Formulaction
Smart scientific analysis

TURBISCAN LAB

Nanoparticles and toxicity

- Establishing more detailed protocol for fate evaluation -

RAW MATERIALS

Introduction

While nanoparticles (**NPs**) are massively released in the environment and consumed by humans in food products, there is a growing concern of a potential hazard of NPs on human health. To assess the NPs toxicity in in-vitro studies, NPs are dispersed in cell culture media and this dispersion is dispensed to cells. However, when dispersed in cell culture media, NP's can undergo many physical destabilizations, e.g. agglomeration and sedimentation. These NPs-media specific interactions are of paramount importance to understand the NPs fate, transport and exposure dose delivered to cells. Hence, to obtain reliable dose-size/cytotoxicity responses, it is mandatory to control NPs size, dispersibility and stability. In this application note, we demonstrate how Turbiscan® device allows to accurately characterize these unique dispersions prior to toxicological studies.

KEY **BENEFITS**

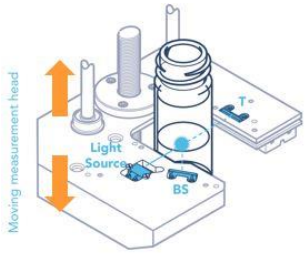
FAST

NO DILUTION

SENSITIVE

Reminder on the technique

Turbiscan® technology, based on Static Multiple Light Scattering, consists on sending a light source (880nm) on a sample and acquiring backscattered (BS) and transmitted (T) signal over the whole sample height.
By repeating this measurement over time with adapted frequency, the instrument enables to monitor physical stability. The signal is directly linked to the particle concentration (φ) and size (d) by the Mie theory knowing refractive index of continuous (n_f) and dispersed phase (n_p):



$$\boldsymbol{BS = f(\varphi, d, n_p, n_f)}$$

Materials & Method

The NPs dispersion preparation was performed in two steps following the NANoREG protocol:

1. NPs batch dispersion was prepared: TiO_2 P25 (Evonik - primary size 21nm) NPs were added to previsouly prepared sterile-filtered BSA-water dispersion to obtain a final NPs concentration of 2.56mg/mL. The resulting dispersion was then sonicated during 16min.
2. The batch dispersion was diluted in DMEM to obtain a final NPs concentration of 0.256mg/mL (diluted 10 times).

Because BSA plays a preponderant role in the dispersibility and stability of NPs in cell culture media, five batches with 0%, 0.05%, 0.1%, 0.2% and 0.5% BSA mass fraction were considered.
The NPs mean particle size, state of agglomeration, sedimentation rate and concentration kinetic have been all evaluated with the Turbiscan® at room temperature.

Results

Initial mean size of the NPS in cell culture media

The initial TiO_2 NPs mean size has been measured for dispersions with different mass fraction of BSA in DMEM, see Figure 1

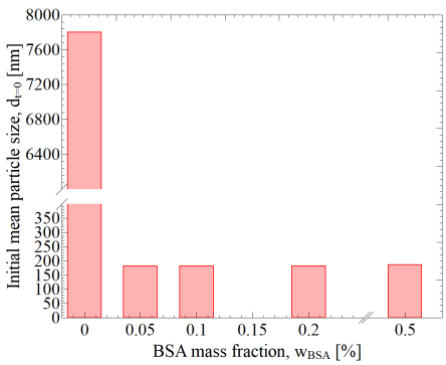


Figure1: Initial mean size of TiO_2 NPs in DMEM for various BSA mass fraction

In absence of BSA proteins, the TiO_2 NPs mean size exceed 7µm proving that the NPs are agglomerated.
By adding BSA, the NPs initial mean size decreases to 180nm for all concentrations (within expected measured range for this specific TiO_2 NPs). BSA proteins are adsorbed into the NPs surface and thus prevent agglomeration.

Sedimentation rate and agglomeration state

The physical stability of all dispersions has been monitored for 24h with a scan every 1min. Sedimentation rate can be calculated - Figure 2(a) - and then converted to mean agglomerate size - Figure 2(b) using Stokes law.

