1.0 Purpose and Scope

This procedure prescribes the Sandia National Laboratories (SNL) Waste Isolation Pilot Plant (WIPP) process for the calibration, operation, and maintenance of the NanoBrook ZetaPALS Zeta Potential Analyzer as part of the laboratory geochemistry research activities to determine the zeta potential of aqueous colloidal suspensions in support of the WIPP Project.

This procedure is applicable only for the NanoBrook ZetaPALS Zeta Potential Analyzer. This document is concise, and is not meant to substitute for, or serve as a reproduction of the manufacturers’ instruction manuals for the Zeta Potential Analyzer. The user is responsible for reading and understanding the appropriate manuals (see references).

1.1 Instrument Description

The Brookhaven NanoBrook ZetaPALS measures zeta potential of charged colloids dispersed in a conductive liquid at different pH values. It incorporates both electrophoretic light scattering (ELS) based on the principle of doppler velocimetry and phase analysis light scattering (PALS) for high conductivity measurements to determine electrophoretic mobility of roughly spherical colloids in an electric field. The high salt tolerance of PALS makes it possible to measure zeta potentials at extremely high ionic strengths or conductivities (salt concentrations up to 3 molar).

Acronyms and definitions for terms used in this procedure may be found in the Glossary located at the Sandia National Laboratories (SNL) WIPP Online Documents website.

2.0 Implementation Actions

2.1 Responsibility

The Principal Investigator (PI) or designee is responsible for implementing the requirements of this procedure.
The Technical Staff is responsible for performing the measurements following the requirements of this procedure and documenting all required information as described in this SP. Technical staff should familiarize themselves with the manufacturer’s manuals for the for an understanding of assembly parts, nomenclature, controls, operation, calibration, measurement, and cleaning methods. The NanoBrook is controlled by software via the host computer.

If the procedure cannot be implemented as written, the user has the responsibility to stop work and resolve all concerns with the PI/designee and/or the SNL Environmental Safety and Health programs (ES&H) coordinator, as appropriate, prior to proceeding with the work.

2.2 Safety

The activities described in this SP shall conform with the SNL Environmental Safety and Health programs (ES&H) as described in the current version of the laboratory TWD/SOP. Users shall review the attendant Job Safety Analysis as well as the NanoBrook manuals before using the instrument.

2.2.1 User Safety

- The NanoBrook comes with:
  - BI-ZR5 Zeta Potential reference material that contains anthraquinone (Permasil Blue FBL 150%). Exercise necessary caution as prescribed by the material safety data sheet (SDS) enclosed with the instrument manuals.
  - The instrument has a 40 mW, 640 nm temperature-controlled semiconductor Class 3 laser. Under normal operation, the laser is considered Class 1 because the red-diode laser source is enclosed.

2.3 Controls

Controls are established by written procedures or instructions prepared in accordance with NP 5-1, Implementing Procedures, and issued in accordance with NP 6-2, Document Control Process, of the Sandia National Laboratories WIPP Quality Assurance Program.

2.4 Instrument Start-Up

1. Flip the switch on the back of the NanoBrook ZetaPALS to the on position. A green LED light on the front of the instrument (bottom left) will illuminate.
2. Open the BIC Particle Solutions program located on the computer desktop.
3. Enter an Operator ID (name of technical staff).
4. Verify the instrument is connected. Under Instrument Information, ELS and PALS should be highlighted in blue (Figure 1). If they are not, click Update to sync the computer and instrument.

![Figure 1. BIC Particle Solutions main screen](image)

2.5 Clean Electrode

Always clean the BI-SREL electrode before use and after each measurement. The palladium plates at the bottom of the electrode should be rinsed between samples – even repeat measurements of the
same sample. Do not submerge the electrode in water (electronic component is located at top of electrode). Note: gloves should be worn when handling the electrode to keep it free from oils and dirt.

1. Rinse the electrode 3x with a deionized (DI) water squirt bottle.
2. Gently flick off water droplets.
3. Dry the electrode with a Kimwipe, making sure to also thread the wipe through the palladium plates.

Typically, DI water will suffice. If a black film is observed on the parallel plates, rinsing with alcohol may facilitate its removal. For extensive cleaning, see instructions in the included BI-ELECCK electrode cleaning kit.

2.6 Clean Cuvette/Cell

Disposable plastic (polystyrene or acrylic) cuvettes are used for aqueous solutions and simple polar liquids. Reusable glass cuvettes are used for organic solvents. Note: gloves should be worn when handling cuvettes to keep them free from oils, dirt, and scratches.

1. Rinse the cuvette 3x with DI water prior to use.
2. Shake out residual water inside the cuvette, wipe the exterior with a Kimwipe, and allow the cuvette to dry. If high purity air is available, it can be used to remove residual water droplets (or dust) in the cuvette.
3. Check for scratches or dust as these can alter results.
4. Clean glass cuvettes after use.

2.7 Sample Preparation and Running a Measurement

Note: steps should be taken to mitigate dust exposure as necessary, including but not limited to filtering the dispersant solution used to prepare aqueous samples and dilutions (if needed) and washing all glassware/containers. As mentioned above, rinse out the electrode between each sample to reduce contamination and rinse cuvettes.

