New Developments and Applications in Inline Size Analysis for Nanodispersions Based Processes

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ABSTRACT

Nanodispersions are taking an increasingly prominent role both in the pharmaceutical (e.g. vaccines, cancer therapeutics) and in the chemical industry (e.g. for catalysts, inks, coatings). In both cases, inline monitoring of size characteristics during processing has important advantages for rapid process/formulation development in R&D, upscaling and in controlled manufacturing. Specifically in pharma, the need for real time particle size information during manufacturing of nanodispersions is also increasingly emphasized by regulatory bodies, but has been strongly limited by lack of suitable technology [1].

The NanoFlowSizer (NFS) [2] with novel Spatially Resolved DLS technology was recently introduced to overcome these limitations. In the present paper several new examples of inline real-time monitoring using this instrument are described, for both 'bottom-up' and 'top-down' manufacturing methods. As 'bottom-up' examples, the crystallization of ibuprofen and titanium dioxide nanoparticles was monitored at lab scale, showing the benefits for rapid insight and efficiency gain in optimizing growth conditions. To show the applicability of the NFS in 'top-down' manufacturing methods, the instrument was coupled to different high-pressure homogenization (HPH) processes of emulsions, where the size reduction/increase of nanodroplets was tracked at real time.

The suitability of the NFS to monitor (cycled) processes requiring a specific endpoint and for continuous nano-dispersion manufacturing technologies, (i.e. continuous liposome manufacturing platforms) opens new possibilities for process control -and quality control.

Keywords: nanoparticle analyzer, process analytical technology, vaccine development, lipid-nanoparticles, liposomes, crystallization.

1. TECHNICAL BACKGROUND

Spatially Resolved Dynamic Light Scattering is based on low coherence interferometry of backscattered broadband light (multiple wavelengths instead of a single wavelength laser). Similar as in conventional DLS, intensity fluctuations due to Brownian motion of nanoparticles are recorded and translated to diffusion rate and particle size. In addition to conventional DLS intensity fluctuations are obtained simultaneously as function of depth in the sample up to about 3mm. Depth resolved data enables effective and automatic filtering of single and multiple scattered light which results in a significantly wider accessible turbidity range compared to conventional DLS. As a result, many nanosuspensions up to relative high concentrations or turbidity can be measured without dilution. The spatially resolved data also allows to measure and correct for flow, flow profiles are recorded for every measurement as well. Additional movement of particles due to flow is filtered out to obtain the Brownian motion component only for particle size characterization. Measurements are performed non-invasively directly in the process through any glass interface such as a flow cell, flask or specific container type or sample vials for measurements under static conditions (Figure 1).

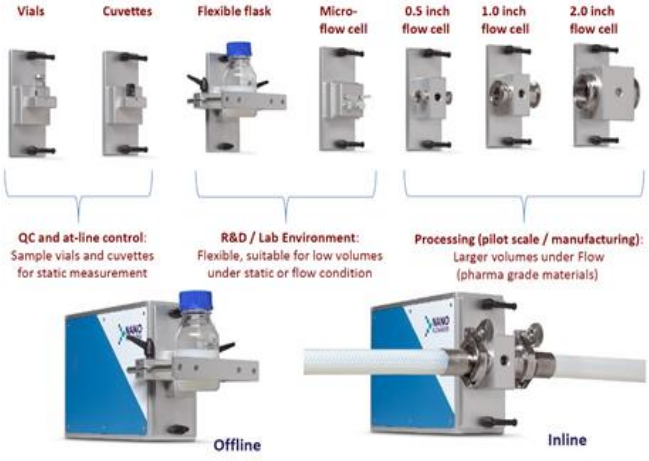


Figure 1. Images of the NanoFlowSizer showing the different configurations (bottom) and modules (top).

