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1 Description of task

'The SbD concept aims at reducing potential health and environmental risks at an early phase of the innovation process. Within the safe-by-design concept the **functionality** of a nanomaterial and its **toxicity/safety** are therefore considered in an integrated way. Such an approach maximizes resources use and expedites the development of products containing nanomaterials and new nanomaterials that are safe(r) by design. In this task, the key parameters important for functionality will be described. The chemists in this Work Package (WP) differentiate from those in WP2 as they are material scientists rather than analytical chemists. The results of the activities in this task will not only help to prevent marketing of unsafe nanomaterials but will also help identifying characteristics of safety concern for already marketed nanomaterials. The precise design will help to simulate characteristics of the same material as present in bulk, in a product (to be) marketed, etc. thereby supporting extrapolation (WP3) and risk assessment along the value chain (WP1).

The Safe by Design approach will require close collaboration between material experts dealing with functionality and characterization, analytical chemists dealing with characterization and toxicologists/risk assessors for toxicity testing and interpretation of the results. Moreover, liaisons with safe nano design initiatives (e.g. the NIOSH Prevention through Design) should be established in order to get overview of hurdles that block multidisciplinary/multistakeholder collaboration (process driven) as well as getting overview of scientifically driven hurdles, before the safe by design concept can be beneficial for all stakeholders. This task is composed exclusively of advanced materials engineering, testing and development, but with links to regulatory requirements. This will require the following activities:

• Initial development of a decision tree based on the safe window of (eco)tox parameters. Test and validation of the decision tree in a Value Chain Case Study (Task 1.6). Compilation of the decision tree as a tool for the NANoREG tool box.

• Establishing a first set of relationships between certain functionalities and triggers for testing specific (eco)toxicity endpoints, for example by an extensive programme of material development, characterization and testing.

• Inventory of topics of shared interests between certain stakeholders/experts in the safe by design process, compilation of the interests to a recommendation as part of the NANoREG tool box.

2 Description of work & main achievements

2.1 Summary

There is an ongoing growth in Nanotechnology where manufactured nanomaterials (MNMs) are more often coming into contact with humans and the environment. The interface between any type of nanomaterial with the surrounding environment either proteins/cells in a culture medium or bound to a matrix/composite or in a solvent depends on colloidal forces as well as dynamic biophysicochemical interactions. The development of predictive relationships between structure (key parameter) of nanomaterials and activity (functionality) are determined by nanomaterial properties such as size, shape, surface chemistry, aggregation, and the test medium. The understanding of these relationships is very important from the perspective of safe use of nano materials. It is not possible to describe all the biophysicochemical interactions at the interface, but we made an effort to assemble knowledge and information to provide a framework to guide this exploration. D6.6 is part of the NANoREG project aimed to provide some consensus on which key parameters of MNMs affect specific functionalities in an attempt to establish a relation between them and to propose a workable decision tree. This is one of the first attempts to identify the main key properties of the MNMs that in relation to their functionalities play a dominant role on the safe by design concept. This proposed data model is considered as one of the possible approaches towards a decision tree strategy for (re)designing safe MNMs for humans and the environment. The optimal design of safe nanomaterials is a challenge with multiple compromises between functionality and safety characteristics.

2.2 Background of the task

Due to the rapid expansion of nanotechnology and the increasing range of MNMs under production and development, it is essential that the potential impacts on human and environmental health are addressed at an early phase of the innovation process. The identification of any potential deleterious effects is therefore necessary in order to prevent any potential environmental or human health adverse effects. The linking of physicochemical characteristics of nanoparticle (NP) to their biological behaviour and its functionality is a first step towards achieving this goal. It is widely accepted that much work is still needed to advance knowledge in the area of physicochemical characterisation of nanomaterials, and how characteristics and properties of these nanomaterials influence their fate and behaviour in the environment and their potential to induce toxicity in different environmental receptors [1].

Rapid growth in nanotechnology is increasing the likelihood of MNMs coming into contact with humans and the environment. Nanoparticles interacting with proteins, membranes, cells, DNA and organelles establish a series of nanoparticle/biological interfaces that depend on colloidal forces as well as dynamic biophysicochemical interactions. These interactions lead to the formation of protein coronas, particle wrapping, intracellular uptake and biocatalytic processes that could have biocompatible or bioadverse outcomes. For their part, the biomolecules may induce phase transformations, free energy releases, restructuring and dissolution at the nanomaterial surface. Probing these various interfaces allows the development of predictive relationships between structure and activity that are determined by nanomaterial properties such as size, shape, surface chemistry, roughness and surface coatings. This knowledge is important from the perspective of safe use of nanomaterials [2].

The generation of a prescribed list of requirements should recognise the limitations of resources and capabilities, but it should also be mindful of achieving scientific robustness in the context of the objectives of a particular study.

Main aim and main activities:

The main goals of Task 6.3/D6.6 are:

1. To identify, categorize and prioritize the physicochemical characteristics of MNMs in relationship to functionality

2. To demonstrate the relation between different functionalities

3. To propose a data model/decision tree demonstrating the effect of the physicochemical properties on performance characteristics

As reported above, this deliverable focuses on the initiation of a data model demonstrating the effect of the physicochemical properties on performance characteristics. NANoREG project is linked to NanoReg2 through the D6.6 since the data model is the initial idea towards the formation of a decision tree demonstrating the effect of the physicochemical properties on performance that will be performed during NanoReg 2.

Safe by design (Definition by D1.10): MNMs can be engineered to be Safe By Design. This means a design maximizing the benefits for functionality while posing minimal risk to human health and the environment at an early phase of the innovation process. The 'safe by design' concept aims at reducing potential health and environmental risks. Such concept aims at creating an integrated research strategy, which enables the consideration of safety aspects for humans and the environment in the design process of a product/material to eliminate or minimize the risk of adverse effects during its life cycle including construction, use, maintenance and deconstruction. Within the safe-by-design concept the functionality of a nanomaterial and its toxicity/safety are therefore considered in an integrated way. Such an approach maximizes resources use and expedites the development of products containing nanomaterials and new nanomaterials that are safer by design.

Functionality: The relationship between the MNMs properties and their practical use is defined as Functionality (common term with NanoReg2). Specifically, nano-relevant materials can come into contact with cells and tissue or can be taken up by them (depending on the specific MNMs properties) and can cause effects therein as a function of their potential action (based on their practical use). The potential action of nanomaterials on health and the environment is estimated by: the dissolution/dispersion, hydrophobicity, catalytic activity; the conductivity (electrical and thermal); magnetic properties etc.

This deliverable complements the other deliverables (D6.3-D6.5) of the task by covering the impact of the physicochemical properties or key parameters of MNMs on functionalities. The proposed data model (NANoREG) and decision tree (NanoReg2) is envisaged to be a useful tool for (re)designing safe MNMs for humans and the environment.

2.3 Description of the work carried out

2.3.1 Methodology

D6.6 is a literature review focusing on the physicochemical properties of MNMs (mainly at pristine state) and how these properties affect functionalities (except toxicity). Toxicity is described in D6.3 of WP6.

In an attempt to collect only relevant and specific literature, a priority list was formulated focusing **on the seven different groups of MNMs** (core nanomaterials of NANoREG project); the **key physicochemical parameters** as shown in the Overview of the key parameters per risk potential for safety screening of MNMs (see poster by Cornelle Noorlander entitled 'Task 6.2: Safe by design: lessons learned from drug development testing'); and **the publication year** (we tried to focus on literature from 2010 up to now).

In order to set up and structure the literature review, we had to decide whether to categorize the literature sections based on functionalities or on physicochemical properties. It was apparent that because the physicochemical properties of pristine MNMs are inter-related it would have been clearer to categorize the literature based on functionalities.

It was suggested by the Task Leader to create a definitions list of all the key parameters (shown below in alphabetical order).The general definitions of the physicochemical parameters are as follows [by the Consolidated Framework for EHS of Manufactured Nanomaterials under the framework of ERA NET project "Safe Implementation of Innovative Nanoscience and Nanotechnology (SIINN)"]:

Agglomeration State (Aggregation): Attractions that hold together a collection of nanostructures; agglomeration is formed by clusters of structures held together by electrostatic interactions; aggregation is the formation of covalently fused or sintered particles not easily separated;

Aspect Ratio: The ratio of the longest Feret's diameter of a particle to the shortest perpendicular;

Composition: is an intrinsic property in terms of elemental composition and chemical structure;

Crystal structure: is composed of a pattern, a set of atoms arranged in a particular way, and a lattice exhibiting long-range order and symmetry;

Particle Size: It is the size of a particle determined by a specified measurement method;

Porosity: It is a measure of the void spaces in a material, and it is a fraction of the volume of voids over the total volume;

Roughness: is a component of surface texture/pattern or the state of an engineered surface;

Shape: It is the variation of the hydrodynamic radius between spherical particles and oblong ones with the same mass, which triggers a variation in their mobility and diffusion in both gas and liquid phases;

Surface area: It is the measure of how much exposed area a sold object has expressed in square units; Surface reactivity: It is closely related to surface area; by increasing surface area, the reactivity and sorption behavior increase;

Surface charge: It is the electric charge present at an interface;

Surface Chemistry: It is often used in the context of surface chemical composition; it includes elements of solubility, equilibrium, catalytic properties, surface charge, and surface adsorption/desorption;

Surface Coating or modification: Modification can either be done by coating, functionalization, or other means, which may be chemical (organic, inorganic or both) or physical (eg. irradiation, surface attrition);

Test medium (Only water): Medium/solvent conditions to affect particle dispersion and agglomeration state of nanoparticles;

Topology: It is the mathematical study of shape.

The main definition of Functionality is given above. The Functionalities of D6.6 include Solubility/dissolution, dispersion, Catalytic activity, Hydrophobicity, Electronic and Optical properties, extensively defined and discussed during the results section. We decided to group these functionalities into two classes; i) **properties or performance characteristics** and ii) **applications.** The Functionalities properties/performance are the dispersion, solubility/dissolution, and hydrophobicity since these are affected directly by the physicochemical properties of the pristine MNMs but at the same time are themselves properties/characteristics that they can

have an effect on the MNM practical use (functionality definition). The other Functionality class is the Applications (except toxicity) such as cell uptake, and optical, electronic, magnetic and catalytic properties.

The results section comprises of the following:

- There are five subsections of literature on each one of the five functionalities addressed in D6.6;
- All the above literature was categorized into tables (Tables 1A and B), and on Table 2 for an easy access summary of the properties and their effect on functionality.

How are the Tables created?

Table 1 is based on five categories of key parameters of MNMs such as Geometrical, Chemical, Morphological characteristics as well as Coating characteristics, Dispersibility and Others. This categorization is based on an attempt to organize/tidy-up the properties and not to have them in alphabetical order on the y axis of each table. The importance and priority were formulated to justify the fact that a key parameter might be a priority for some functionalities while only of importance for other functionalities; Table 2 exhibits the priority list and some statistics of the review; and

What is the route used for the data model?

The data model will act as a first attempt towards a decision tree for the Safe by Design concept that will be used by industry and regulators during the innovation/development of MNMs. The data model was formed taking into account Tables 1 and 2 and their statistical observations as well as the discussion points of each section of the Functionalities. The data model focused on the key parameters groups where in bold are the key parameters of priority for their effect on functionalities. There are two arrows, a red one representing YES which means that depending on the practical use and for the minimal hazard, the MNMs need improvement, modification, (re)design in respect to their properties. On the other hand, the black arrow NO indicates that there is no effect/no hazard on functionalities i.e. the use of an MNM for a specific application has the appropriate conditions/parameters for its safe use. It is very interesting to observe that specific MNMs' key parameters can be tuned in order to enhance the functionalities as shown in Fig.1.

2.4 Results

D6.6 shows the vast range of different studies reporting on the MNMs key parameters and how these affect the functionalities.

