European Journal of Pharmaceutical Sciences 133 (2019) 205–213

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

New unique PAT method and instrument for real-time inline size characterization of concentrated, flowing nanosuspensions

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ARTICLE INFO

Keywords:
Nanoparticle sizing
Process analytical technology
Nanosuspensions
Nanoemulsions
Liposomes
Dynamic light scattering
Quality by design

ABSTRACT

With the rise of nanotherapeutics -and nano based products in general-, there has been an increasing need for better understanding and control of nano-particle (NP) synthesis and formulation processes. Size characteristics are often primary, if not critical, quality attributes of nanodispersions. Process Analytical Technology (PAT) tools for inline size characterization during dispersion processing are therefore highly desired. Traditional methods for NP sizing -based on Dynamic Light Scattering (DLS) – are typically ill-suited for direct inline application: (i) typical dispersion turbidities in process conditions often exceed by far the application limits for DLS (ii) agitation/flow typical for process conditions is incompatible with standard DLS and (iii) direct and convenient inline application requires a non-invasive PAT tool giving measurements on process relevant time scales.

In this article we describe a new non-invasive PAT instrument – the NanoFlowSizer (patent pending)- which provides continuous, real-time, inline size and PSD characterization of concentrated and/or flowing nanodispersions in process environments. The instrument employs Fourier Domain low coherence interferometry, yielding path length resolved dynamic light scattering data of nanodispersions. Particle size characteristics can be analyzed from these data while effects of flow and/or multiple scattering are simultaneously characterized and accounted for. As first application examples we describe (i) real-time monitoring of NP size characteristics by remote measurement of mono and bi-disperse suspensions at different turbidities in a stirred beaker (ii) real-time monitoring of NP size characteristics using an online sampling loop with a micro-flow cell and (iii) real-time inline monitoring of size characteristics of a pharmaceutical nanoemulsion during industrial pilot scale nano-emulsification and for a pharmaceutical NP suspension during circulation, at flowrates ranging up to ~l/min.

1. Introduction

1.1. Nano particle analysis

Synthesis and processing of nanoparticles (ranging in size from approximately 1–1000 nm) has become widespread in various industries over the last few decades due to the unique advantages nanoparticle (NP) products and formulations can offer. E.g. in the pharmaceutical industry, therapeutics formulated as NPs, nanodroplets or liposomes may offer better pharmacokinetic properties, controlled release and targeting (Tinkle et al., 2014; Tinkle, 2010) (Lovelyn, 2011) (Samad et al., 2007) (Allen and Cullis, 2013). In food, cosmetics and e.g. paints/coatings, nanocolloids are also abundant, either occurring naturally or present through formulation. Due to increasing NP product development and production efforts as well as increasing demands for monitoring processes involving NPs, there is a growing need for continuous inline methods to characterize NPs during these processes (Stenger et al., 2005). Direct inline measurement of critical process parameters or monitoring and control of NP size and distribution to ensure quality during routine production, has so far been strongly limited or impossible due to restrictions of currently available NP sizing methods (Wang et al., 2009). In many cases, NP size and particle size distribution (PSD) are primary, if not critical, quality attributes. Methods for inline size characterization of nanodispersions during processing are therefore highly desired.

The common current practice for monitoring NP size characteristics in a process involves manual sampling of dispersions followed by off-line analysis. This has important disadvantages, e.g. excessive feedback times for process adjustments and significant uncertainties regarding measurement representativeness due to limited representation of actual process dynamics and possible changes in NP size characteristics or poor dispersion stability after sampling and sample preparation. In addition, for many continuous nanodispersion processes, sampling is not favored since processes may have to be stopped or sterility may be

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https://doi.org/10.1016/j.ejps.2019.03.024
Received 2 November 2018; Received in revised form 10 February 2019; Accepted 26 March 2019
Available online 28 March 2019
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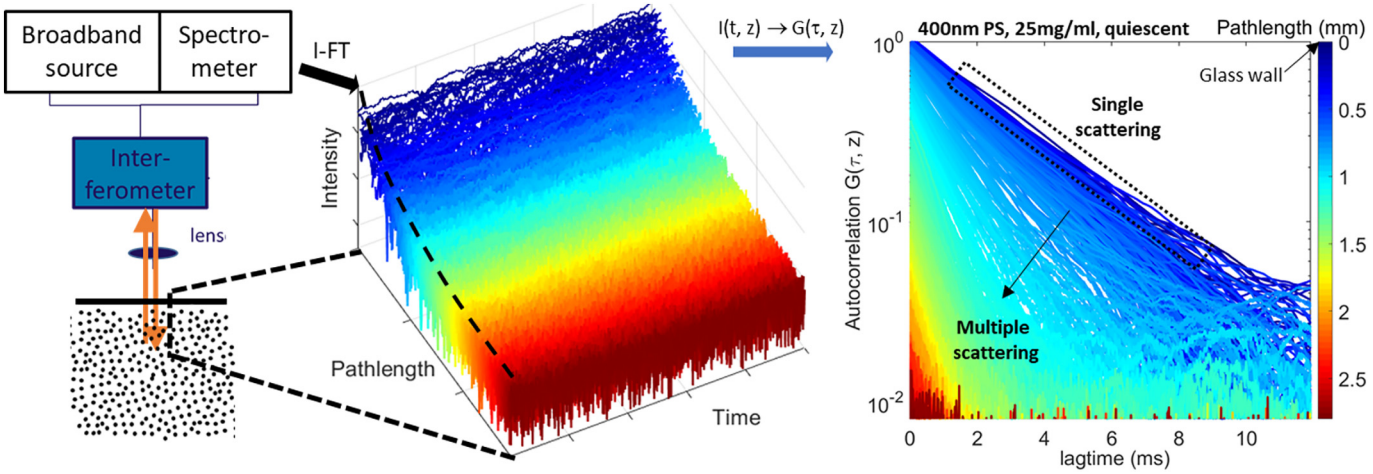

Fig. 1. Schematic of Spatially Resolved DLS using FDLCI. Data are for a quiescent, highly turbid sample, with scattering coefficient ~5 mm^{-1} at 1300 nm.

compromised. For unstable dispersions, offline analysis may even not be feasible altogether. Sampling may also entail product loss and corresponding high costs, further increasing the need for noninvasive, inline NP size characterization.

