pH meters and their electrodes: calibration, maintenance and use

Following consideration of centrifugation and liquid handling in the February and March issues, respectively, of *The Biomedical Scientist*, Peter Riddle now directs attention to pH meters, their electrodes and the measurement of hydrogen ion concentration.

As living processes are dependent on the unique ionising solvent we know as water, and as much of classical chemistry was concerned with the study of reactions in aqueous solution, the ubiquity of the pH meter in present-day biomedical laboratories is scarcely surprising. The concept of electrolytic dissociation was introduced over a century ago by Arrhenius, and in 1909 Sorenson demonstrated the importance of hydrogen ion concentration on enzyme activity and coined the term 'pH'. Although that same year saw the first systematic study of the glass electrode, it was not until the development of convenient electronic techniques capable of accommodating the high electrical resistance of the glass electrode that interest in electrometric pH determination and its importance in biological systems became clear.

The pH meter is designed to measure the concentration of hydrogen ions in a solution. Basically, three parameters are involved in the measurement: the actual molar concentration of hydrogen ions, the dissociation constant of the acid (pKa), and temperature. pH is defined as the negative of the logarithm to the base 10 of the hydrogen ion concentration ($pH = -log10\ [H^+]$).

The hydrogen electrode was the first electrode to be used for pH measurement but it required a supply of hydrogen gas and thus its application was limited. The quinhydrone electrode appeared in the early 1920s and

around 1928 the first commercial pH meter was produced by the Cambridge Instrument Company. Both electrodes were subject to interference from the components of redox systems that reduced the effectiveness of these electrodes to measure the potential response due to hydrogen ions. Membrane electrodes, including the glass electrode, are not subject to these limitations and

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lithium-based glass electrodes are now used exclusively for pH-responsive glass electrodes.

POTENTIOMETRIC MEASUREMENT

Almost all conductors of electricity are metal or an electrolyte, with the current being carried by either electrons or ions. When current passes from metal to electrolyte, or from electrolyte to metal, the type of carrier usually changes suddenly, and whenever there is an interface between the metal and ions of that metal in a solution then an electric potential is produced. This potential is called the electric potential of that metal. An electrode potential is also produced when different concentrations of an ion are separated by a membrane that is semipermeable to that ion. Non-metallic elements such as hydrogen also have electrode potentials.

In order to measure an electrode potential, another voltage source (such as another metal/solution interface) is needed to measure it against. Each of the electrodes is called a half-cell. The two half-cells arranged together constitute an electrochemical cell, in which one of the half-cells maintains a constant voltage. The electrode in the half-cell with the constant voltage is called the **reference electrode**, whereas the variable voltage portion is termed the **indicator electrode**. It is possible to measure the
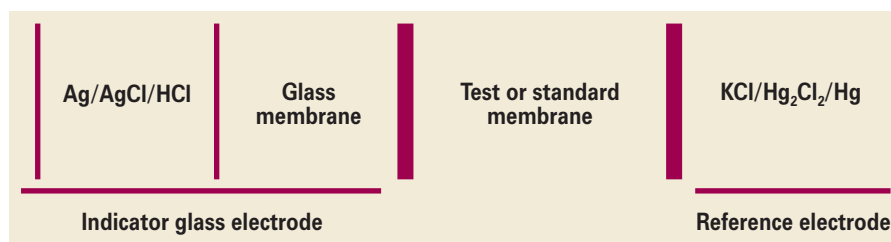

Ag/AgCl/HCl	Glass membrane	Test or standard membrane	KCl/Hg$_2$Cl$_2$/Hg
Indicator glass electrode			**Reference electrode**

Fig 1. The composition of the two half-cells that permits the difference in potential to be detected between them.

Fig 2. The reference electrode.