2.4.1 Key Parameters and how they affect Functionalities_Properties

A. Dispersion

The state of dispersion is one of the most important characteristics of a nanoparticle system, yet it is one of the most difficult to quantify [3]. The aggregation of MNMs has been shown to depend on particle properties (e.g., size, shape, surface roughness, surface charge, and concentration) and on the physicochemical properties of the media (e.g., pH, ionic strength and presence of organic macromolecules) [4]. In the absence of surface coating (engineered or incidental), aggregation/disaggregation is mainly governed by **particle intrinsic properties such as size, ξ potential, and solution ionic strength** as described by DLVO theory proposed by Derjaguin, Landau (1941) and Verwey and Overbeek (1948).

In a number of works, the effect of the dispersion media has been evaluated since it is known that nanoparticles agglomerate immediately in cell culture media. Proteins, serum, and chemical surfactants are often used to enhance nanoparticle dispersion and stabilization. For example, the dispersion of titanium oxide (TiO_2) nanoparticles in six different cell culture media including Bronchial Epithelial Growth Medium (BEGM), Dulbecco's Modified Eagle's Medium (DMEM), Luria-Bertani Broth (LB), Tryptic Soy Broth (TSB), Synthetic Defined medium (SD), and Yeast Extract Peptone Dextrose medium (YPD) was investigated [5]. All six culture media had high ionic strength (50-270 mM) and conductivity (3-17 ms cm-1). Under the optimum sonication conditions, the particle size in water was ~200 nm which was much larger than the hydrodynamic diameter of the primary particle size suggesting that the TiO_2 (P25) sample consists of some hard aggregates that are not easily broken up by ultra-sonication. When suspended in cell culture media without any dispersing agents, TiO_2 nanoparticles showed much poorer dispersion and the agglomerate size varied from 770 to 1052 nm depending on the type of medium. Consistent with the dramatic size increase, the zeta potentials of all suspensions also dropped to ~ -10 mV. The pHs remained similar to those of the nanoparticle-free media. Unlike in water, where particle size remained similar in a wide range of nanoparticle concentrations (2-100 μg mL-1), the TiO_2 agglomerate size increased with increasing nanoparticle concentration in all cell culture media. To improve the TiO_2 nanoparticle dispersion, BSA as a model protein and FBS as a protein rich serum were selected. The **concentration of each dispersing agent** was adjusted to achieve the best TiO_2 nanoparticle

dispersion. However the effectiveness varied from medium to medium, mainly due to the different **water chemistries**, and thus, different **protein-nanoparticle interaction mechanisms** in the media whereas phosphate ions can play an important role in the nanoparticle dispersion. FBS appeared to be the best dispersing agent of these studies for stabilizing TiO_2 nanoparticles in all six cell culture media, which was attributed to the synergistic effect of various proteins in FBS.

The dispersion of selected MNMs in natural river water was examined as well and the effect of the water chemical composition was investigated. Fullerenes (C60), nanosilver (nAg), and nanocopper (nCu) were used [6]. Nominal ranges of particle diameters as provided by the manufacture were 15-45 and 20-30 nm for nCu and nAg, respectively. The particle size of the received C60 determined by DLS averaged 35.8 nm. Aqueous suspensions of the MNMs were prepared in both deionized water and filtered natural river water samples collected from the Suwannee River (SR) basin, to emphasize differences in dissolved organic carbon (DOC) concentrations and solution ionic strengths (I). Results obtained from exposure studies show that water chemistry affects the suspension / solubility of MNMs as well as the particle **size distribution**, resulting in a wide range of biological responses depending on the type of toxicity test used. The effect of Solution Chemistry on MNM Particle Suspension and Dissolution was investigated as well, without discrimination between particulate and truly dissolved fractions. A rather complex response of nAg to solution chemistry was observed in contrast to Cu in tested waters.

Ultrafine and fine carbon black and TiO_2 were suspended in a variety of suspension media including phosphate buffered saline (PBS), rat and mouse bronchoalveolar lavage fluid (BALF), dipalmitoyl phosphatidylcholine (DPPC), albumin, or the combinations of DPPC-albumin [7]. The results of this study show that PBS is not a satisfactory medium to prepare nanoparticle suspensions. However, acellular BALF is effective in dispersing the nanoparticles without masking the biological activity of the surface. BALF as a suspension medium significantly reduces agglomeration of nanoparticles in solution. The use of PBS containing protein or DPPC alone, in concentrations found in BALF, did not result in satisfactory particle dispersion. However, PBS-containing protein plus DPPC was satisfactory, although less effective than BALF.

The dispersion of different CNP (sources and types) in various media has been investigated as well [8]. Fullerene carbon spheres (C60CS), single-walled nanotubes (SWNT) and multi-walled nanotubes (MWNT), from two different sources each, were examined in seven different media These media were: 100% fetal calf serum (FCS), 7.5% bovine serum albumin (BSA) in phosphate buffered saline (PBS), RPMI media with 10% fetal calf serum (FCS), 100% delipidated FCS, 1% tween 80 in PBS, 100% dimethyl sulfoxide (DMSO). CNP agglomerates are present in all dispersing vehicles to some degree. A general observation is that the degree of aggregation increases with going from the first to the last medium. The vehicle that contains some protein, lipid or protein/lipid component disperses the CNP best, producing fewer large agglomerates in contrast to vehicles absent of lipid and protein that produce the largest agglomerates. The **source of the CNP** is also a factor in the degree of particle agglomeration within the same vehicle.

In another work, stable aqueous dispersions of fullerenes, C60 and C70, were prepared by simply injecting into water a saturated solution of fullerene in tetrahydofuran (THF), followed by THF removal by purging gaseous nitrogen [9]. Fullerenes are dispersed as monodisperse clusters in water, 60 nm in diameter and the dispersions thus obtained are of excellent colloidal stability even though no stabilizing agent is used. It was found that the **surface of the cluster is negatively charged and the electrostatic repulsion** between the negatively charged cluster surfaces is important for the stability of the dispersions.

Al_2O_3 nanoparticles having size of the primary nanoparticles of 30nm were found to form temporarily stable small aggregates both in Deionized (DI) water and EG [10]. CeO_2 nanoparticles with median size of 70nm were shown to form a more stable dispersion in water than in fish medium. In the former medium the nanoparticles were measured to be ~160-200nm whereas in the latter larger than 1000nm. Moreover, sedimentation was clearly observed in the fish medium [11].

The size distribution of gold nanoparticles of 10, 25, 50, and 100 nm in diameter, capped with a carboxylic acid functionalized hydrocarbon agent (500 Da), at fixed mass concentration in DI water and DMEM supplemented with FCS were measured using dynamic light scattering (DLS) technique [12]. In DI water, particle size distributions exhibited peaks around their nominal diameters. However, the gold nanoparticles suspended in DMEM supplemented with FCS formed complexes around 100 nm, regardless of their nominal sizes. DLS and UV–vis spectroscopy indicate gold nanoparticle agglomeration in DMEM that is not supplemented by FCS. The suspension dispersion quality is judged by its deviation from the nominal size. Accordingly, dispersion quality of the samples can be sorted as follows: DI > DI + FCS > DMEM > 124 mM NaCl > DMEM without FCS. The **size of the agglomerates** was found to increase with **increasing nanoparticle concentration** as well.

When the dispersion behaviour of citrate capped silver nanoparticles in aqueous matrices was studied, it was observed that the aggregation tendency was more pronounced in sea water compared to lake water with

hydrodynamic diameters being comparatively less in the latter. The different behaviour in fresh water was attributed to the presence of Natural Organic Matter (NOM) and less ionic strength in fresh water when compared to sea water [13]. A decrease in the measured hydrodynamic diameter was observed with the increase in pH, as well.

The dispersability of CuO and ZnO nanoparticles was tested in different mineral and complex test environments [14]. Both CuO and ZnO NPs were remarkably unstable and tended to sediment. Their agglomeration/sedimentation was especially high in mineral media—media that are used for key regulatory ecotoxicological assays (crustaceans, algae). In contrast, the components of the complex test media (defined as the test environment with organic components) dispersed NPs and prevented their sedimentation.

In another work, the dispersion of Al-based, Ag-based, Cu, TiO_2, SiO_2 and carbon nanomaterials was investigated in water, cell culture media only, and/or cell culture media with serum [15]. All the Al-based particles tended to form agglomerates of similar size when dispersed in either water or cell culture media. The Al_2O_3 (30 and 40 nm) particles formed similar agglomerates ~210-250 nm in water and media with serum whereas it formed considerably bigger agglomerates in cell culture media without serum (1000-1500nm). Similar trend was found for Al particles of 50, 80, and 120nm, showing that the effect of the size of the primary particle on the agglomeration is weak, if any. Ag (80nm), Hydrocarbon Coated-Ag (15nm and 25nm), and PolySaccharide-Ag (10, 25–30, 80 nm) showed the same trend by agglomerating at nearly the same size when dispersed in either water or media with serum, whereas higher agglomeration sizes were seen in the media without serum samples. The only exceptions were Ag 80 nm and PS-Ag 80 nm that did not follow this trend. The Ag 80 nm particles in media without serum showed approximately a 50% decrease in agglomeration when compared to the water sample. The PS-Ag 80 nm sample showed an increasing trend from 250 nm in water to 743 nm in media and finally 1230 nm in media with serum. Cu 40, 60, and 80 nm particles were observed after dispersion in water, media, and media with serum. Cu 60 and 80 nm exhibited increased agglomeration in media and decreased agglomeration in media with serum when compared with water whereas Cu 40 nm agglomerated highly in water and media without serum, while in media serum, agglomeration sizes decreased. In the case of TiO_2 two different studies were performed, one with varying crystalline structures and one with varying sizes. The TiO_2 crystalline structures evaluated were TiO_2 39 nm (100% anatase), TiO_2 39 nm (61% rutile, 39% anatase), TiO_2 39 nm (40% rutile, 60% anatase), and TiO_2 40 nm amorphous. The amorphous TiO_2 maintained high agglomeration values for all three solvents. The other TiO_2 particles showed somewhat smaller aggregates in water, and only the TiO_2 61% rutile showed a significant decrease after dispersion in media with serum. TiO_2 61% rutile also had the largest zeta potential of the group at 17.7 mV. The sizes of TiO_2 evaluated were 5, 10, 16, 50, and 100 nm. Even with a wide range of primary particle sizes, all particles tested, except TiO_2 10 nm, exhibited high agglomeration in all three solvents. TiO_2 10 nm was measured below these ranges only when dispersed in water. Zeta potentials varied widely for these samples, with TiO_2 10 nm having the largest at 15 mV. The SiO_2 particles and SiO_2-coated fluorophores (35, 51, and 110 nm) were the only groups of particles tested which dispersed at, or very closely to, their primary particle size. Only SiO_2 420 nm particle was not as close to the primary size. SWNT, MWNT-COOH, and CNT formed aggregates in DI water, whereas CB sample showed agglomeration sizes ranged from 396 (water) to 2190 nm depending upon the solvent used.

The **effect of the nanoparticle size** on the dispersibility was investigated, in another work, utilizing gold nanoparticles of 10, 50, 100 and 250 nm in aqueous suspension [16]. The gold suspensions were 10% diluted by adding one part of 10 times concentrated phosphate buffered saline (10 x PBS) to nine parts of the gold suspension, in order to obtain a physiological solution. Directly after PBS addition, in nanoparticle dispersions of 10, 50 and 100 nm the formation of nanoparticles agglomerates/aggregates was observed, whereas in the case of the 250 nm particles was not. Additionally, TEM show that the 10 nm sample showed mainly individual nanoparticles and some clusters in which up to 60 nanoparticles. The nanoparticles in the cluster were loosely arranged and individual nanoparticles could be easily recognized, indicating that the clusters consisted of agglomerates with weakly binding forces. In samples of nanoparticles of 50, 100 and 250 nm individual nanoparticles and some clusters of 2-8 nanoparticles were observed.

