Nanotechnology For Drug Delivery

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Introduction

Drug delivery, the ability to make sure that a pharmacologically active substance arrives at a relevant *in vivo* location while minimizing toxicity, has become a central topic in pharmaceutical research and development. Achieving such a task requires that the problems presented by undesirable physicochemical properties such as low solubility or high lipophilicity are overcome using formulation technology. That candidate molecules have such properties is not surprising, or even avoidable, given the location that many biological targets have in the body, *i.e.*, highly hydrophobic cell membranes or behind alternating layers of hydrophilic and hydrophobic tissues. In addition, the similarity in active sites among likely targets, *e.g.*, kinases, means that the structural differences between a compound that is usefully active and one that is toxic are very slight – to the point where targeted delivery is the only way a practical therapeutic index can be obtained.

In order to properly describe the impact of nanotechnology on the delivery of drugs some definitions are necessary. For the purposes of this discussion, nanotechnology can be defined as structures that:

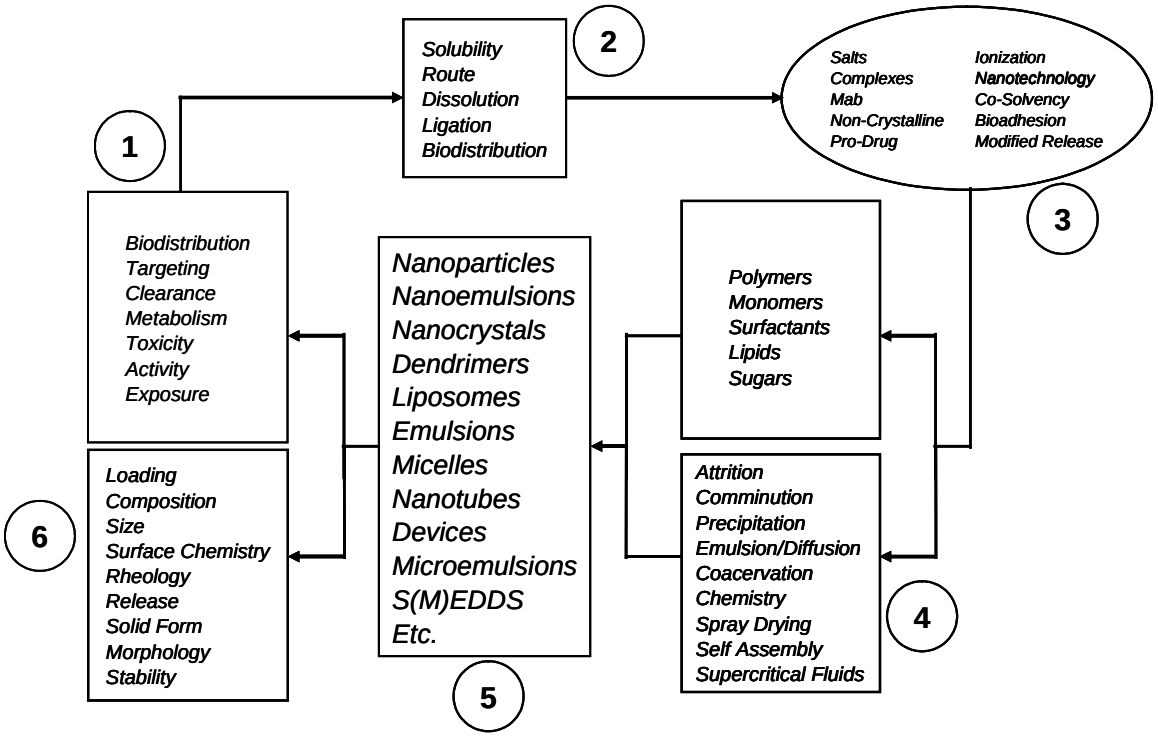
- Have at least one length dimension less than or equal to 500 nm.
- Exhibit novel and unique chemical, physical, or biological behavior because of their small size.

