MONITORING THE FATE OF A LIPID/ZNO EMULSION IN ENVIRONMENTAL WATERS

INTRODUCTION

Characterization of particles with heterogeneous complex structure is an essential task in the formulation process of a cosmetical, cosmeceutical, and pharmaceutical products. Traditional technologies provide an average estimate of particle size and fail in detecting differences in particle composition or structure if no relevant differences in size distribution are present. The task is even more complicated in real heterogeneous systems, e.g., during stability study of particles dispersed in target biological or environmental fluids. In fact, particle-particle and particle-medium interaction with complex matrix can change particle characteristics, as well as their long-term behaviour.

In this application note, we take advantage of EOS Classizer™ ONE based on the innovative patented Single Particle Extinction and Scattering (SPES) optical technique. The unique ability of SPES to distinguish between particles of different composition exploiting their differences in optical properties opens new opportunities for particle stability characterizations in target context.

As reference material, we focus on Zinc Oxide (ZnO), that is very widely used as an additive in a variety of industrial application including plastics, ceramics, cement, lubricants, paints, adhesives, pigments, and foods. ZnO is encapsulated in an oil/lipid phase (ZnO-LP) and dispersed in aqueous phase. Empty lipid particles (LP) are studied as control. Each sample is suspended in water with different characteristics (ultrapure water, reservoir water, sea water, peatbog water). Analytical replicates are processed at determined timepoints up to 40 days of incubation.

Thanks to its unique properties, SPES provides sample information on peculiar behaviours in particle aging and degradation, even in environmental complex medium.

PARTICLE ANALYSIS METHOD

Among the several methods currently adopted, optical ones have unique advantages, and therefore, have brought light scattering into the forefront of analytical methods in many scientific and industrial applications. Unfortunately, the number of parameters typically affecting the scattering properties of a given particle is such that the basic measure of the scattering power (or even the power removal from a light beam -extinction- from one particle) is far from being enough to recover something more than a rough estimate of its size. Things change appreciably when considering a collection of many scatterers, with the immediate drawback of introducing the need for mathematical inversion and ill-posed problems to interpret experimental real data.

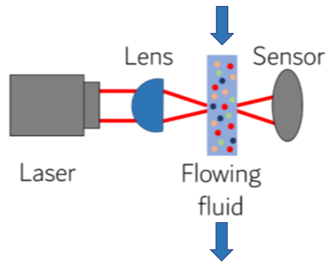
EOS Classizer™ ONE particle analyser is based on patented Single Particle Extinction and Scattering (SPES) method. It introduces a step forward in the way light scattering is exploited for single particle characterization.

Figure 1 EOS Classizer™ ONE – front view

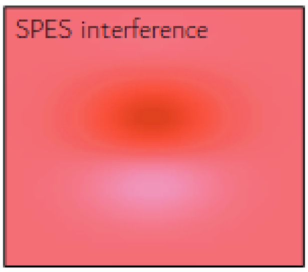
EOS Classizer™ ONE provides data that go beyond the traditionally optical approaches. EOS Classizer™ ONE discriminates, counts, and analyses single particles through their optical properties. It retrieves to the user several pieces of information such as: particle size distribution of the single observed populations, absolute and relative numerical concentrations, particle stability, information on optical particle structure and oversize. Classizer™ ONE works offline and online/real-time, enabling to verify consistency of intermediate and final formulations with target QbD, SbD, and Quality Control target expectations.

SPES TECHNOLOGY IN A NUTSHELL

The patented Single Particle Extinction and Scattering (SPES) method is based on a self-reference interferometric measurement of the scattered wavefront in the forward direction by a single illuminated particle.



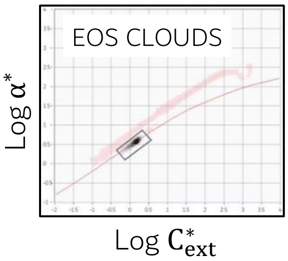
Particles are driven by a laminar fluid flow (liquid or gas depending on the application/CLASSIZER™ version) through the waist region of a tightly focused laser beam.



