

Environmental control technologies specific to nanomaterials are needed to ensure nanomaterials releases do not negatively impact the environment. The application of traditional manufacturing and industrial dust control technologies may be sufficient to prevent nanoparticles from being released into the environment; however, additional testing will be needed to verify the efficacy of these control technologies to prevent nanomaterial releases from industrial manufacturing processes.

ISO Standards are needed to develop and disseminate “Best Known Methods” on nanomaterial manufacturing processes, applications, disposal methodologies and environmental control design criteria.

Product safety and consumer use is another area in which future ISO standards can help foster the safe use of products containing nanomaterials. New analytical methods are needed to determine if nanomaterials are released during the anticipated use of the product and during subsequent disposal.

Specific product labeling and safety warnings guidance needs to be developed in order to ensure consistency and accuracy of product content warning labeling.

The possibilities of nanotechnology are exciting and promise many benefits to mankind. The ISO standards development process can provide the procedures necessary to identify the potential risk of nanotechnologies and to develop effective control programmes that will ensure the safe introduction of these materials into commerce and the environment.

The development of ISO environmental health and safety nanotechnology standards will also provide the mechanism for the sharing and communicating of best EHS practices with all countries involved in nanotechnology development. ■

## Medical opportunities of nanotechnology

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**E**ngineered nanomaterials offer revolutionary improvements for industrial applications, as described elsewhere in this issue. These opportunities are just as dramatic for medical applications of nanomaterials.

Biological processes that contribute to both health and disease occur at the nanoscale – the size scale of proteins, nucleic acids, pores, cellular membranes, and other biomolecules. Engineered nanomaterials, or “nanoparticles”, are now successfully being used for therapeutic and diagnostic

applications – due to their small size and the tailorability of their surfaces.

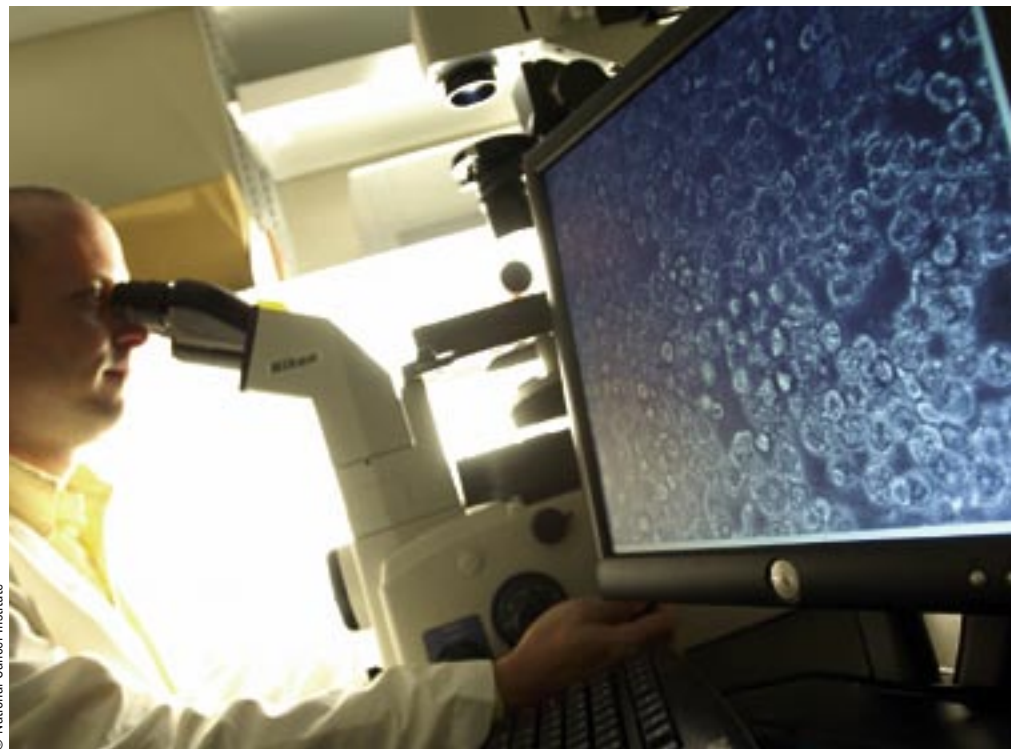
Patients have actually benefited from the improved efficacy and reduced side-effects of nanoparticle-based pharmaceuticals since the early 1990s – several hundred nanotechnology-based products entered US pharmaceutical pipelines in 2006.

