REPORT

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Evaluation and Assignment of Nanoparticle Dispersion/Characterisation Methodologies, to be Developed under PROSPEcT.

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ABSTRACT

This report presents findings from a literature review, conducted to identify and evaluate tools used for nanoparticle dispersion and characterisation; the end goal is to further develop methods for dispersion and characterisation, to be agreed amongst the partners in the PROSPEcT project. Key findings, displayed mainly as summary tables, provide an overview of the tools, detailing the background technology, their merits and disadvantages. This information is matched against a given a list of criteria considered most relevant to the needs and stated objectives in the project. In the light of our findings, we conclude that:

a)   To achieve a stable dispersion, the nanoparticles must first be sufficiently de-agglomerated and subsequently stabilised by the dispersant; stability is dominated by the nature of the dispersant;

b)   De-agglomeration of nanoparticles requires high shear energy and thus the use of ultrasonic probes in the dispersion protocol is highly recommended;

For nanoparticle characterisation, it is recommended that both "as received powders" and "as dispersed in liquid media", should be characterised. Choosing a variety of techniques that provides complementary information is important, such that different physico-chemical properties of the nanoparticles can be probed.

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1 INTRODUCTION: Scope

An important endpoint in the ecotoxicological research of nanoparticles is the ability to link "physicochemical parameters" with "biological activity", in order to answer the question of which parameters play a key role in the mechanisms of toxicity. To do this with some degree of confidence relies on the provision of validated standard operating procedures (SOPs) for nanoparticle dispersion and characterisation.

In this report, we will present findings on the basis of a literature review, to provide:
   a) An overview of the various processes involved in the dispersion of nanoparticles in liquid, and subsequent stabilisation;
   b) The nanoparticle parameters identified to be of some ecotoxicological importance;
   c) A summary of the currently available de-agglomeration tools for dispersion, and analytical tools for characterisation;
   d) A list of criteria identified as being particularly useful for evaluating the tools for dispersion and characterisation (given in (c));
   e) Tools/methodologies to be recommended and further developed in the PROSPEcT project.

Overall, the report seeks to answer the following questions concerning nanoparticle dispersion and characterisation:

   a) What tools/methodologies are currently available for nanoparticle dispersion and characterisation?
   b) What criteria can be identified that will allow us to identify suitable tools for dispersion and characterisation, bearing in mind the requirements for ecotoxicological testing?
   c) From findings in a) and b), what tools/methodologies can be recommended, or require further development, under the PROSPEcT project?

In summary, this report is mainly based on a review of literature to identify suitable methods that can be further developed for nanoparticle dispersion and characterisation (to eventually be agreed by all partners in PROSPEcT). This will thus form an important part of UK's contribution towards a key OECD study for ecotoxicological testing of CeO_2 and ZnO nanoparticles. Material for this literature review was taken from various sources including peer reviewed articles, books and reports (from organisations active in nanoparticle standardisation work e.g. ISO, OECD and NIST).

2 NANOPARTICLE DISPERSIONS

2.1 Background

As depicted in Figure 1, the dispersion of solids into liquid suspension typically involves the following three key steps[1]:

   a) Step 1: Wetting of nanoparticle powder to form a paste. The purpose of this step is to substitute solid-air interface with solid-liquid interface, such that the particles are sufficiently "wetted". The efficiency of wetting will thus depend

on the comparative surface tension properties of the nanoparticles and the liquid media, as well as the viscosity of the resultant mix. It has been recommended by ISO [2] that this can be achieved by mixing the powder with drops of suitable liquid media slowly until it forms a thick paste.

b) Step 2: De-agglomeration (including de-aggregation) using a de-agglomeration tool. It is inevitable that nanoparticle powders undergo uncontrolled agglomeration and aggregation. Sufficient shear energy is needed to break down aggregates/agglomerates, preferably to their optimum primary particle size; what is often termed as a "good dispersion" will thus consist chiefly of primary particles with only a minimum of loose aggregates and agglomerates . During the de-agglomeration step, higher surface areas become exposed to the liquid vehicle and larger amounts of the liquid vehicle are thus required to wet out the newly formed surfaces. Overall, the amount of free liquid vehicle diminishes and thus the viscosity of the dispersion increases [1].

