Rapid Communication

# Taylor Dispersion of Inorganic Nanoparticles and Comparison to Dynamic Light Scattering and Transmission Electron Microscopy

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## ARTICLE INFO

## ABSTRACT

Taylor dispersion analysis (TDA) is an analytical method that has so far mainly been utilized to determine the diffusion coefficient of small molecules, and proteins. Due to increasing interest in nanoscience, some research has been done on the applicability of TDA towards characterizing nanoparticles. This work aims to expand this knowledge and give insight into the range for which TDA can be used for nanoparticle characterization, focusing on various materials and sizes. The TDA setup shown in this work was successful in characterizing all engineered metallic, non-metallic nanoparticles, and proteins tested in this work. Results were compared to dynamic light scattering and electron microscopy, and were in good agreement with both methods. Taking into consideration the wide range of nanoparticle sizes that can be characterized, the minimal sample preparation, and sample volume, required and the simplicity of the method, TDA can be considered as a valuable technique for nanoparticle characterization.

When looking at the huge array of properties that nanoparticles (NPs) possess, it is not surprising that they have found their way into a multitude of scientific research areas, and industrial applications [1]. Interesting NP phenomena are mostly governed by their size, and for many applications it is imperative that the NPs not only have a very specific size but also display a narrow particle size distribution [2]. For example, the heating properties of superparamagnetic iron oxide nanoparticles (SPIONs), which are being investigated as mediators of hyperthermia in cancer treatment, are dependent on their size and size distribution. Therefore, the characterization of nanoparticle size is crucial to ensure their functionality [3]. For this purpose, several analytical methods such as dynamic light scattering (DLS), NP tracking analysis (NTA), UV–Vis spectroscopy, field-flow fractionation, analytical ultracentrifugation, and transmission electron microscopy (TEM) have been utilized to characterize NP sizes and size distributions [4–8]. Each method has its advantages and disadvantages, and a combination of techniques is typically recommended to adequately characterize NPs [4]. For example, TEM provides information about NP core sizes, but cannot evaluate NP hydrodynamic diameters. Conversely, DLS and NTA can evaluate particle hydrodynamic diameter and colloidal stability, but are limited by the quality of light scattering and require a deeper knowledge of the theory and model-fitting to properly analyze the raw data. With scattering-based techniques, the limit of detection for NPs

depends on the sensitivity of the detection of scattered light, and factors such as the material refractive index, particle size, shape and the wavelength used for detection. Furthermore, standard DLS measurements struggle with analyzing NPs in complex environments (e.g. protein-crowded suspensions, high particle concentration etc.) or samples where only limited sample preparation is possible [9–11]. There are possibilities to overcome some of these problems, for example by the use of depolarized dynamic light scattering, as described by Balog et al. [12]. These methods, however, require either an advanced level of data analysis or expensive instruments.

We show here that Taylor dispersion analysis (TDA) is very promising as a NP characterization technique and, interestingly enough, has not been more routinely used for this purpose. Our work includes a systematic variation of NP size and core material to determine the confines in which TDA can be used for NP analysis. We focused primarily on metal NPs (gold), metal oxide (superparamagnetic iron oxide, SPIONs) NPs, and silica NPs, and have also included the protein, bovine serum albumin (BSA), which is a standard for protein characterization. We measured all particles using the setup shown in Fig. 1. For comparison, we used standard TEM and DLS and show the cases where these methods reach its limitations to point out the advantages for the use of TDA.

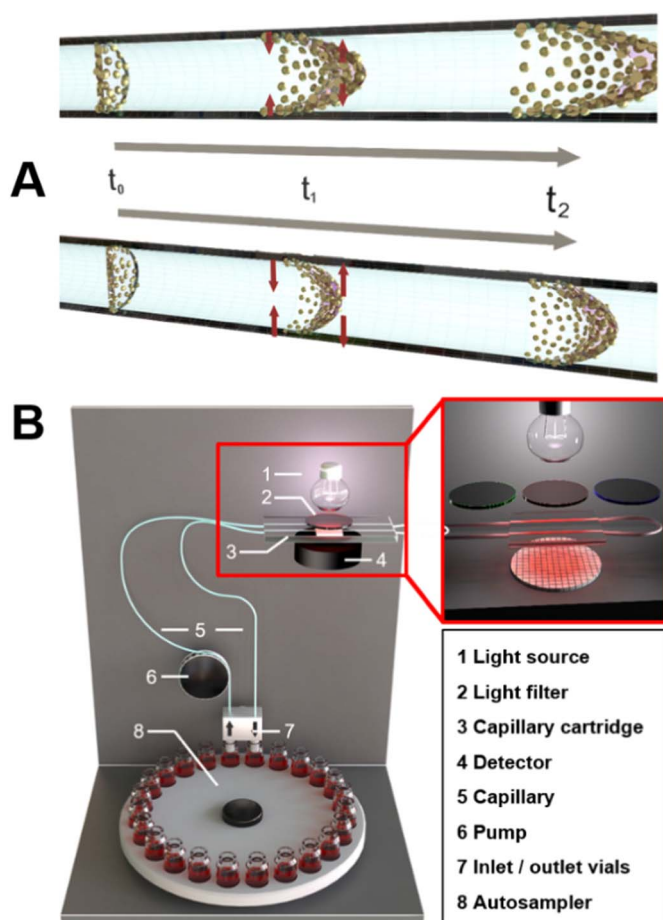TDA, originally described by Taylor in 1953 and further developed

