Analysis Category	SIZE	SIZE	SIZE
ENP Measurement Technique	**Dynamic Light Scattering (DLS)**	**Hydrodynamic Chromatography (HDC)**	**Nanoparticle Tracking (NT)**
Brief Description	Also known as photon correlation spectroscopy, this technique uses scattered light to measure the rate of diffusion of NPs. It provides information about size distribution in terms of hydrodynamic diameter. DLS uses a laser beam which passes through a liquid suspension containing the analyte particles. They scatter the incident laser at different scattering angles. The Brownian motion of the detected particles induce the shift in light frequency, which varies with different particle sizes (Langevin et al., 2018). The shift in light frequency would result in time-dependent fluctuations in the intensity of scattered light. These fluctuations contain information about particle size and shape (Verma and Maheshwari, 2018). The hydrodynamic radius and size distribution of measured particles are calculated using a function of the scattered light signal over time in tandem with the Stokes-Einstein equation (Lim et al., 2013).	A solution-phase liquid chromatographic separation method for particle size determination in the range from 10 nm to 1000 nm (Lespes andGigault, 2011). HDC separates analytes (e.g., particles or bubbles) based on the size differences. After the sample enters the packed column filled with solid beads, a velocity profile is established among the channels between the adjacent beads. According to the mechanism of Brownian motion, for the particles in the range from 10 nm to 1000 nm, their traces are more likely to be disturbed by the media grain, since small particles receive increasing impacting effects from collisions with the media grains (Karatzas and Shreve, 1998; Small, 1974). Under the influence of the viscous force, fluid velocity is greatest at the centre and decreases toward the wall (Small, 1974). The larger analyte is kept near the centre of the tube and passes through the streamline quickly, whereas the smaller analyte migrates closer to the channel wall and passes slowly through the streamline because of the hydrodynamic effect and Van der Waals interactions (Striegel and Brewer, 2012).	Involves the application of intense laser light to illuminate free diffusing particles to track their Brownian motion with monochrome imaging (Bhattacharjee, 2016). A laser beam is passed through a prism edged glass flat within the sample chamber into the particle suspension (Carr and Wright, 2008). When the particles in suspension appear in the scattered light path, they can clearly be visualized and recorded frame by frame using a high-sensitivity camera or charged coupled device (CCD) detector. The motion trail of the particles is obtained and analysed through the recorded frames by means of a microscope. Further calculation of hydrodynamic sizes is completed with the average distance between each particle, temperature, and solution viscosity through the Stokes-Einstein equation (Boyd et al., 2011).
Advantages	a) A non-invasive technique that works on a wide range of liquid samples with relatively low sample volumes (Stetefeld et al., 2016). b) DLS is a useful tool for determining the hydrodynamic diameter and particle size distribution in suspension and for investigating colloidal properties of nanoparticles. c) It can also measure zeta potential of NPs. d) DLS technique is quick and user-friendly. e) DLS is better relative to NTA in detecting larger aggregates (Hou et al., 2018).	a) HDC involves rapid separation of particles based on size differences (Striegel and Brewer, 2012). b) HDC is useful for separating the interference signals of dissolved species from analyte particles in water. c) It is also efficient in separating polymers and organic nanoparticles. d) Analytical repeatability and easy operation are some of the other benefits of HDC.	a) Unlike DLS, NT can measure particle-by-particle size. b) NT provides individual particle intensity as well as motion videos (Hou et al., 2018). c) NT is better relative to DLS in detecting smaller aggregates (Hou et al., 2018).
Limitations	a) Scattering intensity can be affected by the presence of other suspended material, such as dust, in the sample which can therefore interfere with the size measurements (Tiede et al., 2008). b) DLS can only measure the average size; not particle-by-particle size. c) DLS neither provides individual particle intensity nor motion videos (Hou et al., 2018). d) It is not suitable for gravitationally settling particles.	HDC yields low selectivity and poor size resolution, which can cause low efficiency of separation for multi-component nanoparticles.	a) Due to Brownian motion phenomenon, larger particles move slower than the smaller ones. So, if particles are too close together in terms of size, accuracy of measurement can get affected. b) The motion trail of some particles which might be out of the camera view cannot be measured. c) Set-up parameters need to be adjusted carefully to obtain high accuracy.

