SPES CLASSIFICATION OF PROBIOTIC FORMULATIONS

INTRODUCTION

Probiotics are live helpful microorganisms that are intended to have health benefits when consumed. They can be found in yogurt and other fermented foods, dietary supplements, and beauty products. Many of the microorganisms in probiotic products are the same as or similar to microorganisms that naturally live in our bodies. The most common are bacteria that belong to groups called Lactobacillus and Bifidobacterium. Other bacteria or yeasts may also be used as probiotics. Different types of probiotics may have different effects. Products based on probiotics may be in the form of liquids, as e.g., yogurt, or as solid composition, as e.g., tablets. Probiotic products may be in the form of complex formulations based on a mix of spores of nonpathogenic bacteria and other compounds as the cellulose derivatives, which are useful in and for the pharmaceutical, veterinary and nutrition fields. In this application note, the classification, the quantification, and the characterization of a commercial product via patented SPES technology and EOS Classizer™ ONE are presented.

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.

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.

For a general introduction to SPES data please refer to the Application Note AN001/2021, available online along with other application notes and example of applications at EOS website: www.eosinstruments.com/publications/

APPLICATION EXAMPLES

The commercial probiotic product considered and studied for the purpose of this application note is a liquid mix of bacillus clausii and microcrystalline cellulose employed for the management intestinal bacterial flora. The product is advertised to contain 4 billion of live lactic ferments per vial (5mL vial, corresponding thus to an expected numerical concentration of 8E8 probiotic/mL). It is known to also contain a population of micronized cellulose. A small aliquot of the product is dispersed in filtered water and directly measured flowing the sample thought the Classizer™ ONE. The standard SPES/Classizer ONE data are represented in Figure 1.

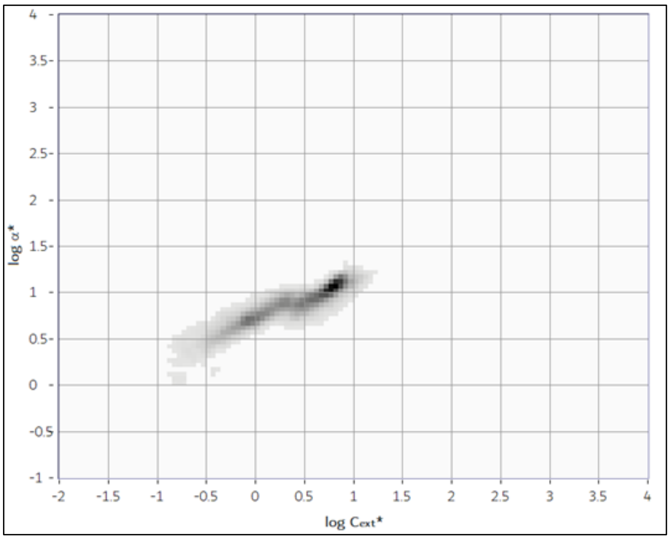


Figure 1 EOS CLOUDS of the commercial probiotic product. Two separate clouds are observed and correspond to two population of particles in the sample. The classification of the two clouds brings information on optical properties and numerical concentration of the two populations in the mix.

The distinctive feature of the SPES technique allows the particles to be separated in the EOS CLOUDS histogram according to their respective optical properties. Using the EOS Classizer™ software, it is thus easy to select the first population and focus the analysis to that subset of the totality of measured particles, as presented in Figure 2.

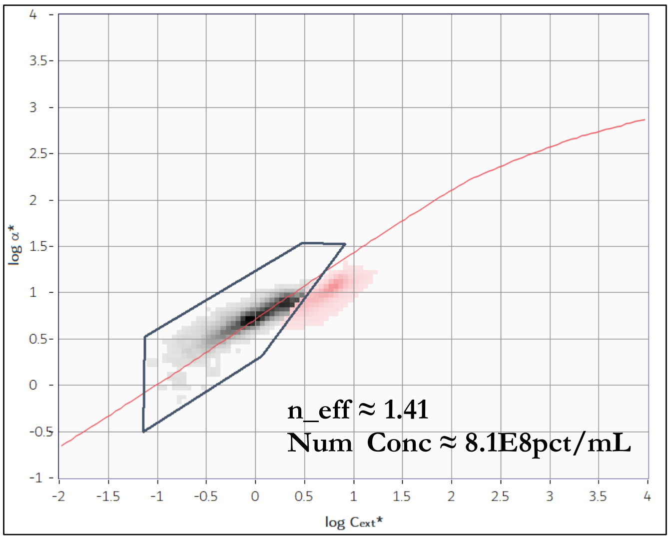


Figure 2 Selection in the EOS CLOUDS histogram of the optical signals corresponding to the particles of the first population. An effective refractive index of 1.41 and a numerical concentration of about 8.1E8 ptc/mL. are retrieved.

The EOS Classizer™ software estimates an effective refractive index of 1.41 by directly comparing the position of the experimental data with the expected ones for particles of different refractive index. The numerical concentration of about 8.1E8 ptc/mL is estimated from the signals counted and represented in the selected area. In Figure 3 the Numerical Particle Size Distribution of the selected area in Figure 2 is shown. The particle size distribution is derived from the measured extinction cross sections, benefiting from the estimation of the effective refractive index of these particles and using the tailored Mie method integrated in the EOS Classizer™ software.

