NANOSIGHT

White Paper

Nanoscale Material Characterization: A Review of the use of NTA

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seeing is believing

White Paper

Introduction

Nanoscale materials, in the form of nanoparticles, play an important and growing role across a range of different applications and industries who seek to exploit the significantly enhanced properties exhibited by such materials (e.g. greatly increased surface area, number concentration). The overall properties and stability of many manufactured products often depends upon the ability to repeatedly produce particle populations within fine tolerances, without the presence of contaminants or aggregates. The concentration of particles within a suspension is another factor that may have an effect upon the desired outcome of a product. It is clear then that there is a real need to characterize a variety of different properties when analysing nanoparticles in order to fully understand the relationship between the formulation and the overall bulk characteristics of the materials (Fedotov 2011). Similarly, Paterson et al (2011) have reviewed the requirement for quantified nanoparticle concentrations in environmental media in order to appropriately assess the risks to biological species due to potential nanoparticle exposure.

There are many techniques available for the analysis of particle size and size distribution, of which the most common include Dynamic Light Scattering (DLS), Electron Microscopy, Atomic Force Microscopy and Analytical Ultracentrifugation. However, each of these techniques comes with a unique set of benefits and limitations. Electron Microscopy (EM) and Atomic Force Microscopy (AFM) both offer users images of the particles themselves with high resolution information about both the size and morphology of the particles present, but both techniques also require time consuming preparation of samples, which could be potentially damaging and require the user to spend considerable time on analysis (Syvitski, 1991).

Ultracentrifugation again provides high resolution information on the size of particles within a sample but the technique requires a degree of previous knowledge of the composition of the material, is time consuming and the apparatus can be costly (Mächtle, 2006).

Ensemble methods based on light scattering and which simultaneously interrogate a large number of particles in a suspension are ideally suited for the analysis of monodispersed systems but have a limited capability to analyse those that are polydispersed. Furthermore, being ensemble methods they are unable to provide users with quantitative results regarding the number concentration of their systems. Foremost of such techniques for the analysis of nanoparticles is dynamic light scattering DLS (alternatively known as Photon Correlation Spectroscopy (PCS) or Quasi Elastic Light Scattering (QELS)) which utilises a digital correlator to analyse the timescales of fluctuations in intensity of light scattered by a suspension of nanoparticles moving under Brownian motion and has been extensively reviewed (Pecora, 1985). Through analysis of the resultant exponential autocorrelation function, average particle size can be calculated as well as the polydispersity index. Furthermore, as the relationship between the size of particles and the amount of light that they scatter varies strongly as a function of radius to the power of 6, the results will be significantly biased towards the larger, higher scattering particles within the sample. The resulting intensity weighted average particle size and poor particle size distribution information available can therefore be seriously misleading when analysing polydispersed samples.

The recent development of the technique of Nanoparticle Tracking Analysis (NTA) offers the ability to directly visualise, size and count nanoparticles in liquid suspension. Due to the fact that this technique can simultaneously analyse a population of nanoparticles on an individual basis, it is ideally suited for the real-time analysis of polydispersed systems ranging from 10-20nm up to 1-2 micron in size (depending on particle type). Additional parameters and measurements also allow users to acquire information on nanoparticle concentration, zeta potential, relative intensity of light scattered and also to visualise and analyse fluorescently labelled particles. (NanoSight, 2011, Carr et al 2009).

Nanoparticle Tracking Analysis: Principles and Methodology.

NTA utilises 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 a prism edged glass flat within the sample chamber, the particles in suspension in the path of this beam scatter light in such a manner that they can be easily visualised via a long working distance, x20 magnification microscope onto which is mounted a camera. The camera operates at 30 frames per second (fps) which captures a video file of the particles moving under Brownian motion within a field of view of approximately 100µm x 80µm x 10µm (Fig 1).