Analysis Category	MORPHOLOGY	MORPHOLOGY	MORPHOLOGY
ENP Measurement Technique	**Transmission Electron Microscopy (TEM)**	**Scanning Electron Microscopy (SEM)**	**Atomic Force Microscopy (AFM)**
Brief Description	TEM characterisation involves the transmission of a high energy electron beam through the sample. Electrons are transmitted through the sample to acquire an image (Peters et al., 2011). When the beam is passed through a food sample, inorganic NPs can be detected as they appear as dark dots on a lighter background. This is because density of the inorganic NPs is relatively higher compared to the background in the food matrix.	A low-energy electron beam is used for scanning the surface of the sample to generate images. The characterisation of the NPs is done through the detection of secondary or back-scattered electrons (Dudkiewicz et al., 2011; Liu et al., 2012).	Consists of an oscillating cantilever arm that measures electrostatic forces between the tip of the arm and the sample surface as the arm scans across the sample. AFM operates by scanning across the sample surface with a tiny tip (typical end diameter 5-10 nm). As the tip approaches the sample surface, the force produced by the tip-sample interaction causes the cantilever deflection, which is measured with a photodiode detector and transferred to obtain the surface information (Butt et al., 2005; Webb et al., 2011).
Advantages	a) TEM is widely used as it is effective in determining the morphological properties of NMs. b) TEM is one of the most suitable nanoscale imaging techniques for the characterization of NMs < 200 nm in food and agricultural samples. c) TEM images can provide the morphology of NMs with an accuracy of ± 5% (Burleson et al., 2004). d) This technique is capable of imaging NPs with a resolution of 0.5 nm (Tiede et al., 2008). e) Soil and sediment samples containing NMs with unique properties, such as light absorption, fluorescence, or those containing a rare metal, can be effectively analysed by TEM technique (von der Kammer et al., 2006). f) TEM images also help in providing additional information about particle size and aggregation state.	a) It provides a high-resolution image of a sample surface in a distinctive three-dimensional appearance. b) SEM can be employed for detecting larger particles (achieving a spatial resolution of 500 nm) (Burleson et al., 2004). c) It is employed to effectively observe the morphology of polysaccharide NPs, protein NPs, and the liposomal NPs (Luykx et al., 2008).	a) The original sample can be preserved during AFM analysis as the measurements can be operated in liquid and air (or vacuum) with desirable conditions. b) AFM enables holistic characterization of surface morphology (Bieletzki et al., 2010). c) In addition to determining morphological property, AFM analysis can also provide information about surface and aggregation characteristics. d) Compared to the other techniques, AFM analysis requires relatively simple sample preparation.
Limitations	a) As TEM involves the transmission of beam through the sample, there is a need to prepare very thin samples for analysis (Dudkiewicz et al., 2011; Liu et al., 2012). b) This technique is not helpful in detecting organic NPs, as carbon is the major element in both organic NPs and the food matrix, which makes it difficult to distinguish (Peters et al., 2011). c) As TEM involves operation under high-vacuum conditions, samples containing water cannot be imaged. Analysis can be done only after dehydration and chemical fixation. d) Techniques which involve electron microscopy require sufficient quantities of the particles to be analysed in order to generate results that are representative of the full sample (Tiede et al., 2008).	a) Sample may accumulate a static charge if they fail to possess conductive property during analysis. To prevent this, if sample is coated in a conducting material, it can result in loss of some surface information which can lead to faulty results (Tiede et al., 2008). b) As SEM involves operation under high-vacuum conditions, samples containing water cannot be imaged without dehydration and chemical fixation. c) Techniques which involve electron microscopy require sufficient quantities of the particles to be analysed in order to generate results that are representative of the full sample (Tiede et al., 2008).	a) Before analysing liquid samples, they must be immobilised to prevent interference with the probe (Tiede et al., 2008). b) In order to acquire high quality images, AFM usually scans samples at relatively slow rates (<1 Hz, up to 1 µm per second). c) The probe has restricted motion flexibility (i.e., the probe's z-axis motion is limited to 5 µm). d) The tip-sample interactions or the image-processing processes may introduce artifacts (Ricci and Braga, 2004; Ukraintsev et al., 2012).

