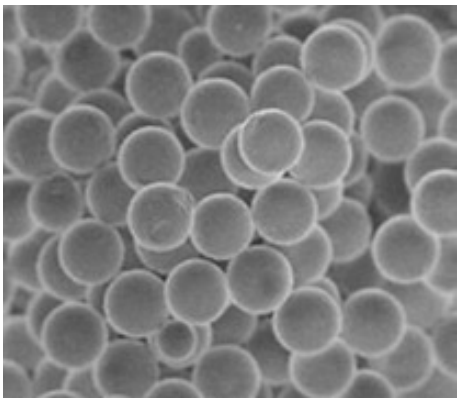
Particle size analysis of polystyrene beads with UV-Vis spectrophotometer and NanoCuvette™ S

APPLICATION NOTE AN-6-101



Challenge

Particle size analysis (PSA) of nano and microparticles is a crucial technique used in various fields such as materials science, pharmaceuticals, biotechnology, and environmental monitoring. However, today PSA techniques require expensive instrumentation, which can be a significant barrier to their widespread adoption. In addition, PSA techniques have limitations in terms of the size range of particles they can measure accurately. How to use other instrumentation?

Solution

The NanoCuvette™ S is a low-cost alternative to other PSA techniques such as Dynamic Light Scattering (DLS) or Laser Diffraction. Building on traditional UV-Vis spectroscopy and a standardized protocol, it requires minimal preparation and can measure particle sizes ranging from 100 nm to 20 µm, making it suitable for a wide range of particle applications here illustrated with polystyrene latex beads.

Intended Audience

Particle or powder producers, researchers, and students.

1 Introduction

Particle size is a crucial parameter in the characterization of particulate materials and plays a vital role in many industries including food, pharmaceuticals, cosmetics, semiconductors, and more. Accurate measurement of particle size and concentration is directly correlated with the mechanical strength, density, and electrical and thermal properties of finished products in these industries. Poor control of particle size and size distribution can result in significant production losses due to high rejection rates [1].

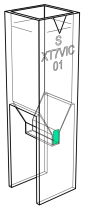


Figure 1: NanoCuvette™ S combining absorbance, refractive index and particle/cell size via static light scattering (SLS) quantification in a conventional UV-Vis spectrophotometer with spectrum analysis performed in SpectroWorks™.

Particle size and concentration are critical parameters to characterize materials in many industries. Dynamic Light Scattering (DLS) is the current gold standard method for obtaining size distribution of small particles and polymers in liquid samples. However, DLS has inherent limitations when characterizing dilute liquid suspensions containing particles. DLS

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apparatuses are often expensive and complicated, limiting their mainstream applicability. Futhermore, estimating the refractive index of the fluid sample introdues errors in the obtained size distribution. It is also seen that for samples containing particles of a varying size, DLS may provide imprecise estimates. Finally, DLS is limited to particles smaller than between 1 micrometer and 500 nm depending on the sample.

In contrast, the NanoCuvette™ S consumable , see Figure 1, offers a user-friendly measurement procedure for estimating particle sizes and concentration based on static light scattering at a fraction of the cost compared to DLS measurements. It utilizes existing spectrophotometer instrumentation commonly found in laboratories, see Figure 2. With its unique photonic crystal technology, NanoCuvette™S provides real-time monitoring of particle sizes. As a low-cost alternative it enables efficient and accurate measurement of particle size and concentration, making it an essential tool for industries that require precise particle analysis.

Figure 2: Examples of UV-Vis spectrophotometers. NanoCuvette™ S is inserted into a spectrophotometer to obtain the A, B and D side spectra.

NanoCuvette™ S creates instant value in any laboratory and quality control station. It relies on static light scattering and the existing spectrophotometer instrumentation in laboratories. Therefore, as a DLS instrument is not needed,

Figure 3: NanoCuvette™ S data is analyzed using SpectroWorks™ cloud software at https://spectroworks.com/.

the measurement can be performed without upfront investment, calibration or training.