Fig 3. The indicator (glass) electrode.
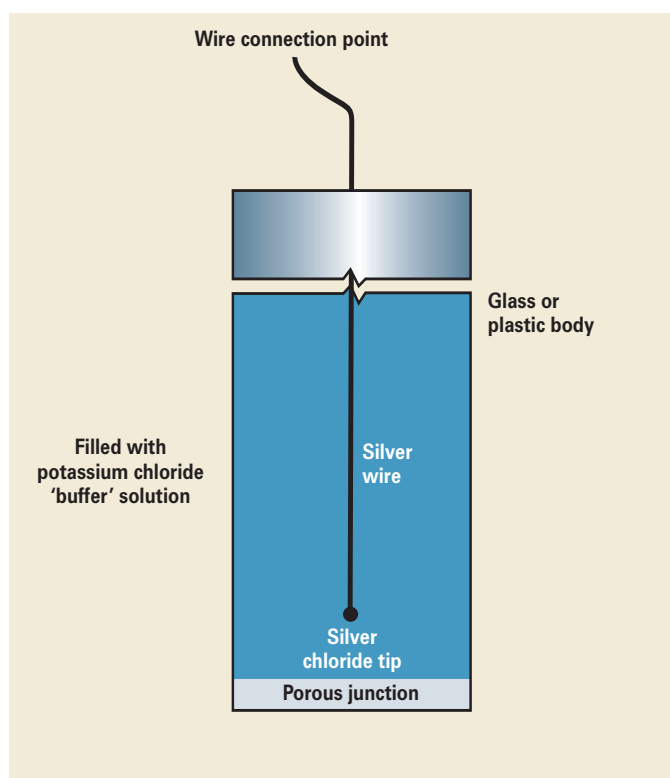
potential difference between these two electrodes and calculate the concentration of ions in the solution of the indicator (measuring) electrode.

For example, if a silver wire is immersed in a solution of silver chloride, ionisation of the silver metal occurs, with the formation of silver ions (Ag^+) and electrons. An electric potential now exists between the wire and the solution. If two half-cells are used, each with a silver wire or foil immersed in a different silver solution, and the two solutions are connected through a meter, a difference in potential can be detected between them (Fig 1). As the potential of each solution depends on the concentration of silver ions in it, the concentration of ions in one solution can be predicted if the value for the other solution and the difference in potential between them are known. A temperature difference between the two half-cells would affect the reproducibility of measurement. Other minor technical factors, such as coating (eg protein), will affect the measurements.

Potentiometric methods are based on the quantitative relationship between the potential of a cell as given by the following distribution of potential:

$$E_{cell} = E_{reference} + E_{indicator} + E_{junction}$$

Because the reference and junction potentials are constant, the indicator potential can be determined. The potential of the indicator electrode can then be related to concentration. Electrode systems used in biomedical laboratories have precalibrated read-out devices that give results in concentration units.

The **cell potential** is related to

concentration through the **Nernst equation**, which, in its simple form, is $E=KT \log (C1/C2)$ where $K = Constant\ 2.3\ (R/zF)$ and if either C1 or C2 is known then the concentration can be calculated from the measurement of the potential developed.

In making an electrical connection between the reference electrode and the sample solution via a salt bridge, a negligible but reproducible potential is produced. This potential develops at the interface between two non-identical solutions and is called the liquid–liquid junction potential. A saturated potassium chloride solution is normally used as a salt bridge in the reference electrode because many ions diffuse from the salt bridge against which the sample ions must diffuse. Thus, diffusion of ions from the sample to the junction is negligible and junction potential is ≤1 mV and is reproducible.

