

NANOPARTICLES IN PHARMACEUTICAL MANUFACTURING

The pharmaceutical field has long been a model for industry, creating best practices in R&D, manufacturing, marketing and other disciplines. As such, it also has recorded historic levels of profitability. But an evolving marketplace, changing regulatory standards, increased manufacturing costs and a number of other factors have begun exerting pressure on pharmaceutical producers. One of the bright spots for the industry is nanoparticle technologies, which offers seemingly unlimited potential to help solve health problems and thus grow the pharmaceutical business.

Nanoparticles are loosely defined as substances with a product dimension of less than 100 nanometers (nm, $0.1\mu\text{m}$ or 10^{-7}m), but the category, particularly in the area of pharmaceuticals, includes substances that are as large as 500 nm. For a point of comparison, a human hair is approximately 80,000 nm wide.

First discovered by British botanist/surgeon Robert Brown in 1827, nanoparticles find application in many states, including solid particles in suspension, powder, dust, liquid drops in emulsion, fog, spray and foam.

Nanoparticles have four broad areas of application in the combined pharmaceutical/healthcare industry:

- Drug delivery
- Diagnostic products
- Product packaging
- Biomarker discovery

By 2014, spending on nanoparticle research and development is projected to exceed \$2.5 trillion annually.

In the discussion of nanoparticles, the dividing line between healthcare and pharmaceuticals applications is often hard to distinguish. Diagnostic products, for example, aren't true pharmaceuticals in that they don't directly affect change in disease or illness. Yet those diagnostic products that employ nanoparticles are typically researched and manufactured in processes similar to drugs; they are delivered internally or applied to sick patients; and manufacturers operate under many of the same rules and regulations. Products for biomarker discovery are similar in these regards. Thus, they will be included in general terms in this white paper.

The pharmaceutical industry already uses nanoparticles. They find use as carriers in medicated topical creams and lotions, in what are called nanoemulsions, which show better penetration and more even delivery compared to traditional emulsions. Microfluidic systems transport nanoliter volumes of fluid for nucleic acid and protein analysis, and micro-array platforms manipulate sub-nanogram quantities of genetic materials in genomics and proteomics.¹ Nanotechnology is also currently working in cancer and anti-tumor medications.

Small is Big Business

Nanotechnologies are booming and will, by all accounts, continue to grow at impressive rates. According to a Lux Research study reported in *Small Times* (March 8, 2007), US\$12.4 billion was spent on nanotech R&D worldwide in 2006. More than \$50 billion worth of nano-enabled products were sold globally that same year. Projections by the U.S. National Science Foundation in 2004 estimate spending to exceed \$2.5 trillion annually by 2014.

The United States is currently the largest purveyor of nano research and products. In 2001, the U.S. government launched the National Nanotechnology Initiative (NNI), an interagency program that coordinates federal R&D efforts in this field. The NNI projects its expenditures will exceed \$1.4 billion in fiscal year 2008, an increase of 212 percent since the program's inception.



Within the all-encompassing category of nanotechnology, the combined healthcare products/pharmaceuticals sector is generally estimated as the second largest category by dollar value (trailing electronics). Market evaluations project that nanobiotechnology will exceed \$3 billion with an annual growth rate of 28 percent by 2008.² In a 2007 study, the Freedonia Group Inc., a Cleveland-based research firm, concluded that demand for nanotech-based medical supplies and devices in the U.S. market will exceed \$110 billion by 2016. Additionally, nano-enabled products currently on the market carry an 11 percent price premium compared to equivalent conventional products.³

When the NNI was initiated, its charter listed 10 R&D targets to be addressed by 2015. Of those 10, five are relevant to drug development and delivery:

- No suffering and death from cancer when treated;
- Advanced materials and manufacturing;
- Pharmaceutical synthesis and delivery;
- Converging technologies form the nanoscale;
- Life-cycle biocompatible/sustainable development.

Nanostructure Classes and Development

The nanotechnology industry is still in its infancy, and the development of nano-sized particles and structures has a long way to go. Particles for pharmaceutical use are available in many different classes with advantages in various applications.

Carbon fullerenes and nanotubes are made of carbon 60 atoms and have numerous points of attachment. Nanotubes are among the most utilized particles because they offer strength and excellent electrical properties. They can be single-walled or multi-walled structures.

Ceramic nanoparticles are commonly derived from silica, titania and alumina. The particles' porous nature makes them suitable for drug delivery, particularly in cancer treatment.