2 Method and Instrumentation

Instead of re-inventing a new optical apparatus, the NanoCuvette™ S is an unique cuvette for use in spectrophotometric experiments and with an advanced optical analysis available in the online cloud software SpectroWorks™, see Figure 3.

NanoCuvette™ S allows for measurements of absorbance, refractive index and static light scattering of particles for size and concentration determination with a spectrophotometer, see Figure 1.

The NanoCuvette™S contains an optical filter inside the cuvette that contains a nano-scale photonic crystal with very high angular resolution. Both the absorption steptrum and the scattering of the sample can be analyzed.

Measurements are recorded with the absorbance side (side A), the photonic crystal (side B), and the opposite side of the photonic crystal (side D) as shown in Figure 4. The results are absorbance, refractive index and an accurate size determination of particles in the nanometer range with known and widely used spectrophotometers together with a cloud-based analysis software, see Figure 3.

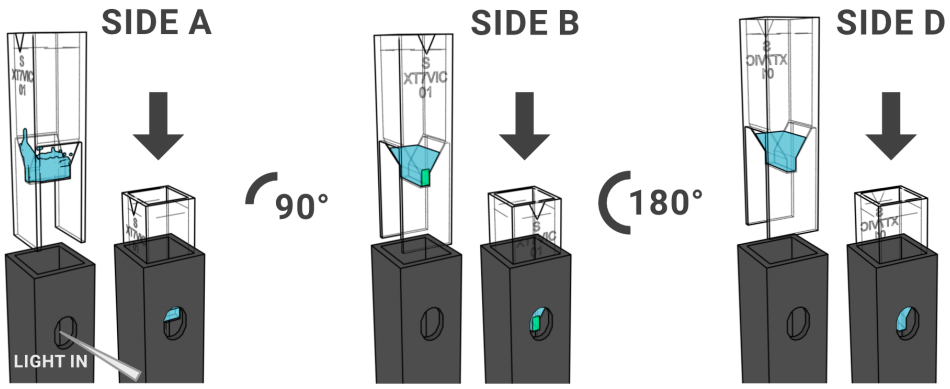


Figure 4: Method for use in a UV-Vis spectrophotometer. Side A, B, and D positions and orientations of NanoCuvette™ S for use with 1 cm path length cuvette-based UV-Vis spectrophotometers. First measure the Side A position by inserting NanoCuvette™ S into the instrument sample cuvette holder such that the optical filter is NOT in the light path (Side A). Then turn the NanoCuvette™ S 90 degrees into the side B position, such that the optical filter is facing towards the light source when illuminated (Side B). Finally, turn the cuvette 180 degrees into the Side D position such that the optical filter is facing towards the detector when illuminated (Side D). Please see our UV-Vis Knowledge Base for more information on the specific orientation of light source and detector for different UV-Vis instrument models.

3 Theory and Working Principles

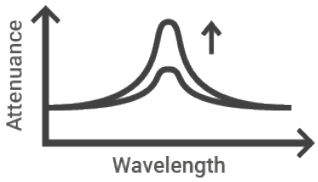
NanoCuvette™S is a breakthrough concept that combines accurate and reliable measurements of:

- **Technology 1:** Absorbance.
- **Technology 2:** Refractive index.
- **Technology 3:** Concentration and size determination of nano- and microscale objects (particles or biological cells).

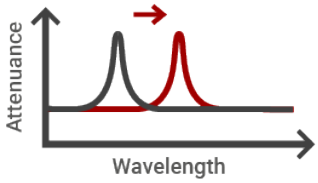
The three next sections decribe the underlying technologies and measuring principles combined with the NanoCuvette™ S consumable.

3.1 Technology 1: Absorbance via Absorption Spectroscopy

For background, the Beer-Lambert law [2, 3, 4] is a well-known principle used in absorption spectroscopy, which allows for the determination of the concentration of a substance in a solution based on its ability to absorb light at a particular wavelength, see Figure 5.



(a) Technology 1: Beer-Lamberts law.



(b) Technology 2: Refractive index, photonic crystal.

