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Determination of nanoparticles in biological matrices

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1. ABSTRACT

Human exposure to nanoparticles has increased considerably due to anthropogenic activities dominated by coal and diesel oil fuel combustion. Not only the inhalation of nanoparticles, which nowadays is considered to be the most significant via of exposure to nanomaterials, but also the gradually more employment of nanoparticles in products such as cosmetics, deodorants, textiles, or even food is broadening the exposure to those materials. Developing the previous applications will obviously require the use of analytical methodologies to analyse biological matrices in order to assess potential risks in their use and take appropriate corrective actions. This chapter describes a general overview of the most important analytical techniques for the analysis of nanoparticles in biological matrices.

2. INTRODUCTION

Nanomaterials are defined as products with at least one dimension less than 100 nm. A number of common products and processes currently use or produce nanomaterials. This increasing use of engineered nanoparticles in research and product development engenders a growing need to understand their properties and behaviors as well as the health, safety, and environmental impact of them in both their synthesized form and when they evolve through application or environmental interaction.

Millions of tonnes of such materials are entering the commercial market in products ranging from cosmetics, catalyst, semiconductors, fillers and drug carriers or as by-products of human activity. Commercial nanoparticle-based

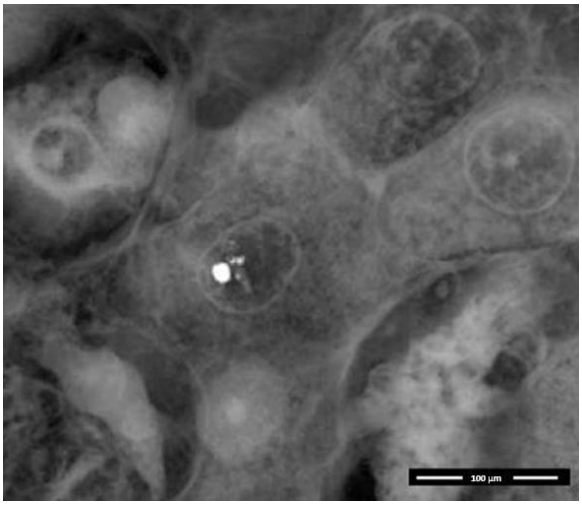

Figure 1. Electron microscopy image of a cancerous tissue of liver with a living cell containing metallic nanoparticles in the nucleus. Reproduced with permission from (1).

products include titania nanoparticles for sunscreens, paints or self-cleaning windows, silica nanoparticles used as solid lubricants, protein-based nanomaterials for soaps and detergents, metal nanoparticles for environmental remediation, carbon nanoparticles for composites, and nanoparticles in electronic devices or pharmaceuticals. On the other hand, by-products in the form of nanoparticles typically come from emissions due to incomplete combustion of diesel fuel or nanosized minerals resulting from acid drainage in mining operations.

It is widely recognized that as particle size decreases to the nanometer scale, their physical and chemical properties differ from those shown in their bulk form. Because of their size and those unique physical and chemical properties, determination of nanomaterials represents a challenge. The increasing use of nanoparticles in industrial applications will inevitably lead to the release of such materials into the environment. Accurately assessing the environmental risks posed by nanoparticles requires using effective quantitative analytical methods to determine their mobility, reactivity, ecotoxicity and persistency, many of which have still to be developed. Moreover, their accumulation in the environment might have major implications for both human and environmental health. Studies have indicated that some nanomaterials can travel through the human body, deposit themselves in target organs and trigger injury to the cells; while other nanomaterials are being designed to enter the body in order to deliver drugs to a specific location.

Human exposure to nanoparticles has considerably increased due to anthropogenic activities dominated by coal and diesed oil fuel combustion. As example, Figure 1 shows the image of a metallic nanoparticle located in a liver cell (1). Furthermore, the exponential growth of nanotechnology is contributing with another source of aerial pollution via engineered nanomaterials. Not only the inhalation of nanoparticles, which nowadays is considered to be the most significant via of exposure to nanomaterials, but also the gradually more employment of nanoparticles in products such as cosmetics, deodorants, textiles, or even food is broadening the exposure to those materials. Consequently, there are being carried out several studies of the ecotoxicity of nanoparticles and the impact of them on human health since there are many medical advances that imply the use of nanoparticles. The possible secondary effects of their use are a controversial issue. Anyway, there is a lack of methods currently available to do reliable measurements in the presence of small particulate contaminations present in biological fluids.

Reliable methods of characterization of nanoparticles in biological fluids and tissues are necessary for development and use of precise drug delivery technologies with nanosized carriers. While more work was devoted to nanoparticles investigation in water suspensions, there is still a lack of characterization techniques in biological liquids and tissues.

Effectively monitoring nanoparticles in biological samples entails meeting several requirements. One is using analytical methods capable of detecting biologically relevant concentrations, and another is avoiding the potential interference of small particles present in biological fluids. A drawback associated with the determination of nanoparticles is the heterogeneous nature of biological fluids, such as serum, containing for example serum albumin, lipoproteins, inmuno-, γ- and macroglobulins, and oleic acid, which can give significant background signals, whereas sizing techniques based on conventional light scattering require minimum levels of background noise for generation of accurate results. This make necessary in most of the cases the extraction or separation of the nanoparticles from their matrix.

2.1. Type of nanoparticles

The general term nanoparticle (NP) is used to define any particle less than 100 nm in at least one dimension. Nanoparticles can be generally classified as natural, antrophogenic or engineered in origin.

Nanoparticles are naturally present in the environment, commonly formed as either weathering byproducts of minerals such as biogenic products of microbial activity, or by growth in super-saturated fluids. Many inorganic growth mechanisms are responsible for nanoparticle formation, for instance crystal growth, aggregation (2), and redoxtriggered crystallization based on changes in mineral solubility (3).

Natural nanoparticles can be biogenic, geogenic, atmospheric or pyrogenically produced. For example, there are fullerenes with interstellar origin that have been brought to earth by comets or asteroids (4), the majority is believed to have formed from polycylic aromatic hydrocarbons (PAH) derived from algal matter during metamorphosis at temperatures between 300 and 500 ºC and in the presence of elemental sulfur (5), or during natural combustion processes

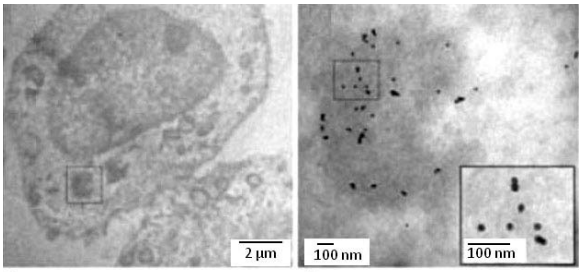

Figure 2. Electron micrographs at different magnifications of a cell containing nanoparticles. Cells were exposed to nanoparticles for 24 h to gold nanoparticles. A) Image at 8000x magnification of a representative cell with nanoparticles subcellularly localized. The small box represents the area magnified in (B) image at 60000x magnification of gold nanoparticles within cells. The inset is a 150000x magnification of the gold nanoparticles. Reproduced with permission from (11).

Inorganic NPs are present everywhere in soils and geologic systems (6, 7). NPs are also ubiquitous aerosols in the atmosphere and they are precursors for the formation of larger particles, which are known to strongly influence global climate, atmospheric chemistry, the visibility and regional and global transport of pollutants (8). Primary atmospheric NPs can be, for example, soil dust and sea salt, although the major mass fraction is composed of coarse particles. The average particle size of airborne mineral dust, based on mass, is between 2 and 5 mm. However, based on the number of particles, the average size is approximately 100 nm with a considerable number below this value (9).

Biogenic nanoparticles are sometimes formed directly by the organism as a metabolic requirement, such as the case of magnetite which is formed by a bacteria in response to a need of mobility (10); or indirectly as a result of the microbial activity. Nanocrystalline mineral phase may precipitate during the redox transformation of a metal induced by a microorganism. Other routes of mineralization are promotion by other metabolites or by microbial cell surfaces acting as organic templates. Anthropogenic nanoparticles are those which are unintentionally produced as by-product of the human activity. These nanoparticles can be also internalized in the cells (see Figure 2) (11). They are predominately combustion derived, formed by automobile exhaust gases or as consequence of the industrial fabric. Some of those combustion-derived nanoparticles, such as diesel exhaust particles, carbon black and fly ash are environmental hazards. Diesel exhaust particles account for up to 80% of the mass of PM10 collected in urban areas. They contain toxic metals and organics and this, in combination with their large surface area, leads to the production of reactive oxygen species. Carbon black is a low-solubility particle produced industrially from the incomplete thermal decomposition of hydrocarbons. Moreover, fly ash is a generic term for particulate matter from mineral and metal contaminants of organic fuels (12).

Another class of unintentionally produced NP is composed of platinum and rhodium containing particles produced from automotive catalytic converters. Although most Pt and Rh are attached to coarser particles, about 17% was found to be associated with the finest aerosol fraction (<0.43 mm) (13).

The design of novel NPs has been the basis of many advances in technology for the last decade. Nanoparticles can be produced by a huge range of procedures which can be grouped into top-down and bottom up strategies. Top-down approaches are defined as those by which NPs or well-organized assemblies are directly generated from bulk materials via the generation of isolated atoms by using various distribution techniques (14). The majority of the top-down strategies involve physical methods such as milling or attrition, repeated quenching and photolithography (15). Bottom-up strategies involve molecular components as starting materials linked with chemical reactions, nucleation and growth process to promote the formation of more complex clusters (16).

Carbon nanotubes (CNT) represent one of the best examples of novel nanostructures derived by bottom-up chemical synthesis approaches. Carbon nanotubes can be considered to be hollow graphitic nanomaterials comprising one (single-walled carbon nanotubes, SWNT) or multiple (multi-walled carbon nanotubes, MWNT) layers of graphene sheets. The lengths of the nanotubes can range from several hundred nanometers to several micrometers and the diameters from 0.2 to 2 nm for SWNT and from 2 to 100 nm for coaxial MWNT (17). Depending on the synthesis method, the technique used for the separation from the amorphous by-products, subsequent cleaning steps, and finally different functionalizations, a variety of different CNT are obtained that have very different properties (18). Most CNTs reported to date have been prepared by using arc discharge (19), laser ablation (20), or chemical vapor deposition (CVD) techniques (21). The former two use a solid-state carbon precursor for nanotube growth. On the other hand, CVD uses hydrocarbon gases as sources for carbon atoms and metal catalyst particles as seeds for CNT growth.

The high potential of CNTs has promoted much study aimed at elucidating their properties (particularly those of SWNTs). Based on structure, CNTs possess nonpolar bonds and high aspect ratios (length to diameter ratio) and are thus insoluble in water. This results in the spontaneous aggregation of CNTs. This affinity to aggregation combined with their high flexibility increases the possibility of bundling and close packing (22). As opposed to aqueous solutions, hydrophobic CNTs are expected to be wetted by organic solvents and, therefore, to assemble less in bundles and ropes. However, CNTs were shown to exhibit a sufficient solubility only in a limited number of solvents, such as dimethyl formamide, dimethyl acetamide, and dimethyl pyrrolidone (23). Like other organic molecules, CNTs can be functionalized covalently (24). SWNTs possess a good surface area to volume ratio and an excellent thermal stability in inert atmospheres. They also possess a π complex both above and below the plane containing the carbon atoms that is the origin of their high electron mobility and electrical conductivity (25).

They present excellent plasticity and elasticity and the sharp tip of the end of a SWNT results in a locally boosted electrical field upon application of a potential.