The intense transmitted beam interferes with the faint scattered wavefront in the far field, thus superimposing the two waves with the same curvature. This causes the interference pattern to exhibit intensity modulations on the spatial scale of the beam itself.

Two scattering features are sampled to follow the evolution of the intensity modulations during the passage of each single particle through the beam: i) the global attenuation given by the particle which removes a small fraction of the incoming power; ii) the fringes given by the partial constructive and destructive interference, proportional to the amplitude of the complex forward adimensional scattered field S(0). These features are directly related to the real $\Re e\, S(0)$ and the imaginary $\Im m\, S(0)$ components of S(0), as stems from the Optical Theorem [H. C. van de Hulst, Light Scattering by Small Particles, 1981].

The Extinction Cross Section $\Re e\, \mathbf{S(0)} = C^*_{ext} = \frac{k^2}{4\pi} C_{ext}$ and the Polarizability $\Im m\, \mathbf{S(0)} = \alpha^* = k^3\, \alpha$, where $k = 2\pi n/\lambda$ is the wave number in the medium n at wavelength λ, **are thus retrieved for each single detected, validated, and counted particle** thanks to a robust Pulse Shape Analysis scheme and proprietary algorithms, without adopting ill-posed problems, like the inversion or deconvolution (other optical parameters could be alternatively retrieved, eg. particle optical thickness ϱ).



In a few minutes SPES/Classizer™ creates the unique **EOS CLOUDS**: a 2D histogram which is the optical fingerprint of the sample. A heterogeneous sample produces simultaneously different clouds for each particle population, which can be individually selected, analyzed, and compared. Particle size distribution, numerical concentration, oversize, and other insights are retrieved accordingly to the selection, to the whole sample, or for each time frame acquired in CFA mode. Statistical approaches as PCA are furthermore viable to extract unique information typically inaccessible nowadays.

Added-value information is provided thanks to **SPES** and **EOS Classizer™ ONE** unique data and analysis libraries:

- **Optical Classification,** Absolute **Particle Size Distribution, Numerical Concentration** of each single population irrespectively of polydispersity/composition.

- Quality Control of particle **porosity, wetting**, **aspect ratio**, **payload**, **impurities**, **scraps**, and **shelf-life without intermediate steps** (purification/filtration).

- Measurement of **particle behavior and formulation stability** directly in real **heterogeneous non-filtered target biological, industrial, or environmental fluids**.

- Hi-Resolution **Continuous Flow Analysis**, also coupling SPES information with other analytical devices as cFFF separators, small chemical reactors, and pilot line.

- Statistical approaches as **Oversize Measure** and **PCA** for Hi-Quality Batch-2-Batch analysis and out-of-specifics identifications in product formulation and production.

Depending on the system configuration and sample, EOS Classizer™ ONE covers a dynamic range of 0.1 – 20 µm, concentration range of 1E5-1E7 ptc/mL @ 0.5-5ccm. External sample manager and autosampler are available.

This document presents representative examples of applications of EOS Classizer™ ONE and does not cover all the cases where the patented SPES method solves the particle identification, classification, and characterisation of challenges in heterogeneous samples and complex liquids. EOS software release SW1.4.42 is used for the data analysis and generation of the figures.

For a general introduction to SPES data with standard samples, as polystyrene spheres, please refer to the Application Note AN001/2021, available for free online at EOS website: www.eosinstruments.com/publications/

APPLICATION EXAMPLES

Materials and Sample Preparation

Commercial ZnO nanoparticles are encapsulated as emulsion in an oil phase (ZnO-LP) and dispersed in aqueous phase. Empty LP were studies as a control. Each sample is let acclimatize at room temperature and ultrasonicated for 2 minutes before proceeding with suspension in environmental waters at a final concentration of 100 mg/L.