Emulsions are oil-in-water mixtures stabilized with surfactants. These have been in use in the personal-care field for some time in anti-aging creams and lotions.

Liposomes comprise lipid bilayer membranes around an aqueous interior. The drug's active compound can be positioned in the aqueous center if it is water soluble or in the lipid layer if it is fat soluble.

Metallic particles include magnetic compounds, such as iron oxide, and gold- and silver-coated particles. Magnetic compounds are being used for targeting drugs. Gold-coated particles display excellent optical properties needed for diagnostic imaging. Silver-coated particles show outstanding anti-infection properties.

Polymers, including chitosan, and newer water-soluble hybrid polymers are used in drug delivery. When attached to proteins, polymer nanoparticles improve the protein's stability and reduce antigen-induced immune responses.

Quantum dots are fluorescent particles that are being researched for use in tumor detection via spectroscopy, as well as other diagnostic applications.

In loose terms, there are four main groups of these structures, only two of which have been realized to levels suitable for possible commercialization. At a Society of Manufacturing Engineers conference in 2005, David Keenan characterized the following groups.

Passive nanostructures are essentially individual particles that are integrated into products to enhance the performance or characteristics of other molecules. [These are already in widespread use in pharmaceutical manufacture.]

Active nanostructures are building blocks that are manufactured to specific shapes and sizes, with predetermined interfaces and positions. [These component parts are currently used in many pharmaceutical R&D projects. While a tiny number of active nanostructures may be commercialized, most are still in clinical trials or still in research.]

Nanosystems combine passive and active nanostructures into simple machines. Molecular nanosystems, the fourth phase of development, build on nanosystems to create synthetic biology. Researchers have not yet achieved these levels.

Pharmaceutical Applications and Advantages

As outlined above, the four major areas of research and development for nanoparticles are product packaging, biomarker discovery, diagnostic products and drug delivery.

Packaging

While critical to products and the overall pharmaceutical market, packaging is not core to drug research and development, and is usually developed and manufactured by companies other than the drug producers. As such, this is a topic deserving of its own white paper. Thus, the section herein will be brief.

Polyvinyl Chloride (PVC) and Polyvinylidene Chloride (PVDC) and its copolymers are among the most common products that incorporate nanoparticles to improve pharmaceutical packaging. PVC and PVDC are commonly incorporated between cellophane, paper and plastics as a barrier to gasses, odors and humidity that could foul the drug.

Manufacturers use nanoparticles as cross-linking and reinforcement mechanisms in the polymer chains. This provides physical strength at light weights. Nanotechnologies also improve packaging's optical properties, helping make it UV resistant and/or improving the clarity of package windows.

Biomarker discovery

Biomarkers are molecules that can be measured in blood, body fluids and tissues to assess the presence of disease or its state of development. Prostate-specific antigen, or PSA, the indicator for prostate cancer, is perhaps the most widely known biomarker in the general public. As early as 1998, nanoparticles were being employed in successful in-vitro biomarker diagnostics for diseases such as skin cancer.⁴ When used in proteomics and genomics technologies, nanoparticles have been shown to help amplify biomarkers and protect them from degradation.

Four major areas of nanoparticle R&D include drug delivery, diagnostic products, biomarker discovery and packaging.

In 2006, the National Cancer Institute reported that researchers at the Nanomaterials for Cancer Diagnostics and the Therapeutics Center for Cancer Nanotechnology Excellence (CCNE) at Northwestern University developed an ultra-sensitive method that can detect as few as 100 molecules of PSA in a drop of blood that is six orders of magnitude higher sensitivity than conventional assays.

Similar results continue to be generated in many areas of biomarker research, making this one of the brightest spots for nanomedicine.

Diagnostic Products

Like biomarkers, diagnostic imaging products find use in early disease detection. They are also used to track disease metastasis and now, thanks to nanotechnology, drug targeting and distribution. Nanoparticles are being used and tested to achieve clarity, targeting and range previously unavailable in processes such as magnetic resonance imaging (MRI), ultrasound, fluorescence and tomography.

Iron oxide particles are improving the effectiveness of MRI imaging. Nanoemulsions using gadolinium complexes dramatically improve ultrasound imaging.

Contrast agents bound to nanocarriers used for drug delivery are helping target drugs to specific locations, such as tumors, and track the drug's transmission through the site. Such diagnostics help clinicians know when to administer the next dose.

