

Direct visualization, sizing and concentration measurement of drug delivery nanoparticles using Nanoparticle Tracking Analysis (NTA)

NANOSIGHT RANGE

Visualize and Measure Nanoparticle Size and Concentration



PARTICLE SIZE



PARTICLE CONCENTRATION

Introduction

The use of nanoparticles in drug delivery continues to grow rapidly. Nanoparticles offer excellent pharmacokinetic properties, controlled and sustained release, and targeting of specific cells, tissues or organs. Interest in nanoparticle drug delivery is also driven by the diminishing rate of discovery of new biologically active compounds that can be exploited therapeutically to treat disease. With fewer new drugs entering the market every year, interest in the use of nanoparticles' versatile and multifunctional structures for the delivery of drugs is swiftly increasing. All these features can improve the efficacy of existing drugs (Malam et al., 2011).

Nanoparticles used in drug delivery have been defined as colloidal systems of submicron size that can be constructed from a large variety of materials in a large variety of compositions. Commonly defined nanoparticle vectors include: liposomes, micelles, dendrimers, solid lipid nanoparticles, metallic nanoparticles, semiconductor nanoparticles and polymeric nanoparticles. In their many guises, nanoparticles have been extensively employed to deliver drugs, genes, vaccines and diagnostics into specific cells/tissues. (Ram et al., 2011).

When considering a nanomaterial drug delivery system, size of the nanoparticle is a key parameter as it directly influences the processes of delivery, uptake, degradation and clearance from the body. For example, nanoparticles in the range of 30 nm to a few hundred nm in diameter can passively accumulate at the site of tumours due to leaky vasculature, phagocytosis favours particles >500 nm, whilst biliary and renal clearance occurs with particles <30 nm and <8 nm respectively. In addition, the liver has a lower uptake of smaller particles (25 nm and 50 nm) compared to larger particles (200 nm and 300 nm). Accurate measurement of the particles being administered is therefore imperative to many systems and processes.

Analysis of drug delivery systems by NTA

Liposomes

Liposomes (Figure 1) have been the subject of significant research and development efforts for many years and are currently the most common targeted drug delivery system. Liposomes have been approved as a delivery system for amphotericin B for

fungal or protozoal infections, doxorubicin for breast cancer treatment, and for vaccines for hepatitis A and influenza. The use and potential of liposomes in drug delivery continues to grow in importance. The reasons are clear:

- Therapeutics delivered via liposomes may be protected from the actions of metabolizing enzymes
- Lipophilic substances may be made soluble by the use of liposomes
- Therapeutics can be targeted to specific areas by attaching specific ligands to the liposome
- Liposomes are readily absorbed by cells
- The rate of release may be controlled by the selection of liposome
- Using liposomes as a delivery vehicle allows potentially lower or less frequent doses to be used, potentially reducing toxicity and side-effects
- Liposomes can carry biological substances such as proteins and DNA

The size of the liposomes used is increasingly being recognized as an important factor in treatment efficacy. A drug delivery liposome's size may affect its circulation and residence time in the blood, the efficacy of the targeting, its rate of cell absorption (or endocytosis) and, ultimately, the successful release of its payload. Such size considerations are hugely important to all nanoscale drug delivery systems.

