



NIST - NCL Joint Assay Protocol, PCC-13

Measuring the pH of Nanoparticle Suspensions

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

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1. Introduction

This document provides a protocol for measuring the pH of dilute nanoparticle suspensions of composition similar to NIST Reference Materials (RMs) 8011, 8012, and 8013, which contain gold nanoparticles in a dilute electrolyte solution. The pH is measured by combination semi-micro glass electrode using bracketing (2-point calibration). Traceability is to the pH values of the respective standard buffers. The measurement as described may be applied to small volumes (4 mL to 5 mL) and is therefore applicable to the contents of a single ampoule of each of the above RMs.

To minimize consumption of the nanoparticle suspension, this protocol for pH measurement may be performed following the measurement of the electrolytic conductivity, κ , of the same sample, provided that κ is measured according to the corresponding protocol listed on this Web site (PCC-12). However, seepage of KCl from the reference junction of the pH electrode during the measurement of pH contaminates the sample with K^+ and Cl^- ions, thereby increasing the κ value obtained subsequently. For this reason, in a serial measurement of pH and κ on a single sample, the κ measurement must be performed first.

Nanoparticle suspensions of significantly different composition from NIST RMs 8011, 8012, and 8013 may require substitution of different standard pH buffers to account for pH values outside the range 6.86 to 9.19. If such a substitution is performed, the two buffers should bracket the value of the sample being measured.

2. Reagents and Equipment

CAUTION: PERSONAL PROTECTION EQUIPMENT SUCH AS SAFETY GOGGLES, LAB COAT, AND RUBBER GLOVES (LATEX OR NITRILE) MUST BE USED WHEN OPERATING UNDER THIS PROTOCOL.

2.1 Reagents

- 2.1.1 Standard pH buffer, nominal pH 6.86 at 25°C, NIST Standard Reference Material® (SRM) 186 g or equivalent
- 2.1.2 Standard pH buffer, nominal pH 9.19 at 25°C, NIST SRM 187e or equivalent
- 2.1.3 Deionized water, resistivity at delivery $>17 \text{ M}\Omega\cdot\text{cm}$

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- 2.1.4 Ethanol, American Chemical Society (ACS) reagent grade, 95 %, Chemical Abstracts Service (CAS) reg. no. 64-17-5
- 2.1.5 Compressed dry air or nitrogen (ca. 1 L/min flow rate)
- 2.2 Equipment
 - 2.2.1 Semi-micro combination pH electrode, Corning^a 476156 or equivalent (“pH electrode”)
 - 2.2.2 pH/mV meter, resolution 0.1 mV or 0.001 pH
 - 2.2.3 Test tubes, 13.5 mm inside diameter, Corning 9800-16 or equivalent. Size may vary from that specified, provided that the glass bulb and reference junction of the pH electrode each remain below the surface level of the solution being measured at all times during the equilibration and measurement period (see Section 3.7)
 - 2.2.4 Water or oil bath, minimum volume of bath fluid 5 L, temperature controlled at $(25.0 \pm 0.1)^{\circ}\text{C}$
 - 2.2.5 Frame to hold test tubes (Section 2.2.3) vertically in the bath fluid
 - 2.2.6 Analytical balance (capacity 100 g, readability 0.0001 g)
 - 2.2.7 Top-loading balance (capacity 1.1 kg, readability 0.01 g)

3. Experimental Procedure

- 3.1 If using NIST SRMs as calibrants, prepare the two pH buffers according to certificate instructions. Pre-prepared commercial standard pH buffers may be used in place of the NIST pH SRMs with a small (likely insignificant) increase in the uncertainty, in which case equipment items 2.2.6 and 2.2.7 are not required.
- 3.2 Clean and dry sufficient test tubes to equal the number of samples to be measured. Use ethanol and a compressed air or nitrogen stream to dry the insides of the test tubes after rinsing.
- 3.3 Transfer 4 mL to 5 mL of the sample to be measured into separate clean, dry test tube(s).

