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pH Measurement

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The measurement of pH is arguably the most widely performed test in the chemical laboratory, reflecting the importance of water as a ubiquitous solvent and reactant. In the 90 years since the first use of an electrode to determine hydrogen ion concentration, the glass electrode and its variants have matured into routine tools of analytical and process chemists. Yet, there continue to be developments that promise to broaden the scope and reach of these measurements. Among recent developments are miniature pH-sensitive field-effect transistors (pHFETs) being incorporated into pocket-sized pH “pens,” metal/metal oxide pH sensors for measurements at high temperatures and pressures, and flexible fiber-optic pH sensors for measuring pH within the body. This chapter discusses electrochemical and optical methods for pH measurement, and is by necessity limited in scope; readers interested in a more comprehensive treatment may wish to refer to the recent text by Galster [1].

71.1 Definition of pH

In its most common interpretation, pH is used to specify the degree of acidity or basicity of an aqueous solution. Historically, pH was first defined as the negative logarithm of the hydrogen ion concentration (*pondus Hydrogenii*, literally hydrogen exponent), to simplify the handling of the very small concentrations (on the order of 10^{-7} moles liter⁻¹) encountered most commonly in nature. This definition of pH is expressed as Equation 71.1, where $[H^+]$ is the molar concentration of solvated protons in units of moles per liter.

$$\text{pH} = -\log[H^+] \quad (71.1)$$

As a greater understanding of the behavior of ionic solutes in solution developed, chemists recognized that the measurement techniques used to determine hydrogen ion concentration were in fact measuring the hydrogen ion activity, often referred to as the “effective concentration.” This led to the adoption of the more rigorous definition of pH as the negative logarithm of the hydrogen ion activity in solution,

$$\text{pH} = -\log a_{H^+} = -\log \gamma[H^+] \quad (71.2)$$

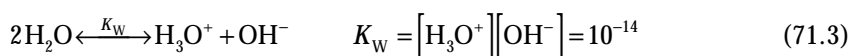
TABLE 71.1 pH of Common Substances

Substance	pH
Soft drinks	2.0–4.0
Lemon juice	2.3
Vinegar	2.4–3.4
Wine	2.8–3.8
Beer	4.0–5.0
Cow's milk	6.3–6.6
Pure water	7.0
Blood	7.3–7.5
Sea water	8.3

Note: D. R. Lide (Ed.), *CRC Handbook of Chemistry and Physics*, 74th ed., Boca Raton, FL: CRC Press, 1993.

where a_{H^+} is the hydrogen ion activity and γ is the activity coefficient. The two definitions expressed in Equations 71.1 and 71.2 are equivalent in dilute solution where concentration approximates activity, a_{H^+} . In practice, the routine measurement of pH is not accomplished by the direct determination of the hydrogen ion activity. Rather, pH is determined relative to one or more standard solutions of known pH.

The hydrogen ion activity of common substances ranges over many orders of magnitude, as evidenced from the tabulation presented in Table 71.1. Water, in the absence of other chemicals that might alter its equilibrium, contains 10^{-7} molar hydronium ions $[\text{H}^+]$ and 10^{-7} molar hydroxonium ions $[\text{OH}^-]$ at 25°C . Under these idealized conditions, water has a pH of 7.0, and is said to be neutral.



The addition of acids or bases to pure water increases or decreases the hydrogen ion activity, respectively. The resulting pH depends on a number of factors, such as the concentration of the added acid or base and the strength as quantified by its dissociation constant [2].

71.2 Electrochemical Methods of pH Measurement

Electrochemical measurement of pH utilizes devices that transduce the chemical activity of the hydrogen ion into an electronic signal, such as an electrical potential difference or a change in electrical conductance. The following sections review electrochemical pH measurement, with emphasis on the glass membrane electrode. Also discussed is the operation of hydrogen ion-selective pHFETs, metal/metal oxide electrodes, and other approaches used in specialized applications. Table 71.2 provides a listing of sources, features, and key properties of selected, commercially available pH electrodes.

The Glass Membrane Indicator Electrode

The most widely used method for measuring pH is the glass membrane electrode. As illustrated schematically in Figure 71.1(a), a pH meter measures the electrical potential difference (voltage) that develops between a glass membrane pH *indicator* electrode and a *reference* electrode immersed in the sample to be tested. The indicator and reference electrodes are commonly combined into a single, functionally equivalent, probe, referred to as a combination electrode. The glass membrane of the indicator electrode develops a pH-dependent potential, as a result of ion-exchange between hydrogen ions in solution and univalent cations in the glass membrane. The sensitivity of the glass electrode membrane potential to changes in pH is small, so a suitably designed reference electrode and a high input impedance meter are required in order for the potential to be precisely measured.

The construction of a typical pH indicator electrode is illustrated in Figure 71.1(b). The glass membrane at the tip of the electrode, which acts as the transducer of pH, is approximately 0.1 mm thick. One