REFERENCE ELECTRODES

The standard **hydrogen electrode** is the international standard but is seldom used for routine work because a supply of hydrogen gas is required and more convenient types (together with reliable calibration buffers) are available. The **saturated calomel electrode** is a widely used reference electrode that contains an inert element (eg platinum) in contact with mercury, mercurous chloride (calomel) and a solution of potassium chloride of known concentration. However, in view of environmental considerations, the mercury electrode is rarely used these days and such electrodes are not suitable for varying temperatures or temperatures above 60˚C (Fig 2). When a high degree of accuracy
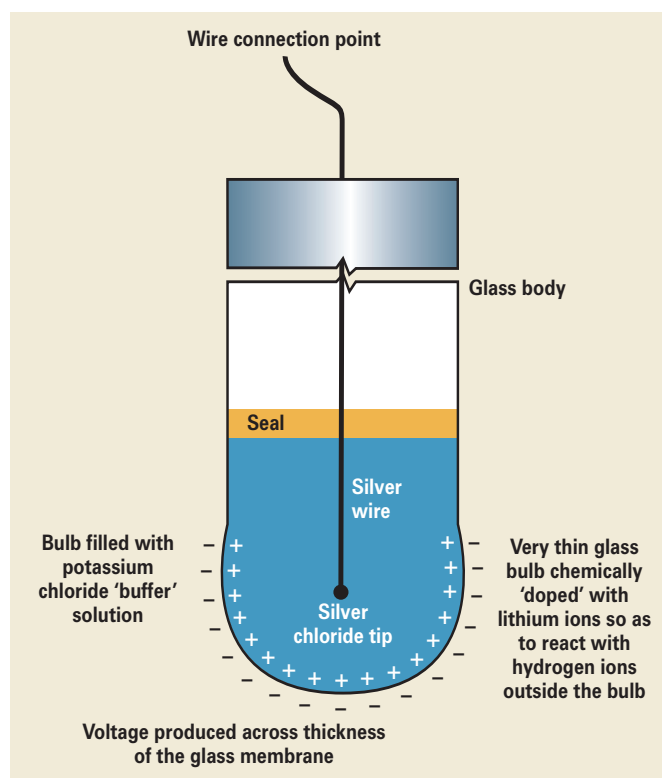
is desired, a 0.1 mol/L or 1 mol/L potassium chloride solution electrode is preferred because it reaches its equilibrium potential more quickly and its potential depends less on temperature than does the saturated type. The silver–silver chloride electrode is a very reproducible electrode. The solution surrounding the electrode should be saturated with potassium chloride and silver chloride.

INDICATOR ELECTRODES

The glass electrode was the first and still most commonly used electrode for measuring hydrogen ion activity. A glass electrode consists of a small bulb of special glass that contains a solution of known hydrogen ion concentration (eg 0.1N HCL or KCL 'buffer solution') and an internal reference electrode (usually calomel or silver–silver chloride; Fig 3). The sensor is normally a platinum wire.

The pH electrode is manufactured with different properties, depending on the application. Those used in biomedical laboratories should be linear at about pH 7.0. Wide-range pH electrodes (1–14) tend to be non-linear towards the ends of the range.

Some are designed to be linear for low pH values, while others are linear for high pH values. These properties are controlled by the glass formula.

COMBINATION ELECTRODE

It is inconvenient in many applications to have to contend with two measuring electrodes (reference and indicator). Combination electrodes are available into which both the reference (usually Ag–AgCl) and indicator electrodes are incorporated.

The pH membrane is usually recessed in, and protected by, a plastic housing. Such electrodes will withstand more physical abuse than will conventional pH electrodes.

The pH of a solution is a function of its temperature. Voltage output from the electrode changes linearly in relation to changes in pH, and the temperature of the solution determines the slope of the graph. pH applications therefore require some form of temperature compensation to ensure standardised pH values. Some manufacturers use a temperature-compensating electrode. This is a resistance thermometer that is inserted into the solution under test, together with the glass and reference electrodes.

The electronic mechanism of the amplifier is designed so that when the pH meter is used in conjunction with the resistance thermometer the pH is independent of temperature, so long as the temperature range is within $\pm 5\,^{\circ}C$ of the temperature of the buffers used to calibrate the instrument. Other instruments use electronic compensation for temperature changes. In these instruments the temperature of the calibration buffers or sample is set with a control on the instrument.

