Characterisation of nanoparticle drug delivery carriers

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ABSTRACT

As the number of small molecule drugs coming through the pipeline continue to diminish and the established large blockbusters continue to come off patent there is a desire within the pharmaceutical sector to look for alternative ways in which they can drive revenues. This in turn is creating a drive for the use of nanotechnology in improving the effectiveness of drug delivery.

Critical to the efficacy of drug delivery vehicles is a consistency in their manufacture and drug loading. This in turn puts a demand on improvements in manufacturing processes and the associated characterisation activity[1].

Within this context and background we review some of the latest development activity using light scattering techniques and separations devices aimed at addressing the critical characterisation requirements on drug delivery carriers.

Keywords: nanomaterials, drug delivery, dynamic light scattering, resonant mass measurement, nanoparticle tracking analysis.

1 INTRODUCTION

As the pharmaceutical industry matures, meeting their investor aggressive growth expectations becomes increasingly difficult. Many companies will continue to grow via merger and acquisition activity, and through internal development of high value compounds.

However we have started to enter the era within which blockbuster drugs are going off patent, which will result in an estimated loss of $30-$40 billion in annual product revenues as generic drugs enter the market. This change is accelerating the industry's constant need to "build the pipeline" of new compounds, and to rapidly enter new markets.

Although concerning for pharmaceutical developers, these trends are actually creating new challenges and opportunities in drug discovery. It is also a significant driver of change in Drug Delivery Systems (DDS), which are increasingly being used to alleviate some of the industry's concerns by extending product and patent lifecycles via reformulation of existing and/or orphaned compounds.

When you add in the massive amount of nanotechnology and pharmaceutical R&D funding now available worldwide, the drug delivery industry is evolving rapidly and is nearly unrecognizable from its humble beginnings in the 1960s. This $40-billion market has seen double-digit growth for the past two decades, and the industry's success has spawned intense competition. It is within this environment that product differentiation through development and/or manufacturing quality becomes even more critical [2].

2 NANOTECHNOLOGY BENEFITS

Before looking at the critical parameters that are important to monitor on drug delivery carriers, it would be useful to understand the benefits that nanoparticle carriers can offer in the arena.

An initial benefit of nanoparticlebased drug delivery systems is their ability to quickly affect a target site. This is due in part to novel encapsulation technologies, coupled with a potentially rapid dissolution rate in the human body. To help quantify the potential benefit, a 10-micron particle could have a surface area of 2-5 m2/g, whereas a 3-5nm nanoparticles may reach of 400-500 m2/g and beyond. This increased surface area allows for an improved dissolution of drug compounds in the body.

They can offer a greater targeting ability of drug products within the body. The increased dosing efficiency afforded by nano-enabled drug delivery systems may lower the overall need for a drug, potentially lowering costs and undesirable effects in the human body. By way of example, ALZA has developed a unique lipid nanoparticle delivery system with a polyethylene glycol (PEG) coating, dubbed Stealth®. This technology has demonstrated an ability to evade certain immune system responses, enabling precise delivery of drugs to targeted areas. Ortho Biotech Products' Doxil® is the first marketed product to incorporate this technology, for treatment of ovarian cancer. Other methods involve the use of external magnetic fields to accurately deliver coated magnetic nanoparticles.

Nanotechnology can also offer consumer-friendly end products. Perhaps most important benefit of nanotechnology is that it may make life easier for - the driving force behind the pharmaceutical industry - the customer. Nano-enabled drug delivery methods may

present an answer to the ever-present demand for increased user-friendliness and convenience.

2.1 Types of nanoaprticles in drug delivery

Nanoparticles can consist of a number of materials, including polymers, metals, and ceramics. Based on their manufacturing methods and materials used, these particles can adopt diverse shapes and sizes with distinct properties.

Many types of nanoparticles are under various stages of development as drug delivery systems, including liposomes and other lipid-based carriers (such as lipid emulsions and lipid-drug complexes), polymerdrug conjugates, polymer microspheres, micelles, and various ligand-targeted products (such as immunoconjugates) [3], [4].

3 DRUG DELIVERY NEEDS

3.1 Characterisation of nanoparticles

As discussed drug delivery carriers can come in a variety of guises, however they all require good characterisation. A good physicochemical understanding of the (nanoparticle) formulation is an absolute necessity for rational formulation design and properly interpreting in vivo results. Size, surface characteristics, particle morphology, structure, drug loading and drug release are all relevant topics within the characterisation needs. Within the context of this paper we will focus on size and drug loading.

