Assessing Nanoparticle Toxicity in Waste Water using Nanoparticle Tracking Analysis

PARTICLE CONCENTRATION

PARTICLE SIZE

Introduction

At the same time as an increasing interest in, and rapid development of, a wide range of materials and products containing nanoscale structures and engineered nanoparticles, awareness has grown that the longer term potential toxic effects of such materials and their potential environmental impact are poorly understood. Existing methods have been assessed and new methods sought by which such materials could be analyzed on a routine basis during development and manufacture.

The use of Nanoparticle Tracking Analysis as a rapid and information-rich multi-parameter nanoparticle characterization technique which allows the user to obtain number frequency particle size distributions of polydisperse nanoparticulate systems has resulted in its rapid adoption as an interesting new technique in a wide range of sectors within environmental and toxicity studies. This white paper addresses some of the latest work in the literature in which NTA has been proposed, used and assessed in the study of nanoparticle toxicity and environmental impact.

NTA has found use in a variety of investigations researching the toxicity and environmental impact of nanoparticles. As well as being used to determine the size of particles in investigations into the toxicity of carbon nanotubes and nanoparticulate metals, NTA has also been used in investigations on the interactions of nanoparticles with organisms at a cellular level and the development of methods for the testing of toxicity. NTA has proved to be a useful tool in determining both particle size and concentration of nanomaterials in waste water analysis.

Nanoparticle Tracking Analysis (NTA) Overview

NTA utilizes the properties of both light scattering and Brownian motion in order to obtain the particle size distribution of samples in liquid suspension. A laser beam is passed through the sample chamber, and the particles in suspension in the path of this beam scatter light in such a manner that they can easily be visualized via a 20x magnification microscope onto which is mounted a camera. The camera, which operates at approximately 30 frames per second (fps), captures a video file of the particles moving under Brownian motion within the field of view of approximately 100 µm x 80 µm x 10 µm (Figure 1).

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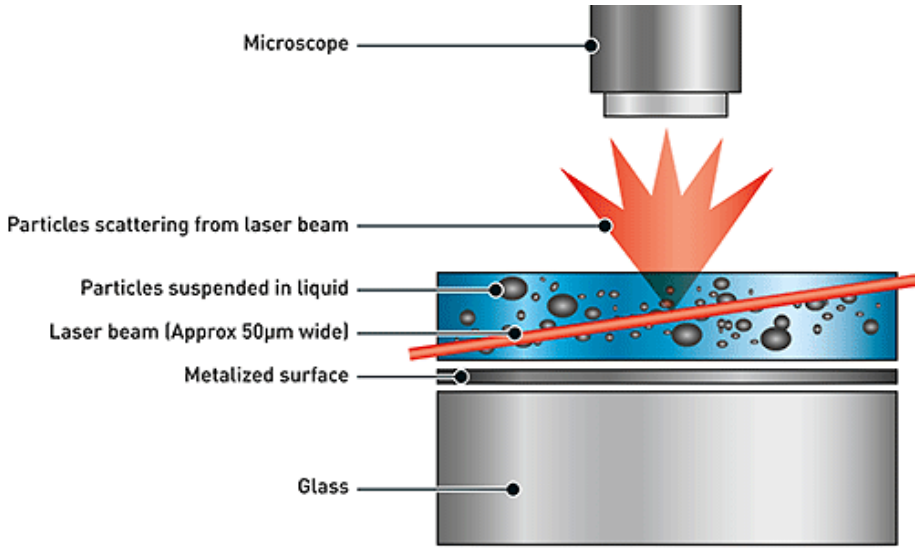

Figure 1: Schematic of the optical configuration used in NTA.

The movement of the particles is captured on a frame-by-frame basis. The proprietary NTA software simultaneously identifies and tracks the center of each of the observed particles, and determines the average distance moved by each particle in the x and y planes. This value allows the particle diffusion coefficient (Dt) to be determined from which, if the sample temperature T and solvent viscosity η are known, the sphere-equivalent hydrodynamic diameter, d, of the particles can be identified using the Stokes-Einstein equation (Equation 1).

$$Dt = \frac{TK_B}{3\pi\eta d}$$ Equation 1

where K_B is Boltzmann's constant.

NTA is not an ensemble technique interrogating a very large number of particles, but rather each particle is sized individually, irrespective of the others. An example of the size distribution profile generated by NTA is shown in Figure 2.

