Patrick HOLE[a], **Pierre PEOTTA**[a,b]*, **Bob CARR**[a]
[a] *Malvern Instrument, NanoSight, Minton Park, Amesbury, UK - Tel: +41 1980 676060*
[b] *Malvern Instrument, 30 rue J. Rostand, Orsay, FRANCE - Tel: +33 1 69 35 18 00 – pierre.peotta@malvern.com*

NANOSIGHT

Assessment of the efficiency of encapsulation of a fluorescent drug using NanoSight's NTA (Nanoparticle Tracking Analysis)

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. When considering a nanomaterial drug delivery system, size is clearly a key parameter as it directly influences the processes of delivery, uptake, degradation and clearance from the body.

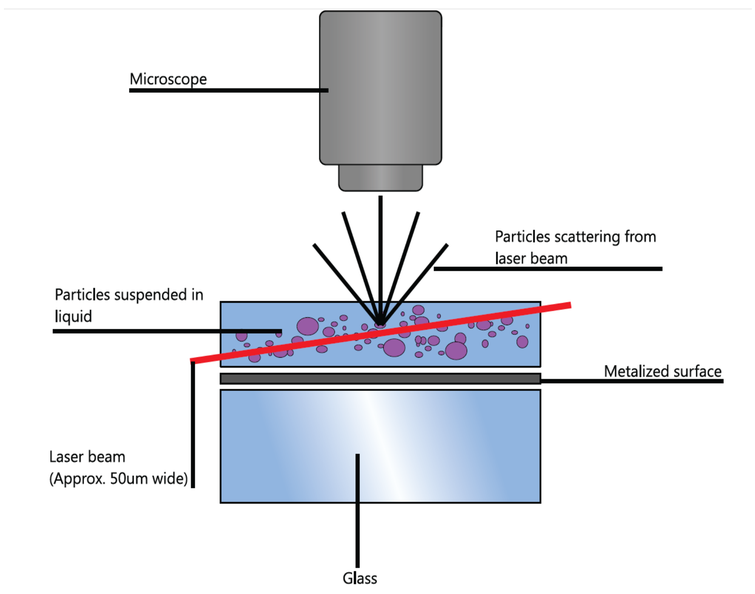
However, it is also imperative to have an accurate measurement of the number based concentration of nano-objects and more importantly the concentration of nano-objects loaded with the drug of interest.

This presentation outlines an example where the methodology of Nanoparticle Tracking Analysis (NTA) is used to characterise nanoparticles for drug delivery purposes. Complementary to classical light scattering techniques, NTA allows nanoparticles as small as 30nm to be sized on a particle-by-particle basis, enabling high resolution profile. On the same analysis, NTA also delivers concentration measurement through a direct count (particles per ml), helping the understanding of aggregation or other particle behaviour in complex systems. Finally, a fluorescence mode allows differentiation of suitably labelled particles.

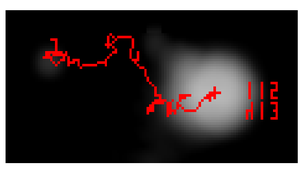
Introduction

Traditional size analysis techniques for nanoparticles have significant limitations, which affect their ability to provide complete information on the true size distribution of a sample. In comparison, NTA has the ability to provide characterisation for bio-nanoparticles (including **viruses, VLPs, protein aggregates, exosomes, and liposomes and other drug delivery nanoparticles**) without significant bias due to inclusion of small numbers of aggregates or sample preparation requirements.

A laser is used to pass a finely focused beam through a sample chamber containing nanoparticles in liquid suspension.



The light scattered by the particles is collected using optical microscopy components, allowing a direct visualisation of their Brownian Motion. A video file of this movement is captured and the NTA software tracks the individual particle movement upon video analysis.