ISO can facilitate the development and commercialization of these nanotechnology-based drugs by establishing characterization standards for use by nanotechnology developers and regulatory agencies.

### Opportunities

Nanotechnology offers many advantages to traditional drug design and delivery, and to medical diagnostics. A nanoparticle coated with hydrophilic molecules can be an effective carrier for otherwise insoluble drugs (*see Fig. 1*).

Similarly, attachment to a nanoparticle can alter a drug’s pharmacokinetics and distribution in ways that can improve efficacy and reduce adverse side effects.



Nanotechnology Characterization Laboratory (NCL) scientist, Dr. Stephan Stern, looking for signs of cytotoxicity in fullerene-treated rat hepatocytes with a fluorescent microscope, as part of an NCL in vitro nanoparticle toxicity assay (<http://ncl.cancer.gov/>).

The utilization of these capabilities is especially evident in the cancer research community. It is now well-established that nanoscaled particles accumulate in tumors of soft tissue and epithelial cell origin.

This phenomenon is referred to as the enhanced permeability and retention (EPR) effect, and is due to the leaky vasculature and incomplete lymphatic system surrounding tumors.

Many nanoparticle-based drug strategies now exploit this “passive targeting” mechanism to concentrate cytotoxic agents within cancerous regions. Preclinical and clinical trials of nanotech-based drugs taking advantage of EPR are already demonstrating pronounced improvements in efficacy over legacy therapeutics.

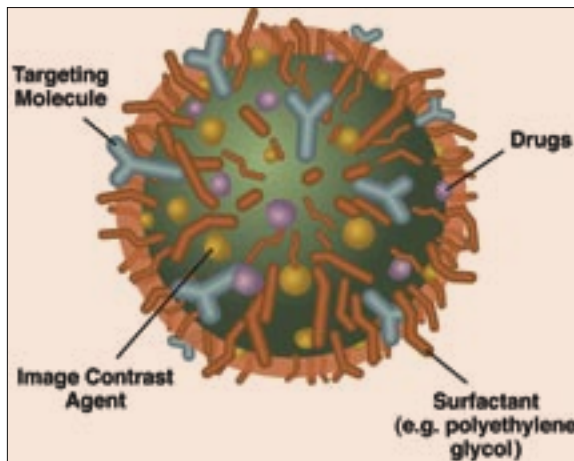
Concentration of the drug at the tumor also decreases side effects (i.e. toxicity) which result from non-specific, systemic exposure. The improved outcome is that patients suffer less adverse effects – such as nausea, anemia, weight loss, neutropenia, hair loss, etc. – when treated with the nanoparticle-based chemotherapeutic compared to conventional treatments.

**“Engineered nanomaterials, or “nanoparticles”, are now successfully being used for therapeutic and diagnostic applications.”**

Cancerous cells often overexpress “biomarkers” that identify a tumor as malignant and distinguish it from the surrounding healthy tissue. Examples of these biomarkers include membrane receptors and mutated cellular proteins.

Biochemical moieties, such as monoclonal antibodies, can be attached to the nanoparticle and facilitate active targeting of tissues expressing those biomarkers – a “zip code” for drug delivery. Similar to its passive counterpart, active targeting significantly lowers a drug’s adverse effects by minimizing its systemic exposure to healthy tissues and organ systems.

Active targeting is just one example of how a nanoparticle can be modified or otherwise engineered to achieve a particular biological effect, such as increased biocompatibility.



