Application Note

TrupH

TrupH Calibration

TrupH measurement systems require proper handling during storage, cleaning, and calibration. In order to optimize the accuracy of a TrupH measurement, care must be taken when calibrating the pH measurement system. This application note describes the proper calibration procedures for TrupH electrodes. For storage and cleaning procedures, please refer to the application note entitled “TrupH Electrode Storage, Cleaning, and Aging”.

Standard and Technical pH Buffer Solutions

A prerequisite for accurate TrupH electrode calibration is the availability of a suitable buffer solution. pH buffer solutions are mixtures of weak acids and the strong base salt of these acids, or mixtures of weak bases and the strong acid salt of these bases. Buffer solutions are characterized by the fact that they resist change in their pH value regardless of additions of small quantities of acids or bases. Their hydrogen ion activity is stable over a wide range of dilution or concentration.

Standard Buffer Solutions The National Institute of Standards and Technology (NIST) recommends using nine different buffer solutions for the exact calibration of pH measuring systems. These buffer solutions also serve as reference points for the pH scale, as it is impossible to prove the activity of single hydrogen ions by measurement. All buffer solutions produced according to the NIST formulas are called *Standard Buffer Solutions*.

As the activity of hydrogen ions is temperature dependent, so also is the pH value of any buffer solution. The temperature dependence of NIST standard buffer solutions is given in table 1. Standard buffer solutions have accuracies better than ±0.005 pH units between 0°C and 60°C. Between 60°C and 95°C their accuracies are less than ±0.008 pH units. Reference laboratories exclusively use NIST buffer solutions.

Technical Buffer Solutions For bioprocess use, where the demand for absolute accuracy is normally not as high as in an analytical laboratory, so-called *Technical Buffer Solutions* can be used.

Technical buffer solutions are more stable than standard buffer solutions and are easier to manufacture. Their accuracies are given as ±0.02 pH units in the best cases, but can differ from manufacturer to manufacturer. The temperature dependence of technical buffer solutions is normally printed on their container by the manufacturer (Temperature dependence of the technical buffer solutions is also posted on our website: www.finesse-inc.com). Conventional technical buffer solutions have a limited shelf-life (typically one year in a sealed bottle). Once opened, their shelf-life is reduced to a few months. Alkaline buffer solutions are especially affected by CO_2 contamination from the atmosphere.

DURACAL pH buffer solutions (Product Number: PHS-BUF-## where ## = 01, 02, 03, …10, 11, 12) offer state-of-the-art stability in pH value for bioprocess applications. These patented technical buffer solutions are guaranteed to provide high accuracies for 5 years after the date of manufacture. The pH=9.21 and pH=10.01 buffer solutions remain stable, even if exposed to air (see Figure 1).

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3350 Scott Boulevard #1
Santa Clara, CA 95054

9351 Irvine Boulevard
Irvine, CA 92618

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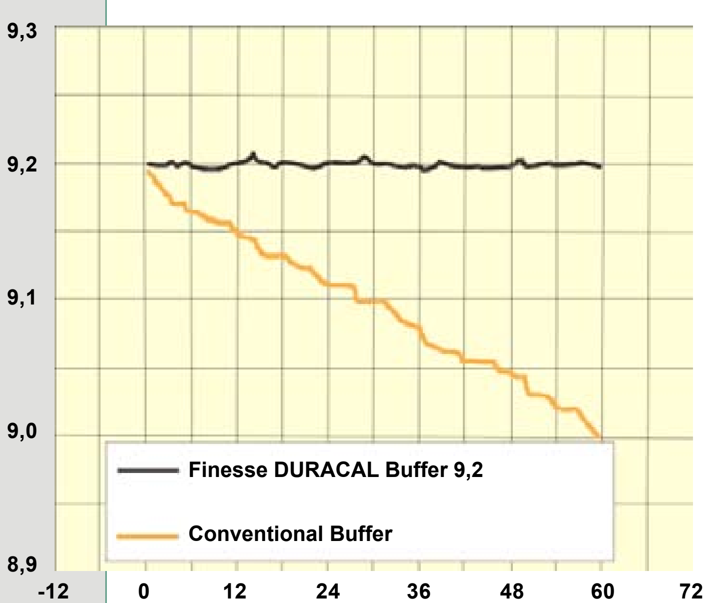
pH Values of Standard Buffer Solutions

Table 1 Temperature dependence of standard buffer solutions traceable to NIST standards.

