

Protocol: Particle size distribution by Photon Correlation Spectroscopy (PCS)

1. Method

The instrument measures the fluctuations in light intensity due to the Brownian motion of the particles in order to deduce the particle size. The measurable size ranges from 2-3 [nm] to 500 nm – 1 micrometer. Dilute suspensions, on the order of 0.0001 to 1.0 [%] v/v are prepared, using suitable wetting and/or dispersing agents. A small ultrasonic treatment is sometimes useful in breaking up loosely-held agglomerates. A few [mL] of suspension are required to carry out the measurements. Just a few minutes are required for the sample and cell to equilibrate with the actively controlled temperature environment inside the instrument. Please note that avoiding dust is essential to carry out these measurements. It is often necessary to filter the solution of dispersant used for preparing the various dilutions and to wash all vessels in order to avoid the presence of dust. This is particularly important for particles with sizes below 50 [nm].

2. General recommendations for particle size distribution measurement

PCS measurements are very sensitive to dust and sedimentation. It is very important to filter the solvent used with filter of 0.2 μm and to work with stable colloidal suspension.

The count rate should not be more than 500 kcps to avoid retro-diffusion.

3. Equipment

- Measurement device: Brookhaven BI-9000AT (more info from <http://www.bic.com>)
- Software versions (9kpsdw32.exe: 4.14; bi_+kapiw32.dll: 2.5; bi_zetapw32.dll: 3.4; Eclectic: 4.4; bi_nnlsw32.dll: 1.8)
- Disposable, acrylic square cells (4 faces) are used for aqueous and simple alcohol suspensions;
- A special glass cell can be used with aggressive solvents;
- Ultrasonication bath in MXD 313

4. Preparations of the samples

- The ideal concentration range for the measurement is 0.1-10 [mg]/[mL] in 0.2 μm filtered solvent.
- Put your suspension in ultrasonic bath for 5-15 minutes
- Approximately 1.5 mL of this suspension is then transferred to the specific cell of measurement (4 faces).
- **If the sample is prepared in an aggressive solvent, use a specific glass electrode**
- Add the cell inside the ZetaPals and cover it with the circle metallic cover.
- It is recommended to perform 3 measurements per sample

5. Operations

- Switch on the instrument (using the main switch or behind instrument).
- Type the username: ZetaPals. There is no password.
- Click twice the icon “BIC Particles Sizing Software”
 - o One can hear a “clic” which indicates the laser has started
 - o Before starting a measurement a delay of minimum 5 minutes must be respected to stabilize the laser and the temperature in the cell

- Select the menu “File”, then “Database”, double click on the file name you want to start with, then select exit.
 - o If you don’t have any user name you can add it on create folder.
- Click on “Clear” before starting a new measurement
- In the bottom of the ZetaPals main window, click on “Parameters”, and fill the table as described with the example of SPION

Sample ID	SPION-MB19
Operator ID	LM
Notes	100 ug/mL ; NaCl 10 mM
Runs	10 (between 1 to 10)
Temperature	25°C (it is advised to work at room temperature to improve the sample stability, but this can be changed if necessary)
Liquid aqueous	(viscosity, refractive index are automatic) You can add your own liquid if it is not in the list.
Angle	90°
Batch number	From 1 to 99999
Run duration	Minutes and Seconds
Refractive index	Real: 2.42 Imaginary: 0
Shape	Uniform spheres <input checked="" type="checkbox"/> Thin shells
Dust Cutoff	30
	Select Auto save results (Very important) <input checked="" type="checkbox"/>

- Click on “Start”
- For the first measurement, the software will optimize the count rate.
- It is better to optimize the count rate for each measurement:
 - o Restart the software for each measurement
- During the measurement:
 - o The Current or Average count rate must be between 100 and 500 kcps
 - o If it is not the case you should dilute or up-concentrate your sample.
 - o The “Polydispersity” indicates the broadness of the size distribution. Below 0.05, the particle size distribution is quasi monodisperse.
 - o The effective diameter is the diameter given in Lognormal distribution.
 - o You can choose the graph on the main window on top right: Lognormal, MSD (Multimodal Size Distribution) or correlation function.
 - **It is better to work with MSD model if the population is polydisperse (Polydispersity > 0.05)**
 - o The Lognormal or MSD graph could be setup in Setup/Lognormal or MSD size distribution and chose between:
 - Intensity
 - Volume
 - Surface Area
 - Number
- At the end of the measurement, it is possible to delete some runs which are far from the mean value of the zeta potential, by double clicking on them.
- Particle size higher than 1000 nm is not precise for the PCS measurements.

- **Please fill in the ZetaPals notebook.**

6. Presentation of the results, data storage and data treatment

Save your results in my_documents/my_folder. If you don't have any, please create one.

6.1. Print the results

It will create a Pdf report with **the effective diameter (in lognormal distribution)**.

- File/Print File/Report Print Options: choose the options
- File/ Print report
- The PDF creator will open
- Choose the name of the report and click on "Enregistrer"
- Save your pdf file in my_documents/my_folder

6.2. Create a report

It will create a text file which could be import in Excel.

- File/Report File Settings: choose the options
- Create Report File From Saved Measurements
- Choose the measurement you want to create a report
- Save the report as text file (.txt) in my documents/my_folder

6.3. Save the distribution file

It will create a text file of the population size in intensity, volume, surface area or number.

- Click on Zoom
- Click on Lognormal or MSD then summary
- Choose intensity, volume, surface area or number
- Copy to SpreadSheet
- Paste in a text file
- Save the report as text file (.txt) in my documents/my_folder
- **It is better to save results in Intensity, Volume and Number.**

If you click on "Autosave results", all your results will be saved on the ZetaPals computer.