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ARTICLE

Nanometrology - challenges for health regulation

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ABSTRACT

The relationship between metrology, nanotechnology and nanoscience and sanitary regulation is discussed from the point of view of its importance and the interrelationship between the themes for the development of products and services involving nanotechnology. The discussion involves the main techniques for measuring dimensional, chemical and biological properties of materials, and presents some of the challenges for the future. Issues such as processes of standardization and regulation in Europe, U.S. and Brazil are also addressed, providing an overview of how these processes are related to sanitary regulation.

KEYWORDS: Nanotechnology; Metrology; Nanobiotechnology; Health Regulation

Introduction

Metrology

Metrology is the science covering all theoretical and practical concepts involved in a measurement suitable for providing results with greater accuracy and metrological reliability. In any area in which a decision is made from a measurement result, paramount attention is given to all metrological concepts involved. Particularly, whenever possible, it is desirable that the measurement can be converted to the International System (IS).

The credibility of analytical data has never attracted so much attention from society as it does today. According to the requirements of the ABNT NBR ISO/IEC 17025[1] standard, a measurement is reliable if it shows evidence of its traceability, and its metrological reliability. These evidences are established as important for evaluating the quality properties of a measurement.

A fundamental principle for the quality and reliability of results is the comparability between laboratories on an international basis. For comparison, the analytical results must be submitted together with a statement of their measurement uncertainty and must be traceable to common primary references. In the industrial area, the metrological reliability of a measurement result is the basis for generating products and services of higher quality. This feature directly influences the decisions of national and international business transactions, often contributing to overcome technical barriers.

Nanotechnology

The term nanotechnology refers to the application of scientific knowledge to manipulate and control matter at the atomic, molecular, or macromolecular scale, and for at least one dimension, range between 1 and 100 nm and results in structures, devices, or systems with new properties and functions due to its size[2]. The minimum internationally accepted definition[3] for nanomaterial is a body having at least one of its dimensions between 1 and 100 nm (Figure 1). This definition is adopted by the *Food and Drug Administration* (FDA)[4]; however, the European Community[5] adopted a more detailed definition:

"Nanomaterial" is defined as a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions range from 1 to 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness, the number size distribution threshold of 50% may be replaced by a threshold between 1% and 50%. By derogation from the above, fullerenes, graphene flakes, and single-wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

Norio Taniguchi was the first to use the term nanotechnology to describe the technologies involved in generating products and developed the nanoscale concepts of Richard P. Feynman shown in a 1959 lecture to the American Physical Society. The following decades were governed by impressive advances in miniaturizing and manufacturing technologies, generating a wide variety of products with dimensions and properties at that scale. Simply to illustrate, in the area of controlled release of drugs, the global market in 2012 reached U.S. $22.5 billion with estimates of U.S. $43.3 billion in 2017[6].

Considering nanotechnology as a carrier technology for the future and its challenges, the Ministry of Science, Technology and Innovation established the SisNANO network by Ordinance no. 245, April 5, 2012, which is regulated by the Normative Instruction no. 2 of June 15, 2012. The regulation stipulates a multiuser system targeted to support research, development and innovation (R, D & I) in nanosciences and nanotechnology laboratories. It also aims to structure and expand access to researchers and companies to the laboratory infrastructure, stimulating R, D & I in nanosciences and nanotechnologies areas.

However, just as conventional products and processes are gaining quality and comparability through metrology, nanotechnology also requires these means to become competitive and reliable. Nanometrology originated from this need.

Nanometrology

Nanometrology discusses measurements of species or events at the nanoscale, such as dimensions at that scale or interactions between molecules or biomolecules. The greatest