NBS Code	A	B	H	C	D	E	F	I	G
Temp. C	Potassium tetraoxalate	Potassium hydrogen tartrate	Potassium dihydrogen citrate	Potassium hydrogen phthalate	Phosphate	Phosphate	Borax	Sodium carbonate/ sodium hydrogen carbonate	Calcium hydroxide
0	-	-	3,863	4,010	6,984	7,534	9,464	10,317	13,423
5	1,668	-	3,840	4,004	6,951	7,500	9,395	10,245	13,207
10	1,670	-	3,820	4,000	6,923	7,472	9,332	10,179	13,003
15	1,672	-	3,802	3,999	6,900	7,448	9,276	10,118	12,810
20	1,675	-	3,788	4,001	6,881	7,429	9,225	10,062	12,627
25	1,679	3,557	3,776	4,006	6,865	7,413	9,180	10,012	12,454
30	1,683	3,552	3,776	4,012	6,853	7,400	9,139	9,966	12,289
35	1,688	3,549	3,759	4,021	6,844	7,389	9,102	9,925	12,133
38	1,691	3,584	3,755	4,027	6,840	7,384	9,081	9,903	12,043
40	1,694	3,547	3,753	4,031	6,838	7,380	9,068	9,889	11,984
45	1,700	3,547	3,750	4,043	6,834	7,373	9,038	9,856	11,841
50	1,707	3,549	3,749	4,057	6,833	7,367	9,011	9,828	11,705
55	1,715	3,554	3,750	4,071	6,834	-	8,985	-	11,574
60	1,723	3,560	3,753	4,087	6,836	-	8,962	-	11,449
70	1,743	3,580	3,763	4,126	6,845	-	8,921	-	-
80	1,766	3,609	3,780	4,164	6,859	-	8,885	-	-
90	1,792	3,650	3,802	4,205	6,877	-	8,850	-	-
95	1,806	3,674	3,815	4,227	6,886	-	8,833	-	-

Each bottle of PHS-BUF (DURACAL) buffer solution is certified by the original manufacturer, and clearly indicates both the actual pH value and the expiration date. This certification is traceable to primary standards from NIST. Table 2 lists the suite of pH values offered by Finesse for TrupH calibration.

Figure 1 Stability Comparison of DURACAL and Conventional Alkaline Buffer Solutions when expose to blowing air



Used buffer solutions should always be discarded and never be returned to their original storage bottle. PHS-BUF buffer solutions have a unique storage bottle that provides a calibration compartment with a non-return valve at the bottom, thereby preventing any used buffer solution from ever reaching the storage bottle. This CALPACK bottle eliminates the need for a separate calibration container, and uses only the minimum required amount of buffer solution (figure 2).

Measurement accuracies better than the accuracy of the buffer solutions used for calibration cannot be achieved. Thus, the resulting worst case accuracy of TrupH probes calibrated using PHS-BUF buffer solutions will be ±0.02 pH units for typical bioprocess operating pH values that range from 5 to 9.