Particle concentration ranges are sample specific, but ideal concentration ranges for measurements are around 0.1 to 1% by volume depending on particle size (smaller particles may require a more concentrated sample).

Suspensions should be equilibrated after preparation and prior to conducting an analysis (allowing to sit for a minimum of 24 to 48 hours depending on the sample). Suspensions should be homogenous, well-mixed and particles re-dispersed before analysis. Depending on the suspensions, this may involve vortexing for 30 seconds and then sonication in an ultrasonicator bath for at least 5 to 15 minutes prior to dispensing for measurement. Always check for bubbles in solution before measurements as they may impact results.

For suspensions in high ionic strength (>1 M salt concentration) solutions, the electrode assembly may need to be conditioned to create a uniform black coating on the electrodes. An example conditioning procedure: inserting the electrode in 1 M NaCl and running 350 cycles with frequency and voltage set to auto.

1. Transfer ~1.5 mL of liquid suspension into a cuvette. Cap the cuvette and gingerly move back and forth to ensure homogeneity. Remove the cap.
2. Carefully insert the electrode into the cuvette while holding the cuvette at a 45-degree angle until snug. The plates on the electrode must be completely submerged in liquid with no visible
air bubbles. Gently tap the cuvette on the bench top if air bubbles are observed in solution or on
the surface of the cuvette and electrode plates.
3. When placing the sample into the ZetaPALS instrument, connect the cable plug to the jack and
insert the cell holder with plug assembly to the right (see Figure 2). Push down gently to ensure
the sample is completely seated.
4. Close the sliding door.

Figure 2. Cell holder and electrode placement

2.7.1 Features of the electrophoretic light scattering (ELS) and Phase Analysis Light
Scattering (PALS) SOPs

1. Select the measurement type (e.g., “PALS Zeta Potential Measurement”) from the “New
Measurement” drop down menu and then click the New button (left). Software will initialize and
open a measurement window. Refer to Figure 3.

Figure 3. Main software window menu bar

2. In the measurement window (Figure 4), click the SOP button and then Load at the bottom left.
Create an SOP and save as a new procedure, which can be recalled later. Or review and make
changes to an existing SOP.
3. To the left side of the SOP window, you will see a tree with the following parameter options to populate (Figure 5). Note: the blue "i" icon provides information about the fields.

![PALS measurement window](image1.png)

**Figure 4. PALS measurement window**

![SOP measurement window](image2.png)

**Figure 5. SOP measurement window**
4. The options which should be specified in the SOP:

- Identification in which the user sets the Sample ID and inputs any Notes describing the analysis to accompany the saved data. Every time a new measurement is run, change the Sample ID.
- Instrument Parameters in which you select the Cell Type (i.e., cuvette) and Electrode Assembly (the BI-SREL (1,250 μL), which is solvent resistant).
- Measurement
  - Parameters in which the user sets the Temperature (typically 25 °C) either a Number of Cycles per measurement (typically 30), or a Target Residual value for the raw data fit (recommended for low mobility samples).
  - Advanced Settings
    - Equilibration Time may be used to invoke a delay after temperature has stabilized and before measurement data acquisition begins. 60 seconds is the least amount of time, 300 seconds (5 minutes) is typically sufficient for samples, 600 seconds (10 minutes) if longer equilibration is required (perhaps if a trend is observed during repeated measurements of the same sample). Allow the laser and temperature to stabilize at least 5 minutes before running samples. 10-minute equilibration may be used for an initial measurement or validation.
- Automation
  - Time Dependent in which the user indicates the number of Total Measurements (typically 5) as well as Time Interval Between Measurements. Time Interval Between Measurements is typically 0 unless a time course is required. The interval is the number of seconds between the end of one measurement and the beginning of the next.
- Sample Parameters
  - Liquid in which the dispersing fluid is specified. The default solvent is water. The dropdown menu contains several common solvents. If particular solvent information is not pre-populated, select Unspecified in the dropdown menu and input the solvent Viscosity, Refractive Index, and Dielectric Constant to obtain meaningful data. pH may also be specified but will not affect the measurement.
  - Particle in which particle characteristics are defined (e.g., Refractive Index and Concentration).
- Data Analysis
  - Model in which the user specifies how the raw data are fit. For aqueous samples and higher saturated brines (electrolytes with at least 1 mM salt), the Smoluchowski approximation is recommended. The model may also be changed between Smoluchowski, Hückel, and Henry after measurements.

5. Press Start. A clicking noise indicates the laser has initialized, a signal optimization screen will appear, and the measurements will begin. It could take up to 10 minutes initially to reach sample stability. Do not escape out of the measurement screen while it is running. The window can be minimized, or the main screen selected to review results while measurements continue in the background.