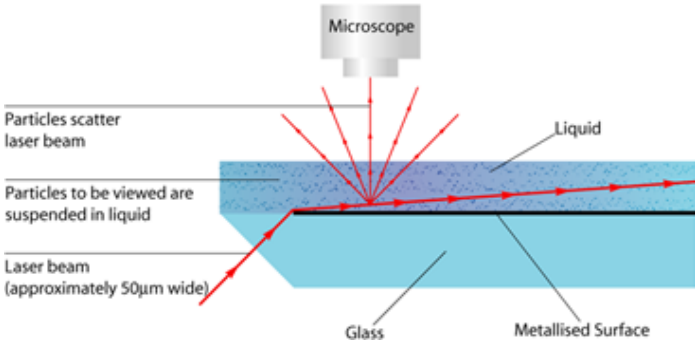
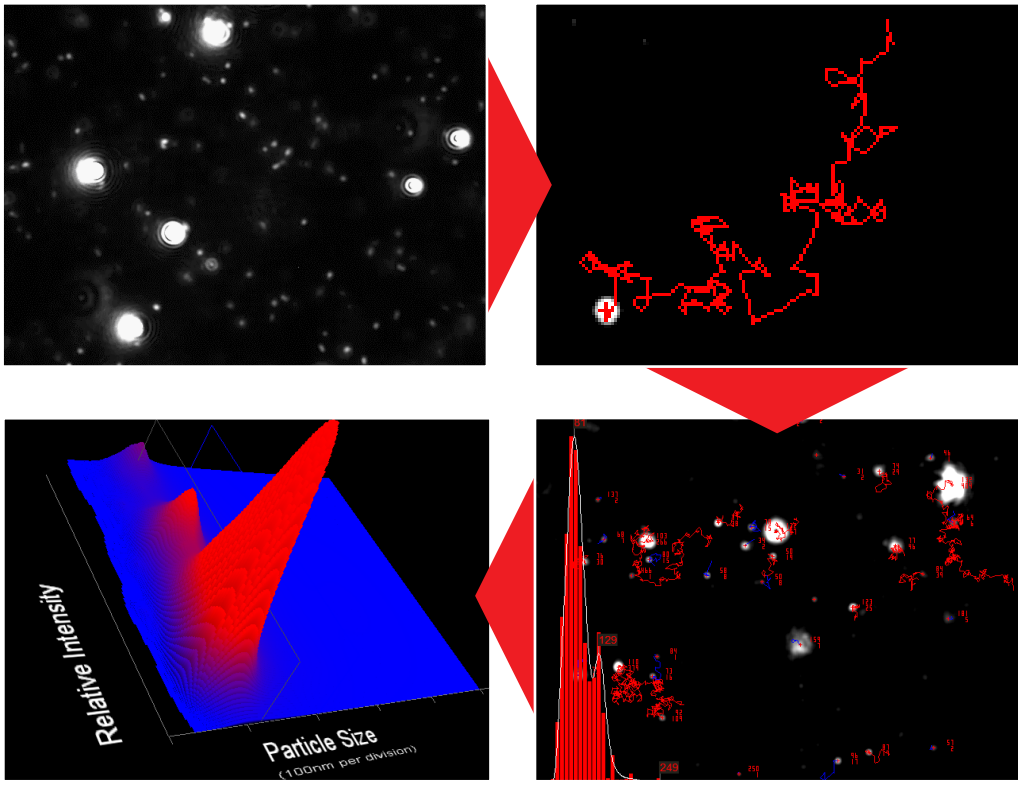


Figure 1: Schematic of the optical configuration used in NTA.

NTA captures a video of particles moving under Brownian motion.



NTA automatically locates and follows the center of each and every particle and measures the average distance moved per frame.

NTA consolidates the particle-by-particle size measurements with 3D intensity vs. concentration vs. size plots.

This is done simultaneously for all particles whilst NTA reports particle size vs concentration distributions.

Figure 2: Flow Diagram of the NTA Process

Concentration ranges measureable

NTA is not an ensemble technique, interrogating a very large number of particles, but rather each particle is sized individually, irrespective of the others. As such, it is important that a sufficient number of particles are analysed within the sample time to ensure that the data produced is statistically viable. A concentration in the region of 107 to 1010 particles per ml provides the user with statistically sound and repeatable particle size distributions within a timescale of typically 30-60 seconds.

