

Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment – issues and recommendations

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Abstract

Studies designed to investigate the environmental or biological interactions of nanoscale materials frequently rely on the use of ultrasound (sonication) to prepare test suspensions. However, the inconsistent application of ultrasonic treatment across laboratories, and the lack of process standardization can lead to significant variability in suspension characteristics. At present, there is widespread recognition that sonication must be applied judiciously and reported in a consistent manner that is quantifiable and reproducible; current reporting practices generally lack these attributes. The objectives of the present work were to: (i) Survey potential sonication effects that can alter the physicochemical or biological properties of dispersed nanomaterials (within the context of toxicity testing) and discuss methods to mitigate these effects, (ii) propose a method for standardizing the measurement of sonication power, and (iii) offer a set of reporting guidelines to facilitate the reproducibility of studies involving engineered nanoparticle suspensions obtained via sonication.

Keywords: Environmental assessment, toxicology, nanoparticle, nanomaterial, ultrasonics, sonication, dispersion, suspension

Introduction

Nanotechnology, and more specifically the emergence of engineered nanoscale particles (ENPs), shows promising potential for the development of advanced materials and devices in the fields of medicine, biotechnology, energy, and environmental remediation, among others (Zhang 2003; Salata 2004; Raimondi et al. 2005). Similarly, ENPs are migrating into a wide range of consumer products, many of which are now in the global market place (Project on Emerging Nanotechnologies 2010). However, some of the properties that make ENPs attractive may also pose environmental and health hazards (Colvin 2003; Gwinn and Vallyathan 2006; Kreyling et al. 2006; Dobrovolskaia and McNeil 2007; Kahru and Dubourguier 2010). As both public and private funding for application-oriented nanomaterial research continues to grow and nanoparticle-based consumer products reach the market, there is an increasing demand for a more comprehensive scientific understanding of the interactions of ENPs

in biological and environmental systems (Maynard 2006; Barnard 2006).

Moreover, the assessment of potential risks associated with ENPs requires the evaluation of their behavior in environmentally and biologically relevant matrices (e.g., cell media, serum, whole blood, standardized seawater). When ENPs are not already in a fully dispersed form, the test suspensions are typically obtained by dispersing dry powders or stock suspensions of the source material into the desired test medium prior to *in vitro* or *in vivo* evaluation (Murdock et al. 2008; Jiang et al. 2009; Labille et al. 2009). In these situations, the use of ultrasonic energy, a process commonly referred to as ‘sonication’, is arguably the most widely used procedure.

Sonication refers to the application of sound energy at frequencies largely inaudible to the human ear (higher than ≈ 20 kHz), in order to facilitate the disruption of particle agglomerates through a process known as cavitation. Ultrasound disruption is more energy efficient and can achieve a higher degree of powder fragmentation, at constant specific energy,

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than other conventional dispersion techniques (Park et al. 1993; Hielscher 2005; Mandzy et al. 2005). Sonication is a convenient, relatively inexpensive tool that is simple to operate and maintain. Consequently, sonication has found extensive application in toxicological and environmental studies, where it is used to break down powders or re-disperse stock suspensions to their nanoscale constituents and allow for the evaluation of the properties and behavior of the dispersed nanoparticles in relevant systems.

However, the inconsistent application of ultrasonic treatment across laboratories and between individual operators, combined with the lack of accepted process standardization, has likely contributed to variability in observed results. Furthermore, the complex physical and chemical phenomena that occur during sonication – and which may significantly alter the dispersed material's properties – are frequently underappreciated, perhaps resulting in the use of suspensions that may not meet the intended experimental design, or potentially leading to artifacts that could compromise conclusions as to how the observed ENP's ecological or toxicological behavior relates to the material's inherent properties. It is the authors' contention that the inconsistent application and reporting of sonication is a potential leading contributor to variability in test results in which sonication is utilized; this conclusion has been echoed by the results of recent interlaboratory studies (Roebben et al. 2010).

Moreover, while not the main focus of this work, it is important to note that many of the sonication-induced physicochemical effects and recommendations addressed herein are equally relevant to other ENP research areas where sonication is utilized, in particular those involving the fate and transport of nanomaterials in environmental systems.

The objectives of the present work were to: (i) Highlight salient aspects of the ultrasonic disruption process and survey potential sonication-induced effects that may alter the physical, chemical or biological properties and behavior of aqueous nanoparticle suspensions (within the context of toxicological testing), (ii) propose a method for standardizing the measurement of sonication power, and (iii) offer a set of reporting guidelines to facilitate the reproducibility of studies involving engineered nanoparticle suspensions obtained via sonication.

The authors aimed not only to identify and discuss key sonication issues relevant to ENP dispersion, but also to address them by offering practical recommendations. For this reason, some discussions will follow a traditional literature review format, while others are more prescriptive in style. The authors also recognize that additional sample-specific preparation steps are often required to achieve the desired state of

dispersion, but their consideration is beyond the scope of the present work.

Terminology

We define at the outset several critical terms, which are used throughout the text, in order to avoid ambiguity, as these terms can have different meanings in different scientific and technical circles. For the most part, we follow definitions relevant to nanotechnology as set forth in the standard E2456 (2006) by ASTM International (formally the American Society for Testing and Materials). Additional guidance is derived from recommendations of the International Union of Pure and Applied Chemistry (IUPAC 2009).

For the purposes of this publication, a *nanoparticle* is defined as a sub-classification of ultrafine particle that is characterized by dimensions in the nanoscale (i.e., between 1 and 100 nm) in at least two dimensions, with the recognition that the more rigorous definition of nanoparticle also requires that these particles exhibit novel size-dependent properties. A *primary particle* is the smallest discrete identifiable entity associated with a particle system; in this context, larger particle structures (e.g., aggregates and agglomerates) may comprise primary particles. An *aggregate* is a discrete assemblage of primary particles strongly bonded together (i.e., fused, sintered, or metallically bonded), which are not easily broken apart. While the distinction is often blurred, aggregates might also be differentiated from *agglomerates*, which are assemblages of particles (including primary particles and/or smaller aggregates) held together by relatively weak forces (e.g., Van der Waals, capillary, or electrostatic), that may break apart into smaller particles upon processing (e.g., using sonication or high intensity mixing). Commonly, agglomerates exhibit the same overall specific surface area (SSA) of the constituent particles, while aggregates have a SSA less than that of the constituent primary particles. In a related fashion, *agglomeration* and *aggregation* refer, respectively, to the process by which agglomerates or aggregates form and grow. It should be noted that, although here we define them as distinct entities, the terms aggregate and agglomerate are often used interchangeably to denote particle assemblies. Our usage is consistent with previous recommendations published in this journal (Oberdörster et al. 2007), but is by no means universally adopted.

Additionally, in the present work, a *suspension* is defined as a liquid (typically aqueous) in which particles are dispersed. The term *dispersion* is used in the present context to denote the process of creating a liquid in which discrete particles are homogeneously

distributed throughout a continuous fluid phase (e.g., to obtain a suspension), and implies the intention to break down agglomerates into their principal component particles. *Dispersion* is also used as an adjective in combination with terms relating to this process or relating to the state of the resulting product.

Sonication basics and equipment

This brief overview highlights the basic aspects of ultrasonic generation and propagation relevant to particle dispersion, and serves as background for the ensuing discussions; while it is not a comprehensive review of the topic, readers can refer to the cited references for a more in-depth treatment of the subject. During the process of ultrasonic disruption, sound waves propagate through the liquid medium in alternating high and low pressure cycles at frequencies typically in the 20–40 kHz range. During the low-pressure cycle (rarefaction), microscopic vapor bubbles are formed in a process known as cavitation (Figure 1). The bubbles then collapse during the high pressure cycle (compression) producing a localized shock wave that releases tremendous mechanical and thermal energy.

Ultrasonic waves can be generated in a liquid suspension either by immersing an ultrasound probe (transducer horn) into the suspension (direct sonication), or by introducing the sample container into a liquid that is propagating ultrasonic waves (indirect sonication). This is shown schematically in Figure 2.