The first part of this definition is relatively straightforward insofar as it uses small size as its central element. It is important to point out that only one dimension need be on such a scale. Nanotubes, while they can have a diameter of 10 nm, can have lengths on the order of tens of microns. The second part of this definition is more complex since it specifies that the object not just be small, but that they behave in a unique fashion as a result. In some cases, this relationship between size and function is clear. That surface area and thus dissolution rate increase as the particle size drops, or that solid drug can only be administered by certain routes when presented as submicron colloids are two examples. However, there are more subtle aspects as well: the interpretation of nano-object behavior depends upon the scale of observation. For example a suspension of nanoparticles behaves like a molecular solution on the macroscopic scale, *i.e.*, flow, but on the smallest scale behaves like a solid substance, *i.e.*, the drug may not be freely available. And in this example, there may be a benefit at both scales in that the drug can be freely injected, but will not become bioavailable until arrival at the site of action.

Nanotechnology has had a significant impact on the science of drug discovery and development. Some examples are:

- Achieving intracellular delivery of drugs via the use of spontaneously forming nanotubes.
- Submicron lipid complexes that allow intravenous administration of antifungal agents with reduced toxicity.
- The existence of 3 commercial products in which nanoparticles are used to increase exposure and reduce the sensitivity to fed/fasted effects.
- Submicron (ca. 200 nm) triglyceride emulsions that serve as a source of parenterally administered nutrition.
- The acceleration of wound healing via the use of silver nanoparticles as antimicrobial agents embedded in dressings.
- The use of quantum dots in the diagnosis of respiratory syncytial virus (RSV).

There are several items of note in this list. First, is the breadth of application. Exposure enhancement, reduction in toxicity, cell-scale delivery, diagnostics, and wound healing are all impacted by the use of submicron materials. The second item is the diversity of physics. Quantum dots, nanoparticles, and nanotubes all qualify as embodiments of nanotechnology, but are vastly different from one another in terms of their fundamental nature, let alone their composition and means of manufacture. Finally, it should be noted that, in spite of its current high profile, the application of nanotechnologies, particularly nanoparticles, has been around for some time. Indeed, the second item, known as Ambisome, was approved for use in the US in 1995 and the fourth item, Intralipid, has been commercially available since 1966.



The task of the drug delivery scientist can be described via Figure 1. The first step is to identify the problem that needs addressing. This problem can then be formulated into a statement such as, "Can the exposure of compound X be improved?" or "In what way can the toxicity of compound Y be reduced?" Next is to determine what needs to be changed such that the desired modification can be obtained. In the case of the aforementioned compound X, it might mean increasing the solubility or dissolution rate of the corresponding solid, whereas solving compound Y's problem might require achieving control over its biodistribution, *i.e.*, targeting.

The third step is to identify what drug delivery technology is most appropriate to solve the problem at hand. Given the topic of this discussion, we will focus on nanotechnology, but it is important to remember that such approaches constitute only one of the many drug delivery options that are available. Within nanotechnology the choice then comes down to one of components and processing. That is, by what method will the formulation be generated, *e.g.*, comminution, self-assembly, etc., and what materials, including the drug, will be used as major and minor components. It is the combination of components and process that will determine what the actual formulation is, some examples of which are presented in step 4 of Figure 1.

Once the formulation has been identified and made, the fifth step is to use *in vivo* testing to determine if the original goal of exposure or toxicity has been attained. In parallel with this task is characterizing the formulation itself. As will be discussed later, there are a variety of measurement tools and techniques that are available, some of which are specific to nanotechnology and require significant levels of expertise and experience to properly apply. The best analytical program is one that will probe as many parameters as possible, *e.g.*, size, structure, release, etc., and focuses on those that will allow the *in vivo* results to be interpreted usefully. A simple example would be determining the effect of particle size on the biodistribution of injected nanoparticles.

So how do we translate this new science of small scale into a practical reality for drug delivery? The remaining portion of this discussion will focus on leveraging the knowledge of nanotechnology categories, production, characterization, and use for this very purpose.