c) Step 3. Stabilisation of the Dispersions. The goal here is to achieve and maintain stability after the nanoparticles are sufficiently wetted and de-agglomerated. Stability in this step is dominated by the choice of the liquid dispersant employed in Steps 1 – 2. Ultimately, it is the surface properties of the nanoparticles and their interaction with the aqueous media (as measured by parameters such as zeta-potential, hydrophobicity and adsorption capacity to specific analytes in the liquid media) that will determine stability; it is not surprising therefore that the addition of surfactants, for example, can lead to marked changes in its interfacial properties and thus stability [2].
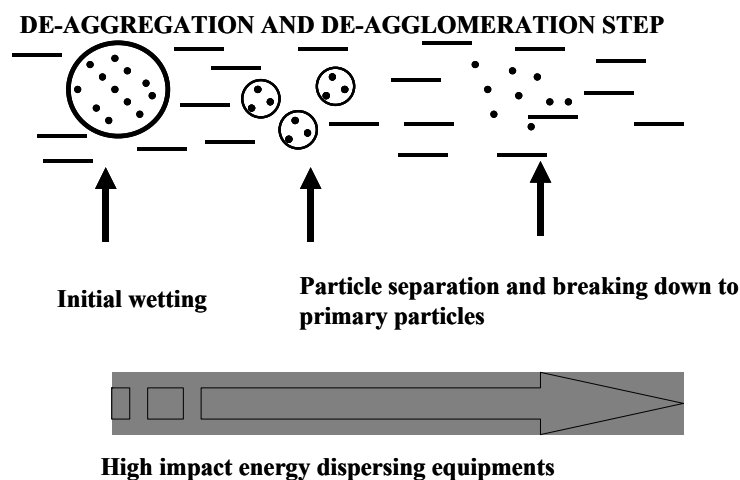

Figure 1. The Dispersion Process. The image sequence above shows de-aggregation and de-agglomeration of particles. The image above is adapted from [1].

2.2. De-agglomeration Tools

As discussed, the use of a de-agglomeration tool to overcome the inherent agglomeration problem inherent with nanoparticles is of paramount importance for successful dispersion. Table 1 below shows a summary of commercially available de-agglomeration tools. The table provides a brief description of the background technology as well as the advantages/disadvantages of employing such a tool to aid nanoparticle dispersion. The relative amount of energy provided by the tool is also

given; it is hoped that this will give some indication as to the effectiveness of such a tool to efficiently de-agglomeration/de-aggregate.

De-agglomeration Tools	State of Nanoparticles (NPs)	Principle of Operation	Advantages	Disadvantages	Shear Energy Provided/ References
Mills (to include ball, stirred media, centrifugal and jet mills)	Mainly suitable for dry/ wet powders	Involves ultrafine grinding process	Useful for large batches	Slow/ inefficient – ball milling may take days in some cases. Grinding motion can lead to significant breakdown of nanoparticle architecture. Can be difficult to clean; contamination likely	Medium [3]
Stirring (magnetic or overhead stirring)	NPs in liquid media	The use of magnetic stir bar or an overhead-stirring paddle, having rotational speed that is sufficient to create a vortex. Overhead stirring has a much higher speed than the magnetic counterpart	Rarely results in attrition or breakage of nanoparticles Cheap/ affordable	Inefficient Rarely results in de-agglomeration and often-employed in order to improve homogeneity of dispersion. Cannot prevent particles from aggregating or agglomerating.	Low. [4].
High speed Homogeniser	NPs in liquid media	The use of a rotor stator generator probe; the rotor acts as a centrifugal pump to re-circulate the liquid and suspends the solids through the generator, where it will subjected to shear, impact collision and cavitations	Suitable for large liquid sample up to 2500 ml	Never tested for nanoparticle dispersion	Unknown as never tested for nanoparticle dispersion

