INTERNATIONAL COOPERATION ON COSMETIC REGULATION
REPORT TEMPLATE

REPORT FOR INTERNATIONAL COOPERATION ON COSMETIC REGULATION

Report of the Joint Regulator - Industry Ad Hoc Working Group:
Currently Available Methods for Characterization of Nanomaterials.

Report is:

Prepared For ICCR   **X   ICCR Report**   ICCR Guidance

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TABLE OF CONTENTS

1. PURPOSE

Provide more detailed background information on the analytical methods presented in the ICCR-4 report and their applicability domain.

The International Cooperation on Cosmetic Regulation (ICCR) held its fourth annual meeting (ICCR-4) July 13-15, 2010 in Toronto, Canada to discuss issues related to cosmetics and cosmetic-like drug/quasi-drug products.

Specific to nanotechnology the meeting concluded[1]:

•   Regulators and industry discussed the report of the ICCR Ad Hoc Nanotechnology Working Group (Nano WG) that was formed in December 2009 to develop criteria for identification of nanomaterials within the context of cosmetic regulation.

•   Regulators will publish the report on their respective websites for consideration in future nanotechnology-related activities in the four jurisdictions.

•   This Nano WG has concluded its work with the finalization of the report. A new Nano WG will be formed to examine safety approaches.

With respect to the new report, ICCR also asked that additional information be provided on the characterization elements. To that end, new Terms of Reference[2] were prepared, endorsed by the ICCR members and a new Joint Regulator - Industry Ad Hoc Working Group on Characterization (WG Characterization) formed with the purpose of providing more detailed background information on the analytical methods presented in the report and their applicability domain.

2. SCOPE

To provide an introduction to the most relevant methods for the characterization of nanomaterials.

The new Ad Hoc Working Group (WG) was directed by the ICCR to provide additional information on those methods identified in the ICCR-4 nanomaterial report *"Report of the ICCR*

Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection"[3] (the Report) presented at the July 14, 2010 meeting in Toronto Canada. More specifically the WG would direct itself to those parameters identified in Table 1 *"Currently Available Methods for Characterization of Nanomaterials"* of the report.

In consideration of those directions the WG undertook a survey of analytical methods most relevant to characterization of those parameters included in the report. In order to provide the data in a more user friendly format it was agreed that the parameters and analytical methods would be grouped and presented in a Table format and that each method or group of methods based on common principles, would then be described following a common template:

1. Measured Properties;
2. How it Works;
3. Sensitivity;
4. Notes; and
5. References

The authors understand this level of detail can only provide the reader with an introduction to the method. More detailed descriptions including information on the theoretical backgrounds of the methods, instrumentation, sample preparation, calculations, etc. may be found in the supplied references but this is beyond the scope of this report.

It is also important to note that it is beyond the scope of this document to identify any particular method as the "most" appropriate method to characterize a nanomaterial or a certain property. Indeed the adequate characterization of a nanomaterial for a particular purpose may require investigating several of its properties which as a consequence involves using one or more methods, used separately or in combination. This is entirely consistent with the approaches of other authoritative bodies. For example, the OECD Working Party on Manufactured Nanomaterials lists several properties to be taken into account when testing specific manufactured nanomaterials for human health and environmental safety[4]. Likewise, for a certain property it may be appropriate to investigate it with more than one method based on different physical principles in order to get a comprehensive understanding.

Finally, the authors are aware that there are a very large number of methods cited in the literature that could be used to characterize nanomaterials and may be relevant in particular situations. However, in consideration of the intent to remain within the scope of the 2010 Report, the authors did limit the number of methodologies included to those that are sufficiently mature and most relevant. Regardless the authors are confident that all of the currently most relevant methods for the characterization of nanomaterials including those in the solid state or as aqueous dispersion are included.

3.   ACRONYMS AND DEFINITIONS

1.	AAS	Atomic Absorption Spectrometry
2.	AFM	Atomic Force Microscopy
3.	AUC	Analytical Ultracentrifugation
4.	BET	Brunauer Emmett Teller method
5.	CHDF	Capillary Hydrodynamic Fractionation.
6.	CFM	Chemical Force Microscopy
7.	CPS	Centrifugal Particle Sedimentation
8.	Criteria Report	Report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection, July 14, 2010 Toronto Canada.
9.	DLS	Dynamic Light Scattering
10.	DMA	Differential Mobility Analyzer
11.	EDX	Energy Dispersive X-ray Spectroscopy
12.	FFF	Field Flow Fractionation
13.	GE	Gel Electrophoresis
14.	ICCR	International Cooperation on Cosmetic Regulation
15.	ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
16.	LD	Laser Diffraction
17.	LDE	Laser Doppler Electrophoresis
18.	PALS	Phase Analysis Light Scattering
19.	PCCS	Photo Cross Correlation Spectroscopy
20.	SAXS	Small Angel X-ray Scattering
21.	SEC	Size Exclusion Chromatography
22.	SEM	Scanning Electron Microscopy
23.	SMPS	Scanning Mobility Particle Size
24.	SNOM	Scanning Near Field Optical Microscopy
25.	SPM	Scanning Probe Microscopy
26.	STEM	Scanning Transmission Electron Microscopy
27.	STM	Scanning Tunnel Microscopy
28.	TEM	Transmission Electron Microscopy

29. ToR	Terms of Reference
30. VSSA	Volume Specific Surface Area
31. WDX	Wavelength-Dispersive X-ray spectrometry
32. XDC	X-Ray Disc Centrifuge
33. XPS	X-ray Photoelectron Spectroscopy
34. XRD	X-ray Diffraction
35. XRF	X-Ray Fluorescence analysis

4. RESPONSIBILITIES

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5. DISCUSSION

5.1 Introduction[5, 6]

Nanotechnology holds considerable promise in many different technological areas and it is already a large sector of industry. In the nanoscale regime, some materials exhibit additional or different properties as compared to bulk materials with the same composition. These materials are now used in a wide range of innovative applications and products, including consumer end-products such as cosmetics and personal care products. Some properties of

nanomaterials can be extrapolated and predicted from their bulk form whereas other properties cannot. Since nanomaterials may have novel properties it is important to gain a comprehensive understanding of the nanomaterial and the interaction with its environment. For that purpose it is essential to characterize the nanomaterial's physical and chemical properties experimentally using appropriate techniques.