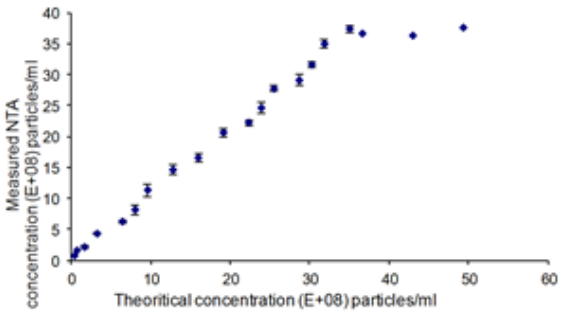


Figure 3: NTA reported concentration vs. actual sample concentration for 100nm latex particles. Error bars show +/- 1 SD. Note that non-linearity in NTA concentration estimation appears at approx >3-4x10^9/ml

Under normal conditions when analyzing optimal concentrations of nanoparticles exhibiting similar optical characteristics such as monodispersed polystyrene, counting accuracies can be as good as 5-10% if the sample is diluted to a suitable concentration range.

Particle size determined combined with Particle's I_{Scat}

While the size of nanoparticles is determined by NTA through measuring their Brownian motion, one of the unique and beneficial features of NTA is the ability to simultaneously measure the amount of light it scatters (I_{scat}) and plot the two measureands as a function of each other. This allows particles which may be of a similar size but different composition/refractive index to be successfully discriminated.

A mixture of 30nm Au, 60nm Au and 100nm polystyrene can be resolved in a 3D plot of Size v. Intensity v. Number (Fig 6) and in which the smaller but high Ri 60nm Au particles can seen to scatter more light than the larger 100nm polystyrene (100PS).

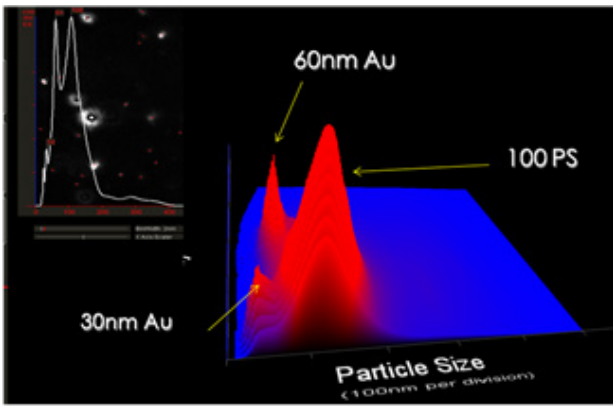


Figure 4: A 3D plot of 30nm and 60nm gold, and 100nm polystyrene particles in which the smaller but higher refractive index 60nm gold particles can be seen to scatter more light than the larger 100nm polystyrene.

The American Society For Testing And Materials (ASTM) have recently published a standard guide for the measurement of particle size distribution of nanomaterials in suspension by NTA, through adoption of which users of the technique can achieve standardisation of results (ASTM E2834 – 12, 2012).

NTA in the analysis of nanoparticles applied in industry.

Given the recognized importance of the subject of nanoparticles and their analysis and the fact that nanoparticles are already used in several consumer products including food, food packaging and cosmetics, and their detection and measurement in food represents a particularly difficult challenge, the European Commission published in October 2011 its recommendation on the definition of 'nanomaterial'. This will have an impact in many different areas of legislation, such as the European Cosmetic Products Regulation, where the current definitions of nanomaterial will come under discussion regarding how they should be adapted in light of this new definition. This new definition calls for the measurement of the number-based particle size distribution in the 1–100nm size range of all the primary particles present in the sample independently of whether they are in a free, unbound state or as part of an aggregate/agglomerate. Recently, Linsinger et al (2012) have analyzed the requirements on measurements for the implementation of the European Commission definition of the term 'nanomaterial'.

Calzolai et al (2012) have subsequently reviewed methods for measuring nanoparticles size distribution in food and consumer products. They gave an overview of the current state of the art, focusing particularly on the suitability of the most used techniques for the size measurement of nanoparticles when addressing this new definition of nanomaterials illustrating the problems to be overcome in measuring nanoparticles in food and consumer products with some practical examples. In assessing NTA and in comparison the other such techniques, they acknowledged that NTA was effective in overcoming the inherent weaknesses of the DLS and SLS methods when confronted with mixtures of relatively similarly sized particles and had a number of important advantages including relatively low instrument cost and high sensitivity which can detect nanoparticles at concentrations as low as low as 10^6 particle / cm^3.