Three different environmental waters are used to simulate different scenarios: reservoir water (representing neutral to alkaline environment), peat bog water (representing acidic environment), and sea water (representing high ionic strength environment). Ultra-pure water (MilliQ grade) is also considered as a reference control. All the water samples are filtered with a 0.2 µm filter before performing the experiment, to avoid the presence of larger particles and planktonic microorganisms, including bacteria.

To ensure a homogeneous suspension, ultrasounds are applied for 2 minutes. Samples were then kept in constant agitation in an orbital shaker at room temperature (50 rpm). At determined timepoints (0, 0.125, 1, 5, 10, 20, 40 days), each sample is gently shaken by hands, and an aliquot is taken for the SPES analysis. A selection of data from representative time points is here presented.

Before performing SPES analysis with EOS Classizer™ ONE, the sample aliquots are diluted with ultra-pure water (MilliQ) in a proportion 1 to 300 to ensure correct numerical concentration as required for the system in use. Analytical replicates are processed for each sample.

Results and Discussions

Two samples (ZnO-LP, LP) are analyzed in ultrapure water, to avoid spurious signals overcoming the environmental particles.

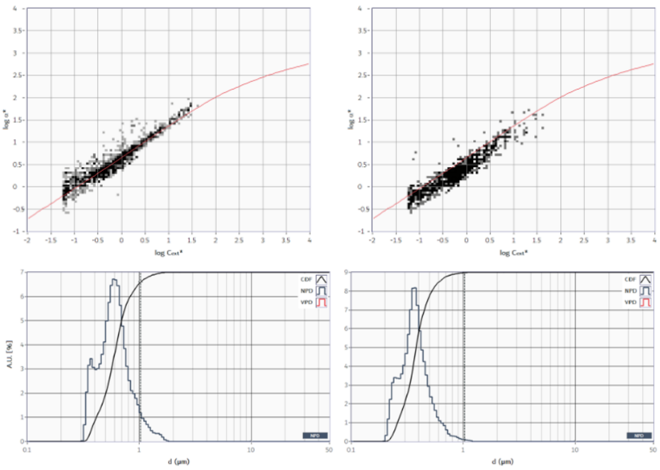
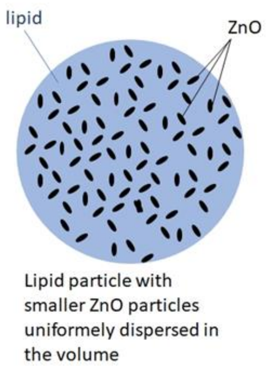


Figure 2 (top) 2D histogram for samples LP (left) and ZnO-LP (right) as suspended in ultrapure milliQ grade water, without incubation. Red lines correspond to theoretical SPES values for spherical dielectric particles with refractive index n = 1.43 @ λ640nm. (bottom) Numerical particle size distribution of LP (left, best n = 1.43) and ZnO-LP (right,best n = 1.57) particles.

As shown in Figure 2, SPES clearly classifies the two samples: due to their different physical nature and structure, they generate signals in different position of the 2D EOS CLOUDS histogram. LP signals correspond to an average refractive index n = 1.43, as expected from literature for a pure emulsion of homogeneous droplets of this material. ZnO-LP particles exploit an effective refractive index n = 1.57 which is an intermediate value between the refractive index of lipid material and the one expected for pure ZnO (n = 2.00). Modelling the particles as heterogeneous spherical structures with smaller ZnO particles uniformly distributed in a LN matrix, a rough evaluation of the particle structure is possible following the Lorenz-Mie approach and assuming the Mean-Field Approximation (MFA) (Chylek et al. 1988; Bohren and Huffman 2008). Data analysis provides a rough estimate of 20-30% w/w of ZnO encapsulated in LP matrix. More complex modelling, eg. via Discrete Dipole Approximation, may lead to more in depth insight on internal particle structure or external particle shape comparing [C_{ext}, α] experimental data with theoretical expected values for different data models.