Types of Nanotechnology

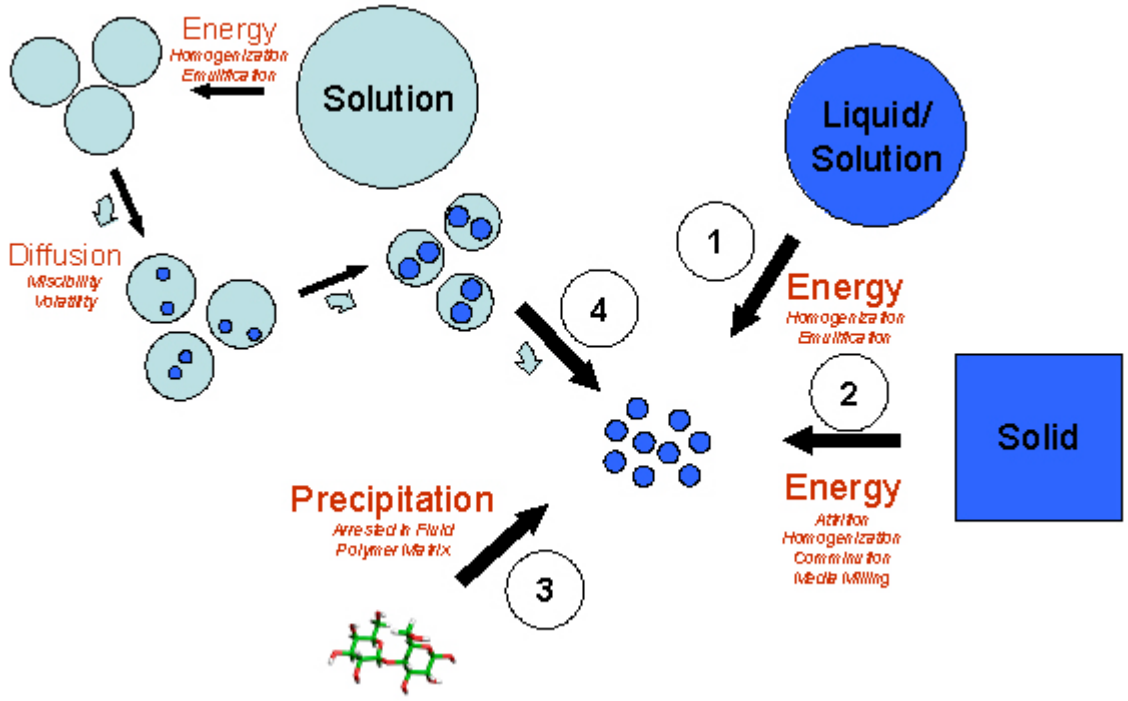
Objects relevant to nanotechnology can be subdivided into two categories, those that form spontaneously, and those that need to be fabricated. The former category includes micelles, microemulsions, some liposomes, and nanocochleates – all structures that self-assemble from their component materials under the proper conditions. In most all cases they are thermodynamically stable. While all of these species fall under the term "nanotechnology" as defined above, they also have a long history of use as agents of drug delivery and hence will not be covered further here. The second category can itself be broken down into objects that are deliberately crafted devices, such as nanomachines manufactured using lithographic approaches, and objects that are produced by more of an ensemble approach, such as nanoparticles. The remaining discussion will focus on the latter group of objects.

Manufacture of Nanoparticles

Emulsification

There are a number of ways to make nanoparticles as described in Figure 2, below.
The first approach starts with a macroscopic aqueous dispersion of a water immiscible liquid. The drug is located in the discontinuous phase and thus the first limitation of this approach is

noted: the drug must have an affinity for the component(s) of that phase. Mechanical or sonic energy is then introduced into the system in order to reduce the particle size to the point where the dispersion becomes kinetically stable, which is generally less than 300 nm. In addition a surface-active agent is required to reduce the interfacial energy as a means of assuring that such stability can be maintained for timescales on the order of years. It is the choice of components and optimizing process conditions that presents the technical challenge to the pharmaceutical scientist. In many cases the immiscible phase is a lipid, which if it is a liquid during processing but a solid at room temperature, results in formation of solid lipid nanoparticles.



- Advantages: Uses common manufacturing equipment (homogenizers); use of lipid improved biocompatibility for oral and parenteral administration and may enhance exposure; provides protection from ambient lighting.
- Challenges: Limited to maximum of 30% discontinuous phase; overall drug load can be low; crystallization/freezing of liquid leads to destabilization of nanosuspension; maintaining physical stability; high energy used in manufacturing can lead to chemical decomposition.