1.2. Current methods for characterizing nanodispersions

Methods currently available for offline (sample) analysis of particle size and PSD of nanodispersions are highly diverse but offer limited or no opportunity for noninvasive use during dispersion synthesis or processing. Examples such as Differential Centrifugal sedimentation or other separation-based methods like size-exclusion chromatography (Bootz et al., 2004) (Anderson et al., 2013) are intrinsically invasive. Methods that are most frequently used for offline nanosizing, despite their lower resolution compared to separation based methods (Anderson et al., 2013), are based on optical detection of the Brownian diffusion of suspended colloids, from which size characteristics can be obtained via the Stokes-Einstein relation. Best known among these is Photon Correlation Spectroscopy (PCS) (Pecora, 2000), covering various methods in which the Brownian diffusion -and thus hydrodynamic size- of an ensemble of suspended NPs is characterized by measurement of the temporal correlations of light scattered from the dispersion. Other such techniques -more recently developed- are Differential Dynamic Microscopy (Cerbino and Trappe, 2008), and Nanoparticle tracking analysis (Filipe et al., 2010).

1.3. Limitations of currently available methods for nanosizing

The above described methods are essentially restricted to off-line analysis since (i) they require dispersions with low turbidity level -different from turbidity levels often encountered in industrial processes- to prevent unwanted effects of multiple scattering (ii) the methods typically require absence of flow in the dispersions, which is a major obstacle for inline analysis and (iii) analysis time for these methods is in the order of minutes, often too slow for process monitoring and control. A solution exists for automated *at-line* measurements based on standard DLS (Zetasizer AT, Malvern) but is based on a sampling circuit/dilution robot coupled to traditional DLS and thus lacks advantages of inline process monitoring. To circumvent turbidity limitations in DLS-type size characterization, alternatives such as cross-correlation DLS (which suppresses the influence of multiple scattered light) and Diffusing Wave Spectroscopy (DWS, which exploits multiple scattering, but yields only mean particle size) (Weitz et al., 1993), exist but so far no instrumentation for direct inline measurements exists based on these methods. Use of heterodyne backscatter DLS methods has also led to improved capabilities for characterizing turbid dispersions and has been employed in an inline probe with mechanical compartmentalization to perform inline measurements (De Kanter et al., 2016). Recent developments employed Low Coherence Interferometry (Lee et al., 2012) (LCI, the basis of Optical Coherence Tomography, OCT (Fercher et al., 2003)) to enable spatially resolved DLS (SR-DLS), as this opens wider possibilities to characterize diffusion and flow in turbid nanodispersions, as described further below.

1.4. NanoFlowSizer: spatially resolved dynamic light scattering (SR-DLS)

The recently developed NanoFlowSizer (NFS) is the first instrument to employ SR-DLS based on Fourier Domain Low-Coherence Interferometry (FDLCI), and thereby provides new possibilities for non-invasive and continuous inline measurement of size characteristics in flowing and quiescent nanodispersions.

In LCI, the sample is illuminated by low coherence light (coherence length L_c ~1–10 μm) from a broadband source, and back scattered light interferes with light split from the source with a specific optical path length. This provides a spatial 'coherence gate' allowing to resolve backscattered light for specific path lengths z in the sample with a resolution ~L_c. Fourier Domain-LCI (FDLCI) employs the fact that the path length resolved scattered intensity profile I(z,t) at each time t is encoded in the acquired spectrum of the interference signal and can be resolved 'instantaneously' using Fourier analysis. By measuring I(z,t), at ~ μs time intervals, temporal intensity fluctuations due to Brownian diffusion and/or flow of suspended NPs are characterized simultaneously over a broad range of pathlengths in the sample (0–3.5 mm in the present NFS system). The resulting auto-correlation functions (ACFs, see Fig. 1) at each pathlength contain information both on diffusion and flow, as described in detail in e.g. (Lee et al., 2012; Weiss et al., 2013). The NFS instrument has proprietary algorithms to accurately separate and analyze the path-length dependent flow contribution and diffusive contribution (the latter providing the size characteristics) in the ACFs. Together these features bring strong advantages for nanoparticle sizing: (i) highly turbid dispersions can be measured due to the ability to selectively analyze singly scattered or multiply scattered photons (ii) intensity decorrelation due to flow can be quantified and compensated for during size characterization (iii) total scattering volume over which signal is acquired can significantly (~10× or more) exceed that in other DLS methods. (iv) high data-information content allows very fast measurements, ~few seconds. (v) the backscatter geometry and optics allows for easy integration as noninvasive process monitoring PAT tool.

The present paper describes measurements using this new technology on a range of nanodispersions, from standard reference suspensions to show validity of the measurements, to inline measurements

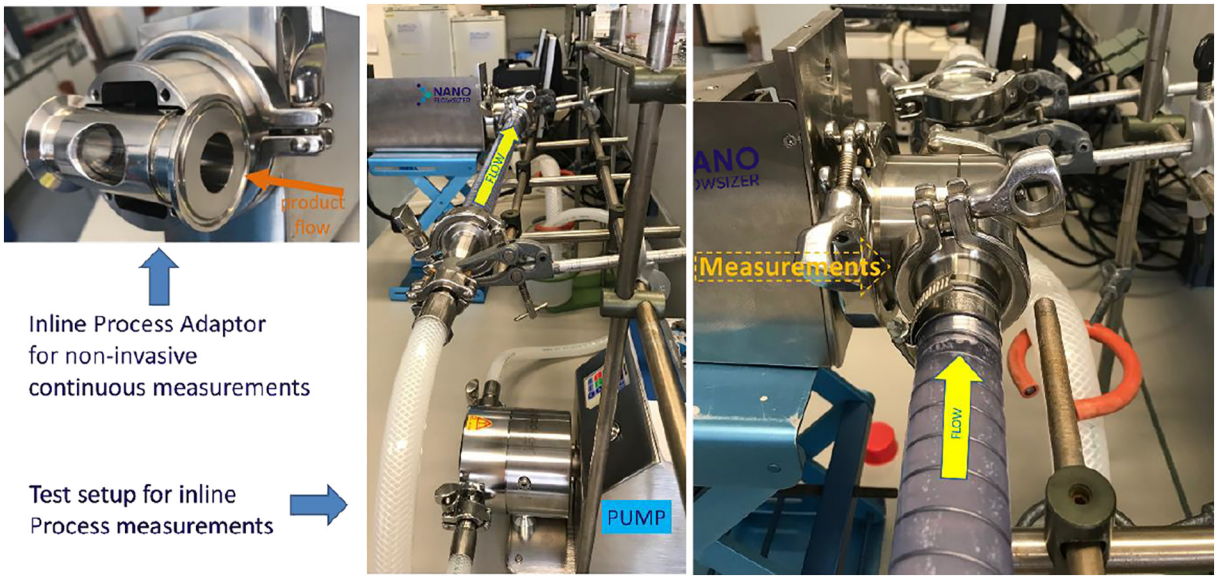

Fig. 2. Experimental setup for inline SR-DLS measurement under flow, including 1 in. inner diameter pharma-grade flow-cell with adaptor connected to the FDLCI measurement head.

of (intermediate) nano-pharmaceutical formulations, using a range of measurement configurations. It should be noted that even for existing standard DLS-based nanosizing techniques, besides their limitations regarding inline/high turbidity measurements, high resolution measurement of complex polydisperse nanodispersions, and particularly correspondence between results of different measurement techniques is a significant challenge in itself and subject of active research (Anderson et al., 2013) (Kestens et al., 2016). While the NFS has successfully been employed to characterize also such complex dispersions (from nano-milled suspensions to e.g. milk, without dilution), the focus in the present paper is on measurement of mono/bidisperse model systems and relatively clean but significantly turbid nano-pharmaceutical formulations.