In a sonication bath or a so-called cup horn sonicator (indirect sonication), the sound waves must travel through both the bath or cup liquid (typically water) and the wall of the sample container before reaching the suspension. In direct sonication, the probe is in contact with the suspension, reducing the physical barriers to wave propagation and therefore delivering a higher effective energy output into the suspension. Bath sonicators typically operate at

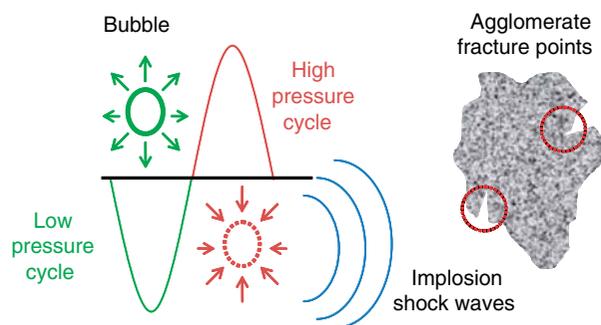


Figure 1. Schematic illustration of ultrasonic wave-induced cavitation and agglomerate fracture.

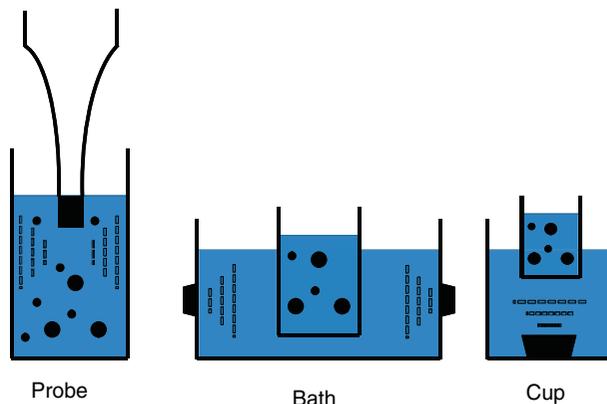


Figure 2. Schematic illustration of direct (left) and indirect (middle and right) sonication configurations as described in the text.

much lower energy levels than are attainable using a probe or cup horn. In the case of bath sonicators, the transducer element is directly attached to the outside surface of the metal tank, and the ultrasonic waves are transmitted directly to the tank surface and then into the bath liquid. In a cup horn, the radiating surface of the horn is inverted and sealed into the bottom of a (typically) transparent plastic cup into which the sample container is immersed.

At ultrasound frequencies, the alternating formation, growth and subsequent shock waves produced by the collapse of bubbles result in extremely large localized temperatures up to 10,000 K, rapid temperature changes ($> 10^5 \text{ K s}^{-1}$), pressure bursts on the order of several MPa, and liquid jet streams with speeds reaching $400 \text{ km} \cdot \text{h}^{-1}$ (Mason 1989; Hielscher 2005). Such massive, local energy output is the basis of the disruptive effect of sonication. It must be noted that these considerable pressure and temperature differentials are a result of the cavitation process and occur at the local interface of the exploding bubbles; consequently, these effects are *inherent* to the sonication process and will occur whether the sonicated container is cooled or not, or the treatment performed using a bath, cup horn, or a probe sonicator.

In all cases, the ultrasonic device transforms electrical power into vibrational energy by means of a piezoelectric transducer that changes its dimensions in response to an applied AC electric field. In direct sonication, the transducer horn serves to transmit and focus the ultrasonic waves into the targeted liquid sample. For direct sonication, the following equation relates acoustic vibrational energy to probe and medium parameters (Contamine et al. 1995):

$$P = \frac{1}{2} \rho c A^2 (2\pi f)^2 a \quad (1)$$

where P is the acoustic power (W) of the ultrasound source, a is the ‘emission area’ (m^2), which is the surface area of the emitting ultrasound source, ρ is the liquid density (kg m^{-3}), A is the amplitude (m) of oscillation of the ultrasound probe, c is the speed of the acoustic wave in the liquid medium (m s^{-1}) and f is the vibration frequency (Hz).

The amplitude of oscillation per unit time determines the pressure difference between cavitation rarefaction and compression cycles. Larger oscillation amplitudes yield larger high-low pressure gradients and consequently greater energy outputs. The probe’s oscillation amplitude is in turn determined by the amount of energy transferred by the instrument’s generator to the ultrasonicator probe, which can be regulated using the sonicator power settings.

Conventional direct sonicators operate by self-adjusting their power consumption from the electrical source in order to maintain the vibration frequency of the transducer (e.g., quartz crystal) at a constant value (usually 20 kHz). As it oscillates, the sonicator probe – connected to the vibrating transducer – will experience a resistance from the sonicated medium that will be transmitted back to the vibrating element and detected by the internal control unit of the instrument. The instrument’s control unit will in turn adjust the power consumption of the instrument to maintain a constant vibration frequency. Highly viscous media will exert a greater resistance to the oscillating probe, and will therefore require greater power consumption to maintain a constant oscillation frequency. For example, to produce an oscillation with an amplitude of 3 μm at 20 kHz in deionized water, a sonicator may consume ‘N’ watts, while to produce the same amplitude at 20 kHz in oil, it will consume considerably more power.

The oscillation frequency value is usually fixed for a given instrument and cannot be changed. Changing the instrument power setting translates to a change in the vibrating *amplitude* – not the frequency – of the probe; that is, increasing the power setting results in larger probe oscillation amplitudes. To maintain a constant oscillation frequency, larger oscillation amplitudes, as well as higher medium resistances,

require greater power consumptions from the electric source.

A sonicator’s stated maximum power (e.g., 600 W) refers to the maximum theoretical power it would be able to consume, *should it require so*; it does not reflect the actual amount of ultrasonic energy delivered to the suspension. That is, for the same frequency and amplitude (and thus the same sonicator setting), the sonicator would need to consume more power to treat a high viscosity suspension than it would for a low viscosity suspension, and if so, it could consume up to a maximum of 600 W. It is therefore erroneous to assume that selecting the highest setting value will result in the delivery of the instrument’s nominal maximum power to the sonicated suspension. For low viscosity media, even at the highest setting value, the delivered power will be a significantly lower fraction of the instrument’s maximum power rating.

Similarly, the power value (measured in W) usually shown on the instrument display reflects the power that the instrument is *consuming* from the electrical source to produce the desired oscillation amplitude (from the selected setting value) in the sonicated medium. The consumed (i.e., displayed) power, however, does not necessarily reflect the power that is actually *delivered* to the sonicated suspension, which is affected by the probe’s oscillation amplitude in the medium (see Equation 1). Herein lies the deficiency with reporting either the manufacturer’s stated maximum power, the adjustable output power reading provided by the instrument, or the chosen setting level. The flowchart in Figure 3 illustrates the energy transformations that occur in a conventional probe sonicator.

The net fragmentation effect from applying ultrasonic energy to a suspension is dependent on the total amount of energy delivered to the sonicated medium. However, not all of the produced cavitation energy is effectively utilized in disrupting particle clusters. The delivered energy is consumed or dissipated by several mechanisms, including thermal losses, ultrasonic degassing, and chemical reactions, such as the formation of radical species. Only a portion of the delivered energy is actually used in breaking

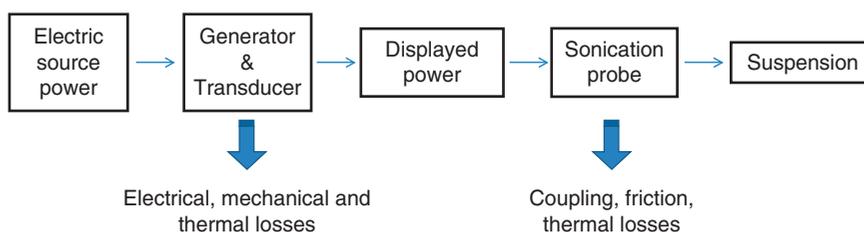


Figure 3. Flowchart illustrating the energy transformation process in a conventional probe (direct) sonicator.

particle-particle bonds to produce smaller particle aggregates, agglomerates, and primary particles. Moreover, an excessive energy input can potentially result in agglomerate formation or re-agglomeration of previously fragmented clusters, as well as induce a variety of physicochemical alterations on the material's surface or to the constituents of the suspending medium. Such sonication-specific side-effects are addressed in the following sections, but first we examine the particle disruption process as it is the primary objective of ultrasonic treatment.

Sonication effects on particle disruption and agglomeration

The properties associated with ENPs, and consequently their distinct biological and environmental interactions, are strongly dependent on the particle size of the processed material. For instance, the size of the particles in suspension dictates – along with the particles' surface chemistry – their biodistribution, elimination, cellular uptake, toxicity, and environmental transport behavior (Rejman et al. 2004; Lanone and Boczkowski 2006; Pan et al. 2007; Powers et al. 2007; Li and Huang 2008; Sonavane et al. 2008). Additionally, the agglomeration state

determines whether the particles will settle out or remain in suspension, which directly impacts assay efficacy and delivered dose. Numerous other examples of size-dependent behavior can be found in the recent literature.

