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Nanoelectrospray Ion Mobility Spectrometry Online with Inductively Coupled Plasma-Mass Spectrometry for Sizing Large Proteins, DNA, and Nanoparticles

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Recently it has been demonstrated that nanoelectrospray (nES) in conjunction with macro-ion mobility spectrometry (macroIMS) and condensed particle detection can be used to size various types of nanoparticles, including large biomolecules (proteins, DNA, etc.), having elecrophoretic mobility diameters ranging from 3 nm to well over 100 nm. The technique is extremely sensitive; however, it lacks specificity as a result of the detector used. To explore the possibility to overcome this limitation, we demonstrate the direct coupling of the nES-macroIMS system to an inductively coupled plasma mass spectrometer (ICPMS). Technical challenges involving the coupling of the air-based nES-macroIMS with the argon-based ICPMS are addressed and overcome. The resulting novel hyphenated technique is used to determine the elemental composition of nanoparticles resulting from the electrospraying of solutions containing inorganic salts and acids (CsI and dimethylarsinic acid). Even though the sensitivity of the used ICPMS does not allow for the simultaneous sizing of proteins and the determination of their metal, metalloid, or halogen content, we have shown that it is feasible to detect and accurately size proteins at femtomole levels by adding CsI to their solutions and detecting the resulting Cs adducts. This is also possible with DNA molecules. A linear relationship between protein amount and ICPMS response for $^{133}Cs^{+}$ is observed, thus hinting at the possibility of further developing the technique for quantitative analysis of large biomolecules.

Over the past decade, considerable progress has been made in the development of analytical techniques for sizing large biomolecules. One of these techniques involves the use of nanoelectrospray (nES) coupled with macro-ion mobility spectrometry (macroIMS) with online condensation particle counting (CPC).[1–6] In several of the relevant publications, the term GEMMA (gas-phase electrophoretic mobility molecular analyzer) is used to describe this complete system.[2,4–6] In brief, the principles of the analytical technique are as follows. The nES converts a dilute analyte solution into a series of multicharged droplets, each ~100−200 nm in diameter,[6] which are driven through a neutralization/charge reduction chamber[7] where they are converted into neutral and singly charged solvent-free nanoparticles. The latter are sized in accordance to their gas-phase electrophoretic mobility in the macroIMS, which in fact is a differential ion mobility spectrometer.[1,8,9] As the macroIMS voltage is ramped, nanoparticles of increasing size sequentially exit the macroIMS and enter the CPC detector where they are counted.[10,11] Typically, the macroIMS used in bioanalytical studies are suitable for sizing biomolecules and nanoparticles having an electrophoretic mobility diameter (EMD) ranging from 3 nm to well over 100 nm or alternatively ranging in relative molecular mass from 8 kDa to 80 MDa.

Overall the technique has been used with success to determine the size of macromolecules of biological origin. In initial studies, Kaufman demonstrated the separation of a mixture of proteins having an EMD ranging from about 3 to over 16 nm.[12] He also demonstrated the direct relationship between EMD and relative molecular mass and thus the possibility to indirectly determine the latter for various types of macromolecules. Recent work in this area has improved our understanding of the existing relationship between EMD and relative molecular mass for large proteins and protein complexes.[5] Furthermore, Thomas et al. described the technique's use for determining the size of intact viruses.[13] The sizes determined were close to those previously obtained

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using X-ray crystallography. Using this technique, Mouradian et al. successfully analyzed synthetic single- and double-stranded DNA oligomers ranging from 6.1 to 300 kDa.[14]

The combination of the nES source, with its capability to generate gas-phase ions of large biomolecules, cellular components, and viruses, in combination with the sizing capabilities of a macroIMS, has introduced unique possibilities for the size determination of macromolecules, macromolecule complexes, and engineered nanoparticles. Although novel adaptations of conventional mass spectrometric techniques are being developed for detecting large macromolecules (10^{14} Da) and nanoparticles,[15] such a task still remains an analytical challenge for most conventional mass spectrometric techniques. The universal CPC detector is extremely sensitive, capable of rapidly analyzing nmol/L macromolecule solutions.[12] Typically, a gas electropherogram can be obtained in 30−300 s with extremely low sample consumption, i.e., femtomoles. The possibility of using this technique for macromolecule quantification has also been explored.[1] However, disadvantages still exist, in particular relating to the inherit nonspecificity of the CPC detector. Even though it is efficient and extremely sensitive for counting nanoparticles, it provides no element composition information for the analyzed particles, in particular their metal, metalloid, or halogen content. It is also unable to differentiate between different macromolecules having the same or similar electrophoretic mobilities and is thus not suitable for the quantification of macromolecules in biological mixtures.

