How to Measure pH
In Protein-Containing Samples

Summary
Measuring pH in protein-containing samples can be challenging as protein can foul both the pH sensing glass and classical ceramic junctions. In order to obtain accurate pH readings, both of those components must be maintained in optimal condition.

Protein Fouling of Sensing Glass

In any aqueous solution, acid is present in the form of hydronium ions. The sensing membrane in a pH sensor is specially designed to interact with these hydronium ions to generate a voltage potential, which is then converted into a useable pH value. In order for this interaction to happen, the sensing glass of the sensor must be free from contamination, such as protein residues. Any contamination present on the glass will limit the surface area available for interaction with hydronium and slow the reaction of the sensor.

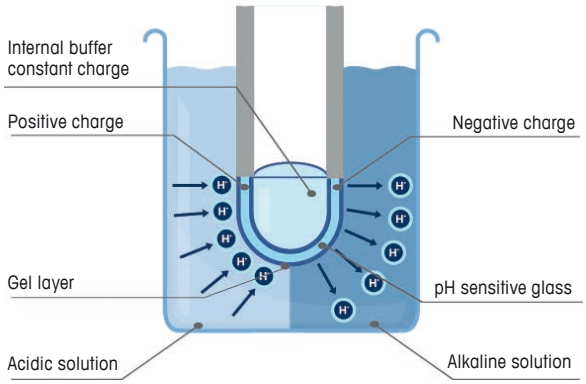

Figure 1: Creation of potential at glass membrane

A slow sensor is more than just an inconvenience. The pH meter to which the sensor is connected needs to find a mathematical endpoint based on the change in millivolt signal per a time unit. Since the change in millivolt signal is derived from the interaction of the hydronium in solution with the sensing glass of the sensor, protein contamination can have an effect on the value, introducing measurement error. When a sensor is free of protein residue the voltage potential changes very quickly over time as it acclimates to the new hydronium ion concentration. After a few seconds, this change in potential per second decreases, and when it decreases below the "stability criterion" for the meter, the final pH value is captured.

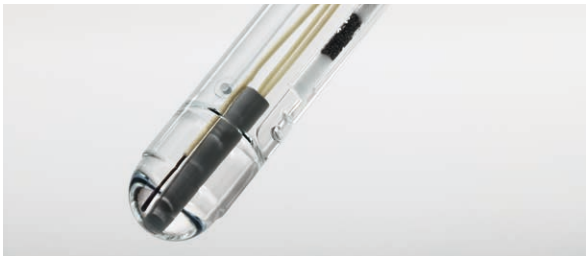

Figure 2: Clean, residue-free sensor

Due to the decreased available surface area of the sensing glass, the initial change in mV potential per unit time is smaller. In the same manner as the clean sensor, the contaminated sensor will also produce smaller changes in millivolt potential with time as the system reaches equilibrium. However, whether the sensor reacts quickly or slowly to a change in solution pH, the pH meter has the same "stability criterion."

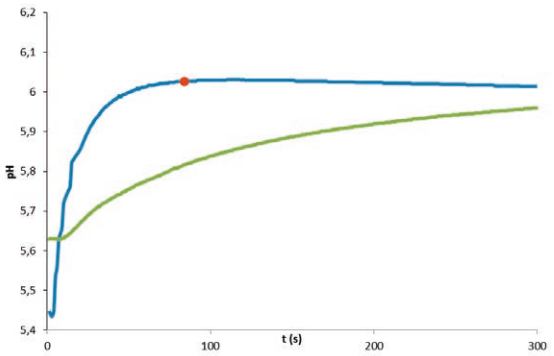

Figure 2: Response time of a clean vs. a contaminated sensor.
Clean membrane (blue) pH=6.026, Endpoint Time: 84s
Contaminated membrane (green) pH=6.022 Endpoint Time: 374s

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Protein Blockage of Ceramic Junctions

Just as with the sensing glass of the sensor, the junction is susceptible to protein fouling. A classical ceramic junction is a frit located just above the sensing glass of the sensor. The frit is comprised of small pores, designed to allow liquid electrolyte to flow out of the sensor and into the sample. The flow of electrolyte is critical to obtaining an accurate pH reading – it produces a stable reference potential and closes the circuit of the sensor. Without steady electrolyte flow into the sample, error in the reading is unavoidable.

Liquid electrolyte is a concentrated salt solution. Many times, when protein solutions are exposed to brines, the protein will precipitate to form a solid. When a solution is subjected to pH measurement, a salt gradient is formed, with the highest concentration of salt being located at the pH sensor's junction. This makes protein precipitation in the sensor's junction a likely scenario. As proteins precipitate in the small pores of a ceramic junction, electrolyte flow is slowed and eventually halted, introducing error into the pH reading.

How to Remove Protein Contamination

Protein contamination can be removed from both the pH sensing glass and the ceramic junction. Sensors contaminated with proteins should be cleaned by immersion in a solution of 5% pepsin and 0.1 mol/L hydrochloric acid for several hours. The solution can digest the protein deposits and leave both the junction and the sensing glass clean. A proactive approach to sensor maintenance can prevent the buildup of proteins in critical components of a pH sensor.

Figure 3: Left to right: clean junction (white "dot") is visible in front of black temperature probe and contaminated junction (black "dot")

After measuring concentrated protein solutions, a 15 minute soak in the same pepsin/hydrochloric acid solution will clean the sensing glass and junction. This maintenance routine will contribute to more accurate results throughout the life of the sensor.

Finding the Right Sensor

METTLER TOLEDO offers sensors designed for use in biological applications. Susceptibility of sensors to protein fouling can be significantly reduced with the right combination of sensing glass and junction.

InLab® Routine Pro-ISM

InLab Routine Pro-ISM is the standard pH sensor used in many labs and employs a ceramic frit. With the correct cleaning (Pepsin/HCl), this sensor provides very good results in proteinaceous samples.

InLab Max® Pro-ISM

The InLab Max Pro-ISM is similar in design to the InLab Routine Pro-ISM, with the introduction of a fixed sleeve junction instead of a ceramic frit. The electrolyte flow is fast, the junction is difficult to block, and the sensor is still very easy to handle.

InLab® Science Pro-ISM

The InLab Science Pro-ISM pH sensor uses A41 sensing glass, which is designed to resist protein fouling and is ideal for biological media. The sensor also employs a moveable glass sleeve junction instead of a ceramic fritted junction. The glass sleeve junction can be easily rinsed, and cannot be blocked by precipitated proteins.

Figure 4: (Left to right) InLab Science Pro-ISM (movable glass sleeve), InLab Max Pro-ISM (fixed glass sleeve) and InLab Routine Pro-ISM (ceramic frit)

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