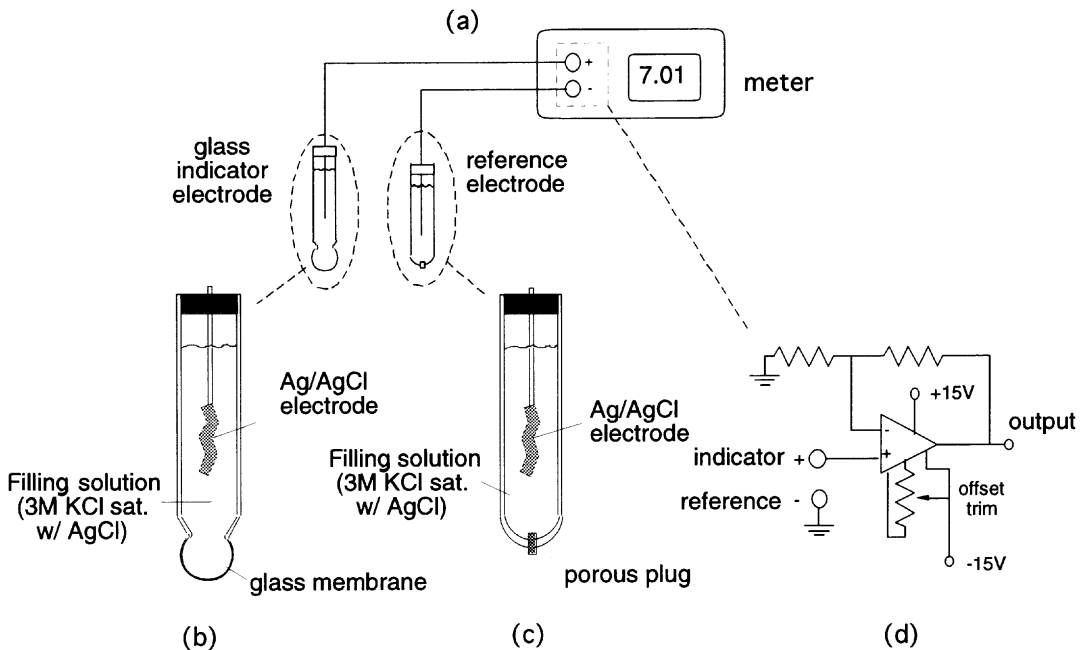


FIGURE 71.1 pH measurement using a glass membrane electrode: (a) measurement system comprising a pH meter, indicator, and reference electrodes; (b) indicator electrode construction; (c) reference electrode construction; and (d) amplifier circuit.

side of the membrane contacts the sample while the other contacts the electrode filling solution, an electrolyte of defined composition and pH. A reference element (e.g., a silver chloride coated silver wire) immersed in the filling solution makes a stable electrical contact between the potential measuring circuitry of the pH meter and the electrolyte in contact with the inner side of the glass membrane.

The reference electrode illustrated in Figure 71.1(c) provides a means of making electrical contact between the meter and the sample that is in contact with the external side of the indicator electrode's pH-sensitive glass membrane. Like the indicator electrode, the body of the reference electrode is filled with an electrolyte into which a reference element is immersed. The electrode also has a means, such as a porous ceramic frit, by which the reference electrode filling solution can make physical and electrical contact to the sample. The importance of this "liquid junction" cannot be understated, as it is a potential source of sample contamination, measurement errors, and reliability problems.

The electrical potential difference between the pH indicator electrode and the reference electrode provides a measure of pH. Figure 71.2 provides an illustration of the potential differences, the origin of which will be discussed below, that contribute to the measured potential. The main function of the pH meter of Figure 71.1(a) is to measure this difference, with a precision of 0.1 mV or better. The amplifier circuit of Figure 71.1(d) illustrates how this may be done. Due to the high electrical resistance of the indicator electrode's glass membrane, the meter must have a correspondingly high input impedance. Most pH meters currently sold contain built-in microprocessors that simplify pH measurement by performing and storing calibrations, doing diagnostics, and implementing temperature compensation.

Glass Membrane Indicator Electrode Construction

The hydrogen-ion selective glasses used to construct pH indicator electrodes are formed by fusing silica, alkali metal oxides, and alkali earth oxides. The silica component, SiO_2 , makes up approximately 70% of the glass. The alkali metal oxide contributes mobile ions such as sodium or lithium, which act as electrical charge carriers, or are exchanged for protons in the hydrated glass layer. Components such as calcium oxide (CaO) are added to incorporate multivalent ions into the glass; these act to modify the network structure of the glass, imparting characteristics such as processability and chemical resistance.

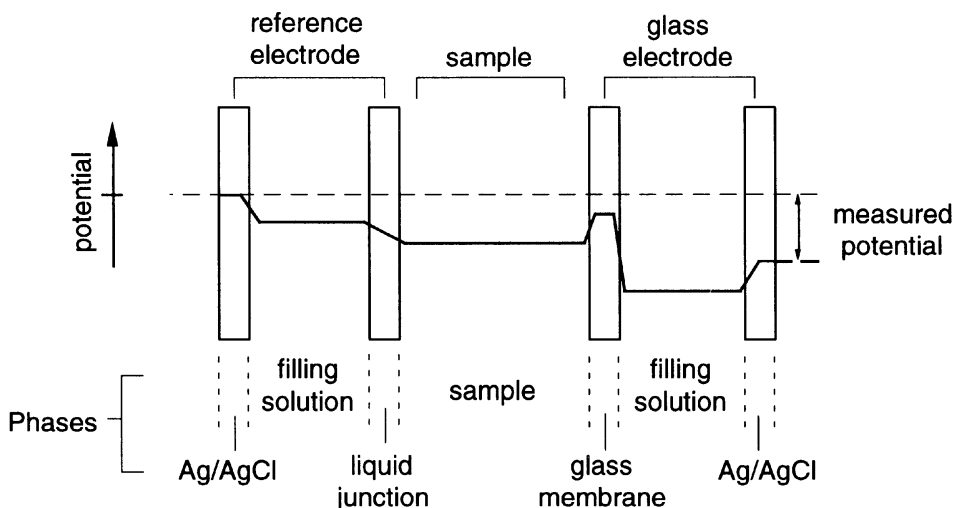


FIGURE 71.2 Schematic illustration of potential differences contributing to the measured potential between a glass membrane electrode and a reference electrode.

Glass Membrane Indicator Electrode Response to pH

When the membrane glass is immersed in aqueous solution, a gel-like hydrated layer on the order of 100 nm thick forms at the surface of the glass. Within this hydrated layer, the exchange of sodium ions in the glass for hydrogen ions in the solution produces a potential difference between the hydrated layer and the solution which depends on the hydrogen ion activity. The net potential developed across the entire glass membrane, ϕ_{net} , is the sum of boundary potentials, ϕ_b , at the inner (reference element side) and outer (sample side) extents of the membrane.

$$\phi_{\text{net}} = \phi_{b,\text{inner}} - \phi_{b,\text{outer}} \quad (71.4)$$

Under conditions where, at the outer surface, the exchange of sodium ions for hydrogen ions is complete (typically for pH less than 10), the hydrogen ion activity at the surface of the gel layer will be constant. The boundary potentials will then depend in a logarithmic manner on the hydrogen ion activity of the solution contacting the membrane, a function known as the Nernst equation.

$$\phi_b = \text{constant}' + \frac{RT}{F} \ln a_{\text{H}^+} \quad (71.5)$$

The net potential developed across the membrane is then proportional to the log of the ratio of the hydrogen ion activities of the sample and the electrode filling solution. The hydrogen ion activity of the filling solution is constant, so the membrane potential will depend linearly on pH.

$$\phi_{\text{net}} = \text{constant}'' + \frac{RT}{F} \ln \frac{a_{\text{H}^+, \text{sample}}}{a_{\text{H}^+, \text{inner}}} = \text{constant}''' - 2.3 \frac{RT}{F} \text{pH} \quad (71.6)$$

The dependence of membrane potential on pH is proportional to absolute temperature and, at 25°C, the factor 2.3 RT/F is equal to 59 mV per pH unit. Many pH meters that perform two point calibrations calculate a slope and display it as a percentage of this theoretical value. For an electrode in good condition,

this will be in the range of 90% to 105%; values considerably different from this indicate a problem with the electrode.