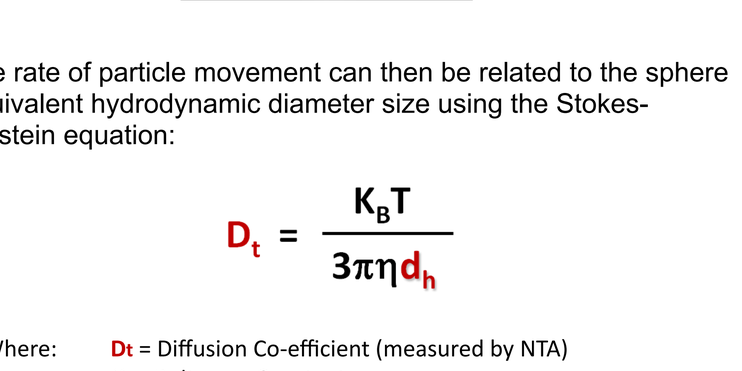
The rate of particle movement can then be related to the sphere equivalent hydrodynamic diameter size using the Stokes-Einstein equation:

$$D_t = \frac{K_B T}{3\pi\eta d_h}$$

Where: **Dt** = Diffusion Co-efficient (measured by NTA)
K_B = Boltzman Constant
T = Temperature
η = Viscocity
dh = Sphere equivalent hydrodynamic diameter particle size

Fluorescence capabilities

NTA only uses light scattering properties to detect, visualise and track nanoparticles. However, it is possible to go further into the characterisation, by using a particle labelled with a fluorophore.



By fitting the device with the appropriate laser (matching the excitation wavelength of the fluorophore) and filter (matching the emission of the fluorophore), it is possible to visualise and characterise the fluorescent particles only.
(for further information, please refer to the Fluorescence Technical Note)

Available Laser Wavelength	405 nm	488 nm	532 nm	635 nm
Standard Filter Supplied (long-pass)	430 nm	500 nm	565 nm	650 nm

Material & Methods

Polylactic Acid particles (provided by Adjuvatis) have been designed and loaded with a drug of interest conjugated to a fluorescent label (Coumarin6).

The purpose of this experience is to evaluate the efficiency of encapsulation of this drug into the PLA nanoparticles.

The device used was an LM10 HSBF comprising a sCMOS camera, 405nm laser and 430nm long-pass filter. Analysis has been performed under NTA software 2.3.5 (Nanoparticle Tracking Analysis). The sample has been diluted 100x.

Results

In the sample, a mixture of labelled and non labelled particles is expected. Thanks to its ability to track, count and measure nanoparticles in liquids, NTA analyses this preparation both under a scatter mode (Fig.1) and a fluorescence mode (Fig.2).

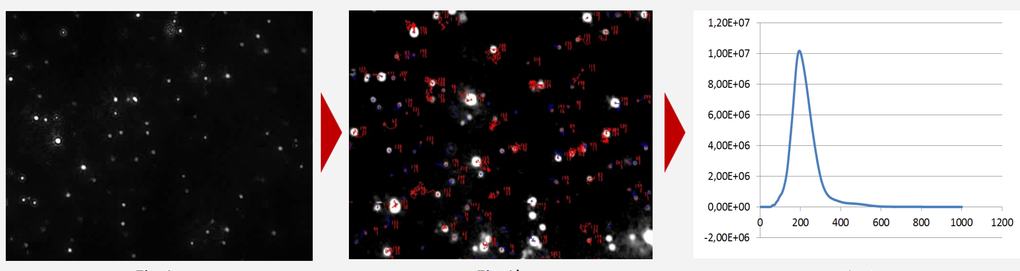


Fig.1a Fig.1b Fig.1c

Mean: 214 nm
Mode: 199 nm
Measured concentration: 1.23e+009 particles/ml
Dilution factor: 100 times

Final concentration : 1.23e+011 particles/ml

Table 1

Fig.1. Measurement of the size and concentration under scatter mode (all particles)

Fig 1a displays footage of the sample under scatter mode (size is approx 100*80µm). The particles moving under brownian motion are tracked and counted by NTA 2.3.5 software (Fig.2b). From this tracking, NTA calculates for each particle the Diffusion Coefficient and gives a high resolution size distribution profile (Fig.1c) (x axis = diameter in nm, y axis = concentration in particles per mL) - Table 1 gives the relevant size distribution information

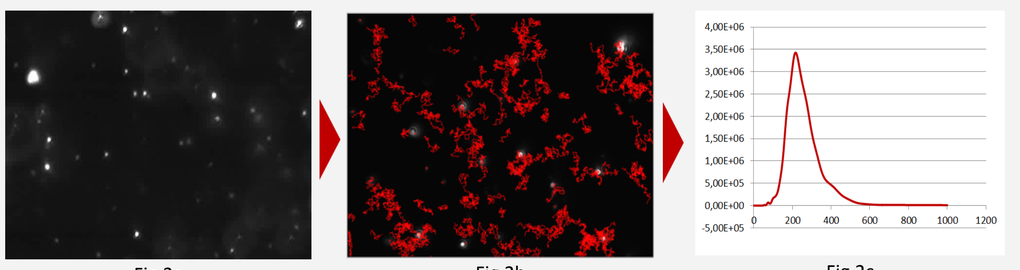


Fig.2a Fig.2b Fig.2c

Mean: 259 nm
Mode: 223 nm
Measured concentration: 5.31e+008 particles/ml
Dilution factor: 100 times

