

Particle Size and Shape Characterization of Nano- and Submicron Liquid Dispersions

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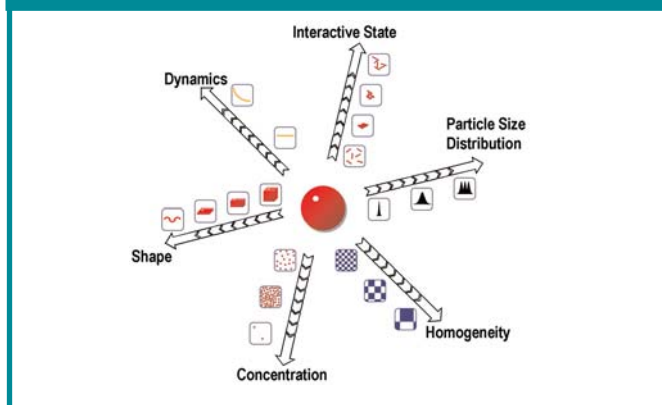
In the pharmaceutical industry, pharmaceutical dosage forms are getting increasingly important as a basis for making more effective drugs. In the successful development of a new dosage form, formulation development typically plays a crucial role in the introduction and application of state-of-the-art pharmaceutical technology, such as for example direct compression, solid dispersion or depot formulation technology. The manufacturability and/or bio-availability of many of these dosage forms regularly depend on the particle characteristics of the individual ingredients. For this reason, particle engineering has become a key aspect in formulation development, meaning that one needs to specify on forehand the required particle characteristics in terms of particle size and shape (distribution) [1], dissolution and/or solid state in order to assure the required profile of the drug. Furthermore, particle engineering requires manufacturing of the particles according to the defined specifications, also meaning analytical control of the relevant aspects of the particulate system [2].

One of the current challenges in pharmaceutical product development is dealing with low solubility and/or low permeability compounds, i.e., BCS class II, III, and IV active pharmaceutical ingredients (API) [3]. A common strategy here is to go to smaller particle sizes, thereby increasing the specific surface area (SSA) and solubility of the product [4]. For the manufacturing of very small particles with a nano [5] or submicron size either a bottom up or a top down approach can be applied [6]. Solid dispersions (e.g. melt extrusion or spray drying) are typically manufactured according to a bottom up approach, while liquid dispersions can be manufactured based on either a bottom up approach (e.g. spray freezing) or a top down approach (e.g. milling). With this publication, the authors would like to share their opinion on the capabilities of some of the most common analytical technologies that are available on the market for the particle size distribution (PSD) analysis of solid nano- and submicron particles in suspension.

PSD analysis of nano- and submicron liquid dispersions is quite often not an easy thing to do. As Figure 1 tends to point out, one should generally take into account six (6) criticalities that may affect the reliability (i.e. accuracy) of the generated analytical data. More precisely, this figure schematically explains that in general the PSD characteriza-

tion of a liquid dispersion is most straightforward for homogeneous suspensions, that consist of a narrow distribution of spherical particles that are well dispersed, present at a suitable concentration, and that are stable in function of time. However, this ideal situation is often far from reality, as is illustrated in Table 1 for the criticality evaluation of a product that has been analysed in function of this publication. Practice shows that particle size data obtained with different PSD technologies

Figure 1. Criticalities in the PSD analysis of nano- and submicron liquid dispersions



are generally less easy to compare for products of which the criticality has increased for either of the six aspects: homogeneity, PSD, morphology, interactive state, concentration, and/or dynamics. As a consequence this means that for products with a high(er) criticality one often may not simply rely on the analytical data as obtained with one single PSD technology, and verification by means of other (orthogonal) techniques is recommended. In practise, the acquired data will demonstrate that no single PSD technique is (in)accurate as such, but that each technique is merely (in)accurate for products with a certain criticality profile. In relation to this, one should always be alert not to confuse a lack

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of knowledge on the fundamentals of signal generation and data processing with an assumed inaccuracy of the technique.

