Characterisation of ZnO nanoparticle suspensions for toxicological applications

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Abstract. ZnO nanoparticles are used in a variety of commercial products including sunscreens. The potential for accumulation in the aquatic environment has driven investigation into the toxicity of nanoparticulate ZnO. Thorough characterisation of the physicochemical properties of nanoparticulates is required in order to gain insight into the key factors which result in a nanoparticle type exhibiting a toxic response. This includes measuring the agglomeration state of the nanoparticles when dispersed in solution. This work focuses on the synthesis and characterisation of diethylene glycol coated ZnO nanoparticles (average particle size ~ 40 nm) suspended in water. Agglomeration measurements by light scattering techniques are compared to that by TEM imaging of nanoparticle dispersions plunge frozen in liquid ethane. The two techniques produce similar size distributions suggesting that the ZnO is polydispersed in the solution with an average agglomerate size of 50-70 nm.

1. Introduction

The increased use of nanomaterials in commercial applications such as cosmetics and pharmaceuticals has led to concerns surrounding their fate and potential toxicity in the aquatic environment [1][2]. ENNSATOX is a European NMP FP7 small collaborative project that aims to relate the structure and size of a set of well characterised engineered nanoparticles to their biological activity in environmental aquatic systems (www.ennsatox.eu). The interaction of the nanoparticles with a series of biological models is being investigated including with supported phospholipid membranes, in vitro models of cell and tissue cultures and in vivo models of several different species of key indicator organisms. Zinc Oxide is one set of nanoparticles that are being investigated.

Zinc Oxide (ZnO) is a wide band gap semiconductor that has excellent ultraviolet (UV-) light absorbance. ZnO nanoparticles <100nm are being increasingly used in the manufacture of sunscreens due to their increased absorbance in the UV-A, UV-B and even into the UV-C range [3]. As well as the benefits to the safety of the sun screen user, there are also those associated with aesthetics because ZnO particles smaller than 200nm are virtually transparent in thin film coatings that are applied as a sunscreen [4].

For toxicological testing, ZnO NPs are dispersed in a range of different media and characteristics, such as degree of agglomeration and solubility need to be understood fully in each of the different media in order to ascertain whether any response is induced by the primary particles or secondary agglomerates, or whether they are a result of released free zinc ions which are already known to be toxic to aquatic organisms [5][6].

Dynamic Light Scattering (DLS) is the technique most commonly employed for investigating the agglomeration state of a dispersion of particles in solution. However; there are intrinsic limitations to
the technique. Firstly, DLS calculates the size of suspended particles by measuring Brownian motion and calculating the hydrodynamic diameter associated with the translational diffusion coefficient [7]. DLS therefore works on the assumption that all of the particles suspended in the liquid are spherical. As well as this, it is not capable of distinguishing between two different particles in a medium and it also relies on knowledge of the refractive index of the suspended particle as well as the viscosity and concentration of the suspension medium [8]. DLS is more sensitive to the larger size fraction of a polydisperse suspension due to the intensity of scattered light being proportional to \(d^6\) (where \(d\) is the diameter of the scattering ‘particle’) [7]. Finally, if the dispersing media contains a solid fraction of undissolved macromolecules, there is a chance that the DLS will measure these as suspended particles if they are significantly more concentrated than the particles of interest.

Transmission electron microscopy (TEM) is often employed to look at the primary particles in dispersions however, just dropping the solution onto a support film and air-drying before imaging in the TEM (drop-casting) does not give a representative view of the particles in suspension because of drying effects such as nanoparticle coalescence.

With this in mind, an alternative sample preparation technique has been investigated for the TEM [9]. This preparation route involves the blotting of the suspension on the TEM support film, immediately followed by plunging it into liquid ethane cooled by liquid nitrogen. This produces an electron transparent thin film of the vitrified dispersant with the nanoparticle dispersion trapped in the film. We have shown that when the specimens are warmed under the vacuum conditions of the TEM the dispersant devitrifies and sublimates leaving the dispersion of nanoparticles on the carbon film unaltered [10]. Consequently for this work, thin film ZnO-suspensions prepared for TEM by plunge freezing have been warmed under rotary vacuum conditions and 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.

2. Experimental

Colloidal ZnO nanoparticles were prepared based on the polyl method [11] [12]. 5g of Zinc Acetate dihydrate (ZnAc.2(H2O)) (Sigma Aldrich Reagent > 99%) is added to 50 ml of diethylene glycol (DEG) (Sigma Aldrich ACS Reagent 99%) and 1ml of triple distilled milliQ water. The mixture is then heated under reflux at 140°C for 1 hour and for a further 2 hours at 180°C, after which a milky white suspension is observed. The suspension is then diluted with milliQ water to a concentration where the ZnO NPs are 0.1% w/v with respect to water. The DEG concentration is 1.25 % v/v with respect to water. The suspension is then sonicated for 20 minutes. Samples for TEM were prepared by two methods:

(i) Plunge frozen TEM specimens were prepared by placing a 3.5 \(\mu\)L droplet on a glow discharge treated carbon film, the grid is then blotted and immediately plunge frozen in liquid ethane [9]. The grid was then allowed to warm to room temperature under rotary vacuum conditions.

