Examining the Exciting Potential of Electrical Asymmetrical Field-Flow Fractionation

Introduction

In recent years the industrial applications of nanoparticles have been growing immensely. Medicine, healthcare, electronics, information technology, energy, and environmental remediation are a few examples among the major industries that nanoparticles help improve or revolutionize [1]. The application of nanoparticles in medicine has produced solutions for disease prevention, clinical diagnosis, and therapy [2]. Extensive research is being conducted in cancer treatment where nanoparticles are utilized as drug carriers [3]. This allows researchers to find new therapeutics which, by delivering the drug directly to the cancerous cells, reduce the risk of damage to healthy tissue. Nanoparticle uptake by cells is highly size and surface-charge dependent [4]. Therefore, size and charge characterization of nanoparticles is very important. Batch mode analytical techniques such as dynamic light scattering and electrophoretic light scattering are widely used to measure nanoparticle size and charge. Although they can provide rapid information, their use is mostly limited to monodisperse and homogeneous suspensions [5].

In addition to the need for characterization of engineered nanoparticles, another emerging application is analysis of nanoplastics. Typically formed by the weathering and breakdown of plastic materials in the environment, nanoplastics are challenging to separate and characterize by commonly used techniques such as dynamic light scattering or size exclusion chromatography [6].

Asymmetrical Flow Field-Flow Fractionation (AF4) is a high-resolution separation technique for characterization of nanoparticles, suitable for both monodisperse and polydisperse suspensions. In addition to the primary separation field (crossflow), an electrical field can also be applied across the separation channel in AF4 which shifts the elution time of charged species based on their charge polarity and magnitude.

In this whitepaper, the Electrical Asymmetrical Flow FFF (EAF4) system was utilized to separate and characterize:
- The electrophoretic mobility of silver nanoparticles with different surface coatings
- A nanoplastic (polystyrene) sample
- The electrophoretic mobility of the NIST mAb RM8671

How is Surface Charge Measured Using EAF4?

Particles introduced into the AF4 channel and moving through it are forced toward the accumulation wall (semi- permeable membrane) with the velocity induced by the field (crossflow) as shown in Figure 1. The field-induced migration is counter-balanced by the diffusion of the particles. The elution time of the particles is mainly related to the ratio of the field- and diffusion- induced migrations. In EAF4 the accumulation wall has a net surface charge induced by the electrical field which shifts the elution time of a charged particle based on the direction and strength of the electrical force applied on the particle. For example, a repulsion between the channel bottom and the nanoparticles will cause the nanoparticles to have a higher probability of being located in the faster flow streams further from the channel bottom and be eluted earlier.

The electrophoretic mobility of the particles can be calculated from the shift in the retention time due to the electrical field. The analysis requires at least three runs, where particle net drift velocity (related to the retention time shift) is plotted against applied electrical field. The electrophoretic mobility can then be calculated from the slope of the linear fit.

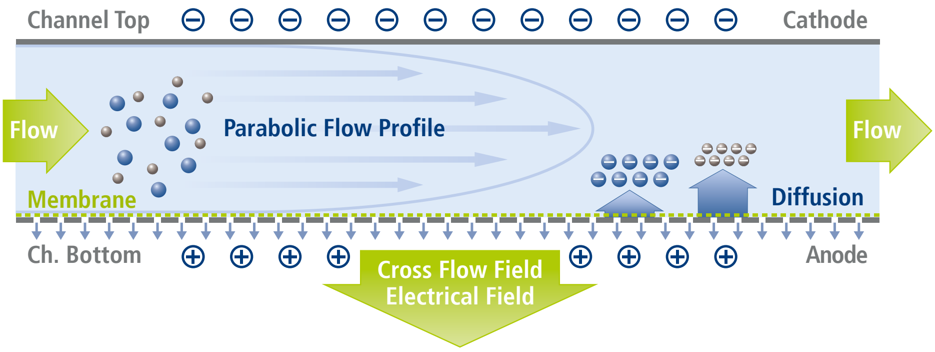

Figure 1. Schematic representation of an Electrical Asymmetrical Flow FFF channel used for analysis of negatively charged particles where the accumulation wall (membrane) was chosen to be the anode. The polarity of the channel walls can be changed readily according to the particle charge type and desired direction of the electrical field.