Special attention needs to be paid to the 'interactive state' [7] of the suspended particles as it is called here. With the manufacturing of nano- and submicron suspensions the particles generally are stabilized in order to assure physical stability of the product. As a result, one initially may expect the criticality of the product with regard to its interactive state as being 'low'. However, in practice this criticality is often not as low as one may think, since in function of time product instability may occur thereby leading to phenomena, which can be difficult to evaluate due to the sample manipulation. Particularly, for flocculated systems sample preparation and analysis may cause rupture of the clustered particles thereby leading to incorrect information on the actual dispersion state of the product. In addition to this, sample preparation and analysis may induce flocculation of the primary particles. Provided that electrostatic repulsion is the mechanism of stabilization, for stability evaluation purposes, the zeta-potential of the particles may give a good indication of their stability in the dispersion medium that is used during analysis [8].

Based on the various arguments mentioned above, one should be aware that the choice for a piece of analytical equipment is not arbitrary. And in function of the criticality profile of the product, one should be able to make an estimate on the suitability of the various PSD technologies that are commercially available on the market. For this reason, in function of the criticality of the product in Table 2, the authors present a schematic evaluation regarding some of the most common PSD technologies. One should keep in mind that this evaluation is merely meant as a guidance, as for certain brands of equipment the actual situation may sometimes be (slightly) different. Since one may not always know beforehand the exact criticality profile of a product, screening by means of a series of (orthogonal) PSD technologies is often required. Only when the complete criticality profile of a nano- or submicron liquid dispersion is known, the true product characteristics can be defined, and preferred PSD technology can be identified in order to monitor and control the critical variations in product quality.

From a historical perspective, the most common technologies for the PSD characterization of nano- and submicron liquid dispersions typically are Laser Diffraction (LD) [9] and Photon Correlation Spectroscopy (PCS) [10]. Both are so-called ensemble techniques that calculate rather than measure the PSD of a product. For LD the latter is calculated from the scatter intensity in function of the angle of scattering, while for PCS, the dynamic fluctuation of the scattered light intensity is the basis for calculation of the average particle size. Though the

Table 1. Example of a criticality evaluation for a submicron dispersion

Product characteristic	Criticality			Evaluation
	Low	Medium	High	
Particle Morphology			×	Plate-like morphology of particles may lead to different results for different PSD techniques
Particle Size Distribution			×	Broad unimodal or bimodal distribution with small fraction of coarse particles
Interactive State		×		Possible rupture of small fraction of agglomerates during measurement procedure
Homogeneity		×		Small fraction of coarse particles may lead to sampling problems and thus to variability in PSD data
Concentration		×		Required dilution of concentrated product may change the initial state of the particles
Dynamics		×		The product is extremely stable in function of time

analytical capabilities of the techniques justify application in this area, their limitation is first of all the not necessarily quantitative response for (small) portions of undersized or oversized particles. Further concerns for LD are (a) the requirement for using the correct optical model, (b) the low scatter intensity for small(er) particles, (c) the low angular information of the scattered light for small(er) particles, and (d) the manufacturer specific approach in extending the size range for detection of small particles (i.e. ca. < 400 nm). Some major concerns for PCS are (a) its calculation of average particle size and standard deviation instead of PSD, (b) the manufacturer specific approach in correlation of intensity fluctuation and particle size, (c) the interference from large(r)