Dry powders consist of particles that are bound together into macroscopic structures by both weak physical Van der Waals forces and stronger chemical bonds including particle fusion (Zachariah and Carrier 1999; Mandzy et al. 2005; Jiang et al. 2009). Commonly, these powders contain micrometer – and submicrometer – scale aggregates, which are in turn held together by physical forces to constitute larger agglomerates. This is depicted schematically in Figure 4. For powders consisting of nanoscale particles and aggregates, and therefore having substantial interfacial contact areas per unit volume, the nominally weaker Van der Waals forces can be extremely large, requiring the use of techniques such as sonication to effectively break down or disrupt powder agglomerates. In contrast, micrometer size primary particles can often be dispersed with moderate mixing or stirring.

Many of the commercially available metal oxide ENP source powders (e.g., silica, titania and alumina) are synthesized by high temperature vapor phase

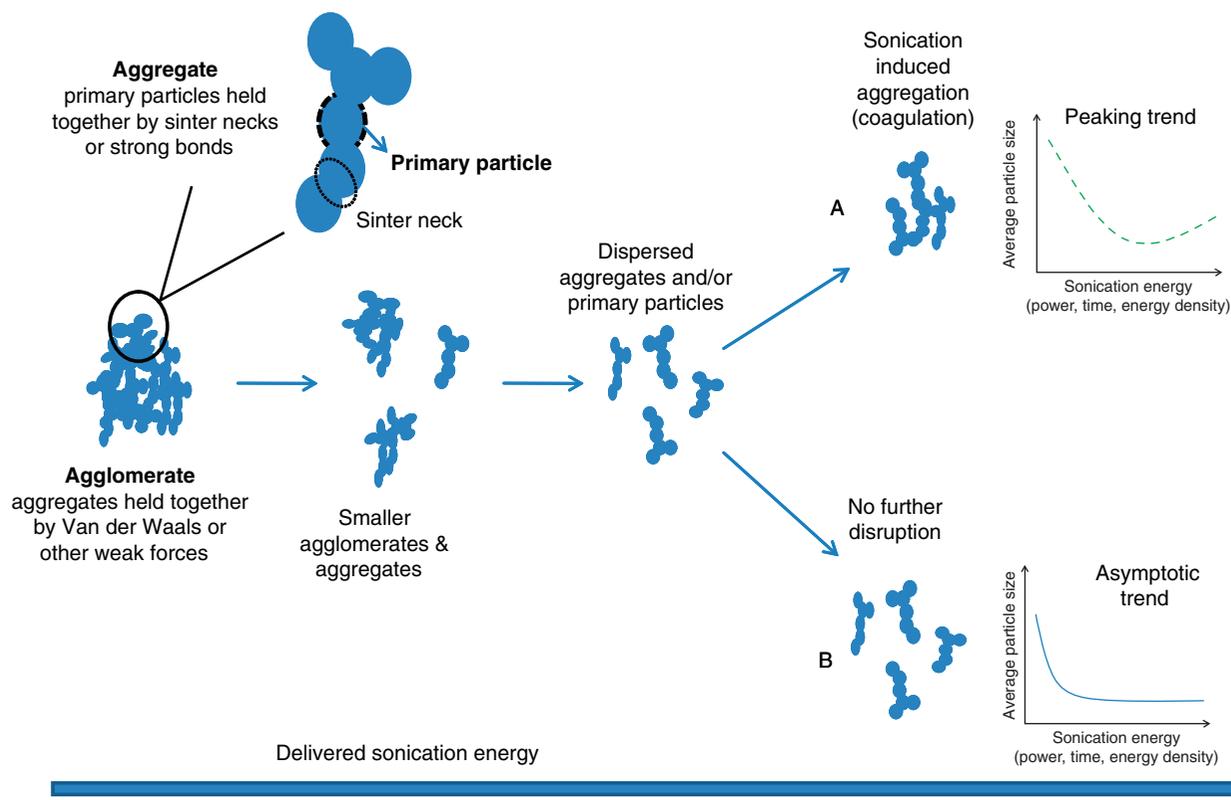


Figure 4. Schematic depiction of particle structures referred to in the text, and illustration of the typical effects of sonication on particle size as a function of delivered sonication energy: (A) Asymptotic behavior (solid line) versus (B) peaking behavior (dashed line).

processes (Kraft 2005; Isfort and Rochnia 2009). In such processes, chemical precursors react at high temperatures to produce small ‘hot’ nanoscale droplets of the desired material. As these hot droplets pass through the reaction zone, they collide and may coalesce fully or partially, depending on the local temperature. Full coalescence leads to a larger primary particle, while partial coalescence leads to the formation of an aggregate of fused primary particles (bound via sinter necks), which later become ‘solid bridges’ upon particle solidification (Figure 4). Particle coalescence and growth continue until the reactants are consumed or the process temperature is quenched (Singhal et al. 1999; Zachariah and Carrier 1999; Teleki et al. 2008; Isfort and Rochnia 2009).

Metal oxide and other ceramic powders frequently show this configuration, wherein the ‘primary particle’ represents the solidified globules that constitute powder aggregates (frequently chain-like structures). Yet, it must be noted that the solid bridges that hold the primary particles together in the dry powder aggregates are often too strong to be broken via sonication or other moderate dispersion processes. Therefore, in some cases, it can be unrealistic to expect sonicated powders to break down to their reported, nominal primary particle size. As a result, powders will be effectively de-agglomerated to aggregates of several primary particles (i.e., ‘primary aggregates’), rather than isolated primary particles. These aggregates should be thought of as the *effective* primary size for the ENP. Although the underlying formation processes in solution phase synthesis (e.g., via sol gel or precipitation) differ from the vapor phase process, the resulting structures often express similar physical properties to the above described structures (i.e., particle fusion, presence of aggregates and agglomerates), though typically to a lesser extent (Brinker and Scherer 1990).

During sonication, shock waves from cavitation collapse are principally responsible for powder breakage. The particles, in fact, serve as nuclei to initiate the cavitation process, which partly explains why sonication is so effective (Suslick 1988). When ultrasound energy is applied to powder clusters in suspension, fragmentation can occur either via erosion or fracture. Erosion or ‘chipping’ refers to the detachment of particles from the surface of the parent agglomerates, while fracture or ‘splitting’ occurs when agglomerates partition into smaller agglomerates or aggregates due to the propagation of cracks initiated at surface defects (Figure 1). These fragmentation effects may occur simultaneously or in isolation, depending on the powder, its environment and the energy levels involved.

For a given material in powder form, depending on the physicochemical properties of the solid material

and method of manufacture, the occurrence of surface defects in the powder clusters, the material’s surface toughness, and the bonding nature (e.g., neck diameter or interfacial contact area) of aggregates and agglomerates, there is a system-specific acoustic energy threshold that must be crossed in order to achieve agglomerate and aggregate breakage. It is thus worth noting that two powders of the same material, but obtained following different synthetic routes, may show significantly different breakage behavior. Material properties also affect the rate at which fragmentation occurs, and, consequently, the amount of time it takes at a given ultrasonic output power and for a given amount of material to reduce all of the powder to the *smallest achievable dimensions*.

Thus, in principle, the cavitation process can effectively break down powder agglomerates. Yet, under certain conditions, the applied acoustic energy can conversely induce particle agglomeration or even lead to the formation of aggregates. Coagulation in ultrasonic fields can occur from enhanced particle-particle interactions due to the increased collision frequency as well as the favorable reduction in free energy that accompanies the resulting reduction in the liquid-solid interface. As noted previously in discussing the basics of ultrasonic disruption, cavitation gives rise to extreme local pressure and temperature gradients, as well as shock waves and jet streams of the order of hundreds of meters per second. Under such conditions, sonicated particles in the neighborhood of a collapsing bubble collide with each other while simultaneously experiencing localized intense heating and subsequent cooling cycles. Depending on the effective energy delivered to the particles and the thermal properties of the material and medium, these concomitant effects can lead to re-agglomeration or even thermally induced inter-particle fusion; that is, sonication-induced aggregate formation.

Published studies have demonstrated the existence of this phenomenon, which is often observed as an increase in particle size beyond a given sonication energy threshold (Aoki et al. 1987; Vasylykiv and Sakka 2001). In other cases, an asymptotic behavior is observed, wherein sonicated powders break down to particles of a given size value, beyond which no further significant size decrease is obtained even at higher ultrasound energy inputs (Bihari et al. 2008; Murdock et al. 2008). The latter behavior further confirms that, for some powders, primary particles can be held together by bonds that cannot be broken by sonication.