While the membrane potential responds principally to changes in hydrogen ion activity, it also responds to sodium and other monovalent cations. These effects are most noticeable at high pH where hydrogen ion activity is small, and is referred to as alkaline error. A more detailed analysis of the membrane response, which considers the effects of interfering ions, can be found in reference texts [3].

Reference Electrodes

A reference electrode, such as that pictured in Figure 71.1(c), is used to make a stable, low resistance electrical contact between the external measuring circuit and the sample, establishing a reference potential against which the indicator electrode can be referred. In the metallic conductors of the pH meter circuitry, current is carried by electrons, while in the sample electrolyte current is carried by ions. The difference between reference electrodes is primarily the oxidation or reduction reactions that effect charge transfer across the reference element/electrolyte interface. The species participating in these reactions must be present in the electrolyte contained within the body of the reference electrode. As a result, the composition of the reference electrode filling solution is, in general, different from that of the sample, and the physical contact between the two dissimilar electrolytes forms what is known as a liquid junction. The two phase boundaries (reference element/filling solution and filling solution/sample) necessary to make electrical contact to the sample each introduce an additional potential, which adds to that of the indicator electrode, but proper design of the reference electrode ensures that these potentials remain constant and can be calibrated out.

Reference Elements

The most widely used reference electrode for pH measurement is the silver/silver chloride electrode. The electrode is constructed from a silver wire that has been coated with silver chloride and immersed in an electrolyte saturated with silver chloride. Current is readily passed across the electrode/electrolyte interface by the reduction of silver chloride to form silver metal and chloride ion.



At equilibrium, the phase boundary potential developed at the interface depends in a Nernstian manner on the chloride ion activity of the filling solution. Maintaining a constant chloride ion activity in the filling solution ensures that this potential remains constant. This type of electrode is easily and reproducibly constructed, and the phase-boundary potential has a smaller temperature coefficient than the calomel reference electrode. The principal disadvantages of the Ag/AgCl electrode include the possibility that samples, biological samples in particular, may be contaminated with silver ion, and that precipitates (silver sulfide and nitrate) may form that can clog the liquid junction.

Another commonly used reference electrode is the calomel electrode, based on the reduction of mercuric chloride to mercury.



Like the silver/silver chloride electrode, the potential of the calomel electrode depends on the chloride ion activity of the filling solution. It is more stable than the silver/silver chloride electrode, due to favorable reaction kinetics, and is therefore preferred for high-precision electrochemical determinations. However, the operating temperature of a calomel reference electrode is limited to 70°C, compared to greater than 100°C for the silver/silver chloride electrode.

A number of reference electrodes use a platinum reference element. Ross described a reference electrode based on the reduction of iodine to iodide [4]. The nature of the redox reaction differs from the Ag/AgCl and Hg/HgCl₂ electrodes, in that both the iodine and iodide are soluble in the filling solution, yielding

an electrode potential that is relatively insensitive to temperature. The hydrogen reference electrode consists of a platinum electrode immersed in aqueous solution, over which hydrogen gas is bubbled. The platinum catalyzes the reduction of hydrogen ion to hydrogen gas. Historically, the standard hydrogen electrode has served as the primary reference electrode for precise electrochemical determinations. The role played by the hydrogen electrode in pH measurement, however, is primarily in its use as an indicator electrode for the determination of the hydrogen ion activity of primary pH standard solutions.

Liquid Junctions

The necessary contact between the filling electrolyte of the reference electrode and the sample forms a liquid junction, and results in the development of a junction potential that depends on the compositions of the two electrolytes. The junction potential can be minimized by filling the reference electrode with an electrolyte having high concentrations of an anion and cation of comparable diffusivities. For Ag/AgCl and calomel reference electrodes, potassium chloride at concentrations ranging from 3M to saturation is most commonly used as the filling electrolyte, as the diffusivities of the two ions are approximately equal. The stability of the liquid junction potential depends on the constancy of the interface between the filling electrolyte and the sample. A number of approaches exist to allow the filling electrolyte to controllably leak into the sample for this purpose. These include cracked bead, porous ceramic, and annular sleeve junctions. Leak rates range from less than $1 \mu\text{L h}^{-1}$ (cracked bead) to greater than 100 (annular sleeve) $\mu\text{L h}^{-1}$.

Selection, Use, and Care of Reference Electrodes

Reference electrode selection is dictated by the sample to be studied and the measurement conditions, such as temperature. Silver/silver chloride electrodes are preferred over calomel for general-purpose use, and at high temperatures (70°C or greater). Calomel electrodes are preferred for high-precision determinations, and where silver contamination of the sample presents a problem. An Ag/AgCl reference electrode incorporating a double junction is also another means of preventing silver contamination of the sample. The type of liquid junction and its flow rate is important; a high flow rate may contaminate the sample and deplete the electrode, while a low flow rate may lead to clogging. Viscous, semisolid samples or low ionic strength samples require a high flow rate junction. The reference electrolyte fill port should remain open during use of the electrode to insure adequate flow through the junction. Leaving the fill port open at all times will prevent any contaminants from entering the body of the reference electrode and reduce the likelihood of junction clogging, at the expense of more frequent refilling. "Low maintenance" reference electrodes use a polymer gel saturated with electrolyte within the body of the electrode, and will perform properly if stored in filling solution.

