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Single particle extinction and scattering optical method unveils in real time the influence of the blood components on polymeric nanoparticles

Tiziano Sanvito, PhD[a,*], Paolo Bigini[b], Maria V. Cavanna[c], Fabio Fiordaliso[b], Martina B. Violatto[b], Laura Talamini[b], Mario Salmona[b], Paolo Milani[d,*], Marco A.C. Potenza[d,*]

[a]*EOS srl, Milano, Italy*
[b]*IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy.*
[c]*Fondazione Filarete, Milano, Italy*
[d]*CIMAINA and Dipartimento di Fisica, Università degli Studi di Milano, Milano, Italy*

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Abstract

Here we report the quantitative in situ characterization of size distribution evolution of polymeric nanoparticles incubated in murine serum, filtered and unfiltered murine blood. We used an analytical optical approach, named Single Particle Extinction and Scattering (SPES), which relies on the measurements of two independent parameters of single particles. SPES is based on a robust self-reference interference optical scheme which allows a rejection of the spurious signals coming from the background caused by the medium. We employed polystyrene nanoparticles as reference system and polydisperse poly(lactic-co-glycolic acid) nanoparticles. Our results demonstrate that SPES can be used for carrying out ex vivo analysis of nanoparticles to evaluate the modifications that NPs undergo in vivo following different routes of entry. Conversely, Dynamic Light Scattering is not able to provide reliable results for these systems due to the presence of the biological components in solution.

Key words: Polymeric nanoparticles; Light scattering; Blood-nanoparticle interaction; Protein corona; In line characterization

Drug carriers based on nanoparticles (NPs) are receiving a considerable attention as effective tools for the modulation of both the pharmacokinetic and pharmacodynamic profiles of drugs.[1] Organic and inorganic NPs are actively fabricated and characterized to assess their capability of increasing drug stability in vivo, and of controlling the drug biodistribution in order to enable effective targeted therapies.[2]

The transport of a drug by a carrier may highly impact its therapeutic efficacy for a variety of reasons,[3] such as: i) the physical protection of the compound (for example peptides, antibodies or MiRNAs that undergo to a sudden degradation after administration); ii) the increased half-life in the bloodstream and the slower clearance by renal filtration; iii) the tropism toward target organs, due to the functionalization of the carrier with specific ligands, and therefore the reduction of side-effects and the increase of the therapeutic index; iv) the non-invasive tracking through the link with contrast agents.[4–10]

One of the major points of weakness for the therapeutic use of NPs resides on the lack of knowledge about the behavior of a nanocarrier after reaching the bloodstream.[11] The interaction between biodegradable nanocarrier and biological fluids should be done before the studies of kinetics, efficacy and toxicity. Nanocarriers that reach the systemic circulation undergo to a variety of interactions with blood constituents and cells that may significantly modify their original physico-chemical features.[12–14]

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*Corresponding authors.
E-mail addresses: tiziano.sanvito@eosinstruments.com (T. Sanvito), paolo.milani@mi.infn.it (P. Milani), marco.potenza@unimi.it (M.A.C. Potenza).

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For this reason, understanding the nature of these interactions is crucial to predict their efficacy or toxicity.[11]

Although the characterization of nanoparticle-based carriers in biologically-relevant conditions is necessary for the assessment of their efficacy, this is usually carried out by ex situ tools such as atomic force microscopy (AFM) and transmission electron microscopy (TEM) that require dried samples.[15,16] TEM or AFM analysis can provide useful information on the shape and size distribution of NPs; however, these imaging methods are expensive, labor intensive, and unsuitable for large-scale and high-throughput analysis.[17] Most importantly, the above-mentioned techniques cannot follow dynamically the interaction of NPs with the biological components of different blood fractions.

An alternative to ex situ characterization methods is the dynamic light scattering (DLS). This is an indirect method based on the measurement of the temporal fluctuations of the intensity of light scattered by a suspension.[18–20] DLS is widely and routinely used to characterize nanoparticles in suspension, although these determinations are carried out in distilled water or buffered saline solutions[15]; in more complex fluids such as serum or blood, its reliability is reduced by plasma proteins or cell debris[21–23]; therefore that it cannot be considered a valid technique for the characterization of NPs in biological fluids.