Interestingly, both asymptotic and coagulation behaviors (see Figure 4) as a function of different sonication parameters have been observed for the *same* source material (e.g., TiO₂, Mandzy et al.

2005; Jiang et al. 2009), illustrating the system-specific dependence (i.e., source powder, dispersing medium, ultrasound input) of powder sonication procedures. Asymptotic and peaking (coagulation) behaviors upon sonication have been reported for a wide variety of engineered nanomaterials. The following are some examples, where the respective asymptotic or peaking energy thresholds are also indicated in each case: TiO₂ (rutile) – 60 s at 7 W (Bihari et al. 2008); TiO₂ (anatase and rutile) – (100–300) s at 750 W (Jiang et al. 2009); ZrO₂ – 100 s at 160 W (Vasylykiv and Sakka 2001); Au – 60 min at 40 W · cm⁻² (Radziuk et al. 2010); Ag – 10 s at (35–40) W (Murdock et al. 2008). It must be noted that in these cases the reported delivered energies do not conform to the guidelines specified herein and therefore may not accurately reflect the actual energy densities to which the ENPs were subjected.

Furthermore, while the disruptive effect of sonication is in most cases instrumental to the achievement of nanoscale particles, it can also result in either desired or undesired physical changes that may alter the inherent properties of the dispersed material. A mainstream example of this phenomenon is the sonication induced scission of carbon nanotubes (Forrest and Alexander 2007; Huang et al. 2009; Lucas et al. 2009).

While it may not be possible to completely eliminate all of the aforementioned phenomena, it is critical to optimize the sonication procedure in order to minimize the undesirable effects, while attaining the desired level of particle disruption. The recommended optimization steps are detailed in a subsequent section.

Chemical effects of sonication

As with size, surface chemistry – and consequently surface charge – plays a critical role in governing the environmental and biological interactions of ENPs. Surface chemistry, whether it is that of the pristine material or includes the effects of a surface coating or capping agent, largely determines the adsorption behavior at the solid-solution interface, the organic/inorganic phase partitioning, colloidal stability in aqueous media, reactivity with other medium components, and affinity towards cell membrane walls (Guzman et al. 2006; Li and Huang 2008; Jiang et al. 2009; Kittler et al. 2010; Walczyk et al. 2010), to name just a few examples. Surface chemistry is thus a key parameter that affects the fate, transport, bioavailability, and bioaccumulation behavior of ENPs. It is therefore critical to consider the potential for changes in surface chemistry in ENP suspensions arising from the application of ultrasonic energy.

The extreme localized temperatures and pressures generated by the cavitation process can yield highly reactive species within a sonicated medium, in a process known as ‘sonic activation’. As an indication of the importance, variety, and complexity of sonication induced physicochemical effects in liquid media, in recent years, *sonochemistry* has emerged as a distinct discipline devoted to the study of a wide range of sonication induced chemical reactions in both homogeneous and heterogeneous solid and liquid systems (Mason 1989; Mason and Peters 2003).

An example of sonic activation of particular relevance to environmental and toxicological studies is that of water sonolysis. Sonicated water partly dissociates into hydrogen and hydroxide radicals, with concentration and lifetime dependent on the sonication conditions (e.g., sonication time and energy density) (Makino et al. 1983; Yanagida et al. 1999), that subsequently recombine to form hydrogen peroxide (Brown and Goodman 1965; Mason and Peters 2003). Any materials or chemical species present in sonicated water will thus be exposed to low (and variable) concentrations of highly reactive species, and as a result may undergo oxidative and other chemical transformations.

Uncoated, sonicated ENPs may undergo changes in their surface chemistry due to the formation of sonolysis radicals. As previously mentioned, the sonication of water can yield reactive radicals that can initiate a variety of chemical reactions. The generated hydrogen and hydroxyl radicals, and peroxide molecules, can interact with the material’s surface, changing its oxidation state or inducing the hydroxylation of the materials’ surface, potentially altering the material’s hydrophilicity and stability, as well as its chemical compatibility with other medium components. Chemisorbed hydrogen radicals generated during sonication could potentially lead to the irreversible disintegration of sonicated carbon nanomaterials, such as fullerenes and nanotubes, via ‘hydrogen-induced exfoliation’ (Berber and Tomanek 2009).

Other changes in the material’s surface chemistry, such as oxidation or hydroxylation, may result in an increased aqueous solubility and result in an enhanced leaching of ionic or soluble species into the medium. The potentiality of sonication-enhanced leaching should be given special consideration when testing the biological interactions of ENPs with known toxicity in their molecular or ionic form; a case in point is nanoscale silver (Wijnhoven et al. 2009; Elzey and Grassian 2010), in which the oxidized Ag⁺ form is well documented to exhibit biocidal properties.

Moreover, if the dispersion medium is not just water, but incorporates other chemical species, the

extent and degree of formation of radicals from both water and other medium components, and the complexity of their interactions with the dispersed material can be further magnified. Such potentiality for sonication induced changes in the surface chemistry, and in particular its oxidation state or the hydroxylation of the material's surface, should be given special consideration in light of recent findings that attribute ENP's toxic effects to oxidative stress and reactive oxygen species (ROS)-mediated events, among other mechanisms (Unfried et al. 2007; Wang et al. 2009a; Kim et al. 2010).

While radical induced changes in the pristine material's surface might be restricted to the period of sonication treatment and be potentially reversible – although to the best of our knowledge this has yet to be determined – it is unquestionable that sonication-specific, radical-mediated changes have been observed as a result of sonication, as discussed below, and can significantly alter the suspension medium properties or the dispersed material's surface chemistry, and consequently influence its environmental and biological behavior.

If organic molecules are present when sonicating ENP suspensions, either as components of the dispersing medium or as a surface coating on the ENP itself, they can be irreversibly degraded via direct sonolysis from the dissipated cavitation energy (Williams 1983; Wang et al. 2009c). Furthermore, sonication-induced active radicals, such as those produced from water sonolysis, have been shown to cleave polymers creating smaller oligomers and to activate polymerization reactions (so called sonopolymerization), producing new polymeric species and irreversibly modifying the organic molecules present in the suspension (Brown and Goodman 1965; Mason 1989). Sonication can also induce covalent bonding of organic functionalities to the ENP surface (Liu et al. 2005). The irreversible degradation and/or transformation of organic molecules due to sonication is a potentiality that should be considered when preparing ENP suspensions for environmental or biological testing.

As a result of sonolysis, the sonicated medium may not retain its native properties. Degraded medium components, such as denatured proteins, will likely interact differently with the ENPs or the intended assay, than they would in their pristine, non-sonicated form. Sugars and proteins are two major biological and environmental media components. Basedow and Ebert studied the molecular degradation of dextran as a function of exposure time under 20 kHz, low intensity (10 W cm^{-2}) sonication (Basedow and Ebert 1979), while in a later work Lorimer et al. (1995) extended the study to

account for the effect of sonication intensity, temperature, and sugar concentration. In all cases, a significant decrease in the sugars' molecular weight at increasing sonication times and powers was observed.

Protein and enzyme sonolytical degradation has been broadly studied as well. Wang et al. (2009c) examined the sonication-induced damage to bovine serum albumin (BSA) both in the presence and absence of metal complexes. In all cases, even at sonication powers as low as 20 W, ultrasonic irradiation was shown to damage the BSA structure, the extent of which increased with increasing sonication time. Coakley et al. (1973) measured the sonolytical inactivation of alcohol dehydrogenase and lysozyme exposed to a 20 kHz ultrasonic field as a function of sonication time (at 90 W), and measured a 70% inactivation of alcohol dehydrogenase and lysozyme after 10 and 25 min of sonication, respectively. A more comprehensive review and discussions on sonication induced alterations of relevant biological compounds (e.g., enzymes, DNA, biological membranes) can be found in the works of Williams (1983) and Riesz and Kondo (1992).

Alterations in the molecular structure of common organic compounds (e.g., sugars, proteins) as a result of sonication may elicit specific biological responses in the animal model or assay upon inoculation with the sonicated suspension, thereby yielding false positives or negatives that could incorrectly be attributed to the ENPs, rather than the degraded medium. To account for this possibility, we recommend using a supernatant control in which the medium, in the absence of ENPs, is subjected to the sonication procedure and tested in parallel with the ENP suspensions.