The effect of **solution pH and ionic strength (IS)** on nanoparticles dispersion was investigated in a series of works. In one [17], anatase TiO_2 (H) nanoparticles with primary particle sizes of 15 nm and specific surface area of 102.1 m^2/g were utilized to determine the effect of the IS and of the pH on dispersion characteristics and more specifically on the hydrodynamic size and on the surface charge. The nanoparticles were in one case, dispersed in NaCl solution with different molar concentrations. Since the addition of NaCl does not change the solution pH, TiO_2 dispersions with different ionic strength had the same pH value, 4.6. To determine the pH influence on the state of dispersion, the TiO_2 nanoparticles were dispersed in solutions with the same ionic strength (0.001 M) but different pH, which was adjusted by adding HCl, NaCl, NaOH, or a combination. In the first case, the average hydrodynamic diameter was found to increase dramatically with increasing solution IS. When TiO_2 was dispersed in deionized water (IS~10-5 M) and 0.001 M NaCl, the average hydrodynamic diameters were similar (~90 nm) and the dispersions were stable, as the electrostatic

repulsive force is dominant over the attractive force, suppressing agglomeration under such conditions. A modest increase in IS to 0.005 M resulted in a substantial size increase to approximately 156 nm. At a NaCl concentration of 0.1 M, the attractive force between particles became dominant over the repulsive force, resulting in an unstable, highly agglomerated dispersion. The zeta potential and the average diameter of the TiO_2 dispersions as a function of pH with ionic strength held constant for all dispersions at 0.001 M were measured. The measured isoelectric point for TiO_2 is approximately 6.0. Particles have a positive zeta potential when pH is lower than 6, while the zeta potential is negative when pH is higher than 6. A strong correlation between the zeta potential and average size was observed. When pH is far from the isoelectric point, the absolute value of zeta potential becomes higher. The electrostatic repulsive force is then dominant over the van der Waals force, such that agglomeration is suppressed. When pH approaches the isoelectric point, the repulsive force is weakened due to low surface charge, and the hydrodynamic size increases beyond which it is measurable. Under these conditions, large flocs were formed which settled out of the solution due to gravitational forces in a short time. Similar results were observed in another work, where the effect of concentration of the Suwannee River Fulvic Acid (SRFA) and the roles of pH and ionic strength in the aggregation of TiO2 were evaluated [18]. Aggregation of the bare TiO_2 nanoparticles increased for pH values near the zero point of charge. **At any given pH, an increase in ionic strength generally resulted in increased aggregation**. Furthermore, conditions which favored adsorption of the SRFA resulted in less aggregation of the TiO_2 nanoparticles, presumably due to increased steric repulsion.

Dynamic light scattering results show that 4-5 nm TiO_2 particles readily form stable aggregates with an average diameter of 50-60 nm at pH ~4.5 in a NaCl suspension adjusted to an ionic strength of 0.0045 M [19]. Holding the pH constant, but increasing the ionic strength to 0.0165 M, leads to the formation of micron-sized aggregates within 15 min. At all other pH values tested (5.8-8.2), micron-sized aggregates form in less than 5 min (minimum detection time), even at low ionic strength (0.0084-0.0099Mwith NaCl). In contrast, micron-sized aggregates form within 5 min in an aqueous suspension of $CaCl_2$ at an ionic strength of 0.0128 M and pH of 4.8, which is significantly faster than observed for the respective NaCl suspensions indicating that divalent cations may enhance aggregation of nano-TiO_2.

In another work, the effect of Natural Organic Matter (NOM, alginate humic and fulvic acids) on the aggregation of anatase TiO_2 nanoparticles was evaluated. Changes in the particle size were measured at different concentrations of three electrolytes: NaCl, $CaCl_2$ and Na_2SO_4, and at different solution pH values [20]. In general, all additions of electrolytes without NOM followed the DLVO theory. Low coverage of NOM on top of bare titania particles were found to induce aggregation but further coverage could protect them from aggregating even at high ionic strengths. The degree of coverage is governed by the concentration ratio between NP and NOM and different approaches in the process of mixing can lead to different states of aggregation. The **ionic composition** strongly influences the aggregation behaviour, with divalent anions and cations destabilizing more strongly positively and negatively charged titania, respectively. The pH of the suspension is a major influential factor that will determine the lower absolute value of the surface charge of the particles, with direct consequences on stability. Positively charged NP at low pH were more prone to destabilization by SO_4^{-2} compared to Cl^- while the opposite holds for Ca^{+2} compared to Na^+ at high pH. If the pH of the suspension is far from the isoelectric point (IEP), the particles have a higher charge (either positive or negative) and this leads to slower aggregation rates at a similar particle concentration than when the pH of the particles is closer to the IEP. The addition of NOM at concentrations which generated stable suspensions increased the stability of the systems with respect to NaCl and Na_2SO_4 but did not have much influence on the $CaCl_2$ systems.

The **aggregation of silica nanoparticles** in aqueous suspensions was investigated for different pH and salt concentration, as well [21]. It was found that changing the pH does not affect the aggregation in the absence of electrolyte for the range of pH studied whereas the addition of different types of salts (NaCl, $CaCl_2$, $MgCl_2$, and $BaCl_2$) causes aggregation of the silica nanoparticles. The aggregation was found to depend on the kind of cations with divalent cations Mg^{2+}, Ca^{2+}, and Ba^{2+} being more effective in destabilizing (i.e., causing aggregation) the nanoparticle dispersion than the monovalent cation Na^+.

The effect of pH and sodium dodecylbenzene sulfonate concentration on the size of Al_2O_3 and Cu nanoparticles in water was investigated. Optimized values of pH (pHalumina ≈ 8.0, pHcopper ≈ 9.5) and SDBS concentration (SDBSalumina≈0.10%, SDBScopper≈0.07%) were found, at which the values of particle size take a minimum value Dalumina ≈ 240 nm, Dcopper ≈ 320 nm, respectively [22]. Particle size distributions with more than one order of magnitude smaller sizes were observed for Cu/water suspensions in the presence of hexadecyl trimethyl ammonium bromide [23].

Enhanced **dispersion and stabilization of CNMs** in water is a critical challenge [24]; while dispersion degree depends on the dispersing agent, generally, CNMs aggregate more at low pHs, due mainly to relatively less negative charge. The dispersion of CNMs can also be influenced significantly by the presence of background ions in water. CNM aggregation increases with increasing ionic strength. However, once ionic strength

becomes high, no additional increases in aggregation occur, indicating that electrostatic repulsive forces are successfully shielded. CNM stability increases with increasing temperature, presumably due to disruption of weak interaction forces, increased Brownian motion/collisions, and decreased zeta potential. Among various natural and synthetic dispersing agents, Natural Organic Materials have been studied widely. The stability and dispersion of CNMs is significantly enhanced in water bodies with NOM, since the hydrophobic surfaces of CNMs facilitate their interaction with NOM. Surfactants also enhance the stabilization of CNMs in water through their adsorption via hydrophobic and π– π interactions. Ionic surfactants, stabilize dispersions of CNMs by electrostatic repulsion between the hydrophilic head groups, with both cationic and anionic surfactants showing similar ability to sufficiently disperse CNMs.

Surface modification of nanoparticles is one of the most common methods to improve their dispersion stability [25]. It requires a design of the surface structure based on the type of nanoparticles and the liquid media. Surfactants are generally used to improve colloidal stability in water because many nanoparticle syntheses are performed in organic media. Furthermore, monomeric, inorganic as well as polymeric stabilizers have been utilized [26]. Adsorption of a polymeric dispersant on nanoparticles is one of the simplest surface modification techniques to improve the stability of the dispersion. Neutral polymers such as polyethylene oxide or dextran are employed as stealth coating agents to improve colloidal stability and pass physiological barriers, while proteins, enzymes, antibodies, or nucleotides are the most common cell targeting agents [27]. When dispersing hydrophilic nanoparticles in aqueous media or in organic solvents with high polarities, anionic or cationic polymer dispersants are widely used to generate the steric repulsive force originating from the polymer chains and to increase the surface charge. Among anionic surfactants, various types of polycarboxylic acids and their salts including polyacrylic acid (PAA), polyacrylic acid sodium salts (PAA-Na) and co-polymers of polyacrylic acid and maleic acid are used to disperse oxide nanoparticles such as $BaTiO_3$, TiO_2, Al_2O_3, MgO and Fe_2O_3. A common example of cationic surfactants is polyethyleneimine (PEI). The relationships among pH of suspension, solid fraction of the suspension, dissociation ratio of polymer dispersant, molecular weight of polymer surfactant, surface charge of nanoparticles and the particle size affect the **adsorption ratio of surfactants and the degree of steric repulsive force**. It is expected that the molecular weight of the polymeric surfactants will affect their ability to improve the dispersion stability of the suspension. Further than the molecular weight, the structure of the surfactant can affect the dispersion stability. For example it is expected that a loop-train structure can be controlled by tuning the ratio of the hydrophilic and hydrophobic sites. Comb polymers have been applied to improve the stability of various oxides, such as $BaTiO_3$ and Al_2O_3 in a wide range of pH and ion concentrations. Copolymers with a hydrophilic group and a hydrophobic group are often used in anionic surfactants when dispersing hydrophobic nanoparticles, such as SiC, carbon nanotubes (CNTs), and coal, in aqueous media. The hydrophobic segments facilitate adsorption of dispersant on hydrophobic particles; moreover an aromatic compound such as styrene may improve the adsorption via hydrophobic and π–π interactions. The hydrophilic segments are added for compatibility with aqueous media; they also play an important role in the generation of an effective repulsive force by the electrical double layer. When using cationic polymers, PEI can be applied to hydrophobic particles to improve the stability of SiC and CNTs in aqueous media. Chemical modification of the particle surface is also a useful technique to improve the stability of nanoparticles in various liquid media. Silane coupling agents are used to modify oxide nanoparticle surface. Various reactive groups such as amines, epoxides and vinyls are first introduced on the particle surface by silane coupling agents, and then polymers are grafted from or grafted to the particle surface.

In a previous work, the acidic form of sophorolipids (SL) was utilized to functionalize magnetic iron oxide nanoparticles [28]. If no SL were employed, a black, water-demixed, precipitate was obtained and no stable dispersion was achieved whereas when SL were employed, samples that corresponded to a stable colloidal solution of maghemite nanoparticles in coexistence with a black/brown precipitate were obtained; the latter was probably due to nanoparticle aggregation before SL-addition and/or insufficient complexation by SL. At 80°C, the solution was clearly darker than in the experiment performed at RT, suggesting that either SL functionalization is more efficient or aggregation phenomena among nanoparticles before SL surface coating are reduced. The good colloidal stability of sophorolipids-functionalized nanoparticles was shown both in pure water and in 0.01 M and 2M KCl solutions whereas the amount of dispersed nanoparticles in solution increased with increasing nanoparticle–sophorolipids mass ratio. When an ethanol–water mixture was used as dispersing medium the hydrodynamic diameter increased for all samples proving that a sophorose layer coats the nanoparticle since carbohydrates are insoluble in alcoholic media.

Synthesized ZnS-coated CdSe nanocrystal QDs were coated to possess single -COOH, -NH2, -OH or dual -OH/COOH, and -NH_2/OH functional groups [29]. ζ-potential measurements showed that QD-COOH and QD-OH/COOH were highly negatively charged, whereas QD-NH2 and QD-NH2/OH were positively charged. QD-OH was less negatively charged than the carboxylic acid groups. QDs with both hydroxyl and carboxyl/amine groups had median charge in both groups. Amino-QDs showed a broad particle distribution around 40 nm. In contrast, QDs of carboxyl groups had a narrow distribution around 20 nm. Processing the QD surface with

hydroxyl group resulted in improved dispersion and stability under hypertonic conditions. In contrast, all of the Quantum Dots (QDs) were stable in nonelectrolyte solutions. All of the modified QDs were stable under weak alkaline conditions, whereas only QDs of the amine groups were stable under acidic conditions.