1.1. 'Bottom-up' processes: Crystallization

Commonly applied 'bottom-up' approaches in which nanoparticles are crystallized or precipitated lack a real time monitoring tool to follow initial stages of particle growth. Although some particle size monitoring tools are available for larger above micron size particles, the submicron area remains a challenge to monitor in real time. Since kinetics of 'bottom-up' approaches are important for understanding and control of these processes, there is a general desire to monitor the initial stages of particle growth as early as possible. To probe the applicability of the NFS to monitor the growth of nanoparticles during the first stages of crystallization and its ability to distinguish between 'real' particle growth and particle aggregation some examples are shown.

1.1.1. *Crystallization of Ibuprofen nanoparticles*

In this section the growth of ibuprofen nanoparticles was tracked at real time during antisolvent crystallization. The initial stage of crystallization includes nucleation which relates to the transition from a (over saturated) liquid phase to a solid phase. After nucleation, particles start to growth and new particles will be formed in parallel by ongoing nucleation. As a result of the increasing solid concentration the driving force to nucleate due to super saturation will decrease and particle growth will become the dominant mechanism. In Figure 2 the particle size distributions measured by SR-DLS are shown during the antisolvent crystallization of ibuprofen in a glass vial (solvent: 2-propanol, antisolvent: water, solvent to antisolvent ratio = 1/3).

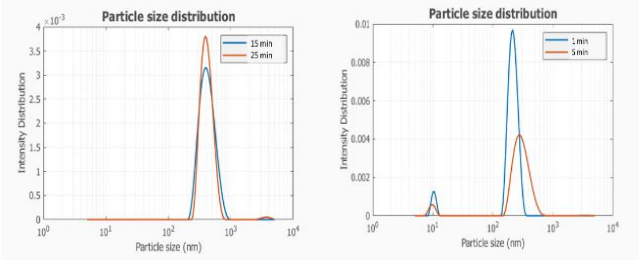


Figure 2. Real time particle size distribution information at different time points, during the antisolvent crystallization of Ibuprofen.

After 1 and 5 minutes the PSD fit shows a small fraction of around 10 nm particles most likely corresponding to formation of new particles in the nucleation stage. The fraction of small particles disappears in time and particles of a few 100 nm start growing in time.

Additional studies with ibuprofen in 2-propanol were executed to monitor the effect of anti-solvent (in this case water) ratio and presence of surfactant (tween 80). In Figure 3 some examples of the monitored crystallization process in real time are shown. The real time data shows a direct effect of different solvent/anti-solvent ratios to particle size (the more anti-solvent, the smaller the particles). The effect of the surfactant is also significant, particle size increases much faster without surfactant which is most likely due to formation of aggregates.

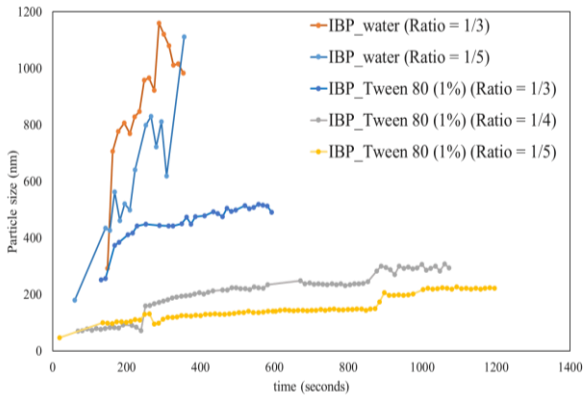


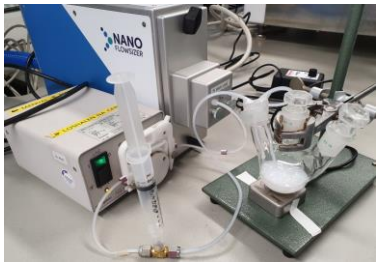
Figure 3. Real time information about the growth of particles during the antisolvent crystallization of ibuprofen from a 2-propanol solution using different amounts of water (with or without Tween 80) as anti-solvent.

