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Sedimentation of Nanoparticles in *in vitro* Toxicity Assays

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INTRODUCTION: Nanoparticle toxicity assessments face specific challenges because nanoparticles settle, agglomerate and diffuse in liquid media, depending for example on particle size, shape, surface chemistry, and media viscosity. For reliable toxicity assays it is therefore necessary to assess the influence of nanoparticle-specific properties. In this contribution we specifically analyse particle sedimentation and its influence on the effective cellular dose relevant for *in vitro* assays.

METHODS: Cobalt ferrite ($CoFe_2O_4$) nanoparticles of different dimensions (reference P601 and P703) were provided by Colorobbia Italia s.p.a. UV-VIS measurements were used to characterize particle sedimentation in cell culture medium without phenol-red. Cyclic UV-VIS spectra were recorded every 30 min, for up to 72 h.

RESULTS: We consider the sedimentation of nanoparticles in a liquid column under a gravitational field and assume that the particles move at their terminal velocity in z-direction and that the particle concentration c is a function of z only. The concentration of such particles can be described by the following partial differential equation:

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial z^2} - B\frac{\partial c}{\partial z} \quad . \qquad (1)$$

D is the diffusion coefficient and $B = \frac{2g\delta r^2}{9\eta}$, where δ is the density of the particles minus the density of the liquid, r is the particle radius and n is the liquid viscosity.[1] Assuming an initially homogeneous particle concentration and considering that the particles move at low speed without convection and little turbulence, the analytical solution of equation (1) can be used to calculate the concentration of nanoparticles at any position of the column as a function of time.

Depending on their size and mass particles accumulate with time at the bottom of the liquid column. We show that the sedimentation of $CoFe_2O_4$ nanoparticles of different size, as measured by UV-VIS spectroscopy at different height positions in liquid medium, can be well described with this model. For experiments that use cells attached to the bottom of a culture dish,

e.g., in the colony forming efficiency assay, this can affect the effective dose reaching the cells. Hence, as a consequence of sedimentation the effective concentration of nanoparticles at cell cultures increases with time, and depends on the nature of the particles (size, material) and the specific experiment (type of Petri dish or well, height of the liquid column above the cells). The example in figure 1 shows that for $CoFe_2O_4$ particles the effective nanoparticle concentration n at the bottom of a dish, i.e., at the location of the cells, depends sensitively on the size of the nanoparticles and the duration of the experiment (n_0 = initial concentration).

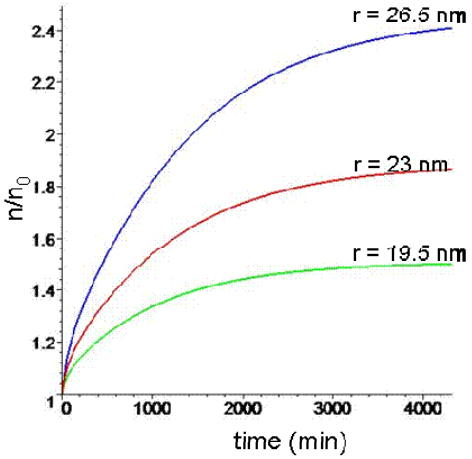

Fig. 1: Accumulation of nanoparticles at the bottom of a Petri dish for different particle radii r as a function of time.

DISCUSSION & CONCLUSIONS: For *in vitro* assays to study nanoparticle toxicity which use cells located at the bottom of a liquid column, sedimentation effects can considerably increase the nanoparticle concentration reaching the cells. As a consequence, if cell cultures are exposed on a time scale of days to the same nanoparticle-containing test medium, sedimentation effects should be considered in order to get a proper dose-effect relationship.

REFERENCES: [1]M. Mason, W. Weaver (1924) *Phys. Rev.* **23**: 412.