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Letter

Bipolar Corona Discharge-Based Charge Equilibration for Nano Electrospray Gas-Phase Electrophoretic Mobility Molecular Analysis of Bio- and Polymer Nanoparticles

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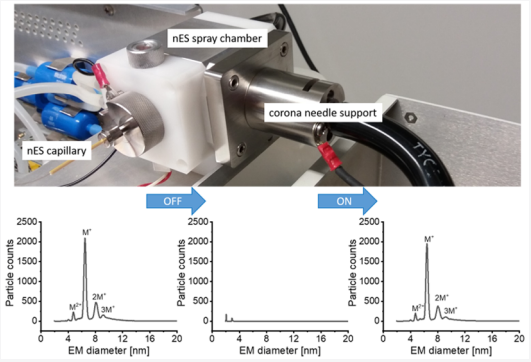
ABSTRACT: Separation of polydisperse, single-charged analytes in the nanometer size range in a high laminar sheath flow of particle-free ambient air and a tunable electric field based on the respective particle electrophoretic mobility diameter (EMD) can be achieved via gas-phase electrophoresis. In order to transfer analytes from a volatile electrolyte solution to the gas-phase as a single-charged species, a nano electrospray (nES) process followed by drying of nanodroplets and charge conditioning reaching Boltzmann charge equilibrium is a necessary prerequisite. In the case of a so-called nES gas-phase electrophoretic mobility molecular analyzer (nES GEMMA, also known as nES differential mobility analyzer, nES DMA), charge equilibration is based on bionanoparticle interaction with a bipolar atmosphere induced, e.g., by a radioactive α-particle emitter like ^{210}Po. It was the aim of our investigation to examine whether such a radioactive source can be easily replaced in the same nES housing by a nonradioactive one, i.e., by an AC corona discharge unit. The latter would be significantly easier to handle when compared to radioactive material in laboratory day-to-day business, waste disposal, as well as regulatory confinements. Indeed, we were able to combine a standard nES unit of our nES GEMMA instrument with a commercially available AC corona discharge device in a novel setup via an adapter. Our results show that this replacement yields very good results for a number of chemically different nanoparticles, an exemplary protein, a noncovalent protein complex, a virus-like particle, a polymer, and a liposome sample, when compared to a ^{210}Po based bipolar charge equilibration device.



Gas-phase electrophoresis describes a technique separating single-charged analytes in the range from single-digit to several hundred nanometer size in the gas-phase at ambient pressure according to their electrophoretic mobility diameter (EMD). A first instrumentation was described in 1996 by Kaufman and colleagues for the analysis of globular proteins.[1] Over the years, this setup was termed differently, nano electrospray gas-phase electrophoretic mobility molecular analyzer (nES GEMMA), nES differential mobility analysis (nES DMA), LiquiScan ES, macro ion mobility spectrometer (MacroIMS), or scanning mobility particle sizer (SMPS). Analytes included proteins and biospecific noncovalent protein complexes,[2−4] intact viruses,[5−8] virus-like particles (VLPs),[9−11] liposomes and lipid-based nanoparticles (NPs) of biological origin,[12−15] organic[16,17] as well as inorganic NPs.[18,19]

Overall, gas-phase electrophoresis is based on the separation of polydisperse, surface-dry, and single-charged NP material in a high laminar sheath flow of particle-free air and an orthogonal tunable electric field. Variation of the electric field strength leads to deviation of particles from their flow-imposed trajectory. Hence, at a given voltage, only NPs of a certain EMD can pass the size separator and are counted as a monomobile fraction as they pass a focused laser beam (ultrafine condensation particle counter, CPC). Therefore, analyte detection is particle-number based as suggested by the European Commission for NP characterization (2011/696/EU from October 18th, 2011).

Transfer of the NPs from the liquid to the gas-phase is a necessary prerequisite. One possible approach is based on a nES process, in which NPs are electrosprayed from a volatile electrolyte solution via a cone-tipped capillary. Application of a sheath flow of particle free, compressed ambient air and CO_2 helps to carry nanodroplets through the spray/charge equilibration chamber and to the separation part of the instrumentation. At the same time, drying of nanodroplets occurs. In the case of the electrospray ionization (ESI) process, this leads to an increasing number of charges located on a shrinking surface of the liquid nanodroplet. Ultimately, the number of charges is too high for the confined space and

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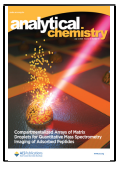