Nanoparticle Design and Production

The effects of ball milling time on the synthesis and consolidation of nanostructured WC–Co composites was investigated by high energy milling in a horizontal ball mill by Hewitt and Kibble (2010) using NTA to determine particle size distribution and which showed that the number of nano size (<0.2 μm) particles increased with milling time. The onset of the WC–Co eutectic was lowered to 1312°C through an increase in milling time.

Kendall (2011) discussed problems of particle aggregation in ceramics presenting three types of problem to illustrate the thesis that small inter atomic forces between ceramic particles have a major influence on the aggregates formed during processing and on the final ceramic products microstructure and strength. The first is a theoretical problem of ceramic particle aggregation to define the weak inter atomic forces between spheres. The second concerns the better processing that can be applied to dispersed particles to deliver improved ceramic properties by adding polymer to ceramic dispersions to reduce particle attractions which lead to aggregation. The last is the application of polymer extrusion to make improved ceramic fuel cells which can start up in a short time to provide auxiliary power to new applications.

Reduction in the formation of aggregates by the use of surfactants has been investigated using NTA and other techniques. Accordingly, Pollet et al (2011) used ionic and non-ionic surfactants for the control of platinum nanoparticle aggregation in proton exchange membrane fuel cells. Pt nanoparticles were prepared in aqueous dispersion using tetradecyltrimethylammonium bromide (C14TAB), cetyltrimethylammonium bromide (C16TAB) and nonylphenolethoxylate (NP9). The aggregation behaviour of the nanoparticles was studied using Transmission Electron Microscopy (TEM), NTA and Dynamic Light Scattering (DLS). NTA was used specifically to characterize the aggregate's particle size distribution profile. In further work, the same group used NTA to study the aggregation behavior of these materials to help them conclude that the surfactant molecule selection is vital in obtaining effective fuel cell catalyst (Newton et al (2011).

Herrington et al (2010) have studied the effect of the size and size distribution of BaTiO3 nanoparticles on the electro-optic properties of nematic liquid crystals and

Jawor-Baczynska et al (2012) have shown 250nm glycine-rich nanodroplets are formed on dissolution of glycine crystals but are too small to provide productive nucleation sites, both studies using NTA, amongst other techniques for determining nanoparticle size and number.

Monitoring and Treatment of Wastes and Contamination

Sachse et al (2012) have studied the effect of nanoclay on dust generation during drilling of polymer nanocomposites, using NTA to follow particle size distribution and quantity. While, there is currently a lack of information available in the literature on the nano and ultrafine particle emission rates from these, it was shown that the influence of nanoclay on mechanical drilling of PA6 composites, in terms of dust generation, has been reported with more particles in the size range between 175 and 350 nm being generated, during drilling of the nanocomposites, and these particles deposit in a shorter time. In a similar type of application Njuguna et al (2011) have investigated the nanoparticles generated from nanofiller reinforced polymer nanocomposites during structural testing.

Rezić (2011) has reviewed analytical techniques for the characterisation of ENPs on textiles. In this context, the increasing number of nanomaterial based consumer products raises concerns about their possible impact on the environment. In assessing of the effluent from a commercially available silver nanowashing machine Farkas et al (2011) used inductive coupled mass spectrometry (ICP-MS) and TEM to confirm the presence of an average of 10nm silver nanoparticles but employed NTA to determine that 60–100 nm particles were also present. The effluent was shown to have negative effects on a natural bacterial community as its abundance was clearly reduced when exposed to the nanowash water and they suggested that if washing machines capable of producing AgNPs become a common feature of households in the future, wastewater will contain significant loadings of AgNPs which might be released into the environment.