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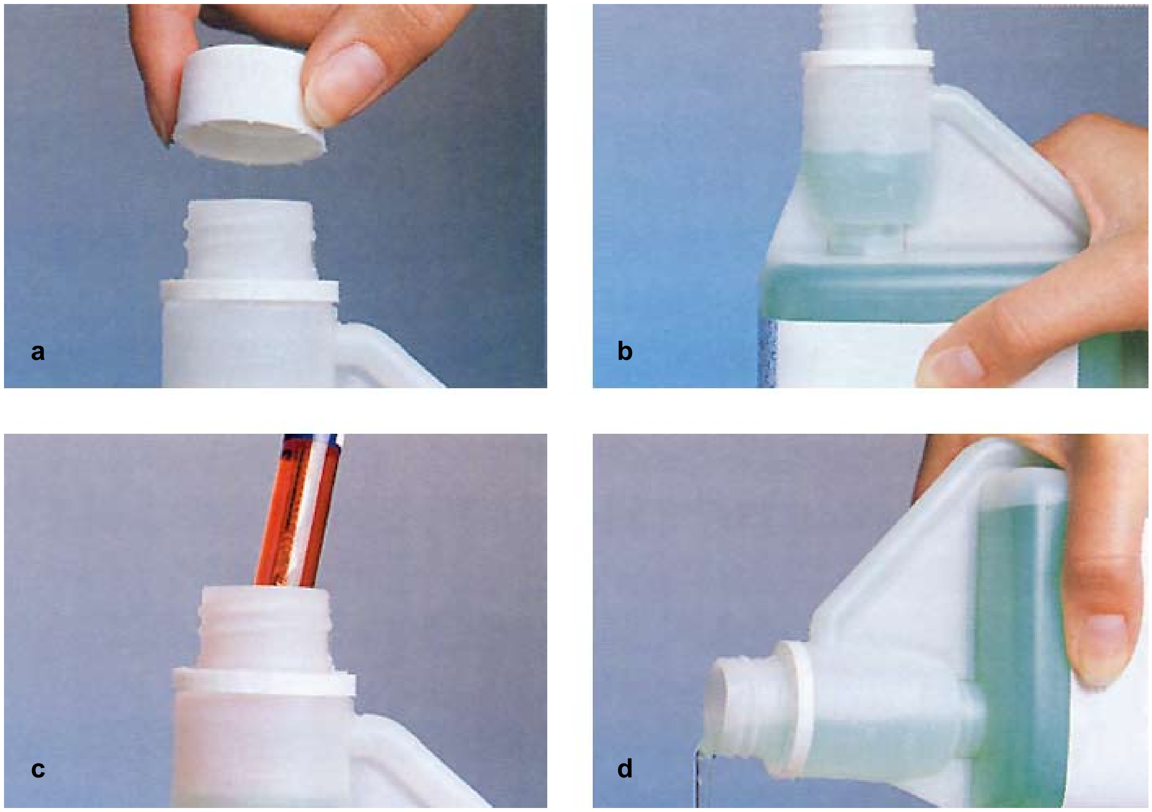

Figure 2 **CALPACK Calibration Bottles for easy and instant calibration. Four steps to calibrate a pH electrode, a) remove cap, b) tilt bottle to fill calibration reservoir, c) insert probe and calibrate, and d) discard contents of calibration reservoir into chemical waste.**

Table 2 **Values and Accuracies of DURACAL buffer solutions.**

Buffer Solution	pH value	Accuracy	Stability in months
PHS-BUF-001	1.09	+/- 0.02 pH	60
PHS-BUF-002	2.00	+/- 0.02 pH	60
PHS-BUF-003	3.06	+/- 0.02 pH	60
PHS-BUF-004	4.01	+/- 0.01 pH	60
PHS-BUF-005	5.00	+/- 0.02 pH	60
PHS-BUF-006	6.00	+/- 0.02 pH	60
PHS-BUF-007	7.00	+/- 0.01 pH	60
PHS-BUF-008	8.00	+/- 0.02 pH	60
PHS-BUF-009	9.21	+/- 0.02 pH	60
PHS-BUF-010	10.01	+/- 0.02 pH	60
PHS-BUF-011	11.00	+/- 0.05 pH	24
PHS-BUF-012	12.00	+/- 0.05 pH	24

The Calibration Procedure

As detailed in the technical note entitled “pH Measurement Systems”, pH electrode output voltages usually do not precisely follow the ideal Nernst potential equation, and their response changes over time. Therefore, in order to use a TrupH electrode to effectively perform accurate and repeatable pH measurements, the pH meter/transmitter used in conjunction with the electrode must be adapted to the ever-changing characteristics of the electrode; specifically, the entire pH measurement loop must be calibrated on a regular basis.

Note that a calibration procedure does not modify the electrode performance *per se*, but rather compensates for any electrode changes through the instrument that actually measures the electrode output voltage. In other words, the calibration process uses the electronic meter/transmitter to compensate for irregularities or aging in the pH electrode output. This compensation procedure relies on obtaining the correct output of the complete pH measurement loop (electrode + cable + meter/transmitter) for a series of buffer solutions having pre-defined pH values, and acting as reference standards.