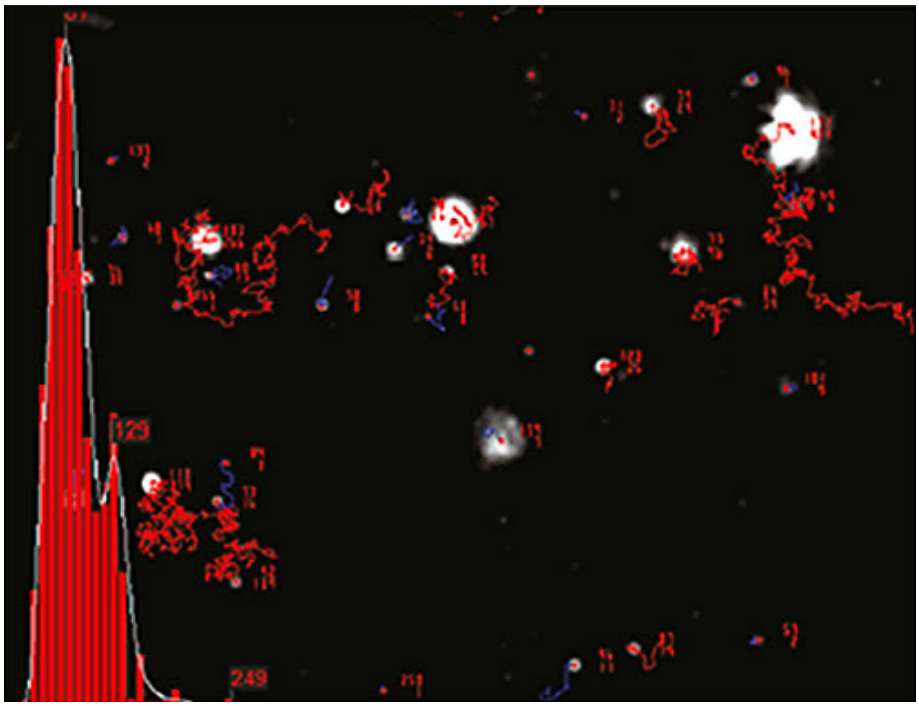

Figure 2: An example of the size distribution profile generated by NTA. The modal size for this sample is found to be approximately 70 nm, with larger sized particles also present.

In addition, the particles' movement is measured within a fixed field of view (approximately 100 µm by 80 µm) illuminated by a beam approximately 10 µm in depth. These figures allow a scattering volume of the sample to be estimated; by measuring concentration of the particles within this field of view and extrapolating to a larger volume it is possible to achieve a concentration estimation in terms of particles per mL for any given size class or an overall total.

Cytotoxic studies

At a cellular level, NTA has proved useful in studying the genotoxicity of cobalt NPs in human peripheral leukocytes (Colognato et al. 2008) and mouse fibroblasts (Ponti et al. 2009a). The ability of nanoparticles to cross the human placenta (Wick et al. 2009) including the transport of SiO_2 nanoparticles through human skin (Staroňová et al., 2012). Similarly, Filton et al. (2012) reported on human skin penetration of cobalt nanoparticles through intact and damaged skin suggesting that Co applied as NPs is able to penetrate the human skin in an in-vitro diffusion cell system.

An increasing number of studies exploiting NTA address the potential hazards of different metal species in a variety of cellular and aqueous systems. These include the effect of gold (Gosens et al. 2010) silver (MacCuspie et al. 2011, Bouwmeester et al. 2011) and copper and chrome oxide nanoparticles (Studer et al. 2010, Khatoonet al. 2011).

An understanding of the dispersion distribution of nanoparticle sizes prior to their introduction to cellular systems for cytotoxilogical testing is crucial and NTA has proved useful in this regard compared to other nanoparticle characterization techniques such as Dynamic Light Scattering (DLS) (Kendall et al. 2009, Patel et al. 2010, Munaro 2010, Karlsson 2010).The chemical interactions of nanoparticles of different types with various matrices of biological origin such as serum (Treuel et al. 2010) and organic

pollutants (Ben-Moshe et al., 2009) and dithiothreitol, (Sauvain et al., 2008) have also been studied.

The toxicological effects of cobalt nanoparticle (Co-NP) aggregates were examined and compared to those of cobalt ions using six different cell lines representing lung, liver, kidney, intestine and the immune system. The overall findings were in line with the hypothesis that the toxic effects of aggregated cobalt NPs are mainly due to cobalt ion dissolution from the aggregated NPs. NTA was used to determine particle size distribution profiles (Limor et al., 2011).