2. Materials and methods

2.1. Instrumentation and method

The NFS measurement head (see Fig. 2 and Fig. 4) includes a fiber coupler, interferometer and other optics including objective lens and is connected through optical fiber up to several meters in length to the broadband light source (center wavelength 1300 nm) and spectrometer (Thorlabs). In Fig. 2 the measurement head is coupled to an adaptor which connects to a 1 in. inner diameter pharma-grade flow cell with sight glass; for other configurations dedicated adaptors are also available (off-line measurements using sample vials, or at-line/in-line configurations using a micro-flow cell, see Fig. 4). Focus and alignment are automatically adjusted to these specific configurations. Measurements are possible through various transparent windows, e.g. glass with thickness up to at least 5 mm. Accessible optical path lengths range from the glass-suspension interface up to ~3.5 mm in the sample, accessed simultaneously by the FDLCI-method.

The final autocorrelation functions resulting from the flow correction algorithms of the instrument – reflecting only Brownian diffusion-were analyzed using the cumulants method (Frisken, 2001) to obtain *Z*-average (intensity weighted harmonic mean) hydrodynamic diameter and polydispersity index (PDI). Regularized inverse Laplace analysis (CONTIN (Provencher, 1979)) was employed to obtain intensity weighted Particle Size distributions (PSD). Volume based size distributions (and mean) can be calculated from this using Mie theory implemented in the algorithms but requires accurate knowledge/assumptions on the optical properties of particles/droplets and on inter/intra-particle uniformity of these properties. Since this is a well-known source of uncertainty, here analyses are restricted to Intensity based data. Appropriate temperature and host solvent viscosities of the dispersions were employed in the analysis to convert measured diffusion constants to hydrodynamic size using the Stokes-Einstein equation.

2.2. Polystyrene NPs with known particle size

Suspensions of monodisperse polystyrene (PS) particles with a mean diameter of 120 nm (Standard Dev. 5 nm) and 620 nm diameter (Standard Dev. 20 nm), as analyzed by disc centrifuge method, were purchased from Sigma-Aldrich. The original suspensions (concentration 10 mg/ml) were prepared at the concentrations described below using deionized water.

2.2.1. Direct remote measurement

In a beaker glass a 120 nm PS suspension at a concentration of 0.05 mg/ml was prepared. The SR-DLS measurement was performed remotely by positioning the beaker in front of the NFS system at the appropriate working distance ~4 cm, see Fig. 3. The suspension was measured continuously in quiescent condition (A) at an acquisition rate resulting in *Z*-average particle size data every ± 8 s. While continuous measurements proceeded, the suspension was agitated by a propeller mixer at 50 rpm (B). Thereafter, suspension of identical 120 nm polystyrene NPs was added (C), increasing the concentration to 0.5 mg/ml resulting in a more turbid suspension (D). Finally, a 620 nm polystyrene NP suspension was added to create a bimodal size distribution (E) with a total PS concentration of ~1 mg/ml.

2.2.2. Online micro flow-cell measurement

To a turbid, stirred 120 nm polystyrene suspension (0.5 mg/mL) in a beaker, a concentrated 620 nm PS NP suspension was repeatedly added until the total concentration was ~1 mg/ml. An online sampling loop was connected to a micro-flow cell (2 mm pathlength along the optical axis) fitted in an adaptor connected to the NFS instrument, see Fig. 4. A peristaltic pump provided continuous flow (~10 ml/min) in the loop.

After each addition of the 620 nm PS suspension, the mixture was first circulated for two minutes to ensure uniform composition in the sampling loop, after which actual particle size data recording was started while flow in the loop was maintained. After 10 and 25

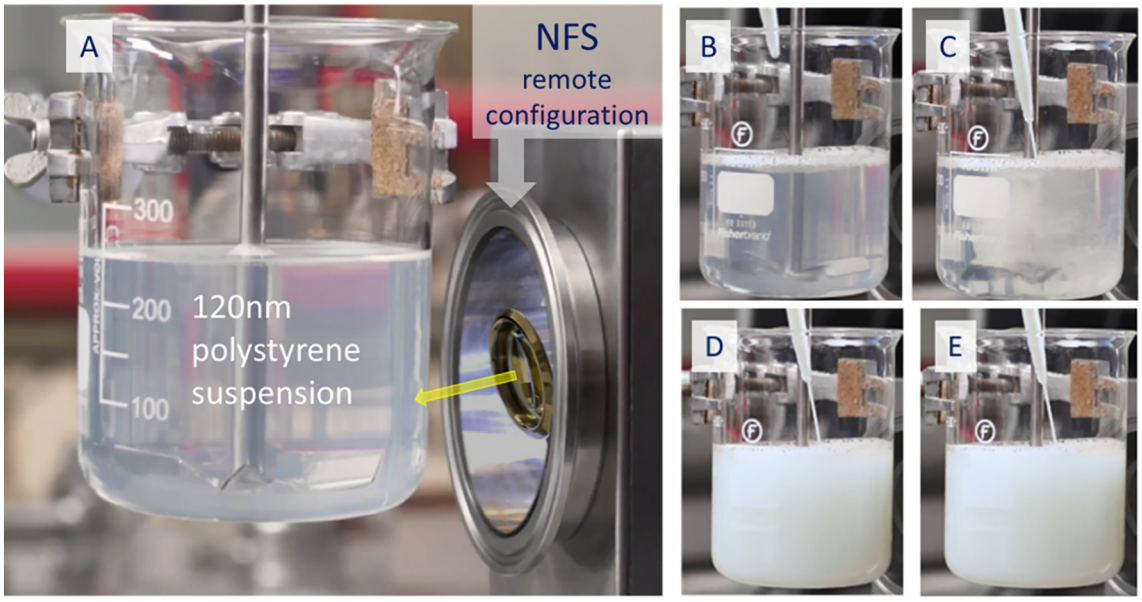

Fig. 3. Remote setup for SR-DLS analysis of quiescent and agitated suspensions: A: unagitated 120 nm PS nanosuspension at low turbidity, B: agitated 120 nm suspension, low turbidity, C: addition of 120 nm PS particles under continuous stirring, D: stirred 120 nm suspension, high turbidty (0.5 mg/ml) at fixed concentration, E: addition of 620 nm PS NPs under continuous stirring.

