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Research review paper

Techniques for physicochemical characterization of nanomaterials

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ABSTRACT

Advances in nanotechnology have opened up a new era of diagnosis, prevention and treatment of diseases and traumatic injuries. Nanomaterials, including those with potential for clinical applications, possess novel physicochemical properties that have an impact on their physiological interactions, from the molecular level to the systemic level. There is a lack of standardized methodologies or regulatory protocols for detection or characterization of nanomaterials. This review summarizes the techniques that are commonly used to study the size, shape, surface properties, composition, purity and stability of nanomaterials, along with their advantages and disadvantages. At present there are no FDA guidelines that have been developed specifically for nanomaterial based formulations for diagnostic or therapeutic use. There is an urgent need for standardized protocols and procedures for the characterization of nanoparticles, especially those that are intended for use as theranostics.

Contents

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1. Introduction

The emerging field of nanomedicine utilizes nanomaterials to improve diagnosis, prevention and treatment of diseases (Duncan and Gaspar, 2011). According to the Nanotechnology Characterization Laboratory (NCL) at the National Cancer Institute, National Institutes of Health nanoparticles (NPs) have a size range between 1 and 100 nm (McNeil, 2005). Nanomaterials have at least one dimension in the range of sub-nanometer to 10 nm. Small molecules and certain naturally occurring biological materials are not usually referred to as nanomaterials, even though they may be in the range of 1 to 100 nm. Research on manmade nanomaterials and engineered nanomaterials in the 1 to 100 nm range has gathered momentum because of their potential for a diverse array of applications in science, technology and medicine (Webster, 2006). Some examples of nanomaterials include liposomes, dendrimers, carbon nanorods, carbon nanotubes, fullerenes, graphene derivatives, titanium oxides, gadolinium nitride nanowires, silver NPs, gold NPs, platinum NPs, magnetic NPs and quantum dots (Duncan and Gaspar, 2011; Mahajan et al., in press; Singh and Sahoo, in press; Wong et al., in press).

When a solid is split, it exposes two new surfaces; with every subsequent cut, newer surfaces emerge. As any material is broken down to very small particles, the surface area per unit mass increases dramatically. Nanomaterials are characterized by a relatively large surface area per unit mass. Since the surface area of a solid depends on its shape, *e.g.* a sphere has the smallest surface area per unit mass, the surface area of nanomaterials depends on the size as well as shape. Changes in size or shape of nanomaterials can affect their physicochemical and physiological properties.

The physiological interactions in the body influenced by the biodistribution, passage, phagocytosis and endocytosis of nanomaterials through tissues may differ from those of conventional medicines (Gref et al., 1994). In order to realize the full potential of nanomedicines, it is necessary to develop robust standards for characterizing the engineered/fabricated nanomaterials, for example, to provide a guidance for ensuring quality control and assessing the safety as well as toxicity of nanomaterials (Pleus, 2012). Characteristics such as molecular structure, chemical composition, melting point, boiling point, vapor pressure, flash point, pH, solubility, and water octanol partition coefficient have to be determined for nanomaterials in the same manner as they are for larger non-nanomaterials. In addition, nanomaterial characterization places special emphasis on parameters such as size/size distribution, porosity (pore size), surface area, shape, wettability, zeta potential, adsorption isotherm (adsorption potential), aggregation, distribution of conjugated moieties and impurities.

At present there are no U.S. Food and Drug Administration (FDA) guidelines developed specifically for nanomaterial based formulations for diagnostic or therapeutic use. However, the agency has issued two product-specific draft guidance documents to address the utilization of nanotechnology in the food and cosmetics industries (http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm301093.htm). This can be a stepping stone towards detection or characterization of nanomaterials, although currently there are no standardized methodologies or regulatory protocols. Still, the NCL, serving as "a national resource and knowledge base" to assist the regulatory review of nanotechnologies and the development and translation of nanoparticles and devices for clinical applications, characterizes the physicochemical properties, *in vitro* biological properties and *in vivo* compatibility of nanoparticles (http://ncl.cancer.gov/about_mission.asp). The assay cascade protocols at the NCL include a number of methods to investigate nanomaterials' characteristics, such as size, molecular weight, aggregation, purity, chemical composition and surface properties. The NCL protocols also include methods for determining sterility, drug release and toxicity *in vitro*, and efficacy, disposition and immunotoxicity *in vivo* (http://ncl.cancer.gov/working_assay-cascade.asp). Similarly, the European Union has formed the unit of Registration, Evaluation, Authorization and Restriction of Chemicals, by which nanomaterials are regulated.

Many methods have been used for evaluating manufactured nanomaterials, including techniques in optical spectroscopy, electron microscopy, surface scanning, light scattering, circular dichroism, magnetic resonance, mass spectrometry, X-ray scattering and spectroscopy, and zeta-potential measurements, as well as methods in the categories of thermal techniques, centrifugation, chromatography, and electrophoresis (Sapsford et al., 2011). In this review article, we briefly describe the principles, applications, strengths and limitations of a variety of modalities commonly used to investigate the physicochemical characteristics of nanomaterials (Table 1).

2. Overview of physicochemical characteristics

Typically, engineered materials with dimensions in the nanometer scale are intermediates between isolated small molecules and bulk materials. Nanomaterials, which are similar to biological moieties in scale, can be used as diagnostic and therapeutic nanomedicines (Del Burgo et al., in press; Hachani et al., 2013; Kim et al., 2010). Compared to their bulk material counterparts, the distinct physicochemical properties of the nanomaterials, such as size, surface properties, shape, composition, molecular weight, identity, purity, stability and solubility, are critically relevant to particular physiological interactions (Table 2) (Patri et al., 2006). These physiological interactions may provide benefits in medical applications, including improvements in efficacy, reduction of side effects, prevention and treatment (Farokhzad and Langer, 2006; Hall et al., 2007).

Impact of nanomaterials on their physiological behaviors will influence the therapeutic efficacy and/or diagnostic accuracy of nanomedicines. In this context, it is important to understand how the different physicochemical characteristics of nanomaterials affect their *in vivo* distribution and behavior. This demands reliable and robust techniques for studying the different physicochemical characteristics of nanomaterials in general and nanomedicines in particular. The different techniques used for characterization of nanomaterials, based on their different features, are described in the following sections. A rigorous but practical approach to reliable characterization of nanomaterials is essential for quality assurance and safe, rational development of nanomedicines and theranostics (Akhter et al., 2013; Kim et al., 2013).

2.1. Size

In engineered nanomaterials, size is a crucial factor that regulates the circulation and navigation of nanomaterials in the bloodstream, penetration across the physiological drug barriers, site- and cell-specific localization and even induction of cellular responses (Feng, 2004; Ferrari, 2008; Jiang et al., 2008). In general, the size of a nonspherical nanomaterial is defined as an equivalent diameter of a spherical particle whose selected physical properties, *e.g.* diffusivity, are equivalent to those of the nanomaterial in the same environment (Powers et al., 2006; Shekunov et al., 2007). One frequently adopted example is the hydrodynamic diameter of a molecule, which is the effective size