Practical pH Measurements

In this technical note, the various voltage sources within a pH combination electrode are identified, and the contribution of each to the overall pH loop output voltage is reviewed. Sources of deviation of the pH electrode response from the ideal Nernst equation such as zero offsets and asymmetry potentials are presented. Sources of error caused by temperature dependence and acid or alkaline errors at the pH range extremes are also discussed.

Characteristics of a pH Measurement Loop

The characteristics of a pH measurement depend on the individual properties of the measuring and reference electrodes. Most electrodes in use to-day are combination electrodes. Finesse TrupH electrodes are also combination electrodes. This technical note will therefore be focused on com-bination electrodes only. Nonetheless, this infor-mation can be applied to measuring and refer-ence electrodes as well.

**The Different Potentials
of a Combination Electrode**

Figure 1 Six different potentials that form the total out-put potential of a pH electrode.

When a combination electrode is immersed in a sample solution, a potential, E_1, develops at the outer gel layer of the glass membrane. This outer gel layer forms a phase boundary between the glass membrane and the sample solution and the resulting potential depends on the pH value of the sample solution. The E_1 potential is therefore of primary interest. Unfortunately this potential can-not be measured directly. Only the total potential, E_{total}, which is the sum of many contributing poten-tials ($E_{total} = E_1 + E_2 + E_3 + E_4 + E_5 + E_6$) from other phase boundaries within the pH electrode, can be measured. The gel layer potential, E_1, must then be deduced from this composite total value.

As can be seen from figure 1, there are six po-tentials which develop within a TrupH electrode, but only one potential – E_1 – is proportional to the pH value of the sample solution. Ideally the other potentials, E_2 through E_6, should stay constant, in order to isolate the measurement of the relevant potential, E_1.

E_2 is called the "asymmetry potential" of the glass membrane. If the measuring and reference elec-trode use the same internal electrolyte, and if the electrode is immersed into a buffer solution having the same pH value as the internal electrolyte, then the potential difference between the inside and the outside of the glass membrane should theoretically be 0 mV. In reality, even a new pH electrode will show an asymmetry potential of a few millivolts. The magnitude of the asymmetry potential de-pends on the different gel layer thicknesses, and on the thickness of the glass membrane itself.

E_3 is the potential which develops on the inner gel layer of the glass membrane and is depen-dent on the hydrogen ion concentration of the in-ner buffer solution. As the pH of this inner buffer solution does not change in value, the potential E_3 should be constant at all times.
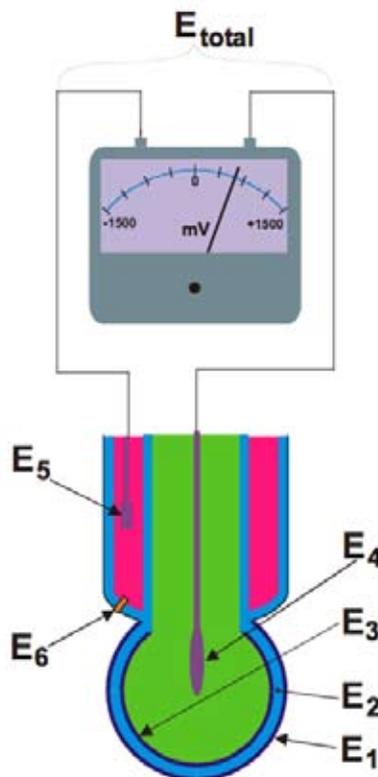
E_1 Potential developing on the outer gel layer

E_2 Asymmetry potential of the glass membrane

E_3 Potential developing on the inner gel layer

E_4 Potential developing on the internal conductor electrode.

E_5 Potential developing on the reference electrode

E_6 Diffusion potential of the diaphragm

E_{total} The total potential mea-sured by a pH electrode assembly is the sum of potentials E_1 to E_6

E_4 is the potential that develops at the metal and buffer solution phase boundary in the measuring electrode, while E_5 is the potential that develops at the metal and electrolyte phase boundary in the reference electrode. If both metal conductor systems are identical, and the buffer solution and electrolyte have the same chloride ion activity, then E_4 and E_5 are equal in magnitude but opposite in sign and cancel one another. Therefore, the sum of E_4 and E_5 does not contribute to the total potential output of the pH electrode.