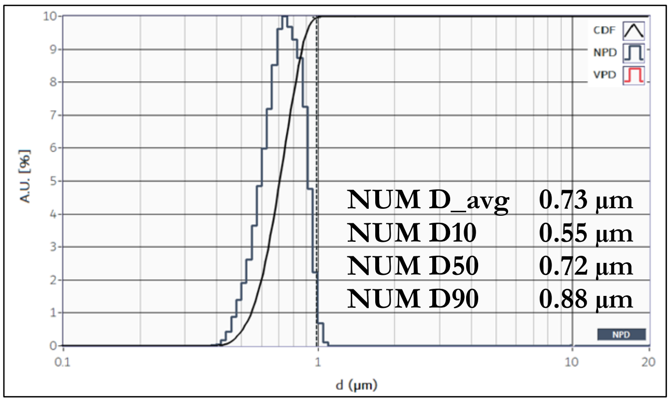


Figure 3 Numerical PSD of the bacillus clausii population.

The technology allows a classification of the particles without requiring calibration. These experimental results correspond and agree with expected ones for the bacillus concentration per vial (mL). The optical properties of bacillus clausii agrees with expected values from literature.

A further step in order to complete the study of the product is the analysis of the second population, which likely correspond to the microcrystalline cellulose suspended in the liquid. In Figure 4 and Figure 5 there are represented the selection of the second population and its corresponding numerical particle size distribution. The expected refractive index of the micronized cellulose is about 1.504 and agrees with measured values.

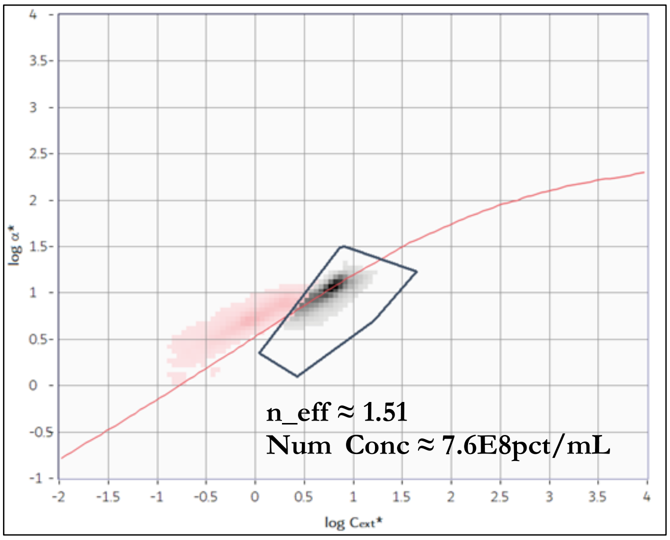


Figure 4 Selection in the EOS CLOUDS histogram of the optical signals corresponding to the particles of the second population. An effective refractive index of 1.51 and a numerical concentration of about 7.6E8 ptc/mL. are retrieved.

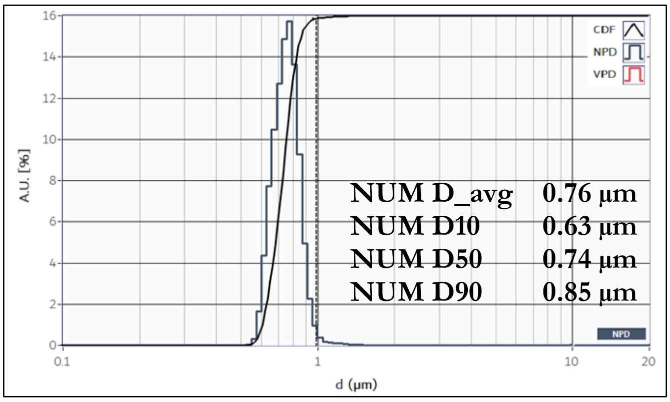
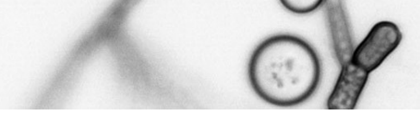


Figure 5 Numerical PSD of the micronized cellulose population.

CONCLUSIONS

The capability of EOS Classizer™ ONE and SPES patented method in discriminating single particle basing on their optical properties fits the need of a value-added application in the characterization of heterogeneous probiotics based on mixes of particles. Each of the information provided by SPES is crucial to improve the knowledge and the quality of the probiotic formulates as well as to monitor the quality of the batches measuring the relative and absolute numerical concentration, the optical properties and numerical size distributions of the suspended particle population before and after a mix.



RELEVANT PUBLICATIONS AND REFERENCES

Presentation of Single Particle Extinction and Scattering (SPES) method for particle analysis
AN001-2021 Analysis of Polymeric Particle Mixes via SPES Technology – an 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

AN015-2022 Classification of Oil and Oil Mixes Emulsions via SPES Technology

Examples of SPES application to particle analysis and behavior characterization in biotech applications
AN011-2021 Quantitative Classification of Particles in Biological Liquids via SPES Technology

AN017-2022 SPES Classification Of Probiotics Formulations

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)

AN013-2022 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)

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