Attrition

A second approach to making nanoparticles is very similar to the above, but applies when the material to be dispersed is a solid under processing conditions. As a result, attrition approaches, such as bead milling, now become possible. While the surface of solid particles are obviously stable to coalescence, surfactants and/or polymers are required to maintain the stability of the colloid as a whole. In addition, breaking down solid particles takes more energy and time than does forming nanoparticles from liquid droplets.

- Advantages: Straightforward concept; since the nanoparticles are almost all drug, the load can be quite high and is limited, mostly by choice of processing technique, to 60% (w/w); approach validated by the successful introduction of several commercial formulations; works best with low solubility compounds.

- Challenges: Identifying suitable stabilizing excipients; excipient choice and processing conditions vary from drug to drug; substantial amounts of heat can be generated; avoiding intellectual property conflicts; obtaining precise control over particle size; particle size limited to greater than 100 nm.

Arrested Precipitation

The third means of production is arrested precipitation, a process that involves driving solubilized material out of solution via use of a pH, temperature, or solvent shift and then stopping the process almost immediately while the particles are still small. Precipitating from highly supersaturated conditions leads to the formation of many small particles rather than a few large ones, and using large amounts of highly surface active excipients deactivates the highly energetic particle surfaces as a means of arresting particle growth and avoiding subsequent aggregation. In many cases, the only way to adequately control the process and size is to coprecipitate the drug with a suitable polymer. For obvious reasons, directly forming a high concentration formulation with this approach is problematic with respect to process control, but the final concentration can be elevated via the use of diafiltration techniques. This approach can be generalized in that the precipitation can take place from a solid solution (to make a solid dispersion of nanoparticles), from a supercritical or cryogenic fluid, or from an aerosol, *e.g.*, spray drying.

- Advantages: works well with oils and high LogP compounds; produces particles less than 100 nm; very low energy input.
- Challenges: effectively stopping particle growth is not easy; requires the removal of large amounts of solvent; low drug load; long term stability of particle structure; compound must be rendered soluble in a water-miscible solvent or through pH control; subtleties of fluid dynamics makes scale-up difficult.

Emulsion–Diffusion (Template)

The fourth means of making nanoparticles is really a combination of two of the above. In this approach, a drug-containing emulsion is produced though in this case using a solvent with significant volatility or water miscibility. After preparation, the drug-solubilizing discontinuous phase is removed through diffusion either via evaporation or dilution, which in turn leads to drug precipitation within the emulsion droplet. Unlike the direct precipitation approach in which particle formation occurs throughout the solution, in this case precipitation is limited to the discontinuous phase so that the droplet serves as a template for nanoparticle formation. Note that if the drug load in the original emulsion is not very high, then nanoparticles can be formed even if the droplet itself is several microns in size because most of the latter is solvent. In addition, precipitation from an emulsion is a more controlled process thus allowing the concentrations of stabilizers to be lowered, though not eliminated.

- Advantages: slower, spatially distributed precipitation leads to more effective control of the precipitation process; less stabilizer required; fewer scale-up issues; use of a volatile solvent facilitates its removal during processing; useful with oily drugs.
- Challenges: requirement of volatility or partial water miscibility limits the choice of available solvents and hence the drugs to which this method can be applied; multi-step process more complicated/costly then direct size-reduction approaches; use of dilution to remove solvent from droplet leads to large batch volumes.

Additional Processing Steps

Most of the above methods result in the formation of an aqueous suspension of nanoparticles. In some cases, such a presentation is acceptable. However, many times what is desired is a solid dosage form, such as a tablet or lyophilized cake. There is little point in making 100 nm particles only to have them form irreversible micron-sized agglomerates upon further processing. Thus the task becomes one of converting the nanosuspension into a form from which it can be dispersed

back upon reconstitution. The formulator has two tools at his/her disposal to solve this problem: controlling the processes employed, *e.g.*, spray drying, and employing stabilizing excipients, *e.g.*, charged surfactants. Another consideration is the make up of the particle itself and the effect it can have on overall behavior. For example, crystallization of lipids used in solid lipid nanoparticles can lead to irreversible aggregation. One way this problem has been solved is to use mixtures of lipids, rather than pure components, since they are more difficult to crystallize. It should be noted that when a dry powder form of nanoparticles is desired, there is a real advantage to using methods, such as supercritical fluid processing, which lead to direct production of nanoparticles in the solid state.