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 nanoparticle system at hand [5].

3.2 Dynamic Light Scattering

The variation with time of the scattered laser light intensity at some defined angle by a collective of particles, dispersed in air or in a transparent liquid, is measured. The rate of change of this intensity is related to the diffusion coefficient of the particles, which in turn is related to particle size by the Stokes-Einstein equation. Several mathematical methods are used for conversion of the intensity-time relationship to a PSD. The fact that this conversion is ill-conditioned and has a poor signal-to-noise ratio limits the amount of PSD information that can be obtained. Thus, usually the simple “cumulants” method is favored for data analysis; it leads only to a mean size and a value for PSD width. Other methods claim to lead to a PSD, but often results are not stable. There are two versions for measurement. The conventional technique operates usually at an angle of 90°, or another specified angle, at very low concentration. New techniques, such as fiber-optics quasi-elastic light scattering (FOQELS) and diffusive wave spectroscopy, use back-scattered light and may operate at higher concentration. Then, particle-particle interactions often influence the particle movement and, thus, the sizing result.

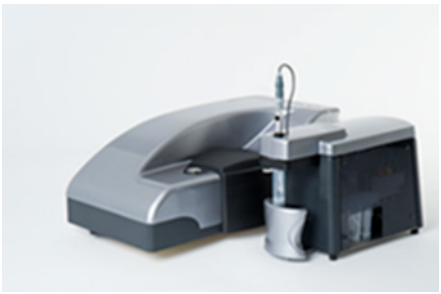

Fig.1 Zetasizer (DLS) product with titrator

3.3 Field Flow Fractionation

Asymmetrical flow FFF is frequently used in the high-resolution separation of nanoparticles and proteins. Laminar flow of the eluent through a thin separation channel results in a parabolic flow velocity. For asymmetrical flow FFF, the field is generated by pumping eluent through a permeable lower plate, which is covered by an ultrafiltration membrane.

This applied field-flow (cross-flow) is perpendicular to the channel flow, driving injected particles toward the membrane (accumulation wall). In a typical separation scheme, particles are constantly driven toward the accumulation wall by the cross-flow while thermal motion of the particles (i.e., Brownian motion) counteracts the field. As elution proceeds, smaller particles assume a higher mean steady-state distance from the wall and thus elute at a faster rate. In contrast, larger particles (with lower diffusion coefficients) occupy regions closer to the accumulation wall and show greater retention. The eluting particles may be conveniently detected by DLS, which is a powerful means to assess the size of the individual eluting particles.

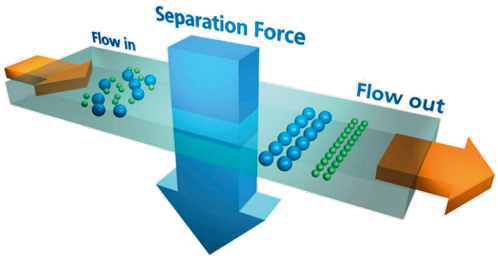

Fig.2 FFF Schematic

3.4 Nanoparticle Tracking Analysis

NTA (Nanoparticle Tracking Analysis) products derive the hydrodynamic size and under some circumstances other parameters (concentration, zeta potential and speciation) of particles in the size range from 10-2000 nanometres. This instrument uses a microscope to observe the light scattered by individual particles suspended in an optical cell and illuminated with a laser and then tracking their diffusion under Brownian motion.

The light scattered by the particles is captured using a scientific digital camera and the motion of each particle is tracked from frame to frame. This rate of particle movement (diffusion coefficient) is related to a sphere with equivalent hydrodynamic radius as calculated through the Stokes-Einstein equation. This is exactly the same approach which is used to calculate size from the diffusion coefficient measured by Dynamic Light Scattering (DLS). However, as NTA calculates particle size on a particle-by particle basis it can offer advantages over DLS, for instance in terms of size resolution and the ability to provide absolute concentrations. Also, since video clips form the basis of the analysis, accurate characterisation of real-time events such as aggregation and dissolution is possible. One other major advantage of NTA technology over DLS is the ability to discriminate between different populations even when the size is equivalent. This may simply be achieved by monitoring the intensity of light scattered by the different populations or through the use of fluorescent labelling of the different entities combined with different wavelength lasers and optical filters.