E_6 is the diffusion potential of the diaphragm, and reflects the state of the boundary between the two electrolytes. This potential is non-zero when the two electrolytes differ in ionic concentration and composition. The magnitude of E_6 is determined by the amount of diffusion that occurs across this boundary owing to different ionic polarity and mobility.

As stated earlier, the potentials E_2 through E_6 should ideally be constant, so that changes in the total electrode potential only reflect changes in the pH potential, E_1. However, the individual potentials (E_2 through E_6) are subject to non-ideal deviations, thereby producing a zero point error in the pH electrode output. For this reason, a zero point calibration is always required prior to starting a pH measurement.

Zero point of a pH Electrode

The zero point of a pH electrode is the pH value at which the total electrode potential, E_{total} is equal to 0 mV. In theory, the zero point of a pH electrode is determined by the measuring electrode's internal buffer solution, which typically has the pH value of 7. If the pH value of the sample solution is also 7, then the potential across the pH electrode should be 0 mV.

In practice, however, this situation seldom occurs, because E_{total} is actually the sum of all potentials, E_1 through E_6. Each constituent potential reacts differently to the composition of a sample solution, as well as to any temperature changes during the measurement. Therefore it is difficult, if not impossible, to produce a pH electrode with a zero point that is both accurately defined and reproducible.

For example, the German Industrial Standard (DIN) stipulates that the zero point may, in fact, vary from –30 mV to +30 mV. Many manufacturers of pH electrodes deliberately set their pH electrode zero point at a slightly lower pH value (approximately 6.8 instead of the true 7.0), in order to compensate for electrode ageing: the zero point tends to drift upwards in pH value over the lifetime of the electrode. The repeatability of pH electrode measurements (the uncertainty factor) is seldom stated by manufacturers, because of this zero point drift. However, experience has shown that the repeatability of pH electrodes is seldom better than ± 0.02 pH units ($\approx$1.16 mV).

The exact zero point offset of a pH electrode must be established by the user prior to every new bioprocess run. The zero adjustment potentiometer of the pH meter/transmitter in the measurement loop is used to compensate for the zero point offset and set the loop output voltage to zero. Microprocessor based pH transmitters can adjust the zero point of a pH measurement loop automatically during the calibration procedure.

The zero point check and adjustment must be repeated regularly, because the zero point tends to drift due to:

a Diffusion of the sample solution into the reference electrolyte via the diaphragm. The sample solution can either poison or dilute the reference electrolyte, thereby affecting the chloride ion activity of the electrolyte, and consequently changing the reference electrode output potential.

b A change of the internal buffer solution. During exposure to high temperatures, the glass membrane of the measuring electrode will release alkali hydroxides into the inner buffer solution, gradually increase its pH value, and consequently change the asymmetry potential.

c An increase in electrode plug and cable contact resistance caused by corrosion of the metal contacts.

The Asymmetry Potential

Ideally, the potential difference across the glass membrane of a measuring electrode should be 0 mV if both the inner buffer and sample solutions have the same pH value (normally, pH=7). In practice, however, a potential difference of a few millivolts is measured across the membrane. The potential is called the asymmetry potential.

In reality, the asymmetry potential forms because the inner and outer gel layers experience very different environments over the electrode lifetime. The inner gel layer starts developing on the first day that the glass electrode is filled with inner buffer solution (during its manufacture) and will hardly alter thereafter, because it is isolated from the environment. The outer gel layer, however, is continuously attacked by the environment, such as chemical reactions with the sample solution or simple abrasion during handling.

The asymmetry potential can also be ascribed to small imperfections in the manufacture of the glass membrane. Exposure of the glass membrane to strong acid or alkaline solutions alters the external surface of the glass membrane to the extent that the response of the membrane to the presence of hydrogen ions gradually changes.

The asymmetry potential is specified to not exceed ± 47 mV (± pH 0.8) at a pH value of 7. Like the zero offset, the asymmetry potential can be eliminated using the zero potentiometer of the pH meter/transmitter during the calibration process of the electrode.

The Slope or Sensitivity of a pH Electrode Assembly

The slope of a pH electrode output is defined as the quotient of the potential difference developed per pH unit, namely:

$$Slope = \frac{\Delta U}{\Delta pH}$$

In theory, a pH electrode output slope at 25°C should be +59.16 mV/pH unit for pH values between 7 and 0, and –59.16 mV/pH unit for pH values between 7 and 14.