Different mineral and complex test environments were utilized to test the dispersability Ag and PVP-coated Ag nanoparticles [14]. In all test media coated Ag NPs are remarkably more stable than the uncoated NPs in accordance with the idea that in high ionic strength suspensions uncoated Ag NPs tend to precipitate and sediment within a few hours. In another work, $AgNO_3$ and Ag NPs with similar size ranges were coated with either polyvinylpyrrolidone (hydrophilic) or oleic acid (amphiphilic) [30]. Primary particle diameter was determined by measuring images of the particles taken using TEM. The PVP coated particles had a slightly larger mean diameter (56.35 ± 1.16 nm) than the particles coated with OA (50.60 ± 1.02 nm). DLS in DI water provided similar size distributions to TEM size analysis for the PVP-coated. However, the particles coated in OA had a greater percentage of aggregates which were as large as 200 nm in diameter.

Carbon nanotubes can be dispersed in water when coated by adsorbed surfactants, preferentially with those that have relatively high hydrophyle-lyphophyle balance [31]. The nature of surfactant, its concentration, and type of interaction are known to play a crucial role in the phase behavior of carbon nanotubes. Knowing the surface charge of carbon nanotubes in different media is absolutely essential for understanding the interaction (adsorption) mechanism with ionic surfactants, and to predict the colloidal stability of CNT solutions. The effect of head-group charge was investigated for various CNT-based systems, revealing no clear conclusion on the superiority of either cationic or anionic surfactants in dispersing the tubes. It seems that the adsorption mechanism of ionic surfactants, which is promoted by electrostatic interactions with CNT surface, is heavily controlled by the purification process and wall-functionalization of the tube, which in turn determine its surface charge. The stability of aqueous dispersions of CNTs is usually increased with the aid of SDS [32]. UV–vis spectroscopy has showed that the CNT/SDS dispersion exhibits extreme stability, with the supernatant CNT concentration decreasing only 15% compared with a decrease of 50% for the bare CNTs. The interaction between CNTs and SDS through the hydrophobic segment causes a higher negative surface charge and steric repulsion, which improves the stability of the CNT/SDS dispersion leading to the conclusion that a surfactant containing a single, long, straight-chain hydrophobic segment and a terminal hydrophilic segment is a suitable dispersant for the stable CNT dispersion. Moreover, the dispersion of mwCNTs in aqueous media was increased when the non-ionic surfactant T80 was utilized. In this case, the presence of RPMI and DMEM cell culture media was found to improve the dispersion as well. The stabilization is attributed to steric effects since no change in the zeta potential was observed [33].

B. Solubility/Dissolution

The potential of NPs to dissolve is one of the key parameters that affect their toxicity and their biological response since it determines NPs fate in the surrounding environment and within the body [34]. Solubility/dissolution of NPs and nanomaterials is often confused with the term of dispersibility. According to Born *et al*., dissolution is the dynamic process by which a particle goes into the solution phase to form a homogeneous mixture. During the dissolution process, molecules of the dissolving solid migrate from the surface to the bulk solution through a diffusion layer. Diffusion layer is the region between the particle surface and bulk solution which is enriched with solvated molecules. NPs dissolution is **dependent on solute concentration, surface chemistry, area, morphology and energy, dissolution layer properties, adsorbing species, and aggregation.** Furthermore, together with solubility, dissolution is **size and surface area dependent**, this is the reason that NPs dissolve faster and to a greater extent compared to macroscopic particles of the same material [35]. NPs dissolution is an important characteristic that affects their antimicrobial properties, toxicity, biomedical applications and environmental impact. NP dissolution may lead to the delivery of highly toxic ions in solution such as Zn^{2+}, Cu^{2+}, Cd^{2+}, Ag^{+} etc. However, it is very likely that dissolution in media may produce a complex suspension that involves partially dissolved NPs, free ions delivered from NPs and adsorbed ions on the NP surfaces [36]. The parameters that affect dissolution of NPs and what properties dissolution effects are discussed below.

Generally, dissolution of NPs increases as **particle size** decreases [37-39]. However, particle size is difficult to be determined especially in cases where surface modification NPs has been employed. Studer *et al*. have studied the dissolution of CuO NPs and found that reducing the particle size the dissolution increases [40]. On the contrary ZnO NPs do not appear to have significant dissolution behaviour compared to micron sized particles as studied by Mortimer *et al*. Nano- and micro- particles were added in Osterhout's medium and they both showed 80% dissolution [41]. Cases where reduction of particles size inhibited dissolution have been reported. Tang *et al*. investigated the effect of HA NPs and they found that only larger particles were responding to dissolution process [42]. The combination of particle size together with **surface modification via capping agents or surfactants** also brings changes to NPs dissolution [43]. Kittler *et al*. investigated the dissolution of citrate-stabilized and poly(vinylpyrrolidone)-stabilized silver NPs in water. The NPs were dissolved, however a limited value of silver release was observed, the rate and degree of dissolution was

dependent on the functionalization and storage temperature [44]. Apart from NPs size, their **shape and surface morphology** affect their dissolution. Misra *et al*. investigated CuO NPs of different shapes, spherical and rod shaped and they concluded that **spherical NPs** dissolve more and faster compared to **rod shaped** [45].

Dissolution of NPs is not only affected by their morphology, but also by the characteristics of the **surrounding media** such as **pH, ionic strength, water hardness and the presence of organic compounds** [46]. For instance, the presence of amino acids enriched media leads to complete dissolution of CuO NPs [47], or cysteine can increase the dissolution of Ag NPs [48].

NPs dissolution is highly associated with their **bioavailability, uptake rate and toxicity** [49]. Two actions of NPs may be responsible for their toxic effects: their chemical composition (release of toxic ions, formation of reactive oxygen species ROS) and/or stress or stimuli caused by the **surface, size and shape** of NPs [50]. Small particle size and large reactive area are the most possible parameters that can induce toxicological injury through the **production of ROS**. However, in cases when NPs dissolve during cell culture it is not easy to identify which of the above is the reason for the toxic effects. Tobias *et al*. tested the toxicity of SiO_2, $Ca_3(PO_4)_2$, Fe_2O_3, ZnO, CeO_2, TiO_2 and ZrO_2 in terms of their dissolution. First, they categorized the NPs into soluble ($Ca_3(PO_4)_2$, Fe_2O_3, ZnO) and insoluble (CeO_2, TiO_2, ZrO_2). Then they conducted cytotoxicity studies using two different cells lines. For high dissolution the toxic effect was much higher compared to the no or little dissolution [51]. A number of studies have been conducted for the solubility of ZnO NPs. It has been reported that when ZnO NPs are placed in aqueous suspension they produce ROS such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), hydroxyl radical (OH^-) and organic hydroperoxides (OHPs). Such ROS can damage cells and on the other hand exhibit a strong antibacterial activity [37]. Xia *et al*. studied the dissolution and cytotoxicity of TiO_2, ZnO and CeO_2. Their findings suggest that ZnO NPs induced toxicity in cells. The reason was related to ZnO NPs dissolution under aqueous conditions and the release of Zn^{2+} cations, due to dissolution in culture medium which is associated to high levels of Reactive Oxygen Species (ROS). On the contrary **CeO_2, suppressed ROS production** leading to cellular resistance on the oxidative stress, showing a **cytoprotective behavior**. Finally, TiO_2, did not appear to have any toxic effects on mammalian cells, and was considered as inert [34]. ZnO NPs dissolution has also been examined in seawater in order to evaluate the toxicity in marine diatoms. Toxicity effects were attributed to release of Zn^{+2} ions [52]. Inert NPs are capable of inducing ROS under biological conditions; this is based on the ability of NPS to target mitochondria. Several cellular events are affected by ROS some of them are signal transduction, proliferative response, gene expression and protein redox regulation. When ROS levels are high, the can damage cells by deoxidizing lipids, altering proteins, disrupting DNA, cause cancer due to modulation of gene transcription and many other [53].

Levard *et al*. in a review paper, discuss the relationship of dissolution and toxicity of silver NPs. They suggest that in the presence of oxygen, Ag_2O is formed on the Ag NPs surface and Ag^+ dissolves in aqueous solution. Also, the silver NPs dissolution is enhanced in **low pH and as the particle size decreases** [54]. In general Ag NPs' suspension may contain different forms of Ag including Ag NPs, free/complexed Ag ions and adsorbed Ag^+ on Ag NPs. Elzey *et al*. investigated the state of silver NPs in water and aqueous nitric acid environments over a range of pH 0.5-6.5. Their findings suggest that silver NPs dissolution is **size dependent** since larger particles do not dissolve in nitric acid until the concentration is 4M, also faster reaction rates occurred with increased temperature [55].

NPs that undergo dissolution in the media before being taken up by the organism will have ion channels as the preferred route for cellular entry [36]. For those NPs that resist complete dissolution, there are other routes to influence cells fate, such as endocytosis or ion transportation or both. Dissolution of NPs may also take place inside the cells after cell uptake; the so-called intracellular dissolution. The intracellular dissolution mechanism shows how particles bypass the otherwise good protection of mammalian cells and how heavy metal ions behave inside the cells. Studer *et al*. evaluated the effect of copper NPs dissolution on cytotoxicity. Simulation of internal dissolution after the uptake was conducted at pH 5.5 (pH inside lysosomes). They found that at pH 5.5 copper oxide NPs dissolved. Thus, intracellular dissolution is attributed to pH effects [40].

Dissolution is one of the main contributors to toxicity of NPs. It may happen inside or outside the cells. The main way that NPs dissolve are through the release of ions. These ions may be toxic for a living organism. The parameters that affect dissolution of NPs, may be the **nature of NPs (size, shape, chemistry, coating) and the surrounding environment (type of media, pH, solution properties).**

C. Hydrophobicity

Hydrophilicity and hydrophobicity of NPs and Nanomaterials is one of the key parameters associated mainly with their chemical characteristics (composition, surface charge, chemistry) and their coating (coating characteristics, coating stability, surface reactivity). Wettability of NPs is an essential criterion for biological applicability [56-57] and is often related to their biocompatibility which is induced to NPs in order to improve their dispersion and interaction with bio molecules [58]. In biological systems, hydrophobic interaction is considered to be the strongest of all long-range non covalent interactions. It is beneficial for adsorption of biomolecules, enhances interaction/adhesion with cellular membranes by increasing the uptake of nanoparticles for cellular delivery and tailors the release rate of drugs [59].

A number of methods have been applied to **modify the surface characteristics** of various NPs in order to alter their wetting behavior. This can be achieved by either **surface coating of nanoparticles** with hydrophilic polymers/surfactants or via **formulation of nanoparticles with biodegradable copolymers** with hydrophilic segments such as polyethylene glycol (PEG), polyoxamer, poloxamine and polysorbate 80 (Tween 80). Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or graft copolymers for production of nanoparticles [56]. The surface of most of inorganic NPs is hydrophilic. One of the most common methods to modify their surface is attaching polymer chains onto their surface. Such attachment can be conducted via chemisorption, covalent attachment of end-functionalized polymers to a reactive surface or in situ polymerization with monomer from immobilized initiators [60]. Also coupling agents, which are capable of introducing a certain functional group onto the particle surface such as titanate coupling agents, silane coupling agents and organophosphonic acids [61-62].

Silver NPs are the most biocompatible and with the highest antimicrobial activity among all know NPs. Chudasama et al. have synthesized silver NPs using the thermal reduction of AgNO3 and oleylamine as reducing and capping agent. **Oleylamine chemisorption on NPs surface** makes them hydrophobic. To enhance the dispersability of the hydrophobic NPs in water they developed a facile phase transfer mechanisms using pluronic F-127, a biocompatible block co-polymer. The **hydrodynamic size** of the hydrophilic NPs is 8.2 ± 1.5 nm [57]. Another surface modification method has been reported by Shoults-Wilson et al. In their study, Ag NPs were coated with polyvinyl pyrolidone (PVP) or oleic acid (OA). The Ag NPs coated with PVP are hydrophilic and form stable suspensions in polar solvents, while the OA coated are amphiphilic forming stable suspensions in polar solvents, non-polar solvents or polar/non-polar interface layers depending on ph. [63].