The calibration procedure required will change based on the type of meter/transmitter used: for example, the calibration processes for analog and digital (microprocessor-controlled) pH meters/transmitters are different. Moreover, the calibration procedure will be highly dependent on the manufacturer of the meter/transmitter, so for best results, the calibration procedure described in the operating instructions of the pH meter/transmitter should be used.

A brief description of generic calibration procedures for both analog and digital pH meters/transmitters follows on the next page. For more details about the calibration process for TrupH electrodes using Finesse TruTransmitters, please refer to the TruTransmitter operating manual.

Calibration of Analog pH Meters

The number of buffer solutions required for calibration depends on the pH range of the process itself. If the process pH range is predominantly on the acidic (0 to 7) or the alkaline (7 to 14) range of the pH scale (e.g., measuring range 1 in figure 3), two buffer solutions will suffice. However, if the process pH range is essentially evenly distributed between the acid and alkaline regions of the pH scale (e.g.,measuring range II in figure 3), the use of three different buffer solutions is recommended. In all cases, a zero point buffer solution of pH = 7 is used. The following steps describe a typical calibration process for an analog pH meter:

1. **Buffer Solution selection** The first pH buffer solution is the zero point (pH = 7).

   For measurement range I, select the second buffer solution so that its pH value is as close as possible to the range endpoint (e.g., pH = 2) of the anticipated measuring range, and its pH value differs as much as possible from the zero buffer solution (always at least 2 pH units).

   For measurement range II, the pH value of the second buffer solution should lie in the acid region, while the third buffer solution should lie in the alkaline region of the desired measuring range. Both values should differ as much as possible from a pH of 7. For example, buffer values of pH = 4 and pH = 10 could be chosen for this calibration.

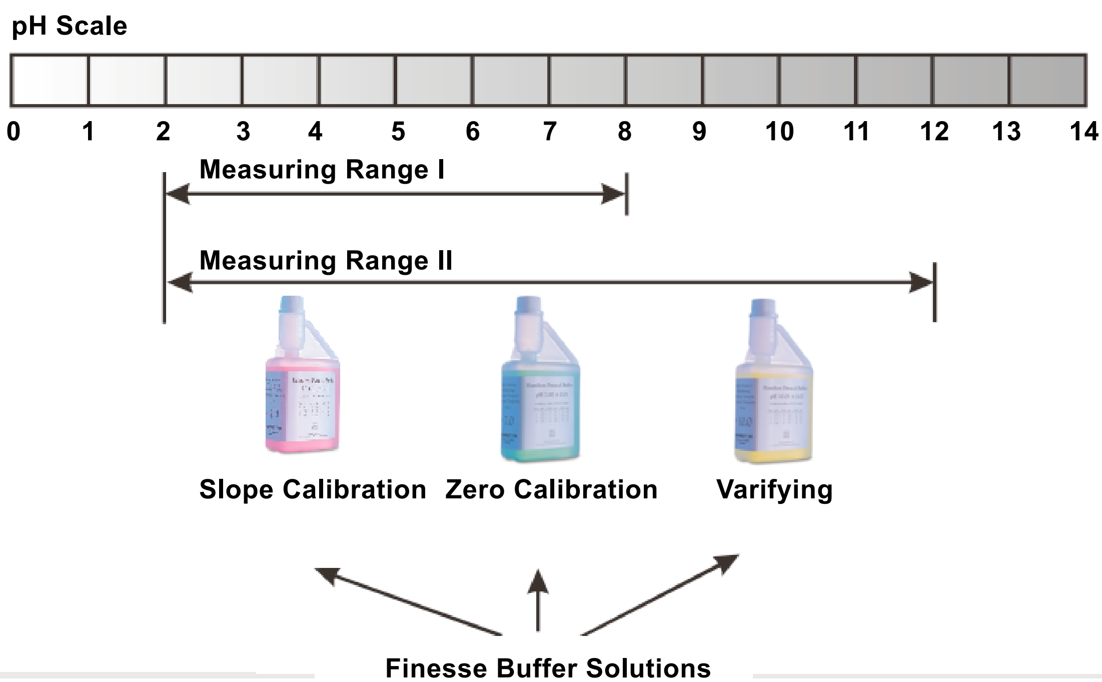

Figure 3 **Selection of Buffer Solutions for a pH electrode calibration procedure.**

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Santa Clara, CA 95054

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Irvine, CA 92618

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2 Temperature Compensation The pH value of any buffer solution is temperature dependent and must be controlled and/or measured during calibration, in order to provide an accurate reference pH value. The buffer solution temperature is the reference temperature for the meter/transmitter.