Christen and Fent (2012) showed that engineered silica nanoparticles and silver-doped silica nanoparticles induced endoplasmatic reticulum stress response and altered cytochrome P4501A activity in human liver cells (Huh7) and Pimephales promelas (fathead minnow) fibroblast cells (FMH) with NTA being used to monitor stability of the particle in nanopure water.

Carbon black and related diesel exhaust nanoparticles have been studied in human epithelial cells (Frikke-Schmidt et al., 2011) while Kadar looked at the enhancement of spermotoxicity of stabilized nanoiron (Kadar et al., 2011). Hemmingson et al. (2011) have used NTA in their studies of metabolic and genetic stress induced in a number of cell types exposed to conventional diesel and biodiesel nanoparticulate combustion products and showed biodiesel to be, on an equivalent mass basis, less toxic than conventional diesel. In other studies on diesel exhausts, Jantzen et al. (2012) looked at oxidative damage to DNA by diesel exhaust particle (DEPs) exposure in co-cultures of human lung epithelial cells and macrophages concluding that exposure of mono-cultured cells to DEPs generated oxidative stress to DNA, whereas co-cultures with macrophages had lower levels of oxidatively damaged DNA than A549 epithelial cells.

Suggesting that the toxicological effects of wood smoke particles are less investigated than traffic-related combustion particles, Forchhammer et al. (2011) compared the expression of adhesion molecules, monocyte interactions and oxidative stress in human endothelial cells exposed to wood smoke and diesel exhaust particulate matter using NTA to determine the particle size distribution of the wood smoke particles and those of standard reference material (SRM) 2975 diesel exhaust particles being used as benchmark particles. Similarly, Vesterdal et al. (2012a) looked at carbon black (CB) nanoparticles and vascular dysfunction in cultured endothelial cells and artery segments reporting that nanosized CB exposure activates endothelial cells and generates oxidative stress, which is associated with vasomotor dysfunction, using NTA to confirm nanoparticle stability during their experiments. Vesterdal et al. (2012) also used NTA to measure particle size in their study on pulmonary exposure to particles from diesel exhaust, urban dust or single-walled carbon nanotubes and oxidatively damaged DNA and vascular function in apoE-/-mice. Vesterdal et al. (2013) have most recently looked at the accumulation of lipids and oxidatively damaged DNA in hepatocytes exposed to particles concluding that exposure to four different carbon-based particles (diesel exhaust particles, fullerenes C60 or pristine single-walled carbon nanotubes) is associated with oxidative stress and steatosis in cultured human hepatocytes (HepG2). Zemanova et al. (2011) previously determined the influence of C60 fullerene derivative nanoparticle size on toxicity and radioprotectivity of water soluble fullerene derivative, while Matthews has also investigated the transport of CNTs across pulmonary epithelium using an isolated perfused rat lung preparation using NTA (Matthews et al., 2010 and 2009).

Using NTA to quantified microparticles in serum, Choudhary et al. (2013) evaluated the effect of cigarette smoke exposure on ventricular function and PA pressures. Progressive effects over multiple weeks exposure, including an impaired RV systolic but without elevated PA pressure and an increase in circulating microparticles (1x108/

mL) leading the researches to speculate that these effects may be due to exposure to circulating CS constituents or microparticles released in response to CS.

Mahmoudi et al. (2011) have discussed the opportunities and challenges surrounding the study of protein-nanoparticle interactions. Bulcão et al. (2012) investigated, for the first time, the toxicity of lipid-core nanocapsules (LNC) containing a polymer wall of poly(epsilon-caprolactone) (PCL) and a coating of polysorbate 80 (PS80) used as drug delivery devices (~245 nm as determined by NTA) in Wistar rats after single- and repeated-dose treatments. The findings were in agreement with earlier reports regarding no appreciable toxicity of biodegradable polymeric nanoparticles, indicating that LNC might be a safe candidate for drug delivery system.

More recently, Prina-Mello et al. (2013) have carried out a multiparametric toxicity evaluation of superparamagnetic iron oxide nanoparticles (SPIONs) by a high content screening technique in their identification of biocompatible multifunctional nanoparticles for nanomedicine. Mwilu et al. (2013) used a variety of methods including Absorbance Spectroscopy, High Resolution Transmission Electron and Scanning Electron Microscopy (TEM/SEM), DLS and NTA to follow changes in silver nanoparticles exposed to human synthetic stomach fluid; specifically the effects of particle size and surface chemistry. They found that generally, the smaller sized AgNPs (< 10 nm) showed higher rates of aggregation and physical transformation than larger particles (75 nm). Also working with silver, Kruszewski et al. (2013) found that oxidative DNA damage corresponds to the long term survival of human cells treated with silver nanoparticles. Silver nanoparticle aggregation was followed by NTA.

