APPLICATION NOTE

pH MEASUREMENT IN TRIS BUFFERS WITH IntelliCAL™ pH ULTRA PROBES

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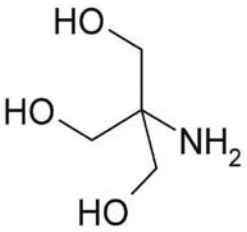


Introduction

Tris-(hydroxymethyl)-aminomethane (C4H11NO3; M = 121,14 g/mol) is a common buffer for biochemical, microbiological and pharmaceutical purposes. TRIS has a wide buffer capacity from pH 7.2 to 9.0, although the acidity constant is temperature dependent (pKs = -0.031 K-1).

Bioscientists and molecular biologists use TRIS frequently since it does not react with inhibiting enzymes, making it excellent for use for *in vitro* experiments. Some commonly used standard mixtures of TRIS contain HCl or EDTA. Due to the high reactivity of the amino group, TRIS is not typically used for chemical applications.

Why Use a Special pH Electrode for TRIS Buffers?

The standard Ag/AgCl reference as part of the normal pH electrode has only one liquid junction and an electrolyte of KCl with traces of Ag+ ions. A problem arises when proteins and related substances come in contact with the Ag+ ions forming precipitates in the liquid junction area and inhibit electrolyte flow. This causes errors in pH measurement due to high liquid junction potentials and can affect response times.

Since the alternative, a Calomel-reference electrode, uses mercury and is not recommended, a double junction reference electrode is the only choice. HACH's IntelliCAL™ pH ultra probe (PHC281) uses a double junction design with an outer electrolyte that is free of Ag+ ions.

To measure performance of the pH ultra probe, an internal test was conducted over 2 weeks with continuous operation in TRIS-HCl buffer solution and IUPAC pH buffers 4.01 and 10.012.