Table 2. Schematic evaluation of some common PSD technologies

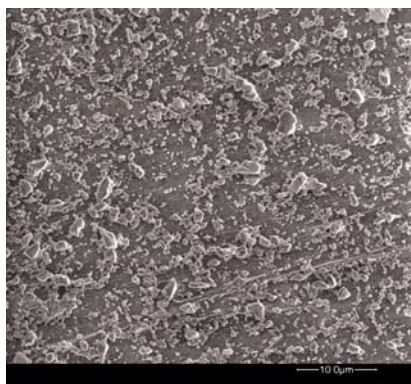
Technology	nm	µm	Shape	PSD	Homogeneity	Interactive State	Dynamics	Concentration
Laser Diffraction (traditional)								
Laser Diffraction (extended range)								
Dynamic Light Scattering								
Back Scattering								
Centrifugal Sedimentation								
Hydrodynamic Chromatography								
Scanning Electron Microscopy								
Optical Microscopy (Static)								
Acoustic Spectroscopy								
Near Infrared Spectroscopy								

particles present in the sample, and (d) the dependency of the particle size with the viscosity of the liquid, buffer concentration, particle concentration, etc. For all these reasons, high criticality samples preferably require the validation of LD or PCS by means of orthogonal technologies.

An important group of orthogonal PSD technologies relate to separation of the particles based on differences in particle size. This analysis principle is typically applied for Centrifugal Sedimentation (CS) [11], Hydrodynamic Chromatography (HDC) [12] and Scanning Mobility Particle Sizing (SMPS) [13]. Contrary to the ensemble techniques mentioned earlier, separation techniques allow a much more quantitative monitoring of multimodal distributions. Though CS and HDC are robust and easy to operate, the techniques show some limitations in terms of (a) a limited size range, (b) relatively long analysis run times, (c) impact of particle morphology on the separation process, and (d) the necessity for an optical model in the detection of the particles. For these reasons, PSD results for CS and HDC may not always be identical to LD and PCS.

The next group of orthogonal PSD technologies relates to the counting and classification of the particles based on their size. This principle is most of all applied for the computerized analysis of digital images as obtained with for instance Electron Microscopy (EM) [14] or Atomic Force Microscopy (AFM) [15]. Very recently, so-called Nano Particle Tracking Analysis (NPTA) in combination with Optical Microscopy (OM) has become commercially available, which allows low cost PSD analysis of nano- and submicron particles based on their Brownian motion [16]. Though the full capabilities of the latter are not known yet, NPTA is expected to be a rapid alternative for the more elaborate SEM and AFM technology. Since EM and AFM methods are not easy to run on a routine basis, until now they are merely used for trouble shooting or verification purposes. Image Analysis (IA) typically offers the advantage of measuring both the size and shape [17] of particles by means of their 2D projected surface area, whereas AFM even allows 3D measurement. In Figure 2, an example is given of the Environmental Scanning Electron Microscopy (ESEM) analysis of a submicron liquid dispersion. For this purpose, the particles in suspension have been filtrated over a 50 nm pore size filter in order to enable evaluation of the morphology of the particles, and to make an estimation of their approximate size. IA is often considered as an absolute and thereby ideal reference technique for the validation of ensemble and separation PSD techniques. Though in theory this may be true, in practise special attention should be paid to the performance of the method and the interpretation of the data [18].

Figure 2. Imaging of submicron particles with Scanning Electron Microscopy



All technologies as discussed so far have one common limitation which is related to the necessity for dilution of concentrated samples prior to analysis. Due to this manipulation of the sample, it is often quite difficult, if not impossible, to monitor the dispersion state of for instance flocculated systems. For this purpose, 2nd generation ensemble techniques such as Back Scattering (BS) [19], Acoustic Spectroscopy (AS) [20], and Near Infrared spectroscopy (NIR) [21] have become commercially available for the analysis of concentrated suspensions. However, since these techniques require calibration by means of reference PSD data, the quality of the final results are never better than the quality of these reference data. The latter is for sure a limitation, but the possibility to measure dynamic processes in concentrated suspensions means that these technologies can typically be used as Process Analytical Technology (PAT) for the in-line monitoring of the product during manufacturing.