Furthermore, sonication-induced transformations in the medium could potentially result in a loss of the medium's stabilizing properties, therefore reducing its ability to maintain the ENPs in suspended non-agglomerated form. For example, species-specific albumin homologs, such as BSA, have been utilized as stabilizing components in dispersion media intended for toxicity testing of ENPs (Bihari et al. 2008; Porter et al. 2008; Kittler et al. 2010; Tantra et al. 2010), while humic acid is commonly used as a medium component and organic stabilizer added to ENP suspensions to simulate environmental matrices (Chen and Elimelech 2007; Pallem et al. 2009; Saleh, Pfefferle and Elimelech 2010). Typically, the albumin or humic acid is present when the suspension is sonicated. Naddeo et al. (2007) demonstrated that humic acid is degraded (either via oxidative mechanisms or physical aggregation) when subject to 20 kHz sonication, the extent of which was found to increase with sonication intensity and organic concentration. Preliminary studies in our

own laboratory on the stabilization effect of BSA on sonicated TiO₂ powders have shown a measurable loss of nanoparticle stability against agglomeration when BSA is sonicated together with the source powder, as opposed to when BSA is added post-sonication. In the latter case, the particles did not exhibit agglomeration over the same time frame. These results will be published as part of a separate research article currently in preparation.

Moreover, the ultrasonic degradation of organic molecules has the potential to irreversibly modify the chemical structure of ENP functional surface coatings (e.g., polyethylene glycol [PEG], and polyvinylpyrrolidone [PVP]), or even a complete loss of the coating itself. Kawasaki et al. (2007) studied the ultrasonic degradation of PEG as a function of sonication time at 28 kHz and 20 W, over a wide range of molecular weights. In all cases, radical mediated sonolysis caused C-O bond scission and molecule breakage. Taghizadeh and Bahadori (2009) measured the degradation kinetics of PVP when subject to 24 kHz 100 W sonication, revealing a dependence of the degradation rate on molecular weight and polymer concentration. Such potential sonication-specific alterations of organic surface coatings may adversely impact the ENP's capacity to remain in suspension, as particle coatings often provide protective electrostatic or steric stabilization. Ultrasonic degradation of organic surface coatings could also modify the ENP's interactions with other medium components, interfering with the ENP's transport, adsorption, or toxicological behavior.

Additionally, ENPs may act as sonolysis catalysts, synergistically enhancing the aforementioned effects when sonicated in the presence of organics. Wang et al demonstrated the sonocatalytic effect of nano-sized silica and titanium dioxide towards the degradation of BSA, showing an enhanced degradation of BSA when the ENPs were present in the sonicated solution, with respect to ENP-free controls (Wang et al. 2005, 2009b).

For these reasons, the chemical complexity of the sonicated suspension and the particle coating should be reduced to the greatest possible extent. Whenever possible, organic compounds should be spared from sonication and the formation of radicals minimized. A proposed approach for the preparation of ENP suspensions is, when applicable, to first sonicate the pristine, uncoated powder in de-ionized water (or other appropriate pH adjusted aqueous solution) and then mix the sonicated suspension into the desired, non-sonicated, dispersion medium, or add the stabilizing agent and other organic additives to the suspension after sonication. This may require significant additional effort to properly formulate and

disperse the ENPs, but the extra effort will result in less perturbation of the chemical species present. The formation of radicals can also be mitigated by minimizing the sonication power, again prompting the need for optimizing the dispersion procedure to minimize undesired effects while obtaining the desired dispersion properties. Furthermore, nitrogen or carbon dioxide bubbling, or the addition of dry ice or other radical scavengers, can quench free radicals during the sonication process and minimize their potential impact (Kondo et al. 1986; Honda et al. 2002).

Measuring and reporting delivered power

A critical parameter for the reproducible preparation of sonicated ENP suspensions is the delivered sonication power. As previously mentioned, only a portion of the total output power is effectively consumed in particle disruption.

The energy loss and consequently the efficiency of the energy transformation (or the 'energy yield') from the electrical source to the acoustic power effectively received by the sonicated suspension is heavily instrument dependent (on the instrument's particular driving circuitry, self-adjusting feedback loops, and its various electrical components). Furthermore, the displayed power omits thermal and mechanical energy losses, which are also instrument specific, that occur as the output power is transferred 'downstream' through the sonication probe and into the suspension (Figure 3).

Therefore, simply reporting the sonicator's power setting value (e.g., 'setting 5' or '60%') or the displayed power does not provide an accurate indication of the *effective acoustic power delivered* to the sonicated suspension, and are as such reporting parameters that do not allow for transferable or reproducible results. Two different instruments operating at a 'level 5', or a '60%' setting can deliver significantly different effective acoustic powers to the same suspension, as they may translate into different oscillation amplitudes for different instruments. Alternatively, instrument 'A' may consume – and display – 50 W from the electrical source to produce a probe oscillation amplitude of 3 μm, while instrument 'B' may produce a probe oscillation amplitude of 2 μm by consuming 50 W, thereby delivering different powers to a suspension even when showing the same displayed power. Published works have discussed and demonstrated that the power reading displayed by the instrument bears poor correlation with the measured power received by the sonicated suspension (Contamine et al. 1995; Kimura et al. 1996; Raso et al. 1999). This issue is broadly known

in the sonochemistry field as ‘the reproducibility problem’ (Mason 1991; Contamine et al. 1995; Kimura et al. 1996).

While there is yet no available standardized procedure for reporting the effective acoustic energy delivered to liquid suspensions, we hereby propose the calorimetric method as a simple procedure to establish a standardized, instrument-independent, transferable and reproducible method for the measurement and reporting of sonication power. It is our belief that by implementing such an approach, variabilities derived from the inconsistent application and reporting of ultrasonic disruption will be reduced.

The calorimetric method

We propose a simple and easily transferable set of experimental guidelines for the determination of calorimetric curves. The calorimetric method has been proposed to standardize sonochemistry studies and used to calculate the amount of acoustic energy delivered to a liquid medium subject to direct sonication (Kimura et al. 1996; Zhu et al. 2009). Calorimetry is a relatively simple, rapid, and inexpensive procedure that allows for the direct measurement of the acoustic energy delivered to a sonicated liquid (Mason 1991). The method is based on the measurement of the temperature increase in a liquid medium over time, as a bulk effect of the cavitation process induced in a liquid by an ultrasound probe.

At a given sonicator setting, the temperature increase in the liquid is recorded over time and the effective delivered power can then be determined using the following equation:

$$P = \frac{dT}{dt} MC_p, \quad (2)$$

where P is the delivered acoustic power (W), T and t are temperature (K) and time (s), respectively, C_p is the specific heat of the liquid ($\text{J g}^{-1} \text{K}^{-1}$) and M is the mass of liquid (g).

By obtaining calorimetric curves following a standardized procedure, users can report and reproduce the power level applied to a sonicated suspension in a manner that is easily transferrable and instrument independent.

The following protocol is proposed for the determination of direct sonication calorimetric curves (Taurozzi et al. 2010a):

- (1) Fill a 600 mL cylindrical borosilicate beaker with 500 mL of de-ionized water (resistivity $>18 \text{ M}\Omega \cdot \text{cm}$);

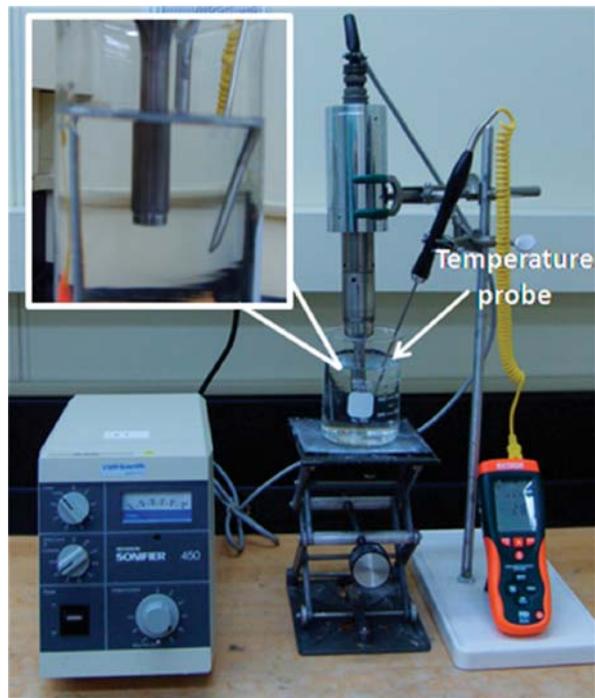


Figure 5. Set-up for the measurement of calorimetric curves. Inset shows sonicator tip and temperature probe immersion depths. Sound protection enclosure was omitted for clarity of the image.