**Figure 1– The Tailorability of Multi-Functional Nanoparticles.** A nanoparticle’s surface can be functionalized with hydrophilic polymers (e.g. PEG--polyethylene glycol) to improve solubility or help the particle evade uptake by the immune system, targeting molecules (e.g. antibodies), drugs, and imaging contrast agents for diagnostics. The interior core of a nanoparticle can be solid (e.g. quantum dots), liquid (e.g. liposomes) or contain an encapsulated drug. Molecules are not shown to scale.

Drugs found to be efficacious under *in vitro* conditions, such as in high-throughput screening studies, are often insoluble and are rapidly cleared from the bloodstream when injected into animals or people.

Hydrophilic molecules such as polyethylene glycol (PEG) are now routinely bound to nanoparticle surfaces, which greatly increases solubility and biocompatibility. Candidate drugs – previously discarded due to insolubility – can be attached to this nanoparticle “platform”.

The drug’s solubility, half-life, and general biocompatibility now depend on the tailorable properties of the nanoparticle, rather than the properties of the drug itself.

Finally, image contrast agents such as gadolinium can be bound to nanoparticle surfaces by the tens of thousands – offering high concentrations and consequently bright signals. Since the contrast agent collocates with the drug,

this multi-functional capability offers a new paradigm for monitoring therapeutic efficacy in near-real time.

**Standards**

Voluntary consensus standards contribute to making the development, manufacturing and supply of products and services more efficient, safer and cleaner.

In the medical and pharmaceutical arenas, ISO standards are used for regulatory evaluation and quality control.

In particular, for several decades, the pharmaceutical industry has used standards to assess material biocompatibility, hemolytic properties, immunotoxicity, purity, and sterility – to name a very few.

Nanoparticle developers and manufacturers leverage these well-established methodologies whenever possible. However, the unique properties of nanomaterials greatly complicate this seemingly straightforward process.

Nanoparticle constructs intended for medical applications consist of a wide variety of nanomaterial categories – including dendrimers, fullerenes, quantum dots, liposomes, metal oxides, gold colloids, and polymers.

Many of these particles, such as gold colloids, scatter light and often invalidate colorimetric assays that rely on absorbance measurements.

**About the author**



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of nanomaterials intended for cancer therapeutics and diagnostics. He received his bachelor’s degree in chemistry from Portland State University and his doctorate in cell biology from Oregon Health Sciences University.

Similarly, quantum dots have very large molar extinction coefficients and their emission wavelengths can yield ambiguous results from spectrophotometers or when using light scattering instruments (e.g. MALLS, DLS) for measuring particle size.

Other nanoparticles, such as dendrimers, can have catalytic properties and often interfere with standardized enzymatic tests, such as those which evaluate endotoxin contamination.

Several nanoparticle formulations include surfactants to promote dispersion (i.e. prevent agglomeration) of the individual components. If not accounted for during sample preparation, these compounds will interfere with conventional characterization methods (*see Fig. 2*).

Impurities and contaminants which absorb to nanoparticle surfaces can also contribute to ambiguous findings. Indeed, there are scientific reports that incorrectly attribute toxicity to nanoparticles – only to later find that the toxicant was a contaminant or dispersant.

These difficulties tend to hamper the development of standards for characterization, and perhaps even the subsequent commercialization of nanoparticles.

Because of these obstacles, nanotech standard developers have to design and validate novel characterization methods to assess safety, toxicity and quality control.

Regulatory agencies must then evaluate data generated from unfamiliar techniques without a substantial history of supporting literature.

This is where ISO has an opportunity; the time to market for nanotech-based drugs would be reduced by the availability of a standardized set of characterization methods.

Of course, crafting characterization standards for nanoparticles is no easy task. Characterization of nanoparticles for medical applications is cross-disciplinary and involves the fields of biology, chemistry, physics, material sciences, engineering, biotechnology, and medicine.

