pH Tips & Tricks

Tips & Tricks

for pH measurement

"Storing sensors and samples in different locations can affect pH results."

Want to know why?

Some time ago, METTLER TOLEDO had launched a series of tips and tricks emails for life science. These tips and tricks covered the areas of weighing, pipetting and pH measurement. Since the emails were very well received, we decided to compile them, to provide easier access to those who are interested.

This booklet is about tips and tricks for pH measurement. It will help you to achieve accurate results, by describing common mistakes and giving advice on how to improve operator technique.

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pH Measurement Tips & Tricks for Life Science

Summary

pH measurement is a frequent task in a lab, the results of which are often dependent on the performance of the instruments used. There are regular check-ups and calibration possibilities for your pH meters, but did you know that your measurements can be influenced by external factors, such as the surrounding temperature or the age of the buffer solutions? Did you know that there are cases where you measure the sensor and not the sample?

Technique directly affects the success and repeatability of your experiments and fortunately there are a few simple tips that will greatly improve your pH measuring performance.

1. Choosing the Right Electrode (Email 1)

The right electrode body and membrane

General purpose glass membranes suit most everyday laboratory applications. Select a plastic body for applications where rough handling and breakage are a major problem. Opt for a glass body for highly corrosive materials and high temperature applications. For more demanding applications, temperature, alkaline and acid resistant glasses are available.

Reference element and junction

Silver/silver chloride is the most common reference element, but chemical reactions may cause silver precipitation and consequently clogging of the reference junction. To avoid this, select an open or diaphragm (ceramic or PTFE (Polytetrafluoroethylene)) junction, depending on the requirements of your application.

Temperature range

pH is influenced by temperature. Selecting an electrode with a temperature sensor embedded in the probe body ensures automatic temperature compensation.

- Search for the right electrode
- Search pH electrodes by application

2. Choosing the Right Buffer Solution (Email 2)

Select the correct range of buffers

Always calibrate the pH meter over the entire measurement range. Outside the calibrated range, linearity is assumed and an extrapolated value is obtained. Always choose calibration standards that bracket the expected measurement range.

Use fresh buffers

Unopened buffers typically have a shelf life of 2 years, opened buffers 3 – 6 months and alkaline buffers (pH 10 or higher) 1 month, as their pH changes noticeably through contact with carbon dioxide in the air. Always use fresh buffer solution, store buffers in closed bottles and never use them after the expiry date.

Use buffers in the correct sequence

When calibrating the pH meter, use pH 7 buffer first, followed by a low pH (e.g. pH 4) or a high pH (e.g. pH 10) buffer for measurement of acidic and alkaline samples respectively. To measure a wide pH range, three point calibration with both high and low pH buffers is necessary.

- Electrode Accessories
- Quality Control Certificates

3. Avoiding Temperature Effects (Email 3)

Automatic and manual temperature compensation

Automatic temperature compensation (ATC) is most effective with sample volumes greater than 10 ml. Sensors with an integrated temperature probe allow ATC by the pH meter, whereas sensors without an integrated temperature probe require a separate probe. Manual temperature compensation is extremely accurate, but can be timeconsuming. Always enter temperatures in the measurement settings of the pH meter, and adjust with every temperature change.

Store sample and sensor at the same temperature

Store the sensor with the samples, as the greatest accuracy is achieved when the pH membrane, reference system and sample are at the same temperature.

Measure the sample, not the sensor

With very small samples, the sensor can take so long to reach equilibrium that its temperature is wrongly interpreted as the sample temperature. Take the time necessary to ensure that you actually measure the sample temperature.

- Guide to pH measurement

4. Micro-volume Containers (Email 4)

Micro pH sensors

Measuring the pH of small volume samples in micro-volume containers, such as 96-well plates or Nuclear Magnetic Resonance (NMR) tubes, can be difficult. The electrode can displace a relatively large volume of sample or it may not fit inside the tube.

Sample pH can affect the analysis and, for example, many protein samples are prepared at very low pH before adding neutralization buffer to raise the pH to 7 for further analysis. The volume of these samples is often less than 500 µl and, if the pH is checked after an experiment, it may be necessary to take measurements in volumes of just 20 - 100 µl. A range of micro and semi-micro sensors is available to help eliminate these problems, enabling pH measurement in sample volumes as small as 15 µl.

- InLab® Micro electrode
- InLab® Ultra-Micro electrode
- InLab® 751-4mm Micro Conductvity Probe

5. Tall Containers (Email 5)

Semi-micro sensors

Measuring pH in tall, narrow containers such as test tubes can be difficult, as the sample volume is still relatively low and the electrode may not fit inside the container. The sample solution level must be above the pH electrode reference junction when the electrode is immersed in the sample. Semi-micro sensors, with a shaft diameter of 5 - 6 mm, are significantly easier to use with test tubes and other small containers and enable pH measurement in small or narrow necked vessels, promoting efficient workflow.

Long shafted sensors

When measuring the pH of large sample volumes in very large containers, for example in 5 l beakers, it can be difficult to measure the pH in the center of the sample rather than on the surface of the liquid. Long shafted – 200 mm – sensors allow the electrode to be placed in the center of the solution being measured.

- Extra long pH micro electrode
- InLab® Flex Micro electrode
- InLab® Micro Pro electrode

6. Avoiding Contamination of the Electrolyte (Email 6)

Sample carry-over

There is always a risk of contamination – either by sample carry-over or by microbiological or genetic contamination – when measuring sample pH. By cleaning the sensor with RNase and DNase decontamination solutions, the potential for biological contamination is significantly reduced. Clean electrodes with RNase and DNase cleansers and autoclave to eliminate biohazards.

Diaphragm clogging

TRIS-based buffers can damage standard pH equipment, as the reference junction on conventional pH electrodes can clog when TRIS reacts with silver ions in the filling solution. This may also occur with protein in the buffer, such as BSA, eventually causing slow or fluctuating readings, or even entirely wrong results. To ensure reliable results and accurate buffer values choose an electrode specifically designed to be compatible with TRIS-based buffers.

- Electrolyte and cleaning solutions

7. Posters

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Technique directly affects the success and repeatability of your experiments. Luckily there are simple measures to ensure that your pH results are always accurate.

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