The LP and ZnO-LP samples are thus dispersed in environmental waters and incubated at room temperature. Comparing the SPES data at zero time, no major differences in optical properties are observed for all the sample respect to milliQ water in Figure *2*. It must be also noted that Classizer™ ONE did not detect any relevant particle coming from environmental water. This can be ascribed to the filtration procedures. Sample behaviors are then analyzed over time. In Figure *3* a representation of the more interesting results and timepoints is provided for LP samples dispersed in the fresh and sea waters.

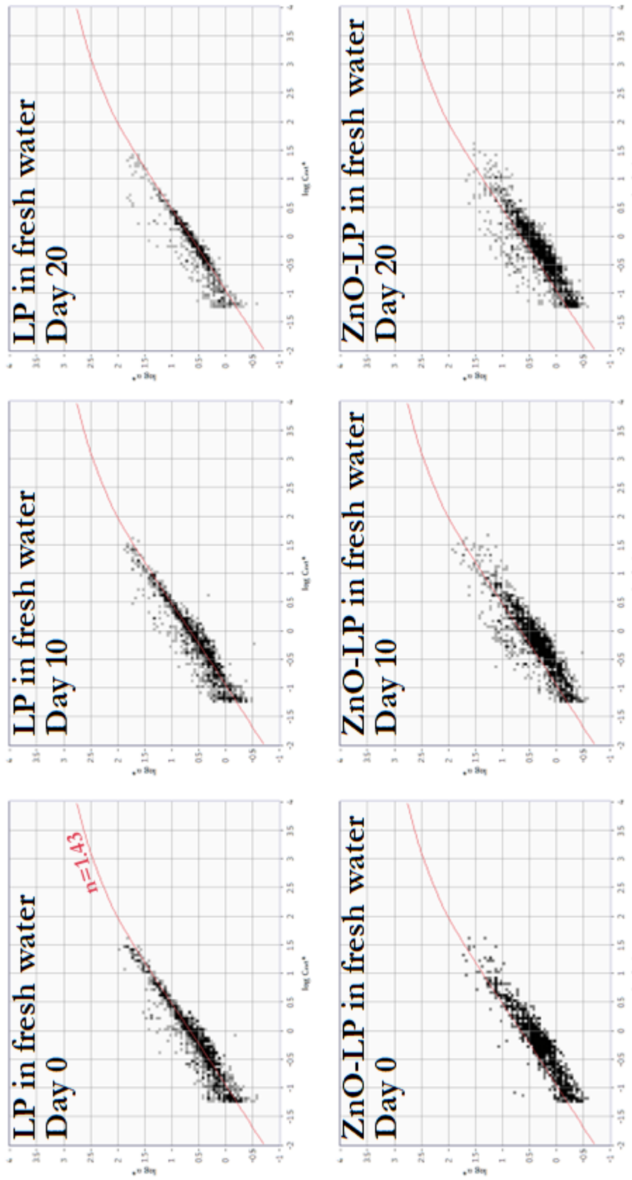


Figure 3 Evolution of LP and lipidic ZnO particles in fresh water at three time steps: day 0, day 10, day 20. Red line corresponds to the expected Mie curve for n = 1.43, reported here as a guide to the eye.

All the samples dispersed in fresh waters show high stability compatible with results in milliQ water. A slight but not significative decrease in the number of particles per milliliter is observed. This could be attributed to the interaction of the particles with the flasks used for the water incubation. No changes in the shape of the EOS CLOUDS allow to conclude that the samples optical properties, therefore both ZnO-LP and LP particles, are stable in these environments over the whole observation.

On the other side, as clear visible in Figure 4, ZnO-LP and LP samples dispersed in sea water show a clear change of their optical properties, therefore stability, over time.