High Pressure Homogeniser	NPs in liquid media	Shear and cavitations provided via increase in the velocity of pressurised liquid streams in micro-channels	Highly efficient	Nanoparticle architecture can be altered; increase of temperature in the dispersion likely. Expensive	High [4].
Ultrasound Sonicating Bath	NPs in liquid media	The use of ultrasound waves and cavitations (i.e. the formation, growth and implosion of bubbles in liquid) activity in a bath.	Cheap/ Affordable	Bath format less effective (less shear) compared to probe format. Can alter nanoparticle architecture; increase in temperature likely if dispersion is sonicated for long period. Highly variable performance at lower end of the market	Medium [4].
Ultrasound probe sonication or ultrasonic disruptor	NPs in liquid media	Similar to ultrasonic bath but aims to deliver more energy density in smaller volume in comparison to the corresponding bath format	Highly efficient	Probe tip disintegration can contaminate samples. Can alter nanoparticle architecture; temperature increase (even for a few minutes) in dispersion highly likely. Highly variable performance at lower end of the market.	High [4]

Table 1. Comparison of de-agglomeration tools, as provided from literature sources.

2.3. De-agglomeration Tools: Research Recommendations

The choice of tools used in PROSPEcT will ultimately depend on the criteria set out for the project. As no clear guidelines are set by any internationally recognised standardisation organisation such as ISO, the criteria identified below will be subjective in nature. The identification of such criteria is seen as a good starting point, as this will enable us to further identify and evaluate suitable de-agglomeration tools. In choosing a suitable de-agglomeration tool, the criteria deemed to be of some importance include the need to have a tool capable of:

a) De-agglomerating the nanoparticles whilst suspended in liquid media; this is of importance as the medium plays a huge role in stabilising the nanoparticles after the de-agglomeration step;

b) Providing sufficient shear to de-agglomerate/de-aggregate and thus rendering the tool as "efficient". As shown in Table 1., the various de-agglomerating tools differ in terms of shear energy that they provide; the more shear energy the more efficient it is at breaking interlock bonds of the agglomerates/aggregates. In terms of shear, de-agglomerating tools can be categorised as low, medium or high shear;

c) Minimising unnecessary breakages/ attrition of the primary particles themselves;

d) Minimising any temperature rise in liquid media during the de-agglomeration step, as this rise in temperature can ultimately result in re-agglomeration;

e) Economical (e.g. below 3,000 GBP) and commercially accessible to the partners.

From criterion (a), it would seem that the main de-agglomeration step should be conducted when nanoparticles are suspended in the appropriate liquid media matrix. In this sense, mills will not be suitable as they are often associated with the pulverisation (or mixing of materials) when in a dry or in wet-paste form. Although mills will not be used as the main de-agglomeration tool in PROSPEcT, mills may be beneficial in the first step i.e. making a paste prior to the main de-agglomeration step. Hence, the use of mills should be explored in PROSPEcT to further test whether they can be useful to be used in conjunction with other de-agglomeration tools. Criterion (b) indicates that tools should be capable of providing sufficient shear energy to break up the agglomerates/ aggregates. Table 1 indicates that only two tools are considered to be sufficient in this regard i.e. the ultrasonic probe and high-pressure homogeniser. The main disadvantage of such tools is that they will not be able to satisfy criteria (c) and (d). These high shear energy tools have the potential to result in nanoparticle breakage/attritions as well as increase the temperature of the dispersion. However, the extent of this will be highly dependent on the inherent nature of the nanoparticles as well as the protocols adopted during the dispersion; literature indicates that high aspect ratio nanoparticles in particular (such as carbon nanotubes) are particularly sensitive to such attritions and rise in temperature [5]. Potentially, non-fibrous particles are less sensitive, and such effects can be minimised by having better control during dispersion i.e. by minimising de-agglomeration time as well as monitoring temperature rises using a temperature probe. From our own earlier work on this (data not yet published), high shear ultrasonic tools such as ultrasonic probes did not result in damage of non-fibrous nanoparticles, such as TiO_2 and ZnO. In addition, our data

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have shown that the rise in temperature is not problematic if short de-agglomeration exposure time is adopted in the protocol. Out of the two tools identified as suitable so far, only ultrasonic probes satisfy criteria (e) i.e. they are economical, with some models available commercially under £3,000. Hence, the ultrasonic probe will be the de-agglomeration tool of choice in the project. The performance of this tool will be compared to other tools of interest, in particular overhead mixing and high speed homogeniser tools. From Table 1, it is evident that these tools have not been sufficiently investigated as de-agglomeration tools for nanoparticles.

