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A method for assessing nanomaterial dispersion quality based on principal component analysis of particle size distribution data

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ABSTRACT

Seemingly contradictory findings between studies are a major issue in nanoecotoxicological research and have been explained as a result of the lack of comparability between assay methods, with dispersion of nanomaterials being identified as a key factor. Here we show the use of a multivariate method, principal component analysis (PCA), as a tool in protocol development and categorization of dispersion quality. Results show the significance of particle concentration within a protocol, and its effect on repeatability. Our results suggest that future studies should involve the use of PCA as a powerful data exploration tool to facilitate method development, comparability and integration of data across different laboratories.

Introduction

The implementation of an established protocol ensuring the reasonable dispersion of powdered nanomaterials in the appropriate medium is one of the most critical steps in nanoecotoxicological investigations (Tantra & Shard, 2013; Tantra et al., 2011). The need to have a clear and comprehensive guidelines for dispersion of nanomaterials has led to several published documents (Taurozzi, Hackley, & Wiesner, 2012). Researchers have highlighted the importance of well-controlled sonication protocol in a dispersion protocol (Tantra, Gohil, & Cumpson, 2009; Taurozzi, Hackley, & Wiesner, 2011). Undoubtedly sonication is an important parameter that may contribute to the variation in dispersion quality but there are also other potential sources of variability to consider, e.g. particle concentration, age of the dispersion, and subsampling (Tantra, Jing, Gohil, Popov, & Brebbia, 2010; Tantra, Sikora, Hartmann, Sintes, & Robinson, 2015). Therefore, it is important to identify factors that make the most significant contribution to the quality of dispersion.

In order to assess the magnitude and source of data variability associated with the dispersion step, a comparative analysis of repeatability is required; this would mean the need to acquire and process large volumes of data. Subsequently, a procedure that will result in effective data processing in order to understand relationships between controlling factors and dispersion quality is needed. One approach in relation to how data should be processed is in the use of a statistical procedure, principal component analysis (PCA). PCA is a multivariate statistical tool that searches for patterns and relationships. PCA has the advantage in that it provides a visual aid for identifying homogeneity and differences amongst large datasets, displaying detectable patterns in an unbiased way. The method works by taking complex datasets with multiple interrelated variables and reducing them down, with minimal loss of information, to simpler uncorrelated datasets known as principal components (PCs). The ability of PCA to analyze complex data has seen its application in many fields; this ranges from multivariate data analysis (Daszykowski, Kaczmarek, Vander Heyden, & Walczak, 2007), sensing (Lu, Partridge, Meyyappan, & Li, 2006), genetics (Slonim, 2002) to process control (Boonkhao & Wang, 2012; MacGregor & Kourti, 1995). Furthermore, it has also been used to analyze particle size distributions data, even though this has not been used in nanoecotoxicological setting (Boonkhao & Wang, 2012; Chan & Mozurkewich, 2007; Chan & Mozurkewich, 2007; Mattila, Saloheimo, & Koskinen, 2007). In relation to nanotoxicology however, PCA (as a statistical tool) is of limited use. Some recent

Abbreviations: PCA, principal component analysis; PC, principal component; DCS, differential centrifugal sedimentation; (Q)SAR, (quantitative) structure–activity relationship.

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examples on the use of PCA in nanotoxicology has been its application to isolate a number of nanomaterial properties that influence toxicity (Wang et al., 2014) and to identify the negative effects on earthworm metabolism resulting from TiO_2 nanoparticle exposure from nuclear magnetic resonance (NMR) spectra (Whitfield Åslund et al., 2011).

The limited use of PCA in nanoecotoxicology is unlike (Q)SAR ((quantitative) structure–activity relationship), which has been more frequently used by researchers to find certain relationships e.g. correlating physicochemical properties (such as particle size) with toxicological response (Epa et al., 2012; Fourches et al., 2010; Puzyn et al., 2011). The popularity of (Q)SAR in nano-safety research has become more prominent since the encouragement of EU's REACH (registration, evaluation, authorization, and restriction of chemicals) regulation on the use of in silico techniques for the risk assessment of manufactured nanomaterials. As the name suggests, the ultimate aim of the (Q)SAR analysis is to establish a mathematical equation in which the biological activity of a class of compounds is expressed as a function of its physicochemical characteristics. Although the (Q)SAR approach has been proven to be useful for variety of applications in the case of classic chemicals, the traditional (Q)SAR algorithm needs serious reconsiderations to account for the novel properties of nanomaterials.