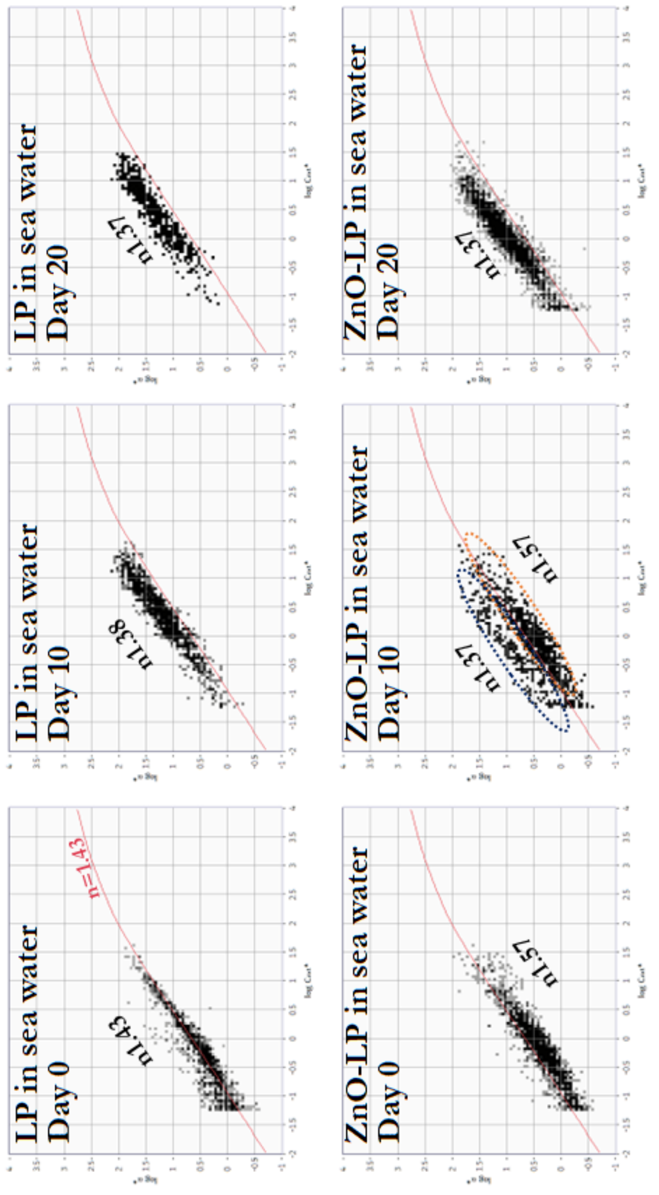


Figure 4 Evolution of LP and lipidic ZnO particles in sea water at three time steps: day 0, day 10, day 20. Red line corresponds to the expected Mie curve for n = 1.43, reported here as a guide to the eye.

At day 10, LP sample showed a completely different data distribution in the 2D EOS CLOUDS histogram corresponding to a reduction in the average refractive index (average from 1.57 at day 0 to 1.37 at day 10) and a sharp increase in particle size distribution (mode from 0.62µm at day 0 to 1.20µm at day 10). A similar distribution was observed at day 20, with a slight reduction in particle concentration (ranging from 1.1E6 ptc/mL at day 10 to 3.4E5 ptc/mL at day 20).

The sample ZnO-LP started to degrade at day 10 as well, but shows a peculiar behavior. The mode size apparently increases slightly (from 0.43 to 0.51 µm), while average *n* reduced from 1.56 to 1.50. By looking at the 2D EOS CLOUDS histogram, however, two different particle populations are detected and a dedicated analysis for each population is necessary. The former, evidenced by the orange circle in Figure *4*, is compatible with a ZnO-LP particle population as observed at time 0. The latter, blue circle in Figure *4*, shows a lower effective refractive index 1.37 and a higher size distribution (average 0.95 µm), thus indicating a strong change occurred in the particles. This change appears to be almost abrupt, instead of continuous as it may be expected. At day 20 the process reaches an equilibrium, with one population compatible with the latter measured at day 10. Noticeably, this final population has optical properties compatible to data resulting from LP sample after degradation in sea water at the same time point. We can argue that the physico-chemical properties of the sea water degraded similarly both LP and ZnO-LP particles. ZnO nanoparticles are thus freed in the environments, as detected using another independent analysis method. Modelling the degraded LP / ZnO-LP particles as mesoporous structures of lipid swelled particles in water, a rough evaluation of the particle structure is possible by applying MFA. Experimental data are compatible with an effective refractive index of 1.37, thus we can estimate of about 40% w/w of water absorbed in the LP matrix, which lowers the whole LP / ZnO-LP particle refractive index.