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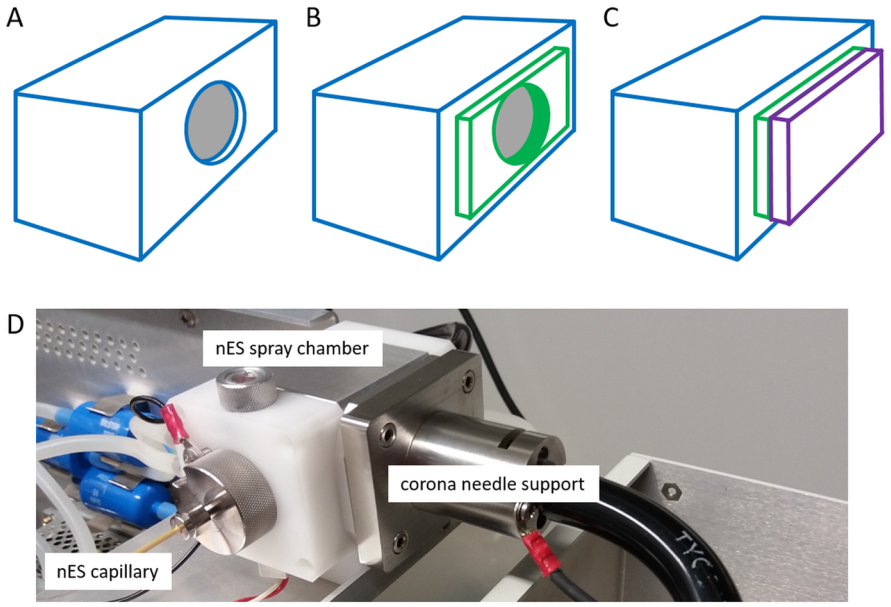

Figure 1. Setup of the described instrumental combination. The ionizer retainer of the spray chamber (blue) is removed, leaving a circular, threaded recess (gray, A). Subsequently, a specially manufactured mounting for the corona discharge needle support is attached (green, B). The corona discharge needle carrying plate is then attached to the support (violet, C). Part D displays a photograph of the described setup.

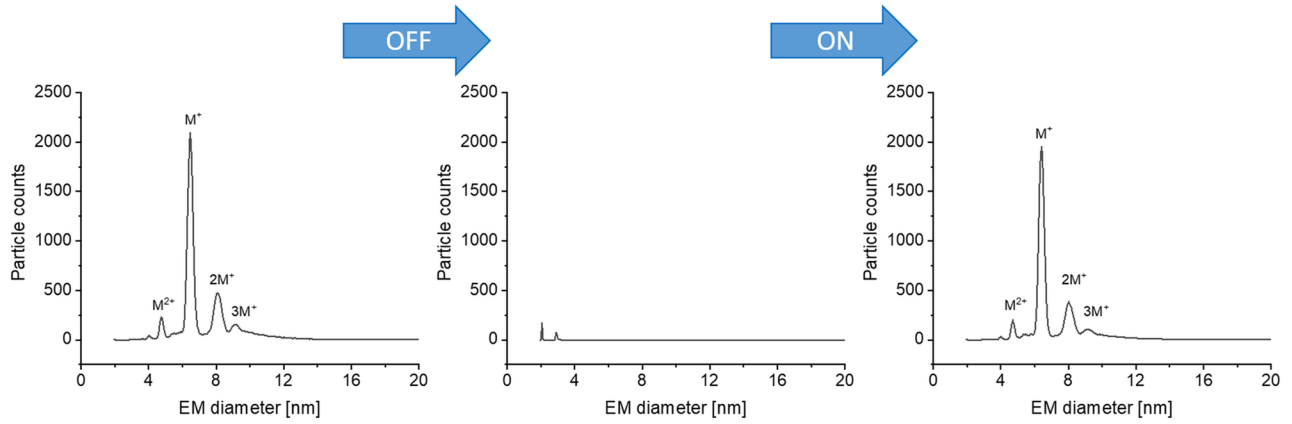

Figure 2. Obtained signals are sensitive to the corona discharge process. Switching off the corona discharge leads to the loss of the signal recorded for ovalbumin. Upon powering up the corona discharge process again, the signal is regained.

nanodroplets are fragmented in an uncontrolled process until solvent-free multiple charged NPs are generated.

Gas-phase electrophoresis on the other hand is based on single-charged NPs. Hence, multiple charges originally located on nanodroplets formed at the tip of the cone-shaped capillary have to be reduced. Boltzmann charge equilibration as described by Wiedensohler and Fissan[20] in a bipolar atmosphere under certain reaction conditions leads to a known charge conditioning. Finally, a majority of NPs is neutral, and a smaller percentage is only carrying one single charge. Hence, analysis of single-charged NP material can be achieved.