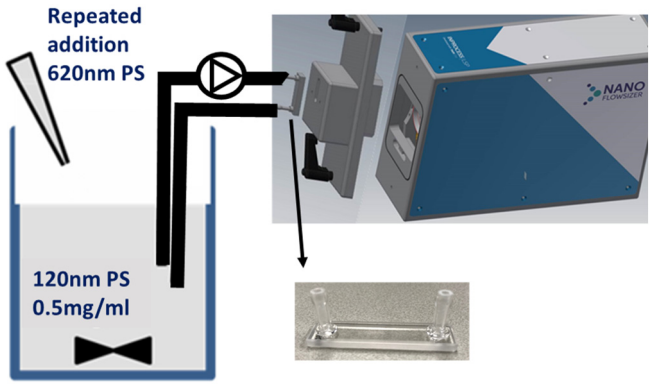

Fig. 4. Schematic of online sampling loop with the NFS instrument connected to a micro-flow cell adaptor.

measurements the peristaltic pump was switched off to record data of the suspension without flow, for comparison with measurements performed under flow. The *Z*-average size and PDI was monitored in real time. At the start, during addition of the 620 nm PS suspension and after the additions, the PSD was calculated as well.

2.3. Inline measurement of nano-emulsification and nanosuspension circulation

A challenge for characterization of various nanoemulsions, e.g. by sampling, is the intrinsic instability and coalescence behavior these emulsions may display (Lovelyn, 2011). This may result in rapid changes of droplet size and PSD when the emulsion is not agitated. Hence, nanoemulsion samples taken during a homogenization/size reduction process may rapidly change and size characteristic obtained using off-line measurements may not be representative for the actual characteristic during processing. In the present case, actual inline size measurements with the NFS integrated in an emulsification loop (see Fig. 5) are performed to monitor droplet size reduction during homogenization/flow (and coalescence during stopped flow) in a pharmaceutical emulsion. NFS inline measurements were also performed for a suspension of polymer- drug-conjugate nanoparticles at different flow rates.

2.3.1. Process description

As the tested products were highly turbid (see below), they were unsuitable for standard direct DLS characterization. In both the emulsification and suspension-circulation experiment, the measurement system was coupled with the 1 in. flow cell with sight glass, integrated in the process circulation loop for inline measurements of droplet or particle size. In the nano-emulsification experiment, the circulation loop contained a high-pressure homogenizer and the typical flowrate was ~200 ml/min. During the suspension-circulation experiment, the employed flowrate ranged up to 2000 ml/min. For the employed 1 in. inner diameter flow cell, this corresponds to a bulk Reynolds numbers of Re~1800; therefore, during all experiments flow in the measurement cell was laminar.

2.3.2. Nano-emulsification

The emulsion consisted of organic-solvent (in which API is dissolved) as dispersed phase (~3% volume fraction) and water being the main component of the continuous phase. For the present purpose, the relevant characteristic of the emulsion is its turbidity (or, similarly, its scattering coefficient), which is comparable to that of a ~6 mg/ml suspension of 120 nm PS particles in water (Scattering coefficient $\mu_s(1300\,\text{nm}) \sim 1/16\,\text{mm}^{-1}$, no significant multiple scattering for pathlengths < 16 mm). The droplet size (*Z*-average) of the emulsion was analyzed by the NFS in real time during homogenization with continuous flow in the measurement cell. Measurement of a single data point took ~15 s maximum, including data-acquisition and processing. The software then provides a continuous trace of droplet size data during monitoring. This allows treatment and representation of the data as usual in statistical process control. Temperature is monitored using a sensor integrated with the flow cell and thus accounted for. Two different data acquisition times were applied: 0.50s and 0.15 s, the latter providing more frequent but possibly less precise update of particle size information. The conducted emulsification runs consisted of continuous homogenization and circulation of the emulsion from a reservoir through the NFS flow-cell, followed by a diversion in which flow in the NFS loop-segment was stopped while NFS monitoring continued, after which the recirculation was continued. Note that due to the unstable

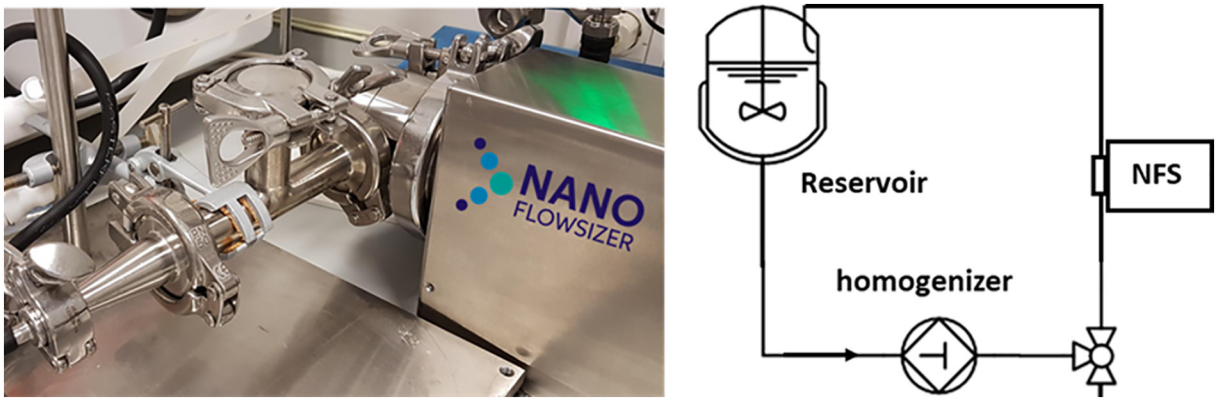

Fig. 5. Left: experimental setup for inline measurement during the nano-emulsification process, with the NFS instrument connected to an adaptor with pharma-grade flow cell integrated in the circulation loop. Right: schematic of the homogenization circuit with the NFS integrated.

nature of the emulsion no reliable offline measurements of the droplet size during processing could be made (see Section 3.2.1).