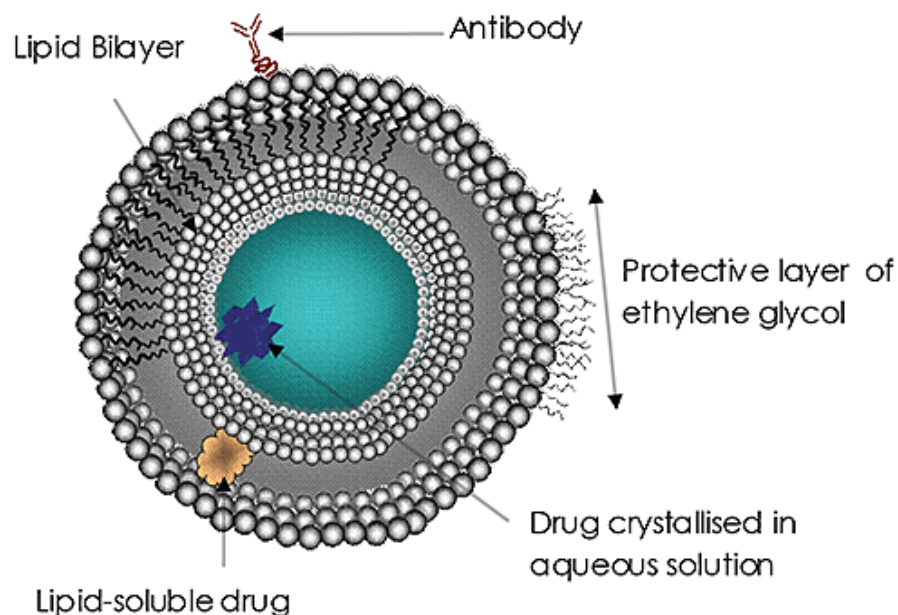


Figure 1. Typical Liposome Structure

Sizing liposomes with NTA

Malvern's NanoSight instrument range accurately and rapidly sizes liposomes and measures their concentration in water and other solvents. Only small volumes and very little sample preparation is required. The instruments enable individual liposomes in suspension to be visualized and their Brownian motion tracked – enabling particle size distributions, based on individual particles, to be built up in a matter of seconds.

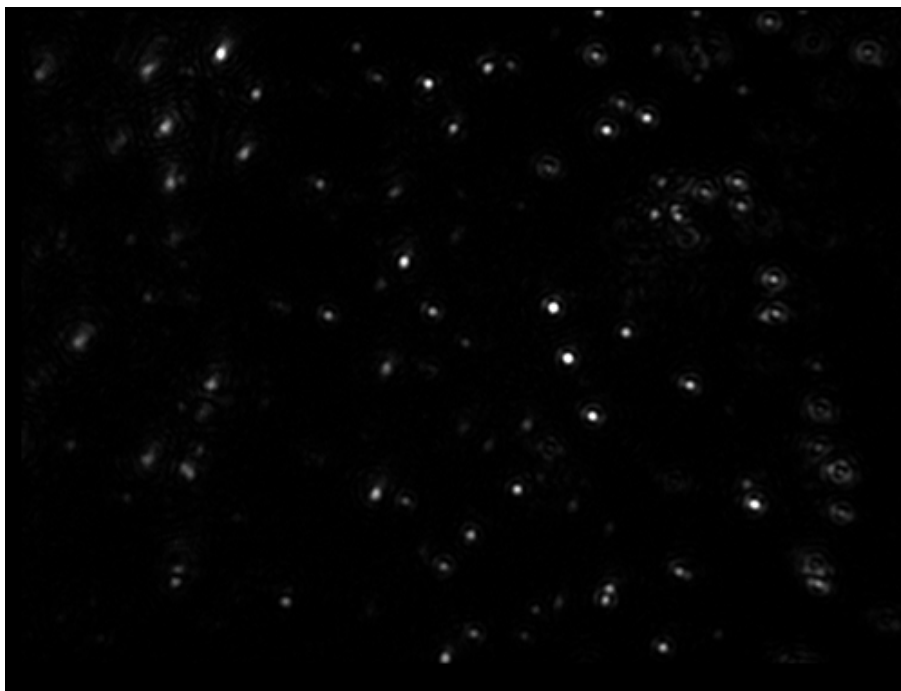


Figure 2. Typical image of liposomes provided by a Malvern NanoSight instrument

Simultaneous multi-parameter analysis of nanoparticles in real-time

In addition to size and concentration, NTA also provides the following parameters, simultaneously and particle-by-particle:

- Scatter intensity, which resolves adjacent populations of particles and differentiates materials of sufficiently-differing refractive indices.
- This unique ability potentially allows the user to probe whether nanoscale drug delivery structures such as liposomes vary in their contents, i.e. empty liposomes may have a lower refractive index (light scattering power) than those loaded with a higher refractive index material. This would allow them to be differentiated even though they may be of very similar sizes.
- A fluorescence detection capability allows differentiation of suitably labeled particles from complex backgrounds. NanoSight's fluorescent capabilities are described in a further application note.
- NTA has been used to investigate the effect of serum on the stability of different liposome structures in research into photodynamic cancer therapy (Reshetov et al., *Photochem Photobiol.* 2012 Sep-Oct;88(5):1256-64. doi: 10.1111/j.1751-1097.2012.01176.x).