In the area of nanomaterials characterization, there is a need for clarity in the identification of measures related to several materials properties, including those corresponding to endpoints in the Criteria Report. Various methods may be available to measure a certain property (e.g., "size"), but in fact it can be the case that different methods may yield different results because they do not measure the same quantity (e.g., "hydrodynamic size" vs. "aerodynamic size"). This must be kept in mind when comparing results on materials properties originating from different studies.

The dimensions of these nanomaterials, which are in the realm of 1 nm and 100 nm (Reference 3), may require method sensitivity on the order of 0.1 nm, while ensemble of nano-objects sensitivity on the order of nanometers may be sufficient.

It is important to realize that some of the physical-chemical parameters differ considerably when the nanoparticles are in dry form, liquid suspension or aerosol. For example, the aggregation state, the surface charge and other properties may change in different solvents. Furthermore, there are different types of particle size to measure: primary particle size, hydrodynamic size, and aerodynamic size.[7] All these three measurements give valuable information regarding the nanoparticle's physical behaviour, but it is important to realize under which conditions the measurements were made.

It is therefore clear that, for purposes of characterization properties of a manufactured nanomaterial, the state of the material and its behaviour in a certain environment are critical. For instance, in the specific case of nanoparticles, it is not generally realized that particle size distribution is not a fundamental property of the material being studied, but a temporary state of dynamic equilibrium between dispersion and agglomeration in suspensions or aerosols,

and this state can change drastically in different media, or with pH. Thus if a researcher simply aims at the preparation of the optimal formulation state and then measuring these preparations with the most sophisticated measurement methods scientifically valid result may be obtained that may however have little to do with the properties of a manufactured nanomaterial as provided by the supplier.

Manufactured Nanomaterials such as TiO_2, CeO_2, ZnO, polystyrene (PS), Ag, carbon nanotubes (CNT), or carbon black (CB) may be *nanomaterials* for regulatory purposes, but they are not necessarily *nanoparticles* as defined by other experts like ISO. For instance, even after optimal dispersing of fumed products, such as carbon black, fumed silica, alumina or titanium, only a small fraction of nanoparticles will remain, while the rest can be regarded as nanostructured materials (Reference 8) with outer dimensions of up to micrometers or, in a dry state, up to millimeters. This, and numerous other differences between perception and reality, present tremendous challenges in such a high-profile subject area.

It should also be mentioned here that some methods used for characterisation of manufactured nanomaterials are mature (however, new aspects may have to be considered when addressing properties of nanomaterials), whereas other techniques were developed recently and some are still in the development phase. For this report, the Working Group chose to limit the methods to those that were considered sufficiently mature.

In particular, methods for characterisation of manufactured nanomaterials in complex matrices such as preparations, final formulations or consumer products, generally are in the stage of development or in the prototypical stage. Hence, if it is deemed important to also characterize a final product (lotion etc) for its content of manufactured nanomaterials, it is recommended to analyze the state of potentially suitable methods and the associated special needs in a future report.

5.2   Methodology[8]

A review of the literature identified four fundamental approaches to the characterization of nanomaterials.

5.2.1   Spectroscopy

Spectroscopy is the use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes. The matter can be atoms, molecules, atomic or molecular ions, liquids or solids. The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules.

- Absorption: A transition from a lower level to a higher level with transfer of energy from the radiation field to an absorber, atom, molecule, or solid.
- Emission: A transition from a higher level to a lower level with transfer of energy from the emitter to the radiation field. If no radiation is emitted, the transition from higher to lower energy levels is called nonradioactive decay.
- Scattering: Redirection of light due to its interaction with matter. Scattering might or might not occur with a transfer of energy, i.e., the scattered radiation might or might not have a different wavelength compared to the light incident on the sample.

5.2.2   Chromatography

Chromatography is a group of separation methods which rely on differences in partitioning behaviour between a flowing mobile phase and a stationary phase to separate the components in a mixture. As the components elute from the column they can be quantified by a detector and/or collected for further analysis. Examples of pairing separation and detection methods include gas and liquid chromatography with mass spectrometry (GC-MS and LC-MS), Fourier-transform infrared spectroscopy (GC-FTIR), and diode-array UV-VIS absorption spectroscopy (HPLC-UV-VIS). The more recent Field Flow Fractionation (FFF) techniques are also separation techniques, but there a field is applied to a fluid suspension or solution pumped through a long and narrow channel, perpendicular to the direction of flow. This causes separation of the particles present in the fluid, dependent on their differing mobility under the force exerted by the field. The field can be transverse flow, electrical or of another type. In the report, FFF is also grouped under chromatography. The elute from FFF is further analyzed by detection or characterization

techniques, e.g., Dynamic Light Scattering (DLS). Electrophoresis, a separations technique that is based on the mobility of ions in an electric field, is also included in the chromatographic techniques.

5.2.3   Microscopy

Microscopy uses radiation and optics to obtain a magnified image of an object. The resolution of the imaging is limited by the minimum focus of the radiation due to diffraction. For light microscopy, the diffraction limit is approximately 1 μm (10^{-6} m) and for high-resolution transmission electron microscopy the limit is approximately 1 Å (10^{-10} m). Several comparatively new imaging techniques, referred to as Scanning Probe Microscopy (SPM), do not use radiation and optics, but use very sharp tips or probes to achieve up to atomic-scale resolution (AFM, STM), or to obtain optical images below the diffraction limit (SNOM).

5.2.4   Other Physical Methods

These include:

- Methods based on centrifugal force that measure particle size distributions by determining the rate of sedimentation of particles across a rotating disc or along sample cells in a rotor due to centrifugal force.
- Gas Absorption, a method based on the adsorption of an inert gas on a surface.
- Mobility of charged airborne particles in an electric field.
- Scattering of monochromatic light and analysis of the scattering pattern.