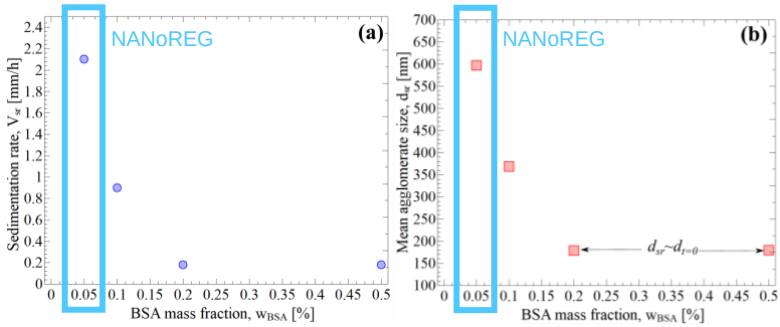


Figure 2: (a) Sedimentation rate and (b) mean agglomerate size of TiO_2 NPs in DMEM for various BSA mass fraction.

The sedimentation rate decreases with the increase of BSA mass fraction until reaching a constant value for $w_{BSA} \geq 0.2\%$. The same trend is seen on agglomerate size: the TiO_2 NPs agglomerate size decreases from 600nm to 180nm approximately for BSA mass fraction (w_{BSA}) between 0.05% and 0.2%. Thus, for:

- **w_{BSA}<0.2%**, the quantity of BSA is not optimal to coat all the surface of TiO_2 NPs.
- **$w_{BSA} \geq 0.2\%$**, no diminution of the agglomerate size is observed which proves that the TiO_2 NPs are optimally coated.

In addition, the agglomerate size is in good correlation to the initial mean size calculated previously (180nm) proving that no agglomeration is occurring.

Concentration kinetics at the sample bottom

Finally, the NPs concentration kinetic in a 5mm slice taken at the bottom of the sample was evaluated for all dispersions, see Figure 3.

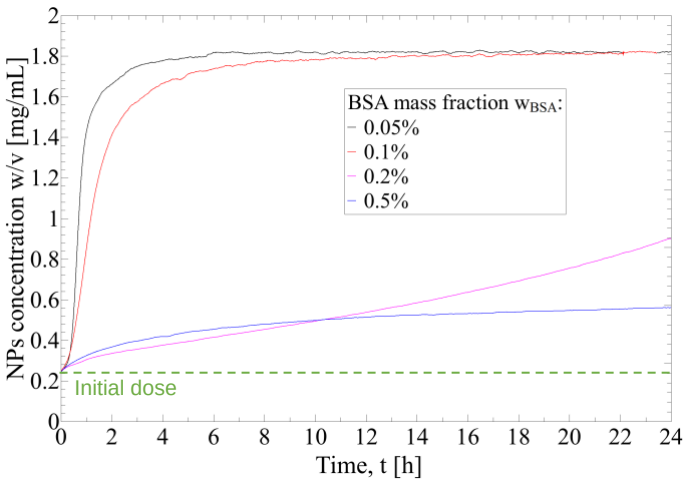


Figure 3. Concentration kinetic of TiO_2 NPs in DMEM at the sample bottom for various BSA mass fraction

As established previously samples with BSA w_{BSA}<0.2% present agglomerates, this results in a strong increase of the NPs concentration in the bottom layer. In addition, as TiO_2 NPs are not agglomerating for BSA $w_{BSA} \geq 0.2\%$, it is within the expectations that the concentration increase is slower.
After 10 hours, at the bottom of the cell:

- for **w_{BSA}<0.2%**, the available dose of NPs has increased **10x**.
- for **$w_{BSA} \geq 0.2\%$,** the available dose of NPs has increased **2x.**

This demonstrates the impact of the BSA concentration on the dispersion state and stability of the NPs in cell culture media. BSA mass fraction higher than 0.2% must be used to obtain the best TiO_2 NPs stability and dispersibility in high glucose DMEM allowing more accurate characterization.
Indeed, during the evaluation of TiO_2 toxicity toward adherent cells, samples prepared at the same concentration and in the same cell culture medium could result in drastically different cytotoxicity responses depending on time and depth of sampling in the cell due to occurring sedimentation and thus different local concentration of NPs in the cell.

CONCLUSION

As the NPs dispersion characterization in the biological media is of extreme importance to ensure cells exposure to the desired particles size and dose, in order to avoid misleading toxicity results, only direct stability measurements in native and concentrated media should be considered.
The Turbiscan® has proved to be valuable tool to understand the fate and transport of NPs by providing quantified information of the NPs initial mean size, agglomeration state, sedimentation rate and delivered dose as a function of time.