Figure 5: Working principles for Technology 1 (Absorbance via Absorption Spectroscopy) and Technology 2 (Refractive Index via Label-free Spectroscopy) used with NanoCuvette™ S.

With absorption spectroscopy, the Beer–

Lambert law relates the attenuation of light to the properties of the material through which the light is traveling:

$$A = \varepsilon C l + A_0,$$

where A is the absorbance, ε is molar extinction coefficient, C is the concentration, l is the path length and A_0 is the background absorbance. The NanoCuvette™ S measures traditional absorbance with the side A spectrum, see Figure 4.

3.2 Technology 2: Refractive Index via Label-free Spectroscopy

In label-free spectroscopy, light is used to quantify the sample in question. NanoCuvette™ S uses a new type of optical filter (optical chip or photonic crystal) which is inserted into the light beam and the spectrophotometer measures the refractive index by measuring a wavelength color shift on the x-axis at fixed intensity that is proportional to the concentration or sample change in the NanoCuvette™S, similar to Hands law for proteins [5].

When light interacts with the photonic crystal, it creates a resonance at a specific wavelength that is related to the refractive index or concentration of the material near the crystal's surface. In the simplest linear model, the resonance wavelength, λ_R, can be described using the equation:

$$\lambda_R = n_D \beta + \lambda_0,$$

where λ_0 is a reference wavelength, β is a coefficient, and n_D is the refractive index of the sample. By measuring the resonance wavelength, the refractive index can be determined. A comprehensive computational model for calculating refractive index based on UV-Vis spectra is built into SpectroWorks™. The NanoCuvette™ S measures refractive index with the side B spectrum, see Figure 4.

3.3 Technology 3: Particle Size via Static Light Scattering quantification

Absorption and light scattering are the two major physical processes that contribute to the visible appearance of most objects. A spectrophotometer measures the combination, known as attenuation, of absorption and scattering, but it cannot distinguish between them. Figure 6 is a schematic representation for the classification of absorption and scattering media.

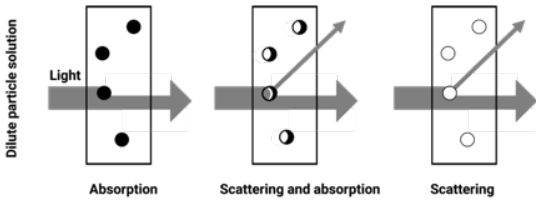


Figure 6: Classification of absorption and scattering in a dilute sample.

The optical filter inside the NanoCuvette™S consists of a nano-scale photonic crystal capable of measuring very high angular resolution. The absorbance spectrum and the scattering of a dilute sample can be analyzed, and particle size and concentration can be determined with an advanced optical model available on the online cloud software SpectroWorks™. The NanoCuvette™ S measures particle and cell size by combining information from the side A spectrum, the side B spectrum and the side D spectrum, see Figure 4.

Different instruments use different scattering information in different ways to calculate the particle sizes from samples. Below is a comparison to pinpoint the characteristic feature of how NanoCuvette™ S measures particle sizes:

- **Dynamic Light Scattering (DLS)** conventionally measures scattering over time at a given wavelength.
- **Multi Angle Dynamic Light Scattering (MA-DLS)** conventionally measures scattering over time at a given wavelength for different angles.

- **Static Light Scattering (SLS) with NanoCuvette™ S** measures scattering at different wavelengths in different angles at a given time.

The scattering can be by particles and the molecules in the sample which relates to the size parameter that differentiates the types of scattering involved. There are three types of scattering involved namely Raleigh scattering (when particles are smaller than the wavelength of light), Mie scattering (when the particle size is comparable to the size of the wavelength of light) and Geometric optics or scattering of large particles (when the particles are very big relative to the wavelength of light and can be estimated by their interaction of light using optical principles of a lens or prism), see Figure 7.