2.3.3. Nanosuspension recirculation

A stable suspension of polymer-drug conjugate nanoparticles in water was circulated at various flow rates using a peristaltic pump, while particle size was monitored to study robustness of the NFS flow correction algorithms. For the present purpose, the relevant characteristic of the suspension is its turbidity or scattering coefficient μ_s, which is comparable to that of a ~2 mg/ml suspension of 120 nm PS particles in water: μ_s (1300 nm) ~0.02 mm^{-1}. Flow rates of 800, 1600 and 2000 mL/min were applied, and resulting size information was compared to measurements of the same suspension using the NFS without flow (checked by NFS measurements of the same suspension in vials, undiluted and diluted) and with standard DLS data (using a Malvern ZetaSizer) of a sufficiently diluted suspension.

3. Results and discussion

3.1. Polystyrene NPs with known particle size

3.1.1. Direct remote measurement

Fig. 6 shows the measured *Z*-average values for the individual measurements in the beaker configuration. The Z-average values obtained for the 120 nm polystyrene NPs are very close to the reference values. For the static (A) and agitated (B) suspension at low turbidity similar data was obtained. The average size for static and agitated condition based on 10 measurements are 121.4 nm versus 120.3 nm with a precision of respectively 1.3% and 1.4%. Since *Z*-average particle size of both the static and agitated suspension are measured at high accuracy and precision, it can be concluded that the SR-DLS method as applied performs adequate for both situations. The simultaneously recorded backscatter intensity for each measurement shows a very constant pattern as expected. After increasing the concentration of 120 nm particles, the *Z*-average values are still close to the reference value, while the backscatter intensity increases significantly due to the higher turbidity level. During the process of adding 120 nm particles (C), inaccurate results may be obtained due to transient mixing effects. Once the higher concentrated suspension was homogeneous, stable Z-average size data were obtained. Due to the rapid feedback (~8 s per measurement), the system offers opportunities to measure relatively fast changes in size characteristics of NPs during processing. After addition of the 620 nm particles, the Z-average size showed a significant increase as expected. Note that the Z-average here represents the mean of a bimodal distribution. The backscatter intensity increased further after addition of the 620 nm particles, due to the enhanced turbidity. Backscatter intensity may be used to monitor concentration as well, although variation in particle size will also affect the turbidity level.

In principle the PSD can be processed for each measurement within about 1 min. However, processing time of the PSD is very dependent on desired/required precision and complexity of the distribution and suspensions scattering properties. In this experiment the PSD was processed for the highly concentrated monodisperse suspension of 120 nm (D) and for the 120 nm + 620 nm suspension (E). In Fig. 7 the obtained PSD data is shown. As observed, the PSD in the two cases reflects the single and bimodal nature of the PSD of the suspensions. The narrow PSD of the 120 nm particles is correctly reflected by the measurement. For the bimodal mixture, some deviation of the mean size of the populations is observed, the size of the 120 nm particles being somewhat overestimated and that of the 620 nm somewhat underestimated. Deviations such as these are partly intrinsic to the low resolution nature of DLS-based size characterization for complex (e.g. bimodal) distributions (Filipe et al., 2010) (Kestens et al., 2016).

3.1.2. Online micro flow cell measurement

In Fig. 8a and b the *Z*-average size and corresponding PDI are shown as processed in real time. At the start of the experiment (labeled 'A' in the figure), only 120 nm particles were present in the suspension. The corresponding PDI is below 0.1. Analysis of the PSD, shown in red in Fig. 8c, shows a narrow size distribution around 120 nm. With repeated additions of the 620 nm suspension, the *Z*-average size increases linearly with the fraction of large particles added. The PDI increases strongly during the first additions reflecting the strong sensitivity of this quantity to small amounts of added larger particles. After 15 additions (in Fig. 8 depicted as 'B'), the *Z*-average size is still increasing, while the increase in PDI starts to flatten, due to the smaller effect of addition of large particles to a suspension which already has a quite significant size dispersity. The PSD for composition 'B' displayed in green in Fig. 8(c), shows presence of larger particles in the suspension although the size range is relatively broad (± 400–900 nm). The uncertainty in the obtained PSD data is related to change in obtained correlation function due to the bimodal distribution. In this case, situation 'B' corresponds to detectable change form monodisperse to bimodal, but with a relative low accuracy for actual PSD of the larger particles. After 36 additions a total concentration of ~1 mg/mL was reached (labeled 'C' in Fig. 8a). The Z-average size showed a constant increase while the PDI saturates to a constant high level as expected. The associated PSD shows that the 620 nm population is more accurately resolved in this case compared to situation 'B' due to the higher concentration of 620 nm particles. The data measured without flow confirm the data (both Z-average and PDI) taken during flow.

The NFS offers unique possibilities to monitor particle growth/change in a continuous mode on a timescale of seconds. In principle, corresponding PSD data can be obtained for each measurement, but for higher precision/accuracy, averaging multiple measurements may be required. In practice, PSD data can be obtained in real-time every 1 to