Nanoparticle-containing matrices are being increasingly investigated for the removal of environmental pollutants from industrial process wastewaters. NTA was employed by Prasad et al (2011) in their study of the adsorption of arsenite (As^{3+}) on nano-sized Fe_2O_3 waste powder from the steel industry while Savu et al (2010) earlier assessed the generation of airborne nanoparticulates during pulsed laser welding processes and considered methods for their removal.

Cheng et al (2012) have recently described the synthesis of carbon-coated, porous and water-dispersive Fe3O4 nanocapsules with a diameter of about 120 nm as determined by NTA and their excellent performance for heavy metal removal applications. The heavy metals removal test the employed demonstrated the excellent affinity of nanocapsules, the high efficiency for different metals (>90%), 79 mg g−1 adsorption capacity for Pb^{2+} and ultrafast removal process (Pb^{2+}, 99.57% within 1 minute).

Paper, Inks and Coatings

Lamminmäki and her co-workers have described studies using NTA into the reported short timescale inkjet ink component diffusion as an active part of the absorption mechanism into inkjet coatings (Lamminmäki et al, 2011) and the limitations of current formulations when decreasing the coating layer thickness of papers for inkjet coating. The rate of uptake of inks is strongly related to the number of fine diameter pores in the substrate and a critical parameter in industrial scale printing processes both in terms of speed and coating density. The results showed that, under the external pressure, caused by the surface tension and impact of the ink droplets themselves, the permeability of the coating layer dominates after at least 4 ms from the time of ink application on a high-speed inkjet printing press. She described in detail the various parameters associated with the comparative dynamics of bulk liquid flow and interpolymer diffusion during inkjet ink imbibition in porous coating structures.

Kosmala et al (2011) have also reported the development of high concentrated aqueous silver nanofluid and inkjet printing on ceramic substrates in which the effect of substrates, printing temperature and dot spacing on the size and morphology of printed silver features was investigated. NTA was used in the analysis of silver nanoparticles and zeta potential dependent on pH for the nanosilver powders treated with IPA and acetone. The use of high solid loading inks reduces the number of printed layers required for thick, dense and conductive film thus leading to the reduction of the costs, and high efficiency of the printing process (Kosmala et al, 2011)

Nanocelluloses can be used to fabricate and reinforce hemp fibres. Thus, Dai, Fan and Collins (2012), developed a novel fabrication which was employed to produce nanocelluloses from natural fibers (hemp) and the developed nanocellulose was then used as “coupling agent” to modify hemp fibers themselves. The size distribution of nano-particles (nanocellulose) was measured by NTA and results showed that oxidation–sonication developed nanocellulose had wider size range (29–281 nm) than the average size (100–112 nm). Mechanical testing showed that the nanocellulose modification could improve the mechanical properties of natural fibers significantly. The modulus, tensile stress and tensile strain of nanocellulose modified hemp fibers were increased by 36.13%, 72.80% and 67.89%, respectively.

Filtration

Co-workers Boulestreau and Schulz have carried out extensive studies of filtration using NTA as the primary method for testing filter efficiency and performance. Thus, in describing the online analysis of the nanoparticles to prevent membrane fouling by a secondary effluent, Boulestreau et al (2011a and 2011b) tested NTA in terms of reliability and reproducibility of the device as well as the impact of the prefiltration on the measurements made. They showed that NTA was able to measure the particle size distribution and the absolute particle concentration of particles between 100 and 1000 nm in secondary effluent. Results showed clearly a relationship between the amount of nanoparticles below 200 nm and the filtration behaviour.

More recently Boulestreau has described the on-line use of NTA in which it was used to optimize the ozonation and the coagulation conditions in a filter system. They stated that the fact that the absolute size and concentration of the nanoparticles can be observed within a few minutes allows the user to detect the effect of ozonation and coagulation on the nanoparticles and that the NTA instrument is “a highly capable device to analyze the nanoparticles” (Boulestreau et al, 2012).

Nanobubbles

Seddon has recently and comprehensively reviewed the area of nanobubbles at surfaces and in bulk, and has considered the current understanding of their formation, stability, physicochemical properties and current and future applications (Seddon et al, 2012). . In principle, a nanobubble in the bulk should be less stable than one of the same volume at an interface. The bulk nanobubble has a larger gas/ liquid interface to allow diffusion of gas out of the bubble.