Final concentration : 5.31e+010 particles/ml

Table 2

Fig.2. Measurement of the size and concentration under fluorescence mode (drug containing particles) with the use of a 430nm long-pass filter

Fig 2a displays footage of the sample under fluorescence mode (size is approx 100*80µm). With the use of the 430nm long-pass filter, only the labelled particles are tracked and counted (Fig.2b). Using the same basis (Stokes-Einstein equation), the size is derivated from the Diffusion Coefficient. The size distribution profile is displayed on Fig.2c (x axis = diameter in nm, y axis = concentration in particles per mL) with software information on Table 2

Discussion & Conclusion

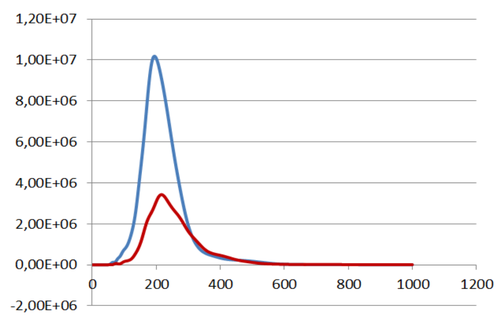


Fig.3. Comparison of labelled (red line) and non labelled (blue line) particle concentrations
(x axis = diameter in nm, y axis = concentration in particles per mL)

Under the scatter mode (no filter), the analysis of the sample (Fig.3 blue line), shows a modal distribution at 199nm with a final concentration at 1.23e+011 particles/ml.

Under the fluorescence mode (filter inserted), the distribution of the labelled population (Fig.3 red line) shows a slightly bigger mode at 223nm with a final concentration dropping at 5.31e+010 particles/ml.

Such data allows a fast evaluation of the amount of particles labelled amongst the total population (approx 43%) and as a result the efficiency of encapsulation of this drug into polylactic acid particles

> NanoSight provides an attractive alternative to more complex methods such as Dynamic Light Scattering or Electron Microscopy

> Real time capture provides high resolution size distributions and number concentration measurements to reveal the true nature of the sample, revealing both qualitative and quantitative information

> Fluorescence capabilities, serve as an alternative option to obtaining data on labelled subpopulations

> Minimal sample preparation is required save dilution of the sample to a concentration between 10^7 and 10^9 particles/mL.

References

Bhise NS, Gray RS, Sunshine JC, Htet S, Ewald AJ and Green JJ (2010) The relationship between terminal functionalization and molecular weight of a gene delivery polymer and transfection efficacy in mammary epithelial 2-D cultures and 3-D organotypic cultures, Biomaterials, doi:10.1016/j.biomaterials.2010.07.023

Bhise NS, Shmueli RB, Gonzalez J and Green JJ (2011), A Novel Assay for Quantifying the Number of Plasmids Encapsulated by Polymer Nanoparticles. Small. doi: 10.1002/smll.201101718

Ghonaim HM, (2008) Design and Development of Pharmaceutical Dosage Forms for Gene and siRNA Delivery, PhD Thesis University of Bath, Department of Pharmacy and Pharmacology, September 2008

Ghonaim HM, Li S and Blagbrough IS (2010) N1,N12-Diacyl Spermines: SAR Studies on Non-viral Lipopolyamine Vectors for Plasmid DNA and siRNA Formulation Pharmaceutical Research, Vol 27, (1) p17-29

Ghonaim HM, Li S, Soltan MK, Pourzand C and Blagbrough IS (2007a), Chain Length Modulation in Symmetrical Lipopolyamines and the effect on Nanoparticle Formulations for Gene Delivery, in British Pharmaceutical Conference BPC2007, Manchester, 10th Sept.

Ghonaim HM, Li S, Pourzand C and Blagbrough IS (2007b), Efficient Nove Unsymmetrical Lipopolyamine Formulations for Gene Delivery, in British Pharmaceutical Conference BPC2007, Manchester, 10th Sept.