Fullerenes are polyhedral carbon cages in which sp^2 carbons are bonded to three carbon neighbors in an arrangement of five-membered and six-membered rings (26). C_{60} has been extensively studied since its discovery in 1985, it present low electron delocalization over the spherical surface with a resonance structure, being a good electron acceptor, so it can form charge-transfer complexes with compounds with donor groups. A drawback is their low solubility in aqueous and organic solvents, which limit their applications and focus lot of research to functionalization (27). Fullerenes have been proposed to be used in fullerene polymer combinations, as thin films, in electro-optical devices and in biological applications (28).

Engineered inorganic NP also cover a broad range of substances including elemental metals, metal oxides and metal salts.

Colloidal gold nanoparticles have been used technologically since ancient times due to their optical properties, in particular for staining glass (29). Gold spheres have a characteristic red color, which is due to the collective oscillation of the electrons in the conduction band, known as the surface Plasmon oscillation. The oscillation frequency is usually in the visible region for gold and silver giving rise to the strong surface plasmon resonance absorption. Therefore, the origins of properties on the nanoscale are different for metal nanoparticles than for semiconductor nanoparticles.

Many applications became possible due to the large enhancement of the surface electric field on the metal nanoparticles surface. The plasmon resonance absorption has an absorption coefficient orders of magnitude larger than strongly absorbing dyes. Anisotropic shapes have plasmon resonance absorptions that are even stronger, leading to increased detection sensitivity. Metal nanoparticles generate enhanced electromagnetic fields that affect the local environment.

Silver nanoparticles have been widely used as bactericide (30), whereas gold nanoparticles are mainly used for optical sensors or to exploit its catalytic activity (31). The use of nanoscale zero-valent iron for groundwater remediation ranks as the most widely investigated environmental nanotechnological application. Nanoparticles having magnetic properties are the most interesting in this field. To this end, metallic iron is very effective in degrading a wide variety of common contaminants such as chlorinated methanes, brominated methanes, trihalomethanes, chlorinated ethenes, chlorinates benzenes, other polychlorinated hydrocarbons, pesticides and dyes (32).

Today, nanoparticulate metal oxides are among the most used NPs (33). Bulk materials of TiO_2, SiO_2 and aluminum and iron oxides have been produced for many years. However, recently they have also been manufactured in nano-sized form and have already entered the consumer market, e.g. ZnO in sunscreens (34). TiO_2 NPs are widely used for applications such as photocatalysis, pigments and cosmetic additives. Two methods are commonly used to prepare oxide-based nanomaterials: stabilised precipitation and flame pyrolysis. (35, 36). Nano-sized zeolites (37), clays (38) and ceramics (39) are other NPs that have been proposed for various catalytic applications. Several non-carbon nanotubes have also been synthesized (40), for example, TiO_2.

Nanoparticles synthesized from organic polymers have gained widespread interest in medicine as carriers for drugs. The possibility to control size, surface charge, morphology and composition make polymers especially well suited for designing NPs with tailored properties based on selective recognition. These NPs are taken up by a wide variety of cells and are studied for their ability to cross the blood-brain barrier (41).

Polymeric nanoparticles have also been developed and synthesized to be used in soil and groundwater remediation. For example, micelle like amphiphilic polyurethane particles have a hydrophilic outer side and a hydrophobic inner core and are therefore very well suited for the removal of hydrophobic pollutants (e.g. phenanthrene) from soils (42). Another realted polymeric nanoscale material is dendrimers that function as water-soluble chelators (43).

Metal and semiconductor nanoparticles in the 2–6 nm size range are of considerable current interest, not only because of their unique size-dependent properties but also because of their dimensional similarities with biological macromolecules (e.g. nucleic acids and proteins) (44). These similarities could allow an integration of nanotechnology and biology, leading to major advances in medical diagnostics, targeted therapeutics, molecular biology and cell biology. Quantum dots (QDs) are inorganic semiconductor nanocrystals with interesting luminescent and electrochemical properties extensively used in numerous bioassays (45, 46). These NPs show broad excitation profiles and narrow emission peaks and can emit in a range of wavelengths by changing their size and composition. Also, they lack photobleaching and have long fluorescence lifetimes. However, QDs can show blinking characteristics when they are excited with high-intensity light, which could be a limiting factor for fast-scan systems, such as flow cytometry. Other limitations are toxicity, size variation, agglomeration and non-specific binding. Surface oxidation of QDs can occur under combined exposure to aqueous/UV-light excitation, which can lead (e.g., in CdSe-based QDs) to the release of cadmium ions, so that these NPs are inadequate for *in vivo* applications, such as *in vivo* drug delivery assays. However, they offer better imaging results than those achieved by organic dyes in cell-based or tissue-based drug studies. Luminescence emission from QDs is detected at concentrations comparable to organic dyes by using conventional fluorescence methods, and individual QDs and QD-bioconjugates are easily observable by confocal

microscopy (47). In particular, CdSe- ZnS core-shell QDs exhibit size-dependent tunable photoluminescence (PL) with narrow emission bandwidths that span the visible spectrum and broad absorption spectra that allow simultaneous excitation of several particle sizes at a single wavelength (48). These nanoparticles have a high quantum yield and a high resistance to photodegradation. Quantum dots have potential in biomedical applications, but concerns persist about their safety.

The particles can be further separated based on their chemical composition into carbon-containing and inorganic NPs. In fact, in order to describe the different approaches for the analysis of nanoparticles in this review we have distinct between metallic and metal oxide nanoparticles, carbon-based nanoparticles and nanodrug or nano food-additives.

2.2. Effects of nanoparticles in biological systems

Human exposure to nanoparticles is inevitable as they become more widely used. This has given rise to a new division of toxicology, namely "nanotoxicology", which involves the safety evaluation of engineered nanostructured and nanodevices. As it has been previously reported, human exposure is not limited to engineered nanoparticles, since there is anthrophogenic sources which unintentionally release nanoparticles to the environment, such as industrial activity or combustion processes. In contrast to nanoparticle exposure through use of consumer products (such as textiles, cosmetics, deodorants), emerging biomedical applications of nanoparticles as drug-delivery agents, biosensors, or imaging contrast agents involve direct ingestion or injection of nanoparticles into the body, therefore understanding the properties of nanoparticles and their effect on the body is crucial before clinical use can occur. A number of authors have reviewed characterization, fate, and toxicological information for nanomaterials and proposed research strategies for safety evaluation of nanomaterials. (49, 50)

A factor influencing potential toxicity in the case of carbon nanoparticles is their complexity and variety in size, shape, charge, methods of production, chemical compositions, surface chemistry/functionalization, and aggregation tendency. Most practical carbon nanomaterial samples are highly complex, containing the desired structure (e.g. single-wall nanotubes) mixed with amorphous or graphitic nanoparticulate byproducts, other nanotube varieties, and metallic catalyst residues of unknown chemical form (metal, oxide, carbide), and in complex association with carbon (within nanotube cavities or as particles partially or wholly encapsulated by carbon shells). The toxicity indicators associated with any given material may just as well be related to these impurities as to the properties of the nanomaterials in question. The catalyst residues in vapor-grown carbon nanotubes include Fe, Ni, Co, and less often Y, Mo, and other metals, many of which would pose health risks of their own in the form of nanophase powders. Ideally toxicological assays would be accompanied by characterization of all the biologically relevant properties of these complex materials—size, shape, surface chemistry (hydrophobicity/philicity), and the amount, form, and encapsulation state of metals. Some of these properties can be measured by routine techniques (overall metal contents by atomic adsorption or emission techniques; size and shape by scanning and transmission electron microscopies) but others are not standard (surface chemistry, metal form and encapsulation) and require experts (51).

Moreover, a given nanomaterial can be produced by different processes which lead to derivatives of the same material, for instance the case of SWNTs which have different physical-chemical properties, namely size, shape, composition and consequently different ecological and toxicological impact (52). Alternative methods for measuring properties of nanomaterials may need to be developed both quickly and cost effectively.

Cytotoxicity studies (53) have demonstrated that in general cells can survive short-term exposure to low concentrations (<10 mg mL^{-1}) of nanoparticles. It is important to remark that the effect is mainly produced by the small dimension of the nanoparticle that its nature. However, at high doses, several groups have found cytotoxic effects to emerge in a dose- and time-dependent manner for carbon-, metal-, and semiconductor-based nanoparticles. The generation of reactive oxygen species and the influence of cell internalization of nanoparticles are two common findings causes for the increase in cell death observed at higher concentrations and longer exposure times.

Gold nanoparticles have been reported to induce little toxicity, around 15% reduction in cell viability, at 200 mg mL^{-1}. (54-56) While higher concentrations could elicit a cytotoxic effect, many substances become toxic at high concentrations. Therefore, it may be reasonable to conclude that the results from cytotoxicity testing of other nanoparticle types suggest low toxicity if those results are similar for gold-nanoparticle solutions containing relatively the same size particles at the same concentration.

Many nanoparticles are not water soluble. However, the most important applications are performed in aqueous media. For that, the addition of a hydrophilic surface coating is required. However, as seen in MWNTs and QDs studies, adding certain hydrophilic molecules results in lower cell viability as the functional groups themselves were toxic (57-60). Although it is difficult to establish a general rule, surface charge also plays a role in toxicity with cationic surfaces being more toxic than anionic, and neutral surfaces being most biocompatible (61) because of the affinity of cationic particles to the negatively charged cell membrane. Therefore, adding a coating that makes the nanoparticle more cationic could make the nanoparticle appear more toxic than it inherently is.

Complexation of metallic nanomaterials may have important effects on biological availability and photochemical reactivity since it reduces the availability by reducing free metal ion concentrations and dissolved metal, for instance iron, which is quantitatively complexed by organic ligands. Solar UV radiation can also interact with

these processes through photoreactions of the complexes. (62) Particle aggregation has also been suggested to be a factor in nanoparticle cytotoxicity. Wick *et al.* aimed to determine how agglomeration influenced SWNT cytotoxicity and (63) find that aggregation occurred in all SWNT fractions except the well-dispersed SWNT bundles. Correspondingly, the SWNT bundles did not induce adverse cellular effects, and as this was the only solution where agglomerates were not formed, this corroborates the hypothesis that SWNT agglomeration leads to cytotoxic effects. However, an earlier study by Tian *et al.*, testing an unrefined SWNT solution and a SWNT solution with the metal catalysts removed, found lower cytotoxicity with the unrefined SWNTs (64) which was proposed to be a result of their aggregation into larger, and therefore less toxic, particles. This contradicts the reasoning of Wick *et al.*, so the effect of SWNT aggregation is still questionable.

3. TOOLS FOR THE ANALYSIS OF NANOPARTICLES IN BIOLOGICAL SAMPLES.

In spite of the increase in the use of nanoparticles, there are still few analytic methods for their quantification in environmental or biological matrix due to their unique physic and chemical properties. Their capacity to form colloidal phases and aggregates, their different ad- and absorption characteristics and their shape and size variety make difficult their quantification.

It is necessary to consider features of biological samples, connected with presence of macromolecules, having the sizes compared with investigated nanoparticles, and various effects of interaction of these particles with the components of biological sample.

Considering the complexicity of biological samples as well as the number of potential interferences and the number of interactions that can be established between the nanoparticles and the sample, liquid-liquid extraction techniques are the most usefulness. The excess of the solvent and chelating agent helps to break the interactions of nanoparticles with biomolecules. To this end, the uses of ionic liquids have been recently proposed for the analysis of gold nanoparticles in tissues. The ionic liquids, especially those derivates from imidazolium groups have a high affinity to interact and solubilize nanoparticles. Preliminary results obtained in our group of research point out that the BMIN-PF_6 can extract gold nanoparticles from liver tissue homogenates. The nanoparticles were directly quantified in the IL by spectrometry and Raman spectroscopy. Other sample treatment systems involve the use of surfactants. Supramolecular complexes of surfactant also have a high tendency to extract nanoparticles from aqueous systems.