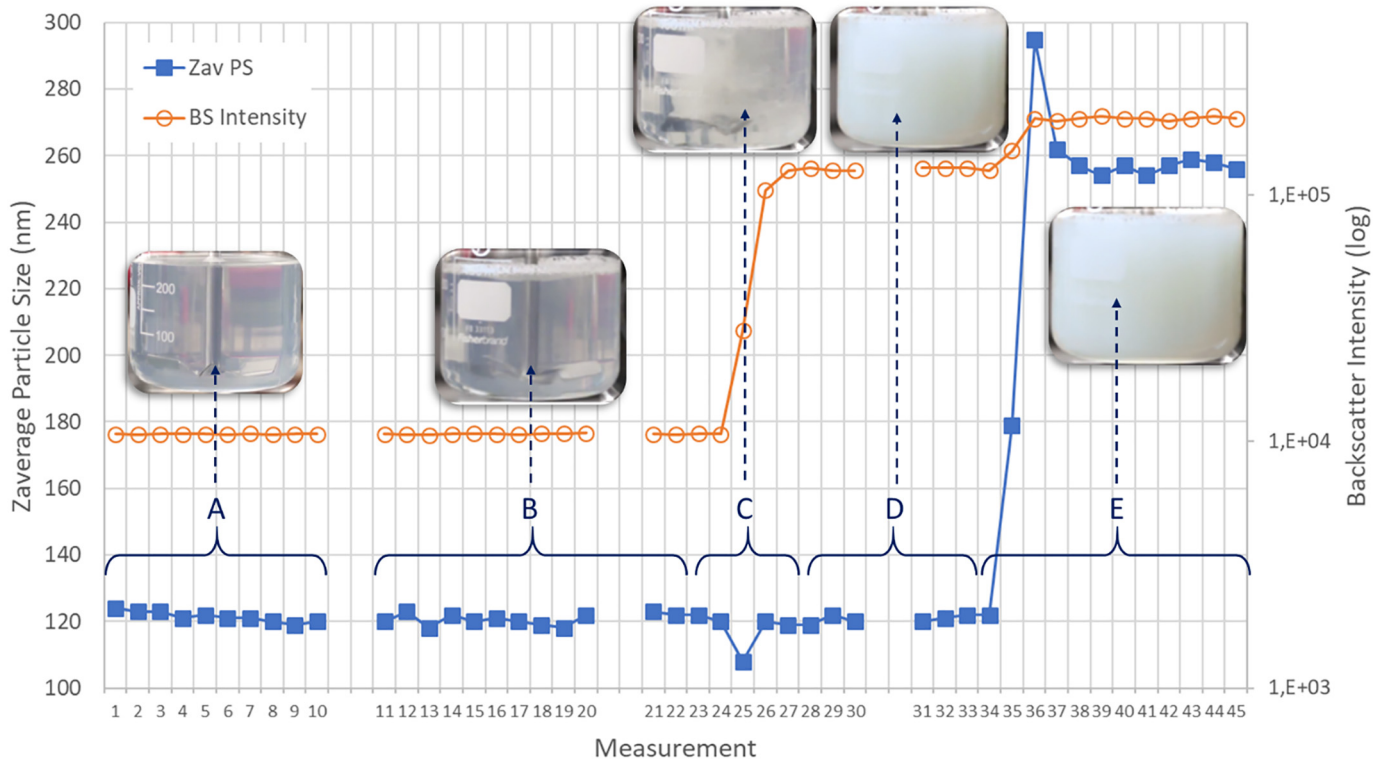

Fig. 6. Z-average particle size of individual measurements of 120 nm polystyrene particles at low turbidity under static (A) and stirred (B) conditions, during (C) and after addition of high concentration 120 nm polystyrene particles (D) and after addition of 620 nm particles (E), all under stirring.

2 min depending on scattering/turbidity properties of the dispersion and complexity of the distribution.

3.2. Inline measurement of nano-emulsification and nanosuspension circulation

3.2.1. Nano-emulsification

Fig. 9. shows data obtained during a nano-emulsification run using the setup shown in Fig. 5, monitored for ~80mins, during which the flow in the NFS section of the loop is halted for a period of ~4 mins. At the start of the process the mean droplet size is ~200 nm. Droplet size subsequently shows a weak decrease on prolonged homogenization, which sets in most clearly after ~20 mins. At t = 39mins when the circulation in the measuring-section of the loop stops, the droplet size measured in that section shows a clear increase from ± 170 nm to ± 350 nm for the duration of the diversion. When circulation is activated

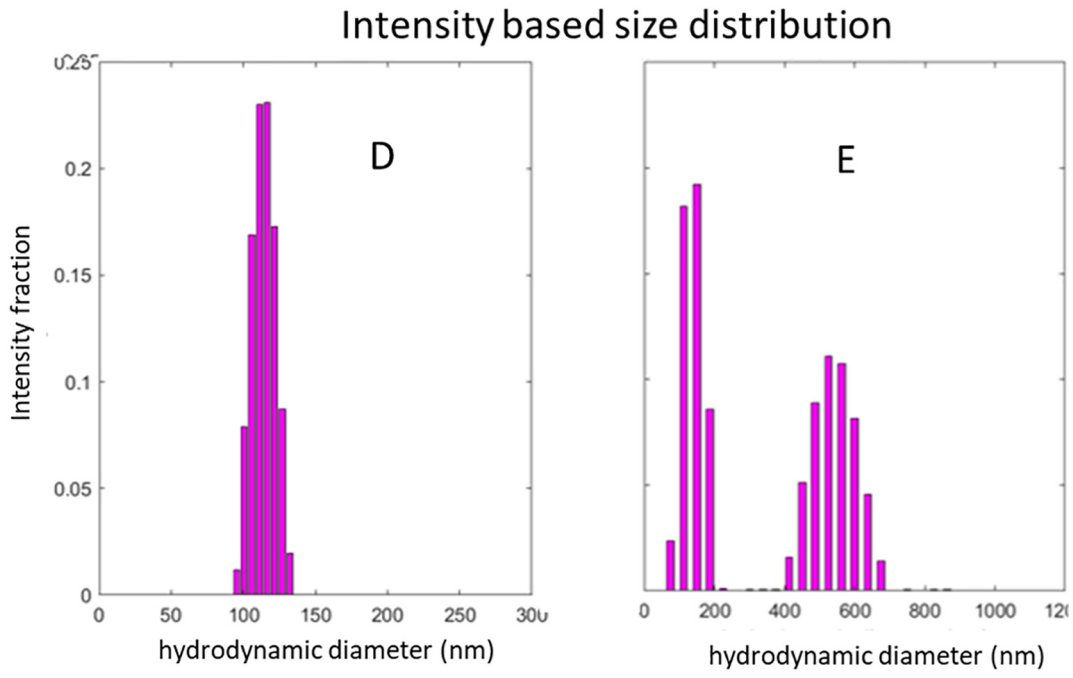

Fig. 7. PSD data of the turbid monodisperse 120 nm suspension (D) and 120 nm + 620 nm suspension (E), both under agitated conditions.