3 NANOPARTICLE CHARACTERISATION

3.1. Background

Once a dispersion has been obtained (as described in Section 2), it is important to characterise the stability of such a dispersion. In order to monitor stability, appropriate characterisation tools must be identified and employed. Measuring dispersion stability is just one of the many important parameters in nanoparticle characterisation; other parameters include (but are not limited to) those listed in Table 2 below. Currently, there is no agreed set of parameters to characterise, as there is not yet a general agreement on the type of physicochemical information that needs to be known for the integrated risks associated with engineered nanoparticles. The purpose of Table 2 therefore is to list all of the parameters deemed to be of ecotoxicological importance. This list was compiled as a result of adopting several guidelines, including those from a recent OECD document [6] as well as review papers [7] [8]. The parameters listed have been suggested by past workers to be associated with mechanisms of toxicity. The table below also indicates some of the most common and appropriate techniques to measure such parameters. These exclude some established techniques, such as Atomic Force Microscopy for size determination, which have no practical benefit over the others that are available.

Parameter	Techniques/ Related Reference
Dispersion Stability	Visual observation/ [9]
	Dynamic Light Scattering (DLS)/ [10]
	Zeta-potential/ [11]
Agglomeration/ aggregation	Scanning Electron Microscope (SEM)/ [10]
	Transmission Electron Microscope (TEM)/ [12]
	Dynamic Light Scattering/ [10]
	Zeta-potential/ [13]
Size	SEM/ [14]
	TEM/ [15]
	DLS/ [10]
	Scanning Mobility Particle Sizer/ [16]
Shape	SEM/ [17]
	TEM/ [18]
Surface charge	Zeta-potential measurement using Doppler Microelectrophoresis/ [13]
Surface Area	Brunauer, Emmett and Teller (BET)/ [19]
Surface chemistry and contamination	X-ray photoelectron spectroscopy (XPS)/ [20]
	Energy Dispersive X-ray Spectroscopy (EDX)/ [21]
	Secondary ion mass spectrometry (SIMS)/ [22]
	Electrochemistry Redox potential/ [23]
Photocatalytic activity	UV-vis spectroscopy/[24]
	Inherent autofluorescence/ [25]
Solubility /Dissolution	Conductivity/ [26]
	Calorimetric assay/ [27]
	UV-vis spectroscopy/ [28]
	Ion exchange chromatography [with suitable detector e.g. conductivity and other electrochemical detector]/ [29]

Table 2. Common techniques that has been identified to measure nanoparticle parameters of ecotoxicological interest

3.2. Characterisation Tools

Table 3 below provides a brief description of the characterisation techniques for: a) nanoparticles and b) liquid dispersants, respectively. The tables provide a summary of the technique, the state of the nanoparticles during analysis, whether the tool addresses single particles or populations, and any notable advantages or disadvantages for nanoparticle characterisation [30]. Note that the under the column "Advantages", relevant documents associated with standardisation are listed; these documents are those that have been published by an internationally recognised standardisation body such as ISO. Although these documents are not focused on nanoparticle characterisation, they will nonetheless give a good basis for development of such protocols.

Techniques (and acronyms)	Principle/ Sensitivity	State of NPs during analysis	Single or Particle Population Method	Advantages (to include relevant standards documents, with references)	Disadvantages
Scanning Electron Microscopy/ SEM	Electron beam to yield secondary electrons to form highly resolved images ~ 2 nm - >1000nm;	NPs on a surface	Single particle	Highly selective and sensitive BS 3406-4(1993)/ [31] BS ISO 16700/ [32]	Requires high vacuum conditions. Nanoparticle must adhere on substrate. Limited number of particles per image. Difficult to achieve representative data. Operator dependent Possible sample damage – if prolonged beam exposure Expensive instrument Potential artefacts from sample preparation and high vacuum conditions Charging effect may require sample to be spin coated with gold film
Transmission Electron Microscopy/	Use of transmitted electrons	NPs on a surface	Single particle	Highly selective and sensitive	Similar but worse than SEM; this is because TEM requires a much higher voltage electron beam and extremely thin samples (to allow high transparency) in