More recently, Dieni et al. (2013) have demonstrated that spherical gold nanoparticles impede the function of bovine serum albumin in vitro. In order to isolate strongly interacting BSA oligomers, irreversible BSA aggregates or strong BSA-nAu complexes induced by recruitment of BSA into the protein corona, BSA-nAu-cap suspensions were subjected to centrifugal filtration and native-PAGE. However, this methodology failed to detect any altered distribution of higher-molecular weight species of BSA compared to control (free of nAu), suggesting that any protein-protein or protein-nAu interactions that contribute to these altered properties of BSA are not irreversible and do not withstand high g-forces and/or electrophoresis.

Physicochemical properties of nanoparticles (NP) strongly affect their influence on cell behavior, but can be significantly distorted by interactions with the proteins present in biological solutions. In a recent study Bartczak et al. (2013) showed how different surface functionalities of zinc oxide (ZnO) NP led to changes in the size distribution as measured by NTA and dissolution of the NP in serum containing cell culture media and how this impacted on NP toxicity. NPs capped with weakly bound large proteins underwent substantial transformations due to the exchange of the original surface ligands to the components of the cell culture media. NTA was also used to determine size of particles in a study of the adsorption of nanoparticles and nanoparticle aggregates on membrane under gravity (Zhu et al., 2013). Wiemann (2013) also showed NTA-derived data in his recent presentation on the agglomeration, uptake, biodistribution and in vivo toxicity of nanosized SiO_2 particles in the rat lung.

Mihaiescu et al. (2013) reported on Fe_3O_4/Salicylic acid nanoparticles behavior on chick CAM vasculature when using a modified ferrite co-precipitation synthesis to obtain core–shell Fe_3O_4/salicylic acid magnetic nanoparticles (Sa-MNP) with well-dispersed aqueous solution properties. They found a reversible and well controlled intravascular accumulation under static magnetic field, a low risk of embolization with nanoparticle aggregates detached from venous intravascular nanoblocked areas, a persistent blocking of the arterioles and dependent capillaries network and a good circulating life time and biocompatibility; all suggesting a possible biomedical

application of these MNPs in targeted cancer therapy through magnetic controlled blood flow nanoblocking mechanism.

Given ecotoxic, non-degradable biocides with a broad protection range are now prohibited in Europe, the paint industry is considering engineered nanoparticles (ENPs) as an alternative biocide. However, there is concern that ENPs in paint might be released in run-off water and subsequently consumed by animals and/or humans, potentially coming into contact with cells of the gastrointestinal tract and affecting the immune system. Accordingly Kaiser et al. (2013) evaluated the cytotoxic effects of three ENPs (nanosilver, nanotitanium dioxide and nanosilicon dioxide) that have a realistic potential for use in paints in the near future. Using NTA to analyze changes in the size (i.e. agglomeration) of nanosilver and nanotitanium dioxide during incubation of gastrointestinal cells (CaCo-2) and immune system cells (Jurkat) in culture media, they showed that the results suggest that paints doped with ENPs do not pose an additional acute health hazard for humans

After passage through biological barriers, nanomaterials inevitably end up in contact with the vascular endothelium and physiological flow and can induce cardiovascular damage. In a recent study the toxicity and sublethal effects of six nanoparticles, including four of industrial and biomedical importance, on human endothelial cells was investigated using different in vitro assays (Ucciferri et al., 2013), NTA being used to analyze the AgNPs used. Broggi et al. (2013) also showed that silver nanoparticles induce cytotoxicity, but not cell transformation or genotoxicity, on Balb3T3 mouse fibroblasts.

In recent years there has been an ongoing discussion whether traditional toxicological methods are sufficient to evaluate the risks associated with nanoparticle inhalation which has led to the emergence of Air-Liquid interface toxicology. Svensson et al. (2013) thus describe the direct deposition of gas phase generated aerosol gold nanoparticles into biological fluids in which they analyze, using NTA as well as DLS and UV spectroscopy, corona formation and particle size shifts. They suggested that their results were important since the protein corona together with key particle properties (e.g. size, shape and surface reactivity) to a large extent may determine the nanoparticle effects and possible translocation to other organs.