Also, the curvature of the surface bubble is greater, thus leading to a greater pressure differential across the interface for a bulk bubble of the same volume. Nonetheless, several groups have presented evidence for their existence. The most startling evidence for bulk nanobubbles is the recent work which reports small nitrogen, methane and argon bulk nanobubbles of radius 50 nm that are stable for up to 2 weeks The bulk nanobubbles, which were produced by mechanical means that led to extreme supersaturating, were imaged from freeze-fracture replicas by scanning electron microscopy (SEM) and were produced in such large quantities that the bulk density of the solution was substantially reduced.

Takaya et al (2011) and Kikuchi et al (2011) described the formation of nanobubbles by water electrolysis and their analysis with NTA while loka at el (2011) investigated their stability and weight having determined their size distribution with NTA.

Uchida et al (2011) used transmission electron microscopic observations of nanobubbles and their capture of impurities in wastewater. They generated a nanobubble solution by introducing pure O2 gas into the ultra-high purity water with a MNB generator and used NTA to count the resulting number concentration, estimated to be on the order of 107 cm-3 of solution under the same sample preparation conditions. Ushida also investigated the efficiency with which nanobubbles could replace detergents in the washing of laundry given it has been estimated that mechanical work has been found to account for 50% of the washing effect and nanobubbles can acheive the same mechanical action. A combination of nanobubbles and reduced detergency resulted in a 10% increase in washing efficiency (Ushida et al, 2011) .

Ushida et al (2012) have recently investigated the drag reduction effect of nanobubble mixture flows through micro-orifices and capillaries in which the nanobubbles-containing mixture was shown to contain 1.0 vol.% nanobubbles by NTA and the results of studies using nanobubble mixtures for water and glycerol which were passed through several sizes of micro-orifices and capillaries suggested that the addition of nanobubbles to a liquid results in excellent drag reduction.

Nanoparticle Production

Silica

Monodispersed spherical silica particles are potentially available for various applications as building blocks for photonic crystals, chromatography stationary phase, and drug support for controlled release. Immobilization of a molecular recognizable unit to the surface of the spherical particles is important in such applications. Okada et al (2012) used NTA in their study of swell able microsphere of a layered silicate produced by using monodispersed silica particles, showing that silica spheres of sub micrometer size were covered by a swell able layered silicate, which plays a role in accommodating cationic species.

Yang et al (2012) obtained relevant particle size distribution to estimate the effects of particle size-matching filling of spherical silica on the flowability of epoxy molding compounds for large-scale integrated circuits packaging.

Zu et al (2012) described the preparation of ultrafine polyethylene-silica composite particle with a core-shell structure, using scanning electronic microscope observation and nanoparticle tracking analysis to determine that the composite particles possess a spherical morphology and the mean size is about 160nm respectively.

Nanoparticulate Silver

Khaydarov et al (2012) used NTA to test the aggregation characteristics of silver nanoparticles in the development

of a novel method of continuous fabrication of aqueous dispersions of silver nanoparticles using cellulose fibres showing that the synthesised colloidal dispersions showed a pronounced antibacterial effect, as evidenced by low minimum inhibitory concentration values obtained for *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* cultures. Hodges (2011) made anti-microbial self-assembling click monolayers utilizing silver nanoparticles for indwelling medical devices, testing her dispersions with NTA.

Ranville et al (2012) analysed metal-containing nanoparticles using single particle ICP-MS (SP ICP-MS) in environmental matrices. Their aim was to develop Sp ICP-MS using spherical monodisperse metal NP "standards" (Au,Ag) and extend this capability to other metal-containing NPs; TiO_2, CeO_2, ZnO, Ag nanowires, and CNTs. The figure right shows their data comparing SP ICP-MS to Disc Centrifuge and NTA data. NTA revealed a broader size distribution than was detected by the other techniques.