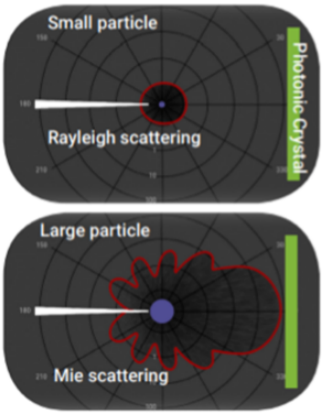


Figure 7: Different types of scattering detectable by the photonic crystal in NanoCuvette™ S.

4 Measurements, Materials and Experimental Methods

This section describes the measurements, materials and methods related to the use of NanoCuvette™ S. Please note the following:

- DI water and sample should be measured in the same cuvette.
- In case one would like to perform the next sample measurement using same cuvette, we recommend a wash step (see our recommended wash protocol on our website).
- For R&D application it is recommended to use a new cuvette with respective DI measurement for each sample measurement.

4.1 Materials and Apparatus

- Latex beads, polystyrene 100nm Product no. LB1-1ML stock from Merck.
- Latex beads, polystyrene 460nm Product no. LB5-1ML stock from Merck.
- Latex beads, polystyrene 600nm Product no. LB6-1ML stock from Merck.
- Latex beads, polystyrene 800nm Product no. LB8-1ML stock from Merck.
- Latex beads, polystyrene 1100nm Product no. LB11-1ML stock from Merck.
- Latex beads, polystyrene 3000nm Product no. LB30-1ML stock from Merck.
- Shimadzu UV-1800 double beam spectrophotometer instrument.
- NanoCuvette™ S used for measurements. For this application note, 15 mm beam height NanoCuvette™ S was used due to the z-height 15 mm of the light beam in the scanning type spectrophotometer.

4.2 Safety Precautions

- Please refer to common laboratory practices.
- This method does not require any additional safety precautions.
- The NanoCuvette™ S is made of PMMA plastic and can disposed as plastic waste.

4.3 Instrument Preparation

1. Start spectrophotometer, warm up for 30 min.
2. Perform Auto zero (without cuvette).
3. Perform System baseline (without cuvette).

Choose methods of scan in the spectrophotometer, with a wavelength range of 1100 nm - 190 nm and the specified parameters ex. Shimadzu UV-1800 "slow-scan-mode" with 2 nm wavelength resolution is used for measurements.

4.4 Reference Measurement

1. Take NanoCuvette™ S.
2. Add 300 µL DI water.
3. Make sure there are no air bubbles.
4. Note the 6 character box number, and the cuvette number.
5. Measure side A of the DI Water.
6. Measure side B of the DI Water.
7. Measure side D of the DI Water.
8. Empty the cuvette.

See Figure 4 for the different orientations of the NanoCuvette™ S.

4.5 Sample Preparation

Mix the stock of Latex beads, polystyrene on a votex mixer. Make a stock solution of each nm size as:

1. Pipet 1998 µL of DI water.
2. Add 2 µL of mixed Latex beads, polystyrene.
3. Mix until the solution is fully dissolved.

Table 1: Example of the dilution for the particle size measurements.

Concentration (%)	Latex beads, Polystyrene (uL)	De-ionized water (uL)
0.01	2 of stock beads	1998
0.001	200 of stock 0.01%	1800
0.003	100 of stock 0.01%	3200
0.0001	200 of stock 0.001%	1800

This stock solution has a concentration of 0.01%. From this stock solution of concentration 0.01% make the following concentration for the assay in de-ionized water, see Table 1. Mix the solutions between dilutions.

4.6 Sample A, B and D Side Measurements

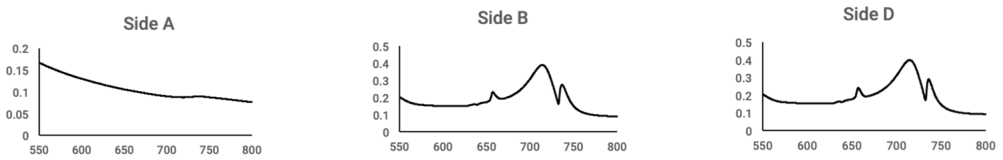
See Figure 4, for the different orientations of the NanoCuvette™ S.