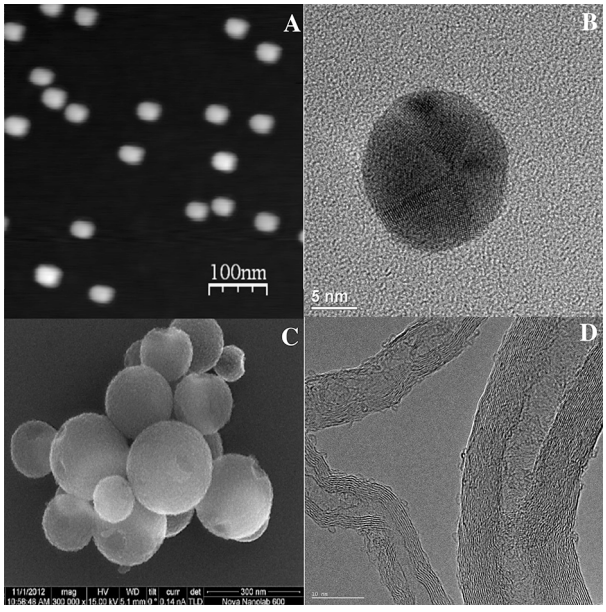

Figure 1. (A) Topographic image of gold nanoparticles obtained by atomic force microscopy revealing the size and distribution of nanoparticles (courtesy of Dr. Giselle Nogueira Fontes). (B) Micrograph of a gold nanoparticle obtained by high-resolution transmission electron microscopy revealing the morphology and size. (C) Micrograph of silica nanoparticles obtained by scanning electron microscopy. (D) Micrograph of carbon nanotubes obtained by transmission electron microscopy.

challenge of this new science is the development and creation of new measurement techniques capable of providing results with high accuracy, which are required to meet the needs of industries dealing with nanotechnology.

In this context, Brazil is considered one of the few countries to invest in nanometrology, and in 2008, the Nanometrology Centre was established at the National Institute of Metrology, Quality and Technology (Inmetro).

The European Coordination of Nanometrology (Co-Nanomet) subdivides nanometrology into several areas according to their specificities[7]: dimensional, chemical, biological, mechanical, electrical, thin films, nanostructured materials modeling, and simulation.

Dimensional

Dimensional nanometrology directly deals with measurements of a material's dimensional properties at the nanoscale. Various applications in nanotechnology require fine dimensional control during manufacturing. Therefore, measurement techniques capable of providing reliable values for dimensional properties of nanomaterials are necessary increasingly.

Techniques currently used for the dimensional characterization at the nanoscale are optical interferometry, atomic force microscopy (AFM), and electron microscopy.

The atomic force microscope is widely regarded as the most appropriate instrument for long-term measurements in nanotechnology. This device uses a tip mounted on a flexible beam that scans the surface to be analyzed and reacts to the interaction between the atoms of the tip and surface forces, thus revealing its topography, as well as other surface properties. The AFM can provide vertical resolution at the atomic scale, while its lateral resolution is typically limited by the tip size in a few nanometers. This equipment has opened a window to the world at the nanoscale level, and is used in imaging, metrology, and surface manipulation. Metrology Institutes in several countries have developed a metrological version of AFM that, if coupled to optical interferometers, paves the way for the traceability of measurements directly to the meter definition by the IS of Units.

These systems can provide measurements on the z-axis with uncertainties at the subnanometer and nanometer scales. However, much remains to be done, such as the expansion of the lateral measure scales to a few millimeters, allowing the accurate measurement of large objects in the subnanometer scale, the development of sensors with higher stability and lower noise, and the correct treatment of the interatomic forces between materials with different interactions[7,8]. In addition, there are groups combining AFM with other techniques of dimensional measurements such as devices measuring coordinates[8]. Other studies are also exploring methods to determine the correct shape of the tip by means of algorithms[9].

The National Metrology Institutes and industry use optical interferometry as the main tool to measure small differences in height because usually, one can work in a wide range (from meters to nanometers) and provides a direct route for traceability through laser frequencies. Interferometry is based on the superposition of electromagnetic waves, which follow different optical paths, and hence interact constructively or destructively, revealing information about the traveled distance.

Currently, interferometric systems reach a resolution in the subnanometer range[7,8] and can be of two types: homodyne (a single wavelength is used) or heterodyne (with modulation in one of the beams, usually by a frequency shift). In the latter, it is possible to achieve considerable increase in the resolution and signal-to-noise ratio. However, the optical interferometer has problems with non-linearity in the nanometer and subnanometer ranges, leading to measurement errors, which can be, to some extent, artificially circumvented by algorithms[8]. Still, the current optical interferometer generation has reached its performance limit with regard to nanoscale metrology. New efforts have been taken to further improve the optical interferometric metrology by various metrology institutes[7,8].