The authors also wish to note Volume Specific Surface Area (VSSA), recently proposed as a criterion for identification of nanomaterials. Kreyling[9], et. al., would regard a material with a VSSA equal to or greater than 60 m^2/g, as meeting the regulatory definition for being a nanomaterial. While it has not been included in this report, there has been sufficient discussion of VSSA to justify it being noted, allowing consideration of VSSA in future discussions.

5.3   Spectroscopy:

5.3.1   Atomic Absorption Spectrometry (AAS)[10],[11],[12]

Measured Properties:

Chemical composition：Qualitative/Quantitative analysis for specified trace elements.

How it Works:

Atomic Absorption Spectrometry (AAS) is a technique for determining the presence and concentration of a particular metal element within a sample. A flame or other atomizers such as a graphite furnace is used to atomize the sample to be analyzed. As each metal has a characteristic wavelength that will be absorbed, the instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance.

As concentration increased, absorbance goes up. The researcher can construct a calibration curve by running standards of various concentrations on the AAS and observing the absorbance.

Sensitivity:

ppm-ppb in the flame AAS

ppb-ppt in the graphite furnace AAS

Notes:

Though AAS can be applied to the determination of most metal elements and also some non-metal elements such as Si and P, a hollow cathode lamp corresponding to each element is needed. As for the measurement of a sample with high concentration of the coexisting compound, adaptation of the standard addition method is more effective than the calibration curve method. A variety of standard materials depending on the analytical sample is commercially available from several companies.

It is required to put the sample into solution for the analysis of nanomaterials used in cosmetic products by the AAS. The dissolution method (acid digestion or alkali fusion) is generally used for the analysis of inorganic compounds and it is also important to select an appropriate dissolution method for each measured sample and element.

AAS is a widely-used and officially-adopted analytical method as the JIS (Japanese Industrial Standards) method and the JP (Japanese Pharmacopoeia). On the other hand, it is recommended to utilize the ICP-AES (Inductively Coupled Plasma Atomic Emission

Spectrometry) which is easy and appropriate for the qualitative analysis of multi-element materials.

5.3.2 **Dynamic Light Scattering** including Photon Cross Correlation Spectroscopy[13],[14],[15]

Measured Properties:

Dynamic Light Scattering (DLS) is an important tool for characterizing nanoparticles and other colloidal solutions. The Brownian motion of sub-micron particles is measured as a function of time from which particle size may be determined.

How it Works:

DLS measures fluctuations in the scattering intensity of a solution as a function of time. Because these fluctuations occur as nanoparticles move through the solution, the timescale over which these changes occur depends upon the rate at which the nanoparticles diffuse. The hydrodynamic diameter (the diameter of a hypothetical nonporous sphere that diffuses at the same rate as the particles being characterized) can be calculated from the time dependence of the scattering intensity measurements.

Sensitivity:

3 to >1000 nm

Notes:

The diameter that is measured in DLS is a value that refers to how a particle diffuses within a fluid so it is referred to as a hydrodynamic diameter. The diameter that is obtained by this technique is the diameter of a sphere that has the same translational diffusion coefficient as the particle. The translational diffusion coefficient will depend not only on the size of the particle "core", but also on any surface structure, as well as the concentration and type of ions in the medium. The strong particle size dependence of the scattered light will also bias the measured size as a small amount of large particles will have such a large influence that smaller particles will be neglected. The method is not suitable for samples with a multimodal particle size distribution. If such samples need to be analyzed it is necessary to apply a separation process first.

DLS is also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS). The hydrodynamic diameter is an important complement to other sizing

measurements such as TEM because it provides information about how particles behave in solution.

5.3.3 Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) [16],[17],[18],[19]

Measured Properties:

ICP-MS is an analytical technique used for qualitative and quantitative elemental determinations.

How it Works:

An ICP-MS combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated by their mass-to-charge ratio. The mass spectrum pattern and isotopic ratio are utilized for qualitative analysis. Individual ion counts are compared to calibration curves for quantitative analysis.

Sensitivity:

ppm – ppt (depends on sample element)

Notes:

The ions formed by the ICP discharge are typically positive ions, $M+$ or $M+^2$, therefore, elements that prefer to form negative ions, such as Cl, I, F, etc., are very difficult to determine via ICP-MS.

This method works well on micro analysis of inorganic substances due to sensitive detection in combination with a mass spectrometer.

Since the method has a very high sensitivity, potential contaminations from the instrument itself (depending on its history) should be considered.

In case of high matrix concentrations ionization of the targeted element may not be complete, so for quantitative analysis the sample should be diluted or corrected for by an internal standard correction method.

To analyze nanomaterials for cosmetics by ICP-MS, complete dissolution of the sample on acid or alkali would be required as first step.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry, where the sample is directly ablating with a pulsed laser beam to form an aerosol may be used for non-volatile

substances. Recently, field flow fractionation (FFF) as separation step prior to ICP-MS has also been considered as an application for nano particle analysis.

5.3.4 Laser Doppler Electrophoresis (LDE)[20]

Measured Properties:

Zeta potential - which is an indication of the strength of surface charge

How it Works:

The movement of charged particles in an electric field is measured utilizing the Doppler effect. Light scattered from a moving particle experiences a frequency shift. The zeta potential is then calculated using the Henry equation

Sensitivity:

No quantitative information could be found

Notes:

One of the biggest practical issues when making zeta potential measurements is that of contamination. If any part of the system has been in contact with a previous sample then the zeta potential, being so sensitive to small changes in the environment can be affected.

5.3.5 Phase Analysis Light Scattering Spectrometry [21] (PALS)

Measured Properties:

Zeta potential - which is an indication of the strength of surface charge

How it Works:

Particle mobility is determined using PALS by doing a phase comparison of the detected signal with that of a reference frequency. The mean zeta potential can be determined from the phase difference.

Sensitivity:

No quantitative information could be found. The technique appears to be more sensitive than Laser Doppler Electrophoresis (LDE).

