An *in vitro* dosimetry dose application for the numerical transport of engineered nanomaterials

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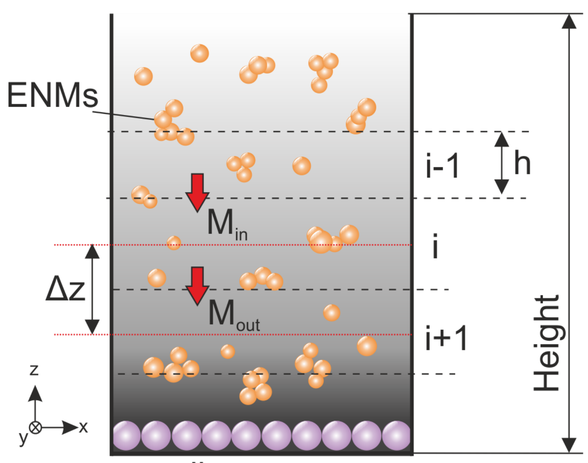
Introduction

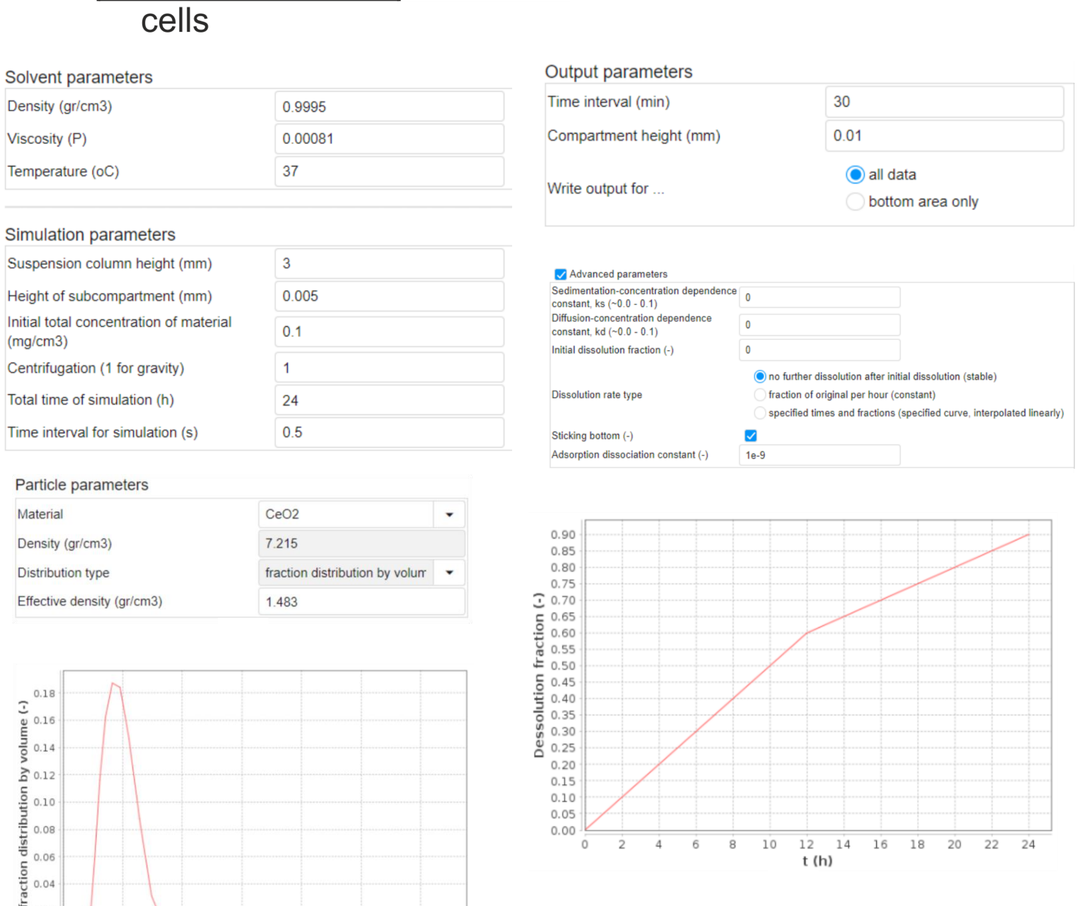
Engineered nanomaterial (ENM) toxicity testing using *in vitro* assays requires the ENMs to be dispersed in cell culture medium, and applied to multiwell cell culture plates. There are numerous techniques and protocols for dispersing ENMs in aqueous media that should be harmonized[1]. Various endpoints are measured during in vitro testing following the exposure, commonly lasting for 24-48 h, and the dose-response relationship is commonly reported[2]. However, the effective dose is not necessarily equal to the nominal dose, since the cells seeded in the plate wells will only react with the ENMs that reach the bottom of the plate. Therefore, for the correct reporting of the ENM dosage regimen, the nominal dose should be adjusted[3]. De Loid et al.[2] addressed the aforementioned issues by developing a multi-step in vitro dosimetry methodology to quantify delivered dose metrics as a function of time which consists of three interconnected parts: 1) ENM dispersion preparation; 2) ENM dispersion characterization; 3) numerical transport modeling to derive the delivered dose metrics. Our work falls into the category of the numerical transport modeling to derive dose metrics. We developed a user-friendly web-based application, termed as "*in vitro* dosimetry application" designed especially for non-expert users.