Another technique worth mentioning is electron microscopy. The scanning electron microscopy (SEM) technique is fairly well established and has great versatility with respect to the depth scale. This indicates that a very specific point of the investigated sample can be observed, starting from a small to a relatively large magnification; this is opposite to AFM, which allows a scan range of only several hundred micrometers. In SEM, the electron beam scans the sample surface, while the image is formed by the detection of secondary electrons emitted in response.

Moreover, electron microscopy also covers the transmission technique, which can reach higher subangstrom resolutions. Here the electron beam passes through the sample, which needs to be sufficiently thin to allow passage of the beam, and an image is recorded by the transmitted signal. However, electron microscopy techniques allow dimensional measurements only in the x-y plane (2D measures) and always need to operate in vacuum. Electron microscopes have been extensively used in the semiconductor industry as a tool for dimensional control at the nanoscale. Metrology institutes can use this equipment for the calibration of transfer of artifacts or nanoparticle (NP) - sized reference measurements.

Table 1 summarizes key information about the above-mentioned techniques. In addition to these techniques, many others are also developed within the area of dimensional nanometrology, such as X-ray interferometry, confocal microscopy, diffraction, and ellipsometry.

In general, the major advances in this area are the setup of instruments with higher resolution and greater accuracy at different scales, geometries, and materials. In the case of AFM, we seek a better understanding of the interaction between the probe/sensor and the sample at the nanoscale.

Finally, it is extremely important that the results of these advances produce calibration services and solutions for nanoscale measurements, and the possibility to ensure the traceability and stability of measurements by means of well-established standards and procedures.

Table 1. Summary of the main techniques involved in dimensional nanometerology.

Technique	Purpose/Application	Characteristics	Limitations
Atomic force microscopy	Dimensional metrology, measurements of mechanical properties (force and tribology), and surface manipulation	Vertical resolution in the atomic scale and lateral resolution in the nanoscale	Measurements of small-scale objects and ferrule shape interference
Optical interferometry	Dimensional metrology and standard block calibration	Main tool for measuring height differences in a wide working range ($1–10^{-9}$ m)	Nonlinear problems in the nanometer and subnanometer ranges
Scanning electron microscopy	Dimensional metrology, chemical, structural, topography, and nanolithography/nanomanufacturing analyses when coupled with the focused ion beam	Nanometer-scale resolution and images with a significant depth of focus	Measurements in the x-y plan (2D) and requirement of vacuum environment
Transmission electron microscopy	Dimensional metrology, chemical, and structural analysis	Subangstrom-scale resolution	Measurements in the x-y plan (2D), requirement of vacuum environment, and careful sample preparation

Chemistry

Chemical nanometrology comprises measurements of chemical species composition, chemical states, or structural properties of nanoscale materials. The techniques involved in this type of analysis are diverse and show complementary information amongst themselves with respect to the chemical characterization of materials. Among the commonly used tools, various spectroscopic techniques can be cited such as X-ray excited photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES), secondary ion mass spectroscopy, energy electron loss spectroscopy (EELS), and X-ray energy dispersive spectroscopy (EDS), the latter is usually coupled with an electron microscope[10].

The XPS technique is used to quantitatively determine the concentration and chemical species present on the material surface. This is a technique that can be used for the characterization of NPs. The Consultative Committee for Amount of Substance has recently developed a project to track thickness of ultrathin silicon oxide films on silicon[11].

Auger spectroscopy or AES is used for identifying the chemical composition of the surface of nanostructures up to 10 nm. Its common applications are the determination of the stoichiometry of semiconductors (including *quantum dots*) and the identification of chemical contaminants on electronic devices.

Mass spectroscopy of secondary ions is another technique for determining the chemical composition of the material surface. In this type of tool, high-energy ion bombardment causes tearing of material that is captured and analyzed by mass spectroscopy, yielding results with high sensitivity. This technique is widely used by the electronics industry and by the geological community for elemental identification.

The EDS technique is coupled with electron microscopy to identify and quantify elements by detecting the energy of X-rays emitted by different materials when they are bombarded by the electron beam. EELS is usually coupled with transmission electron microscopy and measures the amount of lost energy when electrons pass through the sample. This energy difference is directly related to the chemical species present in the sample. In addition, the chemical environment data (type of chemical bonds present) can also be extracted.

Table 2 shows information about the described techniques[8,10]. Other techniques mentioned are those involving optical spectroscopy such as Fourier transform infrared spec-

Table 2. Summary of the main techniques involved in chemical nanometerology.