6. Generally, quality of measurements can be gauged by repeatability/stability of measurements. The user should be mindful of this especially for PALS measurements in brines. For repeat measurements, the less variability (i.e., lower standard deviation/standard error – specifically ≤ 10%), the better the data. If there is high variability, the user may have to dilute highly conductive samples, allow for the sample to equilibrate longer prior to or during the measurement, and/or adjust particle concentration in order to get repeatable results.

7. If a problem occurs, go to measurement window, clear, re-click SOP, load, and then populate. If re-running, the user may bypass stabilization since the instrument has already been stabilized.
2.8 **Instrument Shutdown**

After analysis is complete, close out of the BIC particle solutions software and turn off the ZetaPALS instrument by putting the switch to the off position (from I to O).

2.9 **Maintenance**

Refer to the Basic Guide in the owner’s manual for instructions on how to maintain the instrument.

The manufacturers reported lifespan of the electrode is 3 years. Depending on the use and care, the electrode can degrade faster. Use the reference material to determine when to change the electrode.

There are no user-serviceable parts on the ZetaPALS.

2.9.1 **Instrument References**

Hashmi, Sara M. (2016, January 12), Operating Instruction for the NanoBrook Omni Brookhaven Instruments, Yale University
https://lightscattering.yale.edu/sites/default/files/files/Brookhaven%20Instructions%20Quick%20Start.pdf

Brookhaven Instruments, Instruction Manual for the NanoBrook Series Particle/Protein Size and Zeta Potential Analyzer

2.10 **Calibration**

The Zeta Potential Analyzer is not an instrument that is calibrated using a known standard. The Zeta Potential Analyzer performance can be checked by analyzing the BI-ZR5 reference material.

A calibration check of the instrument will be performed every six months. Follow steps below to carry out an Instrument Standard Preparation and Validation. The results of the validation checks should agree with the following values.

> The standard deviation and standard error should be 10% or less. For validation, a mean zeta potential value of -44 ± 8 mV is acceptable.

2.10.1 **Instrument Standard Preparation and Validation**

Adapted from "Installing your NanoBrook.pdf". Brookhaven BI-ZR5 Zeta Potential Reference Material (anthraquinone) is a dark blue, inorganic powder that is insoluble in water. It is useful in monitoring instrument performance and factors affecting the zeta potential determination process. This solution will be adequate to use for 7 days after preparation.

1. To prepare the ZR5 reference material, start by making or ordering at least 20 mL of a 1 mM KCl solution filtered with 0.2 µm filter paper.
2. Carefully start by adding 6 flakes of the BI-ZR5 to approximately 20 mL of the 1 mM KCl solution in a scintillation vial.
3. Mix by capping and slowly inverting the vial several times until fully dispersed. Do not shake to avoid introducing bubbles.
4. Continue adding ZR5 flakes, as needed, and mixing until the solution develops a light purple color.
5. Rinse a clean cuvette and electrode with DI water and dry using a Kimwipe (as described in Sections 2.5 and 2.6).
6. Using a transfer pipette, dispense ~1.5 mL of the BI-ZR5 solution into the cuvette.
7. Insert the cleaned electrode into the filled cuvette. Check to assure the parallel plates on the electrode are completely submerged and there are no air bubbles. Gently tapping the cuvette can remove small bubbles.

8. Attach electrode and insert into cell holder with plug assembly to the right. Electrode should fit into the cuvette tightly.

9. See Figure 2 to verify the electrode is placed in the correct position. Close the cover.

10. Refer to the particle solutions software. Select a measurement option (ELS or PALS).

11. Select “New”.

12. On the measurement window, open the Performance Qualification SOP. Parameters are set.

13. Select “Load”. You will then return to the measurement window.

14. Press “Start” button to begin analysis. Notice a clicking noise when the optimization signal begins. The solution will take roughly 10 minutes to optimize and 5 minutes to complete measurements.

15. You can then click “Refresh” on the main software window to load your most recent analysis.

16. Under “Summary Statistics” the mean, standard deviation, and standard error will appear at the bottom of the window.

17. The standard deviation and standard error should be 10% or less. For validation, a mean zeta potential value of -44 ± 8 mV is acceptable.

18. New users should refer to “Particle Solutions Quick Start.pdf”

2.11 Reporting

1. On the main software screen, under the measurements tab select the type of measurement whether PALS or ELS. Click the Refresh button to update all samples and data.

![Main menu screen refresh button](Image)

2. Under the Summary Statistics tab you will see the displayed mean, standard deviation, and standard error at the bottom of the screen.

3. As per section 2.10: *The standard deviation and standard error should be 10% or less. For validation, a mean zeta potential value of -44 ± 8 mV is acceptable.*

Once the sample(s) have been measured and analyzed, print the data report, graphs, and/or summary. The report shall be recorded in a Scientific Notebook or a Scientific Notebook Supplement. Data can be exported as a .pdf, .csv, or Excel file (Figure 7).
3.0 Records

The following records, generated through implementation of this procedure, shall be prepared and submitted to the WIPP Records Center in accordance with [NP 17-1](#) (Records):

**QA Record**

- Scientific Notebook (SN)
- Scientific Notebook Supplement (SNS)

4.0 Appendices

Not applicable. There are no appendices in this SP.
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