DLS S.O.P

Instrument:

Zetasizer ZS90 from Malvern Panalytical

Software:

Zetasizer Software from Malvern-Version 7.13

Purpose:

The Zetasizer Nano can measure two characteristics of particles or molecules in a liquid medium:

1. **Particle Size**
2. **Zeta Potential**

The system can also be used to determine protein melting points, perform trend measurements, or conduct auto titrations including pH, additive or dilution titrations.

Particle Size: the diameter of the sphere that diffuses at the same speed as the particle being measured. Size is determined by measuring the Brownian motion (random movement of particles in liquid due to bombardment by the surrounding molecules). Small particles move faster while large particles move slower.

To Begin:

- **Know the specifications of the instrument.**

Specifications	ZS90
Laser	He-Ne, 4.0mW, 633nm
Size Range (DLS)	2nm-3μm
Measurement Technique Size	DLS
Volume (Size)	50 µL*
Size Range (Zeta)	3nm to 10µm
Measurement Technique (Zeta)	M3-*PALS*®
Volume (Zeta)	
Laser attenuation	Automatic, transmission 100% to 0.0003%
Detector	Avalanche photodiode, Q.E. >50% at 633nm
Temperature Range	2°C to 90°C

***Low volume cuvette required (DTS1070)**

Measuring Size

1. Flip the switch on the back right of the machine to turn it on and then click on the Zetasizer icon on the computer. The light source should be allowed to warm up for 5 minutes.

2. To create a new SOP, go to **File** … **New**…**SOP**

3. In the pop up window, click on **Measurement type** and enter **size**

4. Click on **Instrument Configuration**
    - Narrow Band Filter Fitted should say **No**
    - High Temperature Capable should say **No**

5. Click on **Sample**
    - Name your sample
    - Include any notes you would like

6. Click on **Material**
    - Click on **…** and choose your product from the drop down menu
        - If your product is not on the list, click **Add** and type in the product name, refractive index and absorption

7. Click on **Dispersant**
    - Click on **…** and choose your solvent from the drop down menu
        - If your solvent is not on the list, click **Add**
            - ➢ If your solvent is a pure liquid, choose **Simple Dispersant**, and enter the name of the solvent, the temperature (you can also put "any temperature"), the viscosity (if you put "any temperature" for temperature, you can put "varies with temperature" for viscosity), the refractive index, and the dielectric constant
            - ➢ If your solvent is a mixture of two or more liquids, choose **Complex Solvent** and enter the name of the solvent, its overall viscosity, its overall refractive index, the primary solvent, the additive and the concentration of the additive.

8. Click on **General Options**
    - make sure the box "**Use dispersant viscosity as sample viscosity**" is checked

9. Click on **Temperature**
    - **Set Temperature** is usually left at 25□C
    - **Equilibration Time** is usually 2 minutes

10. Click on **Cell** and select the type of cell you are using from the drop down menu

11. Click on **Measurement**
    - You can choose **manual** or **auto**, but usually auto is used
    - For **number of measurements**, 2 is normally used

12. You can skip over **Instructions** (everything should be left as it is)

13. Click on **Advanced**
    - Make sure **positioning method** says "seek for optimum position"
    - **Automatic attenuation selection** should say yes
    - **Extended duration for large particles** is usually left as no
14. Click on **Data Processing**
    - choose general purpose (normal resolution)

15. You can skip **Reports** (don't have to check anything)

16. Click on **Exports**
    - Make sure the **Export Results** box is checked
    - For **Export Template**, choose **Frequency Distribution**
    - For **Output File Name and Path**, search for your folder and save it

17. Click on **File**…**Save**, then close the SOP window (you will have to type in the SOP name and save it a second time

NOTE: Now that you have created an SOP template, you can use it to run your sample. If you have other samples to test, you will have to create a new SOP for each sample unless the product and solvent used in the other samples are exactly the same.

18. Go to **File**…**New**…**Measurement File**, then create a file name in your folder and save it

19. Choose **Size** from the drop down menu
20. Choose **browse for SOP** from the other drop down menu and click the **green arrow** to go to your folder and find your SOP.