Technique	Purpose/Application	Characteristics	Limitations
X-ray photoelectron spectroscopy	Quantitative analysis of chemical species concentration and environment present on the surface	Excellent determination of chemical environments and wide applicability to various types of materials	Lateral resolution is usually limited (on the order of tens of microns)
Auger electron spectroscopy	Quantitative analysis of concentration of chemical species present on the surface	Excellent lateral resolution (on the order of tens of nanometers)	Loading of analyzed samples and their possible damage because of the electron beam
Secondary ion mass spectrometry	Measurement of elemental impurities in the material	Detection of traces of elemental impurities (ppm to sub-ppb)	Absence of chemical or molecular information because of the ionic bombardment process
X-ray energy dispersive spectroscopy	Quantitative analysis of concentration of the chemical species present on the surface	Rapid multi-elemental measurements, usually coupled with electron microscopy techniques	Limited resolution between peaks and relatively low signal-to-noise ratio
Electron energy loss spectroscopy	Elemental analysis and chemical species environment	Excellent lateral resolution (on the order of 1 nm) and determination of the chemical environment	Usually coupled with transmission electron microscopy or scanning transmission electron microscopy and careful sample preparation

troscopy (FTIR) and Raman spectroscopy. The FTIR technique identifies types of connections (or functional groups) in solids, liquids, and gases, and can be used in thin films with thicknesses of tens of nanometers. Raman spectroscopy is complementary to FTIR and very sensitive to the characterization of carbon-based nanostructures (such as nanoribbons, nanotubes, and graphenes), which makes it an indispensable tool for the development of the metrology of such nanostructures.

The metrological aspects of portions of chemical nanometrology have been developed by the *Versailles Project on Advanced Materials and Standards* and also by the International Bureau of Weights and Measures through groups of the *Advisory Committee for the Matter Amount*. However, there is still a significant need with regard to the development of certified reference material able to provide traceability to the measurements performed by using the aforementioned techniques. Moreover, it is still important to improve methods able to amplify the lateral resolution of the chemical analysis, as well as its sensitivity.

The various nanomaterial manufacturing processes, which have currently appeared, require the support of metrology with respect to chemical characterization. For example, it is important to have a better understanding of the first steps of nucleation during manufacturing of these nanomaterials, or how to improve the functionalization of nanomaterials and NPs with respect to the bioanalysis, biology, and medicine fields.

Biology

Nanobiometrology is an emerging science that seeks to understand phenomena, the development of measurement, and data acquisition that promotes biological characterization (quantitatively) at the nanometer scale, thus contributing to the international comparability and reproducibility of the measurements[12,13,14,15,16,17]. Worldwide, nanobiotechnology includes the use of nanotechnology to understand and develop biotechnology, pharmaceutical and medical sciences and has focused on developing nanomedicine, personalized medicine, medical devices, implants, and engineered nanobiomaterials.

Regarding health, nanotechnology aims to contribute to the diagnosis, monitoring and new treatment for diseases. Several new industries have emerged, however established industries also grow by following this area of knowledge and technology, producing new devices, instrumentation, pharmaceutical products, and services. A recent example of the potential of nanobiotechnology was the development of thermometry in living cells at the nanoscale, resulting in mapping of the temperature gradient at the subcellular level by the introduction of nanodiamonds and gold NPs in human embryonic fibroblasts[18]. Therefore, this methodology enables unique applications in life sciences.

Therefore, biological nanometrology is a transverse science applied to various health and life areas supporting the responsible control of the evolution of nanotechnologies and nanosciences, considering ethics, public health, occupational safety aspects, and environmental protection[12,13,14,15,16]. This science is increasingly gaining importance in society because products such as glucose sensors have been commercialized, and products for cancer therapy[19] and multiple resistance to drugs[20] are under development. However, metrology in the biology area is still in its infancy, requiring interdisciplinary efforts for its development as well as new metrology tools.

Metrology institutes from different countries are engaged in metrology activities in bionanometroloy. In Europe, the Co-Nanomet published a report that sets out the priorities and future strategies focusing on the following areas: (a) characterization of chemical, structural, and mechanical properties of bionanostructures and biosurfaces resulting from bionanoengineering; (b) quantification, distribution, structure, and activities of biological materials; (c) measuring and distribution of NPs in biological systems[7].