Analysis Category	MORPHOLOGY	MORPHOLOGY	MORPHOLOGY
ENP Measurement Technique	**Environmental SEM**	**Cryo-TEM/SEM**	**Confocal Laser Microscopy**
Brief Description	Developed to facilitate analysis of wet samples without affecting sample preparation through the use of partial vapor pressure in the microscope specimen chamber. In ESEM, the electron beam is generated under vacuum and is separated from the sample chamber by pressure limiting apertures, allowing the sample to be introduced in its natural state (Doucet et al., 2005; Tiede et al., 2008). Hydrated samples imaging is done when samples are under high vapor pressure.	Also known as low-temperature SEM. It functions under high vacuum conditions and at cryogenic temperatures (between -100 and -175 degrees Celsius) to acquire high-resolution images of biological samples. The lower temperature (typically the vitrified state) allows the life-like appearance of the sample and helps to obtain the micrograph of hydrated and chemically unmodified state of the sample (Dudkiewicz et al., 2011).	Used for detecting nanomaterials (NMs) in agricultural samples, specifically in plant and microbial systems. This technique was used to detect Cerium(IV) oxide and Zinc oxide NPs inside corn plant tissue. Confocal microscope images showed NP aggregates in root epidermis, cortex, and some NP aggregates in the xylem vessels (Zhao et al., 2012a, 2012b).
Advantages	a) Samples with 100% relative humidity can be imaged by controlling the vapor pressure (Burleson et al., 2004; Dudkiewicz et al., 2011). b) ESEM can be used to observe the dynamic changes associated to the sample morphology. c) ESEM is employed to effectively investigate the presence of inorganic micro-sized and nano-sized contaminants in food products (Gatti et al., 2009).	a) Cryo-SEM has a higher resolution compared to ESEM. So, it results in better imagery (Dudkiewicz et al., 2011). b) Cryo-SEM can be applied for effectively imaging NMs in suspensions, and solid lipid NPs.	It yields images with high resolution and depth selectivity.
Limitations	If a sample which is totally submerged in a liquid is analysed using ESEM, contrast of the image gets affected as only the top surface gets imaged.		

Analysis Category	ELEMENTAL COMPOSITION	ELEMENTAL COMPOSITION	ELEMENTAL COMPOSITION
ENP Measurement Technique	**Inductively Coupled Plasma - Mass-Spectrometry (ICP-MS)**	**Single particle ICP-MS**	**Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES)**
Brief Description	One of the well-known element-selective detection techniques. Firstly, the sample is introduced into a plasma flame where the analyte is ionised. The ions generated in the plasma are then introduced into a mass spectrometer for quantification. The number of ions released, and the intensity of the radiation emitted are both related to the concentration of the element of interest in the sample and it is therefore quantified by Mass Spectrometry (Baalousha et al., 2011).	Developed to facilitate chemical characterization of individual nanoparticles (NPs) and to differentiate these particles from the dissolved ions. In SP ICP-MS, a dilute suspension of particles is introduced into the ICP. In the ICP, each NP is vaporised, atomised, and ionised, and so produces a dense cloud of ions. This ion cloud is transferred into the mass spectrometer, where it is guided through the ion optics and mass separation device as a transient pulse of ions with a typical temporal duration between 300 and 500µs. If sufficient number of ions from an NP are passed through the MS instrument, then the NP-produced transient pulse of ions is registered as a signal spike in the time-trace of the mass spectrometer. By counting these NP spikes, particle number concentrations (PNCs) of analyte NPs can be determined. The amount of ion signal in each NP-produced signal spike can be related to the amount (i.e., mass) of an element in the NP. In Single particle ICP-MS, the signal is monitored at a higher time resolution than standard ICP-MS. This allows the discrimination between the ion plumes caused by the particle and the background analyte. The intensity of the ion plume is related to the mass of metal in the NP (Heithmar, 2011).	A common analytical technique used to determine the elemental composition of NMs. Its working principle is based on the fact that ions can absorb energy to move electrons from the ground state to an excited state. Firstly, the sample is introduced into a plasma (usually, argon is used as a source) flame where the analyte is ionised. Then, the radiation emitted at the characteristic wavelength of the element being analysed is measured by an optical emission spectrometer. The number of ions released, and the intensity of the radiation emitted are both related to the concentration of the element of interest in the sample and it is therefore quantified by Optical Emission Spectrometry (Baalousha et al., 2011). 'Beer Lambert' law is the basis as it establishes relationship between light intensity and concentration of the element.
