acid present. Both situations may indicate a problem with product quality. Measurement of titratable acidity can be a very useful way to detect a problem in either direction.

4.8.1 Principle of the Measurement

Titratable acidity is a measure of all the available hydrogen ions in a solution, both those which are free in the solution and those bound in organic acid molecules. A solution of a strong base, NaOH, of known concentration is added to a sample as the sample is stirred. The hydroxyl ions from the base react with free hydrogen ions to make water. As this occurs, bound hydrogen ions are released. The pH of the solution gradually rises as the bound hydrogen ions are released and react with hydroxyl ions. When all bound hydrogen ions have reacted with hydroxyl ions, only a few free hydrogen ions remain in the sample. When a little more NaOH is added to react with the remaining hydrogen ions, the pH rises quickly. The end point of the titration is considered to be reached when the pH of the sample rises above 8.2. The usual way to determine the titration end point is by addition of a few drops of phenolphthalein solution (1 percent phenolphthalein in 70 percent ethanol) to the sample before beginning the titration. Phenolphthalein is a pH indicator dye that changes from colorless to pink at pH 8.2. Therefore, when a pink color first becomes visible throughout the sample and persists for 30 seconds,

titration is finished. Since pH 8.2 is the end point of a titration, a titration can also be done while the pH of a solution is being measured with a pH meter. The titration is complete when the pH meter reads 8.2.

Notes

4.8.2 Standard Sodium Hydroxide (NaOH) Solution

To calculate the percent acid in a sample from a titration, it is necessary to know three things: (1) the exact concentration of standard NaOH solution used for the titration; (2) the volume or weight of sample that is titrated; and (3) the volume of NaOH solution required to reach the titration end point, as determined by formation of a pink color from phenolphthalein or getting a pH 8.2 reading on a pH meter. A solution of NaOH of accurately known concentration that is used for titration is called a **standard solution**. The concentration of a base solution used to do titrations is expressed in terms of **normality (N)**. The normality of an NaOH solution is equal to the moles per liter of hydroxyl ions the solution contains that are available to react with hydrogen ions in the samples. Table 3.1 shows the molecular weight of NaOH is 40. Since each NaOH molecule has only one hydroxyl group, a solution that contains exactly 40 grams of NaOH per liter (a 1 molar solution) has exactly 1 mole of hydroxyl groups per liter, so it is a 1.00 normal solution. This is commonly written as 1.00N. It is usually convenient to titrate food samples with 0.10N NaOH solution. To make this solution, it is necessary to accurately add 4.00 g of NaOH into water to make exactly a liter of solution.

Unfortunately, there are some practical problems with making and using standard NaOH solutions. It is impossible to buy crystalline NaOH of sufficient purity to directly weigh out dry NaOH, dissolve the correct amount in water, and use it to make accurate titrations. The reason for this is that dry NaOH very easily absorbs both water and carbon dioxide from the air. Absorbed water changes the weight of the NaOH, making it difficult to prepare a standardized solution. Carbon dioxide gas can also react with NaOH pellets or solutions to make sodium carbonate, which reduces the amount of NaOH available for titration. Due to these problems, special procedures are required to prepare standard NaOH solutions suitable for doing accurate titratable acidity measurements. Procedures for making standard NaOH solutions are given in the 15th edition of *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)*, Volume 1, 1990, section 936.16, pages 644-645. Standard solutions of NaOH ready for use for titrations can be purchased from chemical suppliers.

4.8.3 Sample Preparation

Sample collection and preparation is the same for titratable acidity determinations as for pH measurements. Care must be taken to obtain samples that are representative of the product.

Notes

4.8.4 Titration Procedure

An accurately measured amount of sample is put into a beaker. If the sample is liquid, it can be pipetted into the beaker with a properly calibrated pipette. The sample volume is recorded. If the sample is a slurry that cannot be accurately pipetted, it can be added to the beaker and the exact weight recorded. Addition of distilled water to a sample after it is measured does not interfere with titration. It may be useful to add water to make a slurry easier to stir during titration, or to dilute a colored sample so it is easier to see the pink color change when phenolphthalein is used as the end point indicator. If samples are so highly colored that it is not possible to see the color change at the end point of the titration, a pH meter can be used to determine when the pH rises to 8.2.

While the sample is being stirred with a magnetic stirring bar, standard NaOH solution is added from a burette calibrated in milliliters. When the end point of the titration is reached, the volume of NaOH solution required to reach the end point is recorded.