CONCLUSIONS

The capabilities of EOS Classizer™ ONE and SPES patented method provide unique added value time resolved classification of particles suspended in environmental fluids. Thanks to its unique properties, SPES was able to investigate, in a deeper way with respect to traditional light scattering technologies, the sample composition as well as other peculiar behaviors in sample aging and degradation, even in target environmental waters.

RELEVANT PUBLICATIONS AND REFERENCES

Presentation of Single Particle Extinction and Scattering (SPES) method for particle analysis
AN001-2021 Analysis of Polymeric Particles via SPES Technology – a general introduction to SPES method

AN006-2021 Multiparametric Classification of Particles as a Pathway to Oversize Analysis in Complex Fluids via SPES Technology

Potenza MAC *et al.*, «Measuring the complex field scattered by single submicron particles », AIP Advances 5 (2015)

Example of CFA application of SPES technology
AN002-2021 Continuous SPES Flow Analysis CFA-SPES

Example of PCA application of SPES technology
AN005-2022 Multiparametric Principal Component Analysis of Heterogeneous Samples via SPES Technology

Classizer™ ONE with Sample Manager Autosampler
AN008-2022 Automatic Liquid Sample Management, Dilution, and System Cleaning with EOS Sample Manager

AN009-2022 Standardize SPES Operative Procedure of Liquid Samples Analysis via EOS Autosampler

Example of SPES application to aggregates
AN003-2021 Addressing the Issue of Particle Wetting and Clustering by means of SPES Technology

Potenza MAC *et al.*, «Single-Particle Extinction and Scattering Method …», ACS Earth Space Chem 15 (2017)

SPES application to non-spherical particles
AN004-2021 Addressing the Classification of Non-Spherical Particle by means of SPES Technology

Simonsen MF *et al.*, «Particle shape accounts for instrumental discrepancy in ice …», Clim. Past 14 (2018)

Example of SPES application to emulsions w/o payload in environmental waters
AN012-2021 Monitoring the Fate of a Lipid/ZnO Emulsion in Environmental Waters

Examples of SPES application to particle analysis and behavior characterization in biotech applications
AN011-2022 Quantitative Classification of Particles in Biological Liquids via SPES Technology

Sanvito T *et al.*, «Single particle extinction and scattering optical method unveils in real…", Nanomedicine 13 (2017)

Potenza MAC *et al.*, «Single particle optical extinction and scattering allows real time quantitative…», Sci Rep (2015)

Example of SPES application to oxide particles, abrasives, and industrial slurries w/o impurities
Potenza MAC *et al.*, «Optical characterization of particles for industries», KONA Powder and Particle 33 (2016)

AN002-2021 Analysis of Abrasives via SPES Technology

Example of SPES application to ecotoxicity analysis
Maiorana S *et al.*, «Phytotoxicity of wear debris from traditional and innovative brake pads», Env Int., 123 (2019)

Example of SPES application to aerosol analysis
Mariani F *et al.*, «Single Particle Extinction and Scattering allows novel optical …», J Nanopart Res 19 (2017)

Cremonesi L *et al.*, «Multiparametric optical characterization of airborne dust …. », Env Int 123 (2019)

Visit EOS website for further applications
www.eosinstruments.com

EOS S.r.l. – viale Ortles 22/4, 20139 Milano (Mi) – Italy
email: info@eosinstruments.com Phone: +39 02 56660179

Distributed by