Advantages	a) A precise and accurate technique to analyse inorganic NPs. b) Usage of element-selective detection techniques like ICP-MS is the correct approach to detect silver NPs as they offer benefits like high speed, precision, sensitivity, and large linear range (Heithmar, 2011; Liu et al., 2012). c) ICP-MS is an integral part of dual isotopic fingerprinting which is done to differentiate between engineered and naturally occurring Silica NPs.	a) Single-particle ICP-MS (SP ICP-MS) is useful for analysing ENPs with coexisting ions. b) It is used for measuring inorganic NPs at low number concentrations (10^2 - 10^6 particles / mL). c) Rapid SP ICP-MS methods are effective in quantifying the concentration, size distribution, and dissolved metal elemental concentration of NPs (Titanium dioxide, Cerium(IV) oxide, Gold, and Silver) in surface water and treated drinking water.	a) ICP-OES is effective in determining elemental composition of nanomaterials in complex samples. b) It is also capable of detecting multiple elemental compositions simultaneously.
Limitations	a) The complex procedure and high-level expertise required for users may limit the application of ICP-MS. b) The particle size distribution cannot be determined by most element-selective detection techniques, including ICP-MS (Heithmar, 2011). c) During chemical quantification of silver NPs, it can only provide results for the total Ag content of the suspension and cannot distinguish the NPs from the ionic or complexed silver (Heithmar, 2011).	a) The complex procedure and high-level expertise required for users may limit the application of Single particle ICP-MS. b) It is difficult to evaluate the coincidence probability for polydisperse ENPs by a statistical method in SP ICP-MS.	a) The particle size distribution cannot be determined by most element-selective detection techniques, including ICP-OES (Heithmar, 2011). b) It is not possible to recover the sample post analysis as ICP-OES is a destructive analytical procedure. Therefore, highly valuable, or rare samples cannot be analysed using this technique.

Analysis Category	**CRYSTALLINE STRUCTURE**
ENP Measurement Technique	**X-ray Diffraction (XRD)**
Brief Description	One of the most popular techniques for the characterization of NPs that provides information about the crystalline structure. As the wavelength of X-rays is on the atomic scale, X-ray diffraction (XRD) is a useful tool to examine structure of nanomaterials. Bragg's law is the basis of XRD analysis. For the XRD analysis, fine powder samples are mounted on the sample holder and the powder is assumed to consist of randomly oriented crystallites. When a beam of X- ray is incident on the sample, X-rays are scattered by each atom in the sample. They are scattered at an angle other than the angle of incidence of the original beam. This is the angle of diffraction. If the scattered beams are in phase, constructive interference occurs, and one gets the intensity maximum at a particular angle. Using Bragg's law, distance between atomic plates is determined based on angle of diffraction and wavelength. This distance between the plates is then used to provide information about the crystalline structure of nanoparticles.
Advantages	a) XRD accurately measures atomic arrangement at interfaces based on the intensities measured by it. b) As this technique is non-contact and non-destructive, it is ideal for conducting in situ studies. c) XRD is best suited for characterization and identification of polycrystalline phases as every crystalline substance gives a unique pattern. d) XRD requires minimum sample preparation.
Limitations	a) Effort is required to ground the material under consideration into a powder as XRD analysis requires few tenths of a gram of material. b) Heterogeneous materials are not suitable for determining unknown crystalline structure.