There is no doubt that sample treatment is a key step in the analysis of biological samples for the analysis of nanoparticles because sample treatment need to provide selectivity in addition to a high capacity to preconcentrate the nanoparticles. In addition to liquid-liquid extraction other treatments such as centrifugation, dielectrophoresis or field flow fractionation have been also applied. In general, it must be point out that these techniques have been applied to the study the interaction of nanoparticles with biomolecules in order to determine and characterize the interaction established between the nanoparticle and the biomolecule. Although these techniques are a good alternative to analyse simple systems, there are not a real alternative for the analysis of complex biological samples. The most reliable attempt to extract and precontrate nanoparticles based on solid phase extraction is with the use of modified filters based on carbon nanotubes. The modified filters have a high capacity to absorb carbon nanotubes. This methodology has been applied to the precocentration of soluble carboxylic carbon nanotubes from waste water (65). It will be also applicable to biological samples, but for that it is necessary the addition of chelating agents to avoid and minimize the interaction of nanoparticles with biomolecules.

A large number of detection techniques have been applied to the analysis of nanoparticles, some of them focus on the assessment of its toxicity (66). A general overview in presented in Figure 3. In general three groups can be distinguished:

i. Microscopic techniques
ii. Spectroscopic techniques
iii. Electrochemical techniques

In general, it can be affirmed that for the detailed characterization of nanoparticles or nanobjectes in general such as subcellular compartments, and other very small biological structures, there is a currently lack of methods for chemical diagnostics and characterization, in particular of the molecular composition. Traditional methods with nanoscale lateral resolution (viz, standard atomic force microscopy, scanning tunneling microscopy and electron microscopy) typically yield very little or no chemical information, whereas traditional methods for the chemical analysis (for example, mass spectrometry, spectrochemical analysis performed with microscope, etc.) are in many cases far from achieving nanoscale lateral resolution.

Microscopy-based techniques capable of analysing nanoparticles include the well-known scanning and transmission electron microscopy (SEM and TEM) and atomic force microscopy (AFM) of the group of scanning probe microscopes. (67) These techniques allow exact information about type and characteristics (shape, size, etc) however one of their most important shortcomings is the low representativity of the results due to the small sample volume that are analysed. Besides, sample preparation involves drying, which might result in aggregation of the sample and thus a false representation of the sample size in tissue. As well, inference of the three-dimensional structure from two-dimensional images is very difficult (68), but can be accomplished in some cases with stereological transformation (69). Eventually, in-situ 3D characterization should be possible by nano-coupled tomography (70). As advantage, by using classifical sample treatments applied to tissues it is possible to analyse biological samples. Although this technique can be applied to a wide number of nanoparticles, its use due to resolution capacity is mainly

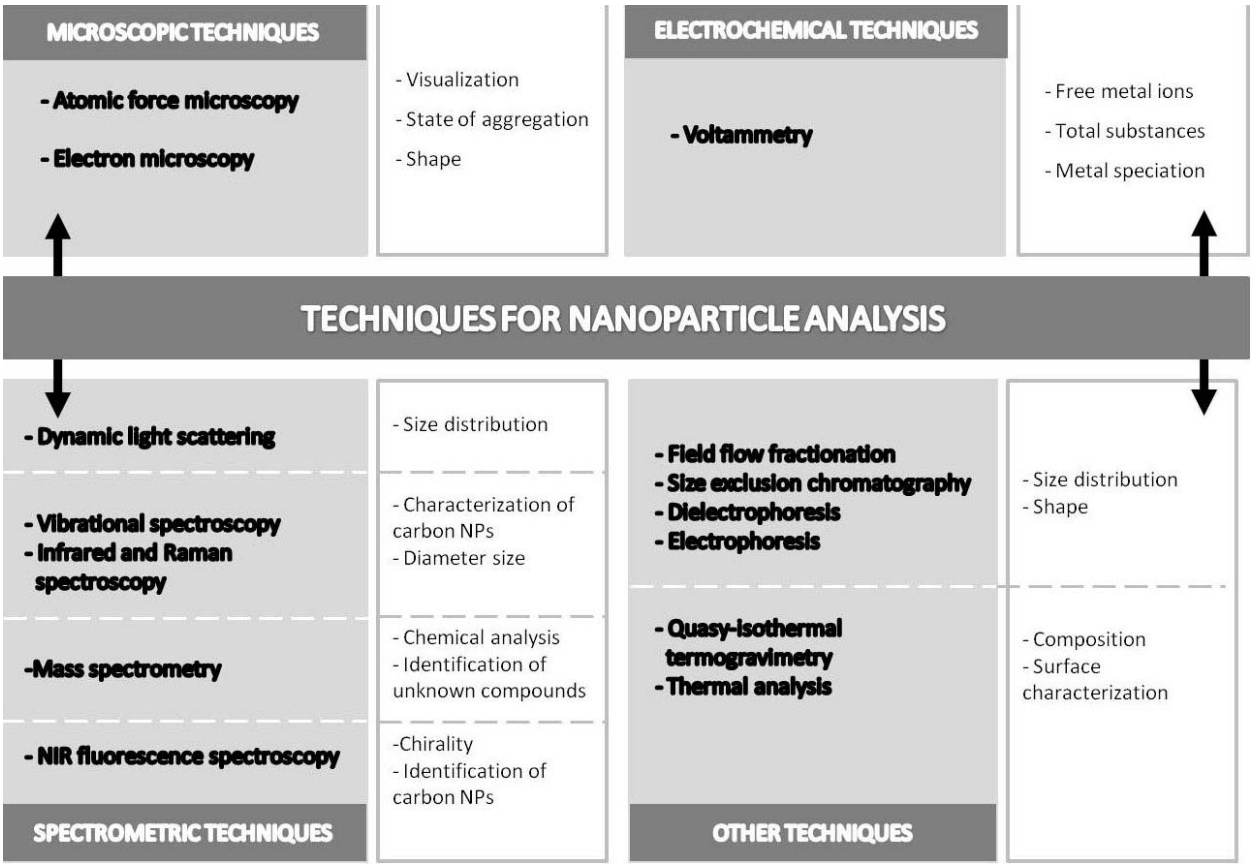

Figure 3. General overview of analytical techniques for the analysis of nanoparticles in biological samples.

limited to the analysis of metallic nanoparticles. Because metals scatter light efficiently, electron microscopy is the technique of choice for physical characterization of colloidal gold nanoparticles. (71) However, many biological compounds (e.g., proteins) are invisible to TEM without heavy metal staining procedures, because these compounds do not sufficiently deflect an electron beam. Atomic force microscopy (AFM) relies on tapping a particle (either in solution or dried to a surface) and so has limited resolution of flexible compounds (e.g., proteins), which may move under the force applied by the instrument tip. Their use for the analysis of carbon nanoparticles in biosamples such as tissue is limited. Apart from providing information on particle aggregation, dispersion, size, structure and shape, for additional elemental information can be obtained by coupling EMs to energy-dispersive spectrometry (EDS), which was employed to confirm the presence of silver nanoparticles within cells (72) or to electron energy loss spectrometry (EELS), used in conjunction with TEM for elemental confirmation of carbon nanotube uptake (73). The combination of these techniques is indispensable when it comes to the characterization of nanoparticles and other nano-structured materials.

Spectroscopic techniques involve a large number of techniques: UV/Vis techniques, fluorescence techniques, NIR-fluorescence, infrared spectroscopy and Raman spectroscopy. These techniques provide a large level of information about nanoparticles. UV/Vis and fluorescence spectroscopy are the most useful for the analysis of metallic, oxide derivates and semimetallic nanoparticles. Fluorescence spectroscopy is useful in both the quantitative assessment of nanoparticle uptake as well as the qualitative assessment of nanoparticle localization. Quantitative assessment can be achieved through use of bulk fluorescence (74) or on a cell-to-cell basis using confocal fluorescence. (75) Non-natively fluorescent nanostructures can still be evaluated using fluorescence methods after applying one of many available labeling techniques in order to study the uptake in toxicological assays, although in the case of natural samples there is no a label that allows their detection. These studies also require additional control experiments to account for effects of the fluorescent surface dyes or incorporated species.

Contrary, Raman spectroscopy is the most important technique for the analysis of carbon nanoparticles. Figure 4 shows the characteristic spectrum of singlewalled and multiwalled carbon nanotubes. The spectrum reported is characteristic of the nanoparticle and its intensity can be related with concentration. Information about interaction and state of aggregation can be also obtained from the spectrum. In the case of single walled carbon nanotubes NIR-fluorescence is a technique with a high capacity to classify the nanoparticles according its

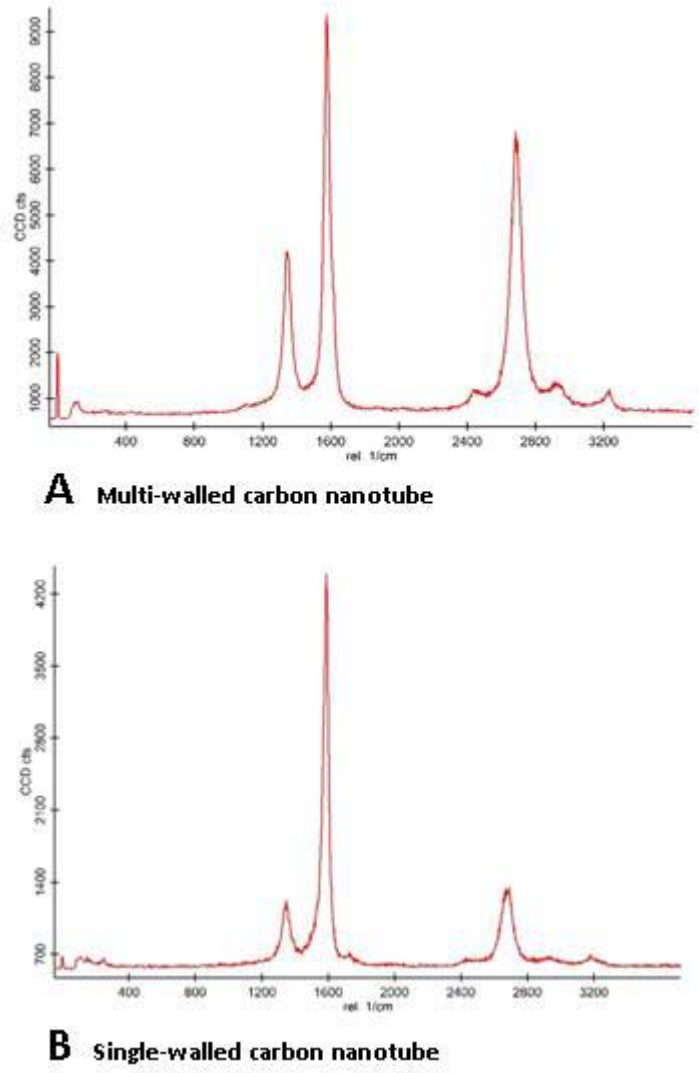

Figure 4. Characteristic Raman spectrum of a A)- multiwalled and B)- single walled carbon nanotubes.

chirality (see Figure 5). (76) Optical-spectral methods are rather informative and practical for nanoparticles characterization. Good representativity of sample, simplicity of sample preparation, high productivity of analysis and opportunity of its automation, price availability of measurement instruments are among the advantages of these methods. The majority of optical-spectral techniques also give the opportunity to measure nanoparticles parameters directly in a liquid. In a number of cases it is necessary to process the results received by optical-spectral methods together with the data of high resolution microscopy.