Instrumentation

The pH meter pictured in Figure 71.1(a) measures the potential developed between the pH indicator and reference electrodes, from which the pH of the sample is determined using a previously established calibration and possibly the sample temperature. The measurement of the potential, which may range in magnitude up to a few hundred millivolts, is complicated by the large electrical resistance presented by the glass membrane. This may range from $100 \text{ M}\Omega$ to greater than $1000 \text{ M}\Omega$, and necessitates the use of high input impedance amplifiers with FET input stages if the glass membrane potential is to be accurately measured. While the relatively low cost and high performance of commercially available meters obviates the need for amplifier construction, Figure 71.1(d) presents a simple amplifier suitable for use with a glass electrode. The amplifier circuitry of commercial pH meters incorporates additional functions to improve the accuracy and stability of the measurement, such as a driven shield to reduce noise pickup and chopper stabilization to reduce drift.

Calibration

The glass indicator electrode is calibrated using standard buffer solutions of known pH. A two point calibration procedure is illustrated schematically in Figure 71.3. A pair of standards is chosen to bracket

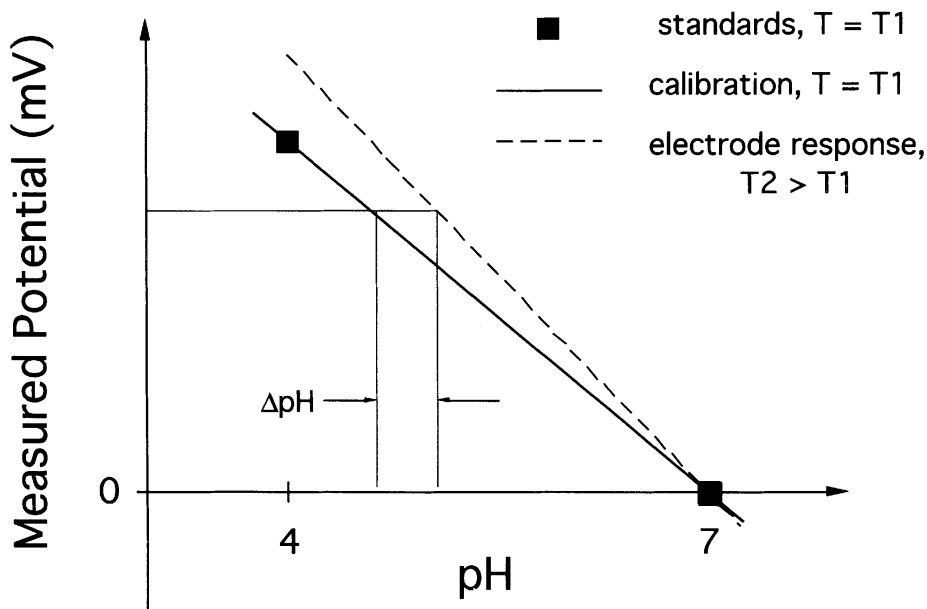


FIGURE 71.3 Calibration and temperature compensation of a glass pH electrode.

the pH range of interest. The response of the electrode is measured in each, and a calibration function is determined by linear interpolation. The slope can be compared to that expected from the Nernst equation to provide an indication of the performance of the electrode. The newest microprocessor-controlled pH meters simplify calibration by automatically recognizing the pH of standards, and constructing calibration curves from as many as five buffers.

Temperature Compensation

The potential developed across the pH indicator glass electrode membrane is temperature dependent, with a temperature coefficient of approximately 0.3% per °C, as follows from Equation 71.6. The effect of temperature on the electrode calibration is illustrated in Figure 71.3. Most pH meters have provision for temperature compensation, which corrects the slope of the measured potential versus pH calibration. Manual compensation permits the user to dial in the temperature at which the measurement is going to be made. Meters equipped with automatic temperature compensation (ATC) use a platinum resistance thermometer to directly measure the temperature of the sample. The instrumentation within the meter then corrects the calibration function such that the millivolt reading is correctly interpreted as the pH of the sample at the measurement temperature.

pHFETs

A relatively recent development in pH measurement is the introduction of systems based on the use of ion-selective field-effect transistors (ISFETs) as the sensing element. ISFETs, of which the hydrogen ion-sensitive pHFET is one variant, are derived from the metal-oxide-semiconductor FET (MOSFET), the basic building block of integrated circuits. These silicon “chips” combine a pH-responsive membrane much like that of the glass electrode with the amplification of a field-effect transistor. The integral amplification and small size have led to the development of inexpensive, battery-powered, pocket-sized pH measurement systems. These devices have found unique and expanding niches, including the food industry where the measurement of pH using breakable glass electrodes presents an unacceptable safety hazard, the measurement of the pH of gels, pastes, and slurries, and for the measurement of strongly alkaline solutions where conventional glass bulbs respond to the sodium ions and give an erroneously low reading. The following

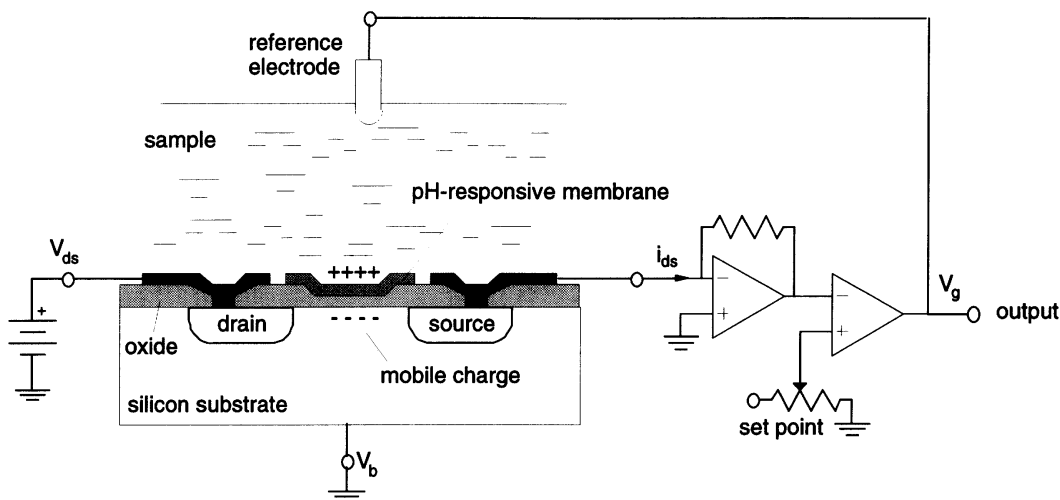


FIGURE 71.4 pH measurement using an ion-selective field effect transistor, including an amplifier circuit for constant drain current operation.

sections cover the basic operation of these devices, instrumentation, and applications. Additional details can be found in reference texts [5].