If the meter/transmitter has manual temperature compensation, the appropriate potentiometer must be set to the buffer solution temperature (figure 4). However, if the meter/transmitter has automatic temperature compensation, using either a separate temperature sensor or one inside the pH electrode, then this temperature sensor must be connected to the pH meter/transmitter and immersed in the buffer solution (see figure 4). In some cases, the temperature dependence of the technical buffer solutions must be programmed into the transmitter.

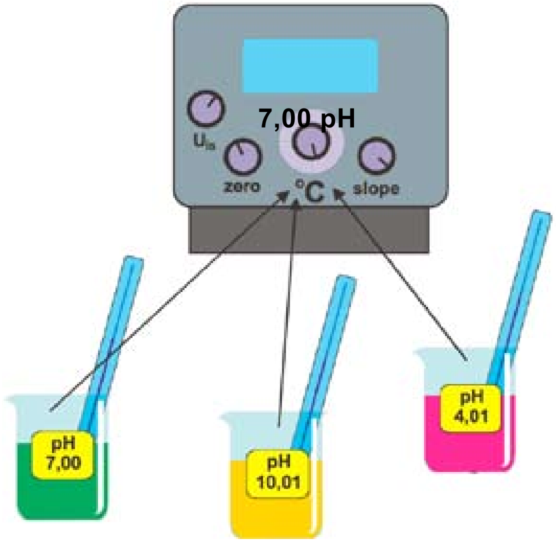

Figure 4 **Manual temperature compensation.**

3 Zero Point Calibration After being rinsed with de-ionized water, the electrode is immersed in the first buffer solution for zero point compensation. Normally the output voltage should stabilize to around 0 mV for pH 7 after no more than one minute. If the measured value doesn't correspond exactly to the target reading for the buffer solution, the meter output must be adjusted with the zero potentiometer, which is usually marked as "**@pH**" or "**pH$_{as}$**".

Note *For analog pH meters it is important that the zero point calibration always be performed first, prior to any slope calibration.*

4 After zero point calibration, rinse the electrode again with de-ionised water, and dry it with tissue paper. When drying the electrode, care must be taken to avoid rubbing the membrane along its length. Gently dab or blot the electrode with lint-free tissue paper (e.g., Kimwipes) until it is dry, in order to prevent the build-up of a static electric charge on the glass shaft of the electrode. Do not rub or twist the electrode through the tissue paper because this will create a static charge. Such static fields can preclude making accurate pH measurements for hours.

Alternatively, a set of" rinse" and "test" buffer solutions can be prepared. The rinse buffer solutions are used to clean the probe before putting it in the test buffers. If calibrating at pH 4 and pH 7, this means that four containers of buffer are required. The probe is first placed in the pH 4 rinse buffer and then moved to the pH 4 test buffer for calibration; when moving to the pH 7 buffer, the probe is first placed in the pH 7 rinse container for cleaning and then moved to the pH 7 container for calibration. This method avoids the potential of static build-up, and helps ensure that the test buffer is not contaminated.

Table 3 **Table of temperature dependence of buffer solutions used for calibration.**

Temperature Dependence of Technical Buffer Solutions

°C	Finesse Buffer Solutions		
5	4.01	7.09	10.19
10	4.00	7.06	10.15
15	4.00	7.04	10.11
18	4.00	7.03	10.08
20	4.00	7.02	10.06
22	4.00	7.01	10.04
25	4.01	7.00	10.01
30	4.01	6.99	9.97
35	4.02	6.98	9.92
40	4.03	6.97	9.86
45	4.04	6.97	9.83
50	4.05	6.97	9.79

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Santa Clara, CA 95054

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5 Slope Calibration The electrode assembly is then immersed in the second buffer solution (acid or base – according to the measuring range). After the pH meter reading has completely stabilized (typically 30 seconds to one minute), the measured value must be adjusted using the slope potentiometer so that it matches the reference pH value of the second buffer solution. The slope potentiometer is sometimes marked as "ΔmV/ΔpH". It is advisable to re-check the zero point calibration at this point.