We are continuing in the same NanoCuvette™ S which was used for DI Water reference.

1. Add 300 µL of the diluted sample.
2. Make sure there are no air bubbles.
3. Measure A side of sample. (Measure without the photonic crystal towards the light path).
4. Measure B side of sample. (Turn the cuvette 90 degrees to the light path and measure through the photonic crystal).
5. Measure D side of sample. (Turn the cuvette 180 degrees away from the light path and measure from behind the photonic crystal).

4.6.1 Data Analysis

1. Log into **https://spectroworks.com/** (online software included in purchase).



(a) (b) (c)

Figure 8: Figure a, b and c shows the three different spectra output from as messaured with the NanoCuvette™S. (a) Spectrum without the photonic crystal of DI water (A-side). (b) Spectrum of DI water with the photonic crystal facing the light path closer to the light source (B-side). (c) Spectrum of DI water with the photonic crystal facing the light path farther to the light source (D-side).

2. Create a login and password.

Create a project, while creating the results parameters:

- *"Mean Particle diameter"*
- *"Protein Concentration"*
- *"Sample angle of incidence"*
- *"Sample mean angle of incidence"*
- *"Scattering fit quality"*
- *"Size distribution fit quality"*
- *"Sample fit quality"*
- *"Reference fit quality"*

Add all these parameters from NanoCuvette™ S to the project. As they are added it will be seen in the final selected results. Then perform the following steps:

1. As a project is created, it will lead to "Get started with full spectrum analysis".
2. Select the NanoCuvette™ S functionality.
3. Enter six character box code and select the cuvette number.
4. Select fluid material, H_2O (Optical parameter for solvent) and accept.
5. Select Particle material, Polystyrene and accept. Click "NEXT".
6. Upload or drag & drop the A-side spectrum from DI water onto SpectroWorks™.
7. Upload or drag & drop the B-side spectrum from DI water onto SpectroWorks™.
8. Upload or drag & drop the D-side spectrum from DI water onto SpectroWorks™.
9. The software will analyze reference data and load results for "Reference fit". Click "NEXT".
10. Upload or drag & drop the A-side spectrum from sample onto SpectroWorks™.
11. Upload or drag & drop the B-side spectrum from sample onto SpectroWorks™.
12. Upload or drag & drop the D-side spectrum from sample onto SpectroWorks™. Click "NEXT".
13. The software will analyze sample data and load results for "Sample fit". Click "NEXT".
14. The software will calculate results. Click "NEXT".
15. Then software will calculate and load results. Click "Finish".
16. Then software will load summary and load results for "Summary". One will get the summary with all results from the measurement. It is possible to add all information about the measurement in the "Sample attributes". Under "Plots" one can see the spectra.

5 Results

The results section presents the validated data obtained from the measurements using NanoCuvette™ S and the analysis performed with SpectroWorks™ software by an external GLP certified laboratory [6].

The NanoCuvette™ S used with a spectrophotometer requires measurements in three specific orientations, which are explained in Figure 4. The input to the online cloud software, SpectroWorks™, see Figure 3, includes spectra from the A-side, B-side, and D-side of both the reference (DI water for most samples) and the sample.

5.1 Water Reference Spectrum Analysis

Example reference spectra from DI water are given in Figure 8a, Figure 8b, and Figure 8c. In the case of DI water, the A-side spectrum shows a flat spectrum because it only interacts with DI water, which does not absorb light in the visible range. However, the B-side spectrum captures a resonant spectrum of light through the photonic crystal, which is composed of periodic nanostructures that interact with light in specific ways. Here resonant peaks can be seen between 750-700 nm *(TE mode - transverse-electric)* and 650-700 nm *(TM mode - transverse-magnetic)*. These are expected behavior due to the interaction with the photonic crystal. Finally, in this case of the D-side spectrum, it would be identical to the B-side spectrum for DI water as there are no particles present in the reference measurements.