Notes

Only one hit was found in a search for PALS and nanoparticle in PubMed, so the technique may not be that widely used. The reference (cited below) found carbon nanotube based coulter counting (CNCC) a more accurate method for surface charge.

5.3.6   X-Ray Photoelectron Spectroscopy[22] (XPS)

Measured Properties:

Elemental composition of the near-surface region, including information on chemical and electronic state ("surface chemistry")

How it Works:

XPS can be used to quantify the surface composition (atomic % of elements) and to quantify the chemical state (oxidation state, bonding configuration etc.) of all elements with an atomic number ≥ 3 (lithium).

In XPS a sample is exposed to characteristic (monochromatized or unfiltered) X-rays. The photoelectrons generated from atomic core level shells and emitted from the sample are counted and analyzed for their kinetic energy. From this the binding energies of these core levels are determined. The atomic core level energies are characteristic for the elements, in addition energy shifts can be used to analyze the chemical bonding state. Quantitative analysis is possible. XPS allows quantification of elements and their chemical state to better than 1%.

Sensitivity:

Quantification better than 1% (depends on the analyzed element). Information depth < 10 nm, i.e., not strictly surface sensitive. For nanoparticles smaller than 10 nm the bulk composition is measured.

Notes:

Ultrahigh vacuum in the region of 10^{-9} mbar or lower is generally required. Specialized instruments allow the analysis of volatile liquids or materials at pressures in the mbar range, but few of these types of XPS systems exist. XPS is a powerful, but expensive and relatively slow technique.

Quantification of nanoparticle surface chemistry can be complicated by shadowing effects.

XPS is also known as ESCA (Electron Spectroscopy for Chemical Analysis)

5.3.7 X-Ray Diffraction (XRD)[23].

Measured Properties:

Crystal size, (often referred to as primary particle size) and morphology.

How it Works:

X-ray Diffraction (XRD) is a powerful non-destructive technique for characterizing crystalline materials. X-ray diffraction peaks are produced by constructive interference of a monochromatic beam of x-rays scattered at specific angles from each set of lattice planes in a sample. The peak intensities are determined by the atomic decoration within the lattice planes. Consequently, the x-ray diffraction pattern is the fingerprint of periodic atomic arrangements in a given material. An online search of a standard database for x-ray powder diffraction patterns enables quick phase identification for a large variety of crystalline samples.

Sensitivity:

XRD: Minimum film thickness for phase identification: ~2 nm. Depth Resolution: ??? Adjustable sampling depth between ~2 nm to 30 micron, depending on material properties and X-ray incidence angles.

Notes:

Cannot identify amorphous material.

No depth information.

5.3.8 Small-angle X-ray scattering (SAXS)[24],[25],[26]

Measured Properties:

Structural analysis for nanomaterials

How it Works:

Small angle X-ray scattering (SAXS) is a technique for studying structural features of colloidal size. It is performed by focusing a low divergence X-ray beam onto a sample and observing a coherent scattering pattern that arises from electron density inhomogeneity within the sample. Since the dimensions typically analyzed are much larger than the

wavelength of the typical X-ray used (1.54 Å, for Cu), dimensions from tens to thousands of angstroms can be analyzed within a narrow angular scattering range. This angular range or pattern is analyzed using the inverse relationship between particle sizes and scattering angle to distinguish characteristic shape and size features within a given sample. Proteins (molecular morphology and size), polymers (size, morphology, lamellar structure, higher-order structure), porous materials (void, morphology), and colloidal solution (size, morphology) are objects to measurement.

Sensitivity:

2 nm to 25 nm (partially ordered systems up to 150 nm).

Notes:

Size and distribution of nano particle are obtained from guinier plot analysis after diffuse scattering measurement. To avoid interference effect, it is necessary to use as dilute a sample as possible.

In case of a sample with far-from-spherical particle or obvious agglomeration, it may differ from particle size or morphological data by TEM. Elimination of agglomerate material or pre-treatment (dispersion) would be useful for accurate measurement.

Crystallite (crystal size) may also be calculated by in half value of diffraction peak X-ray from diffraction (XRD), similar to SAXS. In case of single crystallite (single crystal), this size means theoretically primary particle size, but crystallite is a part of primary particle. It should be noted when using both XRD and SAXS.

5.3.9   X-Ray Fluorescence Analysis (XRF) including Wavelength-Dispersive X-ray and Energy Dispersive X-ray spectroscopy[27]

Measured Properties:

Chemical composition: Qualitative and quantitative analysis of elements

How it Works:

Electrons from atomic core levels are emitted when a material is exposed to X-rays with energy larger than the electronic binding energy, and subsequently electrons from outer shells can fill the resulting core-level holes. The excess energy, i.e., the energy difference between the inner and the outer electronic shell involved in the process, can be emitted as

photons. Those photons are called characteristic X-rays, because their energy is characteristic for each element. XRF is a method of detecting those characteristic X-rays... Quantitative information on the elemental concentration can be obtained from the intensity of the characteristic peaks. In XRF one distinguishes roughly between WDX (Wavelength-Dispersive X-ray Spectroscopy) measuring characteristic X-rays after dispersed spectrum using dispersing crystal by a goniometer, and EDX (Energy-Dispersive X-ray Spectroscopy) which uses a semiconducting detector with a high energy resolution.

Sensitivity:

% - ppm level depending on equipment performance

Notes:

This method is non-destructive, there are little restrictions for the sample geometry, and the measurement time is short. Pre-treatment is unnecessary or minimal so that this method is simple and versatile. EDX can be measured on all elements between Na and U and WDX can be done on Be - U. EDX and WDX with multi-channel detectors can simultaneously analyze a variety of elements. EDX and WDX are used properly according to the purpose. These methodologies can do not only quantitative analysis with standard samples, but also semi-quantitatively analysis without standard samples using the fundamental parameter (FP) method, in combination with theoretical calculations.

For the RoHS (Regulation of Hazardous Substances) regulation in the EU, XRF is used as screening method for monitoring of regulated elements (IEC 62321). If it is unclear from the screening results whether a regulated element is below its maximum tolerated concentration (i.e., in the grey zone), a more precise analysis, e.g. ICP-MS, would be selected.