Note *For analog pH meters, it is important that the slope calibration always be performed second, following the zero point calibration. Otherwise a valid calibration will not be obtained.*

6 Calibration Verification If the operating process covers both the acid and the alkaline regions of the pH scale, then a third buffer solution is used as a verification solution. The pH electrode must first be rinsed and dried (repeat step 4), and then immersed in the third buffer solution. The pH reading must again be allowed to stabilize for about one minute. If the third pH value reading corresponds to the reference value of the pH buffer solution within the targeted accuracy, this verification step is complete. If there is a significant discrepancy between the measured and reference pH values, the zero point and slope calibrations (steps 3 through 5) must be repeated. Be aware of possible acid and alkaline errors that are discussed in the section "Reasons for Calibration Problems".

7 In order to keep temperature-related errors (see the technical note entitled "Practical pH Measurements" regarding errors caused by the diffusion potential and/or the isotherm intersection point) to a minimum, it is recommended that the calibration be executed at or very close to the actual process temperature.

8 After the calibration process is complete, any used buffer solution should immediately be discarded (figure 5). **Never re-use buffer solutions and never return used buffer solution samples to their original storage container.**

Figure 5: Discard all used buffer solution immediately after use.

Calibration of Digital (Microprocessor-based) pH Meters

Digital (microprocessor-based) pH meters/transmitters, such as TruTransmitter, are becoming more widely adopted in both R&D laboratories and manufacturing facilities, owing to their ease of use and feature set. Many digital meter/transmitters will perform a calibration by guiding the user through a sequence of calibration steps and directly adjusting the zero point and slope. By automating the calibration process, digital meters/transmitters reduce the opportunity for user error during the pH loop calibration.

The digital meter/transmitter calibration procedure differs slightly from that previously described for conventional, analog pH meters. Typically, only two buffer solutions are required, and the zero point calibration step using the pH = 7 buffer solution is eliminated. However the aforemen-

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3350 Scott Boulevard #1
Santa Clara, CA 95054

9351 Irvine Boulevard
Irvine, CA 92618

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tioned method using rinse and test buffers can be applied here as well. The two buffer solutions required should as closely as is practical represent the two endpoints of the selected measurement span. The zero point, the slope and even the isotherm intersection point are all determined by the microprocessor during the calibration process.

In general the following calibration procedure is followed:

1 Two buffer solutions of different pH values must be selected. The pH value of the first buffer solution should approximate the most acidic pH value of the desired measurement range, while the pH value of the second buffer solution should approximate the most alkaline pH value of the target measurement range. For example, for bioprocess applications, it is normally appropriate to use pH = 4 and pH = 10 buffer solutions.

After the calibration mode of the pH meter is activated, the calibration menu guides the user through the calibration procedure. In some cases, the buffer solution pH values must be entered manually; generally, however, the meter/transmitter identifies the buffer solution pH values automatically.

2 The temperature of the buffer solutions must be controlled and constant. This temperature is measured with either a temperature sensor already incorporated inside the pH electrode, or with an independent thermometer. The temperature values are either entered manually or, when present inside the pH electrode, measured directly by the meter/transmitter.

Electrode rinsing, cleaning, and drying are performed according to the same instructions as presented in step 4 of the previous section. Similar stabilization times for the pH readings (30 seconds to one minute) after immersion into the buffer solutions should be used.

Re-calibration

pH electrode calibration must be repeated periodically. The exact time interval between calibrations depends on a number of factors such as the:

a measured pH value

b measured solution/media

c temperature of the measured solution/media

d age of the electrode

e required accuracy

f number of sterilization cycles

The calibration interval can vary from hours to days, or even to months, and has to be established individually for each application. Typically, however, a pH electrode must be calibrated prior to first time use in a bioprocess, and typically prior to any sterilization cycle (e.g., autoclave, steam in place, or clean in place). When working with a new media, it is advisable to re-calibrate the pH electrode as often as possible.