5.2 Polystyrene Particle Spectrum Analysis

For samples, the B-side and D-side spectra will be different due to scattering detected near and far from the photonic crystal, respectively, in the sample particles. By using the three different orientation measurements with reference and sample, SpectroWorks™ can analyze particles ranging in size from 100 nm to 3000 nm in dilutions between 0.01% - 0.0001%. Validation of the technology was done by an external company, Particle Analytical ApS, in [6] whom is a GMP certified contract laboratory, and resulted in data comparisons and graphs, which are presented in Table 2 and Figure 9.

5.3 Particle Size Analysis

In Table 2 and Figure 9, a comparison between NanoCuvette™ S based on SLS and the gold standard DLS method for the measured particles sizes can be seen. As earlier mentioned, Particle size analysis (PSA) techniques often have limitations in terms of the size range of particles they can measure accurately and it is known that DLS is often limited to particles smaller than between 1 micrometer and 500 nm depending on the sample.

In the case of the polystyrene bead particles measured here, it can be seen that as the particle diameter increases beyond 500 nm, the DLS method becomes less and less accurate with errors up to approximately 47% whereas the SLS method with NanoCuvette™ S delivers useful results for a wide range of nano- to micron-scale particle sizes, see Figure 10. In addition, the method also provide information about the particle concentration, see Figure 11.

The results from [6] demonstrate the accuracy and reliability of the NanoCuvette™ S SLS technology in determining particle sizes values across a wide range of sizes and dilutions. The strong correlation between the measured and known values, along with the consistency in the measurements, supports the suitability of this technique for various applications in industry and research.

6 Discussion

The PSA results from [6] and provided above illustrate the use of NanoCuvette™ S technology in conjunction with UV-Vis spectrophotometers and SpectroWorks™ software as a reliable and accurate method for measuring the particle

Table 2: Measured poloystyrene latex beads sizes. Specified size from bead manufacterer with uncertainty of one standard deviation. Data from [6].

Specified size from bead manufacturer	Conc. (Vol.%)	Measured DLS (nm)	Measured NanoCuvette™ S (nm)	Error DLS (%)	Error SpectroWorks™ (%)
110 ±10 nm	0.01	108	132	0 - 8	10 - 32
110 ±10 nm	0.0001	-	97	-	3 - 19
460 ±10 nm	0.003	437	414	3 - 7	8 - 12
615 ±15 nm	0.003	648	548	3 - 8	9 - 13
800 ± 50 nm	0.003	717	635	4 - 16	15 - 25
1100 ± 100 nm	0.001	913	1091	9 - 24	0 - 9
Approx. 3000 nm	0.001	1585	3344	Approx. 47	Approx. 11

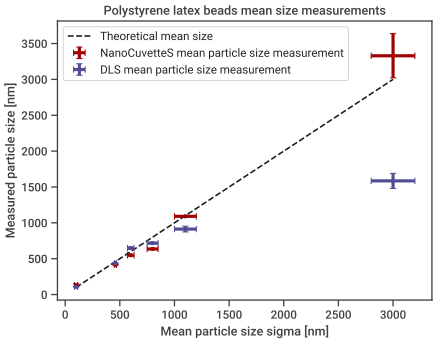


Figure 9: Particle size comparison between DLS and NanoCuvette™ S / SpectroWorks™ validation analysis. Data from [6].

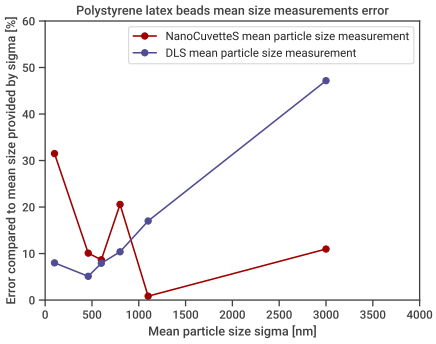


Figure 10: Error on particle size comparison between DLS and NanoCuvette™ S / SpectroWorks™ validation analysis. Note the systemic nature of the DLS error progression. Data from [6].

size of polystyrene latex beads. This method overcomes several limitations associated with traditional PSA techniques like DLS or Laser Diffraction, such as expensive instrumentation, sensitivity to air bubbles, particles or other impurities, limited measurement range, and the need for frequent calibration. By utilizing existing spectrophotometric instrumentation and cloud-based software, this method allows for efficient and precise analysis without significant upfront investments or extensive training.