5.4   Chromatography:

5.4.1   Capillary Hydrodynamic Fractionation. (CHDF)[28],[29]

Measured Properties:

CHDF is a chromatography technique that can separate nanoparticles based on size providing size distribution.

How it Works:

HDC operates similarly to size exclusion chromatography (SEC) and field flow fractionation (FFF), using one (inert) mobile phase and one (hydrodynamic) field. The principle of particles separation is the difference in their transport rates in a capillary, related to their location in the eluent. Large particles are preferentially in the center of the capillary, where the flow rate is at a maximum, so they are eluted faster than small ones, which are closer to the wall of the capillary, where the flow rate is zero. A UV detector is typically used to monitor the elute.

Sensitivity:

15 – 1000 nm

Notes:

To suppress the influence of pressure or temperature fluctuations, a short time after the sample, a marker is injected. The elution time of the marker gives a reference base.

5.4.2   Field Flow Fractionation (FFF)

Measured Properties:

Size separation of nanoparticles in complex samples.

How it Works:

The separation is based on the diffusion coefficient in an open flow channel. It is similar to chromatographic techniques, but solely based on physical separation without relying on a stationary phase. A field is applied to a mixture perpendicular to the mixtures flow in order to cause separation due to differing mobilities of the various components in the field. The field can be gravitation, centrifugal, magnetic, thermal, or a cross flow of fluids. Most of the separation is due to differences in Brownian motion and diffusion after the field has forced the mixture components onto one side. There is a balance between diffusion and the applied force (from the field) on any particular particle in the mixture which results in differing movement perpendicular to the main flow direction. When the flow is turned on, the particles are exposed to a parabolic velocity profile and particles at a higher height from the channel base (i.e., the smaller particles) will travel faster than those at the bottom, resulting in a separation at the exit of the flow channel.

Sensitivity:

FFF is able to fractionate particles in a range of 1 nm - 100 μm

Notes:

The limitations of FFF techniques are potential membrane swelling, membrane interactions, the continuous re-equilibration in the channel, the need (in some circumstances) of pre-concentration, additional concentration of the sample during equilibration and the possibility of aggregation in the channel.

There are different types of FFF including asymmetrical (AsFFF), sedimentation (SdFFF), and flow field flow fractionation (FlFFF).

5.4.3 Gel Electrophoresis[30], [31] (GE)

Measured Properties:

Separation of nanoparticles with a surface charge

How it Works:

The mobility of particles in a gel by electrophoresis is strongly dependent on surface charge density. Therefore charged nanoparticles of different sizes and shapes can be separated. Smaller particles experience less resistance from the gel and can move more rapidly.

Sensitivity: No quantitative information could be found.

Notes: There is a delicate balance between concentration of the gel (pore size), particle charge, and the electric field. Gel density may affect the velocity of migration of nanoparticles and may inhibit movement of very large particles. Good separation of different nanoparticles may require some preliminary studies and possibly confirmation by other methods such as TEM. However, similar methodology has been utilized successfully for different nanoparticles so extensive optimization should not be necessary.

An advantage of gel electrophoresis separation is that multiple runs can be made in parallel on the same gel.

5.5 Microscopy:

5.5.1 Scanning Electron Microscopy[32],[33],[34] (SEM)

Measured Properties:

Electrical equipment to obtain visually ultrafine 3-dimensional information of a surface. The signals that derive from electron-sample interactions reveal information about surface topography, chemical composition and more.

How it Works:

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity. In the most common or standard detection mode, secondary electron imaging or SEI, the SEM can produce very high-resolution images of a sample surface.

Sensitivity:

Sub 0.2 nm image resolution. Contrast of secondary electron image depends heavily on morphology and density of a test material. The appropriate electron accelerating voltage varies according to the purpose of the analysis.

Notes:

SEM cannot obtain information on the interior of a test material.   Due to the requirement for a vacuum (10^{-3} – 10^{-9} Pa), a volatile material or a material containing volatile substances cannot be directly observed. However low-vacuum SEM or Cryo-SEM can be used in some cases. An additional requirement is that the material must be sufficiently conductive due to the use of electrons, and to avoid electrical charging. Conductive materials including Pr, Au, C, W, and Os can be used to coat a test material to make its surface conductive if necessary. A nanomaterial for cosmetic usage tends to be aggregated or agglomerated. For SEM analysis it may be necessary to de-agglomerate or de-aggregate it in order to avoid electrical charging, because the electrons may be trapped in the space of the cohesion inside of aggregation or agglomerate even if the sample as a whole is conductive.

Resolution can vary with properties of the electron beam and by tuning the magnetic lenses of the SEM equipment. It is necessary to select the device according to the purpose. To observe a nanomaterial, a SEM with field emission cathode for high resolution is required. Elemental analysis (EDX or EDS) may be possible by analyzing the emitted characteristic X-rays or the secondary or reflected electrons.

5.5.2 Scanning Probe (SPM) including Atomic Force, Chemical Force and Scanning Tunnel Microscopy[35], [36]

Measured Properties:

Three-dimensional surface topographic imaging, including surface roughness, grain size, step height, and pitch. Scanning Probe Microscopy (SPM) is a branch of microscopy that forms images of a surface by mechanically moving a physical probe in a raster scan of the specimen, line by line, recording the probe-surface interaction as a function of position. Scanning Probe Microscopy (SPM) can provide atomic or near-atomic-resolution. SPM includes Atomic Force (AFM), Chemical Force (CFM), Magnetic Force (MFM) and the first method, Scanning Tunnel Microscopy (STM).

How it Works:

SPM works by scanning a tip over a surface. When the tip is brought into proximity of a sample surface, forces between the tip and the sample lead to a deflection of the cantilever to which the tip is attached according to Hooke's law. Typically, the deflection is measured using a laser spot reflected from the top surface of the cantilever into an array of photodiodes. Other methods that are used include optical interferometry, capacitive sensing or piezoresistive AFM cantilevers.

Sensitivity:

1.5 – 5 nm laterally and 0.01 nm in height.