Sizing of nanoparticles can be done using methods such as scanning and transmission electron microscopy, dynamic light scattering, and size-exclusion chromatography; however, the size values obtained can vary between these methods. A standard technique for measuring and reporting the hydrodynamic sizes of nanoparticles would be valuable. Available technologies for the size fractionation and collection of nanoparticle fractions in liquid mediums include the above mentioned size-exclusion chromatography as well as ultrafiltration and field flow fractionation (77). Field-flow fractionation (FFF) is a high-resolution method that can be an ideal candidate for direct separation, detection, and size characterization of nanoparticles in biological samples, because it allows for the separation of nanoparticles in a liquid matrix of desired composition and as a separation technique, provides a size distribution instead of an average value. FFF has been widely used to separate carbon nanotubes (78), particles (79), cells, bacteria, viruses, and natural organic matter. Unlabeled inorganic nanoparticles extracted from biological media have been characterized, separated and quantified by this technique (80). Probably, the most important shortcoming of this technique is the need of standards to calibrate the FFF equipment. The use of polymeric nanoparticles is the most useful approximation described to data.

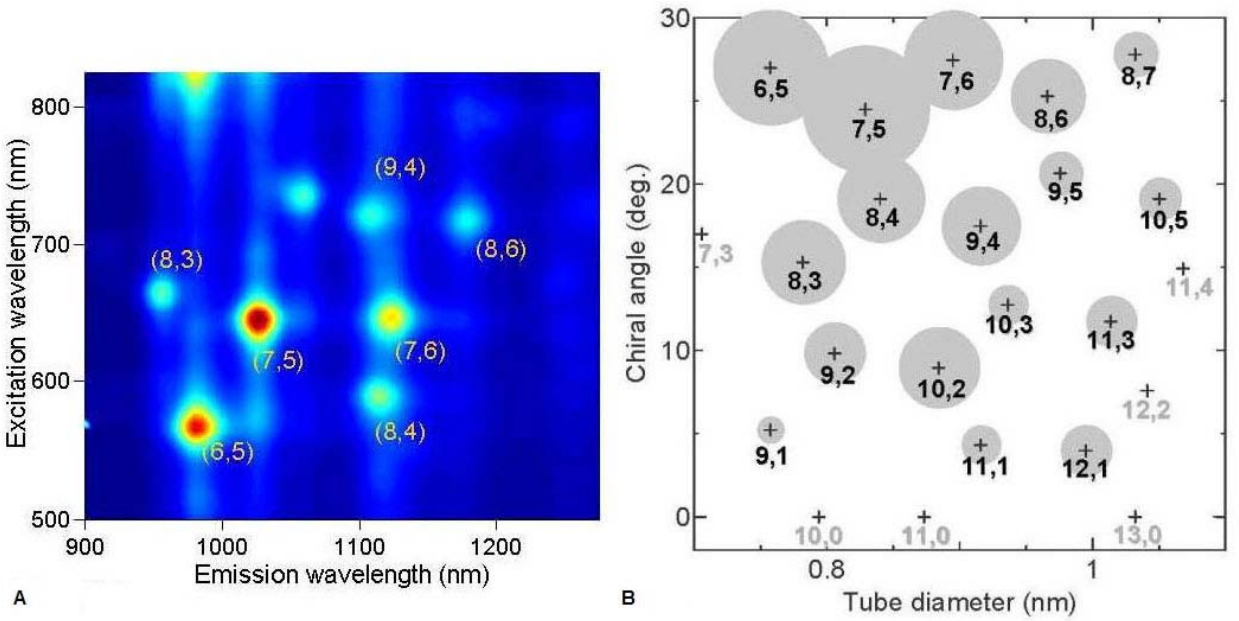

Figure 5. Classification of single walled carbon nanotubes according their chiraly by NIR-fluorescence spectroscopy. A. tridimensional spectrum and B- classification according to carbon nanotube chirality. Reproduced with permission from *(76)*.

On-line particle size analysis in liquid mediums can be done using dynamic light scattering to obtain a particle size distribution (81). Because dynamic light scattering (DLS) measures hydrodynamic diameter, it provides a fundamentally different measure of particle size from TEM or AFM. DLS is very sensitive to "soft" flexible biological molecules such as polymers, proteins, and antibodies because they cause significant frictional drag (82), which can dramatically influence the rate of the particle's motion under Brownian diffusion. DLS is therefore appropriate for measurement of the hydrodynamic size of protein bound nanoparticles. Complementary size characterization by TEM and AFM can be useful in resolving ambiguities due to the different measurement techniques. For example, the DLS-measured size may be influenced by particle agglomeration, and further analysis by TEM is required to ensure that the DLS-measured sizes represent primary particle size (i.e., discrete particle size), and not the size of an agglomerate. (83) For polydispersed samples, the task is more difficult, since the signal intensity has a power relationship with size and the average size can be overestimated if some larger particles exist in the sample. Therefore, although DLS is a common method for measuring nanoparticle size ex-situ, it is not appropriate for nanoparticle analysis in-situ or in a digested biological sample, because of the multiple scattering issues.

Since many nanomaterials are constructed of materials not found natively in the body, uptake of these nanomaterials can be quantified by determining the mass or concentration of these non-native elements within cells. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) is a powerful technique for the quantification of internalized nanoparticle (NP) elemental composition (84, 85) and has been used to quantify nanoparticle uptake. Advantages of ICP-AES are sub-ppb detection limits, high precision, and a dynamic range of five orders of magnitude or more. But this technique is limited in biologica systems to metallic nanoparticles since it is unable to characterize the uptake of carbonaceous nanoparticles (e.g. polymeric NPs and carbon nanotubes) because it cannot distinguish between cellular and nanoparticle sources of carbon. It also cannot differentiate between elements confined within the nanostructure and solvated ions that had leached from the nanomaterial as well as spatial information about the location of nanoparticles. In addition, conversion of the ICP-AES measurement to standard nanoparticle dosing units requires a well-characterized crystalline lattice and nanoparticle monodispersity as well as the assumption that the nanostructure does not change upon exposure to the cellular environment. Using similar procedures, samples can be also analyzed by ICP-MS. Mass spectrometry is a detector with a higher sensitivity than the optical emission spectroscopy (OES). In addition to its higher sensitivity, MS detectors can analyze isotopes and can perform multianalysis in a simple run.

Furthermore, single-particle laser microprobe mass spectrometry (LAMMS) can provide chemical composition data on single particles from a collected fraction. (86)

Determining the concentration of nanoparticles in solution is more difficult. Concentration can be calculated from the optical density using the Beer– Lambert law given the extinction coefficient of the nanoparticle. However, as Yu *et al.* point out, the extinction-coefficient values published for quantum dots differ between groups by an order of magnitude (87). Cryogenic TEM was used as an alternative method of determining concentration. This

method involves direct counting of particles in a relatively fixed volume. As concentration or dose plays a significant role in biomedical applications of nanoparticles, having a standard technique of calculating this value is important.

After this general overview, below several examples of the analysis of different types of nanoparticles in biological matrices are described. In order to facilitate the text to readers, the examples have been classified according to the nature of the nanoparticle because it is clearer than a classification based on the detection system.

4. ANALYSIS OF METALLIC OXIDES AND METALLIC NANOPARTICLES

While a lot of work was devoted to nanoparticles investigation in water suspensions, development of nanoparticles characterization techniques in biological liquids and tissues began more recently. Typically nanoparticles can be detected in cells and tissues, if they have been labeled with radioactive (88), magnetic (89), or fluorescent markers (90). However, the inorganic nanosized powders being produced as bulk chemicals or the ultrafine particles produced by combustion processes do not have a convenient label that allows for their detection. As well, discrimination between nanoparticles, sub-micron particles, and soluble species with the same nominal elemental composition is a very difficult task. This complexity in detection and characterization of unlabeled nanoparticles in biological systems poses a major limitation for current research on environmental and occupational health effects of nanoparticles.

An example of the determination of labeled nanoparticles is the use of fluorescent silica-capped NPs containing a CdSe-CdS core for the study of uptake and loss of these silica nanoparticles in living human lung epithelial cells (91). A number of other luminescent silica NPs have been employed for labeling, and as long as there is not leakage of luminescent materials from the NPs, they have been shown to be compatible for *in vitro* testing. Examples of them are fluorescein isothiocyanate (FITC) in silica (92), FITC–silica coatings for iron oxide NPs (93), and encapsulation of other organic fluorophores for two-photon-fluorescence imaging (94).

A rapid, high-resolution methodology for characterization, separation, and quantification of unlabeled inorganic nanoparticles extracted from biological media, based on sedimentation filed-flow fractionation and light scattering detection (80) have been developed. Silica nanoparticles were added to human endothelial cell lysate and rat lung tissue homogenate and incubated. The nanoparticles were extracted by acid digestion with nitric acid and then separated and characterized by sedimentation field-flow fractionation. Fraction collected at the peak maxima was analyzed by transmission electron microscopy (TEM) to verify their size and shape. It was found a linear relationship between the particle number and the area under the fractogram.

Many techniques have been used to identify and characterize natural inorganic NPs (95). The ones most often used are transmission electron microscopy and scanning probe microscopy; images of individual NP can be obtained with both techniques. However, only a minute fraction of material is characterized, which means that it can be extremely difficult to ensure that a representative sample is examined. Elemental analysis methods such as Atomic Absorption Spectroscopy (AAS) or Mass spectrometry (MS) can quantify nonlabeled nanoparticles, but do not provide direct information on the primary particle size or aggregation state. Besides, sample preparation can involve drying, which might result in aggregation of the sample and thus a false representation of the sample size in tissue.

Dynamic light scattering is another common method for the size characterization of nanoparticles, although may be not appropriate for in-situ measurements or in a digested biological sample, because of the multiple scattering issues. (96) The heterogeneous nature of fluid such as serum can give rise to significant background signals. Moreover, samples of polymer/DNA complexes are typically dilute in nature and often contain large fractions of free polymer (97), which can interact with serum proteins and form aggregates that strongly interfere with size measurements by conventional light scattering techniques (98). However, Xie *et al.* (99) have developed a quantitative method for the estimation of gold nanoshell concentrations in whole blood and estimate the actual delivered dose of intravenously injected nanoparticles using dynamic light scattering. Triton X-100 was added to blood samples containing gold nanoshells to act as a quantitative scattering standard and blood lysing agent. Au nanoshells are similar in size and shape to other types of nanoparticles delivered intravascularly in biomedical applications, and given the pervasiveness of DLS in nanoscale particle manufacturing, this simple technique should have wide applicability toward estimating the circulation tie of other solid nanoparticles. It has some limitations; one is that it is critical that the nanoparticles solution of interest remain unaggregated, because only separate, unattached particles will produce a scattering profile identical to the stock particles enabling concentration calibration. For *in vivo* oncology applications, however, this is often the goal for therapeutic reasons, and this technique primarily quantitates individual nanoparticles that remain in circulation and that are expected to produce a size-dependent accumulation. Also, the use of surfactant Triton X-100 may limit the protocol to solid nanoparticles. Micellular or liposomal systems may be subject to breakdown or aggregation in the presence of this agent, rendering further analysis useless.

Dynamic light scattering has been also employed to evaluate secondary TiO_2 nanoparticles size for *in vitro* toxicity assessment (100). However, when using DLS to determine the hydrodynamic diameter of the nanoparticles in a biological media, it must be taken in account the fact that for example citrate-stabilized gold colloids in the bloodstream are quickly coated by serum proteins. Dobrovolskaia *et al.* (101) demonstrated that after incubation with plasma the intensity-weighted average particle diameters increased, and trypsin digestion of the

particle-bound plasma proteins returns the colloids to approximately their pre-incubation sizes. This measurement can also be influenced by particle agglomeration, so TEM or AFM measurements are useful to determine if the increase could be attributed to a change in agglomeration state.