Silver nanoparticles, synthesized using *Saccharum officinarum* (sugarcane), have been shown to quench and inhibit biofilm formation in *Staphylococcus aureus* by Masurkar et al (2012). NTA measurements revealed that the mean size of synthesised silver nanoparticles was found to be 32 nm with a concentration of 17.4×10^{10} particles/ml. No aggregations or debris were detected on NTA measurements. Similarly, Dhuldhaj et al (2012) demonstrated Tagetes erecta mediated phytosynthesis of silver nanoparticles as an eco-friendly approach for nanomaterials synthesis using NTA and TEM to confirm the synthesis of the polydispersed spherical silver nanoparticles of 20-50 nm, with the average size of 30 nm.

Iron Oxide

Cheng et al (2012) described the synthesis of carbon-coated, porous and water-dispersive Fe3O4 nano capsules of about 120nm (about 50nm cavity) as measured by NTA and claimed excellent performance for heavy metal removal applications. They showed that when protected by a porous carbon layer, the nano capsules displaid excellent acidic resistance and adsorption properties even at pH=3.

The synthesis, solution stability and $64Cu^{2+}$ labelling of magnetite nanoparticles (NPs) coated with different macro cycles has been reported by Barreto et al (2011) using NTA to demonstrate that the NPs formed a stable colloidal suspensions in 0.05M aqueous 2-(N-morpholino) ethanesulfonic acid (MES) buffer, which consist of larger aggregates with a mean hydrodynamic size of about 200nm.

In a systematic examination of the effect of four common polymers on the size, surface chemistry, colloidal stability, and sedimentation behaviour of nanoparticles of none zero valent iron (NZVI) Cirtiu et al (2011) measured the size, surface characteristics and colloidal stability of zero valent iron nanoparticles post- and pre-treatment. TEM images and nanoparticle tracking analysis revealed that iron nanoparticles synthesised in the presence of the polymers were larger in diameter, with TEM mean diameters ranged from 84.5 to 189nm, the bare-NZVI was 59.1 nm, when synthesized with the same initial Fe^{2+} concentration.

When developing efficient water oxidation catalysts based on readily available iron coordination complexes, Fillol et al (2011) carried out different analyses to investigate the possible formation of nanoparticles in solution. Experiments performed include Dynamic Light Scattering (DLS), particle size distribution (from 10 to 1000nm), and particle concentration by real-time visualization and tracking analysis of nanoparticles in a liquid (NTA). Catalytic reactions had very low concentration of nanoparticles in solution (< 0.1 ppm), that was below the limit of detection for DLS and it was not possible to get a reliable size distribution measurement. NTA experiments were shown to be more sensitive in the range of 10nm to 2000µm, and measured values of particles/ml were in the same magnitude order $0.76\text{x}10^8$ particles/ml as the blank experiments.

Other metals, metal oxides and alloys

Vogel et al (2011) have reported a new route for mass production of uniform metal nanoparticles in water by means of laser light induced processes in which NTA showed that pulsed laser ablation from a gold plate in water results in a large amount of nanoparticles with radii in the range of R=75nm with a relatively broad size distribution of sigma=31% but that this broad size distribution had been subsequently narrowed in a single irradiation step to sigma=20% without a significant change of the mean nanoparticle radius utilizing selective laser tailoring.

Carbon and Carbon Nanotubes

To assess the removal efficiency of formaldehyde using nano-size carbon colloid (NCC), which was produced by a comparatively easy and cheap method, Kim et al (2012) produced nano-size carbon colloid based on water by an electro-chemical method. This was then used as a gaseous formaldehyde pollutant. NTA was used to monitor carbon particle size in production. Lv et al (2011) used NTA to determine the size of graphene oxide nanoparticles in the design and production of graphene oxide membranes for possible use in new optical devices

In the case of carbon nanotubes (CNTs), despite their highly asymmetric shape, NTA has been used to determine the sphere equivalent diameter as an indicator of sample monodispersity and behaviour in different

matrices. Thus Schwyzer et al (2012) have studied the influence of the initial state of carbon nanotubes on their colloidal stability under natural conditions over a period of many days. They showed that the initial state of the CNTs (dry vs. suspended) and the medium composition hence are critical determinants for the partitioning of CNTs between sediment and the water column. This work was subsequently extended into a more extended study on the long-term colloidal stability of 10 carbon nanotube types in the absence/presence of humic acid and calcium.

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