^a Certain commercial equipment, instruments, or materials are identified in this procedure to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

- 3.4 Transfer a similar volume of each standard pH buffer into a separate, clean test tube. These test tubes may be rinsed several times with the calibrant or dried as in step 3.2 for the samples. Also fill a similar “rinse test tube” with ca. 10 mL deionized water for rinsing the pH electrode between measurements.
- 3.5 Place the calibrant, sample, and rinse test tubes into the water or oil bath set to 25.0°C. The surface of the liquid in each test tube should be below the bath fluid level. Allow all the test tubes to equilibrate in the bath for at least 15 min before commencing measurements.
- 3.6 Connect the pH electrode to the pH meter.
- 3.7 Perform a pH measurement on a single solution (see below for order of solutions) as follows:
 - 3.7.1 Place the pH electrode into the thermostated water test tube. Allow the pH electrode to temperature-equilibrate in the water test tube for 15 min.
 - 3.7.2 “Wick” excess water off the immersed portion of the pH electrode by touching it to the side of the test tube above the liquid level.
 - 3.7.3 Transfer the pH electrode directly to the solution to be measured.
 - 3.7.4 Gently agitate the pH electrode for 25 s.
 - 3.7.5 Let the pH electrode sit in the quiescent (unstirred) solution for an additional 25 s.
 - 3.7.6 Record the potential (E) indicated on the pH meter in mV.
 - 3.7.7 “Wick” excess solution off the immersed portion of the pH electrode. Transfer pH electrode to the water test tube for rinsing. Do not rinse the pH electrode with non-temperature-equilibrated water (e.g., from an un-thermostated wash bottle).
- 3.8 Perform Step 3.7 for the pH 9.19 standard buffer (S1).
- 3.9 Perform Step 3.7 for the pH 6.86 standard buffer (S2).
- 3.10 Perform Step 3.7 for each sample (X) to be measured. If several samples of similar composition and pH are to be measured, delete step 3.7.1 between such solutions. This omission minimizes unnecessary “shock” to the reference junction.
- 3.11 Perform Step 3.7 for the pH 6.86 standard buffer (S2).

3.12 Perform Step 3.7 for the pH 9.19 standard buffer (S1).

4. Calculations

4.1 Calculate the practical slope (k')^b of the pH calibration curve from the initial E values measured in Steps 3.8 and 3.9 for standard buffers S1 and S2 with respective certified pH values pH_{S1} and pH_{S2} :

$$k' = \frac{E_{\text{S1}} - E_{\text{S2}}}{\text{pH}_{\text{S2}} - \text{pH}_{\text{S1}}}$$

Use the certified pH value for each buffer at 25°C in the calculation. Also calculate the ratio of k' to the theoretical slope, $k = 59.16$ mV at 25°C. The ratio k'/k is frequently referred to as the (electromotive) efficiency of the pH electrode (2).

4.2 Calculate the pH of each sample X, pH_{X} , from the below equation using k' obtained in Step 4.1, where E_{X} is the measured E for that sample:

$$\text{pH}_{\text{X}} = \text{pH}_{\text{S1}} - \frac{E_{\text{X}} - E_{\text{S1}}}{k'}$$

4.3 Repeat steps 4.1 and 4.2 using the final E values obtained in Steps 3.11 and 3.12.

4.4 Average the pH_{X} based on the initial calibration and the pH_{X} based on the final calibration to obtain the final pH value for each sample. Calculate the deviation of the two pH_{X} values for each sample, which indicates the degree of reproducibility expected for the pH measurement.

5. Acceptance Criteria

5.1 In Step 4.1, $k'/k > 0.95$ (95 %).

5.2 The pH_{X} based on the initial calibration and the pH_{X} based on the final calibration for a given sample should agree to within 0.04.

5.3 If conditions 5.1 and/or 5.2 are not fulfilled, recondition the pH electrode per manufacturer's recommendations or replace the pH electrode. Then repeat the calibration and sample measurements with the reconditioned or replaced pH

^b In accordance with ref. (1), the equation for calculating k' is presented such that k' has a positive value.

electrode. New (unused) pH electrodes should be conditioned before use according to manufacturer's recommendations.

- 5.4 Measured pH values for replicated measurements of a given sample should not exceed the expanded uncertainty for pH stated in the certificate for NIST RMs 8011, 8012, and 8013.

6. References

1. R. Buck *et al.*, "Measurement of pH: Definition, Standards, and Procedures", *Pure Appl. Chem.*, 2002, **74**(11), 2169-2200.
2. F.G.K. Baucke, "Thermodynamic Origin of the Sub-Nernstian Response of Glass Electrodes" *Anal. Chem.*, 1994, **66**, 4519-4524.

7. Abbreviations

ACS	American Chemical Society
ca.	circa
CAS	Chemical Abstracts Service
E	potential
κ	electrolytic conductivity
k	theoretical slope
k'	practical slope
kHz	kilohertz
mV	millivolt
M Ω	megohm
NIST	National Institute of Standards and Technology
RM	Reference Material
s	second
SRM [®]	Standard Reference Material [®]