**Fig. 1.** A) Schematic depicting the principle of TDA and the difference in dispersion profile when comparing particles of different sizes. Due to the laminar flow (convection), the initially very narrow band of NPs undergo increased radial migration, causing a continuous band-broadening effect. The diffusion coefficient can be determined by comparing the band broadening at the time points $t_1$ and $t_2$, by implementing calculations explained in the supplementary information (S8). Small particles with higher diffusion coefficients counteract convection better than large particles with lower diffusion coefficients, resulting in comparably narrower band. B) Setup of the TDA instrument used in this work showing the important components. The zoom-in shows the wavelength-selective detection, due to exchangeable wavelength filters, and the pixel-based detection system.

by Aris, is an analytical technique which enables the determination of diffusion coefficients and hence hydrodynamic radii of molecules. This can be extended to virtually any solute, including nanoparticles, provided that the experimental setup is adequate [13]. Here we could determine NP diffusion constants by the band broadening of a solute peak travelling through a capillary. The hydrodynamic diameter can then be calculated from the diffusion coefficient via the Stokes-Einstein equation (Fig. 1). Taylor-Aris dispersion occurs, when an initially homogenous NP concentration band is dispersed due to the convection caused by a pressure-driven laminar fluid flow. Laminar flow results in the well-known parabolic function of the velocity of the flow-profile across the cross section of the tube. This in turn creates a radial concentration gradient at both the front and back of the analyte band, which induces a net (mass) transport of the NPs via diffusion (Fig. 1A). The consequence is that NPs at the front of the band migrate towards the capillary walls, and particles in the rear migrate towards the capillary center. In most Taylor-Aris dispersion experiments, the combination of diffusion and convection results in a band profile that is well described by a Gaussian function, where the width of the Gaussian function contains the information about particle size. The relevant equations and the boundary conditions are found in section SI8

(Supporting information). Depending on the diffusion coefficient of the NPs, the distribution compensation at the concentration front, caused by convection, will vary. In other words, larger NPs will be dispersed more strongly by convection than smaller ones, and by detecting the band broadening of these concentration fronts, and obtaining so-called Taylorgrams of the NPs, one can determine hydrodynamic sizes by fitting the Taylorgram against a Gaussian function [13–15].

In the past, TDA has mainly been used to observe chemical reactions [16,17], or to determine the diffusion coefficient of small molecules, proteins, and polymers [10,11,18–20]. With the increasing interest in nanomaterials, some work has been done in the past [9,13,15,18,21–24] to investigate whether TDA can be used for NP characterization. Previous studies have shown several advantages of this method, e.g. short measurement times [9,11,17], small sample volumes (i.e. nanoliters) [11,25]. Furthermore, the method is not only limited to spherical NPs but can be applied to other shapes, such as rods [18]. However, research focused on NP characterization with TDA has been focused on polymeric samples with a limited number of samples and few different sizes [9–11,18,25]. Due to the scope of this manuscript we opted to compile a list of additional literature concerning Taylor Dispersion and nanoparticle analysis and add it to the supplementary information rather than continue here. (see S19).

The instrumental setup of our TDA experiments is graphically depicted in Fig. 1B. The essential part is the capillary with two detection windows burned at specific areas to insure that the conditions for TDA are met, as described by Taylor [13]. Due to the advantageous setup of having two detection windows instead of one, as shown by Ye et al. [26], fluctuations in flow rate and injection parameters, such as pressure ramp, during repetition measurements, do not affect the analysis as strongly. Thus, the evolution of the dispersion between the two detection windows can be quantified and the NP size can be determined. At this point we would like to point out that the boundary conditions mentioned by d'Orlyé et al. [13], concerning the necessary capillary length etc. required for TDA analysis, was guaranteed for all particle systems, with the exception being the largest silica particles (SiO$_2$-L), which did not reach the $\tau$ limit of 1.4 at the first detection window. The reason for this is explained at a later point in this manuscript.

The sample is pumped through the capillary by applying pressure with a syringe pump to the sample vials. A Xenon lamp generates light (UV–Vis-NIR), which passes through exchangeable emission filters prior to passing through the capillary and reaching the ActiPix UV–Vis area imaging detector, which detects light in such a way as to prevent scattering and false signals due to light scattering at the capillary walls. (see S7 for details). It should be noted here that depending on the NPs measured, different emission filters were used (Fig. 1B).

The first step of the work was the synthesis of 3 types of NPs. The details of the synthesis are reported in sections S1-S3 (Supporting information). Small (S-), medium (M-) and large (L-), respectively silica (SiO$_2$) and gold (Au) NPs were synthesized in addition to two sizes (small, S, and large, L) of superparamagnetic iron oxide (SPIONs). Fig. 2 summarizes representative TEM micrographs, size histograms, and the average mean of NPs used in this study. The synthesized NPs generally show moderate polydispersity, except for the SiO$_2$-S sample. This is attributed to the different synthesis route, where the concentration of L-arginine as catalyst has a strong influence on the polydispersity [27]. We have used BSA throughout our study, since it has previously been used as proof-of-concept for TDA [9–11]. As can be expected, no adequate TEM micrograph could be obtained for BSA due to insufficient contrast.

The following section is divided into segments with increasing particle or measurement complexity, starting with the easily measured BSA. We opted not to convert results obtained by DLS, TDA, and TEM into any weighted distribution system, as suggested by a recent report from the European Commission [28], since this can induce bias. The results of the necessary mathematical equations strongly depend on assumptions concerning BSA particle size distributions. This does not