Notes:

Potential problems with samples those are too rough and oddly shaped. Tip-induced errors are possible.

Depending on the situation, forces that are measured in SPM include mechanical contact force, van der Waals forces, capillary forces, chemical bonding, electrostatic forces, magnetic forces, Casimir forces, solvation forces, etc. Along with force, additional quantities may simultaneously be measured through the use of specialized types of probe (see scanning thermal microscopy, scanning joule expansion microscopy, photothermal microspectroscopy, etc.).

5.5.3 **Transmission Electron Microscopy (TEM)** and Scanning Transmission Electron Microscopy[37]

Measured Properties:

Crystal size, (often referred to as primary particle size) aggregate (particle size) and agglomerate size. Analysis of crystal, aggregate and agglomerate size distribution is possible using TEM in combination with image analysis.

How it Works:

Transmission electron microscopy (TEM) is a technique whereby a beam of electrons is transmitted through a thin specimen, interacting with the specimen as it passes. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device such as a fluorescent screen, for viewing the sample, or onto photographic film, for a record of the sample. Areas of dense material (such as solid nanoparticles) absorb the electrons and appear as dark areas on the viewing screen. Less dense material (such as coating around the nanoparticles) allows the electrons to pass through more freely and appear as lighter areas.

Sensitivity:

Typically a final image will be 200,000 times normal magnification and the TEM is capable of achieving a magnification as high as 1 million, but the quality of the image decreases as magnification increases.

Notes:

Statistical unreliability – small number of particles examined per sample.

Analysis carried out on dry samples – nature of the sample is altered by aggregation and agglomeration.

There is potential that the sample may be damaged by the electron beam, the technique can also be quite time consuming due to the time required to prepare the sample for analysis and developing the photographic film afterwards.

5.6 **Other Physical Methods**

5.6.1 **Analytical Ultracentrifugation**[38] **(AUC)**

Measured Properties:

Particle size, size distribution, particle density

How it Works:

In analytical ultracentrifugation (AUC) experiments, dissolved or dispersed samples inside AUC measuring cells are exposed to high centrifugal force fields (usually larger than 5000 g) induced by a centrifuge rotor spinning at up to 50000 – 60000 rpm. The reaction of the sample to the centrifugal force field is followed by optical detection systems. The change of the sample concentration as a function of distance from the rotation axis and time is given by the Lamm equation. The most important detector systems used are NIR-UV-Vis absorption optics, which simultaneously measure transmission profiles along the centrifuge radius, and Rayleigh interference detectors. The measured profiles can be used to determine the size distribution of the particles. The most important AUC technique, particularly for the determination of the particle size distribution, is the sedimentation velocity experiment, where the concentration change of the sample with time and radius, c(r, t), is measured. Another technique is the sedimentation equilibrium experiment, where a radial concentration gradient is established, sedimentation and diffusion are in equilibrium, and only c(r) is measured.

Sensitivity:

Size range < 1 nm to several μm, resolution 0.1 nm, high statistical accuracy.

Concentrations > 10 ng/ml

Notes:

Very versatile and sensitive technique. Concentration dependent aggregation may take place. Particle size distributions are calculated assuming hard spheres. AUC instruments are rather expensive.

Fractionation technique can be applied to complex mixtures and polydisperse samples and it can be combined with other methods.

5.6.2 **Brunauer Emmett Teller** method based on nitrogen absorption (BET)[39], [40]

Measured Properties:

Specific surface area (m^2/g)

How it Works:

The BET method is based on the adsorption of an inert gas on a surface.

The sample is weighed and placed into a vacuum chamber, which is then evacuated. The sample is degassed and cooled down to a constant temperature by means of an external bath containing a cryogen like liquid nitrogen. A gas (typically N_2) is admitted and adsorbs on the sample surface. From the amount of gas adsorbed at a given pressure an adsorption isotherm is derived. The BET theory is then used to determine the amount of gas necessary to form a monolayer on the surface, also called the monolayer capacity. From the monolayer capacity, the known surface requirement of a single adsorb ate molecule and the mass of the sample, the specific surface area in m^2/g can be calculated.

Sensitivity:

Upper limit of detection: several thousands of m^2/g

Notes:

Dry samples required. Fast and relatively cheap method.

Assumptions in the BET method: (1) the surface is homogeneous, (2) there are no lateral interactions between the adsorbed molecules, (3) the uppermost layer is in equilibrium with the gas phase, (4) the binding of the first (surface) layer to the surface is pronouncedly stronger than for the subsequent layers.

Not all materials have isotherms that can be evaluated using the BET theory.

Other inert gases, such as Ar or Kr, can also be used.

5.6.3 Centrifugal Particle Sedimentation[41] (CPS)

Measured Properties:

Mean aggregate size (hereafter referred to as particle size), mean agglomerate size, smallest particle size (lower cut off), particle size distribution (range of particle size).

How it Works:

The CPS disc centrifuge measures particle size distributions by applying Stokes' Law to the rate of sedimentation of particles across a rotating disc due to centrifugal force. Samples are introduced into the centre of the disc rotating at up to 24,000 rpm and travel through a fluid

in which a density gradient has been established which is suitable to the system being measured. This density gradient aids in the separation of particle sizes as they travel through the fluid to the edge of the disc. Detection of sedimenting particles is by scattering of a monochromatic light source.

When particles settle separated through the viewing zone the intensity decreases. The reduction in intensity indicates the concentration of particles. When all particles have passed the detector the signal returns to the basic value allowing the smallest particles of the sample - the lower cut-off level to be determined. Stokes' law is applied to the data to calculate an intensity weighted particle size distribution, which in turn can be converted to number or volume-weighted distributions by assuming spherical, homogeneous particles.

Sensitivity:

Approximately 20 nm – 2 micron at 8,000 rpm. Disc can be operated up to 24,000 rpm.

Notes:

Measurement in liquid phase.

Dilution of samples is required (in certain cases this can cause agglomeration of aggregates).

Fluid density, viscosity and refractive index and particle density must be known.