Construction and Operation

A schematic cross-section of a pH-sensitive ISFET is presented in [Figure 71.4](#). The pHFET differs from a MOSFET in that the metal gate of the MOSFET is replaced by a pH-responsive membrane material such as silicon nitride, aluminum oxide, or tantalum oxide, which contacts the sample solution directly. As with the glass electrode, electrical contact is made to the sample through a reversible reference electrode. A suitable voltage applied to the reference electrode (relative to the silicon substrate) will charge the capacitor formed by the solution, insulating layers, and silicon substrate, and create mobile charge in the channel region. A potential simultaneously applied between the drain and source electrodes will result in current flow. Using a “charge imaging” model [6], this drain current, i_{ds} , can be described by:

$$i_{ds} = A V_{ds} Q_c = A V_{ds} C_2 \left(V_g - V_T - \frac{V_{ds}}{2} \right) \quad (71.9)$$

where the constant A includes geometrical factors, V_{ds} is the voltage applied to the drain, and Q_c is the mobile channel charge. The mobile charge is a function of V_g , the voltage applied to the reference electrode; V_T , the “threshold voltage” needed to produce mobile charge, and C_2 , the capacitance of the gate region. The threshold voltage includes a number of terms, one of which is the phase-boundary potential at the interface between the sample and the pH-responsive insulating layer. Adsorption of protons at the surface of this layer leads to a Nernstian dependence of this potential on hydrogen ion activity with the result being that changes in pH modulate the drain current of the device.

$$i_{ds} = A V_{ds} C_2 \left(V_g - V_T + 2.3 \frac{RT}{F} \text{pH} - \frac{V_{ds}}{2} \right) \quad (71.10)$$

Instrumentation

Equation 71.10 illustrates the dependence of ISFET current on pH. The preferred method of operation of the pHFET is to operate at a constant drain current. A circuit for doing this is illustrated schematically

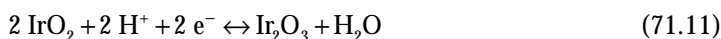
in Figure 71.4. A transconductance amplifier converts the drain current to a voltage, which is referenced against a setpoint. The output of the comparator drives the reference electrode, V_g . Since the mobile channel charge Q_c is constant, as are V_{ds} and V_T , changes in the comparator output directly reflect changes in the hydrogen ion activity. That is, a 59-mV change in output corresponds to a change in pH of one unit. Interface circuitry similar to that of Figure 71.4 allows the device to be connected directly to a glass electrode pH meter. As with the glass electrode, temperature compensation is required if the device is to be used at temperatures different from that of calibration.

Other Electrochemical pH Electrodes

There are a number of other approaches for electrochemical measurement of pH [1]. Two that deserve mention, as they are commercially available, either directly or incorporated within another product, are metal/metal oxide and liquid membrane electrodes.

Metal/metal Oxide pH Sensors

A pH indicator electrode with application to measurement at high temperature and pressure can be constructed from metals coated with an oxide. These metal electrodes may take the form of a wire, polished disk, or sputtered thin film, on which an oxide has been formed through thermal oxidation, chemical vapor deposition, or electrochemical oxidation. Electrodes have been constructed from systems including W/W₂O₃, Sb/Sb₂O₃, Pt/PtO₂, Ru/RuO₂, Pb/PbO₂, and Ir/IrO₂. In aqueous solution, the metal oxide can be reduced to a lower oxidation state with the consumption of a proton in solution. Using an iridium/iridium oxide (Ir/IrO₂) electrode as an example, this redox couple is believed to be represented by an Ir(III)/Ir(IV) half-cell reaction of the form:



The Ir/IrO₂ electrode shows a near Nernstian response of -59 mV per pH unit [7]. The chemical resistance, high temperature and pressure performance, the nonglass construction, and the considerable potential for miniaturization suggests that these pH sensors will find application in areas not typically employed by glass membrane electrodes or pHFETS.

Liquid Membrane Electrodes

Another type of electrochemical pH electrode is based on polymeric "liquid membranes," most commonly used to construct ion-selective electrodes for ions such as potassium [8]. The membranes consist of a plasticized polymer film into which an ionophore has been incorporated. The ionophore is a molecule that selectively binds and transports a given ion across the membrane, making the membrane selectively permeable to the ion of interest. The ionophore N-tridodecylamine binds hydrogen ions and has been used in the construction of a pH sensor incorporated into a disposable cartridge device for measuring blood electrolytes [9].

71.3 Optical Methods of pH Measurement

For some applications, optical methods offer advantages over the use of the glass electrode or other electrochemical devices for the measurement of pH. Organic dye molecules with pH-dependent spectral properties have been routinely used for decades in acid-base titrations and in pH indicator papers. These dyes, many of natural origin, have more recently been put to use as indicators to measure localized pH within living cells and in the development of fiber-optic probes for measuring pH within the body. The following sections cover the basic principles and major applications of optical pH measurement; more detailed treatments may be found in Reference 10.