In practice, however, the output of a new and well hydrated pH electrode can reach, at best, only 99.8% of the theoretical value. With time, the slope will decrease so that the response will diminish further from the theoretical value. Initially, the slope decreases slowly; as the electrode ages, the slope will decrease more rapidly. It is essential to compensate for this reduction in slope during the calibration procedure, using the slope potentiometer of the pH meter/transmitter. As with the zero point adjustment, the slope adjustment must be performed at regular intervals, and always prior to a new bioprocess run.

The slope of a pH electrode output is also temperature dependent (see figure 2). According to the Nernst equation, the slope is proportional to the temperature (see the technical note entitled "pH Measurement Systems"). Therefore, the slope increases with increasing sample temperatures, and decreases with decreasing sample temperature. Furthermore, all temperature dependent slope lines intersect at the theoretical zero point of pH=7 (see figure 2).

Ideal pH Electrode Output Response

In order for a pH electrode to produce an output that follows the Nernst equation as closely as possible, the pH electrode design and implementation must result in the following characteristics:

a The inner and outer gel layers of the glass membrane must produce potentials having identical slopes.

b The internal buffer solution must maintain a constant pH value.

c The asymmetry potential should be as small and as constant as possible.

d The electrode assembly must be symmetrical, i.e. measuring and reference electrode must have identical conducting systems in order to cancel each other's galvanic potential.

Finally, the diffusion potential of the diaphragm should be as small and as constant as possible. In practice, however, the pH electrode output will have both an offset and a non-ideal slope, as illustrated in figure 3. Calibration is therefore required to optimize the electrode performance.
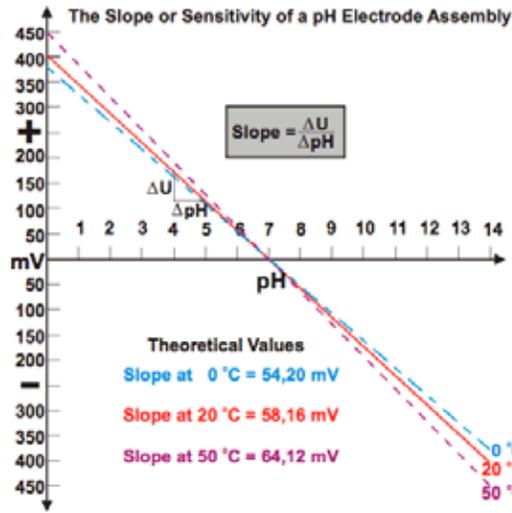
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Figure 2 Relationship between pH electrode output potential and the actual pH value of the sample solution: temperature dependence of slope.

Figure 3 Relationship between pH electrode output potential and the actual pH value of the sample solution: slope and asymmetry potential.

The Isotherm Intersection Point

In the previous section, figure 3 illustrated the temperature dependence of the slope, and the shift of the zero point pH value caused by the asymmetry potential. In this example, all lines still intersected at 0 mV, the theoretical zero output, even though the pH value was no longer 7. The inherent assumption was that all the potentials comprising the pH electrode output have the same temperature dependence. In practice, however, the temperature dependence of each contributing potential is somewhat different, so that the intersection point is not only offset in pH value, but also in voltage (see figure 4). This intersection point is denoted as the isotherm intersection point (U_{is} = isotherm potential).