Figure 1: Slope (%) of pH ultra (PHC281) vs. time in TRIS-HCl

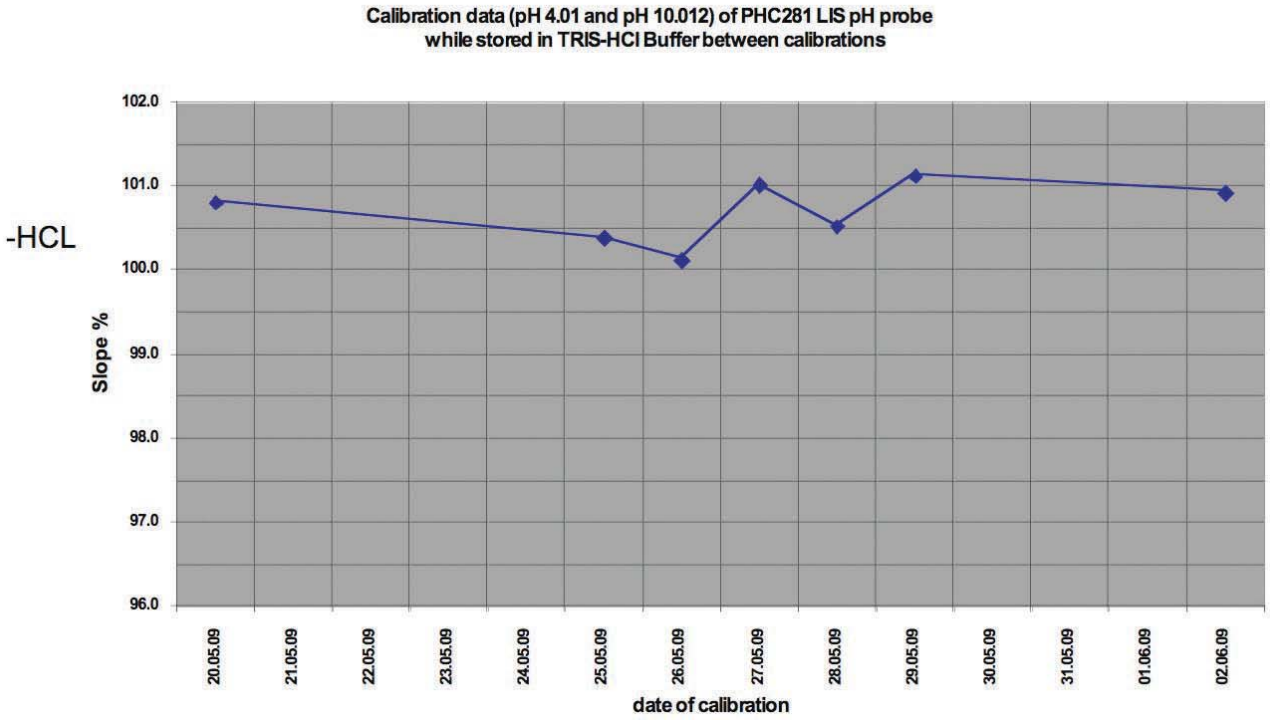
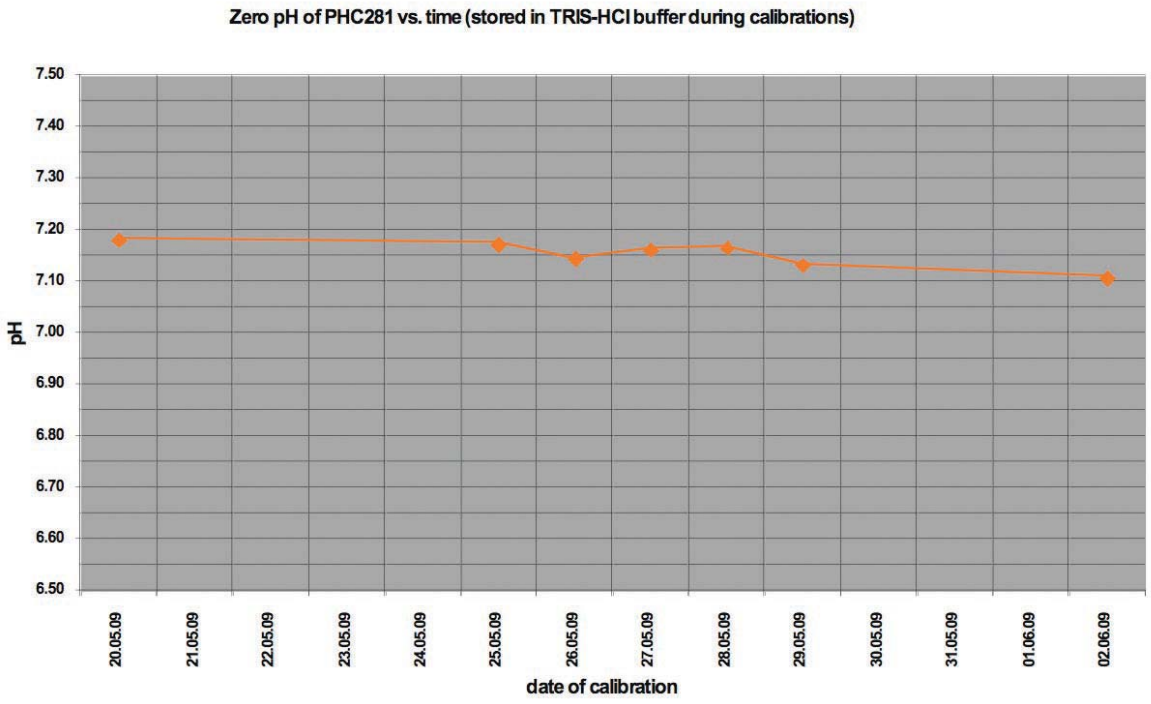


Figure 2: Zero mV of pH ultra (PHC281) vs. time



During 2 weeks of storage in TRIS-HCl buffer solution with pH 9.00, there is no recognizable influence on pH measurement or response time. The calibration data is within normal limits and the pH ultra electrode showed correlating data. No precipitation could be seen inside the electrode nor any recognizable blocking of the junction.

An additional study with the pH ultra electrode in TRIS-HCl buffer (pH 9) with the addition of BSA (a protein), showed after two weeks storage in that solution no precipitation of TRIS-Ag or BSA-complexes building up on the pH ultra probe. There were no changes in calibration data from the beginning of the experiment until the end. In summary, Hach recommends the pH ultra electrode for use in such media.

Maintenance

In order to maintain optimal performance of the pH ultra electrode in TRIS applications, thoroughly rinse the probe after sample measurement with deionized (DI) water. After cleaning, the probe should be stored in the protection cap containing the 2.44M KCl filling solution.

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