Measuring the dispersion of ZnO nanoparticles in solutions for cell viability assays.

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The increased usage of engineered nanoparticles (NPs) in commercial applications such as cosmetics and pharmaceuticals has led to concerns surrounding the fate and potential toxicity of nanomaterials in the aquatic environment [1]. Zinc oxide (ZnO) has excellent UV absorbance (in the UV-A, UV-B and even into the UV-C range) and as such ZnO NPs <100nm are being increasingly included in sunscreens [2]. It is likely that these nanoparticles then end up in the aquatic environment and it is therefore necessary to test the toxicity of ZnO to aquatic biota.

For toxicological testing, ZnO NPs are dispersed in a range of different media and therefore characteristics, such as degree of agglomeration and solubility need to be understood fully in order to ascertain whether any response is induced by the primary particles or secondary agglomerates. Dynamic Light Scattering (DLS) is the technique most commonly employed to investigate the dispersion of NPs in different media however there are intrinsic limitations to the technique. For example, DLS assumes that particles in the suspension are spherical. The technique cannot distinguish between particles of different compositions and relies on the knowledge of the refractive index of the suspended material as well as the viscosity and concentration of the suspending medium. DLS is more sensitive to the larger fraction of a polydisperse suspension of particles as the scattering of light is related to the diameter, d of a particle by d^6 [3]. Finally, if there are macromolecules present in the suspending media DLS may measure these as particles too [4,5]. However, previous papers have shown that this issue is dependent upon the relative concentration of NPs to media [6,7]. Transmission electron microscopy (TEM) is often employed to look at the primary particles in a suspension however this does not give an accurate representation of the particle dispersion when the suspension is drop cast on a grid because drying effects such as NP coalescence take place. With this in mind, an alternative sample preparation technique has been investigated for the TEM [8]. This preparation route involves the blotting of a particle suspension on a TEM support film, immediately followed by plunging into liquid ethane cooled by liquid nitrogen. This produces an electron transparent thin film of the vitrified dispersant with the NP dispersion trapped within. It has been shown that when the specimens are warmed under the vacuum conditions of a TEM the solution devitrifies and sublimes leaving the dispersion of NPs on the carbon film unaltered [9]. This preparation method has already been validated against DLS for diethylene glycol coated ZnO NPs (average particle size ~ 40 nm) suspended in water [10]. In this study, ZnO-suspensions were prepared for TEM by plunge freezing, warming under rotary vacuum conditions and were imaged in the TEM at room temperature. Quantitative analysis of the ZnO agglomerate sizes visible in the TEM images is compared with DLS results from the same sample.

Commercially supplied ZnO NPs (Alfa Aesar, Metals Basis) with an average primary particle size of 45 ± 20 nm, were suspended in 3 different media for 20 minutes of ultrasonication. The ZnO was dispersed at 0.1 % w/v in (i) triple distilled MilliQ water (ii) 50% Dulbecco's modified Eagle medium (DMEM) / 50% triple distilled MilliQ water and (iii) 50% DMEM - 50% triple distilled MilliQ water supplemented with Bovine Serum Albumin (BSA) (5% w/w ZnO). Plunge frozen TEM specimens were prepared by placing a 3.5 µL droplet on a glow discharge treated carbon film, the grid was then blotted and immediately plunge frozen in liquid ethane. The grids were allowed to warm to room temperature under rotary vacuum conditions. Drop cast specimens were prepared by placing a 3.5 µL droplet on a holey carbon support film (Agar) and left to dry in air.

Figure 1 shows TEM images of the ZnO NPs suspended in 50% DMEM - 50% triple distilled MilliQ water and supplemented with Bovine Serum Albumin (BSA) (5% w/w ZnO). TEM samples were prepared by (a) the drop casting method and (b) the plunge freezing method. In the drop cast sample, the NPs were aggregated whereas in the plunge frozen sample they were only found in

small clusters. A histogram of the sizes of the clusters in the plunge frozen specimen, compiled from measurements of 300 clusters, is shown in Figure 1d with the size distribution throughout the sample centred around 135 nm. Figure 2(a) shows the DLS size distributions obtained for all 3 suspensions and indicates a reduction in ZnO aggregate size when BSA is present in the suspension. It is thought that this is due to the adsorption of BSA to the surface of the ZnO NPs which sterically stabilises the suspension. An amorphous coating, likely to be serum proteins, can be seen on the ZnO NPs suspended in the media supplemented with BSA in Figure 1(c). Figure 2(b) displays the size distributions determined by both TEM and DLS.