Shown data was all recorded in a static glass vial mode. Since no sample handling is required and measurements are performed non-invasively through the glass wall of the vial, this type of crystallization studies can be executed conveniently at low volumes in a rather simple manner.

1.1.2. *Synthesis and crystallization of titanium dioxide nanoparticles*

Particle growth of titanium dioxide (TiO_2) particles during its synthesis (by controlled hydrolysis of titanium tetraethoxide in ethanol) and crystallization was monitored online in real time by SR-DLS. A different setup compared to the crystallization of ibuprofen example was applied to show applicability of SR-DLS to measure flowing suspensions as well. An online loop connected to a micro-flowcell sampling module of a NanoFlowSizer with a small peristaltic pump circulated the reaction mixture continuously (Figure 4A), and particle size measurements were recorded every 10 seconds. A number of experiments were recorded in real time for which the amount of added KCl in the reaction mixture was varied to obtain differently sized particles. It was observed that the higher the concentration of salt added the smaller the final particle size of TiO_2 particles was obtained.

(A)



(B)

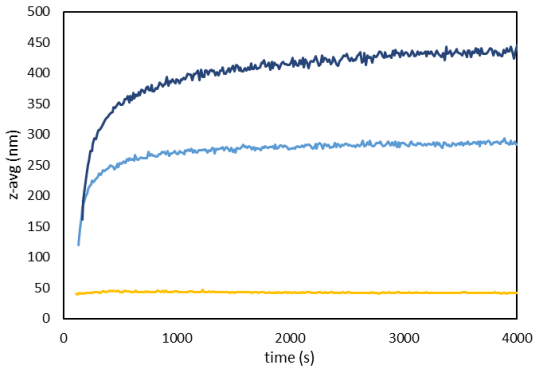


Figure 4. A) Real time monitoring of nanoparticle size during titanium oxide synthesis by SR-DLS via online loop; B) Online real time particle size information of synthesized TiO_2 particles for different KCl concentrations (dark blue: 0.6 mM, light blue: 0.8 mM, yellow: 1.5 mM)

The SR-DLS technology is able to measure over a wide turbidity range which allows continuous and real time measurements up to relatively high concentrations of even highly scattering materials (like titanium dioxide).

1.1.3. Particle growth versus aggregation

In the previous examples, SR-DLS showed to be suitable for real time monitoring of particle growth processes. However, particle size data as such does not distinguish between 'real' particle growth and particle aggregation. Besides particle size information, backscatter intensity as recorded by SR-DLS is obtained simultaneously for each particle size measurement and is directly related to concentration (in principle turbidity) of the suspension. In case of particle growth, the concentration increases, while the concentration remains unchanged in case of aggregation. Both mechanisms may be identified based on backscatter intensity data.

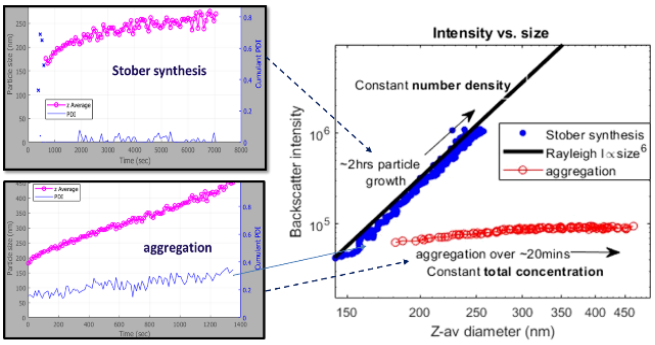


Figure 5. SR-DLS particle size and backscatter intensity of silica particle growth (Stöber) and aggregation

In Figure 5 two processes involving formation of silica particles show increasing particle size in time. One of the processes is based on actual particle growth (Stöber synthesis) and one of processes is based on aggregation only by adding salt to 100 nm silica particles. The backscatter intensity corresponding to the particle growth (Stöber) data shows a close relation with the expected increase in turbidity (based on Raleigh scattering) for a situation in which particles only grow (with constant number density). The obtained backscatter intensity of the silica suspension with added salt shows a nearly constant backscatter intensity indicating no significant change in concentration and therefore indicating aggregation.