Carbon nanotubes (CNT) is another material with unique properties for a variety of biological and biomedical applications. Single-walled (SWNT) and multi-walled (MWNT) carbon nanotubes have diameter from 0.4-2 nm and 2-100 nm respectively. The main technical problem of CNT towards biological applications is the lack of solubility in aqueous media. Several methods have been utilized to functionalize CNT surface in order to alter their hydrophobic characteristics. The most known way is the **functionalization with hydrophilic polymers** since the solubility of CNT is dependent on their **surface functional groups**. Nanotube-bound carboxyl acids generated during oxidative acid treatment enables the defect – targeted functionalization. Amidation, esterification, ionic interaction treatments, and sidewall-targeted functionalization of CNT are conducted mostly by attaching hydrophilic polymeric or oligomeric species on to their surface. SWNT have been functionalized with PEG (polyethylene glycol), PPEI-EI (poly(propionylethylenimine)) and PVA (polyvinyl alcohol). Another way to alter the hydrophilicity of CNT is conducted with **non-covalent and covalent modification** with bioactive species such as carbohydrates, sugar coated CNT and monosaccharide-functionalized SWNT, peptides, proteins and nucleic acids. Eg. Zheng *et al.* have used DNA and RNA to directly disperse individual SWNTs in water, nucleic acid and SWNT interactions in water come from the nucleic acid-base stacking on the nanotube surface with the hydrophilic sugar-phosphate backbone pointing to the exterior to achieve the solubility in water [64]. Hydrophilic CNTs have also been produced using sodium dodecyl sulfate (SDS) as dispersing agent. The **surfactant** contains a sulfate hydrophilic segment and a hydrocarbon hydrophobic segment. The SDS interacts with the CNTs with the hydrophobic segment so interaction between CNTs and SDS through the hydrophobic segment causes a higher negative surface charge and steric repulsion, which improves the stability of the CNT/SDS dispersion [65]. Hydrophilic MWNT decorated with magnetic NP have also been produces using PAA. PAA-g-MWNTs with poly(acrylic acid) grafting ratio of 15% were prepared and were decorated with magnetic NPs. MN-MWNTs (magnetic NP-decorated multi-walled carbon nanotubes) were produced by chemical co-precipitation of Fe^{2+} and Fe^{3+} onto the outer surface of PAA-g-MWNTs. The product showed excellent dispersibility and high magnetic susceptibility [66].

Silica NPs are hydrophilic and biocompatible. However, in some cases it is needed to make them highly hydrophilic. In general, the presence of **silanol groups on the silica surface** makes NPs more hydrophilic, and thus easier to suspend in aqueous solution [67]. Wang *et al.* have added organosilane compounds that contain **polyethylene glycol (PEG)** on silica NPs leading to a highly hydrophilic and easy dispersible NPs [68]. Park et al. modified SiO_2 NPs (14 nm) with water soluble polymers (POEM (poly(oxyethylene methacrylate) and PSSA (poly(styrene sulfonic acid)) . A three step process was followed: activation of silanol group (-OH) on the surface of SiO_2, surface modification to chlorine (-Cl) group and grafting from

polymerization via atom transfer radical polymerization (ATPR). The modified NPs showed better dispersion compared to the unmodified NPs [60]. Furthermore, Sun et al. have prepared polystyrene/silica NPs via radical polymerization of styrene onto silica NPs possessing vinyl groups with benzoyl peroxide (PS-g-SiO_2). Polystyrene was grafted onto vinyl silica NPs via covalent bond. The NPs were added on a Si wafer using the drop casting technique and the resulted surface was hydrophobic [69].

Surface roughness of NPs is another parameter that affects their wettability. NPs with hydrophilic composition but with hydrophobic properties at the nanoscale level have attracted the scientific interest. A group has produced mesoporous hollow silica (MHS) nanospheres with controlled surface roughness [59]. The roughness induced to the MHS (RMHS) showed unusual hydrophobicity compared with the same MHS without roughness and resulted in higher adsorption of a range of hydrophobic molecules and controlled release of hydrophilic molecules. They added smaller silica shell particles with (13-30 nm) onto MHS with larger size (200-400 nm) providing more space to trap air, creating a higher energy barrier and thus more hydrophobicity [59].

Surface modification has also been conducted for hydrophilic barium sulfate NPs (BaSO4) in order to alter their wetting behavior into hydrophobic. BaSO4 NPs have been produced through precipitation reaction in aqueous solution of CaCl2 and (NH4)2SO4 with octadecyl dihydrogen phosphate (n-C18H37OPO3H2, ODP) as a modifying agent. The resulting NPs showed a hydrophobic character due to a thin layer of barium alkyl phosphates that was formed and coated the surface of the NPs [61].

Iron oxide NPs offer a high potential for biomedical applications such as cellular therapy, tissue repair, drug delivery etc. Without any surface coating iron oxide NPs exhibit **hydrophobic** surfaces, therefore, they **agglomerate and form large clusters**. To avoid this, different surface coatings have been used to modify the NPs surface to hydrophilic. Some of them are polyethylene glycol, Dextran, Polyvinylpyrrolidone, fatty acids, Poly(vinyl alcohol), Poly(acrilic acid), Polypeptides, Chitosan, Gelatin and many others. The polymeric shell of NPs can be produced using an inverse microemulsion polymerization process. For example, hydrophilic iron oxide NPs have been produced by encapsulating adsorbed iron oxide nanoparticles onto oppositely charged polystyrene-core/poly(N-isopropylacrylamide) shell. Linker molecules such as EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride), SPDP (N-succinimidyl 3-(2-pyridyldithio) propionate) and MBA (N-hydroxysuccinimide or N, N0 methylene bis acrylamide) can be used to attach the coated molecules to a protein coating in order to facilitate cell attachment [70].

When NPs come in contact with biological fluids they are immediately coated with proteins, and thus cells or tissues never encounter the naked particles [71-72]. The protein-nanoparticle interactions possess a key role in nanomedicine, the so called nanoparticle-protein 'corona' [73]. Plasma protein adsorption onto NPs surface depends on their surface characteristics and is very important for the *in vivo* organ distribution [74]. NPs hydrophobicity is a key parameter that affects plasma protein adsorption amount and composition. Muller et al. have studied NPs with decreasing surface hydrophobicities and the influence on plasma protein adsorption. They used latex particles as model colloidal carriers with different wetting behavior and concluded that decreasing surface hydrophobicity leads to decrease amounts of adsorbed proteins and deteriorates the changes in the obtained proteins adsorption patterns [75]. Lindman *et al.* examined the adsorption of Human Serum Albumin (HAS) to copolymer NPs (70-700 nm) of different hydrophobicities. The most hydrophobic NPs were fully covered with a single layer of HAS. The hydrophobicity was controlled via the co-monomer ratio, N-iso-propylacrylamide/N-tert-butylacrylamide (NIPAN/BAM), showing that the 100:0 NIPAM/BAM was the most hydrophilic. For particles with 25% BAM and below, very little binding of HAS was observed [71].

2.4.2 Key Parameters and how they affect Functionalities_Applications

A. Cell Uptake

At nanoscale, the materials exhibit clearly differentiated or enhanced properties compared with their conventional 'bulk' counterparts, due to their increased surface area that translates into higher reactivity [76]. Nanoscale objects interact with all components of living organisms, often in a manner that is fundamentally different from freely diffusing small molecules or large particles that are recognized by the immune system, [77-80]. Due to their **size**, nanomaterials could interact with the endogenous cellular machinery and can thus enter cells through the active energy-dependence processes [81-88].
So far, several studies have identified that cellular uptake of nanoparticles can depend mainly on **specific aspects of the nanoparticles (including the size and/or shape of the nanoparticle, sedimentation effects of large and dense particles and the composition of the protein corona on the nanoparticle** [89-91, 92-99], and secondly on the state of cell (e.g. the cell cycle phase) [100].
The size effect on cell uptake of nanoparticles is a currently important issue in the field of nanobiology [101]. **Particle size** is an important parameter in designing suitable cell-tracking and drug-carrier nanoparticle systems, because it determines the mechanism and rate of cell uptake of a nanoparticle and its ability to

permeate through tissue [102-103]. In this context, the **size and shape of nanoparticles** are extensively studied.
Jiang *et.al* observed significant differences concerning the uptake of the different size and shaped gold nanoparticles [104]. Specifically, the uptake concentrations for 74 _ 14 nm **rod-shaped** nanoparticles were different than those for 74 or 14 nm **spherical nanoparticles**. The authors attributed these results to the difference in the curvature between spherical and rod-shape nanoparticles which imparts to the rod-shaped nanoparticles can have larger contact area with the cell membrane receptors than the spherical nanoparticles. Generally they suggested that nanoparticle size and shape can mediate receptor-ligand binding constants, receptor recycling rates, and exocytosis.
Yu *et al.* examined the uptake, localization, and cytotoxic effects of well-dispersed amorphous SiO_2 NPs in mouse keratinocytes (HEL-30) [105]. These cells were exposed for 24 h to various concentrations of amorphous SiO_2 NPs in homogeneous suspensions of average size distribution (30, 48, 118, and 535 nm SiO_2) and then assessed for uptake and biochemical changes. Results of TEM revealed that all sizes of silica were taken up into the cells and localized into the cytoplasm.
Carlson *et al.* evaluated size-dependent cellular interactions of known biologically active Ag NPs (15, 30, and 55 nm) in alveolar macrophages [106]. Alveolar macrophages provide the first line of defence against foreign debris in the lung and were studied for their potential role in initiating oxidative stress. In-vitro exposure produced morphologically abnormal sizes and adherence characteristics with significant NP uptake at high doses after 24 h.
In another study polymeric NPs were used to investigate the effects of **particle size and surface charge on cellular uptake and biodistribution** [90]. The results showed that NPs with high surface charge and large particle size were phagocytized more efficiently by murine macrophage. Slight particle size and surface charge differences and different cell lines had significant implications in the cellular uptake of NPs, and various mechanisms were involved in the uptake process. *In-vivo* biodistribution suggested that NPs with slight **negative charges and particle size** of 150 nm tended to accumulate in tumours more efficiently.
In order to avoid the sedimentation of the NPs in a typical cell culture plate, Cho and his colleague used upright and inverted cell culture configurations to show that **cellular uptake of gold nanoparticles** depends on the **sedimentation and diffusion velocities of the nanoparticles** and is **independent of size, shape, density, surface coating and initial concentration of the nanoparticles** [96]. Using this system, they proved that more nanoparticles are taken up in the upright configuration than in the inverted one, and nanoparticles with faster sedimentation rates showed greater differences in uptake between the two configurations. These results indicate that **sedimentation** needs to be considered when performing in-vitro studies for large and/or heavy nanoparticles.
Some reports show saturation of the intracellular nanoparticle concentration within hours [89, 107] others after several day [108-110]. It is also mentioned [89] that kinetics and saturation concentrations are highly dependent upon the physical dimensions of the nanoparticles (e.g., uptake half-life of 14, 50, and 74 nm nanoparticles is 2.10, 1.90, and 2.24 h, respectively) but the saturation rate of uptake may also depend on the **number of available proteins** (that are not adsorbed onto the gold nanoparticles in the DMEM + serum) since these unbound proteins can compete for receptor sites on the cell surface against protein-adsorbed nanoparticles.
As it is already mentioned NPs enter cells through active processes, thanks to their capability of interacting with the cellular machinery. Once the NPs are in contact with biological fluids, such as the cell serum, they strongly adsorb on their surface a very selective layer of proteins and other biomolecules which is called corona [111]. The corona mediates the interactions with cells in situ. As a consequence of this, it has been proposed [111] that the same nanomaterial can lead to very different biological outcomes, when exposed to cells in the **presence or absence of a preformed corona**. In particular, silica nanoparticles exposed to cells in the absence of serum have a stronger adhesion to the cell membrane and higher internalization efficiency, in comparison to what is observed in medium containing serum, when a preformed corona is present on their surface. The different **exposure conditions** not only affect the uptake levels but also result in differences in the intracellular nanoparticle location and impact on cells. Interestingly, results showed that after only one hour of exposure, a corona of very different nature forms on the nanoparticles exposed to cells in the absence of serum. Evidence suggests that these different outcomes can all be connected to the different adhesion and surface properties in the two conditions.
Finally, the role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population has also been investigated [100]. Kim *et.al* showed that the cell cycle phase could also influence the cell uptake of nanoparticles. Although cells in different phases of the cell cycle were found to internalize nanoparticles at similar rates, after 24 h the concentration of nanoparticles in the cells could be ranked according to the different phases: G2/M> S > G0/G1. Nanoparticles that are internalized by cells are not exported from cells but are split between daughter cells when the parent cell divides. This study suggested that in a cell population, the dose of internalized nanoparticles in each cell varies as the cell advances through the cell cycle.