In order to perform accurate pH measurements and properly calibrate the pH measurement loop, the isotherm intersection point must first be established. At least two buffer solutions are required. The position of the isotherm intersection point can then be determined by measuring the pH electrode output for these two buffer solutions as a function of temperature (i.e., heated buffer solutions). The mV outputs of the electrode assembly are then plotted against the actual pH value, and the position of the isotherm intersection point is thereby established. The magnitude and
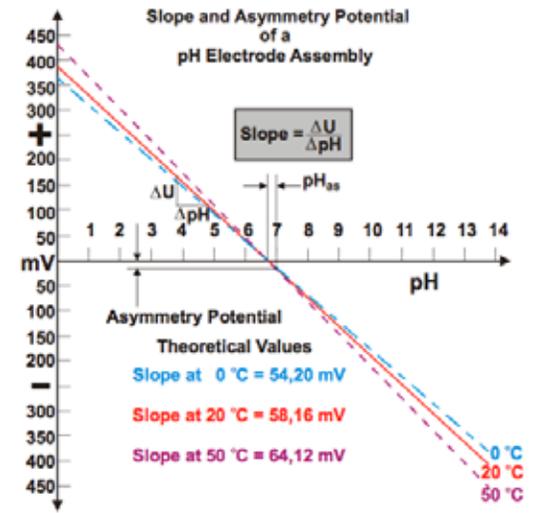
sign (polarity) of the resulting isotherm potential U_{is} can be zeroed if the pH meter/transmitter used is equipped with a U_{is} potentiometer. Most microprocessor based pH meters/transmitters have the ability to normalize the U_{is} potential to zero. New TrupH electrodes require a maximum compensation of 0.1 pH units, when calibrated at 25°C but operated in a solution at 60°C.
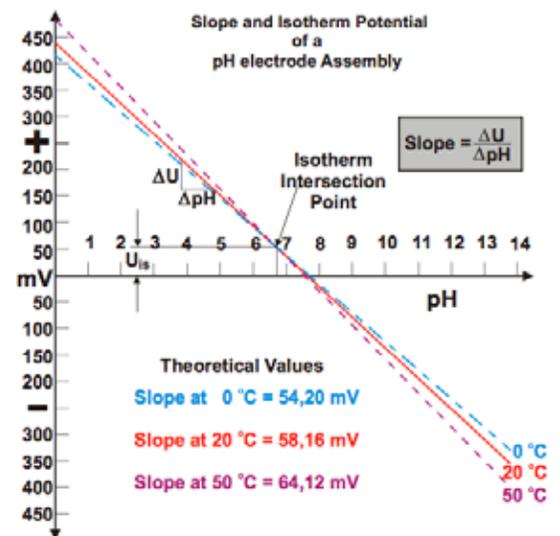

Figure 4 Relationship between pH electrode output potential and the actual pH value of the sample solution: the isotherm potential

Alkaline and Acid Error

Alkaline Error

At high pH values (pH > 10), the gel layer formed at the membrane of a measuring electrode is subject to certain changes which lead to measurement inaccuracies, called "alkaline error". This alkaline error is caused by a high concentration of alkaline ions, such as sodium ions (Na^+). These ions replace, either partly or completely, the hydrogen ions in the outer gel layer of the glass membrane, and by doing so, contribute to the potential at the outer phase boundary. Figure 5(a) illustrates alkaline error as a deviation from the theoretical straight line.
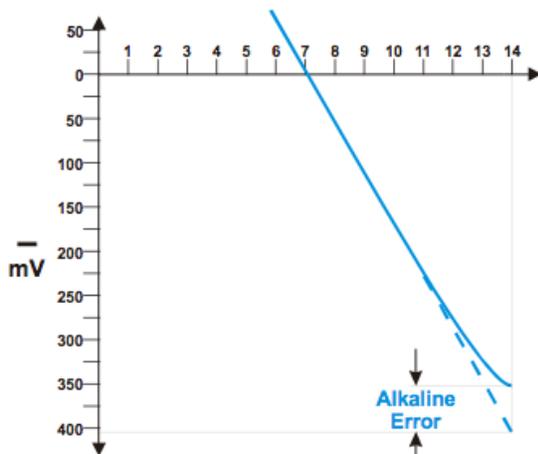
As a result, a pH value that is lower than the actual pH value of the sample solution will be measured. In the past, alkaline errors of glass electrodes began to manifest themselves at pH values as low as 9 and 10. Today, owing to glass membranes that contain lithium instead of sodium, the alkaline error only becomes noticeable at pH values above 12 or 13. The alkaline error increases with increasing pH values, higher alkaline concentrations and increasing temperatures.

In order to minimize the contribution of alkaline errors, pH electrode manufacturers use special membranes for electrodes that are used to measure high (alkaline) pH values.
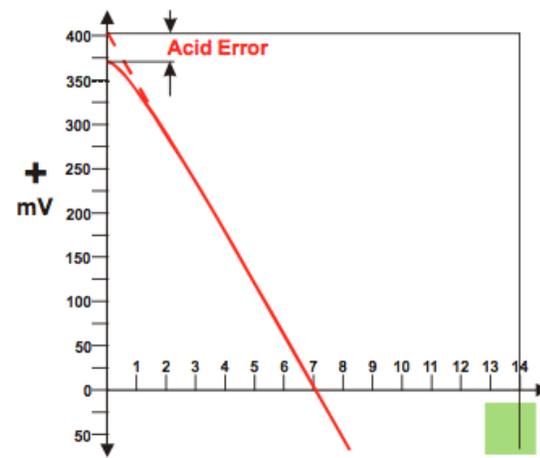

Figure 5 (a) alkaline error and (b) acid error at the extremes of the pH range

Acid Error

At low pH values (pH < 2), the potential difference between the measuring and reference electrodes will not conform exactly to the straight line predicted by the Nernst equation. Experimentally, it has been observed that the gel layer of the membrane will absorb acid molecules at very low pH values.