Drug Delivery

Drug delivery represents what is potentially the largest immediate application for nanoparticles in the pharmaceutical industry and perhaps the widest area of use. There are more than 300 companies in the United States alone involved in developing drug delivery mechanisms.

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According to the Pharmaceutical Research Manufacturers of America (PhRMA), the average total cost to push a new drug through development in the United States is more than \$800 million. The process takes an average of 12 to 15 years, leaving only five to eight years of U.S. patent protection. PhRMA further notes that just one in 5,000 new compounds survives the process to become a new drug in the marketplace.

An estimated 20 percent of those drugs that have made it to market since 1995 do not perform up to their full potential because they have low bioavailability (acceptance and uptake by the body). This can result in poor performance characteristics, such as slow or variable onset, increased side effects and poor compliance, as well as the need for higher dosing.

In addition to newly formulated drugs, nanoparticles can, in many cases, be applied to reformulations to:

- improve the efficacy of current drugs that under perform;
- make viable many compounds that never made it through trial phase to market;
- improve efficacy or limit side effects of older drugs now off the market or off patent, allowing them to be re-introduced;
- change the method of drug delivery to improve customer acceptance or reduce manufacturing costs.

All common delivery vehicles — oral, injection, transdermal, transmucosal, ocular, pulmonary and implant — can effectively use nanoparticles in their formulation. The technology offers numerous potential advantages that may apply in some or all of these delivery forms. These advantages include, but are not limited to, a number of functionalities depending on the drug's specific application and performance goals:

- prolong circulation in the blood;
- accumulate in the pathological area;
- enhance water or lipid solubility;
- respond to local stimuli, such as changes in pH, temperature or light;
- penetrate anatomical features such as cell walls, blood vessels, stomach epithelium and blood-brain barrier; and
- selectively target specific cell types.

Numerous studies show that particles less than 100 μm in size have greater absorption and delivery efficiency in the gastrointestinal, pulmonary and vascular systems, and similar dermal penetration characteristics. This could prove particularly beneficial to BCS class II, III and IV active pharmaceutical ingredients, which typically have low solubility and/or low permeability. A drug is considered to have low solubility at 0.1 mg/L. Industry analysts estimate that about 10 percent of drugs currently on the market have poor solubility.

Nanodelivery has shown tremendous promise in targeting drugs at tumors. Tumor microvasculature typically contains pores 100-1,000 nm in diameter, while healthy heart, brain and lung tissue is 10 nm or less. So by manufacturing nanoparticle-based drug molecules between 75-900 nm, researchers and manufacturers have been able to create compounds that selectively target only malignant tissue.

Recent research with the anti-cancer drug doxorubicin has shown it is more effective on cancerous tumors when delivered via nanoparticle than through traditional delivery mechanisms.

Nanotechnologies have also improved the reach of drugs, offering unique forms capable of carrying RNA and DNA into subcellular structures to repair genetic faults or to alter genetic expressions. Delivering proteins and peptides into cells, an idea previously ineffective for a lack of delivery method, also shows great promise in addressing diseases such as diabetes.

Research in the Journal of Physical Chemistry this year reported steps forward in glaucoma treatment using nanoparticles. Only 1-3 percent of existing glaucoma drugs delivered via liquid drops into the eye actually penetrate the eye, a very poor rate of success. The nanodelivered treatments showed high penetration and less abrasion.

Specific nanoparticle shapes can be used to encapsulate or bind drug compounds to improve drugs' solubility, stability or absorption rates. This allows manufacturers to overcome numerous obstacles that have in the past been research dead ends. In some cases, the problem has been tissue or cellular damage or irritation associated with comparatively large doses of drugs. Nano-sized doses, however, impart significantly less irritation or damage, which the body is able to heal without adverse affect. Examples include drugs dosed via inhalers, which can cause coughing and throat irritation.