1.2. 'Top-down' processes: High-pressure homogenization

Nano-emulsions are widespread as intermediate or end-products in food, cosmetics, pharmaceutical and chemical industries. Among the various 'top-down' manufacturing methods for nano-emulsions, high pressure homogenization (HPH) is very popular due to its flexibility, ease of tuning droplet size and scale-up possibilities. Nevertheless, it is often challenging in HPH processes to achieve in depth formulation and process understanding efficiently, largely due to the absence of effective methods to monitor droplet size in real-time during production. Besides efficiency and process understanding, inline size characterization also allows production of higher quality, 'precision-manufactured' nano-emulsions, and helps to identify and solve processing problems during production.

1.2.1. Real-time size characterization of emulsions.

To illustrate the benefits of the NFS for 'top-down' processes, two examples in which the NFS was integrated in HPH processes of a lipid nanoemulsions will be shown in this section.

1.2.1.1. Monitoring homogenization of emulsions

A highly turbid emulsion (5wt% sunflower oil + 1wt% Tween in water) underwent three homogenization stages, each at a fixed pressure. Figure 6 shows how the NFS was integrated in the HPH process. The flowrate during homogenization was 9 L/hr, for which true inline measurements are easily achieved with the instrument by using a suitable flow cell.

(a) (b)

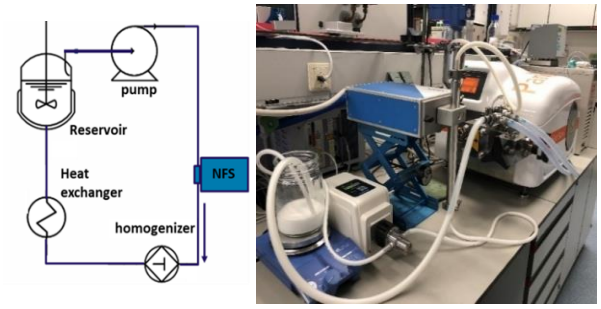
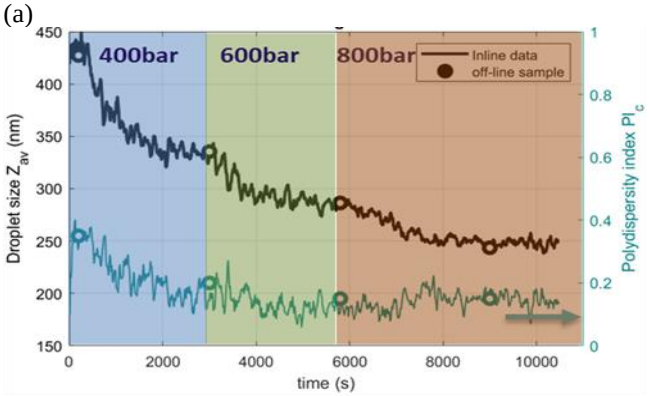


Figure 6. (a) Flow diagram and (b) picture of the set-up employed for lipid the nanoparticle emulsions production.

Figure 7a shows the monitoring results during the stepwise pressure changes during an emulsification process. A fast reduction of the droplet size (Zav) was observed after the pressure rise to 400bar, going from c.a. 450 nm to somewhat below 350 nm in 50 min, as observed in Figure 7a. After an increase of the pressure to 600 bar the droplet size decreased to a size of about 280 nm. Increasing the pressure to 800 bar results in a constant droplet size of 250 nm after about 30 minutes. A decrease in the polydispersity index (PDI), from the beginning to the end of the process can also be observed in Figure 7a. The corresponding PSD at different timepoints is shown in Figure 7b. Over time the PSD becomes narrower and the peak shifts to smaller droplet sizes. These results confirm the expectation of droplet size and polydispersity changes during homogenization. Offline samples taken from the reservoir, measured in a glass vial without flow for comparison, are shown as circles in Figure 7a, and confirm the real-time inline data.