Another post-processing issue that can be problematic for nanoparticles is sterilization. In this case formation of a liquid suspension is an advantage and sterile filtration is a good option if the particles are small enough to allow it, *i.e.*, a d90 less than 190 nm. Aseptic processing is another option, and while maintaining such an environment may be straightforward for precipitation-based approaches, they can become more difficult when high energy processing is involved. The final option is terminal sterilization. When heat is used, loss of physical stability can occur either because of chemical changes induced at the particle surface or because of the large amounts of thermal energy that are introduced into the system. Nanoparticle formulations that appear to be equivalent may turn out to be quite different after terminal heat sterilization; hence the formulation development process must include this step in the cycle. On the other hand some formulations, such as parenteral lipid emulsions, are more stable after heat treatment than before – a process thought to be due to a combination of annealing and chemical modification of the surface.

Characterization of Nanoparticles

A good physicochemical understanding of the formulation is an absolute necessity for rational formulation design and properly interpreting *in vivo* results. Size, surface characteristics, particle morphology, structure, and drug release are all relevant topics each of which will be briefly discussed below.

Size

Size is a central focus of nanotechnology as defined above, so its measurement is significant from that perspective. More importantly the size of a nanoparticle will determine its behavior both *in vitro* and *in vivo*, hence quantitative data on this characteristic is indispensable. Particle sizing can be broken down into three classes, ensemble, counting, and separation. Ensemble techniques, which include many of the spectroscopies such as light scattering and acoustic, make a single measurement of the system and then apply appropriate mathematics to extract a size population. They are very useful because of their speed, accuracy, and convenience, however they are poorly suited to describing the particle population at the edge of a size distribution, and are subject to systematic errors if the data quality is poor or if required parameters such as refractive index are not available. Counting methods, such as microscopy or single particle counting, provide very quantitative results since data is collected from individual particles. However, for the same reasons such methods are slow, frequently require extensive sample preparation or dilution, and are subject to sampling errors. Finally, separation techniques give a good understanding of the shape of a size distribution, but care is required to make sure that the mechanism of separation is completely understood, *i.e.*, is occurring in a manner quantitatively related to size rather than anomalous interaction channel walls. Analytical ultracentrifugation, various forms of field-flow fractionation, and hydrodynamic fractionation are examples of such approaches. Regardless of the analyses employed, a key precept of size characterization is to use more than one method as a

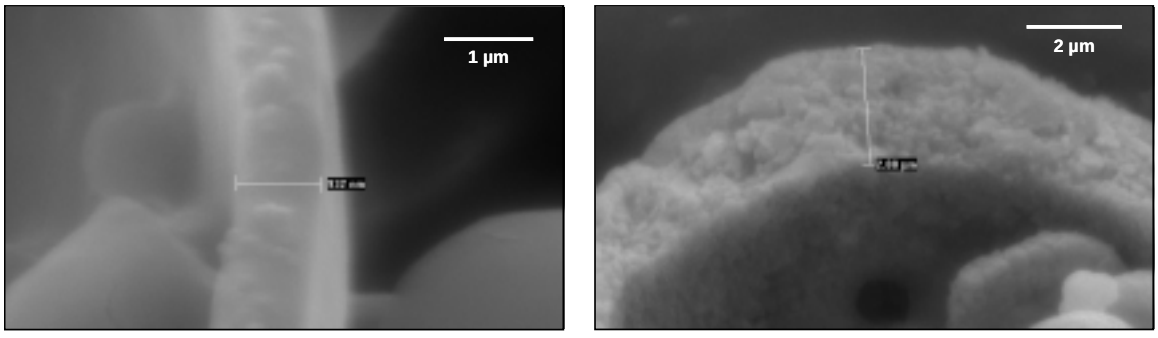
means of obtaining complementary information and thus a fuller understanding of the nanoparticle system at hand.