Several possible sources of bipolar atmospheres have been described to date in association with gas-phase electrophoresis for bio- and polymer NPs, e.g., a ^{210}Po α-particle source, a soft X-ray charger, or similar.[21,22] We now focus on an AC corona discharge device for creation of a bipolar atmosphere, i.e., generating high concentrations of positive and negative ions as done, e.g., by Qi and Kulkarni in a prototype setup.[23] However, in contrast to previous work and as shown in Figure 1, we directly coupled an AC corona discharge needle taken from a commercially available device (MSP 1090, Electrical Ionizer from TSI Inc., Shoreview, MN) with the model 3480 nES sprayer/charge equilibration unit (TSI Inc., Figure 1A, blue) from a standard nES GEMMA instrumentation. Hence, no excessive conversion of already available instruments is necessary. In order to achieve this coupling, a special needle support/adapter was manufactured in house from an aluminum alloy. This needle support replaced in our setup the ^{210}Po source and ionizer retainer of the nES sprayer unit (Figure 1B, green). Subsequently, the direct mounting of the corona needle carrying plate (Figure 1C, violet) of the corona discharge instrument to the needle support was possible. No additional electrical groundings were necessary. O-Rings were employed to seal the setup. Figure 1D displays a photograph of the combined instrumental setup.

Electrospraying an ovalbumin (0.3 μM in ammonium acetate) containing sample resulted in a protein related signal only upon activation of the corona discharge. As seen in Figure 2, an ovalbumin signal is obtained at 6.34 ± 0.07 nm (n = 7)

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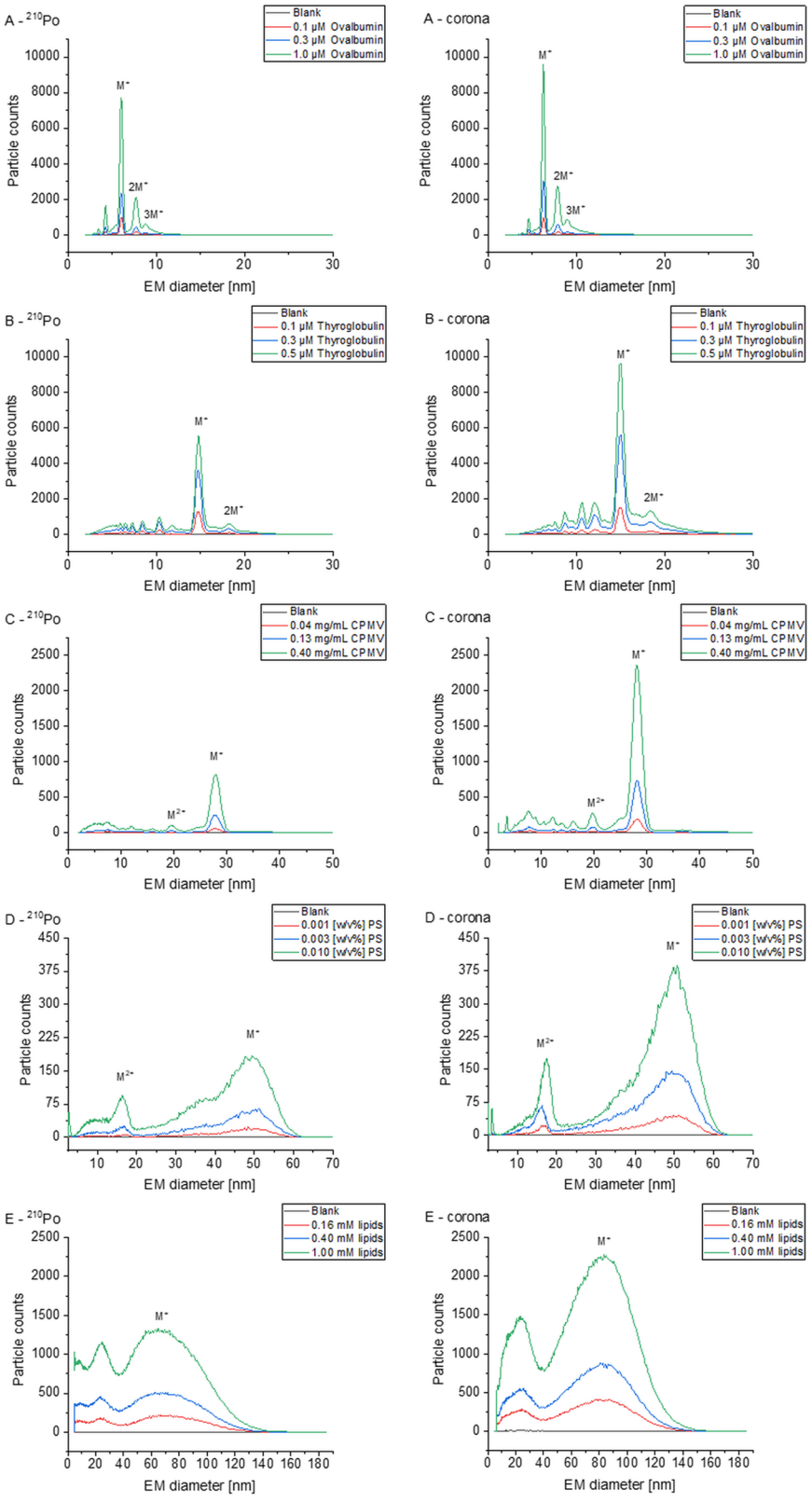