Optical-Spectral methods have been useful for the characterization of nanoparticles in biological fluids and tissues. Absorbance spectroscopy and resonant light scattering spectroscopy were used for determination of nanoparticles number concentration. Gold nanoparticles surface plasmon resonance peaks are clearly visible on absorption and scattering spectra. Levin and coworkers from the Russian Scientific Research Institute for Optical and Physical Measurements in 2009 in the International Nanotechnology Forum Rusnanotech presented an overview on the characterization of nanoparticles in biological fluids and tissues with optical methods. Concretely, they executed measurements for nanoparticles suspensions in water, phosphatic buffer solution (PBS), whole blood and plasma. Directy before measurement the filtration of solution through membrane filters with pore size about 0,2 microns was made. It was necessary to exclude light scattering on red blood cells, for this purpose lysis of red blood cells was made by dilution of blood with surfactant Triton X-100.

In order to know NPs penetration efficiency through biological barriers it is necessary to define their concentration in organs and tissues. A possible method in this case is atomic absorption spectrometry with electrothermal atomization. Such approach is possible, if nanoparticle's composition includes even one of elements, containing in organism not more then on trace level. Penetration of gold nanoparticles through skin of laboratory animals was investigated. Samples of liver and spleen from rats were subjected by autoclave mineralization and gold concentration in mineralized solution was measured using atomic absorption spectrometer with electrothermal atomization.

Electrothermal atomic absorption spectrometry (GFAAS) with Zeeman background correction has been used to measure the Ag concentration permeated through human abdominal full thickness skin (102). Experiments were performed using the Franz diffusion cell method with intact and damaged human skin. Physiological solution was used as receiving phase and silver nanoparticles coated with polyvinylpirrolidone dispersed in synthetic sweat were applied as donor phase to the outer surface of the skin for 24 h. The receptor fluid measurements were performed by electro thermal atomic absorption spectroscopy (ETAAS). Human skin penetration was also determined by using transmission electron microscope (TEM) to verify the location of silver nanoparticles in exposed membranes.

Electrophoretic methods, both gel and capillary electrophoresis are being widely employed for separation of nanoparticles mainly as a function of their size, but in case of functionalized nanoparticles, the charge and pKa of the group attached to the NPs plays an important role in their separation.

For instance, Hanauer *et al*. (103) demonstrated separation of gold and silver nanoparticles according to their size and shape by agarose gel electrophoresis after coating them with a charged polymer layer. The separation was monitored optically using the size- and shape-dependent Plasmon resonance and confirmed by transmission electron microscopy (TEM). In other work, Xu *et al.* (104) also used agarose gel electrophoresis for preparative separation of gold nanoclusters with sizes of 5, 15 and 20 nm. Gold balls, plates and long rods could also be separated according to shape. LC and gel electrophoresis can also be coupled to inductively coupled plasma (ICP)-MS (105) for the separation of gold nanoparticle standards by size.

It has been also report the suitability of CE for the separation of metallic nanoparticles such as Au (106-111), Ag (110, 111), maghemite (γ-Fe_2O_3) (112, 113), semiconductors (quantum dots) (114-116), and mixtures of metal oxides such as TiO_2, Fe_2O_3, Al_2O_3 and Fe_3O_4 (117, 118).

Flow cytometry allows high-resolution size distribution analysis of multimodal populations of nanoparticles directly in biological fluids and a proof of principle for preparative fractionation of polydisperse samples in the submicron range. Flow cytometry integrates light scattering and fluorescence measurements to gather information regarding size, shape, morphology of cells, which are their originally target analytes, although recently this technique have been exploited to study submicron matter, including unilamellar synthetic vesicles, liposomes, viral particles and nucleic acid containing nanoparticles (119). It is also possible to correlate nanoparticle counting directly with cell numbers or sort cells based on nanoparticle uptake using a flow cytometry technique such as fluorescence-activated cell sorting (FACS). (120)

Inductively coupled plasma mass spectrometry (ICP-MS) have been also employed to measure quantitatively the presence of gold distribution in rats (121). Gold suspensions were diluted by adding phosphate buffered saline in order to obtain a physiological solution for intravenous injection. One mL of each freshly prepared solution was injected in the tail vein. At 24 h after injection, blood and the following organs were collected: adrenals, aorta, brain, heart, kidney, liver, lung, lymph nodes (mesenteric and popliteal), spleen, testis, thymus, and vena cava. Organs were weighed, and tissue samples were homogenized and frozen for determination of gold content by inductively coupled plasma mass spectrometry (ICP-MS).

Many *in vitro* nanotoxicity studies have used ICP-AES to assess nanoparticle uptake of gold, (122, 123, 124), cerium-oxide (125), and iron-oxide nanomaterials (126) among others. Generally, sample preparation includes isolation of cells from culture media followed by acidic sample digestion before dilution and ICP-AES analysis. In some instances, the mass concentrations obtained from ICP-AES are converted into nanoparticle numbers after estimating the mass of a single nanoparticle using the

atomic weight, the crystal lattice unit length and the well-defined geometry of the nanoparticle. (127)

The inherent fluorescent properties of some nanoparticles allow for facile analysis of uptake such as the recently reported uptake of quantum dots (QDs) by stem cells. (128) Recent advances in confocal light collection, such as spinning-disk confocal microscopy, can be used to monitor the trafficking of quantum dot trajectories (129) within cells with sub-second time resolution.

5. ANALYSIS OF CARBON NANOPARTICLES

In this section we will focus mainly in the analysis of fullerenes and carbon nanotubes since they are the most used carbon-based nanoparticles. Both carbon nanotubes and fullerenes are poor soluble in water, however, nanoparticles of the fullerene aggregates can be prepared in water solutions and carbon nanotubes are dispersed in water in the presence of surfactants. The concentrations of these nanoparticles in biological media as consequence of systemic exposure could be very low, so trace analysis will be required in order to develop toxicological studies of the nanomaterials.

While a range of methods is accessible for detection and characterization of nanomaterials, a number of challenges will arise when analyzing these materials in biological matrices due to the analytical artifacts caused by sample preparation, lack of reference materials and the matrix of the sample. Few methods have been reported.

One of the proposed methods for trace analysis of fullerenes in biological samples is a simplified liquid-liquid extraction (LLE) prior to high-performance liquid chromatography (130). LLE is a usually choice in method development for complicated biological samples, although it has a longstanding problem, emulsion. Xia *et al.* have demonstrated that the conventional LLE protocols could not be used for trace analysis of fullerenes in biological samples. The large amounts of proteins, lipids and surfactants in the biological samples would create a heavy emulsion that will interfere with the extraction. The emulsion problem is overcome by adding glacial acetic acid in order to solubilize the proteins and surfactants without causing significative analyte losses. Magnesium perchlorate was used to destabilize the nano-C_{60} particles in the water solution and promoted the solvent extraction since it is known that nano-C_{60} in water is difficult to be extracted back into the toluene phase. They found a serious drop in extraction efficiency at low concentrations. Several reasons could cause this problem including protein adsorption, glassware adsorption, or reconstitution loss. The glassware adsorption could cause analyte loss and extraction efficiency drop in trace analysis of small chemicals. Deactivation of the glassware is a routine practice to reduce the analyte loss. It was observed that glassware silanization only provided limited improvement. The extraction efficiency drop could be due to the C_{60} adsorption to the glassware or aggregation during dryness evaporation, which could not be re-dissolved into toluene even under the sonication. The procedures of evaporation to dryness and reconstitution with a solvent are routine practice in chemical analysis; however, it cannot be used for trace analysis of fullerenes in biological and environmental samples. This method was evaluated with samples of BSA media and porcine plasma.

Quantification of C_{60} can be performed by UV-vis measurements for high concentrations, with absorption bands of C_{60} are located at 336, 407, 540, and 595 nm. HPLC is used for the detection of low concentrations of C_{60} (131) but also a method for the direct analysis by electrospray time-of-flight mass spectrometry has been developed (132).

Optical spectroscopies are particularly useful for the analysis of homogeneous samples containing one type of nanoparticles. However, samples subjected to the analysis are often composed of populations of polydispersed nanoparticles. Often it is necessary to study parameters such as size, shape and surface modification. If separation of subpopulations of nanoparticles present in the sample is required, application of techniques such as chromatography or electrophoresis should be considered (133). For example, fullerenes can readily be separated and quantified by LC coupled to ESI-MS (134).

Electrophoresis has been also employed for the separation of single-walled carbon nanotubes, based on tube length (135), or diameter-selective CE separation for bundle and individual carbon nanotubes (136). Gel electrophoresis has been useful for the separation of Nucleic acid-carbon nanotube complexes (137). Recently, Wang *et al.* (138) have developed a method to measure the concentration of SWNTs extracted from biological tissue. They employed polyacrylamide gel electrophoresis (PAGE) followed by quantification of SWNT bands by measure of the intensity from digitized images of these bands, which showed to be proportional to the amount of SWNTs loaded onto the gel. Normal rat kidney cells in culture were allowed to take up SWNTs upon exposure to medium containing various concentrations of BSA-SWNTs for different times and temperatures. When BSA-SWNT dispersions were subjected to sodium dodecyl sulfate (SDS)-PAGE, BSA passed through the stacking gel, entered the resolving gel, and migrated towards the anode; however, SWNTs accumulated in a sharp band at the interface between the loading wall and the stacking gel.

It has been also demonstrated the suitability of the combination of capillary electrophoresis with Raman spectroscopy or with scanning probe microscopy for individual single-walled carbon nanotube analysis (139). This system could be applied to the characterization of nanoparticles extracted from biological samples.

CNT quantification is normally performed by UV-vis spectrometry at high concentrations (140, 141). CNT show strong absorption in the UV and visible region with peaks at 253 nm, 266 nm, 350 nm and strong absorption extending up to 1200 nm (142). Wrapping of CNT with conventional fluorophores, fluorescent polymers or with DNA- oligonucleotides allowed their detection in

biomedical applications. SEC with UV-detection was used for the separation of CNT from impurities and amorphous carbon.

A work evaluated extraction of carboxylic CNTs from surface water using a filter modified with MWCNTs as a preconcentrator (65). To prepare the filters, a dispersion of MWCNTs prepared in Triton X100 was filtered through a nylon filter with a pore size of 0.45 μm. They were then washed with methanol and dried under air stream. MWCNTs can interact with SWCNTs through π-π interactions, showing a high capacity to adsorb SWCNTs. Similar methodologies can be adapted to be applied to biological samples.

6. ANALYSIS OF NANODRUG AND NANO FOOD-ADDITIVES

Two aspects of nanotoxicology should be mentioned, which concerns the safety of nanodrugs. First, the patients will use the nanodrug particles, and it explains the need to examine their toxic profile not only in healthy organism, but also on animal models of particular diseases. Second, the wide spread of the genetic polymorphisms in human population suggests that some part of this population can be extremely sensitive to the action of nanoparticles (143). This hypothesis agrees with the preliminary data on the role of oxidative stress in the realization of the toxic effects of nanoparticles and genetically determined heterogeneity of the antioxidant and other protective systems (144).

7. FUTURE TRENDS

One challenge for analytical chemists is the area of nanoparticle characterization. Firstly, most of the current techniques used for physical nanoparticle characterization provide only limited information before or after engineered nanoparticles are in the biological environment. Next years, it is expected to develop new methods to extract and preconcentrate the nanoparticles from the biological matrix before their physic-chemical characterization. Electron microscopy, scanning probe microscopy, and dynamic light scattering are techniques commonly used for this purpose, which reveal nanoparticle size and distribution but give no information about aggregation/agglomeration that may occur within the biological environment, adsorption of biomolecules, or degradation of the nanoparticle itself (biotransformation), all of which may change during residence within the biological system and would greatly influence nanoparticle uptake and/or behavior. These concerns suggest the future development of a new area among the field of nanotoxicology, which could be called as "nanomaterial speciation". It will be focus in the determination of the nanoparticles taking into account their possible biotransformation. So, it is expected that nanoparticles having different biocoatings will have different effects from the toxicological point of view.