TABLE 71.2 Sources, Features, and Key Properties of Selected pH Electrodes

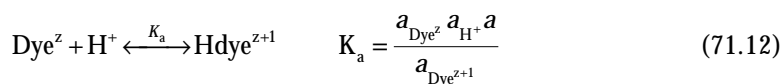
Manufacturer	Reference	Range	Accuracy pH Units	Cost (U.S., 1997)	Key Features
Glass combination electrodes					
ABTECH Scientific, Inc. ^a Miniature Combination pH electrode, CPE 905-X	Ag/AgCl	0–14	±0.1	\$285	Handheld Refillable bulb Min. vol. = 10 µL
ATI-Orion, Inc. ^a Combination Electrode, 8102BN	Proprietary	0–14	±0.03	\$245	0–100°C
Brinkman ^a FUTURA Plus Combination Electrode, 39539	Ag/AgCl	0–14	±0.05	\$132	Benchtop Handheld Refillable bulb
Corning ^a High Performance Comb. Electrode, 476146	Ag/AgCl “scavenger”	0–14	±0.05	\$225	Increased sensitivity TRIS compatible Rapid response
Hanna ^b pHep 3 Pocket pH tester	Ag/AgCl fabric junction	0–14	±0.1	\$52	Handheld, waterproof Auto 2 pt. cal. Auto temp. comp.
Hanna ^c Combination Electrode Research Grade HI1270	Ag/AgCl	0–12	±0.1	\$115	Handheld Field portable Sealed rugged epoxy body
Hach ^c Hach One Electrode with Temperature Sensor, 48600-00	Ag/AgCl	0–14	±0.05	\$185	Benchtop, handheld Field portable, Free-flowing reference junction 0–100°C
Microelectrodes, Inc. ^a Micro-Combination pH Probe MI-410	Ag/AgCl	0–14	±0.1	\$185	Handheld Min. vol. = 5 µL
Radiometer ^a PHC2401	Ag/AgCl	0–12	±0.1	\$185	General purpose
Sensorex ^a Combination Electrode 450CUS	Ag/AgCl	0–14	±0.05	\$65	Epoxy body 0–100 psig Flat tip for use on moist surfaces 0–100°C
ISFET electrodes					
ATI-Orion ^{a,c} pHuture Sure-flow electrode, 616500	Ag wire	0–14	±0.02	\$326 ^d \$393 ^e	Built-in temperature probe
Bioanalytical Systems, Inc. ^b pH Boy, MF8960	Ag/AgCl	2–10	±0.1	\$180	Handheld, stores dry Min. vol. = 50 µL
Corning ^c ISFET electrode 476395	Ag/AgCl	0–14	±0.02	\$320	Handheld, stores dry Min. vol. = 20 µL Auto temp. comp. 0–60°C
IQ Scientific Instruments ^a IQ 200	Ag/AgCl KCl gel	0–14	±0.01	\$229 ^d \$695 ^e	Handheld, sterilizable Auto. temp. comp. –5–+105°C Automatic two point calibration Min. vol. = 50 µL
Sentron ^b S1001	Ag/AgCl fabric junction	0–14	±0.01	\$259 ^d \$695 ^e	Handheld, stores dry Man. 2 or 3 pt. cal Replaceable sensor tip, 0–60°C Min. vol. = 50 µL (1 drop)
Metal/metal oxide electrodes					
Cypress Systems, Inc. ^c METOXY	Ag/AgCl	0–14	±0.01	\$1299 ^b	Auto. temp. comp. 0–100°C Automatic two point calibration Min. vol. = 50 µL

TABLE 71.2 (continued) Sources, Features, and Key Properties of Selected pH Electrodes

- ^a General-purpose meter.
- ^b Integrated sensor and meter.
- ^c Dedicated meter (specific to manufacturer).
- ^d Replacement electrode.
- ^e Electrode and converter for use with general-purpose meter.

Indicator Dyes

Optical measurement of pH is based on the use of organic dye molecules that are weak acids or bases. The loss or gain of a proton changes the electronic structure of the molecule, producing a measurable change in the manner in which the molecule interacts with light. This interaction can be the absorption of light at a particular wavelength, or fluorescence by one form of the molecule. An equilibrium exists between the acid and base forms of the dye, whether free in solution or chemically attached to a supporting membrane, and can be described by the equilibrium:



where z represents the valence of the molecule. The interrogation of the solution or membrane with light will produce a response that is weighted by the relative proportions of the acid and base forms of the molecule, which in turn depends on pH. Inspection of Equation 71.12 shows that the ratio of the two forms, and hence color, varies continuously with hydrogen ion activity. The sensitivity of the measurement is greatest when the acid and base forms of the dye are present in approximately equal concentrations. This will occur when the pH of the solution is close to the $\text{p}K_a$ ($-\log [K_a]$) of the dye. Limitations of the human eye restrict detectable changes in color to a tenfold excess of one species over the other. This corresponds to a change of ± 1 pH unit. Thus, an indicator with a $\text{p}K_a$ of 1×10^{-5} will display a color change if the solution in which it is dissolved changed from 4 to 6 pH units. The pH of interest therefore dictates selection of the particular dye.

An understanding of possible interactions between the indicator dye and the sample is important in the effective use of optical indicator dyes for pH measurement [11]. Factors such as temperature, electrolyte concentration, and the presence of organic solvents may cause a shift in $\text{p}K_a$ of the indicator dye; the shift could be as much as one or more pH units. As weak acids and bases, the addition of indicator dyes to a sample can change the pH, particularly in weakly buffered samples. This effect is important when using pH papers or optical sensors to measure small samples, where the potential exists for the buffering capacity of the sample to be exceeded by the amount of dye immobilized in the paper or on the sensor. As a general rule, potential errors can be minimized by calibrating in solutions similar in composition to the sample to be measured.

Absorption Indicator Dyes

The conjugate acid and base forms of absorption indicator dyes differ in the characteristic wavelengths at which they absorb light energy. A number of common absorption indicator dyes are listed in [Table 71.3](#), and their $\text{p}K_a$ s span the range of pH from approximately 1 to 13. The absorption spectra of a solution of the dye phenol red at a number of different pHs is shown in the inset of [Figure 71.5](#). The acid form of the dye has an absorption maxima at $\lambda_{\text{max}} = 435$ nm, while the base form absorbs maximally at $\lambda_{\text{max}} = 565$ nm. As the pH of a phenol red solution is increased from 6 toward the $\text{p}K_a$ of 7.9, the equilibrium of Equation 71.12 shifts to the basic form of the dye. The relative heights of the absorption peaks change, reflecting a shift in concentrations of the two forms. For quantitative pH measurement, the absorption of light by the indicator is measured by a spectrometer or reflectometer at a specific wavelength or narrow set of wavelengths corresponding to the wavelength of maximum absorption, λ_{max} for the acidic or basic

TABLE 71.3 Acid-Base Indicator Dyes

Indicator	pK _a	λ _{max} (nm)	
		Acid Form	Base Form
Thymol blue	1.7	544	430
Methyl orange	3.4	522	464
Bromphenol blue	3.9	436	592
Bromcresol green	4.7	444	617
Methyl red	5.0	530	427
Chlorophenol red	6.0	—	573
Bromcresol purple	6.3	433	591
Bromthymol blue	7.1	433	617
Phenol red	7.9	433	558
Cresol red	8.2	434	572
Phenolphthalein	9.4	—	553
Thymolphthalein	10.0	—	598

form of the chromophoric dye molecule. Beer-Lambert's law describes the proportionality between absorbance, A , and the concentration of the indicator, c_{dye} , in moles per liter.