Nanoparticle Creation by Media Milling

Media milling, commonly called grinding, is the most well documented and well established method for creating small-scale particles, dating to the first half of the twentieth century. The process of using force shearing to reduce the size of particles into the nano scale has been significantly improved during the last quarter century and, when combined with new dispersion techniques, offers many benefits:

- excellent particle-size control
- comparative cost-effectiveness
- equipment scalability, from benchtop to production
- limited or zero contamination of active
- repeatability of process
- meets cGMP production requirements

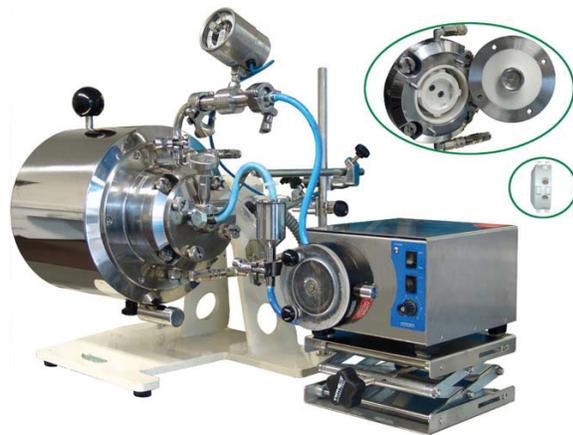


Figure 1. NETZSCH Fine Particle Technology *MicroCer* media mill.

Popular because of their simplicity and scalability, fine bead mills also offer lower costs compared to plasma gas and precipitation process techniques and other alternative technologies. Often used in conjunction with plasma gas process techniques, fine bead mills provide an efficient way to disperse the output to a primary particle size, stable dispersion, assuming the proper stabilizing agents are used (Figure 2).

The principle behind agitator bead mills is based on grinding of suspended solid particles by impact and shearing forces between moving grinding beads. Kinetic energy is transmitted to the grinding media by the agitator shaft within the stator housing.

The achieved fineness is primarily defined by two basic parameters – the stress intensity and the number of contact points. The stress intensity is a function of the kinetic energy in the grinding beads. This energy must be high enough to produce the grinding process. The number of contact points determines how often the media interacts within the grinding chamber. A fine particle size distribution requires a high number of contact points in the grinding chamber. To increase the number of contact points, smaller grinding media must be used. The rule of thumb is that particle size is equal to 1/1000 the size of the grinding media.

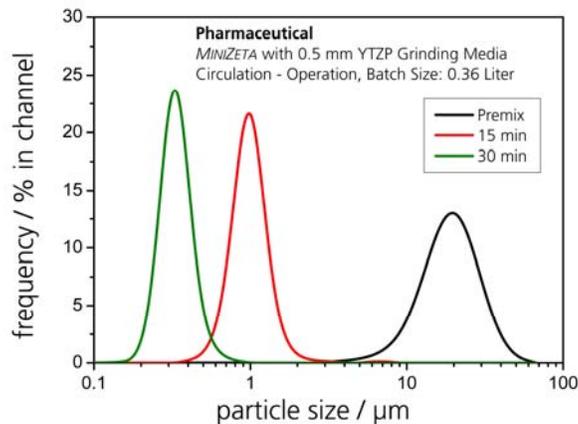


Figure 2. Particle Size Distribution with NETZSCH's *MiniZeta*

Achieving Repeatable Nanoparticle Dispersions

Creating repeatable distributions of submicron particles is essential to using them effectively in formulations. Traditional plasma gas processes promise superior particle uniformity, but do not offer the ability to disperse particles in a solution at their primary size. Precipitation processes produce large crystal sizes and agglomerated, aggregated crystals in the range of 100 nm to 100 µm. A fine bead mill with grinding media on the order of 100 µm to 200 µm is the simplest, most scalable, most cost-efficient way to make and disperse nanoparticles.

But dispersing nanoparticles into liquids can be a challenging undertaking. The tremendous surface area and surface energy which delivers the beneficial effects of nanomaterials also prevents their easy dispersion into liquids. Intermolecular forces increase as particles become smaller, causing cohesive forces (agglomerates, aggregates or primary particles) in the product. Agglomerates are formed by point-focal or linear cohesive primary particles, while aggregates form by laminar binding. Primary particles are crystalline, or amorphous, particles that are separated against each other. The goal is to disperse these particles to their primary particle size. But conventional technologies for dispersing powders into liquids are not sufficient for dispersing these tiny particles as discrete entities.

The production of stable suspensions or dispersions of nanoparticles requires a comminution process, such as a small-media mill provides. Mills using media beads in the range of 70-125 μm can economically grind into the nanometer range.

Mill designs using ultra-fine grinding beads 70-125 μm in size offer an effective solution for creating stable dispersions of these newly formed nanoparticles with outstanding process efficiency as well. Called mild dispersion, this process uses multiple mild contacts instead of one strong contact, which can cause breaking and destruction.