B. Optical Properties / Electronic Properties and Catalytic Activity

The following section reports a series of studies related with the key properties of MNMs and how these affect the functionalities/applications related with the optical, electronic properties and catalytic activity of systems and materials. A single MNM may have different roles in different systems, therefore a careful design of MNMs is essential in order to develop a device with maximum performance, stable, and safe for human use and for the environment. There is not a specific order that this section is written, as the functionalities are inter-related and complemented with the various key properties of the MNMs.

Inhalation of butane causes headache, temporary memory loss, narcosis, euphoria, drowsiness and harmfulness to nerve system [112]. As a volatile organic compound (VOC), butane can be involved in photochemical reactions that create ground level ozone, which significantly affects human health and natural ecosystems. Recently, non-thermal plasma technique combined with catalysis is regarded as a good oxidation method available for the treatment of VOCs [112].

Generally, MNMs have outstanding catalytic properties, compared to bulk materials. ZnO nanomaterials are considered as potential candidates for solar cells, light-emitting diodes, nano-lasers, transistors, photocatalysis, sensors, and antimicrobial agents owing to its low cost, easy availability, **good stability, high excitation binding energy (60 meV) and wide band gap (3.37 eV)** [113]. The **photocatalytic efficiency of ZnO** nanomaterials has extensively been investigated for environmental purification applications.

The observed catalytic activity of ZnO in the oxidative decomposition of butane was strongly **shape-dependent**. It was found that the ZnO NWs exhibited higher catalytic activity than the other nanomaterials and could completely oxidize butane into carbon oxides (CO_x). When using the bare or ZnO NPs-coated ceramic membrane, several unwanted partial oxidation and decomposition products like acetaldehyde, acetylene, methane and propane were identified during the decomposition of butane. When the ZnO NWs- or ZnO NRs-coated membrane was used, however, the formation of such unwanted byproducts except methane was completely avoided, and full conversion into COx was achieved. **Better carbon balance (CB) and CO_x selectivity** were obtained with the ZnO NWs and NRs than with the NPs.

ZnO nanomaterials depend largely on **the particles size, morphology and surface area,** properties significant for **oxidation reactions** [114]. A study showed that the photocatalytic decomposition performance of methylene blue dye depends on the **morphologies, oxygen vacancies and crystal planes of ZnO.** Specifically ZnO nanodisks have better photocatalytic activity due to more population of (0001) crystal plane structures [115]. The photocatalytic treatment of organic pollutants by ZnO nanorods (NRs) with a cone of **small aspect ratio** was reported to be more effective than that of ZnO NRs with a cone of large aspect ratio and that of short-and-fat ZnO microrods [116]. Moreover ZnO nanoflowers and nanosheets showed significantly higher photocatalytic activity for methyl orange degradation than ZnO nanospheres [117].

The **shape, crystal size and structure of the ZnO** nanomaterials are also important for their **antimicrobial performance** [118]. ZnO nanoflowers demonstrated substantially higher photocatalytic activity in the inactivation of Escherichia coli and Staphylococcus aureus than ZnO NRs and nanospheres.

The **shape-dependent catalytic behavior of ZnO nanomaterials** plays a role in the potential applications of VOC purification. All the reactive oxygen species such as oxidation reactions, hydroxyl radicals, superoxide and singlet oxygen, give rise to the oxidation of the organic pollutants [112].

During the past decade, there has been increasing attention to the biosensing field and biosensors such as electrochemical aptasensors (ECASs). ECASs use aptamers selected by SELEX as recognition elements, exhibiting advantages of high sensitivity, fast response, simple operation, low cost [119]. Shortly, the recognition reaction is translated into targeting concentration- or activity-related electrochemical signal by transducers. There are labelled and label-free ECASs.
A careful design of the ECASs and its corresponding detection is important. Mainly the detection strategies focus to clinical diagnosis through DNA analysis, immunoassay or enzymatic sensing as well as environmental monitoring, including ocean and atmosphere pollutants. However, previously reported ECASs tend to analyse the single targeting molecule [119]. In real samples, there are multiple molecules usually existing and thus the detection mode of single analyte is not enough efficient.

The use of carbon nanomaterials (CNMs) to form functionalized composites of ECASs CNMs is one of the current development strategies for ECASs-based sensing platforms. These composites can enhance the sensitivity to target substances with a low LOD, even as low as 10–18 M. Generally, CNMs exhibit excellent electrical conductivity and high specific surface area and their main functions are as electronic conductive matrixes and aptamers immobilization platforms [119]. Using functional compositions to modify CNMs is an effective strategy to overcome the defects, and even can breed new functions. In particular, carbon nanotubes

(CNTs) are widely used as catalyst carriers or backing layers [119]. The main advantage of using CNTs is attributed to the enhanced **electro-catalytic activity** and a giant large surface to volume ratio. The combination of CNT with other materials can improve the carrier content and stability of proteins or enzymes. These excellent properties significantly depend on the **functional atomic structures of different types of CNMs** such as carbon nanotubes, graphene, graphene oxide,etc., **but also the interactions** with other materials, such as gold nanoparticles, SiO_2, chitosan, etc [119]. CNTs are divided into single-wall (SWCNTs) and multi-wall CNTs (MWCNTs), which the latter is more often used in ECASs applications due to the difficulty in controlling the **chirality and diameter of SWNTs**. Another comparison arising here is between CNTs and Graphene (GN). GN has low thermal noise, good chemical stability, simple preparation and low cost. GN is also an excellent conductive material [120; 121]. GN can be distinguished into the original GN and the reduced graphene oxide (rGO) from graphene oxide (GO). rGO is proved to possess **lower electrical conductivity** than original GN by several magnitudes since oxygen-containing functional groups remain on GN surface; but still higher than the GO [122]. Thus, a comparison between these three types of CNMs shows that Gr is the preferable CNM for ECASs developments followed by rGO and then GO. Furthermore, Gr-inorganics composites exhibit a great attention by taking the advantages of both Gr and inorganic elements (e.g. AuNPs). In this case, these composites enable a higher active area and an enhanced rate of electron transfer.

Magnetic nanoparticles (MNPs) are a type of metal oxides NPs, with their main characteristic the use of an external magnetic field. Their chemical, optical, electrical, thermal and magnetic properties are exploited at different analytical steps including sample treatment, chromatographic techniques, and detection, in order to achieve the required analytical properties (accuracy, precision, sensitivity, selectivity, speed and cost) [123]. MNPs can be modified with organic, inorganic and biochemical compounds to increase their physico-chemical properties. For example, hybrid MNMs are thrived by the combination of Fe_3O_4NPs and metallic, silica, carbon and polymeric NPs for the manufacture of electrodes, thereby finding that **some electrocatalytic properties** [124]. These electrodes are important because of their numerous advantages, including **large surface area, low resistance to electronic transmission and the ability to absorb chemically (bio) chemical analytes**, which make them very attractive and useful in the electrochemical classical determinations. The main advantages of using MNPs in this field are the minimization of deterioration of the electrode surfaces, the increase of the electrocatalytic activity and the simplification immobilization process [123].

Engineering the electrochemical sensing interface with functional MNMs leads to novel electro-chemical biosensors with improved performances in terms of sensitivity, selectivity, stability and simplicity [125]. Functional NMs possess **good conductivity, catalytic activity, biocompatibility and high surface area**. These properties are dependent **on their sizes and shapes**, such as, size-dependent **optical properties** of metal nanoparticles (NPs) [126], **electrical conductivity of CNMs** [127], **electrocatalytic properties of metal NPs and nano-carbons** [128], and high surface area.

The hybridization of different types of materials is crucial for enabling versatile and tailor-made properties with performances far beyond those of the individual materials. There is a question arising whether CNMs and their hybrids can provide a new choice for developing a series of electrochemical and analytical devices with high performance. As it is stated at previous sections, it is very challenging to design structures possessing maximum performance and at the same time provide a safe use throughout their lifetime. In the case of GN, there are the GN-based functional hybrids, which are highly desirable for optimizing the **optical, electrical and catalytic** properties of GN and enhancing its performance in different fields such as electrochemistry and analytical chemistry [129]. However, there are two challenges: GN should be incorporated as **individual nanosheets**, and **homogeneously distributed**, into various nanomatrices, and various nanomaterials on the surface of GN should be **accurately tuned into the desirable architectures**, which will most facilitate the performance improvement of hybrids. [130].

Currently, the design and development of multicomponent hybrid nanostructures containing at least one GN component is affected solely by the **particular properties of GN** and synergistic properties induced by different functional nanoscale objects [129]. The main key issues that need to be considered are: (i) GN should exist in the **form of individual nanosheets** in the corresponding hybrids in order to effectively **enhance the function of GN** according to the specific application; (ii) Bifunctional linker or molecular ‘‘glue’’ can be synthesized to adhere NPs onto the surface of GNs; (iii) NPs should be **uniformly distributed on the surface of GNs** with controllable **density** in order to tune the performance of hybrids; and (iv) the **size, morphology and component** of NMs should be carefully controlled [129].

Another main feature of GN is that it effectively **promotes the electron transfer** between electrode and analytes. GN-based electrodes have shown superior performance in terms of **electrocatalytic activity and macroscopic scale conductivity** than CNTs-based electrodes [131].

The electrochemical oxidation of nicotinamide adenine dinucleotide (NADH) is of great interest since it is required in a whole diversity of dehydrogenase-based biosensors [132]. Pyrolytic graphite electrodes have been used to study the electrochemical oxidation of NADH at carbon electrodes and specifically to elucidate the mechanism of oxidation [132]. Furthermore, it is crucial to state that in respect of basal plane graphite electrodes, **electron transfer** may be more facile at samples containing a **higher proportion of edge plane defects**. In a study they have recently demonstrated an electrode wholly formed of edge plane graphite that is a disc of pyrolytic graphite machined to a chosen diameter with the disc surface facing parallel with the edge plane to display high electrocatalytic activity for a variety of electroanalytical tasks, including the oxidation of thiols [133] and gas sensing [134].

Specifically, they compared an edge plane pyrolytic graphite electrode with basal plane pyrolytic graphite, glassy carbon, boron-doped diamond and MWCNT modified bppg electrodes for the sensing of NADH [132]. The main findings are described: i) the **electrocatalytic properties of MWCNTs** modified electrodes toward the oxidation of NADH are attributed to the **edge plane sites/defects** which occur along the tube axis or at the open ends of the tubes, with 'hollow tube' and 'bamboo' type nanotubes giving similar responses; ii) NADH adsorption at CNTs and edge plane electrodes occurs at edge plane sites. Due to the **high density of edge plane sites on CNTs and edge plane pyrolytic graphite electrodes**, they are unsusceptible to electrode passivation [132]. The oxygen functionalities may likely reside here and promote a means for binding the adsorbing materials; iii) electroanalytical sensors by CNTs based electrodes should optimally have a large **proportion of edge plane sites for the best detection limits** and, iv) edge plane pyrolytic graphite electrodes can conveniently replace CNTs modified electrodes due to their low susceptibility to electrode fouling, low detection limit and insensitivity to interference from ascorbic acid (oxidized at similar potentials causing problems at the determination of biological substances) [132].