It comes as no surprise that these disparate fields do not share the same “nanolanguage”. Differences in discipline-specific terminology and techniques make it difficult for interlaboratory comparison of results, controls, and conclusions.

For example, nanoparticle size can influence efficacy, as mentioned above. But the term size might be defined by a material scientist as the particle’s electron density, whereas a biologist may refer to its hydrodynamic diameter.

WG 1 of ISO/TC 229, *Nanotechnologies*, is actively pursuing consensus on terminology and nomenclature for nanotechnology for this reason.

An unconventional but pragmatic approach being promoted by the US delegation is an ontology-based model. In this strategy a core set of nanotech-unique terms are agreed upon by joint committee, but then each discipline maintains its own dictionary of secondary and tertiary terms.

Under an ontology paradigm, knowledge sharing between disci-

plines is facilitated by conceptual relationships between terms, rather than by a composite dictionary of definitions.

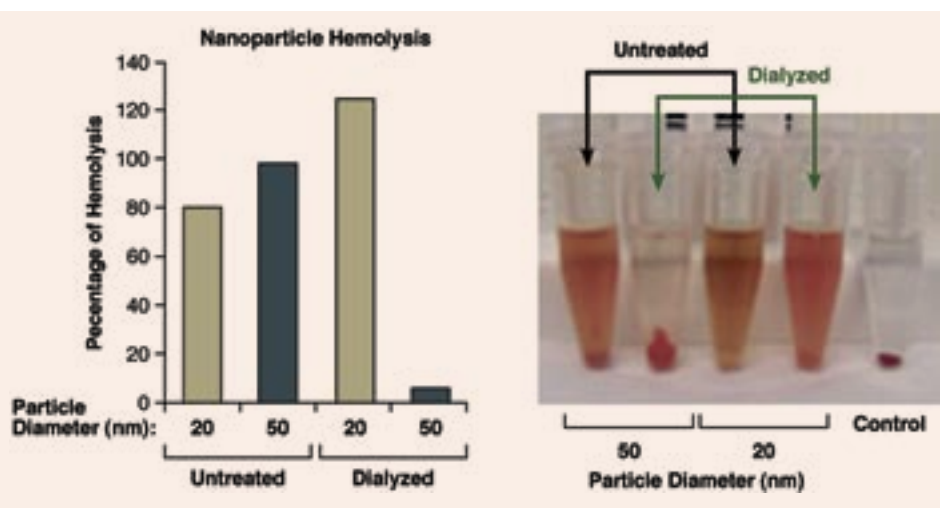
## The Road Ahead

Nanoparticle-based drugs are commercially available in North America, Europe, and Asia. It’s the size and tuneability of nanoparticles which so particularly suits them for use as medical tools – they can interact with cells and biomolecules and achieve a variety of remarkable medical functions.

Standardized methodologies for characterization of nanoparticles intended for medical applications will help alleviate confusion, help dispel ambiguity, speed the translation of nanoparticle drugs from discovery to development, and facilitate regulatory approval. This is an opportunity that ISO should not miss. ■

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**Figure 2 – Nanoparticles Can Interfere with Conventional Characterization Methods.** The graph (left) shows the results of an assay to determine the hemolytic properties of polystyrene nanoparticles. In this commonly used protocol, 20 and 50 nm nanoparticles are incubated in whole blood, the blood is centrifuged to remove undamaged erythrocytes and nanoparticles, and the percent hemolysis is determined by colorimetric detection of hemoglobin in the supernatant. Under these conditions, untreated (i.e. commercially supplied) particles with 20 and 50 nm diameters were strongly hemolytic. In the case of the 50 nm particles, spectroscopic analysis indicated a reduction in hemolysis following dialysis. Visual inspection of the microfuge tubes (right panel), however, showed the dialysed 50 nm particles adsorb hemoglobin (compared to control tube), and the adsorbed hemoglobin precipitates with the particles upon centrifugation – yielding a false negative result.