- (2) Determine the mass of the liquid using a top loading balance. First tare the empty 600 mL beaker;
- (3) Immerse the sonicator probe (horn) approximately 2.5 cm (1 inch) below the liquid surface;
- (4) Immerse a temperature probe connected to a temperature meter and data logger (e.g., an Extech HD 200¹ temperature meter and data-logger coupled to a Type K immersion temperature probe). The temperature probe tip should be about 1 cm from the sonicator probe (see Figure 5);
- (5) Select a sonicator output power setting and, operating in continuous mode, record the water temperature increase for the initial 5 min. During sonication, ensure that the beaker does not shift position, especially when operating at high power settings; this can be accomplished for instance, using a clamp attached to a ring stand;
- (6) Using the recorded values, create a temperature vs. time curve and obtain the best linear fit for the curve using least squares regression.

1. Certain trade names and company products are mentioned in the text or identified in illustrations in order to specify adequately the experimental procedure and equipment used. In no case does such identification imply recommendation or endorsement by National Institute of Standards and Technology, nor does it imply that the products are necessarily the best available for the purpose.

With the obtained slope, calculate the delivered power using Equation 2. The procedure should be repeated for all desired power settings, relating each setting level to the measured power. The power obtained via calorimetry is the value we recommend for reporting power used in sonication-based dispersion procedures. By doing so, laboratories using different commercial ultrasonic disruptors should be able to reproduce a similar delivered power by selecting the appropriate calibrated setting. It is important to note that the intended use of calorimetry curves is not to measure the actual fraction of power utilized for powder disruption under specific dispersion conditions, but simply to establish a standardized procedure to relate instrument setting levels to measurable powers under a fixed set of controlled conditions, and thus allow for the reporting and transference of sonication power levels between laboratories and users.

The robustness of the method was tested under different scenarios, including variations in temperature probe location, water volume, and the use of stirring. While variations in temperature probe location and stirring only resulted in minor variations of the measured power (< 5%), it should be emphasized that using a different water volume, a container of a different material or a different type of horn tip than those recommended here can result in changes in the measured temperature versus time slope for a given power setting due to variations in the rate of heat transference between the sonicated water and the environment. If conditions other than those recommended here are used to measure calorimetric curves,

these modifications to the protocol would need to be reported.

Figure 6 shows calorimetric curves and calculated delivered power at different sonicator settings, obtained using the above method for a Branson 450 analog probe sonicator with a standard $\frac{1}{2}$ -inch probe and tip.

Additional considerations

As noted previously, sonication is a highly system specific dispersion procedure, involving a variety of complex concomitant physicochemical interactions in both the sonicated ENP and the suspension medium. Therefore, for a given system, optimal sonication conditions can only be determined by considering the effects of a variety of sonication parameters on the dispersion state of the suspension under a broad range of relevant conditions. As follows, a discussion of the effects of these parameters is offered along with recommendations for implementation of ultrasonic disruption as a preparatory tool. Two parameters, namely powder properties and sonication power, are not discussed here as they have already been addressed in previous sections.

Temperature

During sonication, the extreme local heating cycles that take place at the micro-scale bubble interface due to cavitation will result in bulk heating of the liquid over time. Excessive bulk heating can cause

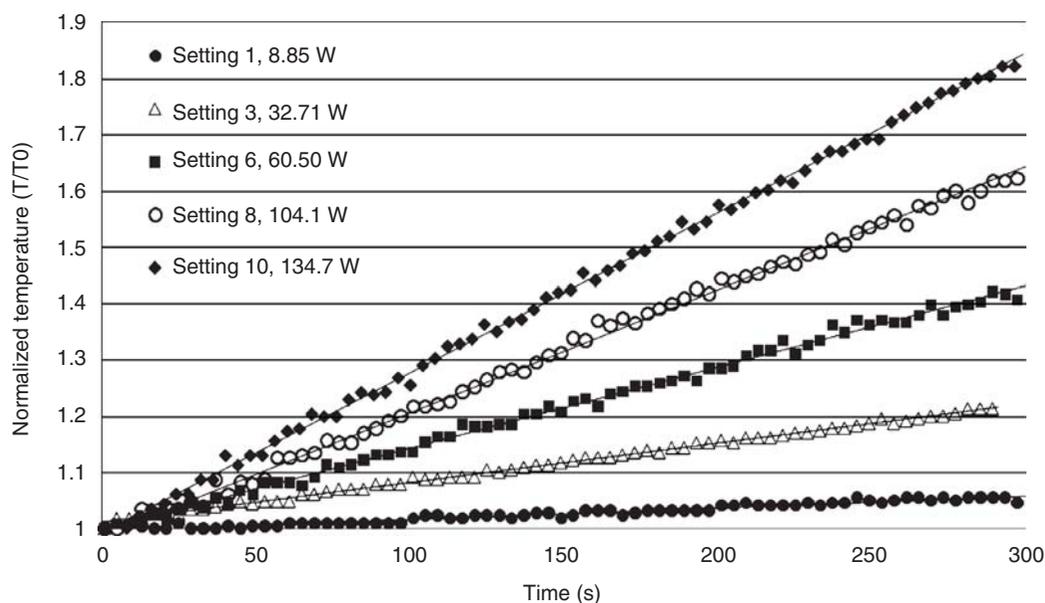


Figure 6. Calorimetric curves, linear fits, and corresponding calculated power values for different operational settings, obtained using a Branson 450 analog sonicator. For ease of illustration, the y-axis shows the recorded temperatures normalized to the initial temperature (T_0) in each case.

evaporative loss of liquid, resulting in changes in the sample volume after sonication. Moreover, high suspension temperatures may lead to degradation of the ENP or other medium components if proper precautions are not employed.

A common approach to minimize temperature driven side effects is to keep the sample from experiencing substantial high-temperature excursions by immersing the suspension container in a cooling bath. The container should be immersed to a level roughly equal to that of the contained suspension.

An ice-water bath is often sufficient to keep suspensions from overheating. If, however, ice water is not sufficient to cool the sample under intense sonication (e.g., the sample volume is too low, the sonication time is too long, or high sonication powers are used) an ice-salt bath could be considered as an option.

Working with containers made of materials with high thermal conductivities ensures a rapid release of the generated heat from the suspension into the surrounding cooling bath. With regard to thermal properties, the following container materials work best, in order of decreasing thermal conductivity: aluminum, stainless steel, glass, and plastic (e.g., polyethylene). When selecting the container, consideration must also be given to the chemical compatibility between the container material and the suspension components, especially under intense local heating conditions; for instance, aluminum is incompatible with acidic suspensions, and glass is incompatible with alkaline solutions.

Additionally, an aluminum foil or thin thermoplastic film with an opening just large enough for the probe to enter untouched, will reduce the evaporative loss of liquid content, especially when sonicating in volatile solvents or for substantial durations. Cooling the sample will also reduce evaporative loss. This cover will also minimize the potential release of aerosols generated by the sonication process.

Sonication time and operation mode

The total amount of energy (E) delivered to a suspension not only depends on the applied power (P) but also on the total amount of time (t) that the suspension is subject to the ultrasonic treatment:

$$E = P \times t \quad (3)$$

Consequently, two suspensions treated at the same power for different times can show significantly different dispersion states.

Ultrasonic disruptors typically can operate in either continuous or pulsed mode. In pulsed mode, ultrasonic intervals are alternated with static (sonication

off) intervals. The duration of on and off intervals can be regulated. Operating in pulsed mode retards the rate of temperature increase in the medium resulting from the cavitation process, minimizing unwanted side effects and allowing better temperature control than with continuous mode operation.

Sample volume and concentration

While sonication power and time describe the amount of energy *delivered* to the suspension, the work done on samples of different volumes and particle concentrations can differ. At constant volume, higher particle concentrations result in an increased particle collision frequency. In principle, an increased collision frequency can enhance particle breakage due to an increase in particle-particle impact events. However, if sufficient local activation and sintering energies are achieved, increased collision frequencies can also induce agglomerate or aggregate formation as particles collide and coalesce (Figure 4). The effect of concentration is thus dependent on both the energy delivered into the suspension and the physiochemical properties of the suspension.

