pH Measurement Basics

How to Measure pH

In Small Samples

Summary

Various applications in life sciences involve very small samples that require analysis by multiple protocols. These samples typically represent costly experiments, and sample manipulation can introduce error in the results. With the right pH sensor it is possible to minimize sample size and manipulation to achieve accurate results.

Some small-volume applications include:

- RNA sample analysis using microarrays
- DNA sample analysis using PCR
- Protein sample analysis using immunoprecipitation

The Challenge of Small Samples

There are three key components influencing the accuracy of the pH measurement: the sensing glass, the reference junction and the temperature input.

The sensing glass is the membrane at the bottom of a pH sensor. It is doped with ions allowing it to generate a voltage potential when it interacts with hydronium ions in solution. The resulting voltage potential is then converted into a useable pH reading. In order to generate an accurate voltage potential, the sensing glass must be in full contact with the solution being analyzed.

Partial exposure of the membrane to air during measurement will introduce error into the result. For this reason, standard sized sensors are not appropriate for use with very small samples. The sample does not completely cover the sensing glass, leaving an air/glass interface.

The reference junction is typically located just above the sensing glass. This allows reference electrolyte to flow out of the sensor and into the sample. Without steady electrolyte flow into the sample, error in the reading is unavoidable. Small samples rarely meet the electrolyte junction of a standard sized sensor, which prevents the flow of electrolyte into the sample and yields erroneous results.

pH results are only correct if the sample temperature is taken into account during the measurement. In very small samples the sample mass is negligible compared with the sensor mass. Thus, the sensor can take so long to reach equilibrium that the sensor temperature is wrongly interpreted as the sample temperature.

How to Measure Small Samples

Because the pH value of a sample represents the concentration of hydronium in the solution, it is frequently impossible to dilute the sample and still yield an accurate pH value. In such cases, the pH value must be obtained from the original sample solution, and specialized sensors can be employed to generate accurate data.

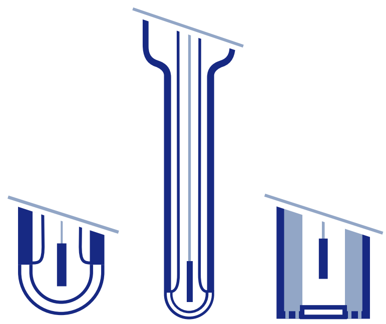

Figure 1: Standard sized sensors pictured next to equivalent micro and surface sensors.

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Micro Sensors

METTLER TOLEDO micro sensors include the same components as a standard pH sensor, configured to optimize measurements in small containers.

Whereas a standard sized sensor requires a few milliliters of sample to measure accurately, a micro sensor has miniaturized components and can be successfully used in much smaller samples. InLab® Micro-Pro-ISM sensor with integrated temperature probe has a small sensing glass and junction that can be completely submerged in samples of only a few microliters in volume. Its 5 mm diameter allows sample measurement in small containers.

For best results, micro sensors should be stored at the same temperature as samples, be it in the refrigerator or incubator, or at room temperature. This ensures that the pH membrane, reference system and sample are at the same temperature, thereby eliminating errors from temperature readings and yielding more accurate results.

Surface Sensors

Some samples are too small even for a micro sensor to measure accurately. In such cases, a surface sensor is the optimal configuration.

Surface sensors, such as METTLER TOLEDO's InLab® Surface-Pro-ISM, utilize flat sensing glass membranes and a ceramic ring junction. In such a case, a drop of the sample is placed on a clean, dry surface. The surface sensor is then placed on top of the drop to spread the sample across the entire pH membrane. With the ceramic ring junction, reference electrolyte is allowed to escape and make contact with the sample to yield an accurate measurement.

Figure 2: Drop measurement with an InLab Surface

This technique also prevents samples from being contaminated with reference electrolyte. The drop of sample used for the measurement is discarded afterwards.

		Minimal sample volume per micro sensor in specific container type		
Container type and typical sample size		**InLab® Nano** 1.7 mm diameter	**InLab® Ultra-Micro** 3.0 mm diameter	**InLab® Micro** 3.0 mm diameter
Small test tubes	> 2 mL	50 µL	100 µL	200 µL
LiteTouch tubes	1.5 – 1.7 mL	20 µL	25 µL	65 µL
Sample tubes	0.5 mL	20 µL	25 µL	65 µL
96 well plates	200 – 300 µL	10 µL	20 µL	45 µL
384 micro plates	5 – 100 µL	5 µL	15 µL	–

Table 1: Minimal sample volume required for measurement with micro sensors in specific container types

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