4.8.5 Calculation of Titratable Acidity

The **percent (%) acid** in a sample is calculated as grams of the predominant acid per 100 grams or 100 milliliters of sample. The equation to calculate titratable acidity is as follows:

$$\% \text{ acid} = \frac{N \times V \times M}{S \times 10}$$

Where:

N	=	Normality of standard NaOH solution used for titration
V	=	Volume of standard NaOH used for titration in milliliters
M	=	Molecular weight of the predominant acid in the sample divided by the number of hydrogen ions in the acid molecule that are titrated
S	=	Sample size in milliliters or grams

(Note:

The 10 is in the equation because the result of calculating $(N \times V \times M)/S$ is grams of acid per 1000 milliliters or 1000 grams of sample. Because percent is grams per 100 milliliters or grams per 100 grams of sample, the result of those four terms must be divided by 10 to get percent acidity.)

Notes

Values for **M** in the titration equation for common acids are 60 for acetic acid (vinegar), 90 for lactic acid, and 64 for citric acid. The molecular weight of citric acid is 192, but a citric acid molecule has 3 titratable hydrogen ions, so 192 is divided by 3 to give 64 for **M**.

Let's calculate two examples.

- (1) The titratable acidity expressed as percent acetic acid of a brine from fresh-pack cucumbers acidified with vinegar is to be determined. Titration of a 20-milliliter brine sample required 30.0 milliliters of 0.100N NaOH solution to reach the end point of the titration. Calculate the percent acetic acid in the brine.

$$\text{acetic acid (\%)} = \frac{0.100 \times 30.0 \times 60}{20.0 \times 10} = 0.9\%$$

- (2) What is the percent citric acid of a thick salsa where tomatoes and peppers are the predominant acid ingredients? Titration of 11.2 grams salsa slurry required 18.3 milliliters of 0.135N NaOH solution to reach the titration end point.

$$\text{citric acid (\%)} = \frac{0.135 \times 18.3 \times 64}{11.2 \times 10} = 1.41\%$$

4.9 SUMMARY

- (1) Procedures for preparing samples and measuring pH and acidity are specified in Section 114.90 of GMPs. These should be followed carefully.

Notes

- (2) All aqueous phases of acidified foods must be at pH 4.6 or below. Therefore, solid and liquid phases may need to be sampled separately for pH determinations.
- (3) The pH meter is a potentiometer which measures a potential difference between the measuring electrode and the reference electrode.
- (4) Temperature affects pH measurements. Therefore, the temperature of standard buffers and samples should be the same.
- (5) The pH-sensing electrode is the glass electrode. The tip of this electrode is made of very thin pH-responsive glass.
- (6) The other electrode is a reference electrode. It is filled with a concentrated solution of potassium chloride. It has a small porous junction which must remain open to allow a small amount of the potassium chloride solution to flow into the sample.
- (7) Combination electrodes have both electrodes in the same body.
- (8) Electrodes and pH meters should be maintained according to manufacturer's recommendations.
- (9) Electrodes should be handled with care and kept immersed in a liquid when not in use, as recommended by the manufacturer.
- (10) Electrodes should be rinsed between samples with distilled water and blotted dry, or rinsed with the next sample.
- (11) When glass electrodes respond too slowly, they may be cleaned by wiping them carefully with tissue paper saturated with methanol, immersing in 0.1 M HCl, rinsing with water, and soaking in storage solution overnight.
- (12) The pH meter must be standardized frequently using two buffers. For acidified foods that are to be equilibrated close to pH 4.6, it may be necessary to check a pH meter using one buffer after each sample.
- (13) If a stirrer is used while measuring pH, it should also be used during standardization of the pH meter.
- (14) The finished equilibrium pH of acidified foods with an equilibrated pH less than 4.0 may be measured by potentiometric methods, by use of colorimetric techniques such as pH indicator paper or dye solutions, by titratable acidity, or by any other suitable method. However, use of a pH meter is preferred.
- (15) Standard solutions of NaOH used for measurement of titratable acidity may be purchased commercially or prepared using official procedures. NaOH solutions need to be

protected from constant exposure to air because they absorb carbon dioxide to form sodium carbonate.

- (16) If a sample is highly colored so that the formation of a pink color from phenolphthalein pH indicator dye cannot be clearly seen, a pH meter should be used to measure the titration end point at pH 8.2.

Notes