Particle concentration can also alter the acoustic properties of the suspension, affecting the medium's viscosity and acoustic conductivity and thus the effectively delivered power. However, at the concentrations commonly used for environmental and toxicological evaluations, typically in the 50–2000 $\mu\text{g} \cdot \text{mL}^{-1}$ range, a significant impact on the medium's acoustic properties is not anticipated. Where particle concentration may alter acoustic properties is when working with concentrated stock suspensions prior to dilution.

The effect of suspension volume (at constant particle concentration) is often measured as *energy density* ($W \text{ s mL}^{-1}$). This magnitude expresses the amount of delivered energy per unit of suspension volume. In principle, at equal power and particulate concentration, higher energy densities (i.e., smaller suspension volumes) will result in a higher disruptive effect. It should be emphasized that when working with small volumes the temperature of the suspension will rise at faster rates and more intense cooling conditions may be required in this case.

Sonicator probe, container geometry, and tip immersion

The sonicator probe is the acoustic element that conducts the acoustic energy from the transducer into the sonicated suspension. The amount of acoustic energy transferred to the suspension will heavily depend on the shape of the sonicator probe and its immersion depth in the suspension.

The vibrational amplitude of the horn at a given power is dependent on the horn taper (Berlan and Mason 1992). Tapered probes act as vibration amplitude magnifiers, and consequently allow for higher delivered powers (see Equation 1). For example, stepped horn probes allow for up to 16-fold power amplifications, while exponentially tapered probes can yield power densities on the order of hundreds of $W \cdot cm^{-2}$ (Mason 1989).

At equal sonicator power settings, microtip probes vibrate with larger amplitudes than conventional flat tip horns. However, microtips are less mechanically robust and often limited in terms of the maximum power setting at which they can be used. The manufacturer of the sonicator will typically specify a maximum power setting (based on a percentage of the maximum power output setting) for use with microtips.

The way in which the sonic energy is distributed within the suspension is also heavily influenced by the container geometry. For example, in one published study (Pugin 1987) acoustic energy distribution profiles measured in round bottom flasks subject to indirect sonication significantly differed from those of flat bottom (e.g., Erlenmeyer) flasks subject to similar sonication conditions. Conical bottom, flat bottom, and round bottom flasks will also show different energy maxima and minima distribution profiles, which will in turn vary significantly for different probe tapers and probe tip immersion depths.

When possible, using the smallest diameter vessel that allows for the probe to be inserted without touching the container walls is probably the best approach. Using smaller container diameters raises the height of the liquid and maximizes the liquid-probe surface area exposed to the acoustic waves, as well as the container wall surface/volume ratio for dissipation of heat by the cooling bath. Cylindrical shaped beakers work well, especially for small volumes.

Probe immersion depths between 2 and 5 cm are generally recommended when operating with standard horns having a flat radiating surface (tip) or with microtips. Probes should be placed no closer than about 1 cm from the bottom of the sample container, and contact between the probe and the container walls should always be avoided.

Aerosoling and foaming

If the sonicator probe is not sufficiently immersed in the suspension it can give rise to surface agitation resulting in nebulization (i.e., the formation and release of aerosols). This could pose a risk if ENPs or other potentially harmful medium components are released in this manner.

Aerosoling may be indicated by fluctuations in the audible sound pitch during operation, fluctuating power readings, or by the appearance of a fine spray in the vicinity of the probe. If aerosoling is observed, it can usually be eliminated by lowering the sonication probe deeper into the suspension.

Additionally, if surfactants are present, the suspension could generate a foam during sonication, and the presence of a stable foam in contact with the horn surface will interfere with the delivery of ultrasonic energy to the suspension in a self-limiting process. Pulse mode operation with long off periods will help avoid foaming in samples subject to this effect. The appearance of foaming may require that operation is ceased until the foam dissipates. Anti-foaming agents can be effective, but would probably not be compatible with toxicological studies.

Tip maintenance

As the source of ultrasonic waves, all sonicator probes are subject to progressive erosion due to the intense cavitation that occurs in the immediate vicinity of the probe tip. Tip erosion is therefore an unavoidable side effect in direct sonication. There are two effects of tip erosion that are relevant to the present discussion. During erosion, microscopic tip residues (typically titanium metal) are released from the tip into the sonicated suspension, introducing an impurity and potentially contaminating the suspension. Operationally, tip erosion also results in a reduced energy output. The extent of potential contamination from tip erosion can be evaluated by performing a control experiment, or by measuring the amount of metal released into the sonicated medium (e.g., via digestion followed by ICP-MS analysis) under relevant sonication conditions. Erosion contamination can be minimized by adequate and frequent tip maintenance procedures, as explained below, or by using indirect sonication.

The extent and occurrence of tip erosion depends on the intensity, duration, and frequency of sonication cycles, as well as the chemical properties and concentration of the sonicated suspensions. If used on a daily basis, tips should be inspected weekly for signs of erosion; tip erosion can be recognized by the appearance of a grayish matting, as opposed to the shiny lustrous appearance of a non-eroded tip (see Figure 7).

A mildly eroded (i.e., matted) tip or horn surface can be reconstituted (dressed) by buffing with a very fine grit carbide paper or emery cloth (refer to the tip or sonicator manufacturer for the correct grit size; do not use conventional sand paper) (Berliner 2010). Substantially eroded tips or horn surfaces (i.e., with



Figure 7. Active surface of removable $1/2$ -inch flat tips, ranging from pristine (left) to matted (center) to eroded with extensive pitting (right).

visible pitting) should be replaced, as these cannot be reconstituted. Standard $1/2$ -inch diameter horns typically have a removable threaded tip, but larger probes are likely to consist of a solid horn and thus greater care may be required to dress the horn's active surface if it becomes matted.

It is also essential to ensure that removable probe tips are tightly coupled to the horn, and do not loosen as a result of prolonged operation; a loose tip will result in a reduced acoustic power conversion and energy yield.

Medium properties

As previously noted, the ultrasonic energy delivered to a suspension is partly attenuated and dissipated by the suspending medium. The amount of energy lost to the medium at a given acoustic frequency is governed by its physicochemical properties and chemical composition.

Generally, the attenuation of acoustic energy in a given medium can be estimated using the following equation (Brown and Goodman 1965):

$$I_x = I_0 e^{-2\alpha x} \quad (4)$$

where I_0 is the acoustic intensity (power/area) at the source, I_x is the intensity at a distance x from the source and α is the absorption coefficient.

The magnitude of the absorption coefficient, α , is dependent on the medium's physicochemical properties and composition, as well as the acoustic frequency. In principle, higher medium viscosities and acoustic frequencies will result in larger α values. For a given liquid, α can be estimated by using the following equation (Brown and Goodman 1965):

$$\alpha \propto \frac{2}{3} \frac{\mu f^2}{\rho} \quad (5)$$

where μ is the dynamic viscosity of the liquid ($\text{kg m}^{-1} \text{s}^{-1}$), f is the acoustic frequency (s^{-1}) and ρ is the liquid's density (kg m^{-3}).

As shown by Equation 5, acoustic attenuation increases with frequency for a given medium. For this reason, to achieve equivalent energy densities, higher power inputs are required when operating at higher frequencies. For example, to achieve an acoustic intensity of $20 \text{ W} \cdot \text{cm}^{-2}$ at a distance of 10 cm, would require approximately 54% greater source intensity at 10 MHz compared with that required at 10 kHz (Mason 1989).

Additionally, higher viscosities will dampen the cavitation process, requiring higher power inputs to achieve dispersion. The medium's density and acoustic wave speed (c) will also impact on the amount of vibrational energy that is transformed into acoustic energy (see Equation 1).

The medium's viscosity, density, acoustic wave speed, and chemical composition are all important parameters that impact on the amount of delivered energy that is effectively utilized to disrupt powder clusters. For media relevant to environmental and biological testing, the salt content is likely to have only a very small impact on the acoustic properties relative to pure water, as the presence of physiological salt levels will slightly alter the density and viscosity of the solution. Overall, at ultrasound frequencies, these effects are probably insignificant compared to other factors discussed previously.

Yet, as noted previously, the medium's chemical composition is of particular relevance in terms of the occurrence of potential side reactions during sonication (e.g., radical activation, sonopolymerization) that may alter the physicochemical properties of the sonicated medium and/or the sonicated nanomaterial.

