



AN5001: TiO₂ nanoparticle dispersions in cell culture media characterized by high-throughput DLS

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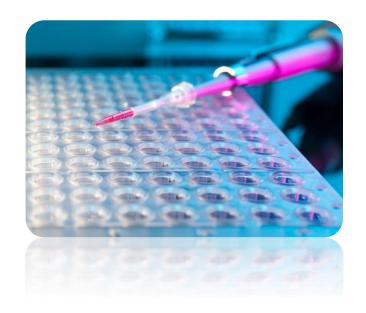
Introduction

Characterization of nanoparticle size and distribution in biological environments and understanding the parameters that affect them are imperative to accurately assess nanoparticle toxicity. In the case of in vitro studies, nanoparticles must be well-dispersed to ensure uniform dosing. Similarly, for in vivo studies, delivering nanoparticles in a well dispersed form is necessary for accurate assessment of their toxicity.

Based on these considerations, the goal of this investigation is to accurately evaluate the ${\rm TiO_2}$ nanoparticle dispersion in different cell culture media using the high throughput dynamic light scattering DynaPro® Plate Reader. To improve the dispersion of these nanoparticles, dispersing agents such as bovine serum albumin (BSA) and fetal bovine serum (FBS) were explored.

Materials and Methods

The concentrations for TiO_2 , BSA, and FBS were 50 µg/mL, 2 mg/mL, and 0.5-5 wt.%, respectively. DLS measurements were acquired in the DynaPro Plate Reader, read and analyzed by DYNAMICS® software. All measurements were conducted in a 384-well plate at room temperature. To ensure reproducibility, samples were loaded in triplicate and five runs were collected for each well. Average hydrodynamic radius (R_h), as determined by the cumulants method, was used to color-code the degree of agglomeration in SpectralViewTM. The DynalsTM regularization non-negative least squares (NNLS) algorithm was applied to estimate size distributions within each well.





Results and Discussion

SpectralView was programmed to display heavily agglomerated samples in black, light agglomeration in red, and no agglomeration in blue. Display of R_h in SpectralView (Figure 1) suggests that BSA at 2 mg/mL is an effective dispersing agent in all media but LB and TSB. By contrast, 5 wt.% FBS (equivalent to 2 mg/mL BSA) resulted in highly dispersed TiO_2 in all media. No noticeable change in nanoparticle dispersion was observed with FBS concentration decreased to 1 wt.% (Figure 1), which implies that FBS is an effective dispersing agent.

In addition to the qualitative analysis, particle size distributions were calculated using DYNAMICS' regularization algorithm. Figure 2 shows an example of TiO₂ particle size distribution in LB, which again confirmed the effectiveness of FBS. Overall, this study demonstrated the use of the DynaPro as a convenient and effective tool for determining TiO₂ nanoparticle size and for evaluating the effects of different dispersing agents much faster than conventional "batch" DLS would have allowed. Currently, more effort is being made to understand the dispersion mechanisms. Kinetic/stability study of TiO₂ suspensions is also being performed.

Learn more about the DynaPro Plate Reader at www.wyatt.com/DynaPro.

More application notes on nanoparticle characterization may be found at www.wyatt.com/AppNotes/Nanoparticles.

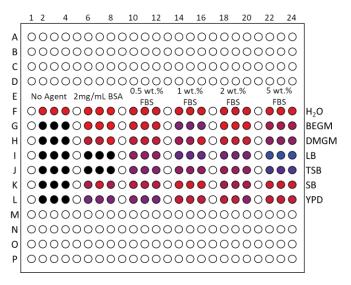


Figure 1: SpectralView display of TiO₂ nanoparticle dispersion in water and six different cell culture media (BEGM, DMEM, LB, TSB, SD, and YPD) suggests severe agglomeration in all media without dispersing agents, improved dispersion in most media using 2 mg/mL BSA, and the best dispersion using ≥1 wt.% FBS.

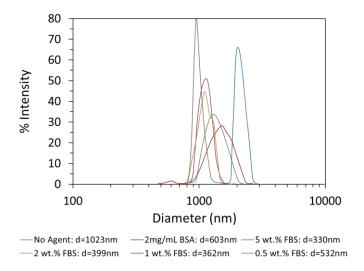


Figure 2: TiO_2 particle size distribution in LB calculated by the built-in regularization algorithm shows the effect of BSA and FBS dispersing agents. The corresponding hydrodynamic diameter of TiO_2 was determined to be 1023 nm, 608 nm, 330 nm, 399 nm, 362 nm, and 532 nm for no dispersing agent, 2 mg/mL BSA, 5 wt.% FBS, 2 wt.% FBS, 1 wt.% FBS, and 0.5 wt.% FBS, respectively.



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