This absorption decreases the activity of hydrogen ions and results in a lower potential at the outer membrane phase boundary. The pH measurement therefore shows a higher pH value than the actual pH value of the sample solution. This effect is known as the acid error and is illustrated in figure 5(b). For these situations, manufacturers supply measuring electrodes with membrane glasses having specifically low acid errors.

Temperature Dependence and Compensation

As noted previously, pH measurements are temperature dependent. Three temperature factors must be considered when making pH measurements:

1 The temperature dependence of the Nernst equation (slope)
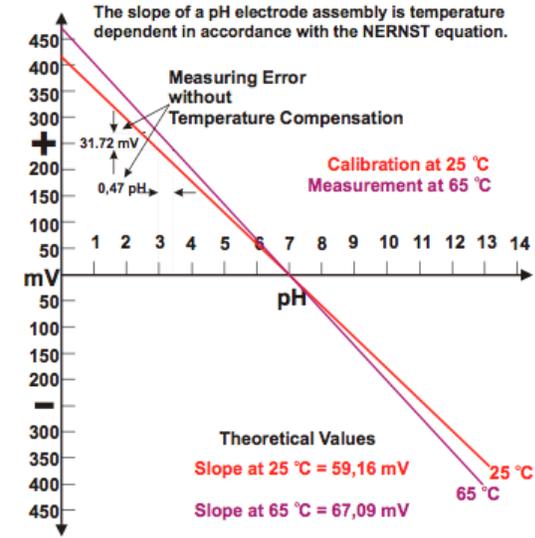
2 The isotherm intersection point

3 The actual effect of temperature on the pH value of the sample solution

The temperature dependence of the slope through the Nernst equation has already been discussed. This temperature effect on pH can be

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Figure 6 Temperature compensation for slope thermal dependence of a typical pH electrode.

accounted for by manufacturers in pH meters/transmitters through manual or automatic potentiometers that compensate using a real-time correlated temperature input (e.g., pH electrode thermistor or temperature sensor input). Figure 6 illustrates the theoretical slope error and the corresponding compensation method.

The isotherm intersection point results from the different temperature dependencies of each contributing potential and has also already been discussed. This temperature effect on pH is already mitigated by constructing electrodes such that the

isotherm intersection point is as close to the theoretical zero point (pH 7) as possible. This design reduces the residual error of conventional temperature compensation. Today, manufacturing processes for pH electrodes are sufficiently well controlled that most analog transmitter manufacturers omit the U_{is} potentiometer entirely. Microprocessor based pH meters/transmitters, however, have retained the ability to correct for the position of the isothermal intersection point (U_{is}).

The third factor is simply the temperature coefficient of the sample solution. Because the dissociation of molecules is highly temperature dependent, any change in temperature of the sample solution will produce a change in the hydrogen ion concentration of that solution, and therefore change its pH value. This pH change is purely physical, and cannot be described as a measurement error. The relationship between pH and temperature remains unknown for many acids and bases. It is therefore of the utmost importance to state the actual temperature of a sample solution when presenting its pH value, otherwise the pH result will become meaningless: for example, when calibrating pH probes, the user must account for the temperature dependence of the buffer solutions (see www.finesse.com for buffer temperature curves).
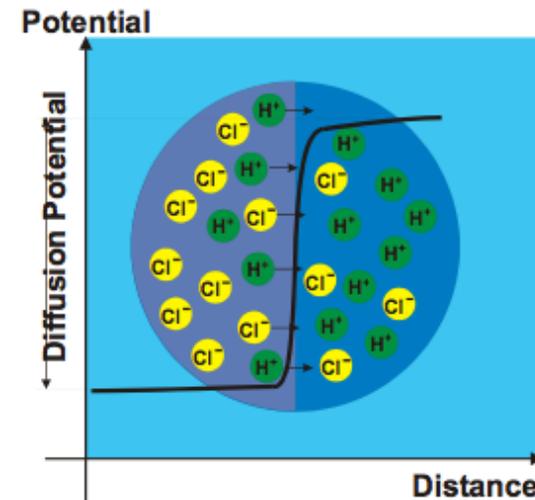
The Diffusion Potential

Figure 7 (left) diffusion potential created between two differenct concentrations of HCl solution, and **(right)** diffusion potentials created between a saturated KCl electrolyte and various sample solutions.