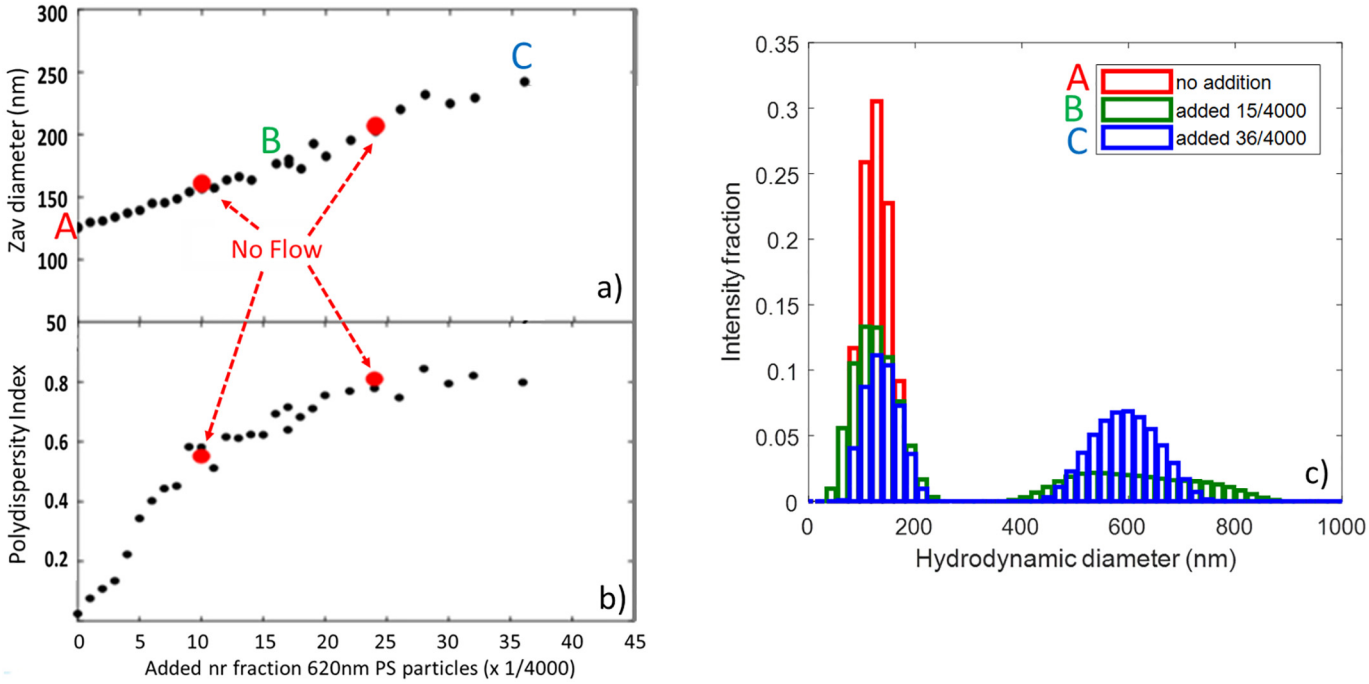

Fig. 8. Continuous online-sampled Z-average size (a) and polydispersity index (b) of the 120 nm suspension with repeated additions of 620 nm particles, measured during flow using the micro flow cell. (c) Measured PSD of the suspension at the three different compositions labeled by 'A', 'B' and 'C' in Fig. 8(a).

again, the z average droplet size almost instantaneously decreases and starts to retrace the slow decreasing trend observed before the diversion. The size increase (reduction) seen before (after) the diversion is *not* a measurement artefact: the NFS algorithms provide separate analysis of the flow and the diffusive (Brownian) motion and the latter provides the size characteristics. This is further confirmed by the absence of any discontinuity at t = 39mins when the flow is suddenly stopped. These data clearly show how the NFS inline monitoring capabilities can be employed for better understanding and control of nanodispersion processing.

3.2.2. *Nanosuspension recirculation*

The data measured during recirculation of the nanosuspension at various flow rates are shown in Fig. 10. Note that the measured size is essentially independent of flow rate, which is an important characteristic for a successful inline measurement method. The data agree with the NFS data measured without flow, measured in a vial and are also in very reasonable agreement with size data measured using standard DLS after dilution of the sample. It should be emphasized that a flow independent size is only expected for a suspension which is highly stable, in which interactions between particles e.g. those causing sticking/aggregation, are essentially absent. Flow-induced reduction of particle size (e.g. shear induced de-aggregation) may well be observed in other,

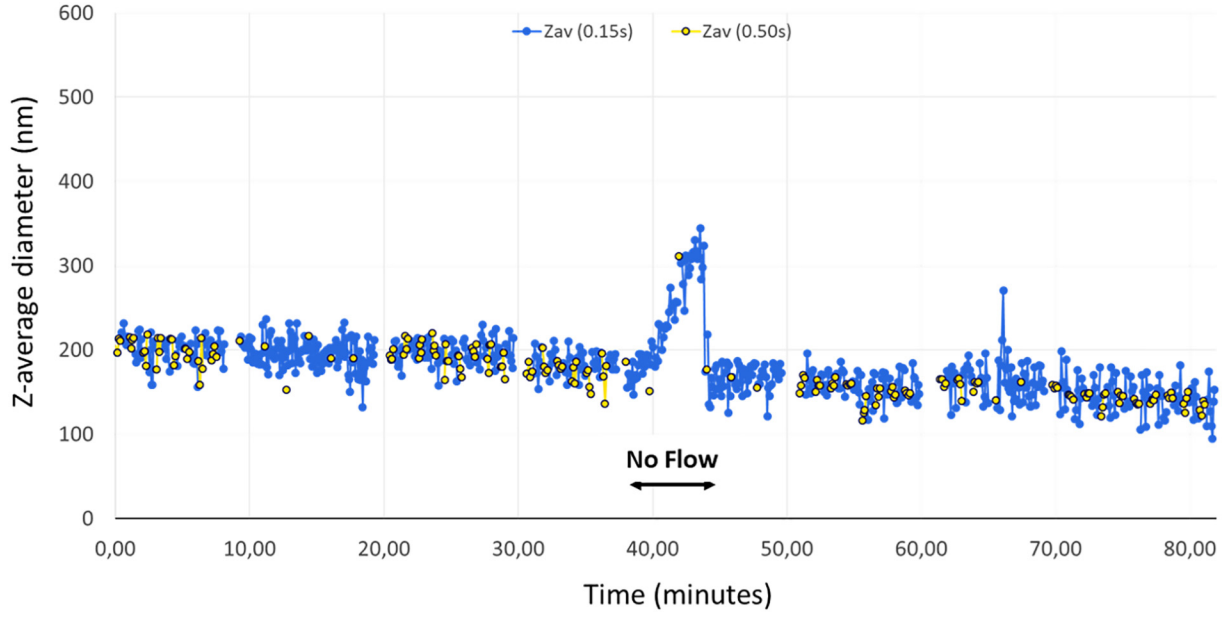

Fig. 9. Mean hydrodynamic diameter of the nanoemulsion measured during processing. At t = 39mins the circulation is interrupted for ~4mins, leading to an immediate increase in droplet size.