TEM	through thin specimen to produce an image. ~ 0.2 nm - >1000 nm			BS ISO 29301/ [33]	order to create an image.
Dynamic Light Scattering (DLS)/ Photon Correlation Spectroscopy/ Quasielastic light scattering.	Measure the time dependent light intensity fluctuations (Doppler shifts in the incident light frequency) that originate from Brownian motion of particles. The diffusion constant is estimated and hence particle size. ~ 1 nm - 7 microns	NPs dispersed in liquid media	Population Method	Suitable for routine Excellent for low particle concentrations i.e. < 1 wt% Excellent for narrow distributions. Best used to monitor aggregation behaviour. Instrument for DLS can have the option for zeta-potential measurement	Difficult to detect presence of a bimodal distribution (unless the components are separated in size by a factor of order 10). Less reliable as size distribution broadens. Subject to contaminants. Insensitive to large aggregates that may settle. Factory-installed algorithms and geometrical models that require post-measurement interpretation related to the validity of the model Needs to consider particle characteristics Limitation interpretation e.g. when using mathematical conversions to volume or number distributions.

				BS ISO 22412/ [34]	
Brunauer, Emmett and Teller/ BET	Use gas adsorption and capillary condensation principles to obtain information about surface area and porosity of solid material. ~ 5 nm - several microns	Dry powders	Population Method	It can measure surface areas as low as $0.01 m^2/g$ using nitrogen as the adsorbate ISO 9277/ [35]	Factory-installed algorithms and geometrical models that require post-measurement interpretation related to the validity of the model Insensitive to macroscopic morphological arrangement of the particles; will fail to indicate the nature of agglomeration.
Doppler Microelectro-phoresis/ Doppler electrophoretic light scattering [Measure of zeta-potential]	Measurement of electrophoretic mobility by analysis of intensity autocorrelation function of the scattered light. ~ 5 nm - >1000	NPs dispersed in liquid media	Population Method	Suitable for routine – rapid measurement	No relevant standard yet published. Sample preparation technique critical e.g. concentration of nanoparticles, concentration of ions in solution (may contribute to Joule heating), fluorescing samples, etc. Factory-installed algorithms and geometrical models that require post-measurement interpretation related to the validity of the model

	nm				
Scanning Differential Mobility Particle Analysis / SMPS	Uses a bipolar charger to impart a known charge distribution on an aerosol sample. Particles are then classified according to their ability to traverse an electric field. Measure electrical mobility to estimate particle size. ~ 5 nm - 2 microns	Aerosols	Population Method	Suitable for sizing spherical particles Suitable for routine – rapid measurement BS ISO 8573-4/ [36]	Errors in the mobility size data interpretation for aggregates, and subsequent calculations of surface area and volume (and hence mass) distributions
X-ray photoelectron spectroscopy (XPS)/ Electron Spectroscopy for Chemical Analysis	Irradiation of material with X-ray beam and simultaneously measuring the kinetic energy (KE) and	NPs on a surface	Population Method	Chemical fingerprinting elemental composition of the surface (top 1–10 nm usually)	XPS requires high vacuum conditions. Expensive/ not suitable for routine

(ESCA).	number of electrons that escapes. Detection limits for most of the elements are in the parts per thousand ranges. Detections limits of parts per million (ppm) are possible, but require special conditions: concentration at top surface or very long collection time (overnight).			BS ISO 15472/ [37] BS ISO 18516/ [38] BS ISO 21270/ [39] BS ISO 24237/ [40]	
Energy dispersive X-ray spectroscopy (EDS, EDX or EDXRF)	Energy dispersive spectrometer to measure emission characteristic X-rays from	NPs on a surface	Population Method	Platform integrated with SEM platform. BS ISO	Requires high vacuum conditions. The majority of the signal does not come from the surface, but from the top few μm in depth.

	specimen upon excitation with incident electron beam. Allows elemental composition of the specimen to be measured. Parts per million sensitivity.			15632/ [41]	
Secondary ion mass spectrometry/ (SIMS)	Sputtering of surface with primary ion beam and collecting/ analysing ejected secondary ions, which are measured by mass spectrometer to reveal composition of solid surface and thin films.	NPs on a surface	Population Method	Most sensitive analysis technique Depth profiling possible; can identify organic molecules on surface and not just elements BS ISO	High vacuum