(ii) Drop cast specimens were prepared by placing a 3.5 \(\mu\)L droplet on a holey carbon support film and allowed to dry in air.

TEM was then performed on an FEI Tecnai F20 FEG-TEM operated at 200 kV and equipped with a Gatan Orius SC600A CCD camera. DLS of the suspension was performed on a Malvern Zetasizer Nano ZS with the data manipulation performed by the DTS Nano software.

3. Results and Discussion

TEM micrographs were obtained for the grids prepared by both drying techniques. The average primary particle size (38 ± 10 nm) was calculated from measurement of 200 particles over a set of mid-range magnification TEM images (e.g. Figure 1 a). The majority of the nanoparticles are faceted with an aspect ratio of approximately 1:1, however a number of the particles have grown along the c-axis to produce elongated nanoparticles with bullet like edges. The average aspect ratio for the
elongated particles is approximately 2.5:1. Ignoring the length and measuring only the width of the elongated particles and the diameter of the faceted particles, the sample has an average size distribution of 30 nm with a standard deviation of less than 5 nm. In comparison to commercially available samples of ZnO nanoparticles, the particles synthesized have a relatively narrow size and shape distribution.

Low magnification images of the two different sample preparation methods are shown in Figure 1 (b) and (c). From the images of the drop cast sample, 100 agglomerates were measured and the average agglomerate size was calculated to be 2.4 ± 2.1 µm however there were agglomerates of up to 10 µm and also some individual particles on the film (Figure 1 (b)). In stark contrast, the particles are dispersed to a far greater degree in the plunge frozen preparation method (Figure 1(c)). There are very few agglomerates larger than 400 nm, and there is also a significant fraction (~50%) of individual particles as well as very small clusters of 2 or 3 particles on the film. The agglomerate size distribution in this specimen shows a distribution centred around ~ 50 nm (Figure 1(d)) which implies that in the drop casting method most of the small aggregates are swept into the big agglomerates in the drying process (Figure 1(b)).

![Figure 1: (a) TEM image of the ZnO suspension drop cast onto holey carbon support film. Primary particles of 30 – 50 nm are clearly visible. Low magnification TEM images of samples prepared by (b) the drop-casting method and (c) by the plunge-freezing method. The maximum agglomerate size in (b) is orders of magnitude bigger than in (c). (d) Histogram showing agglomerate size (nm) vs. frequency obtained by quantifying several TEM images of the plunge frozen specimen seen in (c).](image)

![Figure 2: DLS plot for colloidal dispersion of ZnO nanoparticles showing (a) number (b) volume and (c) intensity plots](image)

![Figure 3: DLS number plot (a) for colloidal dispersion of ZnO nanoparticles with TEM plunge freezing data (b) overlaid](image)

The light scattering profiles of the suspension obtained by DLS are converted into plots of Size (nm) vs Intensity/Volume/Number and all have a principle scattering peak with a maximum at a hydrodynamic diameter that drops from 200 to 70 nm respectively (figure 2). The intensity plot shows the relative intensity of light scattered by each of the size groups. This can then be converted into a volume plot using Mie theory which takes into account the greater light scattering of the larger particles compared to small. This explains why the volume peak is at a lower size than the intensity peak and consequently why the number plot has an even lower average particle size. Both the volume and intensity plots both show a secondary maximum in the 5-10 µm region, this may be a result of scattering from a very large agglomerate or from dust or contaminants in the suspension. Comparing
the number plots from DLS and the TEM plunge freezing data, it can be seen in (figure 3) that the shapes of the curve and hence the overall size distribution is in good agreement even if however, the TEM data peak is at a smaller particle size than for DLS (50 nm vs. 65 nm). This could be due to a number of different reasons.

Firstly, there are a series of assumptions that take place in acquiring data through DLS, the Mie theory approximation as well as the assumption that the particles are spherical will undoubtedly lead to inaccuracies in particle size measurements because of the presence of nanoparticles with elongated morphologies [7]. Secondly, following the synthesis route and even after dilution with water there will be a coating of DEG on the surface of the ZnO NPs that is likely to be fully extended in solution due to its miscibility in water. DLS will measure the hydrodynamic diameter of both the particle and the coating on the surface and so this may account for a small discrepancy in size [13]. Thirdly there will be uncertainty in the TEM measurement due to potentially non representative and insufficient sampling which we are trying to reduce with further work. Either way, the two results are sufficiently similar to suggest that although the current ZnO suspension is polydisperse the average agglomerate length is no more than twice the primary particle length.

4. Conclusions
A highly dispersed sample of ZnO NPs with a reasonably tight size distribution has been produced using the polyol route. The dispersion of DEG-coated ZnO nanoparticles in water has been investigated by DLS and a plunge-freeze TEM method. Both techniques indicate a polydispersion of ZnO nanoparticles with an number average agglomerate length no more than twice the primary ZnO length and with 90% of the agglomerates (in a number based size distribution) having lengths less than 150 nm. Accurate measurement of ZnO agglomeration in solution will assist the interpretation of Zn solubility and toxicity measurements.

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