Response Time

A pH electrode will not produce the correct pH reading immediately after it is immersed in buffer solution. A response time of approximately 30 seconds is normally required by the pH electrode in order to produce a reasonably accurate pH reading, i.e., a reading that is within 0.01 pH units (0.6 mV) of the buffer solution reference pH value. For this reason, it is very important to wait for the pH reading on the meter/transmitter to stabilize during each step of the calibration cycle. Only after the pH reading has stabilized, can the meter/transmitter be adjusted to produce the correct reading. Premature interruption of the electrode output stabilization will result in an inaccurate pH measurement, and invalidate the calibration procedure.

The response time of an electrode assembly can be especially slow if the electrode temperature and the temperature of the buffer solution differ by more than 10°C. Although this situation rarely occurs in a bioreactor or fermentor, where temperature gradients are small and the temperature is ramped relatively slowly, it can occur during calibration, when the buffer solutions are operated well above room tempera-

Finesse, LLC
3350 Scott Boulevard #1
Santa Clara, CA 95054

9351 Irvine Boulevard
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ture to simulate a bioprocess (e.g., 37°C for mammalian cell culture process calibration). In cases where buffer solutions are heated, the electrode should be allowed to reach the temperature of the heated buffer solution before adjusting the meter/transmitter.

It should also be noted that the storage solution used can affect the time response of pH electrodes. For example, storing unused pH electrodes in distilled water can slow down their response time considerably, and the electrode will require regeneration in storage solution for at least 15 minutes. Conversely, 3 mole KCl solution (pH ~4) is the best storage medium for glass electrode assemblies, and these electrodes will respond quickly to a buffer solution.

Most modern digital pH meters/transmitters have an Auto-Read function for early detection of a stabilized reading. This feature is useful during calibration as it ensures that the electrode's response has truly stabilized, so that the pH reading is correct. Verification measurements have shown that the Auto-Read values do not differ more than 0.01 pH units from the final reading of the electrode when dealing with new electrodes that have had sufficient time to stabilize in the buffer solution.

Common Calibration Problems

Three common problems could be encountered when calibrating a pH measurement loop:

1 difficulty in achieving a zero point calibration

2 limited or no success in obtaining a slope calibration

3 very long response times (≥ 3 minutes)

Several different reasons can cause the above mentioned problems. The most frequent ones are:

a the buffer solutions used are either contaminated or have expired. The reference pH value associated with the buffer solution used may be incorrect – never store buffer solutions in unmarked bottles

b the electrolyte of the reference electrode is contaminated and/or the diaphragm is blocked

c a time-worn electrode is utilized

d an electrode which has not been hydrated for a sufficient time (after dry storage or after cleaning with an acid solution) is utilized

e hairline cracks are present in the membrane of the measuring electrode

f electrostatic charge is present on the electrode from rubbing the electrode shaft with cloth instead of gently dabbing it with tissue paper

g a temperature difference between electrode and buffer solution of greater than 10°C

h the zero point of the pH electrode and the meter/transmitter differ. This is seldom the case because both normally have pH 7 as their zero potential. In exceptional cases the pH electrode assembly can have a zero point which differs from pH 7

i the cable (and connectors) between the electrode assembly and meter/transmitter can also cause problems: either an open- or short-circuit in the cable, and/or connection plug (usually due to moisture contamination)

Predicting Achievable Accuracy

The accuracy of a pH measurement depends on many factors, some of which cannot be controlled. In order to minimize the influence of non-controllable factors on the pH measurement, the controllable factors must be monitored and controlled closely.

The calibration of the pH measurement loop is one of the most important factors. To ensure high measurement accuracy, the user must follow the correct calibration procedure, use new and accurate buffer solutions, and wait long enough for stable measurement values to