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9. REFERENCES

1. http://ec.europa.eu/research/quality-of-life/ka4/pdf/report_nanopathology_en.pdf

2. Alexandra Navrotsky: Energetic clues to pathways to biomineralization: Precursors, clusters, and nanoparticles. *Proc. Natl. Acad. Sci. USA* 101, 12096–12101 (2004)

3. John M Zachara, Steve M Heald, Byong-Hun Jeon, Ravi K Kukkadapu, Chongxuan Liu, James P McKinley, Alice C Dohnalkova, Dean A Moore: Reduction of pertechnetate (Tc (VII)) by aqueous Fe (II) and the nature of solid phase redox products. *Geochim Cosmochim Acta* 71, 2137–2157 (2007)

4. Luann Becker, Robert J Poreda, Jeffrey L Bada: Extraterrestrial helium trapped in fullerenes in the Sudbury impact structure. *Science* 272, 249-252 (1996)

5. D Heymann, L W Jenneskens, J Jehlicka, C Koper, E Vlietstra: Terrestrial and extraterrestrial fullerenes. *Fuller Nanotub Carbon Nanostruct* 11, 333-370 (2003)

6. Jillian F Banfield, Hengzhong Zhang: Nanoparticles in the environment. *Reviews in Mineralogy & Geochemistry* 44, 1-58 (2001)

7. Glenn A Waychunas, Christopher S Kim, Jillian F Banfield: Nanoparticulate iron oxide minerals in soils and sediments: unique properties and contaminant scavenging mechanisms. *J Nanopart Res* 7, 409-433 (2005)

8. Cort Anastasio, Scot T Martin: Atmospheric nanoparticles. *Reviews in Mineralogy & Geochemistry* 44, 293-349 (2001)

9. Michael F Hochella, Andrew S Madden: Earth's nano-compartment for toxic metals. *Elements* 1, 199-203 (2005)

10. Dennis A Bazylinski, Richard B Frankel: Magnetosome formation in prokaryotes. *Nat Rev Microbiol* 2, 217–230 (2004)

11. Ellen E. Connor, Jugith Mwamuka, Anand Gole, Catherine J. Murphy, Michael D. Wyatt: Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small* 1, 325–327 (2005)

12. Kelly BéruBé, Dominique Balharry, Keith Sexton, Lata Koshy, Tim Jones: Combustion-derived nanoparticles: mechanisms of pulmonary toxicity. *Clin Exp Pharmacol Physiol* 34, 1044-1050 (2007)

13. Fathi Zereini, Clare Wiseman, Friedrich Alt, Juergen Messerschmidt, Juergen Muller, Hans Urban: Platinum and rhodium concentrations in airborne particulate matter in

Germany from 1988 to 1998. *Environ Sci Technol* 35, 1996-2000 (2001)

14. Christof M Niemeyer: Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science. *Angew Chem Int Ed* 40 (22), 4128–58 (2001)

15. Guozhong Cao: Nanostructures and nanomaterials. Synthesis, Properties & Applications. *Imperial College Press*; London (2004)

16. Vincent Rotello: Nanoparticles: Building Blocks for Nanotechnology. *Springer*, NY (2003)

17. Peter J F Harris: Carbon nanotubes and related structures: New materials for the 21st Century. *Cambridge University Press*, Cambridge (1996)

18. Hongjie Dai: Carbon nanotubes: synthesis, integration, and properties. *Acc Chem Res* 35, 1035-1044 (2002)

19. C Journet, W K Matser, P Bernier, L Laiseau, S Lefrant, P Deniard, R Lee, J E Fischer: Large-scale production of single-walled carbon nanotubes by the electric-arc technique. *Nature* 388, 756-758 (1997)

20. Andreas Thess, Roland Lee, Pavel Nikolaev, Hongjie Dai, Pierre Petit, Jerome Robert, Chunhui Xu, Young Hee Lee, Seong Gon Kim, Andrew G Rinzler, Daniel T Colbert, Gustavo E Scuseria, David Tománek, John E Fischer, Richard E Smalley: Crystalline ropes of metallic carbon nanotubes. *Science* 276, 483-487 (1996)

21. Emmanuel Flahaut, Christphe Laurent, Alain Peigney: Catalytic CVD synthesis of double and triple-walled carbon nanotubes by the control of the catalyst preparation. *Carbon* 43, 375-383 (2005)

22. Linda Vaisman, H Daniel Wagner, Gad Marom: The role of surfactants in dispersion of carbon nanotubes. *Adv Colloid Interface Sci* 128-130, 37-46 (2006)

23. Bumsu Kim, Yun-Ho Lee, Jee-Hyun Ryu, Kyung-Do Suh: Enhanced colloidal properties of single-wall carbon nanotubes in α-terpineol and Texanol. *Colloid Surf A* 273, 161-164 (2006)

24. Vasilios Georgakilas, Konstantinos Kordatos, Maurizio Prato, Dirk M Guldi, Michael Holzinger, Andreas Hirsch: Organic functionalization of carbon nanotubes. *J Am Chem Soc* 124, 760-761 (2002)

25. Ray H Baughman, Changxing Cui, Anvar A Zakhidov, Zafar Iqbal, Joseph N Barisci, Geoff M Spinks, Gordon G Wallace, Alberto Mazzoldi, Danilo De Rossi, Andrew G Rinzler, Oliver Jaschinski, Siegmar Roth, Miklos Hertesz. Carbon nanotube actuators. *Science* 284, 1340-1344 (1999)

26. Miguel Valcárcel, Soledad Cárdenas, Bartolomé M Simonet, Yolanda Moliner-Martínez, Rafael Lucena: Carbon nanostructures as sorbent materials in analytical processes. *Trends Anal Chem* 27, 34-42 (2008)

27. Fred Wudl: Fullerene materials. *J Mater Chem* 12, 1959-1963 (2002)

28. Maurizio Prato: Fullerene Materials. *Top Curr Chem* 199, 173-187 (1999)

29. Travis Jennings, Geoffrey Strouse: Past, present, and future of gold nanoparticles. *Adv Exp Med Biol* 620, 34–47 (2007)

30. Jose Ruben Morones, Jose Luis Elechiguerra, Alejandro Camacho, Katherine Holt, Juan B Kouri, Jose Tapia Ramirez, Miguel Jose Yacaman: The bactericidal effect of silver nanoparticles. *Nanotechnol* 16, 2346-2353 (2005)

31. Mathias Brust, Christopher J Kiely: Some recent advances in nanostructure preparation from gold and silver particles: a short topical review. *Colloids Surf A* 202, 175-186 (2002)

32. Wei-Xian Zhang: Nanoscale iron particles for environmental remediation: an overview. *J Nanopart Res* 5, 323-332 (2003)

33. R J Aitken, M Q Chaudhry, A B A Boxall, M Hull: Manufacture and use of nanomaterials: current status in the UK and global trends. *Occup Med* 56, 300-306 (2006)

34. M N Rittner: Market analysis of nanostructured materials. *Am Ceram Soc Bull* 81, 33-36 (2002)

35. Douglas C Hague, Merrilea J Mayo: Controlling crystallinity during processing of nanocrystalline titania. *J Am Ceram Soc* 77, 1957–1960 (1994)

36. S R Dhage, R Pasricha, V Ravi: Synthesis of ultrafine TiO2 by citrate gel method. *Mat Res Bull* 38, 1623–1628 (2003)

37. O Larlus, S Mintova, T Bein: Environmental syntheses of nanosized zeolites with high yield and monomodal particle size distribution. *Micropor Mesopor Mater* 96, 405-412 (2006)

38. Dana Yaron-Marcovich, Yona Chen, Shlomo Nir, Rene Prost: High resolution electron microscopy structural studies of organo-clay nanocomposites. *Environ Sci Technol* 39, 1231-1238 (2005)

39. Markys Cain, Roger Morrell: Nanostructured ceramics: a review of their potential. *Appl Organomet Chem* 15, 321-330 (2001)

40. Vladimir V Pokropivnyi: Non-carbon nanotubes (review). Part 2. Types and structure. *Powder Metallurgy and Metal Ceramics* 40, 582-594 (2001)

41. Joanna Koziara, Paul R Lockman, David D Allen, Russell J Mumper: *In situ* blood-brain barrier transport of nanoparticles. *Pharm Res* 20, 1772-1778 (2003)

42. Ju-Young Kim, Sun-Bo Shim, Jin-Kie Shim: Enhanced desorption of phenanthrene from aquifer sand using

amphiphilic anionic polyurethane nanoparticles. *J Ind Eng Chem* 10, 1043-1051 (2004)

43. Yinhui Xu, Dongye Zhao: Removal of copper from contaminated soil by use of poly (amidoamine) dendrimers. *Environ Sci Technol* 39, 2369-2375 (2005)

44. Manoj Nirmal, Louis Brus: Luminescence photophysics in semiconductor nanocrystals. *Acc Chem Res* 32, 407-414 (1999)

45. Matthias Seydack: Nanoparticle labels in immunosensing using optical detection methods. *Biosens Bioelectron* 20, 2454-2469 (2005)

46. Jose Manuel Costa-Fernandez, Rosario Pereiro, Alfredo Sanz Medel: The use of luminescent quantum dots for optical sensing. *Trends Anal Chem* 25, 207-218 (2006)

47. Hedi Mattoussi, J Matthew Mauro, Ellen R Goldman, George P Anderson, Vikram C Sundar, Frederic V Mikulec, Moungi G Bawendi: Self-assembly of CdSe-ZnS quantum dot bioconjugates using an engineered recombinant protein. *J Am Chem Soc* 122, 12142-12150 (2000)

48. C B Murray, D J Norris, M G Bawendi: Synthesis and characterization of nearly monodisperse CdE (E = sulfur, selenium, tellurium) semiconductor nanocrystallites. *J Am Chem Soc* 115, 8706-8715 (1993)

49. Kara Morgan: Development of a Preliminary Framewotk for Informing the Risks Analysis and Risk Management of Nanoparticles. *Risk Anal* 25, 1-15 (2005)

50. Joyce S Tsuji, Andrew D Maynard, Paul C Howard, John T James, Chiu-Wing Lam, David B Warheit, Annette B Santamaria: Research Strategies for Safety Evaluation of Nanomaterials, Part IV: Risk Assesment of Nanoparticles. *Toxicol Sci* 88, 12-17 (2006)

51. Robert H Hurt, Marc Monthioux, Agnes Kane: Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue. *Carbon* 44 1028-1033 (2006)

52. Karluss Thomas, Philip Sayre: Research strategies for safety evaluation of nanomaterials, Part I: Evaluating Human Health Implications for exposure to Nanomaterials. *Toxicol Sci* 87, 316-321 (2005)

53. Nastassja Lewinski, Vicki Colvin, Rebekah Drezek: Cytotoxicity of nanoparticles. *Small* 4, 26-49 (2008)

54. Wei Fu, Dinesh Shenoy, Jane Li, Curtis Crasto, Graham Jones, Charles Dimarzio, Srinivas Sridhar, Mansoor Amiji: Biomedical applications of gold nanoparticles functionalized using hetero-bifunctional poly (ethylene glycol) spacer. *Mater Res Soc Symp Proc* 845, 223-228 (2005)

55. Dinesh Shenoy, Wei Fu, Jane Li, Curtis Crasto, Graham Jones, Charles Dimarzio, Srinivas Sridhar, Mansoor Amiji: Surface functionalization of gold nanoparticles using hetero-bifunctional poly (ethylene glycol) spacer for intracellular tracking and delivery. Int J Nanomed 1, 51–57 (2006)

56. Aliasger K Salem, Peter C Searson, Kam W Leong: Multifunctional nanorods for gene delivery. Nat Mater 2, 668–671 (2003)