The characterization of nanoscale structures without changing the initial sample properties is of extreme importance for the development of nanotechnology[21,22]. Several techniques have been developed with an aim to increase the accuracy of analysis of biological structures such as nucleic acids, proteins, cells, and microorganisms[12,15,16,18,19,20,22,23,24,25,26]. Techniques such as the fluorescent resonance energy transfer (FRET), surface plasmon resonance, quartz crystal microbalance, infrared and Raman spectroscopy have been widely used since analyses of biological environments are possible[12,16,22,27,28].

Nanobiometrology has been used in accurate measurements of chemical, structural, and mechanical properties of nanostructured biomaterials, and in understanding the structure, quantification, distribution, and activity of biomolecules for a wide range of industrial applications such as sensors, implants, and regenerative medicine[12,15,16,19,20,23,24]. Although the development of nanosciences and nanotechnologies has numerous beneficial applications for our society, the possible impact on the en vironment and human health is still not well known[29,30,31]. Currently, a wide variety of nanoparticles (NPs) are produced and incorporated in various products with applications in different areas such as the environment, electronics, informatics, biomedical, pharmaceutical, and cosmetics[29,30,31].

As a result, most people are daily exposed to high concentrations of particles that may be harmful to human health. When NPs reach the cells, these can be internalized by the cell membrane through processes such as endocytosis (or by other processes involving specialized lipids), generating reactive oxygen species causing oxidative stress of some cellular organelles and severe cell dysfunction[12,16,29,30]. Importantly, acute manifestations resulting from exposure to NPs are not common. However, chronic effect of exposure to NPs and possible synergy between NPs and environmental factors, such as exposure to UV[32], cigarette smoke, are both not well known. Furthermore, there is much discussion in the scientific community about the correspondence of traditional techniques of toxicology to this new field.

Despite the evidence on potential adverse effects to organisms, the specific legislation for regulating the tests to which those NPs should be subjected to is inexistent. Therefore, there is an urgent need for research the potential harmful effects of NPs to the human body, and it is in this context that nanobiometrology has gained a major role. Different advanced techniques have been applied to perform the characterization, quantification, and distribution of NPs in biological systems (see Figure 2A). However, the absence of a single technique for assessing in real time the internalization of NPs and their location is a limitation in this area[12,16,29,30].

Several events occur when these NPs interact with biological systems. Agglomeration often occurs (Figure 2B) as a result of physicochemical surface characteristics of NPs and their great affinity for biomolecules. This agglomeration phenomenon is the result of several adsorption and desorption events of biomolecules on the NP surface; however, the complexity is even higher as such events are dynamic.

The nature of the NP protein complexes also affects the way NPs interact with biological systems, and are often responsible for the toxicity events involving lysosomal cell damage and subsequent apoptosis[33].

This is one area where nanobiometrology currently plays a key role. The need for precise and traceable quantification methods, evaluation of NP distribution in biological systems, is in exponential growth because of the recent use of NPs in applications involving nanomedicine, such as contrast agents in magnetic resonance imaging equipment and systems for loading and release of drugs (drugs and vaccines) to improve their therapeutic efficacy[33].

Nanometrology and sanitary regulation

The emerging markets for the development of nanotechnology are pharmaceuticals, electronics, and materials. Because of their rapid growth, standardization initiatives and regulation of nanotechnologies increasingly gain importance to assure society that their development is safe, responsible, and sustainable[4,7].

Risk assessment of NPs to human health, which includes the possible pathogenic effects and the induction of genotoxicity, is a current concern of the United States, Europe, and more recently, the Brazilian society (National Health Surveillance Agency, ANVISA). Despite the large number of studies related to NPs, knowledge of their interaction with living organisms and possible effects on human health is still scarce, and regulation is nonexistent[34,35,36,37,38,39,40]. Currently, there are still no reliable methodologies and regulations that establish the difference between the properties at the macroscale and the nanoscale ranges.

Both in Europe and the United States, a considerable amount of research and development projects focused on assessment of potential risks and hazards arising from the expansion and development of nanotechnology were funded by governments in order to assess the potential risks of nanomaterials as well as to generate specific legislation and regulations applied to products containing substances in their composition with dimensions at the nanoscale level[34,35,36,37,38,39,40].