Optimizing sonication for dispersion of ENPs

As discussed in preceding sections, the treatment conditions required to achieve complete ENP dispersion are highly system specific. It is therefore advisable to conduct dispersion optimization studies (e.g., measure particle size as a function of sonication parameters) for the test system (Bihari et al. 2008). While it would be unrealistic and impractical to offer a

unique set of sonication conditions for all possible systems, the ultimate goal of the optimization procedure should be to achieve the desired degree of particle dispersion with the *least* possible energy input, in order to minimize unwanted side effects.

The process to determine such conditions is based on a trial and error approach. The guidelines offered below are intended as tools for the interpretation of the observed behavior at a given set of sonication conditions, and to determine the appropriate parameter modifications to be made in response.

As noted previously, the relationship between dispersion efficiency and the different sonication parameters is not linear. The convolution of delivered energy input and suspension properties can result in either a net agglomerate breakage or cluster reformation effect.

For this reason, the optimization process should scan parameter values covering a range both above and below a starting point. The first trial set of sonication conditions for an untreated powder in suspension should be intended to ensure that dispersion can in fact be achieved. Being the first trial and therefore likely not the optimal set of conditions, it should be designed to confirm that under reasonably attainable sonication conditions, the powder in suspension can be effectively dispersed. The starting point of the optimization should therefore provide a high energy input to the suspension. This is achieved by selecting a relatively large sonication output power level, a prolonged sonication time, and a high energy density (small suspension volume).

Table I offers a general guideline of starting points for power, time, and volume selection, which might be utilized to investigate new ENPs or media/ENP combinations (Hielscher 2005).

The choice for the starting powder concentration is subject to the desired application. It is also possible to start with concentrations higher than the target level, and then if needed dilute the processed suspension post-sonication.

The effect of different processing parameters on the dispersion state of the suspension can be analyzed independently by varying one parameter while keeping the others constant. For each parameter, the effectiveness of the applied sonication conditions

can be evaluated by measuring the particle size distribution (PSD) of the sonicated suspension. In principle, a reduction in the average particle size and polydispersity (breadth of the distribution) are indicative of more effective dispersion. The parameter optimization sequence is explained as follows.

Once a starting set of values for time, concentration, and volume has been selected, and keeping all other processing parameters constant, sonication power is varied. Sonication powers above and below the starting point should be tested. As a general guideline, the effect of power can be scanned in increments of 10 W both above and below the starting sonication power. Using the power value that yields the smallest measured size and lowest polydispersity, then sonication time should be scanned (e.g., in increments of 30 s) above and below the starting point. Operation in pulsed mode is recommended for sonication times greater than 1 min, particularly when using small volumes (i.e., below 50 mL).

For the optimized time, power, and selected volume, the effect of particle concentration both above and below the concentration starting point and down to the minimum acceptable concentration for the desired application, may be evaluated if desired. If the optimal concentration is above the desired value for the particular application, the suspension's PSD should be measured following dilution to ensure there has been no change.

The above described approach for optimizing the sonication conditions for dispersion is both simple and practical, but the authors recognize that there may be other factors to consider or other optimization approaches that work equally well; it should be considered as a recommendation not a prescription for success.

Reporting guidelines

In light of the relevance of the above discussed parameters and their significance in dictating the state and quality of suspensions obtained via sonication, we propose that the following parameters be reported in published work involving the use of ultrasonic energy to disperse ENPs prior to environmental, exposure, or biological testing (Table II). It should be noted that many of these parameters or material characteristics would likely be reported anyhow, or certainly should be according to several recent publications and recommendations (Murdock et al. 2008; Warheit 2008; Minimum Information for Nanomaterial Characterization [MinChar] Initiative 2009; Boverhof and David 2010).

The lengthiness of this list may preclude full disclosure within a typical experimental section in a

Table I. Guidelines for sonication starting points.

Energy density (W · s/mL)	Sample volume (mL)	Power (W)	Time (s)
<100	10	50	<20
100–500	10	50	20–100
>500	10	50	>100

Table II. List of parameters and material characteristics recommended for reporting purposes.

Sonication	ENP or source material	Test medium	Final suspension
Type of sonicator used (bath, probe immersion, inverted cup)	Description of powder or ENP as received (to the extent relevant and known)	Composition including ionic strength or molar salt concentrations	Order in which components were added and whether or not they were present during sonication
Sonicator make, model, rated power output, frequency, probe type/diameter (if used)	For commercial ENPs, product trade name and/or product number together with batch/lot number if available	For commercial media, source, product and batch number(s), as applicable	Post-treatment storage conditions prior to testing (e.g., container material, temperature, exposure to light, exposure to oxygen, time)
Sample container volume and type	Primary particle size and method of measurement (e.g., TEM)	Initial pH (preferably measured, otherwise nominal buffer value)	Whether endotoxin was evaluated or procedures applied to avoid/remove endotoxins (relevant if suspension is to be used for toxicity testing)
Tip/probe immersion depth (if used)	Gross particle morphology (e.g., spheroidal, tubular, fibrous, platelet, aggregate)	Dispersing agent(s) and concentration(s), if used	Conditions of filtration and/or centrifugation used (where appropriate)
Operation mode (continuous or pulsed), if pulsed indicate pulse duration	Specific surface area (for powders) and method of measurement	Source and quality of water if test medium was prepared or diluted on-site	Post-treatment mass concentration of particle phase
Total cumulative treatment time	Identity of coatings or surface functionality if known	Any relevant details relating to storage, dilution, or preparation of medium	Final pH
Power input to sample (as measured for probe sonicators: See above and also Taurozzi et al. (2010a))			Particle size distribution in the relevant suspending medium and method of measurement
Whether cooling bath was used; if not temperature rise during sonication			
Mass of powder and total volume of suspension sonicated			

refereed journal, but could easily be accommodated in the supplemental information supported by most scientific journals. The recommendations may also be viewed as a checklist for identifying issues that *may* need to be addressed during the selection or development of a test material or dispersion procedure. For a more detailed and formal guidance document on reporting, refer to Taurozzi et al. (2010b) or consult the references cited above.

Additional parameters and conditions may need to be reported in order to fully describe the test sample and experiment in their entirety. For commercial sources, steps should be taken, if possible, to ensure that the as-received material reflects the manufacturer's description – for instance, comparison of powder characterization to nominal specifications and typical values. Examples might include moisture content by TGA, primary particle or aggregate size by electron microscopy, or purity determination using analytical methods. Under certain circumstances it may be prudent to test for the presence of contaminants arising from the dispersion treatment process itself, such as additional dissolved species (e.g., metal ions, organic carbon). It may be equally important to test for the loss of species that should be present, such as constituents of the original test medium. The fate of dispersing agents (e.g., surfactants, proteins, sugars) may be relevant for both environmental and biological testing.

Other material-specific parameters that are commonly mentioned as reportable include aspect ratio, crystalline form and content, amorphous content, and zeta potential from electrophoretic mobility or other measure (such as the isoelectric point or zero point of charge) related to the particle surface charge properties; zeta potential is both material and medium dependent, and typically varies with pH for materials that contain acidic or basic moieties, so the pH and ionic strength of the sample should always accompany zeta potential results. It may also be necessary, and certainly prudent to demonstrate that the post-dispersion suspension is stable over the time period of the experiment; for instance, particle size could be monitored in situ (e.g., using dynamic light scattering) over a period of time congruent with the testing protocol. The potential list of reportable physico-chemical endpoints is nearly unlimited, so prioritization within the context of the intended use is critical.

Closing remarks

Sonication has proven to be a simple and energy efficient technique for the disruption of coarse powders and, as such, has become a mainstream tool for the preparation of ENP suspensions for

environmental and toxicological studies. While we do not intend to discourage the use of ultrasonic disruption to produce such suspensions, our goal here is to promote a rational use of sonication, in order to ensure that unintended side effects, such as sonolysis, are minimized and that the results obtained using such suspensions are accurately interpreted. Additionally, by providing a set of guidelines for the standardized measurement and reporting of relevant sonication parameters, we seek to improve the reproducibility of related studies and allow for a comprehensive peer review process, which may be otherwise hindered by insufficient information regarding the preparation of the tested suspensions. We do not attempt to address the nearly infinite range of possible experimental scenarios, but instead offer a starting point towards the reproducible preparation and comprehensive evaluation of ENP suspensions.

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NOTICE OF CORRECTION

The Early Online version of this article published online ahead of print on 15 November 2010 contained an error on page 3. Equation 1 should have read

$$P = \frac{1}{2} \rho c A^2 (2\pi f)^2 a \quad (1)$$

This has been corrected for the current version.