Fig. 1 illustrates the PCA process followed in this study. The original size distribution data (Fig. 1(a)) undergoes pre-processing (Fig. 1(b)) in preparation for covariance or correlation PCA (see "Materials and Methods"). PCA carried out on the pre-processed data involved the extraction of PCs using either covariance or correlation matrices. In covariance PCA the data is mean, centred whereas correlation PCA involves additional auto scaling of the data variation. As a general rule, covariance PCA is used when the scale or units of the variable are the same and correlation PCA when the scale or units differ. The PCs were then plotted against each other as scores plots (Fig. 1(c)), which allow relationships between samples to be visualised, or as loading plots (Fig. 1(d)), which display their relationship to the original variables. An ellipsoid was added to the scores plot (Fig. 1(c)) representing a 95% confidence interval (Hotelling's T^2 statistic), aiding in the detection of strong outliers.

The main purpose of PCA is to reduce the dimensionality of dataset through the extraction of PCs that summarize the data with the minimal loss of information and eliminate the dependency between the original variables. The derived PCs are a new set of variables which are uncorrelated and orthogonal to each other. The first PC accounts for the greatest variation in the data while the second one explains most of the remaining variance. Although the total number of components extracted from the data can be equal to the number of original variables, in most cases, only a few PCs that capture most of the variation in the data are retained. Appendix A aims to further explain the principle of PCA by a simple algorithm, e.g. information about how the fractions were obtained.

In this study, PCA was used to observe variation in dispersion quality, as assessed via particle size distributions obtained from differential centrifugal sedimentation (DCS) analysis. The study is concerned with: (a) assessing variability arising from different dispersion protocols; four protocols were chosen from past literature; (b) identifying the sources of variability within a protocol, i.e., looking at the effects of: dispersion ageing, sonication exposure time, sonication in the presence/absence of an ice bath, material subsampling, particle concentration, and the presence/absence of a pre-wetting step.

In this study, deionized (DI) water is the dispersant liquid of choice. Although it is far from an ideal biologically relevant medium, the study presented here is fundamental in that as a first step, it is important to assess the degree of variability arising from a highly stable dispersion in a simple medium, for the purpose of accurate analytical measurements. In the past, DI water has been used as the ideal liquid dispersant for metal oxide nanomaterials; past workers having shown that dispersion in DI water resulted in extremely stable dispersions when compared to relevant environmental media such as fish or daphnia media (Tantra et al., 2010, 2011).

Materials and methods

Materials

Nanomaterial TiO_2 (NM-105) was supplied by the Joint Research Centre (JRC) through a Framework 7 MARINA (Managing Risks of Nanomaterials) project (ISO 14887:2000 Sample preparation – Dispersing procedures for powders in liquids) and used as received. Deionized water, with a resistivity value of 18.2 MΩ cm was used throughout the study.

Although not fully reported here, a preliminary study to characterize NM-105 was carried out. Particle Feret diameter, as analyzed using SEM (scanning electron microscope, Supra 40, Carl Zeiss GB, Germany), resulted in: mean primary particle diameter ($\pm\sigma$) = (38 ± 10) nm, with D_{10}, D_{50}, and D_{90} oversize percentiles of (50 ± 5), (36 ± 3), and (27 ± 3) nm, respectively. In this case, an oversize percentile D_{10} of 50 nm means that 10% of the particles will have particle diameter of 50 nm or larger. SEM images show highly agglomerated and aggregated particles; this is expected as the powdered nanomaterial is industrially relevant. When dispersed in DI water, the zeta-potential, acquired using micro-Doppler electrophoresis (ZetaSizer Nano ZS, Malvern, USA) was measured as (22.2 ± 1.8) mV, this falls within the typical range for stable dispersions (>±20 mV), suggesting that the dispersed TiO_2 in DI water was sufficiently stable for the purposes of the study.

Dispersion protocols

In order to prepare nanomaterial dispersion, samples were weighed into clean glass vials and dispersed in accordance to one of four protocols, as detailed below.

Protocol A: This protocol specified a particle concentration of 1 mg/mL. The protocol did not specify conditions related to the sonication step, apart from the fact that a 2 min sonication exposure time was required. An ice bath was used during the sonication step.

Protocol B: This protocol specified a 1 mg/mL concentration and a delivered sonication power output of 50 W under a pulsed operation mode (80% on/20% off). The sonication was carried out under ice bath conditions for a period of 15 min.

Protocol C: This protocol specified a particle concentration 0.015 mg/mL. Sonication step did not involve the use of an ice bath and required an input power of 130 W at 90% amplitude for a period of 20 s. The protocol also specified the need for a pre-wetting step prior to sonication, which involved the addition of several drops of DI water into the vial containing the nanomaterial and then mixed using a spatula.

Protocol D: This protocol was a slight modification of protocol C, with the only difference being a change of particle concentration to 0.1 mg/mL. This particle concentration was chosen as it has been recommended for use with the DCS instrument (Aimable & Bowen, 2010). This protocol was studied in greater detail here, in which small variations in the individual steps was carried out. Here, we studied the effects of sonication time (20 s to 15 min), having an ice bath during sonication, pre-wetting the nanomaterial powder, subsampling effects (in which the powders were subsampled six times; this was done either by taking sample from one batch or six separate batches), age of dispersion (dispersion analyzed