A number of scientific reports on security in the development of nanosciences and nanotechnologies have been published since 2000; however, all highlight the persistent scientific uncertainties and gaps in knowledge about environmental impacts and human health. As an example, one can cite the 2004 report on nanos-

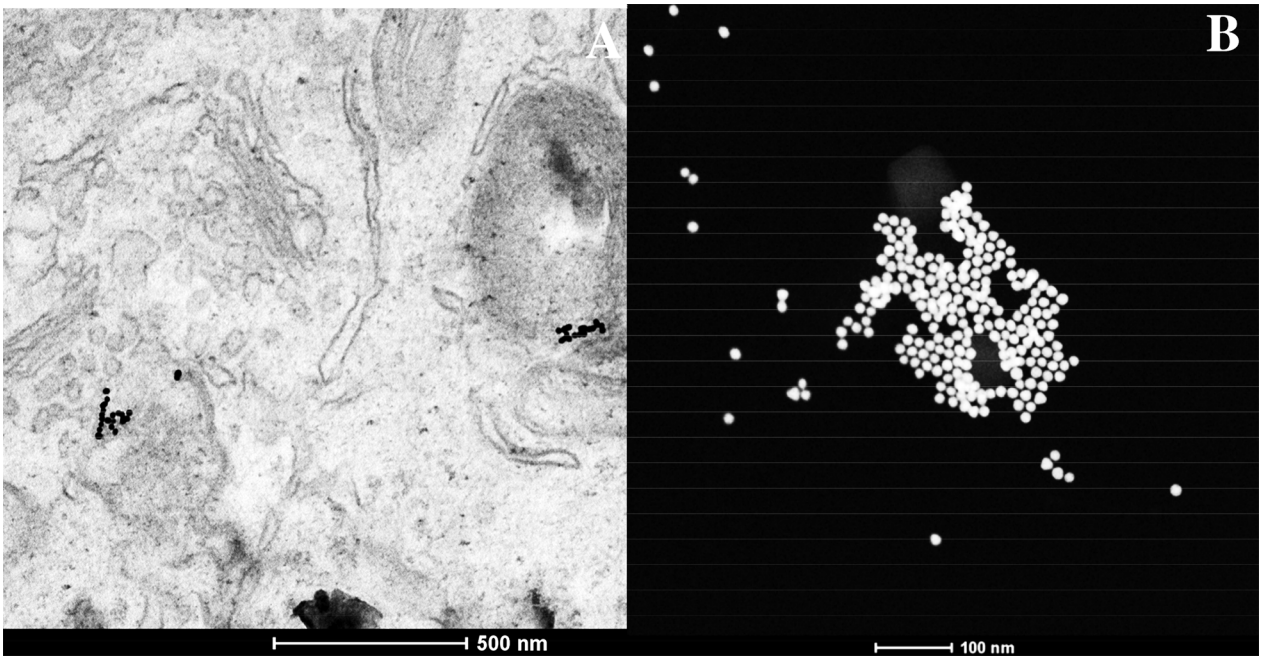

Figure 2. (A) Micrography showing the cellular internalization of gold nanoparticles. (B) Spontaneous aggregation of gold nanoparticles.

ciences and nanotechnologies published by the *Royal Society, Royal Academy of Engineers*, and the American *National Science and Technology Council*, where the harmful effects of nanomaterials on health and environment were evatuated[34].

In the United States, there are several federal entities engaged in nanotechnology regulation, including the *United States Environmental Protection Agency* (EPA), which researches in the area of nanomaterials: (i) identifies the sources; (ii) target; (iii) transport; (iv) exposure; (v) understanding their effects on human health and the environment in order to inform about the risks and test methods; (vi) development of risk assessment approaches; (vii) prevention and mitigation of risks[36]. The FDA is responsible for protecting the public health by regulating and supervising the safety of foods, drugs, vaccines, biopharmaceuticals, medical devices, and veterinary products[41].

The National Institute for Occupational Safety and Health (NIOS) conducts research and makes recommendations for preventing occupational accidents and diseases, while the *National Institute of Health* (NIH) is directed to healthcare, developing diagnostic methods and applied products in nanotechnology-based therapeutics. Despite having a system of non-centralized regulation, the United States sought to setup a coordinated strategy, promoting the creation of the *National Nanotechnology Initiative* (NNI). Launched in 2000, the NNI aims at coordinating 25 different federal agencies engaged in nanotechnology[35].