MEASURING DEVICE

When the indicator electrode and the reference electrode are immersed in a solution containing hydrogen ions, the small

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potential difference between the two half-cells is measured by a very sensitive voltmeter. When the instrument is calibrated against standards and adjusted for the temperature effect, the hydrogen ion activity can be read very accurately. Owing to the high resistance of the glass electrode, electronic amplification is necessary for measurement of the potential and a very sophisticated, stable and sensitive measuring device is required. This is what we now know as a pH meter.

The voltage produced by the electrodes initially is amplified and compared against a standard reference voltage. The output of the voltage comparator goes to the display electronics, which convert the comparator output to a suitable form for the display unit. Both standard moving-pointer meters and digital display units are common, although most new instruments incorporate digital displays. The critical portion of most pH meters is a potentiometer circuit.

INSTRUMENT CALIBRATION

It is essential that pH electrodes are calibrated regularly using the meter and reference electrode with which they are to be used in the laboratory. The procedure is as follows:

1. Turn instrument on and allow adequate time to warm up (15–30 min)
2. Position electrodes in holder and plug into instrument
3. Set function switch to pH
4. Prepare pH buffer solutions for integer pH values. These are available in dry form in foil packets and are prepared using deionised water. Preprepared liquid buffers can also be used
5. Set temperature compensation control for temperature of the buffers (not room temperature). Most modern meters and electrode systems have automatic temperature-compensation correction
6. Insert electrodes in pH 7.0 buffer
7. Adjust calibration control until meter indicates pH 7.0 – modern pH meter models working in calibration mode often recognise the buffer and take necessary action automatically
8. Do not readjust calibration control for steps 9 to 12
9. Remove electrodes, flush with deionised water and blot gently to remove excess water
10. Start with the highest pH buffer and place

electrodes in the solution. Read and record pH value. The highest pH buffer represents the lowest hydrogen ion concentration. Calibration in this manner minimises test solution carry-over between measurements

11 Repeat steps 9 and 10 with each successively lower-value buffer and record all results

12 Plot calibration curve.

Most modern instruments have a built-in automatic buffer recognition facility and will automatically identify and set to the appropriate temperature-corrected calibration values. This can be over-ridden in some instruments to allow free entry of the actual buffer pH.

ROUTINE USE

A considerable variety of instruments from several manufacturers is available and the user is advised to follow the operating instructions supplied with the instrument.

The glass electrode can be used with strong acids; however, it is attacked by strong alkaline solutions. Therefore, glass electrodes should never be left in alkaline solutions for longer than is necessary to measure the pH. The glass electrode responds rapidly to large pH changes in buffered solutions, but the response is slower in poorly buffered, or unbuffered, solutions. Equilibrium is reached slowly and may require several seconds. Poorly buffered solutions should be stirred vigorously during measurement to prevent stagnation at the electrode.

Measurements can be made in partly aqueous solutions but the degree of hydration of the outer surface of the membrane will alter the potential across the membrane. Hence, values obtained in non-aqueous, or highly ionic, solutions will be incorrect.

ELECTRODE CARE AND MAINTENANCE

Many types of pH electrode are available but the standard glass or epoxy-bodied combination electrode is ideal for the majority of tests carried out on aqueous solutions with a reasonable ionic strength at ambient temperatures and with limited use in strongly acidic or alkaline solutions.