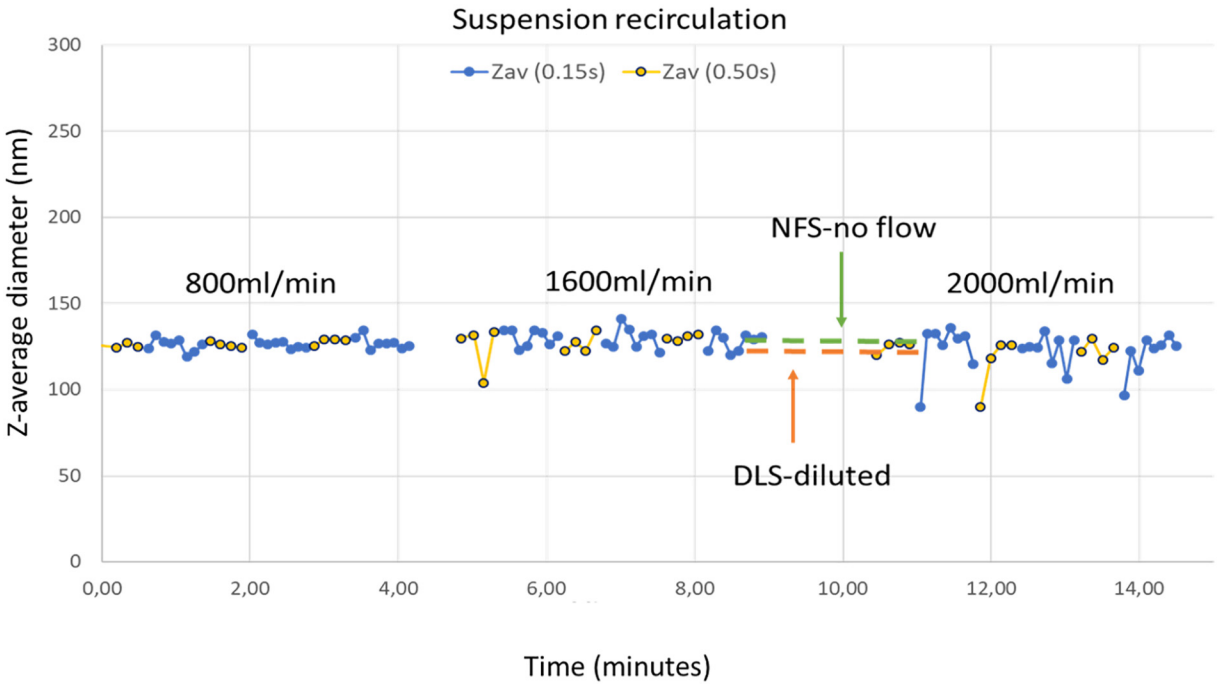

Fig. 10. Particle size during recirculation of a polymer-drug conjugate NP suspension at three different flow rates. Also shown are reference data of the same sample in a vial measured by the NFS (green dashed line) and diluted sample measured using standard DLS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

less stable suspensions, and thus the effective size under flow can in general provide valuable information on stability or other properties of the suspension, further detailed below.

4. Discussion

For the applications described above, the optical properties and flow conditions during measurements, while unsuitable for measurement by standard technologies, are clearly within the working range of the NFS. Yet limitations do exist, both regarding flow speed and turbidity. As for the flow-rate: for the NFS algorithms to be able to extract sufficient information on Brownian motion, the shear rate near the confining wall should not exceed certain limits. Physically this is because, for accurate measurement of diffusion (and thus size) under flow, the passage time during which particles travel though the NFS beam should not be much less than the time to diffuse over the wavelength of light (the 'Brownian diffusion time'). High flow thus first limits the measurement accuracy for large particles. Actual performance characteristics beyond the regimes discussed in the present paper are currently under study. If larger volume flow is required for inline use (e.g. > 100 l/h, depending also on the properties of the dispersion) flow cells with diameter > 1″, in which the wall shear rate is reduced, may be employed.

As for optical sample properties: despite the visible strong turbidity of investigated dispersions, their scattering strength at the NFS wavelength of 1300 nm is limited, and thus multiple scattering is not observed within the NFS pathlength range for the investigated systems. In fact, for SR-DLS analysis of samples with the NFS, dispersions with significantly higher scattering coefficient, exceeding $\mu_s(1300\,nm)$ $\sim 5\,mm^{-1}$ are suitable and have been measured successfully (e.g. 400 nm PS at ~2.5% w/v as shown in Figs. 1). For even higher scattering/turbidity, e.g. when multiple scattering sets in for pathlengths < 100 μm ($\mu_s > 10\,mm^{-1}$), size information can still be extracted from the NFS data using DWS principles (Weitz et al., 1993) which will be discussed elsewhere.

Regarding accessible NP size range of the present NFS: the lower limit is set by the NFS spectral acquisition rate, which sets the maximum measurable diffusion rate. For aqueous suspensions the associated lower size limit is < 15 nm, provided such small NP's have sufficient scattering strength. The upper size limit is several microns, depending on the extent of sedimentation for such large particles.

A few additional comments are in place here. Firstly, for aspherical particles, not considered in the present paper, the size obtained in quiescent conditions is an effective rotationally averaged size (Han et al., 2006), (that of a sphere with the same diffusion constant as the particle) which is well known and commonly applied in DLS. Interestingly, since the NFS (in standard configuration) measures diffusion perpendicular to the flow, shape anisotropy might be detectable by flow alignment which changes the effective size for diffusion. Second, for concentrated possibly strongly interacting samples, the NFS allows to obtain a wealth of additional information, such as general shear induced changes in diffusion of the NPs (e.g. due to reorganization of the nano-structure in concentrated suspensions) (Besseling et al., 2007) (Koumakis et al., 2015), rapid aggregation or particle growth processes in formulation preparation or in NP synthesis, flow induced breakup of NP aggregates, and information on optical properties and heterogeneity of samples. This makes the technique relevant not only as PAT tool in industrial formulation/process development and process monitoring, but also as tool for fundamental research.

5. Conclusions

This paper has introduced a new instrument and measurement method for non-invasive inline, real-time nanoparticle size characterization in flowing, turbid nanodispersion, using Spatially Resolved Dynamic Light Scattering based on Low Coherence Interferometry. Using reference polystyrene suspensions at various turbidity levels it was shown that this novel technology provides accurate and precise measurements under flow conditions at high frequency (~5–10 s per measurement). Additionally, it was shown that the method can monitor size characteristics of a pharmaceutically relevant nanoemulsion and nanosuspension in an industrially relevant pilot scale emulsification process. The instrument shows versatile application opportunities, as illustrated by measurements in various flow geometries at different

scales, such as in a stirred beaker, in a micro-flow cell and, with a process adaptor allowing measurements during flows exceeding 1 l/min. This new technology therefore offers various new opportunities to gain better understanding and control of NP based processes, both in pharma and beyond. The ability to measure at high frequency without sample preparation allows to gain new insight in evolution and dynamics of nanodispersions on time scales and in geometries previously inaccessible. This extends the possible range of applications also to more fundamental nanodispersion research (e.g. in synthesis/stability etc.) as well as for example high throughput characterization.

Conflict of interest statement

The authors are all affiliated with InProcess-LSP, the patent holder/supplier of the Nanoflowsizer instrument. Rut Besseling, Michiel Damen, Gerben Wynia and Ad Gerich are shareholders of InProcess-LSP.

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