21. Wash the cell you will use with Millipore water 3 times (unless you are using a disposable cell that has never been used before). Use the compressed air to blow the cell dry and then add your sample, which shouldn't be too dilute or too concentrated.

22. Insert the cell into the sample holder with the open window side facing you, close the lid and hit the **green arrow start button** in the pop up window. Click on the **Expert Advice** tab to see if the sample is running properly. When the measurement is finished, remember to save it.

<u>Zeta Potential:</u> Most liquids contain ions (+ or -). When a charged particle is suspended in a liquid, ions of an opposite charge will be attracted to the surface of the suspended particle forming 2 layers.

- The inside layer is called the **stern layer**
- The outside layer is called the **diffuse layer**
- The boundary between these two layers is called the **slipping plane**

The zeta potential is the potential created when the stern layer moves with the particle, but the diffuse layer does not.

- The zeta potential is affected by pH because a solution may be negatively or positively charged depending upon its pH
- The **Isoelectric Point** is the point at which the zeta potential is zero.

The zeta potential makes use of the Henry equation:

$$Ue = 2ezf(Ka)/3n$$

Where 3 = dielectric constant, n = viscosity, and f(Ka) = Henry's function where you either use 1.5 or 1.0

Electrophoretic Mobility is measured using the folded capillary cell

- particles move towards the electrode of opposite charge
- The velocity is measured and expressed in units of field strength as their mobility using Laser Doppler Velocimetry (LDV).

The Main Components of the Measurement System

- **Laser:** provides the light source that illuminates the particles in the sample. The laser is split into an incident and reference beam.
- **Cell:** The laser beam passes through the center of the sample cell
- **Detector:** When an electric field is applied to the cell, any paricles that are moving through the measurement volume will cause the intensity of light that hits the detector to fluctuate with a frequency proportional to the speed of the particles.
- **Digital Signal Processor:** receives information from the detector and sends signals to the computer
- **Computer:** the nano software produces a frequency spectrum from which the electrophoretic mobility and hence the zeta potential information is calculated.
- **Attenuator:** reduces the intensity of the laser and the scattering light beam.
- **Compensation Optics:** installed within the scattering beam path to maintain alignment of the scattering beams.

Measuring Zeta Potential

1. Flip the switch on the back right of the machine to turn it on and then click on the DTS(nano) icon on the computer. The light source should be allowed to warm up for 20 – 30 minutes.

2. To create a new SOP, go to **File** … **New**…**SOP**

3. In the pop up window, click on **Measurement type** and enter **Zeta Potential**

4. Click on **Instrument Configuration**

- Narrow Band Filter Fitted should say **No**
- High Temperature Capable should say **No**

5. Click on **Sample**
    - Name your sample
    - Include any notes you would like

6. Click on **Material**
    - Click on **…** and choose your product from the drop down menu
        - If your product is not on the list, click **Add** and type in the product name, refractive index and absorption

7. Click on **Dispersant**
    - Click on **…** and choose your solvent from the drop down menu
        - If your solvent is not on the list, click **Add**
            - If your solvent is a pure liquid, choose **Simple Dispersant**, and enter the name of the solvent, the temperature (you can also put "any temperature"), the viscosity (if you put "any temperature" for temperature, you can put "varies with temperature" for viscosity), the refractive index, and the dielectric constant
            - If your solvent is a mixture of two or more liquids, choose **Complex Solvent** and enter the name of the solvent, its overall viscosity, its overall refractive index, the primary solvent, the additive and the concentration of the additive.

8. Click on **General Options**
    - make sure the box "**Use dispersant viscosity as sample viscosity**" is checked

9. Click on **Temperature**
    - **Set Temperature** is usually left at 25□C
    - **Equilibration Time** is usually 2 minutes

10. Click on **Cell** and select DTS1060C Clear Disposable Zeta Cell from the drop down menu.

11. Click on **Measurement**
    - You can choose **manual** or **auto**, but usually auto is used
    - For **number of measurements**, 2 is normally used

12. You can skip over **Instructions** (everything should be left as it is)

13. Click on **Advanced**
    - **Automatic attenuation selection** should say yes
    - **Automatic Voltage Selection** should say yes

14. Click on **Data Processing**
    - choose **Auto mode**

15. You can skip **Reports** (don't have to check anything)

16. Click on **Exports**
    - Make sure the **Export Results** box is checked
    - For **Export Template**, choose **Frequency Distribution**
    - For **Output File Name and Path**, search for your folder

17. Click on **File**…**Save**, then close the SOP window (you will have to type in the SOP name and save it a second time)

NOTE: Now that you have created an SOP template, you can use it to run your sample. If you have other samples to test, you will have to create a new SOP for each sample unless the product and solvent used in the other samples are exactly the same.