Similar work on nanoparticle protein coronas recently under taken by Hayashi et al. (2013) who considered the proposal that, while cells recognize the biomolecular corona around a nanoparticle, the biological identity of the complex may be considerably different among various species. Using coelomocytes of the earthworm Eisenia fetida from which were extracted E. fetida coelomic proteins (EfCP) as a native repertoire and fetal bovine serum (FBS) as a non-native reference, they confirmed the determinant role of the recognizable biological identity during invertebrate in vitro testing of nanoparticles. Their finding showed a case of species-specific formation of biomolecular coronas which suggested that the use of representative species may need careful consideration in assessing the risks associated with nanoparticles. Data from their presentation shows that NTA is less susceptible to the presence of aggregates than DLS when the sample is measured by the two techniques.

Aquatic and Marine Toxicity

Methods such as NTA could be considered as one of a number of means by which the aquatic environmental impact and potential cellular toxicity of nanoparticles could be studied in the future (Hassellöv and Kaegi, 2009). Indeed, later studies showed that the complexity of interactions between NEPs and aquatic environmental matrices is extremely complex representing a significant challenge in both their quantification and modelling but in which NTA may play a role (Gornati et al., 2009; Hartmann, 2011; Arvidsson et al.; 2011; Howard, 2010, Njuguna et al., 2011; Tran et al., 2009).

Large collaborative research projects began investigating the ecotoxicological effects of a variety of nanoparticles on the freshwater environment (Juhel 2009) including CeO_2 which is used increasingly being used as a catalyst in the automotive industry (Quik et al., 2010; Van Hoecke et al,. 2009) and the effect of silver and gold nanoparticles on fish (Scown et al., 2010) and rainbow trout hepatocytes and gill cells (Farkas et al., 2010, Farkas et al., 2011). Trumsina compared various methods for monitoring nanoparticle detachment from textiles during washing and discussed the relative benefits of DLS and NTA against a new method of gas discharge visualization (Trumsina et al., 2011) and Piccapietra et al. (2011) considered the colloidal stability of carbonate coated silver nanoparticles in synthetic and natural freshwater.

Handy and his co-workers have carried out extensive studies on the effects, in terms of aquatic ecotoxicity, of various metal and metal oxide nanoparticles on fish. They looked at the effects of TiO_2 on the physiology and reproduction of zebrafish (Ramsden et al., 2012) concluding there was limited evidence of toxicity but a discernible effect on reproduction. They also looked at the uptake of titanium from TiO_2 nanoparticle exposure in the isolated perfused intestine of rainbow trout, Oncorhynchus mykiss, (Al-Jubory and Handy, 2012; Shaw et al., 2012 and Al-Bairuty et al., 2012) and studied the histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of the same species. Assessing whether copper nanoparticles are more toxic than traditional forms of dissolved copper they studied the pathologies in gill, gut, liver, kidney, brain and muscle of juvenile specimens exposed in triplicate to either a control (no added Cu), 20 or 100 µg/L of either being dissolved Cu (as $CuSO_4$) or Cu-NPs (mean primary particle size of 87 ± 27 nm) in a semi-static waterborne exposure regime. Overall the data showed that pathology from $CuSO_4$ and Cu-NPs were of similar types, but there were some material-type effects in the severity or incidence of injuries with Cu-NPs causing more injury in the intestine, liver and brain than the equivalent concentration of $CuSO_4$ by the end of the experiment, but in the gill and muscle $CuSO_4$ caused more pathology. In further work, they also showed that subtle alterations in swimming speed distributions of rainbow trout exposed to titanium dioxide nanoparticles were associated with gill rather than brain injury (Boyle et al., 2012). In all of these studies, NTA was used to determine the mean size and particle size distribution of the nanoparticles used. Recently, Windeatt and Handy (2012) have reported NTA work on the effect of nanomaterials on the compound action potential of the shore crab, Carcinus maenas.

In related research, the effects of particle size and coating on nanoscale Ag and TiO_2 exposure in zebrafish (Danio rerio) embryos was studied (Osborne et al., 2012), the results of which showed titanium dioxide nanoparticles (nominally, 4 nm, 10 nm, 30 nm and 134 nm) had little or no toxicity on the endpoints measured while Ag both in nano form (10 nm and 35 nm) and its larger counterpart (600-1600 nm) induced dose-dependent lethality and morphological defects, occurring predominantly during gastrula stage. Of the silver material tested, 10 nm nanoparticles appeared to be the most toxic. More recent work on zebrafish has resulted in reports from Christen et al. (2013)

who show that silver nanoparticles induce endoplasmatic reticulum stress response in zebrafish and compared their data to that obtained when tested on human hepatoma cells (Huh7).