This system implemented in the United States is sufficiently nimble to evaluate the new and emerging risks of nanotechnology. For example, in 2006, the EPA decided to regulate a washing machine as a pesticide and demanded a suitable record, because this equipment contained silver NPs as antimicrobial elements[36].

However, for preanalysis of markets and authorization, post-market surveillance, labeling, and reporting of adverse events considerably vary and solely depend on features and limitations of the legal authority from each agency. In addition, regulatory agencies recognized the knowledge gaps and scientific uncertainties regarding nanomaterials. For example, the EPA identified research needs on the toxicology and ecotoxicology of nanomaterials, and recommended a greater collaboration with other agencies and stakeholders. To complete possible knowledge gaps, the EPA introduced a voluntary initiative of information in the nanotechnology area for companies: invited producers of nanomaterials to write a report on voluntary security with relevant information. A review of the program published in 2009 suggested that companies were reluctant to participate in the scheme, leading the EPA to conclude that the lack of knowledge regarding the safety and environmental health persists; as a result, the agency is now examining the possibility to develop a compulsory notification system[36].

The European government faces problems similar to those in the United States: lack of scientific knowledge and uncertainties in the area of nanotechnology. Laws and regulations in force refer to the areas of chemicals, foods, cosmetics, medicines, etc. The European Union (EU) has been very proactive in developing a strategy and encouraging nanotechnology. With the publication of its first strategic document in 2004 until today, the European Commission has consistently stressed the need for "adequate and timely regulation in the field of public health; consumer and environment protection, to ensure confidence of consumers, workers, and investors"[37,38,39,40].

Initiatives in the field of voluntary safety by researchers and companies have been taken in the field of nanosciences and nanotechnologies to anticipate potential environmental-health and safety impacts. In the EU, nanomaterials are regulated by the *Regulation on Registration, Evaluation, Authorization, and Restriction of Chemicals* (REACH) because these are included in the definition of chemical substance. According to REACH, based on current knowledge, nanomaterials are considered similar to chemicals (some can be toxic and some not), and their risks are related to the type of nanomaterial and its application[42].

Its provisions contain extensive obligations for manufacturers, who are required to produce and evaluate data on chemicals and their safe use, and provide this information (through reports) to regulators. Simultaneously, regulators have a range of tools at their disposal to require additional tests and information, and also can restrict the use of chemicals that are of high concern[38]. The *certification, labeling and packaging - Regulation* requires that marketed substances, including nanomaterials, are notified to the *European Chemicals Agency (ECHA)* in accordance with the classification regarding danger[43].

Given the uncertainty about risks and regulation of nanomaterials, the European Commission presented a systematic review of existing laws and regulations. Published in 2008, the review concludes that "the legislation covers risks in relation to nanomaterials to a large extent"; however, it admitted the lack of knowledge in some areas so that "the legislation could be amended in the light of new information becoming available." Driven by political demands of the European Parliament, recent reforms in the laws of cosmetic and food included provisions that go beyond those implemented in the United States, particularly with regard to the mandatory labeling and pre-evaluation of market security requirements[37,38,39,40].

Currently, three independent Scientific Committees provide scientific advice during the preparation of proposals related to consumer safety, public health, and the environment to the European Commission. The committees are the *Scientific Committee on Consumer Products (*SCCP), the *Scientific Committee on Health and Environmental Risks* (SCHER), and the *Scientific Committee on Emerging and Newly - Identified Health Risks* (SCENIHR). The nanosafety cluster is also a European initiative aiming to maximize the synergies between the "*Sixth and Seventh Framework Programme*" *Programs* (FP6 and FP7) for all aspects of nanosafety, including materials, risk, databases, modeling, dissemination, standardization, and nanotechnology metrology[39].

In 2013, a new law that sets safety rules for cosmetic products entered into force in the EU. The purpose of the new law is to inform consumers about the constituents by formulation on

cosmetics. Specifically herein, nanomaterial is defined as an intentionally manufactured insoluble material in the range from 1 to 100 nm. In this context, various types of nanostructures (for example, liposomes, lipid NPs, and nanoemulsions) already employed in marketed cosmetic products do not fit this definition. In the case of cosmetic products containing insoluble nanomaterials, the manufacturer shall notify the Commission as to the presence of the nanomaterial in the product before commercializing it[35,39].