Silver Nanoparticles

The electrophoretic mobility of 50 nm silver nanoparticles with three different surface coatings: citrate, polyvinyl pyrrolidone (PVP), and tannic acid, was measured using EAF4. Figures 2a-2c show the fractograms of 50 nm Ag NPs coated with citrate, PVP, and tannic acid obtained at a constant crossflow field and varying electrical fields.

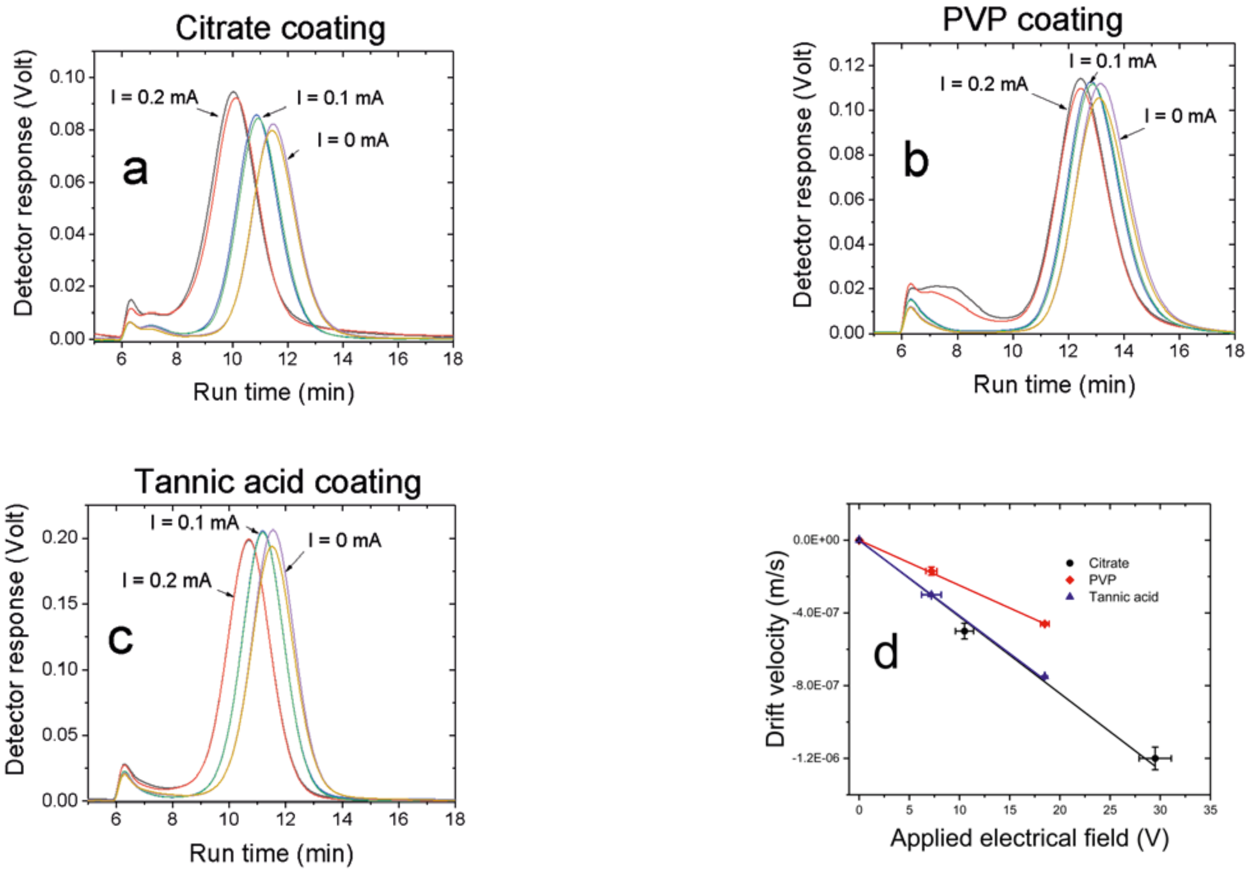

Figure 2. UV-based fractograms of 50 nm Ag NPs with different surface coatings. a) citrate coated, b) PVP coated, c) tannic acid coated, d) plot of net drift velocity versus applied electrical field.

