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▲⟨1153⟩ DRUG PRODUCTS CONTAINING NANOMATERIALS

1. INTRODUCTION

The current interest in nanotechnology and its application to the medical field has historical precedent. Initial products employed general physical chemistry of colloids to produce stable drug suspensions and improve bioavailability. The later introduction of liposomes began the evolution to today's more sophisticated and potentially revolutionary new technologies, which have accompanied the rapid growth in biotechnology product opportunities, notably including targeted drug delivery products.

The term "nanomaterials" by recent convention describes materials that have features or structures that exist on the 1- to 100-nm scale in any of the three spatial dimensions. The existence of a large number of formulations containing nanomaterials that are currently under development or review, or that have already received approval from the United States Food and Drug Administration (US FDA), emphasizes the need to identify important properties of these formulations, as well as methods of measuring these properties to ensure quality and performance. The US FDA has authored guidance on nanomaterials (*1*). In summary, the guidance recognizes materials with one or more dimensions within and outside of the nanoscale range but with properties attributed to its dimensions (less than 1000 nm). These materials often require measurements with specialized instruments (see *Table 1*).

Table 1. Names and Definitions

Name of the Nanomaterial	Subcategory	Definition, Structure, and Description
Dendrimers (*2*)	—	Dendrimers are highly branched star-shaped macromolecules consisting of polymers [e.g., polyamidoamine (PAMAM)]. These structures can be linked to drug substances or used by physical/steric association to encapsulate drug substances.
Drug nanoparticles/Nanocrystals (*3*)	—	Nanoparticles consist of crystalline or amorphous forms of pure drug substance and are used as a formulation strategy to enhance bioavailability by increasing drug solubility as described by the (*4*) Kelvin equation and dissolution rate as described by the Noyes-Whitney equation. Examples of nanocrystal products include those used for cancer (megestrol acetate), organ rejection (sirolimus), and emesis (aprepitant) and dyslipidemia (fenofibrate). Nanocrystal drug products may be solid dosage forms or prepared as aqueous or nonaqueous suspensions. The latter often require stabilization with surfactants or polymeric stabilizers. A common route of administration is the oral route. Nanocrystals are made using milling, homogenization, and precipitation methods, as well as a combination of these methods.
Liposomes (*5*)	—	Lipid vesicles are composed of one or more concentric lipid bilayers enclosing a single or multiple aqueous compartments. Liposomes have been employed to formulate both water-soluble and water-insoluble drug substances. Due to their hydrophilic nature, the former can be incorporated into the internal aqueous compartment(s) of liposomes and the latter into the hydrophobic core of the lipid bilayer. Examples of drug substances used in liposome drug products include anticancer agents (i.e., doxorubicin, daunorubicin, vincristine, irinotecan, cytarabine), antifungals (i.e., amphotericin B, itraconozole), analgesics (i.e., morphine, bupivacaine), and vaccines (i.e., influenza, hepatitis A). Liposomes can be manufactured by extrusion (filtration), homogenization, and micromixing processes. The drug substance can be incorporated during liposome formation or by loading into empty preformed liposomes. Routes of administration include topical; parenteral, with intravenous administration as the most common route; and inhalation.