Here we demonstrate the possibility of the direct in situ characterization of polymeric nanoparticles following incubation in murine serum, filtered and unfiltered murine blood by reporting quantitatively the in situ evolution of NPs size distribution during the incubation by the use of an optical in line analytical approach called Single Particle Extinction and Scattering (SPES).[24,25] SPES relies on the measurements of two independent optical properties of single particles, and it is based on a robust self-reference interference optical scheme, which allows a continuous self-calibration and rejection of spurious signals from the background caused by the medium.[14]

We used SPES to characterize polydisperse poly(lactic-co-glycolic acid) (PLGA) nanoparticles as a typical drug delivery model with clinical relevance due to their biocompatibility and the possibility to finely tune their size and drug payloads.[26] We also used monodisperse polystyrene NPs as a calibration system.

Methods

Materials

We used Polystyrene (PS) nanoparticles typically employed for instrument calibration and validation (diameter 430 nm, Thermo Scientific 5043A, polydispersity <3% CV).[27] PLGA nanoparticles were synthesized by Oil-in-Water (OW) solvent evaporation emulsion techniques. Synthesis and characterization of these particles have been described in detail in Potenza et al.[26]

Biological fluids

For biological fluid collection, 3-month-old male NFR mice were used. Animals were bred and maintained under specific pathogen-free conditions at the Mario Negri Animal Care Facility; they received food and water ad libitum and were regularly checked by a certified veterinarian who is responsible for animal welfare supervision and experimental protocol revision. Experiments involving mice and their care were conducted in conformity with the institutional guidelines at the IRCCS–Institute for Pharmacological Research "Mario Negri" in compliance with national (Legislative Decree n. 26, March 4, 2014; Authorization n.19/2008-A issued March 6, 2008, by the Italian Ministry of Health) and international laws and policies (EEC Council Directive 2010/63, August 6, 2013; Standards for the Care and Use of Laboratory Animals, U.S. National Research Council, Statement of Compliance A5023-01, October 28, 2008). This work was reviewed by IRCCS-IRFMN Animal Care and Use Committee (IACUC) and then approved by the Italian "Istituto Superiore di Sanità" (code: 42/2016-PR).

Healthy mice were sacrificed by cervical dislocation and blood was collected from the retro-orbital plexus. A small volume of blood was added in EDTA (0.5 M, pH 8) pre-treated tubes and either frozen or filtered with Minisart® NML syringe filters, with pore size 1.20 μm (Sartorius) to remove the cellular components. The remaining part of the blood was stored in tubes without EDTA and, after an interval of 30 min at room temperature, centrifuged at 4000 ×*g* for 15 min. The supernatant fraction was then collected and stored in cleaned Eppendorf tubes to obtain the murine serum. All samples were stored at −20 °C until analysis.

Nanoparticle characterization

The Single Particle Extinction and Scattering (SPES) optical method relies on a novel approach to light scattering characterization,[24,25,28] providing single particle measurement of two independent parameters: the *polarizability* α and the optical *thickness* ρ. A two-dimensional plot can represent the result of the characterization of an assembly of particles by SPES where the abscissas and ordinates are the two parameters measured for each single particle. Abscissas indicate the amount of energy removed by the particle when crossing the light beam and are directly related to the volume of the particle (in arbitrary units).[24,25] Ordinates are representative of a specific optical property, i.e. the product $\rho = d\ (m - 1)$, where d is the particle diameter and m is the refractive index relative to the surrounding medium.[29] The refractive index is ultimately related to the degree of compactness of each particle that represents a unique piece of information for saying apart particles with similar hydrodynamic radius but with different compositions.[26]

SPES raw data, obtained with a prototype commercial instrument (EOS Classizer™ One) are reported in two-dimensional plots, ρ versus α, defined above[17]: the plots are histograms where the greytone indicates the number of particles detected within each two-dimensional bin, normalized to the two-dimensional bin with the maximum event number. Due to the single particle approach, the time required for a statistically meaningful measurement (e.g. thousands of particles measured) with the SPES method depends on the numerical particle concentration and on the particle size distribution of the sample. Typically, for a monodisperse polystyrene sample with a numerical particle concentration of 10^6 particles per milliliter, few minutes are required to measure 1 ml of solution.