The retention time shifts were used to generate the plot of net drift velocity versus applied electrical field for the three samples shown in Figure 2d. The results showed that both the citrate and tannic acid coated Ag NPs have a measured electrophoretic mobility of approximately -4 µm cm V^{-1} s^{-1}, whereas the PVP coated Ag NP has a measured electrophoretic mobility of -2.5 µm cm V^{-1} s^{-1}. As these test sample NPs are reasonably monodisperse, we can, in this case, validate the accuracy of the analysis by measuring the electrophoretic mobility of the unseparated Ag NP samples using a traditional batch zeta potential analyzer (Zetasizer Nano, MalvernPanalytical, UK). Figure 3 shows the comparison of the two techniques, where the measured electrophoretic mobility values are in good agreement.

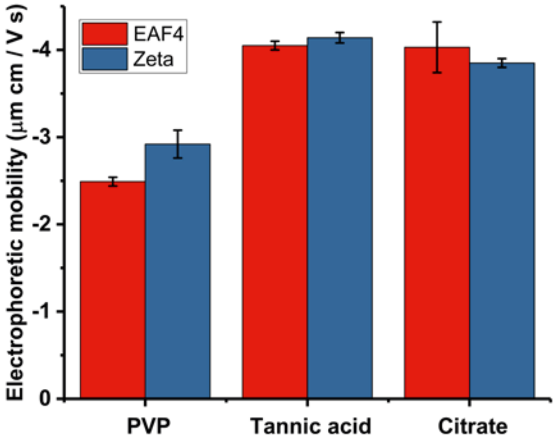

Figure 3. Comparison of the electrophoretic mobility values obtained from EAF4 and zeta potential measurements.

Nanoplastics

Plastic micro- and nanoparticles are increasingly in the headlines, particularly when discussing marine pollution [7], but also with regard to their potential impact on human health [8]. Typically formed by the weathering and breakdown of plastic materials in the environment, nanoplastics are challenging to separate and characterize by commonly used techniques such as dynamic light scattering or size exclusion chromatography. Here we present data on separation of polystyrene nanoplastics, and demonstrate how Electrical Asymmetrical Flow FFF (EAF4) can be used for simultaneous size separation and particle surface charge measurement.

A mixture of two polystyrene latex particles (nominal diameters of 61 nm and 125 nm, respectively) was used as a proxy for a polydisperse nanoplastics system. This mixture was separated by EAF4 using four different electrical field conditions enabling measurement of the electrophoretic mobility and thus the surface zeta potential of both particles. In addition, Multi Angle Light Scattering (MALS) was used as a detector to simultaneously collect information about the size of both particles.

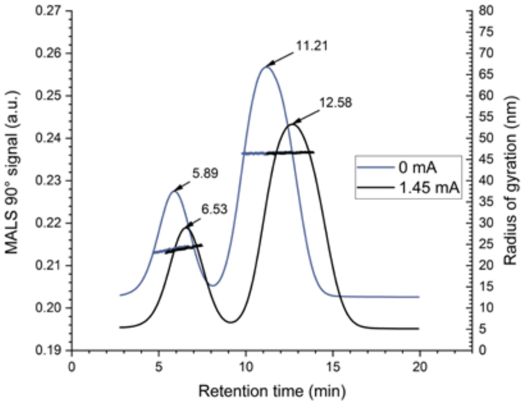

Figure 4. EAF4-MALS fractograms of the investigated nanoplastics particle mixture with and without application of an electrical field (black and blue graph, respectively). The blue and black dotted lines display the radii of gyration obtained from MALS indicating no influence of the electrical field on the particle size.

Figure 4 displays two EAF4-MALS fractograms. In the first fractogram (blue graph) separation was achieved solely by the cross flow field without application of an electrical field (0 mA) while in the second fractogram (black graph) an additional electrical field (1.45 mA) was applied. It can be clearly seen that the electrical field induced a measurable shift in the retention time due to the surface charge of both particles. At the same time, the measured size of both particles (Radius of gyration, R_g, blue and black dotted line) remained unaffected highlighting no influence of the electrical field on the stability of the particle mixture (Table 1).