Still working with zebrafish, Henry et al. (2013) showed that the association of Hg^{2+} with aqueous (C60)n aggregates facilitates increased bioavailability of Hg^{2+} in zebrafish (Danio rerio) using NTA to demonstrate an increase in aggregate size and settlement of nC60 aggregates out of the water column over 24 h. This indicated that aqueous nC60 can sorb Hg^{2+}, transport Hg^{2+} to substrate surface, and increase concentrations of bioavailable Hg^{2+} in organisms located where settled nC60 aggregates accumulate.

NTA has been central to studies on the influence of engineered Fe_2O_3 nanoparticles and soluble ($FeCl_3$) iron on the developmental toxicity caused by CO_2-induced seawater acidification (Kadar et al. 2010). Recently, Tatarkiewicz et al. (2012) have described the use of NTA in the concentration measurement and sizing of colloidal particles in the Arctic Ocean while Stuart et al. (2012) report proof-of-concept measurements relating to the impact of nanoparticles with an electrode potentiostatted at a value corresponding to the diffusion controlled oxidation of silver nanoparticles in authentic seawater media.

The increasing use of nanoparticles in a variety of textiles as antibacterial, antimicrobial, water resistant and protective agents has prompted the use of NTA in the study nanosilver mobilized in washing machine effluents (Farkas et al., 2011). Wang et al. (2012) have also studied the aquatic toxicity of nanosilver colloids to different trophic organisms comparing the contributions of particles and free silver ion.

Using a 15k oligonucleotide microarray for Daphnia magna, a freshwater crustacean and common indicator species for toxicity, to differentiate between particle specific and ionic silver toxicity and to develop exposure biomarkers for citrate-coated and PVP-coated AgNPs, Poynton et al. (2012) determined the degree of aggregation of AgNPs prior to studying their toxicity at the genomic level.

In further work studying the effect of nanoparticles on marine microfauna, Li et al. (2013) have reported on the accumulation of aqueous and nanoparticulate silver by the marine gastropod Littorina littorea concluding that Ag is most bioavailable to L. littorina when in true solution, and that Ag measured in external tissues of the snail following exposure to nanoparticles arises from some physical association that does not result in significant transfer of the metal to internal organs.

Shaw et al. (2013) have proposed a simplified method for determining titanium from TiO_2 nanoparticles in fish tissue with a concomitant multi-element analysis claiming method precision and accuracy were good with coefficients of variation <7% with NTA data being used to confirm, where applicable, aggregate presence and size.

Batley et al. (2012) have recently reviewed the complexities associated with determining the fate and risks of nanomaterials in aquatic and terrestrial environments and Lambert et al. (2013a) have considered the effects of environmental conditions on latex degradation in aquatic systems as well as from products such as natural rubber latex condoms (Lambert et al., 2013b). Samples were immersed in either demineralized water, artificial freshwater and marine water media and exposed for a period of 200–250 days with exposure starting at different times of the year. Effects of pH, agitation and the exclusion of light on degradation were also studied. At the end of the exposure period, recovery of polymer material ≥ 1.6 µm ranged from a low of 22.04% (± 16.35, for the freshwater treatment at pH 5.5) to a high of 97.73% (± 0.38, for the exclusion of light treatment). The disappearance of the bulk

material corresponded to an increase in nanoparticles as measured by NTA and dissolved organic material in the test media. In the case of the condom study, the direct effects of the degradation mixture were investigated using two freshwater organisms with different life cycle traits, the water column crustacean Daphnia magna and the sediment-dwelling larval of Chironomus riparius. Ecotoxicity tests investigated both acute and chronic endpoints and were shown to exhibit no toxic effects. In another recent paper examining the effects of twelve carbon nanomaterials (CNMs) that differ in their core structure and surface chemistry to Daphnia magna over a 21-day chronic exposure Arndt et al. (2013) looked at the effect these materials have on daphnid mortality, reproduction, and growth: They concluded that 1) acute exposure assays do not accurately describe the impact of CNMs to biological systems, 2) chronic exposures provide valuable information that indicates the potential for different modes of action for nanomaterials of differing chemistries, and 3) core structure and surface chemistry both influence particle toxicity.

Recently, Hassellöv (2013) has comprehensively reviewed the occurrence, identification, fate and behavior of engineered nanoparticles and nanoscale pollutants in marine systems including, in addition to synthetic nanomaterials, many other types of micro- and nanoscale pollutants which have recently been identified as potential emerging pollutants, e.g. from road runoff, combustion, mining, waste and industrial processes.

Microbiota and Plants

The effect that nanoparticles have on microorganisms and their ecology has been studied using NTA to determine nanoparticulate properties and behavior.