Use of NTA with other drug delivery systems

Poly(β -amino ester)s (PBAEs) are potential delivery systems for genetic therapies to treat various cancers. They have an advantage over some other systems in that many combinations of polymer with DNA can be made through the combinatorial route. They also have rapid release properties due to hydrolytic degradation, but this creates challenges for dosing, production and storage. Lyophilization is a typical storage method and NTA has been utilized to evaluate the effect of lyophilisation on

aggregation (increase in size) and destruction (decrease in size) of PBAE-DNA nanoparticles (Tzeng et al. 2011 and Sunshine et al. 2012).

Poly(lactic-co-glycolic acid) (PLGA) is an FDA-approved drug delivery system. It breaks down into lactic acid and glycolic acid, both of which are endpoints of metabolic pathways in the body. PLGA has been used as a drug delivery system for amoxicillin and also for gonadotropin-releasing hormone for the treatment of advanced prostate cancer. The immunosuppressant mycophenolic acid has been encapsulated in PLGA with a view to decrease dosing levels and thus reduce toxic side effects. NTA was used to determine the size of these nanoparticles, a critical parameter to ensure good delivery and allow researchers to compare results across studies (Shirali et al. 2011).

The successful transport of molecules across the cell membrane is a key point in their delivery. In many cases, molecules alone cannot penetrate the cell membrane, therefore an efficient carrier is needed. Sokolova et al. (2012) have investigated calcium phosphate nanoparticles (diameter: 100 nm - 250 nm, depending on the functionalization) as versatile carriers for small and large molecules across cell membranes using a number of techniques including NTA, Dynamic Light Scattering (DLS) and Electron Microscopy (EM).

Ohlsson et al. (2012) reported on solute transport in the sub-100 millisecond timescale across the lipid bilayer membrane of individual proteoliposomes using NTA to check liposome stability and integrity.

In research on nanoparticles as gene delivery vehicles, Ghonaim and his co-workers have reported extensively on the use of NTA in their work on the effect of modifications to the chemistry of lipopolyamines and spermines in various non-viral plasmid DNA and siRNA delivery systems (Ghonaim et al., 2007a; Ghonaim et al., 2007b; Ghonaim et al., 2007c; Ghonaim, 2008; Ghonaim et al., 2009; Soltan et al., 2009; Ghonaim et al., 2010). Similarly, Ofek et al. (2010) have employed NTA for the characterization of dendritic nanocarriers for siRNA delivery while Bhise measured particle size and size distribution by NTA in their study of gene delivery polymers in cell culture (Bhise et al., 2010). Bhise further extended this work to develop an assay for quantifying the number of plasmids encapsulated by polymer nanoparticles, in which he used NTA to determine the number density of plasmids per 100 nm nanoparticle (Bhise et al., 2011)

Wei et al. (2012), in exploring the challenges and opportunities in the advancement of nanomedicines, identified the need for robust methods for the accurate characterization of nanoparticle size, shape and composition, as well as particle engineering to maintain low levels of nonspecific cytotoxicity and to increase stability during storage.

Other examples of the importance of sizing and enumerating nanoparticulate drug delivery systems by NTA have been reported (Hsu et al., 2010; Park et al., 2010; Tagalakakis et al., 2010).

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Malvern Instruments Limited
Groewood Road, Malvern,
Worcestershire, UK. WR14
1XZ

Tel: +44 1684 892456
Fax: +44 1684 892789
www.malvern.com

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