Polystyrene latex nanoplastics mixture	Nominal diameter, TEM (nm)	Radius of gyration, MALS (nm)	
		at 0 mA	at 1.45 mA
	61 ± 4	24.0 ± 0.1	24.2 ± 0.3
	125 ± 3	46.4 ± 0.1	46.6 ± 0.1

Table 1: Radii of gyration for both polystyrene latex particles derived from EAF4-MALS with and without application of electrical field (0 mA and 1.45 mA, respectively).

In order to derive reliable data about the electrophoretic mobiliy and zeta potential of a sample, repeated EAF4 measurements under similar cross flow conditions, but different electrical fields, need to be performed.

Figure 5 displays the EAF4-MALS fractograms of the investigated nanoplastics mixture obtained under four different electrical field strengths. By measuring the shift in retention time and relating it to the applied electrical field, the electrophoretic mobility and zeta potential of the particles can be calculated.

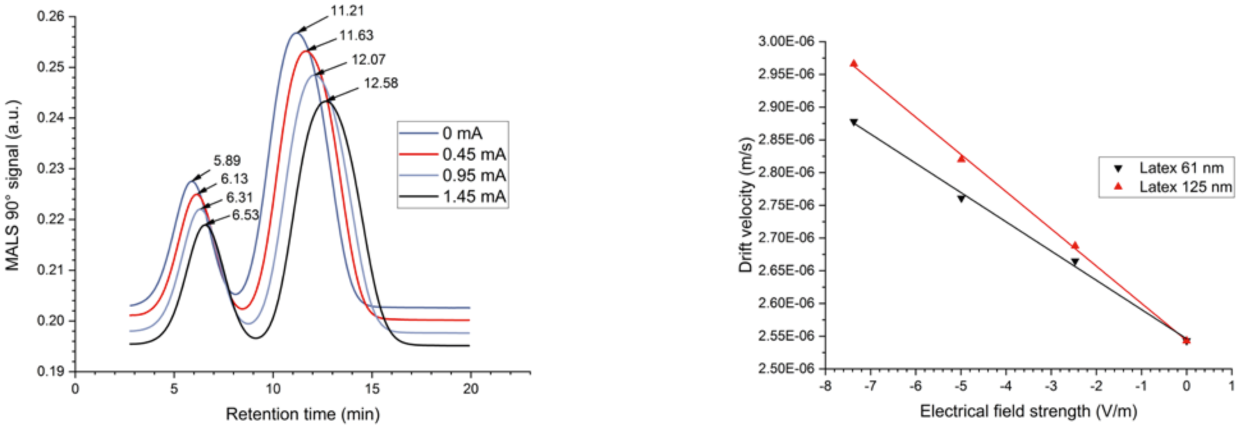

Figure 5: EAF4-MALS fractograms of the investigated nanoplastics particle mixture obtained for four different electrical field strengths (left). Differential velocity versus electrical field strength plot to determine the electrophoretic mobility of the two nanoplastic particles in the mixture (right).

Comparing the EAF4 results with data obtained from bulk zeta potential measurements clearly highlight the advantage of EAF4 for polydisperse samples, particularly when sample constituents exhibit different surface charges (Table 2).

Polystyrene latex nanoplastics mixture	Nominal diameter, TEM (nm)	Electrophoretic mobility, EAF4 (1E-8 $m^2 V^{-1} s^{-1}$)	Zeta potential, EAF4 (mV)	Zeta potential, bulk measurement (mV)
	61 ± 4	-4.31 ± 0.06	-55.2 ± 0.8	-62.1 ± 1.0
	125 ± 3	-5.11 ± 0.23	-65.5 ± 3.0	

Table 2: Overview of the electrophoretic mobilities and zeta potentials of the two investigated nanoplastic particles calculated from EAF4 measurements. Zeta potentials are calculated using the Smoluchowski approximation and compared with data obtained from bulk zeta potential measurements.