Many factors affect the function of mild dispersion, but the main principle is bead size. Decreasing the diameter of the bead yields four primary results:

- The number of beads is increased dramatically.
- Contact of the beads with the product is increased dramatically.
- The weight of one bead is decreased dramatically (weight = diameter³) (Figure 3).
- The energy of one bead is decreased dramatically (mean energy of one bead is equal to the specific energy input divided by the number of grinding beads).

Size of beads (mm)	Weight of a bead
100	1
50	1 / 8
10	1 / 1, 000
1	1 / 1, 000, 000
0. 1	1 / 1, 000, 000, 000
0. 05	1 / 8, 000, 000, 000

Figure 3. Bead size to weight ratios

**Mild dispersion
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The smaller the bead size, the more beads there are per unit volume. For example, if 1-mm beads are loaded into a 1-liter vessel, there are around 1.1 million beads. But with 0.05-mm beads, there are 9.4 billion beads. So, the probability of contacts between particles and beads increases significantly.

As the bead size decreases, the space between the beads decreases too, working as a filtering mechanism that holds back large agglomerates and breaks them apart. A rough calculation indicates the stand-off distance between the beads would be 44 μm for 1-mm beads and about 2 μm for 0.05-mm beads. Mild dispersion provides a dynamic filtering mechanism holding back particles larger than the standoff distance, and it shears them apart to their primary size. Together these changes create uniformity and reduce particle damage while maintaining productive work speeds.

Mill Choices

Selecting appropriate equipment is key to developing a repeatable process and creating efficient workflow in the lab right through to full-scale production.

Obviously, the first requirement is that the equipment be appropriately sized for the work and that it be scalable. New mills allow for the use of beads down to 100 μm in diameter while offering improved functionality that facilitates adequate product throughput at slow, low-energy motor speeds (which prevents damage to the nanoparticles) while providing practical methods for handling and removing the fine grinding media at the end of the process.

The ideal mill would have “plug flow,” so none of the batch is able to bypass the grinding and all the material passes through the machine at the same velocity, producing a uniform grind and residence time distribution; however due to the turbulence in bead mills, this is not possible, so rapid multiple-pass operations are used to achieve a narrow particle size distribution (Figure 4).

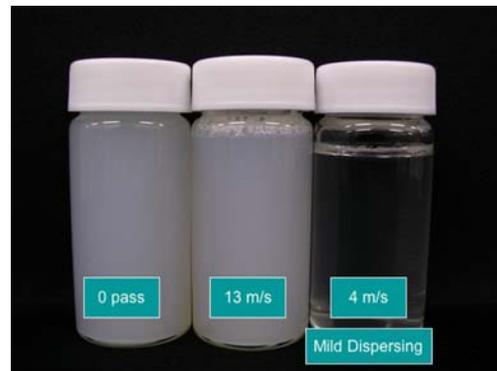


Figure 4. Results of multiple-pass operation

Important for processing nanoproducts in agitator bead mills is the selection of materials suitable for the grinding media and the grinding chamber to avoid contamination of the products. Grinding zone parts manufactured completely with Yttria Zirconia (YTZ), a high strength ceramic, offer metal- and contamination-free grinding. Grinding beads are also available in materials such as plastics, glass, ceramics like Al_2O_3 and ZrO_2 , steel and even tungsten carbide.

A Look Forward

As nanotechnology continues to evolve, so too will its application in pharmacology and related biological health applications. Ongoing research points toward nanotechnology's benefits outweighing potential drawbacks in the majority of applications in which it has been studied. Oncological uses seem to hold early promise, but researchers have only scratched the surface. Nanoproducts research across virtually all biomedical fields is yielding important strides forward.

There is no one nanotechnological solution, just as there is no one nanoparticle to solve every application challenge. Matching technologies to challenges will continue to demand intense research.

New nanoparticle production techniques and equipment will aid in furthering discovery. Mild dispersion is a step in that direction, providing chemists and researchers with a cost-effective method to disperse nanoparticles without damage to the crystal structure.

References

1. Mazzola, Laura, "Commercializing nanotechnology," Nature Biotechnology, Vol. 21, no. 10, Oct 2003.
2. Frontline Strategic Consulting, "Nanobiotechnology opportunities and technical analysis," 2003
3. Lux Research, Inc., "The Nanotech Report 4," 2006.
4. M.K. Bhalgat, et al, "Green and red fluorescent nanospheres for the detection of cell surface receptors by flow cytometry." *J Immunol Methods*, 219: 57-68.