Fig.3 NS300 – NTA type unit

3.5 Resonant Mass Measurement

RMM (Resonant Mass Measurement) is a technique which measures the mass buoyancy of particles to provide information on the size of nanoparticles. At the heart of the instrument is a MEMS (Micro Electronic Mechanical System) sensor within which there is an embedded microfluidic channel. A suspension containing particles of interest is pumped through the microfluidic channel. One by one, particles are introduced to the cantilever structure within the sensor, causing the cantilever's resonant frequency to alter by an amount directly proportional to the particle's buoyant mass in its suspending fluid.

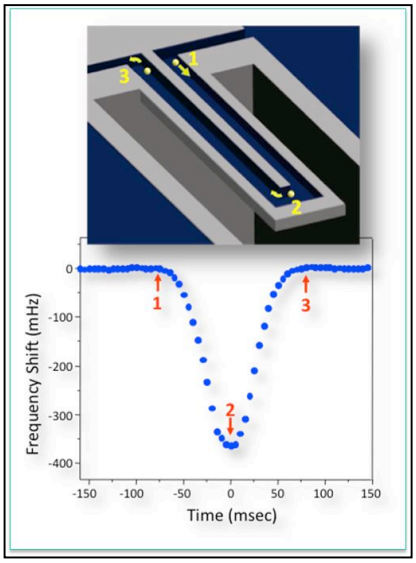

Fig.4 Archimedes MEMS schematic

Fig. 5 shows a schematic of the MEMS sensor at the heart of the Archimedes system indicating the flow of a particle through the channel. As the particle flows through the channel (1 to 3), the oscillating frequncy of the MEMS sensor changes which is directly related to the mass of the particle which can be converted to a size by knowing the density of the particles under study. The technique can distinguish between nanoparticles which are denser than the

fluid in the cantilver and cuase the frequency to shift down and potential air bubbles which are lighter than the fluid and cause the frequency of the cantilver to shift up.

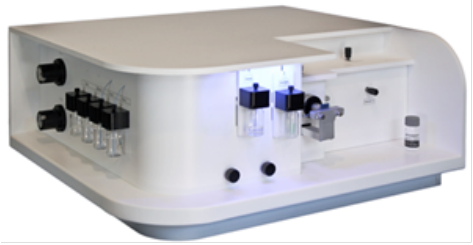

Fig.5 Archimedes system

The system can come with different MEMS sensors which have different size channels embedded in them which allows the system to cater for different particle size ranges. The system has been shown to be both accurate, measuring to within 1% accuracy on NIST traceable standards and also offer high resolution with an ability

4 CONCLUSION

The success of a new developed pharmaceutical formulation is related to the fact that it is able to deliver the active substance to the target organ at therapeutically relevant levels, with negligible discomfort and side effects, increasing the patient compliance to the therapeutics.

Regarding this respect, the route of administration is of major relevance. Topical administration of active substances offers several attractions compared to traditional routes, e.g. it avoids the hepatic first-pass metabolism, it has the potential of long-term controlled release with avoidance of the topical peak-through plasma profiles associated with frequent dosage regiments, Several commercial opportunities exist at the intersection of nanotechnology and traditional pharmaceutical R&D.

Though several companies are generating revenue today, a large degree of uncertainty remains on exactly what affects the industry will see over time. Nanotechnology is rapidly emerging as an answer to pharmaceutical industry formulation challenges, including;

- Solubility enhancements
- Reduction of R&D and manufacturing costs
- Quicker time-to-market (TTM) for new drug candidates
- Greater targeting ability that may allow for lower dosing requirements, potentially lessened side effects, and perhaps an answer to the ever-present customer demand for increased user-friendliness and convenience.

To achieve this requires suitable manaufacturing protocols and characterisation techniques which can offer insight on the size and drug loading of nanoparticle carriers. There are a range of orthogonal techniques that can assist in this arena, however the technology of Resonant Mass Measurement is somewhat unique in its' ability to offer insight into the mass of drug within or coated on drug delivery carriers.

Technique	Size range	Resolution	Speed of analysis	Particle number quantification?
DLS	1 nm to 1 µm	Moderate	Very fast	Calculated from intensity
DLS + Separation (e.g. FFF or SEC)	1 nm to 1 µm	Very good	Slow	Calculated from intensity with improved resolution. Concentration detector option
Nanoparticle tracking analysis (NTA)	30 nm to 1 µm	Good	Fast	Semi quantitative
Resonant mass measurement (RMM)	50 nm to 1 µm or 300 nm to 5 µm	Good	Slow	Quantitative

Fig.6 Comparison of high resolution techniques

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