Stokes equation must be modified to account for the radial variation in the centrifugal force.

Assumes spherical particles.

In order to compare the samples with each other, it is recommended to utilise the same type of equipment for preparation of dispersions prior to measurement.

5.6.4   Laser Diffraction[42] (LD)

Measured Properties:

Mean aggregate size (hereafter referred to as particle size), mean agglomerate size, smallest particle size (lower cut off), particle size distribution (range of particle size).

How it Works:

Laser diffraction, alternatively referred to as Low Angle Laser Light Scattering (LALLS), can be used for the non-destructive analysis of wet or dry samples. In laser diffraction, particle size distributions are calculated by comparing a sample's scattering pattern with an

appropriate optical model. Traditionally two different models are used: the Fraunhofer Approximation and Mie Theory.

The Fraunhofer approximation was used in early diffraction instruments. It assumes that the particles being measured are opaque and scatter light at narrow angles. As a result, it is only applicable to large particles and will give an incorrect assessment of the fine particle fraction.

Mie Theory provides a more rigorous solution for the calculation of particle size distributions from light scattering data. It predicts scattering intensities for all particles, small or large, transparent or opaque. Mie Theory allows for primary scattering from the surface of the particle, with the intensity predicted by the refractive index difference between the particle and the dispersion medium. It also predicts the secondary scattering caused by light refraction within the particle – this is especially important for particles below 50 microns in diameter, as stated in ISO13320-1 (1999), the international standard for laser diffraction measurements.

Sensitivity:

Particles in the size range 20 nm to 2000 micron.

Notes:

Laser diffraction is a non-destructive, non-intrusive method that can be used for either dry or wet samples. As it derives particle size data using fundamental scientific principles there is no need for external calibration; well-designed instruments are easy to set up and run, and require very little maintenance.

Careful preparation of samples is required – optimization of dispersion conditions is essential.

Laser diffraction reports the volume of material of a given size because the light energy reported by the detector system is proportional to the volume of the measured particle. This method is in contrast with counting-based techniques, which report the number of particles of a given size. The differences between number - and volume-based size distributions have been discussed at great length and are well understood. Clearly, the distributions reported by LD techniques will not be the same, especially when polydisperse materials are measured. Thus, although there may be good agreement among various counting-based techniques measurements by LD will not always agree with the results of either volume - or mass-based

techniques. Volume-based distributions will always shift to larger particle sizes in comparison with number distributions

It is difficult to measure polydispaersed samples.

5.6.5 Scanning Mobility Particle Size (SMPS) including DMA – Differential Mobility Analyzer[43], [44]

Measured Properties:

Particle, agglomerate and aggregate size

How it Works:

SMPSs bring airborne particles to a known charge distribution and then separate them according to their electrical mobility. First, particles of the aerosol are charged and enter a Differential Mobility Analyser (DMA). The main flow through the DMA is particle free 'sheath' air. In the DMA particles are separated according to their electrical mobility by using their deviation in an electric field produced by a charged rod. Only particles within a narrow range of electrical mobility have the correct trajectory to pass through a narrow slit at the DMA exit. By changing the voltage of the rod inside the DMA the size distribution can be measured. After that, the particles that leave the DMA are counted by a CPC (Condensation Particle Counter). There, small particles are enlarged by condensation of vapour (n-butanol) on them. The particles grow to >10 µm and become efficient light scatterers. Particles are counted as they pass through a light beam.

Hence, a SMPS consists of a DMA followed by a CPC.

Sensitivity:

Size range 2.5 nm to 1000 nm, resolution ~ 2 nm

Notes:

Particles must be aerosolized. The size range which can be scanned by a single DMA is limited by the voltage which can be applied (too high a voltage causes the insulating properties of air to break down and arcing to occur) and the accuracy with which it can be controlled. Within certain limits the size range to be scanned can be adjusted by changing the sheath flow rate; however the typical dynamic range for a single DMA is about 1:30.

5.6.6   X-Ray Disc Centrifuge (XDC)

Measured Properties:

Aggregate (particle size) and agglomerate size and distribution of size, lower cut off – i.e., smallest particle.

How it Works:

The XDC consists of a glass or polycarbonate rotor mounted with its axis of revolution nearly horizontal. A motor drives the rotor at speeds up to several thousand rpm. In use, the rotor is partially filled with a known weight of sample dispersed in the sedimentation fluid. The rotor is accelerated and the particles which are dispersed in the sedimentation fluid move along the radius at a velocity dependent on their size. An X-ray source generates X-rays which then pass through the dispersion and are detected by a scintillation counter on the opposite side of the rotor. The attenuation of the X-ray beam is proportional to the concentration of the suspension at the measurement radius. As the rotor spins large particles move faster than smaller ones resulting in a change in concentration at the X-ray detector. The largest size present can be calculated by Stokes' law.

Sensitivity:

Typical detection limits 10 nm to 4 micron.

Notes:

This technique will only detect particles containing elements with an atomic number larger than 9 (fluorine).

Suitable for diluted dispersions only, typically 2-4% w/w is required for analysis.

Sample preparation is extremely important. Failure to prepare samples results in erroneous data.

Assumes spherical particles.

Information required carrying out the analysis: Temperature, density of spin fluid and sample, viscosity of spin fluid.

5.7   Tabular Summary of Methods

Table 1 is a summary of the most relevant characterisation methods used for nanomaterials. The methods are arranged in four groups based on the methodologies applied. The properties

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are arranged in three groups: Particle, Surface and Chemical comprising the most important characteristics. The applicability for each method is then highlighted within the table.

Table 2 shows the relative sensitivity ranges of selected methods.

More precise details may be found in the detailed section discussions.