Adequate screening of a liquid dispersion during its (pharmaceutical) development and in function of its clinical performance are the basis for the selection of preferred Quality Control (QC) technology for routine monitoring in production. This technology should first and above all be sensitive for critical variations in product quality, such that it allows the discrimination of good and bad production batches. However, other arguments should be taken into account as well, such as that the technology preferably should be (a) widely accepted, (b) compendial, (c) state-of-the-art, (d) easy to operate, (e) robust, (f) easy to transfer, and (g) CFR 21 Part 11 compliant. For this reason, selection of preferred QC technology generally is a compromise between the various requirements, resulting in a PSD method that may show merely a relative (i.e. qualitative or semi-quantitative) response rather than an absolute (i.e. quantitative) response.

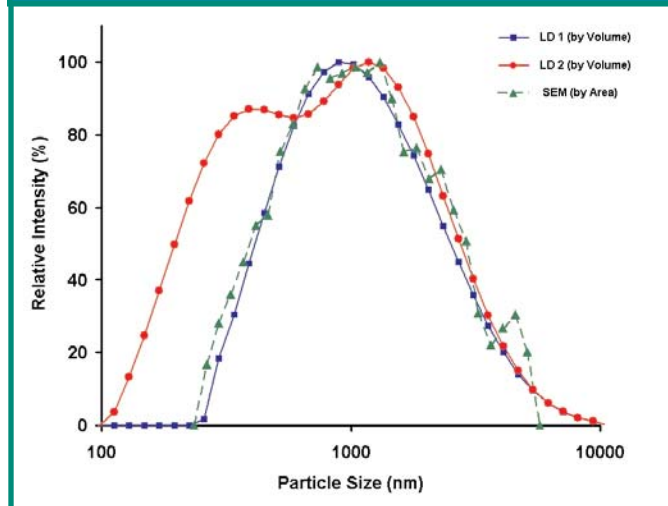
To assure that a PSD method is suitable for the QC monitoring of a liquid dispersion, the method development process should provide in a systematic and stepwise approach, such that at the end a robust and transferable method can be authorised, which is able to detect critical variations in product quality. In an ideal situation these steps should concern (a) screening of production batches by means of various orthogonal PSD technologies, (b) screening of stability samples by means of various orthogonal PSD technologies, (c) identification of critical particle characteristics (i.e., in function of manufacturability, stability and bio-availability), (d) identification of preferred QC technology, (e) development of QC methodology (i.e., including robustness testing), (f) method validation (i.e. accuracy, intermediate precision and reproducibility), and (g) method transfer (i.e., including edge of failure batches). At various time points during the method development process fundamental questions may arise, such as:

1. Is the morphology of the particles still the same?
2. What is the true shape of the distribution?
3. What is the exact size of the particles?
4. Why do the size distributions of different PSD techniques not match?
5. Why are PSD data not in agreement with in-vitro dissolution data [22]?
6. How quantitative is the PSD method for monitoring product instability?

In order to give the right answers to these and other questions, in quite some cases imaging and image analysis appear to be an extremely important analytical tool. The latter is illustrated by means of Figure 3 for the LD analysis of a submicron liquid dispersion. More precisely, for this product, traditional LD analysis shows a unimodal size distribution profile, whereas extension of the size range shows a more or less bimodal profile. As a result, questions did arise on the right detection

configuration setting of the instrument, and the right refractive index and absorption of the MIE optical model. And as one can see from the PSD overlay, quantitative analysis of a series of SEM images really helped to interpret the LD data.

Figure 3. Quantitative image analysis with SEM as a basis for interpretation LD data



Summary

The authors share their opinion on some common analytical technologies that are commercially available for the particle size distribution (PSD) analysis of solid nano- and submicron particles in suspension. The reliability of PSD data is generally affected by the following product criticalities: homogeneity, PSD, morphology, interactive state, concentration, and dynamics. A schematic evaluation is presented on the capabilities of the various ensemble, separation and counting technologies that nowadays are most widely applied. With this publication, the authors intend to help in a systematic and stepwise development of QC methods, that are sensitive for critical variations in product quality.

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