(a)



(b)

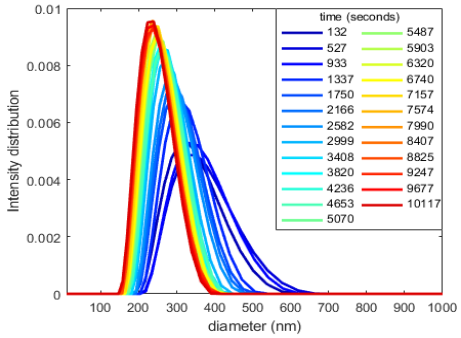


Figure 7. (a) Inline monitoring of size characteristics an emulsion following homogenization at the different pressures. (b) The droplet size distributions at different time points, the different line colors represent different time points (blue: from ±2min to ±60min, green-red: ±70min to ±170min).

1.2.1.2. Tracking the stability of nanoemulsions

A pharmaceutical emulsion consisted of organic-solvent droplets with dissolved active ingredient dispersed in an aqueous solvent was homogenized and the droplet size was monitored using the NFS at a flow of ~20L/hr. The pressure in the HPH was constant during the process. After 39 minutes, the flow past the NFS was stopped for c.a. 4 mins, to check the stability of the emulsion in the absence of flow.

Figure 8 shows the monitoring of the Z-average of nanodroplets during the emulsification of the organic solvent/water emulsion. At the start of the process the mean droplet size was c.a. 200 nm. The droplet size subsequently shows a modest reduction on prolonged homogenization, which set in most clearly after c.a. 20 mins. After 39 minutes, when the flow past the NFS was stopped, the droplet size shows a clear increase from c.a. 170 nm to c.a. 330 nm. When the flow was started again, the droplet size almost instantaneously dropped and started to retrace the slow decreasing trend observed before the diversion.

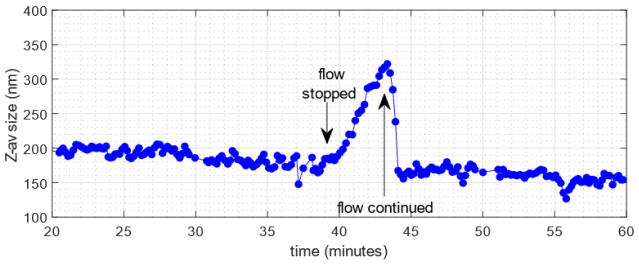


Figure 8. Inline measurement of Z-ave droplet size using the NFS during homogenization and interruption of flow.

This data clearly shows how the NFS inline monitoring capabilities can be employed for better understanding of homogenization of emulsions showing complex, unstable behavior. Due to the unstable nature of the emulsion, no reliable offline measurements of droplet size during processing could be made in practice. The use of a PAT tool for size characterization is thus particularly valuable in this case.

2. CONCLUSIONS

A novel instrument (the NanoFlowSizer) and methodology for inline nanoparticle sizing are presented. The instrument enables rapid and efficient formulation and process development as well as process control. The results described here highlight the ability of the NFS to monitor the evolution of size characteristics at real time, without need of sampling and dilution steps.

The fast data acquisition allows monitoring of particle size and particle size distribution every 5 to 10 seconds. Additionally, due to the ability to measure over a wide turbidity range in static and flowing suspensions, different measurement configurations can be applied to monitor particle size formation during 'bottom-up' and 'top-down' processes.

The ability to obtain nanodroplet/particle size information continuously during processing, for a broad range of dispersions, provides many new opportunities for improved process understanding, efficiency and control in nano-suspension based processes, from R&D to commercial manufacturing.

3. REFERENCES

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