TABLE 1

Most Relevant Methods by Parameter

		Spectroscopy									Chromatography			Microscopy			Physical					
		AAS	DLS	LDE	ICP-MS	PALS	SAXS	XPS	XRD	XRF	CHDF	FFF	GE	SEM	SPM	TEM	AUC	BET	CPS	LD	SMPS	XDC
Particle	Size & Distribution		X				X				X	X		X		X	X		X	X	X	X
	Agglomerate/ Aggregation		X				X							X	X	X			X	X	X	
	Shape						X							X	X	X						
	Stability		X																			
Surface	Area																	X				
	Chemistry							X							X							
	Charge			X		X							X		X							
Chemical	Composition	X			X			X	X	X												

TABLE 2

Most Relevant Methods for Size and Distribution by Sensitivity (size in nm)
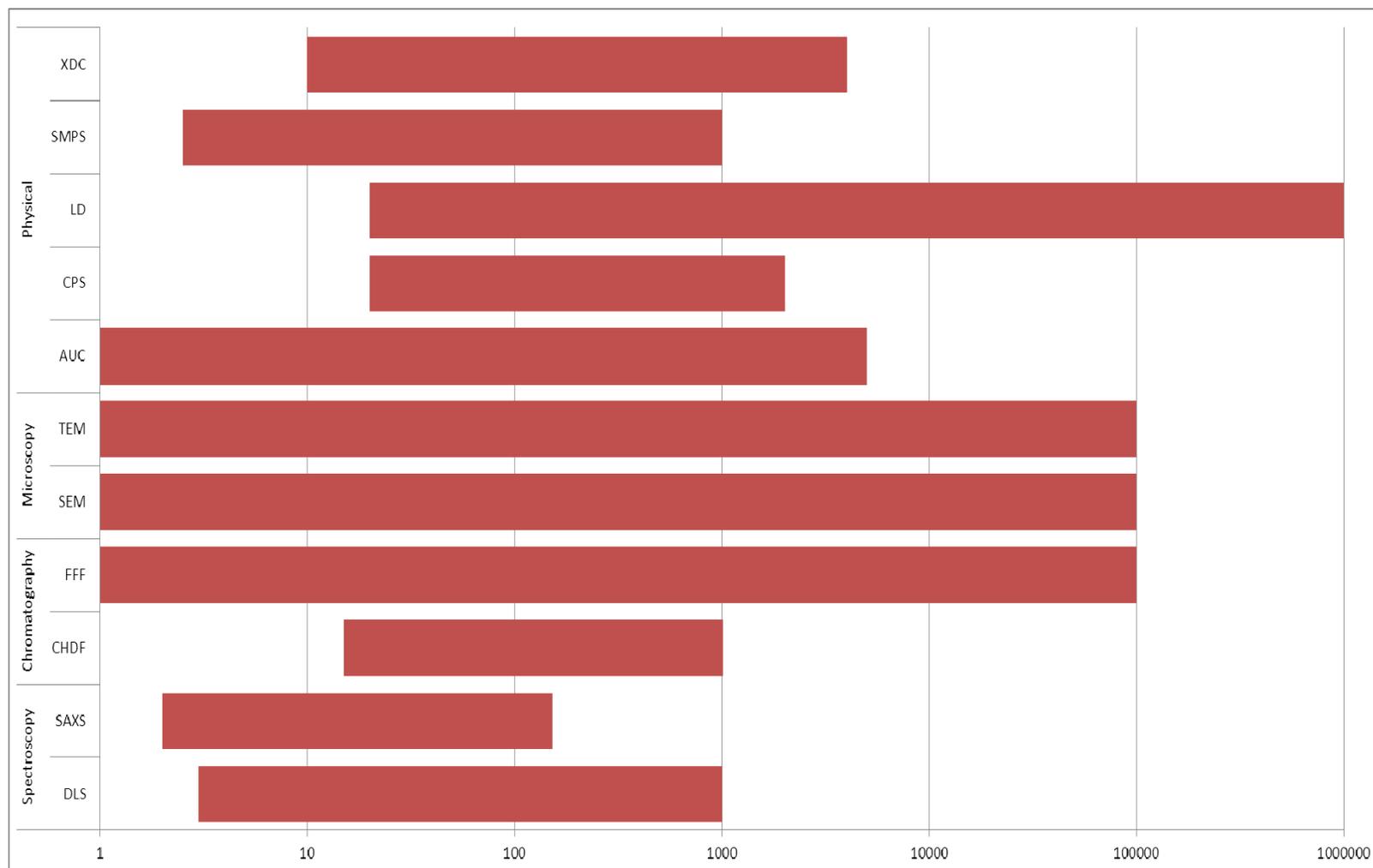
6. **CONCLUSIONS**

In conclusion, the Ad Hoc Working Group on Characterization has provided an introduction to the most relevant methods for the characterization of nanomaterials. No attempt has been made to identify preferred methods and it is understood that there are large number of methods referenced in the literature, ranging from the experimental to those that can be considered mature and well developed, that could have been considered. There are also numerous methods that could be applicable to the characterization of nanomaterials under specialized conditions. However, the Working Group has chosen to include only the most relevant, well-developed methods, for the primary characterization of nanomaterials.

The Working Group also must emphasise that no single method can, in and of itself, fully describe a nanomaterial. Sample preparation, conditions of use, or formulation milieu will all affect the state of the nanomaterial. Thus great care must be taken in the reporting and interpretation of results. Indeed a material that may have one dimension in the nanoscale and be considered a nanomaterial based on one set of definitions may in fact have no nanoparticles under the actual conditions of use.

The Working Group also is aware that while the Terms of Reference specifically requested that the WG would direct itself to those parameters identified in Table 1 "Currently Available Methods for Characterization of Nanomaterials" of the Criteria report, other parameters cited in the Criteria report are not addressed within this report.

Therefore in consideration of the above, the Joint Ad Hoc Regulator Industry Working Group recommends the following actions:

- The current report is accepted and the current Working Group be discharged.
- A new Ad-Hoc Working Group should be formed which specifically addresses methods that are useful to address those properties identified in the Criteria Report. In particular:
  - (i) Solubility

- (ii) Measurement of size in the realm between 1 and 100 nm **in the final formulation**. This is a critical issue because it involves discussing methods for detection and characterisation of nanomaterials in complex media. Related methodologies are still largely in the experimental or prototypical phase, and their applicability should be carefully discussed.
- (iii) Stability and persistence of nanomaterials in biological media

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