The scattering behavior of particles is directly influenced by their size, and this behavior varies across different particle sizes. The size and instrumentation of detectors used in spectrophotometers from different brands can also vary, resulting in different amounts of recorded scattering for different particle sizes and concentrations. Thus, to achieve the most accurate particle estimation with a given instrument, there is an optimal concentration range that must be considered. The results presented in Table 2 were obtained using the VWR UV6300-PC scanning spectrophotometer with a maximum absorbance of 3 A. However, different scanning instruments may have varying internal structures that can influence particle size estimation in SpectroWorks™. Therefore, users may need to optimize their approach based on the respective particle size and the specific instrument being used.

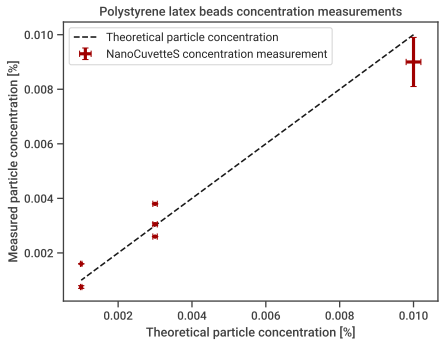


Figure 11: Particle concentration with NanoCuvette™ S / SpectroWorks™ validation analysis. The results do not take into account any dilution errors. Data from [6].

The standard curve of the Latex polystyrene bead particles was obtained by measuring a series of different sizes and dilutions with the NanoCuvette™ S and analyzing the data using SpectroWorks™ software. The resulting particle sizes were determined and are presented in Table 2. Figure 9 shows a graphical comparison of the measured particle size values and the known specified size from the manufacturer, demonstrating a strong correlation between the two.

7 Conclusion

In conclusion, NanoCuvette™ S presents a promising low-cost alternative for accurately and reliably measuring particle sizes of polystyrene latex beads in solutions. With its use of existing spectrophotometer instrumentation and cloud-based software, the PSA method provides a cost-effective and user-friendly solution that does not require extensive training or significant upfront investments.

The SLS technology's ability to overcome the limitations of traditional instrumentation like DLS, both in sensitivity to impurities and the need for frequent calibration, makes it a valuable tool for a wide range of industries, including food and beverage manufacturing and research. Additionally, the method can be adapted to measure other parameters, such as protein concentration, with minor adjustments to the software parameters.

Overall, the NanoCuvette™ S technology offers a powerful and versatile solution for accurate particle size measurements in various applications. The wide particle size range and insensitivity to impurities make this technology particularly beneficial for quality control and research and development purposes across various industries. The NanoCuvette™ S technology is particularly advantageous for particle and powder producers, researchers, and students who require a robust and accurate method to determine particle size in solutions.

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References

[1] J. Stetefeld, S. A. McKenna, and T. R. Patel. Dynamic light scattering: a practical guide and applications in biomedical sciences. *Biophysical Reviews*, 8(4):409–427, 2016. 1

[2] Pierre Bouguer. Essai d'optique sur la gradation de la lumière. *Claude Jombert*, page 16–22, 1729. 3

[3] J.H. Lambert. Photometria sive de mensura et gradibus luminis, colorum et umbrae. 1760. 3

[4] Beer. Bestimmung der absorption des rothen lichts in farbigen flüssigkeiten. *Annalen der Physik und Chemie*, 162(5):78–88, 1760. 3

[5] David B. Hand. The refractivity of protein solutions. *The Journal of Biological Chemistry*, 108(3), 1935. 4

[6] Søren Lund Kristensen Wenbo Wang. Testing and validation of nanocuvette™ s, spectroworks™ together with uv-vis spectrophotometer. Validation report, Particle Analytical ApS, 2021. 8, 9, 10