Diffusion Potentials
which develop between various solutions and a saturated KCl electrolyte

1.0	mole HCl	= 14.1 mV
0.1	mole HCl	= 4.6 mV
0.01	mole HCl	= 3.0 mV
0.1	mole KCl	= 1.8 mV
Buffer pH	1.68	= 3.3 mV
Buffer pH	4.01	= 2.6 mV
Buffer pH	4.65	= 3.1 mV
Buffer pH	7.00	= 1.9 mV
Buffer pH	10.01	= 1.8 mV
0.01	mole NaOH	= 2.3 mV
0.1	mole NaOH	= -0.4 mV
1.0	mole NaOH	= -8.6 mV

Technical Note

TrupH

Another contributor to error in pH measurements is the diffusion potential across the diaphragm, E_6. This potential always develops at the phase boundary if the inner and outer electrolytes have different concentrations or composition. The magnitude of the diffusion potential is determined by the different ionic migration velocities, which depend in turn on the polarity and size of the ion type. Figure 7(left) illustrates the creation of a diffusion potential between two HCl solutions (H^+ and Cl^- ions) having different concentrations.

Assume that the HCl concentration on the right is lower than that on the left. Because H^+ ions diffuse nearly five times faster than the Cl^- ions, any difference in concentration will immediately produce a H^+ concentration gradient and therefore a potential across the boundary of the two solutions. The potential will increase in size as the difference in concentrations between the two HCl solutions increases. Therefore, in order to keep the diffusion potential at the diaphragm of a reference electrode as small as possible, the different ions in the reference electrolyte must have mobilities that are as similar as possible. This situation can be achieved

with a 3 molar KCl solution. Note that for this same reason, 3 molar KCl makes an ideal electrode storage solution (PHS-STO), as it will not deplete the reference electrolyte of H^+ ions.

Figure 7(right) shows different diffusion potentials that can exist between a saturated KCl eletrolyte and various sample solutions. In general it is observed that:

1 The higher the KCl concentration of the reference electrolyte, the lower the diffusion potential.

2 The larger the flow rate of the reference electrolyte through the diaphragm, the smaller the diffusion potential.

3 The more the pH value of the sample solution differs from pH 7, the larger the diffusion potential.

From figure 7(right) it can be seen that different sample solutions will produce different diffusion potentials at the diaphragm of a reference electrode. These deviations must be accounted for when making pH measurements in bioprocess applications.

Diaphragm Contamination through Chemical Reaction

Chemical reactions between the reference electrolyte and the measured solution that occur at the diaphragm must be avoided at all costs. Such reactions will lead to diaphragm contamination, increase of resistance across the diaphragm and false pH readings. The reference electrolyte contains silver chloride, which is prone to chemical reactions, especially with sulphides. For this reason, great care must be taken when measuring pH in solutions containing sulphides, as the diaphragm may be contaminated with silver sulphide deposits.

Silver sulphide contamination can easily be identified by a blackened diaphragm, a substantial increase in the response time, and a greatly increased diaphragm resistance. Furthermore, such a contaminated electrode assembly is often impossible to calibrate.

In order to counteract silver sulphide contamination at the diaphragm TrupH electrodes have a unique reference electrode assembly shown in figure 8. This assembly consists of a silver chloride reservoir from which the silver reference wire leads to the electrode plug. The reservoir is separated from the reference electrolyte by a diffusion barrier consisting of densely packed mesh in a glass tube. The barrier prevents the loss of silver chloride into the reference electrolyte that is otherwise induced by temperature variations.

This unique reference system enhances the stability of the reference potential and extends the life of the combination electrode considerably.

Figure 8 Unique reference system that avoids silver sulphide contamination.

Reference conductor
Silver wire

Reference electrolyte with constant chloride concentration, but free of AgCl

Silver chloride reservoir

Diffusion pipe filled with packed mesh

Diaphragm