18. Go to **File**…**New**…**measurement File**, then create a file name in your folder and save it

19. Choose **Zeta Potential** from the drop down menu

20. Choose **browse for SOP** from the other drop down menu and click the **green arrow** to go to your folder and find your SOP.

21. Wash the cell you will use with Millipore water 3 times (unless you are using a disposable cell that has never been used before). Use the compressed air to blow the cell dry and then add your sample, which shouldn't be too dilute or too concentrated. Avoid getting air bubbles in the folded capillary cell. A good way to do this is to fill a pipet with about 0.8ml of your sample, invert the cell, use the pipet to squirt your sample into the cell until it is filled up halfway (to the bend in the U – shape of the capillary), then turn the cell upright again and fill it the rest of the way. The electrodes should be completely submerged in the sample. Don't forget to put the stoppers in both sample ports!!

22. Insert the cell into the sample holder making sure that the electrodes are touching the electrode contacts, close the lid and hit the **green arrow start button** in the pop-up window. Click on the **Expert Advice Tab** to see if the sample is running properly. When the measurement is finished, remember to save it.

NOTE: Once you are done measuring your zeta potentials, a report will show up in a graph form with all the measured parameters.
- M = Zeta potential
- F(Ka) Value = displays the Henry function or approximation used during the

measurement
  - 1.5 means the Smoluchowski approximation was used
  - 1.0 means the Huckel approximation was used
- Conductivity = shows the ability of the sample to conduct electrical current

Exporting your Data

1. Open the measurement file you wish to export
2. The fastest way to save data is to highlight the grid and copy and paste into notepad.
   a. To save distribution plot data head to the PSD of interest and click on the top **Edit** tab, then select **Copy Size Values** or **Copy Size Graph**.
   b. All figures and tables can be copied this way.

3. If you would like to use the software export function follow the instructions below.

4. Go to **File**… **Export**
   a. Make sure **Export all Records** and **Export to File** are checked.
   b. The path should be **C:\Documents&Settings\Group\Desktop\exported.txt**
   c. Make sure **Append to File** is checked and hit **OK**

5. Click on the **Export Data Macro xls** icon on the desktop to open it. Then go back to the measurement window and highlight all of the data you wish to export and graph.

6. Go back to macro and click **RUN**. A pop-up window will appear, click on **Import Volume Distribution** and your graph along with a list of coordinates should show up in the macro

7. Save the Export Data Macro xls to your USB drive. Then, click on the **Exported** icon on the desktop. Your numerical data from the data table should show up here. Save this to your USB as well.
   i. **NOTE:** I also recommend simply copying your data from the measurement file and pasting it into excel and then saving the excel document on your USB. Once you get to your own computer, you can move the graph from the macro to the excel document along with the numerical data.

Another Option for Viewing Your Data on Your Computer

NOTE: Try the method above first because this method involves downloading software that allows you to graph your data from the company website and once you do that, they will hound you about buying products from them.

1. Go to www.malvern.com

2. You will need to register so click on the **Register** button on the right side of the screen. Once you have registered, go back to the home page and log in.

3. Go to **Support & Education** on the left side of the screen

4. Click on **Software Download**

5. Choose **Zetasizer Nano Series** and click on **more**

6. Choose the newest upgrade **version 7.13** and click on the **arrow button**

7. Save your data to your lap top and open it in excel using the Zetasizer nano series 7.13 software to replot your data.

If there are any further questions, contact:
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Main website: www.malvern.com
Or sign up in the Malvern website and learn through **Interactive Learning**:
www.malvernevents.com