

Project:



Measuring the pH of Nanoparticle Suspensions

Subtitle

AUTHORED BY:	DATE:
Fanny Caputo	10.08.2015

REVIEWED BY:	DATE:
Matthias Roesslein	11.08.2015

APPROVED BY:	DATE:
Matthias Roesslein	18.2.2016

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1 Introduction

Many physical-chemical properties of nanoparticles in liquid state (e.g. size, surface charge, stability) strongly depend on the pH of the suspension. Therefore, the measurement of pH of nanoparticle suspensions in water based media should be performed in parallel to the other physical-chemical measurements in all the particular conditions tested, and pH values should be always associated with their outcomes.

This document provides the protocol for measuring the pH of dilute nanoparticle suspensions. The protocol is very general and should be adapted to the different pH meters available in the laboratories according to the instructions of the different manufactures.

2 Principle of the Method

pH is a measure of the hydrogen ion activity of a solution, which defines the degree of acidity or alkalinity of the solution. pH is defined as the negative logarithm of the hydrogen ion activity:

$$pH = -\log (aH^+) \quad (1)$$

Measure the pH of a solution with a potentiometric cell consisting of a pH-sensitive glass electrode, a reference electrode and a high-input impedance voltmeter. The output of the cell is temperature sensitive in accordance with the Nernst Equation, so a temperature electrode is also included in most systems. Walther Nernst equation for pH, it is:

$$E = E_0 + \frac{2.3 \cdot R \cdot T}{F} \cdot \log (aH^+) \quad (2)$$

where E equals the voltage of the cell, E_0 equals the standard voltage of the cell, R equals the Universal Gas Constant, F equals the Faraday Constant, and T is the temperature in degrees Kelvin. The $2.3 \cdot R \cdot T / F$ is the Nernst number or slope, and at 25° C, equals 59.16 mV/pH.

3 Applicability and Limitations (Scope)

No particular limits exist in the measurement of the pH of nanoparticles suspensions in water based media. pH measurements should be always associated to other measurements in liquid state, such as NP size, surface charge and stability.

To minimize the consumption of nanoparticle suspension two expedients are suggested:

- Use a microelectrode to reduce the volume of the solution you need to test
- Measure pH following the measurement of size by DLS and/or the measure of conductivity

Never measure pH on the same volume before conductivity measurement or before zeta potential measurements, due to the possible interference of K^+ and Cl^- ions that can leak from the pH electrode.

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4 Related Documents

Table 1:

Document ID	Document Title
EUNCL_PCC_001	<i>Measuring Batch Mode DLS</i>
EUNCL_PCC_002	<i>Measuring Zeta Potential</i>

5 Equipment and Reagents

5.1 Equipment

- pH electrode (possibly micro-electrode)
- pH/mV meter with minimum resolution of 0.01 pH or 1 mV. pH meters that allow to perform a self-check of the electrode performances (time response, drifting etc) are strongly recommended.
- Clean tubes/vials (e.g. 1.5 mL eppendorf) for the buffer and the sample

5.2 Reagents

- Standard pH buffer indicated from the manufacture.

Usually standard with nominal pH=4.00, pH=7.00 or 6.86 and pH=9.19 or 10.00 at 25°C are used. NIST standard references or other reliable commercial standards can be used (check the requirement of your system). Store properly the standards, do not contaminate them and do not use after the expiration date.

5.3 Reagent Preparation

- Prepare the standard as indicated by the manufacture
- Dilute the sample in the condition you need to test. Use the minimum volume needed in your pH meter/electrode system.

5.4 Procedure

Follow the procedure reported below

- Prepare the pH buffers according to certificate instructions.
- Calibrate the pH meter with at least two buffers, according to the pH you expect to measure in your sample. If a basic pH is expected in the nanoparticles suspension, then calibrate at least with 7 and 10 buffers. If an acidic pH is expected, then calibrate at least with 4 and 7 buffers. A three points calibration is always to be preferred, especially if you do not know the pH of your nanoparticles dispersions.