Table 1. Names and Definitions *(continued)*

Name of the Nanomaterial	Subcategory	Definition, Structure, and Description
Micelles	—	Nano-scaled colloidal structures are formed by self-assembly of amphiphilic molecules. The constituents of the micelles can be either surfactants or block copolymers (*6*). For block copolymers, there are constructs in which the polymers employ different hydrophobic blocks [e.g., poly(propylene oxide), poly(L-amino acids), or poly(esters). An example of such block copolymers is, poly(ethylene glycol)-block-polylactide (PEG-PLA) polymers]. Polymeric micelles are used to target biological sites and minimize toxicity. As an example, a PEG-PLA polymeric micelle product improves the solubility of paclitaxel. Its route of administration is typically intravenous. Classical micelles (*7*) are thermodynamically stable self-associating surfactant structures used to increase the solubility of sparingly soluble drug substances. Micelles are frequently used for parenteral delivery of anticancer agents, e.g., docetaxel.
Nanobubbles (*8*)	—	Nanobubbles are created using oscillating vibration sources to disperse air in water, usually in the presence of surfactant. Nanobubbles can be employed as contrast agents and also to deliver drug substance by virtue of their incorporation into the stabilizing agent at the surface of the bubble.
Nanoemulsions	—	Submicron oil-in-water or water-in-oil emulsions are stabilized with surfactants and cosurfactants. By definition, they have droplet sizes below 100 nm, but typically ranging less than 1 µm. Nanoemulsions (o/w) are used primarily to administer water-insoluble drug substances. They are made by high-shear emulsification methods or low-energy emulsification methods (phase inversion). Nanoemulsions are used in ophthalmic and oral products, for example, to deliver cyclosporin for dry eyes and for prophylaxis of organ rejection and immune regulation (*9*).
Nanofibers (*10*)	—	Nanofibers are rarely seen in pharmaceutical preparations. Nanofibers are nanoscale in one dimension and are used frequently in electrospun polymer-containing drugs (*11*) or alone, for example, as a wound-healing matrix (*12*).
Nanotubes	Carbon (*13*)	Nanotubes are rarely seen in pharmaceutical preparations. May be manufactured from a variety of substances. Usually an allotrope of carbon that forms into cylindrical carbon structures. These nanotubes have novel properties that make them potentially useful for a variety of pharmaceutical applications (e.g., controlled drug release) and tissue-engineering applications (e.g., as a scaffold for cell growth).
Nanotubes	Self-Assembled	Another nanotube example is the peptide drug lanreotide, which self-assembles at high concentrations to form nanotubes (liquid crystals). The lanreotide nanotube structure extends the bioavailability of the peptide for 1 month following a single intramuscular injection (*14*).
Nanoparticles	Inorganic (*15*)	Inorganic nanoparticles are discrete nanomaterials consisting of inorganic materials (e.g., gold, silver, silica, etc). These inorganic nanoparticles are used as a core substrate to which active moieties, e.g., small molecules or biomolecules, can be attached, particularly for antimicrobial agents, immune therapeutics and vaccines.
		Iron oxide nanoparticles (colloids) are used for diagnostic imaging and iron replacement therapies.
		Titanium dioxide and zinc oxide nanoparticles are used in sunscreens for the prevention of erythema.

Table 1. Names and Definitions *(continued)*

Name of the Nanomaterial	Subcategory	Definition, Structure, and Description
Nanoparticles	Polymeric (natural and synthetic) (*16*)	Solid particles prepared by one of several methods involving either dispersion of preformed polymer or in situ polymerization. Polymers used in preformed particles include various naturally occurring proteins, polysaccharides, and synthetic polymers, e.g., polylactide (PLA), poly(lactide-co-glycolide) (PLG), poly(ethylene glycol)-block-polylactide (PEG-PLA), and other polyesters. In situ polymerization of monomers requires cross-linking. The drug is often incorporated into polymeric nanoparticles by an emulsion evaporation technique (*17*).
Nanoparticles	Solid lipid (*18*)	Polymeric nanoparticles are drug substances embedded in a solid lipid core, which is usually stabilized by a monolayer of phospholipids. Lipid nanoparticles are proposed for use in the delivery of hydrophobic drug substances. This also includes drug complexes with other components such as protein, lipid, polymer, or metal.

General Quality Tests of Drug Products Containing Nanomaterials

The following list is not comprehensive but represents the most common properties characterized for nanomaterials used in drug products.

AERODYNAMIC PARTICLE SIZE DISTRIBUTION (APSD)

Products for inhalation are subject to aerodynamic particle size assessment by multistage cascade impactor in accordance with *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* ⟨601⟩ if intended for administration by inhalation aerosol or powder or in accordance with *Products for Nebulization—Characterization Tests* ⟨1601⟩ if intended for administration by a nebulizing system. The proportion of therapeutic aerosol found in the cascade impactor size fraction is determined by differences in particle inertia. Furthermore, quantification of drug substance mass is possible through chemical assay of the collected subfractions. These differences are related directly to the aerodynamic size, which is indicative of the likely deposition location(s) in the human respiratory tract. Although the size range within which these apparatuses operate is larger than the size range associated with single nanoparticles, their delivery in the formulated drug product to the respiratory tract will likely occur as microaggregated powder particles or as a suspension in liquid droplets. A small proportion of the aerosol may be generated as nanosized particles, for which alternative methods, in particular, low-pressure cascade impactors, might be considered in conjunction with conventional cascade impactors to extend the range within which sizing is undertaken (*19*).

COMPOSITION AND STRUCTURE

Methods of determining composition depend on the nature of the material under investigation. For example, for inorganic molecules, elemental analysis by atomic absorption spectroscopy and atomic emission spectroscopy with flame ionization or inductively coupled plasma mass spectrometry can be employed. For organic polymeric systems, gel permeation chromatography/size exclusion chromatography with refractive index detection may be supplemented with viscosity determinations and application of the Mark-Houwink equation to estimate the molecular weight (*20*). If a multi-angle light scattering detector (MALS) is added the weight average molecular weight can be determined. Photon correlation spectroscopy (dynamic light scattering) also may be used for molecular weight determination. At a fundamental level, surfactants and polymer-based systems can be subjected to elemental analysis or spectroscopy methods. Electron microscopy techniques such as cryogenic transmission electron microscopy may be used for liposome/lipid-based nanoparticle formulations to determine their size and morphology (e.g., lamellarity). Other scattering methods, e.g., small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), may also be used to generate information on nanomaterial size. These scattering techniques are not routine due to their technical complexity and instrument availability but can also provide detailed information on form (shape), surface, and material interactions.