57. Massimo Bottini, Shane Bruckner, Konstantina Nika, Nunzio Bottini, Stefano Bellucci, Andrea Magrini, Antonio Bergamaschi, Tomas Mustelin.: Multi-walled carbon nanotubes induce T lymphocyte apoptosis. Toxicol Lett 160, 121–126 (2006)

58. Arnaud Magrez, Sandor Kasas, Valérie Salicio, Nathalie Pasquier, Jin Won Seo, Marco Celio, Stefan Catsicas, Beat Schwaller, Lázló Forró: Cellular Toxicity of Carbon-Based Nanomaterials. Nano Lett 6, 1121–1125 (2006)

59. Christian Kirchner, Tim Liedl, Stefan Kudera, Teresa Pellegrino, Almudena Munoz Javier, Hermann E Gaub, Sonja Stolzle, N Fertig, Wolfgang J Parak: Cytotoxicity of Colloidal CdSe and CdSe/ZnS Nanoparticles. Nano Lett 5, 331–338 (2005)

60. Akiyoshi Hoshino, Kouki Fujioka, Taisuke Oku, Masakazu Suga, Yu F Sasaki, Toshihiro Ohta, Masato Yasuhara, Kazuo Suzuki, Kenji Yamamoto: Physicochemical Properties and Cellular Toxicity of Nanocrystal Quantum Dots Depend on Their Surface Modification. Nano Lett 4, 2163–2169 (2004)

61. Catherine M Goodman, Catherine D. McCusker, Tuna Yilmaz, Vincent M Rotello: Toxicity of Gold Nanoparticles Functionalized with Cationic and Anionic Side Chains. Bioconjugate Chem 15, 897–900 (2004)

62. Gemma Reguera, Kevin D McCarthy, Teena Mehta, Julie S Nicoll, Mark T Tuominen, Derek R Lovley: Extracellular Electron Transfer Via Microbial Nanowires. Nature 453, 1098-1101 (2005)

63. Peter Wick, Pius Manser, Ludwing K Limbach, Ursula Dettlaff-Weglikowska, Frank Krumeich, Siegmar Roth, Wendelin J Stark, Arie Bruinink: The Degree and Kind of Agglomeration Affect Carbon Nanotube. Toxicol Lett 168, 121-131 (2007)

64. Furong Tian, Daxiang X Cui, Heinz Schwarz, Giovani Gomez Estrada, Hisatashi Kobayashi: Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. Toxicol In vitro 20, 1202–1212 (2006)

65. Beatriz Suarez, Yolanda Moliner-Martinez, Soledad Cardenas, Bartolome M Simonet, Miguel Valcarcel: Monitoring of Carboxylic Carbon Nanotubes in Surface Water by Using Multiwalled Carbon Nanotube-Modified Filter As Preconcentration Unit. *Environ Sci Technol* 42, 6100-6104 (2008)

66. Bryce J Marquis, Sara A Love, Katherine L Braun, Christy L Haynes: Analytical methods to assess nanoparticle toxicity. *Analyst* 134, 425–439 (2009)

67. Andrew D Maynard: Overview of methods for analyzing single ultrafine particles. *Phil Trans R Soc Lond A* 358, 2593-2610 (2000)

68. Barbara Rothen-Rutishauser, Christian Muhlfeld, Fabian Blank, Claudia Musso, Peter Gehr: Translocation of particles and inflammatory responses after exposure to fine particles and nanoparticles in an epithelial airway model. *Part Fibre Toxicol* 4, 9 (2007)

69. Chen-Luh Lin, Jan D Miller: The development of a PC image based on-line particle size analyzer. *Miner Metall Proc* 2, 29–35 (1993)

70. Christian Mühlfeld, Barbara Rothen-Rutishauser, Dimitri Vanhecke, Fabian Blank, Peter Gehr, Matthias Ochs: Visualization and quantitative analysis of nanoparticles in the respiratory tract by transmission electron microscopy. *Part Fibre Toxicol* 4, 11 (2007)

71. Barbara Pergolese, Adriano Bigotto, Maurizio Muniz-Miranda, Giuseppe Sbrana: Gold/palladium and silver/palladium colloids as novel metallic substrates for surface enhanced Raman scattering. *Appl Spectrosc* 59, 194-9 (2005)

72. P V Asharani, Yi Lian Wu, Zhiyuan Gong, Suresh Valiyaveettil: Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology* 19, 255102 (2008)

73. Alexandra E Porter, Mhairi Gass, Karin Muller, Jeremy N Skepper, Paul A Midgley, Mark Welland: Direct imaging of single-walled carbon nanotubes in cells. *Nat Nanotechnol* 2, 713–717 (2007)

74. Vaishali Bagalkot, Liangfang Zhang, Etgar Levy-Nissenbaum, Sangyong Jon, Philip W Kantoff, Robert Langer and Omid C. Farokhzad: Quantum Dot-Aptamer Conjugates for Synchronous Cancer Imaging, Therapy, and Sensing of Drug Delivery Based on Bi-fluorescence Resonance Energy Transfer. *Nano Lett* 7, 3065–3070 (2007)

75. Evangelia Chnari, Jessica S Nikitczuk, Kathryn E Uhrich, Prabhas V Moghe: Nanoscale Anionic Macromolecules Can Inhibit Cellular Uptake of Differentially Oxidized LDL. *Biomacromolecules* 7, 597–603 (2006)

76. Shigeo Maruyama, Yuhei Miyauchi, Yoichi Murakami, Shohei Chiashi: Optical characterization of single-walled carbon nanotubes synthesized by catalytic decomposition of alcohol. *New Journal of Physics* 5, 149.1–149.12 (2003)

77. Bailin Chen, John P Selegue: Separation and Characterization of Single-Walled and Multiwalled Carbon Nanotubes by Using Field-Flow Fractionation. *Anal Chem* 74, 4774-4780 (2002)

78. Jie Liu, Andrew G Rinzler, Hongjie Dai, Jason H Hafner, R Kelley Bradley, Peter J Boul, Adrian Lu, Terry Iverson, Konstantin Shelimov, Chad B Huffman, Fernando Rodriguez-Macias, Young-Seok Shon, T Randall Lee, Daniel T Colbert, Richard E Smalley: Fullerene pipes. *Science* 280, 1253–1256 (1998)

79. J Calvin Giddings, S Kim Ratanathanawongs, Bhajendra N Barman, Myeong Hee Moon, Guangyue Liu, Brenda L Tjelta, Marcia E Hansen: Characterization of colloidal and particulate silica by field-flow fractionation. *Advances in Chemistry* 234, 309–340 (1994)

80. Soheyl Tadjiki, Shoeleh Assemi, Cassandra E Deering, John M. Veranth, Jan D Miller: Detection, separation, and quantification of unlabeled silica nanoparticles in biological media using sedimentation field-flow fractionation. *J Nanopart Res* 11, 981-988 (2009)

81. Pratim Biswas, Chang-Yu Wu: Nanoparticles and the Environment. *J Air Waste Manage Assoc* 55, 708-746 (2005)

82. Kevin W Powers, Scott C Brown, Vijay B Krishna, Scott C Wasdo, Brij M Moudgil, Stephen M Roberts: Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. *Toxicol Sci* 90, 296-303 (2006)

83. Richard C Murdock, Laura Braydich-Stolle, Amanda M Schrand, John J Schlager, Saber M Hussain: Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique. *Toxicol Sci* 101, 239-53 (2008)

84. Joanna Szpunar: Bio-inorganic speciation analysis by hyphenated techniques. *Analyst* 125, 963–988 (2000)

85. Kunnath S Subramanian: Determination of metals in biofluids and tissues: sample preparation methods for atomic spectroscopy techniques. *Spectrochim Acta Part B* 51, 291–319 (1996)

86. P H McMurry: A Review of Atmospheric Aerosol Measurements. *Atmospheric Environ* 34, 1959-1999 (2000)

87. William W Yu, Emmanuel Chang, Joshua C Falkner, Junyan Zhang, Ali M Al-Somali, Christie M Sayes, Judah Johns, Rebekah Drezek, Vicki L Colvin: Forming biocompatible and nonaggregated nanocrystals in water using amphiphilic polymers. *J Am Chem Soc* 129, 2871–2879 (2007)

88. A Nemmar, P H M Hoet, B Vanquickenborne, D Dinsdale, M Thomeer, M F Hoylaerts, H Vanbilloen, L Mortelmans, B Nemery: Passage of inhaled particles into the blood circulation in humans. *Circulation* 105, 411–414 (2002)

89. Jun Sung Kim, Tae-Jong Yoon, Kyeong Nam Yu, Byung Gul Kim, Sung Jin Park, Hyun Woo Kim, Kee Ho Lee, Seung Bum Park, Jin-Kyu Lee, Myung Haing Cho: Toxicity and tissue distribution of magnetic nanoparticles in mice. *Toxicol Sci* 89, 338–347 (2006)

90. Jung-Taek Kwon, Soon-Kyung Hwang, Hua Jin, Dae-Seong Kim, Arash Minai-Tehrani, Hee-Jeong Yoon, Mansoo Choi, Tae-Jong Yoon, Duk-Young Han, Young-Woon Kang, Byung-Il Yoon, Jin-Kyu Lee, Myung-Haing Cho: Body distribution of inhaled fluorescent magnetic nanoparticles in the mice. *J Occup Health* 50, 1–6 (2008)

91. Isaac Stayton, Jeffrey Winiarz, Katie Shannon, Yinfa Ma: Study of uptake and loss of silica nanoparticles in living human lung epithelial cells at single cell level. *Anal Bioanal Chem* 394, 1595-1608 (2009)

92. Xinli Xing, Xiaoxiao He, Jiaofeng Peng, Kemin Wang, Weihong Tan: Uptake of silica-coated nanoparticles by HeLa cells. *J Nanosci Nanotechnol* 5, 1688–1693 (2005)

93. Chen-Wen Lu, Yann Hung, Jong-Kai Hsiao, Ming Yao, Tsai-Hua Chung, Yu-Shen Lin, Si-Han Wu, Szu-Chun Hsu, Hon-Man Liu, Chung-Yuan Mou, Chung-Shi Yang, Dong-Ming Huang, Yao-Chang Chen: Bifunctional Magnetic Silica Nanoparticles for Highly Efficient Human Stem Cell Labeling. *Nano Lett* 7, 149–154 (2007)

94. Sehoon Kim, Haridas E Pudavar, Adela Bonoiu, Paras N Prasad: Aggregation-enhanced fluorescence in organically modified silica nanoparticles: a novel approach toward high-signal-output nanoprobes for two-photon fluorescence bioimaging. *Adv Mater* 19, 3791–3795 (2007)

95. David J Burleson, Michelle D Driessen, R Lee Penn. On the characterization of environmental nanoparticles. *J Environ Sci Health Part A* 39, 2707-2753 (2004)

96. Charlie Yu Ming Hsu, Hasan Uludag: Effects of size and topology of DNA molecules on intracellular delivery with non-viral gene carriers. *BMC Biotechnol* 8, 23 (2008)

97. Jean Pierre Clamme, Joel Azoulay, Yves Mely: Monitoring of the formation and dissociation of polyethylenimine/DNA complexes by two photon fluorescence correlation spectroscopy. *Biophys J* 84, 1960–1968 (2003)

98. Jong-Yuh Cherng, Petra van de Wetering, Herre Talsma, Daan J Crommelin, Wim E Hennink: Effect of size and serum proteins on transfection efficiency of poly ((2-dimethylamino) ethyl methacrylate)-plasmid nanoparticles. *Pharm Res* 13, 1038–1042 (1996)

99. Huan Xie, Kelly L Gill-Sharp, D Patrick O'Neal: Quantitative estimation of gold nanoshell concentrations in whole blood using dynamic light scattering. *Nanomedicine: Nanotechnology, Biology, and Medicine* 3, 89-94 (2007)