The TEM measurement has detected significant numbers of particles below 100 nm in length and yet the DLS has not [Figure 2(b)]. This difference may be critical to cell viability since the preferential cellular uptake of particles by endocytosis is thought to lie in the size range 20-100 nm [11]. As such the results presented here will be correlated to the cyto- and geno-toxic response of A549, HT29 and HaCat in-vitro cell lines; testing against potential exposure by inhalation, ingestion and through the skin respectively. As is, the current results suggest that the plunge freezing TEM specimen preparation route is a promising tool for characterisation of nanoparticulate suspensions used for cell uptake studies and other challenging colloidal systems.

References

[1] Colvin V L, *Nature biotechnol.* **21** (2003) p.1166-1170.
[2] Mitchnick M *et al.*, *J. Am. Ac. Derm.* **40** (1999) p. 85.
[3] Pecora, R. *J. Nanopart. Res.* ***2*** *(2000) p.*123-131.
[4]Sabuncu A C *et al.*, *Colloids Surf.* B **78** (2010) P. 36-43.
[5] Orts-Gil G *et al.*, *J. Nanopart. Res.* **13** (2011)p.1593-1604.
[6] Ji Z *et al.*, *Environ. Sci. Technol.* **44** (2010) p. 7309-7314
[7] Summers *et al., Nature Nanotechnol* **6** (2011) p. 170-174
[8] White, H D *et al.*, *J. Struct. Biol.* **121** (1998) p.306
[9] Hondow N *et al.*, *J. Phys. Conf. Proc.* 2011
[10] Wallace *et al*. *J. Phys. Conf. Proc*. 2011
[11] Zhang, S *et al. Adv. Mat* **20** (2008)p.1-6.

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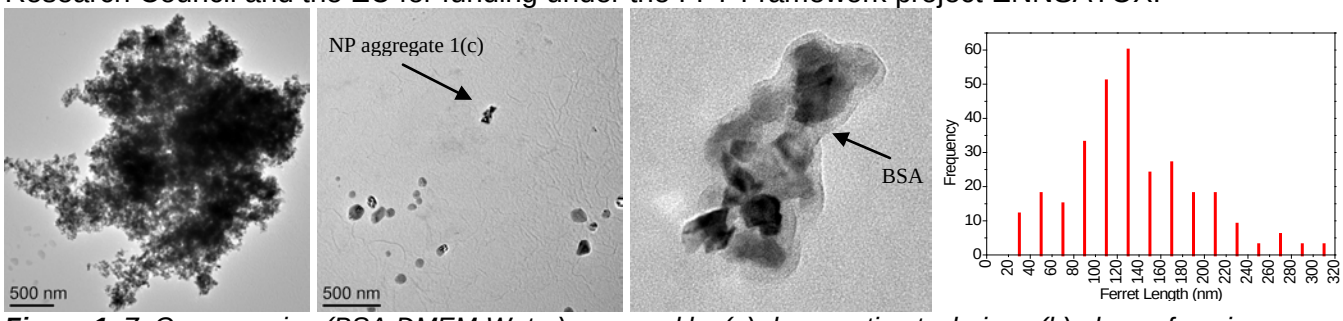

Figure 1*. ZnO suspension (BSA-DMEM-Water) prepared by (a) drop casting technique (b) plunge freezing technique. (c) High magnification TEM image showing the BSA coating on the NPs (d) Histogram of size distribution of aggregates measured from plunge frozen samples.*

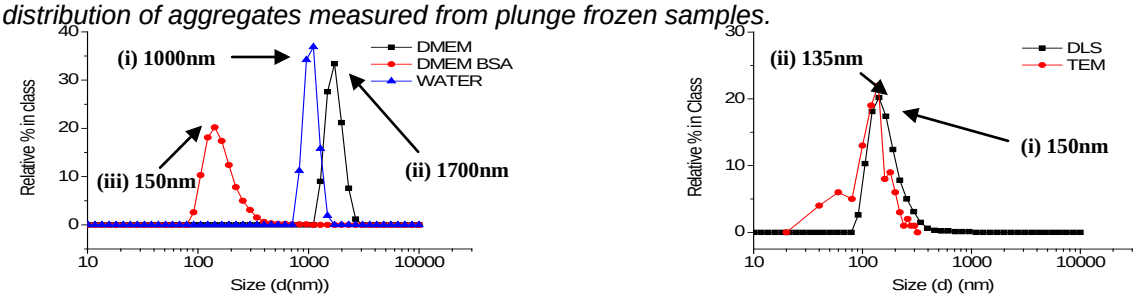

Figure 2. *(a) DLS plot showing size distribution for ZnO suspended in (i) distilled water, (ii) DMEM-Water and (iii) DMEM-Water+BSA and (b) DLS number plot (i) for colloidal dispersion of ZnO NPs(DMEM-Water+BSA) with TEM plunge freezing data (ii) overlaid.*