Figure 3. Bipolar atmospheres for charge equilibration based on ^{210}Po and the AC corona discharge process yield comparable results. Data for two exemplary proteins (ovalbumin, A, and thyroglobulin, B) are shown as well as data for a virus-like particle based on cowpea mosaic virus, CPMV (C), a polystyrene-based size standard (D), and a liposomal vesicle (E). EMD values are comparable in all cases. The corona discharge process yields at least comparable numbers of single-charged NPs if not higher values when compared to the ^{210}Po source. EMD data and a relative plot of spectra are given in the Supporting Information.

EMD. Switching off the corona discharge process while further electrospraying the sample leads to the complete loss of the recorded signal. Likewise, the signal can be immediately regained, once the corona discharge is powered up again. No

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special procedures are necessary to do so. In our experiments, the corona discharge worked for in total approximately 130 h for several months to date with typically 5 h per working day. So far, no significant change in observed signals was recorded.

Finally, we took interest in the performance of the modified nES GEMMA instrument including a corona discharge device in comparison with our ^{210}Po based standard instrumentation under identical conditions. Therefore, we measured several different samples (chemically different as well as in terms of size distribution) on both instruments and compared resulting spectra. As can be seen in Figure 3, investigated samples included ovalbumin, a plain protein molecule (A) and thyroglobulin, a noncovalent biospecific glycoprotein complex (B), as two proteins of different molecular weight (approximately 46 and 660 kDa, respectively). In addition, a VLP, a protein shell without any genetic information content (C), a 46 nm polystyrene size standard (D), and a liposome containing sample (E) were evaluated. Data plotted relative to the highest peak of the respective spectrum is part of the supplement (Supplementary Figure S1). In all cases, both instruments yielded comparable signals for single-charged species in terms of observed EMD values as has already been described.[9,24] EMD value differences were roughly around 1.5% (refer to the detailed data given in Supplementary Table 2). However, especially for liposomes, a significant higher deviation was observed. As described already for polysaccharides and their larger aggregates, analyte heterogeneity influences peak fitting and hence EMD values.[25] In the case of vesicles, double-charged, smaller-appearing species especially detected upon application of a partially decayed ^{210}Po α-particle source impact EMD values in a similar way. The broad peak corresponding to heterogeneous, double-charged species biases the fitting of the peak of likewise heterogeneous, single-charged analytes. In terms of particle counts, the corona discharge based system yielded significantly higher values than the ^{210}Po based one, especially for increasing particle EMD values. This, as well as significantly lower amounts of multiple-charged analyte species, most probably might be accounted likewise for the age of the employed ^{210}Po α-particle source (already exceeding almost four half-lives, 138 days each).

To conclude, we report a novel setup enabling nanoparticle characterization. Replacing a ^{210}Po α-particle source by a commercially available AC corona discharge device is equivalent for creation of a well-defined bipolar atmosphere resulting in a Boltzmann equilibrium in the nES source of a standard nES GEMMA instrumentation. In terms of instrument handling and of recorded spectra (observed particle counts and EMD values, stable charge conditioning), this setup is absolutely comparable to setups including a radioactive ^{210}Po α-particle source. However, replacing an α-particle source by a corona discharge process significantly facilitates direct handling of gas-phase electrophoresis instrumentation in day-to-day laboratory work. Likewise, problems in disposal of radioactive material as well as regulatory requirements are circumvented. Therefore, we are convinced that the application of an AC corona discharge process for Boltzmann charge equilibration in nES GEMMA measurements for (bio-) NPs like proteins, functional protein complexes, intact viruses or virus-like particles, as well as lipoparticles poses a significant instrumental development. It will support the use of gas-phase electrophoresis in routine analysis on standard instrumentations in nonspecialized laboratories.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01904.

Information concerning samples, chemicals, instrumentation, and measurement conditions, EMD data, and relative plotting of spectra presented in Figure 3 of the manuscript (PDF)

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Notes

The authors declare no competing financial interest.

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