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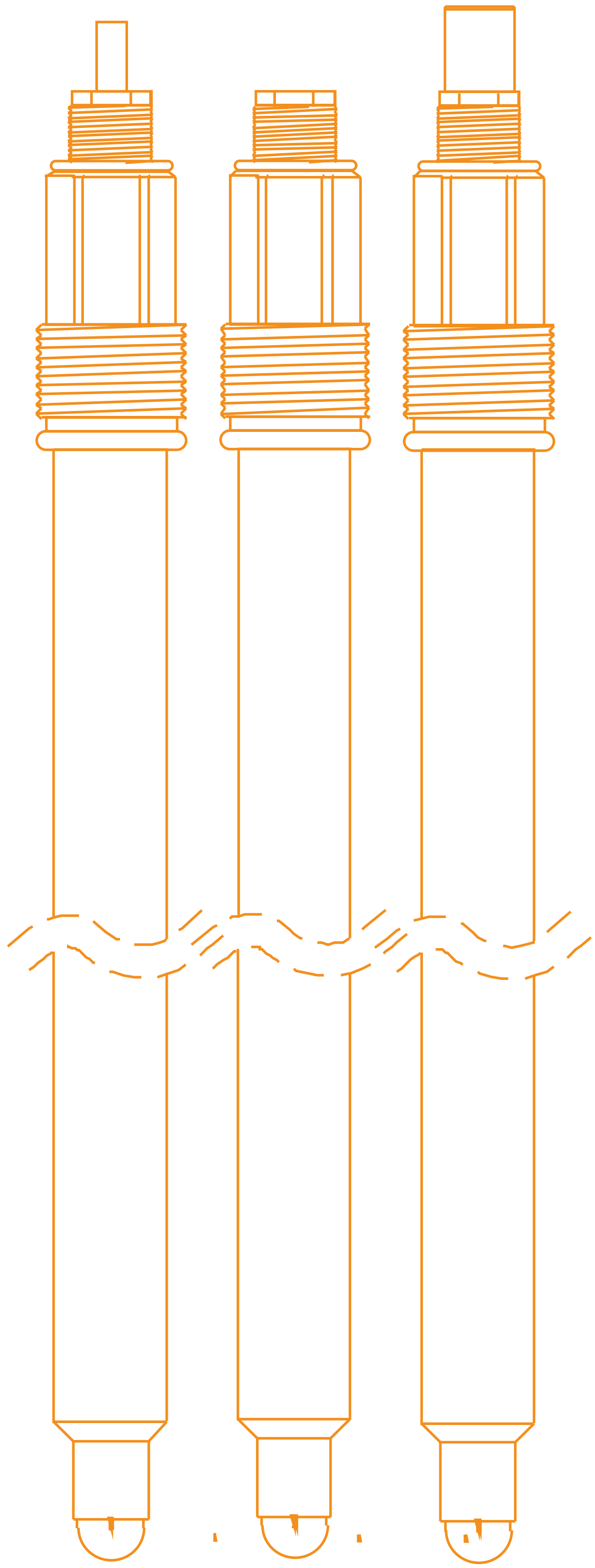
be achieved. Only then can the electrode be properly calibrated. The time interval between re-calibration should be as short as is practically possible. Recall that a pH measurement loop (electrode + cable + transmitter) cannot measure more accurately than the buffers with which it has been calibrated. Thus, the accuracy of pH measurement depends directly on the accuracy and traceability of the calibration buffers used.

It has been noted previously that temperature influences the pH measurement. In order to achieve high measurement accuracy, the temperature difference between the buffer solutions used for calibration and the measured medium must be minimized as much as possible. The temperature of the measured medium must be measured accurately and, if possible, controlled to a constant value, in order to perform effective temperature compensation.

The reference electrode must be kept pressurized to approximately 100 kPa (1 bar) above the pressure of the measured medium to keep the reference electrolyte flowing, and to prevent the ingress of measured solution into the reference electrolyte vessel. The diaphragm must be kept clean at all times. The correct matching of the diaphragm type to the measuring problem will significantly improve the measuring accuracy.

The conduction system and the electrolyte of measuring and reference electrodes must be identical. The membrane of the measuring electrode must be kept clean at all times. Deposits on the membrane glass will reduce the measuring accuracy dramatically and in extreme cases make a pH measurement impossible.

Accuracy expectations should not exceed practical achievable results. Realistic accuracies of between +/- 0.03 pH units and +/- 0.05 pH units can be achieved with a well hydrated and intact electrode when connected to an accurate, state-of-the-art digital pH transmitter having a high impedance of at least 10^{12} ohms. In the laboratory, accuracies of +/- 0.02 pH units are achievable.



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Santa Clara, CA 95054

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