$$A = \log\left(\frac{I}{I_0}\right) = \epsilon b c_{\text{dye}} \quad (71.13)$$

The quantity I/I_0 represents the ratio of the intensity of light transmitted through the sample to that incident on the sample, ϵ the extinction coefficient of the dye molecule, and b the path length. The

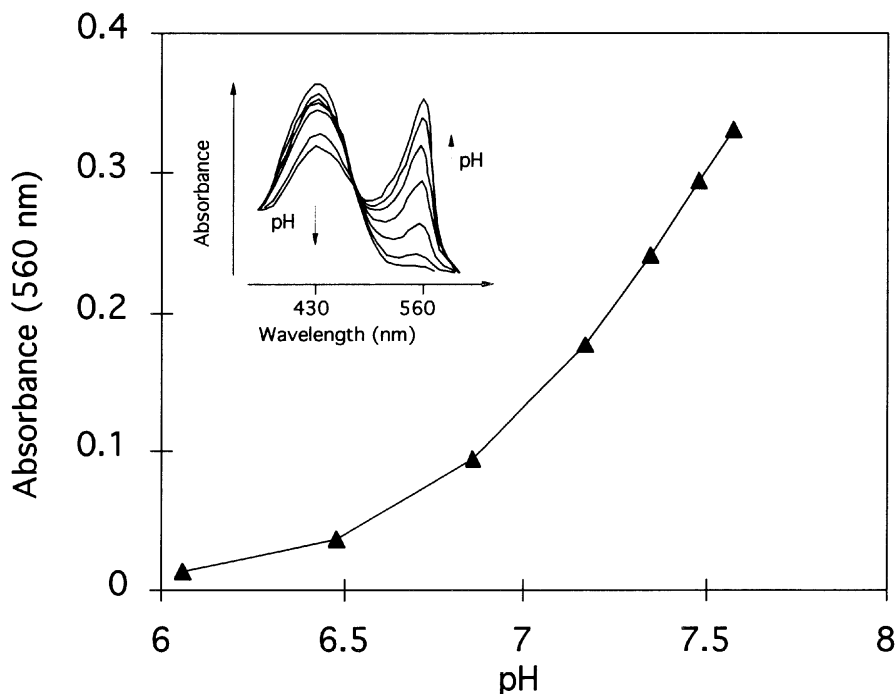


FIGURE 71.5 Magnitude of the absorption of phenol red at 565 nm as a function of pH. Inset: absorption spectra at different pHs.

TABLE 71.4 Fluorescent pH Indicator Dyes (for physiological applications)

Indicator	pK _a	Excitation Maximum (nm)	Emission Maximum (nm)
BCECF ^a	7.0	482	520
Fluorescein	6.4	490	515
HCC ^b	6.9–7.0	410	455
HPTS ^c	6.8–7.3	465	520

^a 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein.

^b (7-hydroxycoumarin-3-carboxylic acid).

^c (8-hydroxy-1,3,6-pyrenetrisulfonate).

magnitude of the absorption peak heights at 565 nm, plotted in Figure 71.5 as a function of pH, reflects the changing concentration of the basic form of the dye as the equilibrium of Equation 71.12 shifts.

Fluorescent Indicator Dyes

Fluorescent indicator dyes absorb light of a particular color (or wavelength) and re-emit some of the absorbed energy as light of a different color. Absorption of light by the indicator promotes the molecule from the ground state energy, E_g , to a higher energy state, E_1 . Subsequent processes such as molecular collisions lead to a transition to a lower energy excited state, E_2 . The molecule can then emit a photon of energy $E_2 - E_g$, resulting in the return of the molecule to the ground state from this intermediate state. The emitted light is at a longer wavelength than the exciting wavelength, and the difference is known as the Stokes shift. In principle, a fluorescence measurement is more sensitive than an absorption measurement because the only light measured by the detector originates from fluorescing molecules. Table 71.4 lists a number of common fluorescent pH indicators used for measurement of pH in the physiological range. Fluorescein is widely used because the absorption maximum at 490 nm of the fluorescent dianion is readily excited by the 488-nm emission of argon ion lasers.

Indicator Papers

Indicator papers are a simple, rapid, and inexpensive means of measuring pH when the precision of an instrumental measurement is not necessary. These are constructed from a strip of paper or plastic that has been impregnated with one or more absorption indicator dyes chosen to span the pH range of interest. The dyes are generally covalently attached to the strip, to prevent contamination of the sample by leaching of the dyes. A strip for indicating pH in the range of 5.5 to 9.0 can be constructed using the dyes bromocresol purple, bromothymol blue, and phenol red [1]. The pH of a sample is determined to a precision of typically 0.5 units by comparing the color of the strip to a color calibration chart provided by the manufacturer.

Fiber-Optic pH Probes

Optical pH sensors, often referred to as optrodes, represent some of the most sophisticated pH sensors, finding use for remote sensing in the body or industrial plants due to their small size and lack of electrical connections. They are typically constructed by immobilizing an indicator dye at the tip of a light guide formed from one or more optical fibers, which are used to couple light between the indicator and the measurement instrumentation, as illustrated schematically in Figure 71.6(a). The resulting probes can be made very small, and with high-quality optical fibers, pH can be measured over considerable distances in electrically noisy environments that would interfere with potentiometric-type electrodes.

Construction and Instrumentation

The pH indicator dye must be immobilized in close proximity to the fiber tip. The method of immobilization impacts the sensor's response, response time, long-term stability, and mechanical integrity. The dye can be immobilized to a solid support, such as a membrane or porous glass bead, which is then