DISSOLUTION/IN VITRO RELEASE

Multiple methods exist for dissolution testing. Importantly, care should be taken to ensure that the nanomaterial does not interfere with or interact with the testing materials or otherwise confound results. For example, an undissolved nanomaterial may pass through filters, resulting in a false elevation of solubility. To address this problem, the use of membrane diffusion and microdialysis has been proposed to separate particles from dissolved material (*21*). Fiber optic probes may also be employed. Some caution may be required when using the fiber optic probe technique with ultraviolet-visible (UV-Vis) detection to accurately estimate the quantity of dissolved material (*22*). However, the use of a fiber optic probe with different detection systems, such as infrared (IR) (*23*) or Raman (*24*) spectroscopy, may be a viable approach. Nanomaterials may also influence the accuracy of results by reacting with the surfaces of the dissolution apparatus. Therefore, novel dissolution methods may need to be developed.

ENCAPSULATION EFFICIENCY

For nanomaterials in which the drug substance is encapsulated in an excipient, e.g., lipid or polymer, the loading and encapsulation efficiency is usually determined. Encapsulation efficiency can be achieved by separating the drug substance in the nanomaterial from the free drug substance, typically using centrifugal filter devices. Size-exclusion spin columns or solid phase extraction (SPE) columns are used to separate free from encapsulated or carrier-associated drugs, performing an appropriate quantitative assay of free drug versus encapsulated drug as a proportion of the total drug present.

PARTICLE SIZE

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) can be valuable tools to measure the particle size of individual nanoparticles. Atomic force microscopy (AFM) also may be used to visualize individual particles and may aid in measuring their size, shape, and surface texture.

PARTICLE SIZE DISTRIBUTION

Dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), can be used to measure the particle size and distribution of nanomaterials. This method measures fluctuations in scattered light intensity from particles in Brownian motion to calculate the sample particle size and distribution. Laser light scattering can also be used to measure the particle size and distribution of nanoparticles, although this technique may be somewhat limited depending on the size of the nanomaterials to be measured.

PURITY

In some cases, the purity of the materials used to generate nanostructures is characterized to assure homogeneity among different batches. For example, the chemical composition of the bilayer of liposomes or micelles or the chemical purity of inorganic particles can be characterized to demonstrate batch-to-batch consistency.

SHAPE

AFM is a useful tool for three-dimensional analysis of nanoparticles. This technique will allow measurement of particle size, shape, and surface texture. SEM and TEM may also be used for morphology information. SAXS and SANS may also be used to generate information on nanomaterial shape. Multiangle light scattering (MALS) may be used for determining shape, as it can also give information on the conformation (e.g., sphere, coil, and rod) of the molecules when coupled with separation techniques.

SOLUBILITY

Typical methods for assessing solubility (e.g., the shake-flask method) may not be suitable for use with nanomaterials because the undissolved particles cannot be readily separated from the solution. Methods to assess the solubility of nanomaterials (i.e., nanoparticles or nanocrystals) sometimes use characterization of the particle size distribution and changes in the particle size distribution as a function of time as a surrogate or complementary data to dissolution data. More generally, solubility assessments for nanomaterials often use highly selective separation techniques (e.g., size exclusion chromatography or ultracentrifugation) capable of differentiating the dissolved and undissolved nanomaterials. To address this problem, the use of membrane diffusion and microdialysis has been proposed to separate particles from dissolved material (*21*). Fiber optic probes may also be employed, although some caution may be required when using the fiber optic probe technique with UV-Vis detection to accurately estimate the quantity of dissolved material (*22*). However, the use of a fiber optic probe with different detection systems, such as IR (*23*) or Raman (*24*) spectroscopy, may be a viable approach.

SURFACE AREA

Gas adsorption techniques (generally using nitrogen or krypton) allow measurement of the nanoparticle surface area. Samples are exposed to varying pressures at which the incrementally dosed analysis gas will adsorb onto the surface of the nanoparticles. The surface area is then calculated. This technique may also be utilized to measure the average particle size.