NIST Monoclonal Antibody

Per the US National Institute of Standards and Technology [9]: "The NIST monoclonal antibody (NIST mAb) reference material, RM 8671, is intended for use in evaluating the performance of methods for determining physicochemical and biophysical attributes of monoclonal antibodies. It also provides a representative test molecule for development of novel technology for therapeutic protein characterization. The RM is intended for a variety of uses that may include system suitability tests, establishing method or instrument performance and variability, comparing changing analytical test methods, and assisting in method qualification."

The NIST mAb was analysed with EAF4-UV-MALS in phosphate buffered saline (PBS) using a control run (no electric field) and two varied electric field strengths (Figure 6). The retention time shifted toward earlier elution with greater negative charge on the channel bottom, indicating the NIST mAb is negatively charged in PBS. The shift in retention time was related to electrophoretic mobility, which was measured to be -1.68 ± 0.05 µm cm $V^{-1} s^{-1}$ under the experimental conditions in this study (Figure 7).

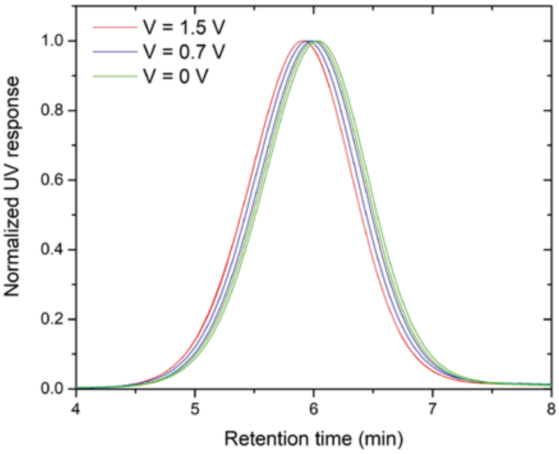

Figure 6. NIST mAb retention time shift with applied electrical field (recorded in voltage mode).

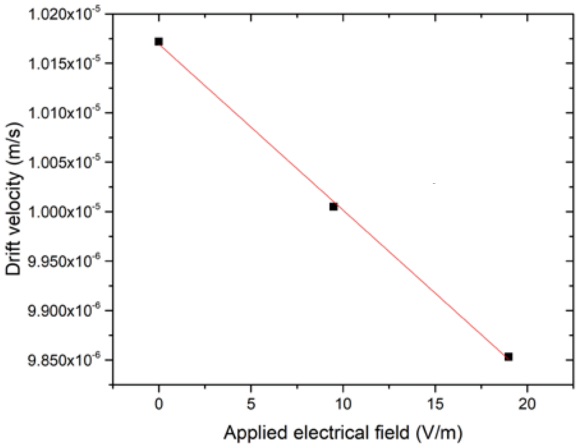

Figure 7. NIST mAb measured electrophoretic mobility.

Conclusion

The above examples demonstrate that EAF4 can be used to analyse a wide variety of nano-sized materials and will be of great importance in those areas where surface charge needs to be determined and batch techniques cannot be used.

In the case of the silver nanoparticles, the electrophoretic mobility values of three different surface coatings were measured successfully using EAF4 and the technique will be very useful for polydisperse nanoparticle suspensions where surface charge distribution measurements are required. In particular, a size-resolved determination of various surface charges among a mixture of different nanomaterials can be performed, in contrast to batch measurements, which only provide average values.

As requirements for the analysis of nanoplastics increase rapidly, high resolution separation techniques will be required for these likely polydisperse analytes. Different polymer materials will have different electrophoretic mobilities, leading to the need for a characterization tool such as EAF4 to provide size and charge information for complex samples.

The NIST monoclonal antibody example demonstrates that biologically active molecules can also benefit from analysis by EAF4 because the system allows both size and surface charge separation, enabling measurement of the distribution of size, molecular weight and electrophoretic mobility in a single experiment. For example, the electrophoretic mobility of the monomer and aggregates can be determined separately. In addition, the open channel design of AF4/EAF4 allows for better recovery of protein/antibody aggregates present in small relative amounts, and represents an advantage of AF4 over SEC for aggregate quantification.

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