The EU and the United States took the lead in international coordination, primarily working through the *Organisation for Economic Co-operation and Development* (OECD) and *International Organization for Standardization* (ISO). The OECD is an intergovernmental organization with the objective of establishing harmonization policies by identifying and discussing common problems and of international interest. In 2005, the OECD has promoted international cooperation to assess the potential implications and safety of manufactured nanomaterials on human health and the environment. In 2006, the OECD has decided to create a working group with the aim of alerting on aspects related to the safety of manufactured nanomaterials in order to help developing a rigorous safety assessment of nanomaterials[44,45]. Recently, the OECD published a compilation of all information about the parameters determining the assessment of nanosafety of manufactured nanomaterials[45].

The standardization activities initiated by ISO and the *International Electrotechnical Commission* (IEC) are the major regulatory actions in the area of nanotechnology. Besides the OECD, ISO has internationally taken the lead in developing harmonized standards and terminology. The technical committee on nanotechnologies (TC 229), created in 2005, has published technical reports and international standards related to terminology and nomenclature; metrology and instrumentation, including specifications for reference materials; testing, modeling, and simulations; and basic science, health, and security as well as good environmental management practices related to the use of nanotechnology[37,38,39,40]. The ISO/TR 12885:2008 focuses on the manufacture and use of engineered nanomaterials, addressing practical definitions in occupational health and safety applied to nanotechnology[40].

In Brazil, Europe, and the United States, no laws and devices can prevent and evaluate the effect of advances in nanotechnology. Laws that are commonly used to authorize the marketing of a particular nanotechnology product for agriculture do not differ from standards for other products. In terms of definitions, the Working Group on Regulatory Framework proposed the adoption of the definition of nanotechnology from ISO TC 229, as well as the definition of nanomaterial from ISO/TR 12885-2008[40].

The ANVISA by Law no. 9782/1999 is competent to regulate, supervise, and control products, substances, and services of interest to health, including health products[46]. Seeking the approval of these products before placing them in the consumer market (process of product registration at ANVISA) and evaluating the safety and efficacy of these products against the requirements of the Resolution ANVISA RDC no. 56/2001 and other specific laws, it is mandatory to present the results of previous analyses or their certificates of conformity assessment during application of product registration at ANVISA[47]. However, ANVISA has not set a specific legislation for nanotechnology, either to register nanomaterials, or for final products containing nanomaterials. Nevertheless, ANVISA has been approving nanotechnology-based products, through case-by-case analysis.

In recent years, Brazil has improved in the development of Science, Technology and Innovation, with concrete results in the scientific, technological, production, and training of human resources in the areas of nanotechnology and nanoscience. Therefore, the development of suitable standards is crucial for the development of safety and reliability of nanomanufactured products. As occurred in Europe and the United States, in Brazil, efforts should be made in order to develop technical standards to establish metrology standards related to nanotechnology, together with investment in accreditation by normalization institutions[40].

Nanometrology plays an important role in the regulation of products derived from nanotechnologies, since there is neither a technique to characterize the size distribution of nanomaterials nor to control the interferences during sample preparation for analyzing methodologies. There is a need for improvement and development of new, highly accurate measurement methods (preferably below 10 nm). In addition, calibration methods and validation of instruments (less than 100 nm range) should be used routinely. New measurement methods, reference materials, and good practice should also be developed to resolve the lack of traceability problem of the international system of measures[12,13,14,15,16,34] .

In general, although there are a large number of scientific papers analyzing toxicological effects of NPs, there is considerable controversy for preparing protocols of the sample dispersion and preparation, and for *in vitro* and *in vivo* assays that are not biased in function of the NPs reactivity. Standard toxicological tests are available to assess the biological response of a chemical substance; however, the standardization for assessing the toxicity of NPs is nonexistent. So far the studies are adaptations of existing standard procedures used with other substances.

Final remarks

Nanotechnology is actually a carrier technology for the future. Its impact on actual and future populations is huge involving many knowledge areas, while showing a deep connection in the production sector. Its regulatory structure is under construction throughout the world, including Brazil. The development of appropriate protocols for the preparation, physicochemical, and biological characterization of NPs is essential to establish the safety and efficacy of these new products.

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