The following general guidelines indicate the care and maintenance required for pH electrodes:

- To dry the electrode, use clean soft tissues and blot the liquid from the electrode.
- Immerse in pH 4 buffer for short-term storage. For longer-term storage use the same solution as the reference electrolyte of the electrode. In most cases this is a 3 mol/L KCl solution. Most manufacturers supply a plastic protection cap which is filled with this solution. Close off the filler opening if there is one. If the electrode will be not used for a long period of time, you may store it dry to prevent ageing (ageing takes place only when the electrode is

wet). This does not apply to combination or gel electrodes, as these must be stored in a concentrated solution of KCl only. Never store your electrode in water (see below). Always rinse thoroughly with deionised water after use. If the response of a glass electrode has become sluggish, the recommended treatment (which should only be performed when other measures have failed) consists of 1 minute in 20% ammonium bifluoride solution, followed by 15 seconds in 6 mol/L hydrochloric acid. Care should be exercised when carrying out this treatment as the risk of the formation of hydrofluoric acid is present. The electrode should then be rinsed thoroughly and soaked for 24 hours in water or in an acidic buffer solution.

- Electrodes that have been allowed to dry out (often indicated by a hard, dry deposit of KCl crystals) should be soaked overnight in warm deionised water. Liquid junctions with fibres or ceramic pins occasionally can become blocked due to crystallisation (eg KCl). If soaking in KCl solution does not solve the problem, raising the temperature to the maximum allowable for the reference system will often help. Other types of blockage can also occur (eg in the form of a precipitate [black] of silver chloride or mercury sulphide in the porous pin). Gentle use of abrasive paper can sometimes remove the precipitate. In other cases, chemical procedures such as soaking the electrode for a few hours in an acidic solution of thiourea (1 mol/L thiourea in 0.1 mol/L HCl) can be used.

- Ensure that the electrode is used and stored within its specified temperature range. Extreme changes in temperature between samples will affect response time, and electrodes used above their temperature range will age rapidly.

- Ensure that air bubbles are not trapped at the bottom of the electrode. If present, bubbles should be removed by holding the electrode vertically and gently tapping the electrode body. If the air bubbles are trapped by KCl crystals, heating the electrode gently to 60 °C (maximum) in a water bath may also prove beneficial.

- Handled carefully – the normal lifetime of glass electrodes is approximately two years.

Occasionally, functional failure occurs before mechanical failure. This is recognised by a gradually increasing electrode response time, with increasingly erratic readings. This is a different effect from electrode shock, which also produces increased response time. Electrode shock is produced by dipping the electrode into a high-concentration solution and then immediately afterwards into a low-concentration solution, or vice versa. Thus, if one tries to measure pH 2 and then pH 11, an increased response time should be expected.

ELECTRODE CLEANING

The solution used to clean pH electrodes depends on the presence of possible contaminants. Mechanically intact electrodes may show slow response due to clogging or coating. Table 1 shows some recommended cleaning solutions for glass electrodes. More detailed information about electrode care and maintenance can be obtained from the manufacturer.

ELECTRODE STORAGE

As a general rule, store the pH electrode in the same solution as the reference electrolyte of the electrode. In most cases this is a 3 mol/L KCl solution. Most manufacturers supply a plastic protection cap which is filled with this solution. Close off the filler opening if there is one. Never store your electrode in water as this will cause ions to leach out of the glass membrane, leading to a sluggish response.

RECONDITIONING ELECTRODES

Older electrodes, or electrodes that have been stored dry, may need to be 'reconditioned'. Recondition an electrode by soaking in pH 4.01 buffer or electrode storage solution for at least 30 minutes.

Table 1. Some recommended cleaning solutions for glass electrodes.

Deposit	Cleaning solution
General deposits	Mild detergent
Inorganic coatings	Commercial glass cleaning solution (not strongly acidic)
Metal compounds	Acid solution (not stronger than 1 mol/L)
Oil or grease	Complexing agent (EDTA) or suitable solvent
Resins, lignins	Acetone, alcohol or detergent (not strongly alkaline)
Proteins, blood etc	Enzyme solutions (eg pepsin in 0.1 mol/L HCl)
Stubborn deposits	Hydrogen peroxide, sodium hypochlorite
CAUTION: Care must be exercised when using solvents to clean electrodes that have a plastic body or a plastic protective skirt.	