100. Haruhisa Kato, Mie Suzuki, Katsuhide Fujita, Masanori Horie, Shigehisa Endoh, Yasukazu Yoshida, Hitoshi Iwahashi, Kayori Takahashi, Ayako Nakamura, Shinichi Kinugasa: Reliable size determination of nanoparticles using dynamic light scattering method for *in vitro* toxicology assessment, *Toxicol In vitro* 23, 927-934 (2009)

101. Marina A Dobrovolskaia, Anil K Patri, Jiwen Zheng, Jeffrey D Clogston, Nader Ayub, Parag Aggarwal, Barry W Neun, Jennifer B Hall, Scott E McNeil: Interaction of colloidal gold nanoparticles with human blood: effects on particle size and analysis of plasma protein binding profiles. *Nanomedicine: Nanotechnology, Biology, and Medicine* 5, 106-117 (2009)

102. Francesca Filon Laresea, Flavia D'Agostin , Matteo Crosera, Gianpiero Adami, Nadia Renzi, Massimo Bovenzi, Giovanni Maina: Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology* 255, 33–37 (2009)

103. Matthias Hanauer, Sebastien Pierrat, Inga Zins, Alexander Lotz, Carsten Sonnichsen: Separation of Nanoparticles by Gel Electrophoresis According to Size and Shape. *Nano Lett* 7, 2881 – 2885 (2007)

104. Xiaoyou Xu, Kenneth Caswell, Elizabeth Tucker, Saswat Kabisatpathy, K Lisa Brodhacker, Walter A Scrivens: Size and shape separation of gold nanoparticles with preparative gel electrophoresis. *J Chromatogr A*, 1167, 35 – 41 (2007)

105. Andreas Helfrich, Wolfram Bruechert, Jorg Bettmer: Size characterisation of Au nanoparticles by ICP-MS coupling techniques. *J Anal At Spectrom* 21, 431 – 434 (2006)

106. Ursula Schnabel, Christian-Herbert Fischer, Ernst Kenndler: Characterization of colloidal gold nanoparticles according to size by capillary zone electrophoresis. *J Microcolumn Sep* 9, 529 – 534 (1997)

107. Fu-Ken Liu, Guor-Tzo Wei: Adding sodium dodecylsulfate to the running electrolyte enhances the separation of gold nanoparticles by capillary electrophoresis. *Anal Chim Acta* 510, 77 – 83 (2004)

108. Fu-Ken Liu, Ying-Ying Lin, Chien-Hou Wu: Highly efficient approach for characterizing nanometer-sized gold particles by capillary electrophoresis. *Anal Chim Acta* 528, 249 –254 (2005)

109. Fu-Ken Liu: A high-efficiency capillary electrophoresis-based method for characterizing the sizes of Au nanoparticles. *J Chromatogr A* 1167, 231 – 235 (2007)

110. Fu-Ken Liu, Fu-Hsiang Ko: Separation and study of the optical properties of silver nanocubes by capillary electrophoresis. *Chem Lett* 33, 902 – 903 (2004)

111. Fu-Ken Liu, Fu-Hsiang Ko, Pei-Wen Huang, Chien-Hou Wu, Tieh-Chi Chu: Studying the size/shape separation and optical properties of silver nanoparticles by capillary electrophoresis. *J Chromatogr A* 1062, 139 – 145 (2005)

112. Natalia G Vanifatova, Boris Ya Spivakov, Juergen Mattusch, Ulrich Franck, Rainer Wennrich: Investigation of iron oxide nanoparticles by capillary zone electrophoresis. *Talanta* 66, 605 – 610 (2005)

113. Chung Keung Lo, Man Chin Paau, Dan Xiao, Martin M F Choi: Capillary Electrophoresis, Mass Spectrometry, and UV-Visible Absorption Studies on Electrolyte-Induced Fractionation of Gold Nanoclusters. *Anal Chem* 80, 2439 – 2446 (2008)

114. Xiangyi Huang, Jifang Weng, Fuming Sang, Xingtao Song, Chengxi Cao, Jicun Ren: Characterization of quantum dot bioconjugates by capillary electrophoresis with laser-induced fluorescent detection. *J Chromatogr A* 1113, 251 – 254 (2006)

115. Mark Pereira, Edward P C Lai, Bryan Hollebone: Characterization of quantum dots using capillary zone electrophoresis. *Electrophoresis* 28, 2874 – 2881 (2007)

116. Ute Pyell: CE characterization of semiconductor nanocrystals encapsulated with amorphous silicium dioxide. *Electrophoresis* 29, 576 – 589 (2008)

117. C Quang, Steven L Petersen, G R Ducatte, Nathan E Ballou: Characterization and separation of inorganic fine particles by capillary electrophoresis with an indifferent electrolyte system. *J Chromatogr A* 732, 377 – 384 (1996)

118. Steven L Petersen, Nathan E Ballou: Separation of micrometer-size oxide particles by capillary zone electrophoresis. *J Chromatogr A* 834, 445 – 452 (1999)

119. E V B van Gaal, G Spierenburg, W E Hennink, D J A Crommelin, E Mastrobattista: Flow cytometry for rapid size determination and sorting of nucleic acid containing nanoparticles in biological fluids. *J Control Release* 141, 328–338 (2010)

120. Yu Pan, Sabine Neuss, Annika Leifert, Monika Fischler, Fei Wen, Ulrich Simon, Guenter Schmid, Wolfgang Brandau, Willi Jahnen-Dechent: Size-dependent cytotoxicity of gold nanoparticles. *Small* 3, 1941–1949 (2007)

121. Wim H De Jong, Werner I Hagens, Petra Krystek, Marina C Burger, Adriënne J A M Sips, Robert E Geertsma: Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials* 29, 1912-1919 (2008)

122. Tanya S Hauck, Arezou A Ghazani, Warren C W Chan: Assesing the effect of surface chemistry on gold nanorod uptake, toxicity, and gene expression in mammalian cells. *Small* 4, 153–159 (2008)

123. B Devika Chithrani, Warren C W Chan: Elucidating the Mechanism of Cellular Uptake and Removal of Protein-Coated Gold Nanoparticles of Different Sizes and Shapes. *Nano Lett* 7, 1542–1550 (2007)

124. Joseph A Ryan, K Wesley Overton, Molly E Speight, Chistine N Oldenburg, Lina Loo, Wayne Robarge, Stefan Franzen, Daniel L Feldheim: Cellular Uptake of Gold Nanoparticles Passivated with BSA-SV40 Large T Antigen Conjugates. *Anal Chem* 79, 9150–9159 (2007)

125. Swanand Patil, Amanda Sandberg, Eric Heckert, William Self, Sudipta Seal: Protein Adsorption and Cellular Uptake of Cerium Oxide Nanoparticles as a function of zeta Potential. *Biomaterials* 28, 4600–4607 (2007)

126. Shy Chyi Wuang, Koon Gee Neoh, En-Tang Kang, Daniel W Pack, Deborah E Leckband: HER-2-mediated Endocytosis of Magnetic Nanospheres and the Implications in Cell Targeting and Particle Magnetization. *Biomaterials* 29, 2270–2279 (2008)

127. B. Devika Chithrani, Arezou A Ghazani, Warren C W Chan: Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells. *Nano Lett* 6, 662–668 (2006)

128. Oleksandr Seleverstov, Olga Zabirnyk, Mattias Zscharnack, Larysa Bulavina, Marcin Nowicki, Jean-Michael Heinrich, Maksym Yezhelyev, Frank Emmrich, Ruth O'Regan, Augustinus Bader: Quantum Dots for Human Mesenchymal Stem Cells Labeling. A size-Dependent Autophagy Activation. *Nano Lett* 6, 2826–2832 (2006)

129. Gang Ruan, Amit Agrawal, Adam I Marcus, Shuming Nie: Imagin and Tracking of Tat Peptide-Conjugated Quantum Dots in Living Cells: New Insights into Nanoparticle Uptake, Intracellular Transport, and Vesicle Shedding. *J Am Chem Soc* 129, 14759–14766 (2007)

130. Xin-Rui Xia, Nancy A Monteiro-Riviere, Jim E Riviere: Trace analysis of fullerenes in biological samples by simplified liquid-liquid extraction and high-performance liquid chromatography. *J Chromatogr A* 1129, 216-222 (2006)

131. J D Fortner, D Y Lyon, C M Sayes, A M Boyd, J C Falkner, E M Hotze, L B Alemany, Y J Tao, W Guo, K D Ausman, V L Colvin, J B Hughes: C_{60} in water: nanocrystal formation and microbial response. *Environ Sci Technol* 39, 4307-4316 (2005)

132. V Kozlovski, V Brusov, I Sulimenkov, A Pikhtelev, A Dodonov: Novel experimental arrangement developed for direct fullerene analysis by electrospray time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom* 18, 780-786 (2004)

133. Mark C Hersam: Progress towards monodisperse single-walled carbon nanotubes. *Nat Nanotechnol* 3, 387 – 394 (2008)

134. Carl W Isaacson, Crystal Y Usenko, Robert L Tanguay, Jennifer A Field: Quantification of Fullerenes by LC/ESI-MS and Its Application to *in vivo* Toxicity Assays. *Anal Chem* 79, 9091 – 9097 (2007)

135. Stephen K Doorn, Robert E Fields, Hui Hu, Mark A Hamon, Robert C Haddon, John P Selegue, Vahid Majidi: High resolution capillary electrophoresis of carbon nanotubes. *J Am Chem Soc* 124, 3169 – 3174 (2002)

136. Stephen K Doorn, Michael S Strano, Michael J O'Connell, Erik H Haroz, Kristy L Rialon, Robert H

Hauge, Richard E Smalley: Capillary Electrophoresis Separations of Bundled and Individual Carbon Nanotubes. *J Phys Chem* 107, 6063 – 6069 (2003)

137. Alexandre A Vetcher, Srimeenakshi Srinivasan, Ivan A Vetcher, Semen M Abramov, Mikhail Kozlov, Ray H Baughman, Stephen D Levene: Fractionation of SWNT/nucleic acid complexes by agarose gel electrophoresis. *Nanotechnology* 17, 16, 4263 – 4269 (2006)

138. Ruhung Wang, Carole Mikoryak, Elena Chen, Synyoung Li, Paul Pantano, Rockford K Draper: Gel Electrophoresis Method to Measure the Concentration of Single-Walled Carbon Nanotubes Extracted from Biological Tissue. *Anal Chem* 81, 2944-2952 (2009)

139. Tatsuhiro Yamamoto, Yoichi Murakami, Jin Motoyanagi, Takanori Fukushima, Shigeo Maruyama, Masaru Kato: An Analytical System for Single Nanomaterials: Combination of Capillary Electrophoresis with Raman Spectroscopy or with Scanning Probe Microscopy for Individual Single-Walled Carbon Nanotube Analysis. *Anal Chem* 81, 7336-7341 (2009)

140. Masahito Sano, Junko Okamura, Seiji Shinkai: Colloidal nature of single-walled carbon nanotubes in electrolyte solution. The Schulze-Hardy rule. *Langmuir* 17, 7172-7173 (2001)

141. Linqin Jiang, Lian Gao, Jing Sun: Production of aqueous colloidal dispersions of carbon nanotubes. *J Colloid Interface Sci* 260, 89-94 (2003)

142. Sandeep S Karajanagi, Hoichang Yang, Prashanth Asuri, Edward Sellitto, Jonathan S Dordick, Ravi S Kane: Protein-assisted solubilization of single-walled carbon nanotubes. *Langmuir* 22, 1392-1395 (2006)

143. A D Durnev: Toxicology of nanoparticles. *Bulletin of experimental biology and medicine* 145, 72-74 (2008)

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