The web application

The *in vitro* dosimetry application is based on the Distorted Grid (DG) fate and transport model[2] and calculates the mass, number and surface area-based concentrations in the cellular microenvironment throughout the duration of the exposure. Details of the DG fate and trsnport model can be found in [2]. In the **Particle parameters**, the user defines the input regarding the particular ENM. The available ENMs are CeO_2, SiO_2, Fe_20_3, TiO_2, CuO, ZnO, Au, Ag, $FePO_4$ (anhydrous) and "User defined …". The user must provide the effective density of the ENM with the solvent, and the fraction distribution by volume (-) or the % number-weighted size (-) vs the diameter (nm) for the specific ENM. In the **Solvent parameters** the user defines solvent related information and in particular density (gr/cm3), viscosity (P) and temperature (°C) while for the **Simulation parameters** simulation related information and in particular the height (mm) of the suspension column, the height, h of the compartment, the initial concentration (mg/cm3) of the ENM, the total simulation time (hours) and the time interval (Δt - sec) of the simulation. In the **Advanced parameters** section the user can define specific advanced input properties concerning the simulation. The latter includes the sedimentation and the diffusion coefficients. In the **Output parameters** the user can define the time interval and the compartment height to save the data and the choice to save the data for all the suspension column height or just for the bottom.



Schematic of the ENMs suspension column. The basic geometrical characteristics are shown along with the compartments *i-1*, *i* and *i+1* and the inflow and the outflow of solute species *j*. The cells are located at the bottom of the suspension column.



Case study

Six different ENMs are used as case studies for the demonstration and proof-of-concept of *in vitro* dosimetry application. All ENPs were designed, prepared and characterized in the IMROH laboratories and included two different gold ENPs, coated with cysteine (CYS) and glutathione (GSH), and four different silver NPs (AgNPs), coated with CYS, GSH, bis(2-ethylhexyl)sulfosuccinate (AOT), and poly-L-lysine (PLL). Preparation, characteristics and toxicity evaluation of CYS- and GSH-coated AgNPs and AuNPs in murine fibroblast cells (L929 cell line purchased by ATCC® CCL- 1TM) have been described recently[4], while AOT- and PLL-coated AgNPs were prepared according the procedure described in [5]. The input parameters for solvent (cell culture medium) were set at the 0.9995 g/cm^3, 0.00081 P and 37°C for the effective density, viscosity and temperature, respectively. The simulation parameters were dependent on the design of cell culture plates used for cell experiments. The experiments were conducted in 96-well plates with 100 μL as total volume of liquid per well and suspension column height of 3 mm. Height of sub-compartment was 0.005 mm and total simulation time was 24 h in all experiments, equal to the duration of NP treatment. In the case of AOT-AgNPs and PLL-AgNPs, time interval for simulation was left at default 0.5 s, while for CYS- and GSH-coated NPs, the value were increased to 2 s to reduce the time required for simulation to complete. The dissolution of NPs was experimentally shown to be negligible, so it was not included in the simulation. For output p*ar*ameters, time interval of 60 min and compartment height of 0.01 mm were set.

Results & discussion

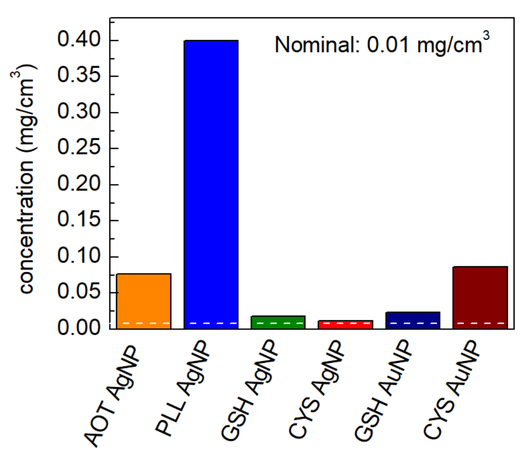


FIG. 1

The results for the ENPs concentrations at the bottom of the suspension column for the various ENPs are shown in Fig. 1. The nominal value for all cases is 0.01 mg/cm^3 which is shown with a white dashed line in Fig. 1. Clearly, besides CYS AgNP where the value of the concentration is almost the same as the nominal, in all other cases the concentration is higher and can be as many as x40.[6]

Conclusions

In vitro dosimetry application is the first freely available web-application for the estimation of the concentration of ENPs in suspension columns. Clearly, comparing to the nominal, the computed concentration is higher in most of the cases which can be a serious drawback as far as the toxicity of the ENPs is concerned.

References

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