In their investigation of the distribution and bioavailability of engineered nanoparticles (silver NP, cerium dioxide NP, titanium dioxide NP) in freshwater periphyton, Kroll and her co-workers used a variety of techniques, including NTA, to monitor material properties such as size, charge, and dissolution (Kroll et al., 2011).

In work exploring the link between chemical composition and molar-mass distribution of the extracellular polymeric substances (EPS) released by the bacterium Sinorhizobium meliloti using chemical, spectroscopic and fractionation techniques, NTA confirmed the size distributions and chemical heterogeneity of such materials as characterized by asymmetrical flow field-flow fractionation (Alasonati and Slaveykova, 2011).

Similarly, Turner et al. (2011) investigated the interactions of Ag nanoparticles with marine microalgae, Ulva lactuca, using NTA to characterize their Ag nanoparticle suspensions.

Finally, Chaudhari et al. (2012) used NTA, TEM and electron dispersive X-ray spectra to assess the effect of biosynthesized silver nanoparticles on Staphylococcus aureus biofilm quenching and prevention of biofilm formation.

Metal-containing nanomaterials have the potential to be used in dentistry for infection control, but little is known about their antibacterial properties. A recent study investigated the toxicity of silver, titanium dioxide and silica nanoparticles (NPs) against the oral pathogenic species of Streptococcus mutans, compared to the routine disinfectant, chlorhexidine. Thus, Besinis et al. (2012) investigated the antibacterial effects of Ag, TiO_2 and SiO_2 nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays using NTA for nanoparticle sizing while Bondea et al. (2012) similarly used NTA in their study of Murraya koenigii-mediated synthesis of silver nanoparticles and its activity against three human pathogenic bacteria, claiming remarkable antibacterial activity

against three human pathogenic bacteria when used in combination with commercially available antibiotics.

NTA was cited in data reported in other studies on bacteria. The antibacterial and water purification activities of self-assembled honeycomb structure of aerosol deposited titania film was studied by Park et al. (2012) and the determination of internalization of chromium oxide nanoparticles in Escherichia coli by flow cytometry Khatoon et al. (2011). Carter et al. (2012) also showed that a bacteriophage cocktail significantly reduces Escherichia coli O157:H7 contamination of lettuce and beef, but did not protect against recontamination.

In a study of the effects of coating applied to zero-valent nano-iron (nZVI) on early life stage development of three key marine invertebrate species, Kadar et al. (2012) used NTA to study the dissolution of nZVI in sea water showing the coating help stabilize the nanometal suspension. Kadar has also studied the effect of NTA-analyzed industrially relevant engineered iron nanoparticles on growth and metabolic status of marine microalgae cultures in which he followed subsequent alterations in their growth rate, size distribution, lipid profiles and cellular ultrastructure (Kadar et al., 2012).

NTA has also been used amongst other techniques to study nanoparticulates transport in fungi (Cunha-Azevedo et al., 2011). Cunha-Azevedo also developed and tested an anti-fungal formulation of PLGA nanoparticles designed to release the active agent itraconazole in which size was considered an important feature and analyzed by NTA as an average of 174 nm (Cunha-Azevedo 2011).

Hartmann et al. (2012) reviewed the challenges of testing metal and metal oxide nanoparticles in algal bioassays using titanium dioxide and gold nanoparticles as case studies. They showed that Au NP coating layers changed over time and TiO_2 nanoparticle aggregation/agglomeration increased as a function of concentration. While NTA was used to determine the hydrodynamic diameter and size distributions of suspended particles, it was found that of three biomass surrogate measuring techniques evaluated (Coulter Counting, cell counting in haemocytometer and fluorescence of pigment extracts) fluorometric methods was found to be most suitable for quantifying biomass, although it is complicated by algae-particle interactions and nanoparticle transformation. She concluded that optimization of the method is needed to reduce further particle interference on measurements.

The anti-microbial properties of nanosilver have been well established and as such much work has been carried out on determining the mechanism and effects of this material on microbial systems.

Piccapietra et al. (2011) used nanoparticle tracking analysis, dynamic light scattering, and ultraviolet–visible spectroscopy to measure changes in the physicochemical properties of silver nanoparticles (AgNP) in their investigation on the fate, mobility, and bioavailability of AgNP in aquatic systems including the influence of pH, ionic strength, and humic substances on the stability of carbonate-coated AgNP of average diameter 29 nm. He extended this work to include studies on the colloidal stability of silver nanoparticles and their interactions with the alga Chlamydomonas reinhardtii (Piccapietra, 2012).