Certain MNMs exhibit unique and interesting optical properties and thus are used as biomolecular labels. Their main function is to amplify biorecognition signals and enhancing the sensitivity of the biosensor [135]. A large variety of NPs, including metal NPs, oxide NPs, semiconductor NPs, and even composite NPs, have been widely used in electrochemical sensors and biosensors. The excellent **catalytic and optical properties**, as well as the **large surface area of NPs**, offer many design challenges for biosensing devices with exceptional performance [136]. The majority of the NPs bear high isoelectric point (IEP) that favors the **electrostatic adsorption of proteins** with low IEP. Therefore, they are promising immobilization supports.

A cholesterol biosensor is fabricated where an interfacial layer of AuNPs has been used for immobilizing ChOx on gold electrode surfaces. Here, AuNPs were shown to provide an environment for **increased electrocatalytic activity of ChO_x** and thus improved the analytical performance of the biosensor in terms of **stability** [137].

AuNPs have been shown to improve the analytical performance of the fabricated cholesterol biosensors. This observation is attributed to the biocompatibility of AuNPs based matrices to help proteins to retain their biological activities for a long time and thus enhancing the biosensor's stability [135]. The increase in the biosensor's sensitivity and selectivity is mainly due to the **electrocatalytic activity of AuNPs**. Here, AuNPs aided in enhancing **electrode conductivity and facilitated the electron transfer** between the enzyme's redox center and the electrode. AuNPs on flat electrode surfaces may also partially penetrate the enzyme matrix and thus may get closer to the redox centre of the enzyme which further aid in the electron transfer pathway.

AuNPs also exhibit outstanding optical properties which are due to a phenomenon called SPR which occurs because of the interaction of light with the collective oscillations of electrons on the AuNPs surface at a definite wavelength of light [135]. This extinction of light due to SPR depends on the **size, shape and aggregation state of AuNPs**. This has found strong applications in detection assays where an alteration in extinction of light resulting from the aggregation of AuNPs upon analyte addition can be used as optical signal [138].

Metal oxide NPs, due to their high **electron conductivity**, assist in achieving low detection limits in the analysis [139; 140]. Also, their better **adsorption capability** for the biomolecules leads to **high stability of the biosensors**. Zinc oxide (ZnO),iron oxide (Fe_3O_4), cerium oxide(CeO2), and titanium oxide(TiO_2) NPs that have been exploited to improve the sensor performance [141; 142; 143]. ZnO has many distinctive properties such as electronic and optical properties, high surface area, high catalytic efficiency, chemical and photochemical stability, optical transparency, biocompatibility, and ease of fabrication.

As mentioned at an earlier section, CNTs possess large aspect ratio, strong adsorption properties and metallic or semiconductive properties. They exhibit quantum confinement of electrons normal to the nanotube axis with which they can transport electrons over long lengths [135]. They have great potential as biomolecules

immobilization platforms. The nanostructuring of electrodes with CNTs/polymer composites to improve the analytical performance of amperometric biosensors has been demonstrated at various studies [144; 145]. These composites display percolation behavior, where **organized nanotube network** results in remarkable enhancement of **conductivity of electrodes**. Another interesting point is that the **electrical and thermal conductivity of CNTs** can be modulated by the doping of CNTs with various elements such as potassium, cesium, boron, nitrogen, phosphorous and silicon etc. [146; 147].

New matrix/hybrid systems (based on nanomaterials) are designed in order to develop efficient and with improved performance cholesterol and other biosensors. Each MNM has its own advantages for the different applications, and therefore it is essential involve synergistic properties of different nanomaterials to complement each other at the desired hybrid system [148; 149]. MNM-based cholesterol biosensors have shown better performance with respect to **wider detection range, high stability, and lower Km**. A single MNM may have different roles in different biosensing systems. In cholesterol biosensor application, **the electronic properties of the MNMs** play a major role to improve the biosensor's analytical performance. Understanding the mechanism behind the enhancement of this performance is also an essential factor that is weakly reported in the studies.

The literature review was interpreted in different ways. A table showing the relationship of the key parameters with functionalities was formulated and categorized as of importance and of priority. In other words a key parameter might be **'a priority'** indicating that it affects significantly the functionality. For example size and composition are two physicochemical properties that are 'of priority' for all the stated functionalities in D6.6. '**Only of importance'** shows the relationship of key parameters to functionalities but not on their own (see Table 1A and B). Here an example is the case of surface area that is of importance when investigating its effect on functionalities such as hydrophobicity, dissolution and optical/electronic applications . In order to draw some statistics a second table was created, from which it became clear which nanomaterials are used in almost every application and therefore an extensive design strategy is required. All the tables and the review were summarized in order to draw the data model.

2.4.3 Literature review in Tables and Statistics

Table 1A: Functionality in terms of Applications in relation to key parameters (* of importance; ** priority); a key parameter is of a priority for some functionalities while only of importance for other functionalities.

FUNCTIONALITY		APPLICATIONS			
KEY PARAMETERS		Cell uptake	Optical Properties	Electronic Properties	Catalytic Activity / Biorecognition
Geometrical	Particle Size (eg. Hydrodynamic radius and polydispersity index-PdI)	**	**	**	**
	Shape	**	**	**	**
	Aspect Ratio (ratio of width over height)	**	*	*	*
Chemical	Composition	**	*	**	**
	Surface charge / Z potential	*	**	**	
	Chemistry /release	**			
Crystallinity	Crystal structure /Crystallinity		*	*	*
Morphological	Topology (eg core shell etc)				
	Porosity	*			
	Surface area		*	*	*
	Roughness	*			
Coating	Surface Coating Characteristics	**	*	*	*
	Surface Coating Stability		*	*	
	Surface reactivity		*	*	*
Dispersibility	Agglomeration State (Aggregation)	**	**	*	*
	Test medium(Only water)	**	**	*	*
	Test medium_pH	*			
	Test medium_Ionic Strength(or chemistry)	*			
Other	Specific Surface area	*			

Table 1B: Functionality in terms of Performance in relation to key parameters (* of importance; ** priority); a key parameter is of a priority for some functionalities while only of importance for other functionalities.

FUNCTIONALITY		PERFORMANCE		
KEY PARAMETERS		Solubility/ Dissolution	Dispersion	Hydrophobicity
Geometrical	Particle Size (eg. Hydrodynamic radius and polydispersity index-PdI)	**	**	*
	Shape	*	*	
	Aspect Ratio (ratio of width over height)	*	*	
Chemical	Composition	**	**	**
	Surface charge / Z potential	*		*
	Chemistry /release	*	*	*
Crystallinity	Crystal structure /Crystallinity			
Morphologic	Topology (eg core shell etc)			
	Porosity			
	Surface area	*		*
	Roughness	*		*
Coating	Surface Coating Characteristics	**	**	**
	Surface Coating Stability	**	**	**
	Surface reactivity	**	**	**
Dispersibility	Agglomeration State (Aggregation)	**	**	**
	Test medium* (Only water)	**	**	**
	Test medium_pH	**	**	**
	Test medium_Ionic Strength(or chemistry)	**	**	**
Other	Specific Surface area			

Table 2: Statistics related with the number of papers/studies of the literature review

STUDIES –LITERATURE REVIEW		
MNM type	No of papers	%
MNMs_TiO2	9	7
MNMs_SiO2	15	11
MNMs_ZnO	21	16
MNMs_CeO2	3	2
MNMs_BaSO4	2	2
MNMs_Ag	13	9
MNMs_SWorMWNTs	32	23
No of other Nanomaterials	MNPs_4; AuNPs_15 CuO_6; Al2O3_3; CdSe_2 and others_11	MNPs_3; AuNPs_11 CuO_4; Al2O3_2; CdSe_2 and others_8

Most of the papers in this literature study are recent review papers, where a major number of studies are included. Basic statistics (%) were used and calculated in order to be able to complement the form of the proposed data model.

2.4.4 Discussion – Evaluation of the results

The main points of the above extensive literature review are summarized by outlining the important key parameters of MNMs that play a role for each one of the functionalities.

Dispersion of MNMs

- Particle chemical composition. **In general, all nanoparticles forms some kind of aggregates or agglomerates in water or aqueous media with the only exception being SiO_2 nanoparticles for which there are cases where the primary particle size is detected.**
- Particle size is not that crucial except between nanoparticles and particles with r>300-400nm or bulk.
- Surface charge affects dispersion
- Surface coating affects the dispersion
- Crystalline vs amorphous nanoparticles or nanoparticle crystal phase affects the dispersion
- Organic moieties e.g. proteins in the solution help the dispersion
- Solution pH affects the dispersion
- The solution ionic strength affects the dispersion

Hydrophobicity of NPs

- The surface of most inorganic NPs is hydrophilic
- Hydrophobicity of NPs and MNMs depends on NPs chemical characteristics (composition, surface charge, chemistry) and NPs coating (coating characteristics, coating stability, surface reactivity
- Is very important criterion for the biocompatibility of NPs
- Is related to dispersability (hydrophilic high dispersability/hydrophobic low dispersability
- Hydrophobicity of NPs can be altered by surface modification using hydrophilic surfactants or hydrophilic polymers

Solubility/dissolution of NPs

- Dissolution of NPs depends on NPs characteristics such as size, chemistry, surface coating, crystallinity, composition, surface area
- Dissolution of NPs depends on solution characteristics such as pH and temperature
- Dissolution of NPs affect their antimicrobial activity and biocompatibility
- NPs dissolution involves the release of ions into the solution

Cell uptake

- Cellular uptake of nanoparticles can depend mainly on the size and/or shape of the nanoparticle,
- Sedimentation of large and dense particles and diffusion velocities of the nanoparticles should be considered when performing in-vitro studies for large and/or heavy nanoparticles.
- The composition of the protein corona on the nanoparticle plays a significant role on cellular uptake.

Optical/Electronic properties and Catalytic activity of MNMs

- Single nanomaterials may play different roles in different types of devices in the biosensor field; depending on the desired functionality their key parameters should be carefully designed and tuned; usually used in composite/hybrid system in order to enhance its performance in terms of detection, stability and long duration.
- Shape dependency functionalities (electrocatalytic and optical applications); better carbon balance and carbon oxide selectivity on ZnO wires > rods compared with NPs
- Size is also a very important parameter for these functionalities
- The organization of the single MNMs on a composite affects the overall performance of the sensors and the other systems.
- The atomic structure and morphology of the MNMs is of crucial importance for all the functionalities of this section; electrical conductivity: GN > rGO > GO due to the presence of the oxygen-containing functional groups.

According to Tables 1A and B, it is clear that the key parameters of size, shape, composition, surface charge, aggregation, test medium (in our case water) are ‘of priority’ parameters that on their own could affect all the functionalities; while the parameters such as crystal structure, surface area, roughness are ‘of importance’ indicating their dependency with the ‘of priority’ parameters. The literature study consisted of about 140 papers, mainly reporting on CNTs; ZnO, SiO2, Ag and then the rest of MNMs as shown in Table 2. The main information we gathered from this table involves the interest and significance of these materials in the fields of analytical chemistry, electrochemistry and the sensors field in energy and medicine/pharmacy.