				23812/ [42] BS ISO 14606/ [43] BS ISO 18114/[44] BS ISO 23830/ [45]	
Fluorescence spectroscopy	Photocatalytic activity through the measurement of auto-fluorescence; fluorescence deals with transitions from excited state to ground state. Detection limit dependent on inherent fluorescent properties and	NPs dispersed in liquid media	Usually population method but technique has single particle capability e.g. in near field and confocal configuration.	Suitable for routine ASTM E 578/ [46] NCCLS I/LA24 A/ [47]	Quenching may result in erroneous results

Above the Fluorescence row, the first column contains:

Able to detect elements present in the parts per billion range.

	instrument set-up				
Electro-chemistry Redox potential/ oxidation-reduction potential (ORP)	Tendency/ affinity to acquire electrons under applied potential ; measurement will probe redox chemistry (detection of particular analyte in solution, of parts per million).	NPs to be embedded in a conductive but inert media to produce electrode.	Population Method	Suitable for routine analysis purposes

BIS IS 13673-5/ [48] | N/A |

Table 3a. Summary of tools to potentially characterise nanoparticle parameters.

Technique	Principle/ Sensitivity	Advantages/ Related standard documents with references	Disadvantages
Ion-exchange chromatography (with suitable detector e.g. conductivity and other electrochemical detector)	Chromatographic separation of ions, achieved by using an appropriate ion-exchange column and eluent (sensitivity of parts per million)	Particularly advantageous if a complete analysis of all of the ions in the sample is needed or if the matrix is highly complex. ASTM STP 195/ [49]	Time consuming. The column resin can be prone to fouling e.g. presence of organic matter.
pH	Measure of acidity or basicity of a solution; this parameter determines stability of dispersant as it will affect zeta-potential values.	Easy to use Suitable for routine Specific IEC TR 62434/ [50] ASTM D 5464/[51] BS 1647-2/ [52]	N/A
UV –Vis spectroscopy	Spectroscopy of photons in the ultraviolet-visible region.	Absolute absorbance SAC GB/T 19267-2/ [53]	Often indirect measurements

	Measures absorbance (electronic transitions from ground state to excited state). Can be used as a platform to measure photocatalytic activity by monitoring photochemical radical production rate constants using radical scavenging dyes e.g. methyl viologen and tetrazolium salts Can be used to measure metal ion concentration due to dissolution, which involves the analysis of light absorbing derivatives of the metal ions (rather than a direct measure of the ions themselves, as none of the common ions in water absorb light in the visible region of the spectrum). Parts per million – subparts per billion; sample dependent		

Table 3b. Summary of tools to potential characterise the liquid dispersant**.**

3.3. Characterisation Tools: Research Recommendations

In order to choose suitable tools for characterisation, it is important to develop a set of criteria. As before, there are no set guidelines on this and so the list of criteria below was developed by combining outputs from a meeting on April 30, 2009 between NPL and Exeter University, and findings from the literature review. Although subjective in nature, this list of criteria is considered to be a good starting point for further development of methods for nanoparticle characterisation. It is proposed that ideal characterisation techniques satisfy the following criteria:

a) High selectivity and high sensitivity [54]. Sufficient selectivity is needed to distinguish the nanoparticles from other components present in the liquid media. In the aquatic environment, liquid media are often complex in nature e.g. they often contain considerable amounts of potentially interfering substances such as humic acid, inorganic components, ions and metals. If at sufficiently high concentration, the presence of such species can mask the nanoparticle signal. Furthermore, nanoparticle concentrations of interest in the environment are extremely diluted, often in the range of sub-milligram/litre to microgram/litre. This is the equivalent of having a tool with sensitivity better than ~ sub-parts per million - parts per billion range. In general, most analytical tools that fall under the umbrella of "population methods" have sensitivities close to parts per million rather than parts per billion. Overall, only "single particle" based techniques have the kind of sensitivity that is very desirable; these are often imaging techniques, which have spatial resolution of few nm or less;

b) Representativity [54]. Although highly sensitive and selective, data arising from so-called "single particle" methods (such as SEM and TEM) can not be considered to be representative of the whole sample and this poses a problem as to the reliability of such methods. Hence, "population methods" are more reliable in that they yield data that are more representative;