Silver nanoparticles were also the subject of a recent study by Schacht et al. (2012) on microbial growth dynamics finding, to their surprise, that their data showed growth stimulation of C. necator at certain Ag(0) nanoparticle concentrations, as well as varying susceptibility to nanoparticles at different growth stages underscoring the need for time-resolved analyzes of microbial growth inhibition by Ag(0) nanoparticles.

Using NTA to determine particle size distribution, Matzke et al. (2013a) recently discussed the effects of selected silver nanoparticles on freshwater microbial communities showing that differences in toxicity could be determined for the different particles with $AgNO_3$ being for almost all cases the most toxic compound, with one exception. The same group subsequently described the toxicity of differently sized and coated commercially available silver nanoparticles to the bacterium Pseudomonas putida. The results indicated that the toxicity is driven by the Ag^+ ions, implying that an environmental hazard assessment for microorganisms based on total silver concentration and the assumption that AgNPs dissolve is sufficiently protective (Matzke et al., 2013).

In NTA-supported in vitro and soil experiments studying the impact of Ag and Al_2O_3 nanoparticles on the soil bacteria, Bacillus cereus and Pseudomonas stutzeri, Fajardo et al. (2013) showed that Al_2O^3 nanoparticles did not show significant toxicity at any dose or time assayed, whereas exposure to 5 mg/L Ag nanoparticles for 48 h caused bactericidal effects. In a microcosm experiment, using two different natural soils, Al_2O_3 or Ag nanoparticles did not affect the Caenorhabditis elegans toxicity endpoints, growth, survival or reproduction. These changes were attributable to both the nanoparticles treatment and soil characteristics, highlighting the importance of considering the soil matrix on a case by case basis.

Tlili et al. (2012) also showed the short-term toxicity of silver nanoparticles on litter-associated fungi and bacteria from streams while Masurkar et al. (2012) showed that Staphylococcus aureus biofilm quenching and biofilm formation prevented activity of silver nanoparticles synthesized using Saccharum officinarum (sugarcane). Masurkar later extended this work to demonstrate biofilm quenching activity of silver nanoparticles synthesized using Bacillus subtilis in his work promoting the green synthesis of silver nanoparticles as a basic need in the field of nanotechnology (Masurkar et al., 2013). Similarly, Dhuldhaj et al. (2012) had previously described an eco-friendly approach via the Tagetes erect-mediated phytosynthesis of silver nanoparticles. Gupta et al. (2013) also investigated the Lawsonia inermis-mediated synthesis of silver nanoparticles and its activity against human pathogenic fungi and bacteria with special reference to formulation of an antimicrobial nanogel using NTA to establish particle size distribution. Raheman et al. (2011) had previously proposed silver nanoparticles as a novel antimicrobial agent synthesized from an endophytic fungus Pestalotia sp. isolated from leaves of Syzygium cumini.

Zhdanov and Höök (2013) have reported on the nucleation in mesoscopic systems under transient conditions with respect to peptide-induced pore formation in vesicles given attachment of lytic peptides to the lipid membrane of virions or bacteria is often accompanied by their aggregation and pore formation, resulting eventually in membrane rupture and pathogen neutralization. The results obtained helped clarify the mechanism of the pore formation and membrane destabilization observed during interaction of highly active α-helical peptide with sub-100 nm lipid vesicles that mimic enveloped viruses with nanoscale membrane curvature.

Using NTA to determine particle size, it was found that alumina nanoparticles substantially increase biomass accumulation of the aquatic plant Lemna minor and that such a stimulatory effect of alumina nanoparticles on growth had not been reported previously (Juhel et al. 2011). NTA has also been used amongst other techniques to study nanoparticulates transport in seed germination (Vajpayee et al. 2011) and to study nanoparticulates transport in surface runoff through dense vegetation (Yu, 2011).

Finally, Schwabe et al. (2013) recently discussed the influence of two types of organic matter on interaction of CeO_2 nanoparticles with plants in hydroponic culture. They

used hydroponic plant cultures to study nanoparticle–plant-root interaction and translocation and exposed wheat and pumpkin to suspensions of uncoated CeO_2–NP for 8 days (primary particle size 17–100 nm, 100 mg/L) in the absence and presence of fulvic acid (FA) and gum arabic (GA) as representatives of different types of natural organic matter. They showed that NP-dispersions were stable over 8 days in the presence of FA or GA, but with growing plants, changes in pH, particle agglomeration rate and hydrodynamic diameter were observed. None of the plants exhibited reduced growth or any toxic response during the experiment.

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