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- Perform the calibration of the system according to the experimental steps indicated by the US-NCL PCC-13 protocol [1]:
 1. Transfer x mL of the buffer solutions (x depend on the system used) to be measured into separate clean tubes/vials
 2. Equilibrate the test tubes (e.g. 1.5 mL eppendorf) containing the calibrant buffer at 25.0°C.
 3. Connect the pH electrode to the pH meter, wash it with de-ionized water and dry it with a clean tissue. Connect the temperature probe and put it into the bath to set the right temperature.
 4. Perform a pH measurement on a single solution (see below for order of solutions) by transferring the pH electrode directly to the solution to be measured
 5. Record the pH measured as indicated into the calibration procedure for your system. You may also take the mV value measure by the electrode to manually calculate the slope (see acceptance criteria). Check that the calibration meet the acceptance criteria (See session 6)
 6. Wash the electrode with distilled water and dry it with a clean tissue before to perform another measurement
- Repeat the 1-6 calibration steps for the other buffers. Every particular system has its own software and may require different actions to set and register the different calibration points. Read the instruction reported in your system and follow them. At the end of the calibration an experimental calibration slope k should be given.
- Check the calibration of your system by evaluating the experimental slope and comparing it with the theoretical slope, $k=59.16$ mV at 25°C, as described in newt session.
- Repeat the steps 1-6 to measure the pH of your samples using the measuring mode of your particular system.

6 Quality Control for calibration

To check the calibration of your system you can record the values $E_2 - E_1$ (mV) measured for standard buffers, the respective pH values $pH_2 - pH_1$ and calculate the ratio of k' to the theoretical slope, $k = 59.16$ mV at 25°C, being k' :

$$k' = \frac{E_2 - E_1}{pH_2 - pH_1} \quad (3)$$

Some pH meter may directly report k' after the calibration has been performed (check instruction manual).

The ratio k'/k is frequently referred to as the efficiency of the pH electrode and should be >0.95 (95%).

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Electrode performances are not reliable if there is a slow response and or a drifting during the measurement. The use of systems that allow to perform a self-check of the electrode performances (time response, drifting etc) are strongly recommended to periodically check the functionality of the electrode:

- To check the electrode response in time, many systems allow to set an acceptable threshold of the electrode time response during calibration (e.g. 60 s), and/or perform a time-response analysis to evaluate the good functionality of the electrodes.
- The absence of electrode drifting should also be checked. Many systems allow to check that drifting of electrode response is below a certain threshold. Check on the instruction manual of your pH meter for more information.

7 Maintenance and cleaning of the electrode

To maintain the electrode functional, routinely perform the following tasks:

- Rinse the electrode with clean water after use before putting it away and store it wet.
- Periodically clean the electrode thoroughly, by leaving it soaking for 10 to 15 minutes in a cleaning solution (see indication from the manufacture).
- Inspect the electrode for any scratches or cracks on the bulb or stem. If any are present, replace the electrode.

8 Flow chart

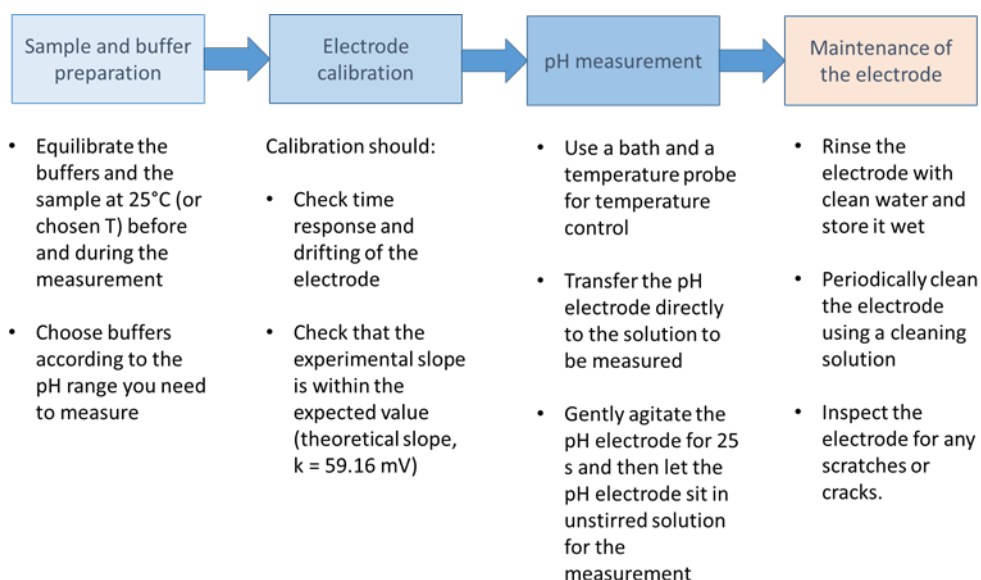


Figure 1. Flow chart that summarize the protocol

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9 Health and Safety Warnings, Cautions and Waste Treatment

Samples should be prepared in a biological safety hood to protect the sample from particulate and to minimize exposure; appropriate safety precautions and protective gear such as gloves, lab coat and goggles must be worn. After the measurement the samples should be discharged as appropriate for nanomaterials

10 References

[1] NCL method PCC-13, Measuring pH of nanoparticles suspensions, February 2008, revised April 2010.

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