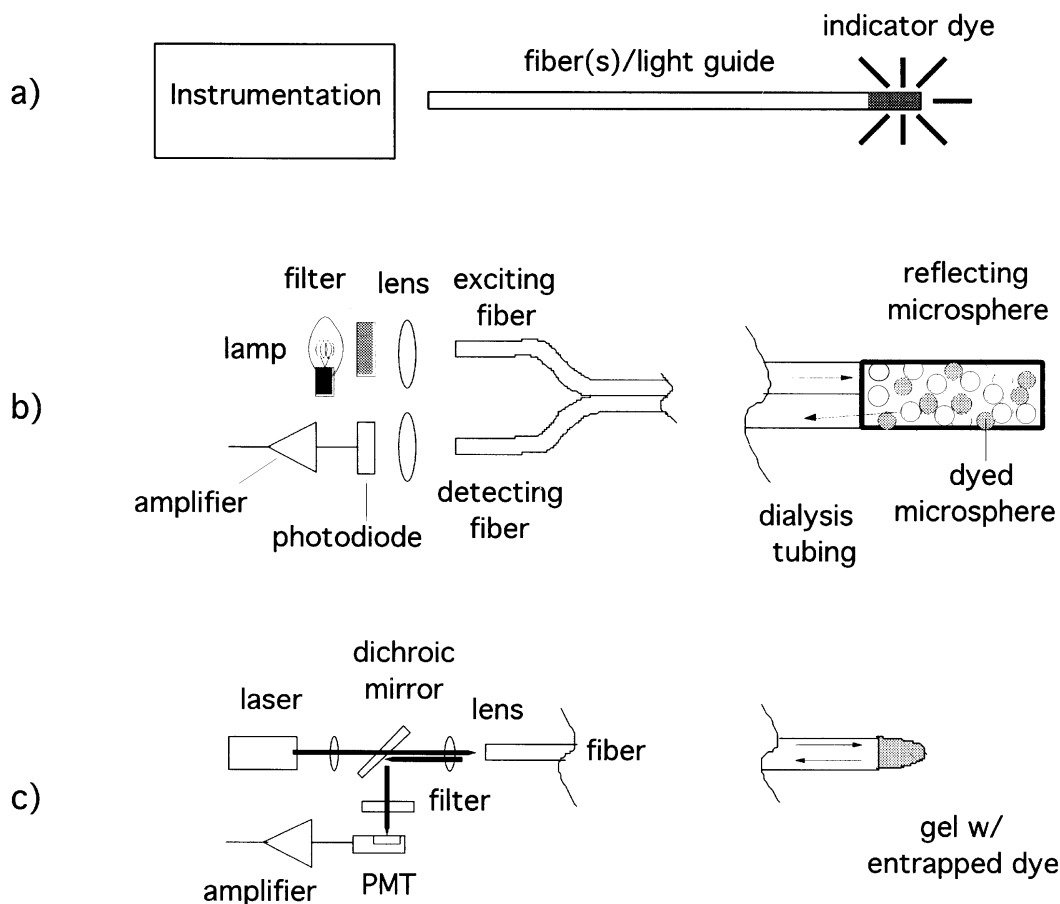


FIGURE 71.6 Optical fiber sensors: (a) generic; (b) based on absorption indicator dyes [13]; and (c) based on fluorescent indicator dyes [14].

attached to the end of the fiber. Indicator dyes have been covalently linked to the tip of glass fibers, or entrapped within polymer films formed at the tip using light energy from the fiber to initiate polymerization. The construction of probe tips and the instrumentation used for a fiber-optic pH probe depend on the type of indicator being used. Unlike the glass electrode, which has remained largely unchanged for more than 75 years, there is considerable variety in the design of fiber-optic pH sensors. While a complete review of the literature is beyond the scope of this work (interested readers may wish to consult Wolfbeis [10] and a recent review of chemical sensors by Janata [12]), the following examples illustrate the approaches to construct optical fiber pH sensors using absorption and fluorescent dyes.

Absorption optodes measure the change in intensity of light returned from the fiber tip/sensing region relative to the interrogating light of specific (or a narrow band) wavelength. These require a separate collection fiber or fibers and a means to reflect the light, such as a reflector or the use of scatterers such as polystyrene beads. In the example of Figure 71.6(b) [13], polyacrylamide beads containing the indicator dye phenol red were mixed with white polystyrene latex microspheres that served as scatterers. The beads were contained within dialysis tubing attached to the end of a pair of plastic optical fibers, one used for excitation, the other to collect scattered light. Light from a lamp was filtered to alternately select the λ_{max} of the base form of the dye (565 nm), and a wavelength (600 nm) where the absorbance is pH independent as a reference. (The isosbestic point at 480 nm could also have served as a reference.) The intensity of light scattered back into the second detector fiber was quantified by a photodiode. The ratio of the scattered intensities at the two wavelengths was then related to the pH of the sample.

Fluorescent indicator optrodes measure the Stokes-shifted fluorescence emission by the indicator and may use a single fiber to both interrogate and collect signal-carrying light. The amount of fluorescent pH indicator at the fiber tip must be maximized since fluorescence is emitted omnidirectionally and only a small fraction of the emitted fluorescence will be captured by the fiber. In the example of [Figure 71.6\(c\)](#) [14], the indicator dye was entrapped within a polymer gel formed at the tip of a glass fiber. The source of excitation light is typically a high-intensity lamp or a laser. If a lamp, its output is filtered to select a narrow band of wavelengths at the absorption maximum of the specific form of the indicator, that is then introduced into the fiber to excite the fluorophore at the tip. Emitted light collected by the fiber exits the fiber and is incident on a dichroic mirror, which reflects the long-wavelength light and passes shorter wavelengths. A filter then selects a narrow band of emission wavelengths, and finally, the light is detected. Due to the relatively small light intensities, the detector is typically a photomultiplier tube rather than a photodiode.

71.4 Frontiers of pH Measurements

Because of its wide-ranging importance, there is likely to be continued innovation in both approaches and opportunities for pH measurements. Some important developments, such as pHFETs and metal/metal oxide pH sensors, have already occurred and are now achieving noteworthy commercialization. Investigators continue to seek new materials for constructing transducers. Examples include electroconductive (electroactive and inherently conductive) polymers such as polyaniline [15] and polyelectrolyte hydrogels [16], which form pH-responsive membranes suitable for the construction of microsensors.

A noteworthy innovation is the application of pH measurement devices as transducers that are integrated into more complex analytical systems directed at other analytes. A pH indicator dye forms the basis of the pCO₂ measurement in an indwelling fiber-optic catheter [17]. Because protons are generated and/or consumed in many biological reactions involving enzymes, pH sensors have found use as transducers in biosensor devices.

An expanding area for pH measurement is likely to be the study of biological systems. The light-addressable potentiometric sensor is a device that measures the pH change resulting from the metabolic activity of cells, with application to examining cellular response to toxins and infectious agents [18]. The measurement of intracellular or subcellular pH using pH-sensitive fluorescent indicators [19] can provide insights into the physiology of the cell.

Acknowledgments

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