Surface Properties

Contact of the particle surface with its environment will determine the particles' interaction with one another and may strongly influence their *in vivo* behavior, *e.g.*, clearance. Charge, usually measured as zeta potential, is a primary descriptor and is determined most easily using techniques such as electroacoustic and electrophoretic light scattering. These particular methods are ensemble in nature and so have the same advantages and limitations as those listed above. Measurements of zeta potential based upon counting also exist. Using electrophoreses to analyze bound proteins extracted from plasma-treated nanoparticles is a useful means of better understanding the interaction of nanoparticles with blood components.

Particle Morphology

In some cases the interest is in the shape of the particle and the nature of its surface. In other cases interest focuses on the particle disposition, such as aggregation state or location within a matrix (see figure). Particularly when the objects to be analyzed have a high aspect ratio, such as the case with nanotubes, an actual picture is a valuable complement to instrumental methods. Given the size of the particles, scanning electron microscopy, either in transmission or scanning mode, are the primary tools of the analyst. The magnifications are much higher in the former mode, but the 2-D appearance of the images and the extensive sample preparation serve as drawbacks. Freeze-fracture sample preparation, where a cast is taken of the sample of interest and then examined in turn is valuable in those instances where the sample is fragile, but it is a laborious procedure that requires a high level of skill to properly utilize. Recently there has been a great interest in applying atomic force microscopy (AFM) to the analysis of pharmaceutically-relevant nanoparticles. Very high resolution images of particles in their native environment can be obtained using AFM, albeit at the expense of time since it is a rastering method. Depending on the interaction established between the probe tip and the sample, a wide range of material characteristics can be mapped on the nano-scale, *e.g.*, electrostatic potential or hardness. The particles must be located on a solid support like mica, so understanding the effect that the latter has on the former, flattening for example, is important, and constitutes one reason that experience of the analyst with AFM is necessary. Optical microscopy can be quite valuable, even though the size of the particles is below the resolution of the technique. For example, dark-field methods allow the presence of nanoparticles, and hence their number-weighted concentration, to be detected even though the particles themselves are not imaged directly.



Structure

The arrangement of components within the nanoparticle can determine its behavior and stability. A number of common methods are appropriate such as differential scanning calorimetry and powder X-ray diffraction if one is interested in overall structural characteristics such as crystal

form or extent of amorphous character. Given the small size of nanoparticles, particular structural features such as layers, may be on the molecular scale. In such cases, establishing the orientation of molecules requires higher energy scattering methods such as small-angle neutron and X-ray scattering. Needless to say such techniques require highly specialized equipment and operators. When the nanoparticle in question has a fluid component or when there is interest in measuring free drug levels, NMR is a good technique since the magnitude of instrumental response depends upon molecular motion. Combining chemical speciation and diffusion measurement in 2-D gradients NMR can be very informative.

Drug Release

A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. In order to obtain such information most release methods require that the drug and its delivery vehicle be separated. The simplest case is separating dissolved from undissolved drug in a traditional solid formulation. In this example, a filtration step is sufficient to produce the separation. However, what happens when the drug containing particles are sufficiently small to slip through the filter pores? This is easily the case with nanoparticles and the result is an inability to distinguish between free (available) and bound (unavailable) drug. In such situations the distinction can be brought about via size discrimination or spectroscopy. The former case is just an extension of conventional methodology although rather than using standard filters, diffusion membranes or ultracentrifugation can be employed instead. While effective at separating released drug from the nanoparticles, this approach takes a long time and thus makes it impossible to measure fast release rates. One can also take advantage of the fact that bound and released drug may have different spectroscopic characteristics. In this way the increase in released drug can be quantified as a measurement of release. Because of its sensitivity to molecular structure and mobility, NMR is one way of taking this approach. On the other hand, the spectroscopic method has to have a sufficient limit of quantitation. Since nanotechnology is frequently applied to poorly soluble compounds, this can become problematic. Note that in either approach it might make more sense to look for the disappearance of drug from the nanoparticle than it does to measure its appearance in the release media.