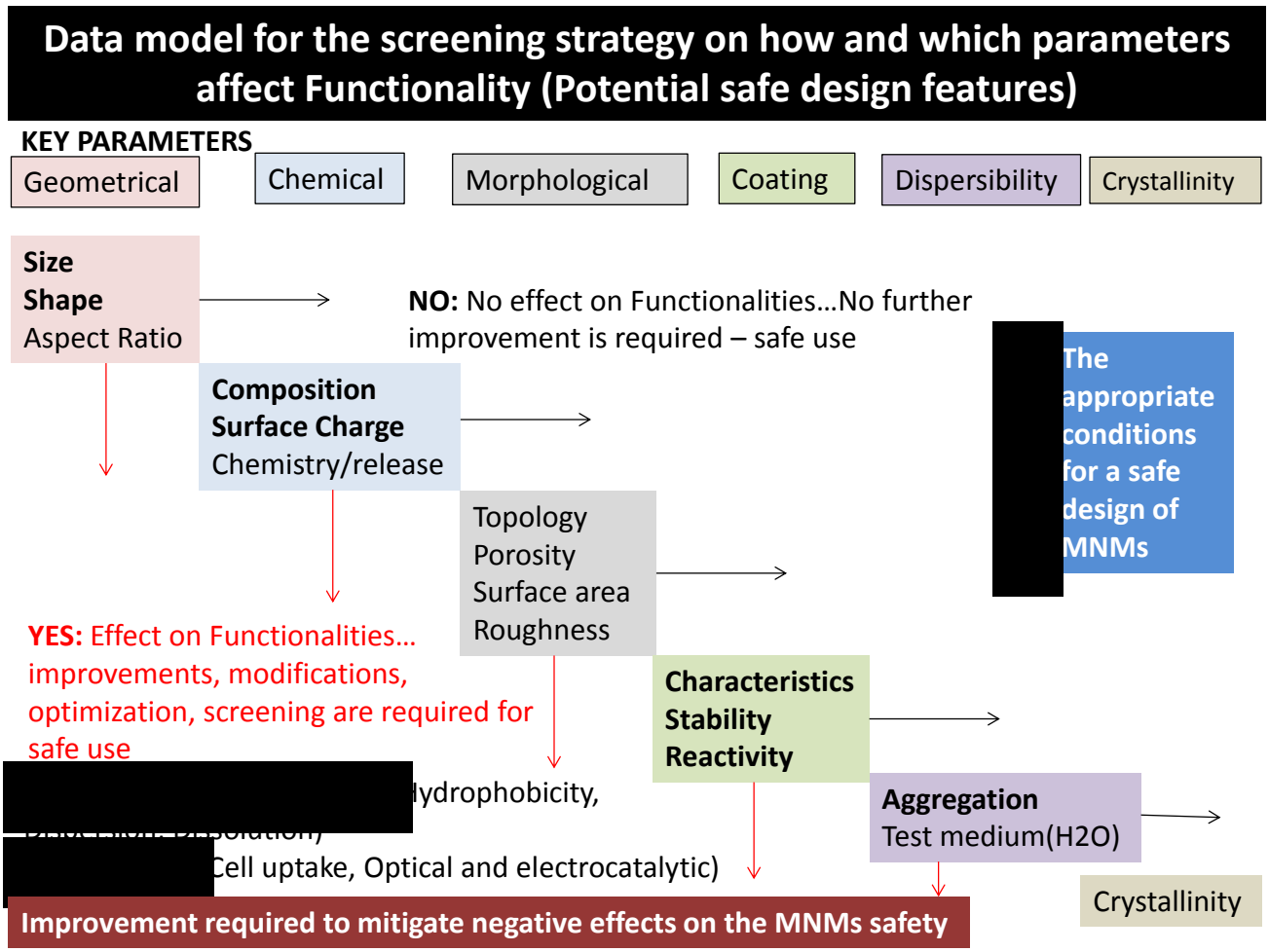

Figure 1: Data model, as a first attempt towards a Decision tree strategy for the Safe by Design of MNMs

How the proposed data model can assist you with your design strategy?

One of the most important functionalities that should be considered as a priority for a design strategy is the dispersion ability of the nanomaterials. Therefore better dispersion characteristics need to be explored. According to the proposed data model in Fig.1, this can be achieved by playing with the size, shape and surface charge of the nanomaterial.

Another route towards this is the use of surface coatings. Stable dispersions are also by the functionalization of nanomaterials e.g. CNTs with small hydrophilic groups. The oxidative state of nanomaterials at the interface is another potential design feature that can be used to mitigate MNMs safety such as toxicity.

It is very important to comment on the fact that there is an intra-relation between the properties and the functionalities and this relation depends to a great extent on the application that the nanomaterials are used for. For example, if you want nanoparticles to aggregate in order to immobilize engineered nanomaterials at disposal sites then you have to modify the size, and surface chemistry of MNMs (intra-relation/dependency between the two properties); on the other hand if you prefer good dispersion ability of nanoparticles to clean up environmental spills you have to modify again their size and surface chemistry (again their dependency) [2]. Furthermore, the Nanotechnology Characterization Laboratory (NCL) at the National Cancer Institute in Maryland Having have assessed more than 130 different nanoparticle types, including fullerenes, metal oxides, polymers, liposomes, dendrimers, quantum dots and gold colloids, and they came to the conclusion that hydrophobicity (Functionality), size and surface charge (key parameters) are the main factors influencing nanoparticle biocompatibility[150].

To improve the design of a nanomaterial, we need to explore innovative tools to probe dynamic biophysicochemical interactions. An integration of characterization methods (both theoretically and experimentally) of traditional bulk material properties with studies of the (nano–surrounding environment)

interface is mandatory in the case of nanomaterials. They also need to be simple and accessible laboratory equipment.

There are many challenges regarding the physicochemical key parameters of nanomaterials; these challenges in the D6.6 report could be characterized as 'data gaps' in the literature and we consider them as significant aspects for enhancing the safe by design strategy. These are mentioned here:

- Bulk properties of nanomaterials (or in pristine state) vs same properties when it is in a solid-liquid environment; dynamic and metastable states present at the interface eg. biological fluid
- Different manufacturing/production routes or modifications as well as different residues
- Different experimental conditions eg protocols, instruments, in-vitro vs in-vivo
- Improvement/enhancement of characterization tools for site-specific or local assessment for nanomaterials eg. high resolution and live imaging, 3D reconstruction, data acquisition process

During the collection of all the above data, it was obvious that much work is still needed to advance knowledge in the area of physicochemical study of nanomaterials, and how their key parameters nanomaterials influence their fate and behavior and their potential to induce toxicity in human health and in the environment.

Preparation of a paper including the literature review, the tables and the data model is under preparation in collaboration with Task Leader and some other partners. Furthermore this deliverable will be communicated and used in WP3 of NanoReg2 regarding the Functionality of Nanomaterials.

2.5 Evaluation and conclusions

There is an ongoing growth in Nanotechnology where MNMs are more often coming into contact with humans and the environment. The interface between any type of nanomaterial with the surrounding environment either proteins/cells in a culture medium or bound to a matrix/composite or in a solvent depends on colloidal forces as well as dynamic biophysicochemical interactions. The development of predictive relationships between structure (key parameter) of nanomaterials and activity (functionality) are determined by nanomaterial properties. The key parameters of size, shape, composition, surface charge, aggregation, test medium (in our case water) are the priority parameters affecting all the functionalities; while the rest of parameters are of importance. The understanding of these relationships is very important from the perspective of safe use of nano materials. It is not possible to describe all the biophysicochemical interactions at the interface, but we made an effort to assemble knowledge and information to provide a framework to guide this exploration. The literature study consisted of about 140 papers, mainly reporting on CNTs; ZnO, SiO2, Ag and then the rest of MNMs; a priority list was formulated focusing on the core nanomaterials of NANoREG project; the publication year in order to have as recent as possible literature data; and the key physicochemical parameters as showed in other deliverables of WP6 including toxicity. The main finding from D6.3 was a list of risk potentials, which in our case involve our functionalities such as solubility/dissolution, that needs to be screened during the development of toxicity testing. D6.6 is part of the NANoREG project aimed to provide some consensus on which key parameters of MNMs affect specific functionalities in an attempt to establish a relation between them and to propose a workable data model. This is one of the first attempts to identify the main key properties of the MNMs that in relation to their functionalities play a dominant role on the safe by design. This proposed data model is considered as one of the possible approaches towards a decision tree strategy for (re)designing safe MNMs for humans and the environment which will be established in NanoReg2 project. The optimal design of safe nanomaterials is a challenge with multiple compromises between functionality and safety characteristics.

2.6 Data management

Nothing to report

3 Deviations from the work plan

FORTH replaced the partner_GeoChem in this project during the final year; approval of the Greek partner at the meeting_12.11.2015 in the presence of Coordinator and Project Manager.

FORTH's involvement in WP6 commenced in December 2015 and continued in 2016 with a series of teleconferences mainly between RIVM (Task 6.3 Leader), and TNO and then at a later stage with Ecamricert. Due to the remaining time the main issue to be addressed within this deliverable was to to find the most appropriate way to describe the relation between physicochemical characteristics of MNMs to functionalities except toxicity . At the Bilbao meeting (June 2016), the first outline of this deliverable was discussed between the related partners. FORTH sent the first draft of D6.6 to the partners beginning of September 2016. The completed version of D6.6 was send for MC approval by RIVM-Task Leader on 30.09.2016.

The work for this deliverable was tuned to activities in NanoReg2 as work was performed during a period of time when NANoREG and NanoReg2 ran simultaneously. The output of this deliverable will directly be taken into account in task 3.2 in WP3 of NanoReg2.

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6 List of abbreviations (optional)

ATPR: atom transfer radical polymerization	NIPAN/BAM: N-iso-propylacrylamide/N-tert-butylacrylamide
BALF: bronchoalveolar lavage fluid	NM: Nanomaterial
BaSO4: barium sulfate	NOM: Natural Organic Matter
BEGM: Bronchial Epithelial Growth Medium	NP: Nanoparticle
BSA: bovine serum albumin	NRs: nanorods
C60CS: Fullerene carbon spheres	NWs: Nanowires
CB: carbon balance	OA: oleic acid
CeO2: cerium oxide	PAA: polyacrylic acid
CNM: carbon nanomaterials	PBS: phosphate buffered saline
CNTs: carbon nanotubes	PEG: polyethylene glycol
COx: carbon oxides	PEI: polyethyleneimine
DI: Deionized Water	POEM: (poly(oxyethylene methacrylate)
DLS: dynamic light scattering	PPEI-EI: poly(propionylethylenimine)
DLVO: Derjaguin, Landau Verwey and Overbeek	PSSA: poly(styrene sulfonic acid)
DMEM: Dulbecco's Modified Eagle's Medium	PVA: polyvinyl alcohol:
DMSO: dimethyl sulfoxide	PVP: polyvinyl pyrolidone
DOC: dissolved organic carbon	QD: Quantum Dot
DPPC: dipalmitoyl phosphatidylcholine	rGO: Reduced graphene oxide

ECASs: Electrochemical Aptasensors	ROS: Reactive Oxygen Species
Fe3O4: iron oxide	SD: Synthetic Defined medium
GN: Graphene	SDS: sodium dodecyl sulfate
GO: graphene oxide	SL: sophorolipids
I: ionic strengths	SR: Suwannee River
IEP: Isoelectric point	SRFA: Suwannee River Fulvic Acid
LB: Luria-Bertani Broth	SWNT: single-walled nanotubes
MHS: mesoporous hollow silica	TEM: Trasmission Electron Microscopy
MNMs: Manufactured Nanomaterials	THF: tetrahydofuran
MNPs: Magnetic nanoparticles	TiO2: titanium dioxide
MWNT: multi-walled nanotubes	TSB: Tryptic Soy Broth
NADH: nicotinamide adenine dinucleotide	VOC: volatile organic compound
nAg: Nanosilver	WP: Work Package
nCu: Nanocopper	YPD: Yeast Extract Peptone Dextrose medium
NIOSH: National Institute for Occupational Safety and Health	ZnO: Zinc oxide

Annexes (optional)

Not applicable