SURFACE PROPERTIES

Chemical composition (for example, as detected by X-ray photoelectron spectroscopy/secondary-ion mass spectrometry), charge, and reactivity influence surface properties. Measurement of the zeta potential and isoelectric point via electrophoretic or electroacoustic methods are often used to help predict whether nanomaterials will tend to aggregate or agglomerate or whether they will remain as discrete particles in the liquid vehicle. A high (positive or negative) zeta potential is one indication of a system in which nanoparticles will repel each other and remain as discrete particles, whereas a system with a low zeta potential (near zero, the isoelectric point) may be more likely to aggregate or agglomerate. Freeze-fracture scanning electron microscopy may also be useful in studying the aggregate or agglomerate morphology.

PHYSICAL STABILITY

Physical stability, in terms of propensity of nanomaterial to aggregate or agglomerate can be assessed using methods such as controlled centrifugation techniques, which apply a force to the system and assess the level of segregation/separation/sedimentation, combined with DLS to assess the apparent particle size (hydrodynamic diameter) distribution. These techniques

may be used as a part of standard stability testing. The integrity of the nanosystem with regard to the retention of drug substance should also be evaluated.

2. GENERAL QUALITY TESTS

The methods shown in *Table 2* relate to the properties of a drug product that may alter due to the presence of nanomaterials.

Table 2

Name	Recommended Methods
Carbon nanotubes	**Dissolution/In vitro release**—New or existing general chapter **Scanning, transmission, and atomic force microscopy**—*Scanning Electron Microscopy* ⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*). **Dynamic light scattering**—*Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size* ⟨1430.2⟩ **Solubility**—*Solubility Measurements* ⟨1236⟩ **Gas adsorption surface area measurement**—*Specific Surface Area* ⟨846⟩ **X-Ray photoelectron spectroscopy and secondary ion mass spectrometry**—Techniques are described elsewhere (*26*). **Zeta potential**—*Determination of Zeta Potential by Electrophoretic Light Scattering* ⟨432⟩ and *Analytical Methodologies Based on Scattering Phenomena—Electrophoretic Light Scattering (Determination of Zeta Potential)* ⟨1430.4⟩ **Sedimentation methods/Centrifugation**—Techniques are described elsewhere (*27*).
Dendrimers	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials* [a]
Nanocrystals and nanoparticles in the amorphous state	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]
Drug nanoparticles	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]
Inorganic nanoparticles	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]
Liposomes	**Dissolution/In vitro release**[a] —*Dissolution* ⟨711⟩ and *Oral Dosage Forms—Performance Tests* ⟨1711⟩ **High resolution microscopy**—⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*). **Dynamic light scattering**—*Analytical Methodologies Based on Scattering Phenomena—Light Diffraction Measurements of Particle Size* ⟨1430.2⟩ **Solubility**—*Solubility Measurements* ⟨1236⟩ **Zeta potential**—⟨432⟩ and ⟨1430.4⟩
Micelles	**Dissolution/In vitro release**—⟨711⟩ and ⟨1711⟩ **High resolution microscopy**—⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*). **Dynamic light scattering**—⟨1430.2⟩ **Solubility**—⟨1236⟩ **Zeta potential**—⟨432⟩ and ⟨1430.4⟩
Nanobubbles	**Dissolution/In vitro release**—⟨711⟩ and ⟨1711⟩ **High resolution microscopy**—⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*) **Dynamic light scattering**—⟨1430.2⟩ **Zeta potential**—⟨432⟩ and ⟨1430.4⟩
Nanoemulsions	**Dissolution/In vitro release**—⟨711⟩ and ⟨1711⟩ **High resolution microscopy**—⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*) **Dynamic light scattering**—⟨1430.2⟩ **Solubility**—⟨1236⟩ **Zeta potential**— ⟨432⟩ and ⟨1430.4⟩
Nanofibers	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]
Polymeric nanoparticles (natural and synthetic)	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]
Solid lipid nanoparticles	All general quality tests identified in *General Quality Tests of Drug Products Containing Nanomaterials*[a]

[a] Where included in an aerosol dosage form, ⟨601⟩ should be consulted.

3. DESCRIPTION OF PRODUCT QUALITY TESTS

- **Dissolution:** ⟨711⟩ and ⟨1711⟩
- **Dynamic light scattering:** ⟨1430.2⟩

- **Crystallinity:** ⟨*696*⟩
- **Gas adsorption surface area measurement:** ⟨846⟩
- **High resolution microscopy (scanning, transmission, and atomic force microscopy):** ⟨1181⟩. Transmission and atomic force microscopy are described elsewhere (*25*).
- **Inertial impaction:** ⟨601⟩
- **Solubility:** ⟨1236⟩
- **Ultracentrifugation (sedimentation):** Techniques are described elsewhere (*27*).
- **X-Ray photoelectron spectroscopy and secondary ion mass spectrometry:** Techniques are described elsewhere (*26*).
- **Zeta potential:** ⟨432⟩ and ⟨1430.4⟩.

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