c) Commercially availability, well established (in terms of standards and standardisation) and suitable for routine analysis purposes. Tables 3 a) and b) show that all of the techniques included are commercially available, with existing guidelines published by ISO already in place for most techniques, although these guidelines are not necessarily specific to nanomaterials. Such guidelines will form the basis of the standard operating protocols to be established in this project, to be further developed so as to render them suitable for nanoparticle characterisation. Note that not all of the techniques in Table 3 are suitable for routine analysis purposes; the "single particle" methods in particular are not suitable, as they are deemed to be highly operator dependent and generally more expensive (both in terms of instrumentation and analysis costs) [7];

d) Minimum sample perturbation. It is important that the "state" of the nanoparticles is maintained; this is achieved by minimising any changes that may occur during sample preparation and analysis. "Single particle" methods such as SEM and TEM are particularly disadvantageous in this sense, as the state of the nanoparticles can potentially be easily perturbed during sample

preparation and during analysis under high-energy electron beam/high vacuum conditions. This is more so for TEM rather than SEM, due to the much higher energy electron beam focused on the sample for the former [55].

It is obvious from our evaluation that due to the demanding criteria for the perfect tool, no single technique can meet all of the criteria listed. As no perfect tool exists, it is the intention of this project to adopt a strategy suggested by Powell et al. [7] and that is to "characterise as much as possible, with as many as techniques as currently available". By this we mean the need to:

a) Further explore the detection limit and selectivity of the various tools – as these will ultimately be governed not only by the inherent specifications of the instrument but also by the nature of the sample and components of the liquid matrix;
b) Use a myriad of different tools, so as to have data that can yield complementary information;
c) **Use standard protocols (with some relevance) as the basis to further develop these methods.**

Lastly, the need to characterise the nanoparticles at various stages of its life cycle, i.e. starting from its life as "received powder" to when inside the organism, is of importance. In addition, not only are we interested in the properties of the nanoparticles themselves, but changes in the nature of the liquid media should also be monitored; for example, monitoring of the dissolution process i.e. the formation of ions or subsequent ionic complexes in the liquid media, which may potentially also result in toxicity [56].

Table 4 below summarises a list of methods to be employed in this project and together with protocols to be developed.

TECHNIQUES	Characterisation of "as received powder"	Characterisation of Dispersed Nanoparticles	Characterisation of Liquid Dispersant
SEM	YES	YES	NO
TEM	NO	YES	NO
DLS	NO	YES	NO
BET	YES	NO	NO
Zeta-potential Using Doppler Microelectrophoresis	NO	YES	NO
SMPS	YES	NO	NO
XPS	YES	YES	NO
EDX	YES	YES	NO
SIMS	YES	YES	NO
Photocatalytic Activity using Fluorescence spectroscopy	NO	YES	YES
Redox potential	NO	YES (the NPs to be embedded in an electrode matrix format)	YES (for background)
Ion exchange chromatography (with suitable detector)	NO	NO	YES
pH	NO	NO	YES
UV –vis spectroscopy	NO	YES	YES

Table 4. A list of methods to be used and protocols to be developed in the PROSPEcT project; refer to Tables 3a and b for further details of the techniques.

4 CONCLUSION

The literature survey conducted here reports a set of tools for nanoparticle dispersion and characterisation, which are commercially available. For successful dispersion, the use of an effective "de-agglomerating tool" is critical; the purpose of this tool is to sufficiently break the interlock bonds of the agglomerates/ aggregates inherent of the "as received powder", whilst minimising changes associated with inherent properties of the primary particles. The use of an ultrasonic probe has been identified as an attractive option and will thus be used to develop appropriate SOPs for nanoparticle dispersion. The effectiveness of de-agglomeration will be evaluated, relative to other de-agglomeration tools, in particular to an overhead mixer and a homogeniser.

For nanoparticle characterisation, the strategy "to characterise as much as possible, with as many techniques as currently available" will be adopted in this project. Characterisation of nanoparticles will be carried out with the "as received powders", as well as when dispersed in solution. When dispersed in liquid media, the nanoparticle as well as the liquid media itself will be characterised and monitored with respect to time. SOP development in this project will thus involve experimental investigations to evaluate the appropriateness of various testing methodologies for the characterisation of ZnO and CeO_2. In particular, sensitivity (detection limit of the instrument) and selectivity will be evaluated.

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