An approach potentially suitable for overcoming some of these limitations would be to couple the nES-macroIMS with a sensitive and element-specific detector such as an inductively coupled plasma mass spectrometer (ICPMS). In addition to providing a new analytical tool for proteomics and metallomics analysis, the nES-macroIMS-ICPMS is expected to ultimately allow for a diverse range of investigations concerning the following: the determination of the element composition of biomolecules, cellular components, and viruses, the study of metal species and macromolecule interactions, and also improved quantification capabilities for the determination of large biomolecules in complex biological samples.

The main objective of this study is to demonstrate the feasibility of coupling of nES-macroIMS online to ICPMS and to determine the size (i.e., the electrophoretic mobility diameter) of large biomolecules (proteins and DNA), as well as inorganic nanoparticles. It is anticipated that the development of this novel hyphenated technique will be further accelerated by introducing it to scientists working in relevant research fields. This aspect is vital in order for the previously mentioned objectives to be further explored and, thus, for the technique to potentially become an important proteomics and metallomics tool.

EXPERIMENTAL SECTION

Chemicals. Cesium iodide (99.999%), human apotransferrin (98%), and DNA sodium salt from salmon testes were purchased from Sigma-Aldrich (St. Louis, MO). Dimethylarsinic acid (>99%), thyroglobulin from porcine thyroid gland, hemoglobin from bovine blood (>90%), ammonia (aqueous solution, puriss. p.a.), and ammonium acetate (MS grade) were provided by Fluka (Buchs, Switzerland). Potassium hydroxide (p.a.) was purchased from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm) was obtained from an Ultra Clear Basic water purification system from SG GmbH (Barsbüttel, Germany). Tanks of CO_2, air, and argon (>99.998%) were obtained from Linde Hellas Ltd. (Athens, Greece).

Instrumentation. A complete nES-macroIMS with CPC detection from TSI Inc. (St. Paul, MN) was used in this study. A batch-type nES source unit (model 3480C), consisting of a fused-silica capillary, used for sample uptake, and a high-voltage platinum wire also dipped into the sample, is operated in a mixture of air (1.0 L/min) and CO_2 (0.1 L/min). For charge reduction purposes, the nES is equipped with a neutralizing α-source (^{210}Po; 5 mCi, model P-2042 Nucleospot local air ionizer, NRD, Grand Island, NY) and connected through silicone conductive tubing to the differential ion mobility analyzer, commercially named macroIMS (Series 3080C), and finally to a 1-butanol-based Ultrafine CPC (Series 3025A). Details regarding this instrumentation have been presented elsewhere.[1,6,12] In brief, the operating principle of the CPC is as follows: a charged nanoparticle emerging from the macroIMS is mixed with a flow of a saturated organic solvent vapor (i.e., 1-butanol), which is subsequently cooled to provide a defined supersaturation. The nanoparticle becomes the nucleus for vapor condensation and a droplet is formed. As a result of the supersaturated atmosphere and continuous 1-butanol condensation, it grows to a final size on the order of 10 μm, which is then detected by using a laser optical device. An X-Series ICPMS (Thermo Fischer Scientific, Winsford, U.K.) equipped with a quadrupole mass analyzer was used as an element-specific detector throughout this study.

IMS version 8.0 (TSI Inc.) software was used for data acquisition with CPC detection and also data analysis. Plasma Laboratory 2003 version 2.3 (Thermo Fischer Scientific) software was used independently of the CPC software for ICPMS data acquisition and analysis.

Procedures. Stock solutions (1000 μg/mL) of all protein and inorganic compound samples were prepared in 20 mM ammonium acetate buffer pH 8 (adjusted with ammonia) and stored at −20 °C. Lyophilized DNA was dissolved in 20 mM ammonium acetate buffer pH 8 at a concentration of 1000 μg/mL, allowed to dissolve at +4 °C for 6 h, and subsequently stored at −20 °C. Before analysis, the stock solutions were thawed, diluted in buffer, and filtered through a 0.45-μm Millipore membrane (Bedford, MA). For comparison purposes, DNA samples were desalted using size exclusion chromatography (300 × 8.0 mm column packed with Toyopearl HW-55S, Tosoh Bioscience) prior to analysis. The samples were placed in Eppendorf tubes into the nES unit. One end of a 25-cm-long, 25-μm-i.d. (150-μm-o.d.), polyimide-coated fused-silica capillary was immersed into the solution, while the other end having a conical shape was located within the nES chamber. The spray was achieved by introducing the sample into the capillary by applying air pressure of 3.7 bar and a voltage between +1.5 and +2.5 kV to a Pt wire, which was dipped into the sample solution. This resulted in a nES flow rate of ~70 nL/min. Air supplied at 1 L/min and CO_2 at 0.1 L/min surrounded the nES spray tip and carried the multiple charged droplets through a ^{210}Po α-radiation source. This source creates primary

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gas ions, which interact with the multiply charged particles causing their charge reduction.[7] This process, in conjunction with solvent evaporation, results in the formation of predominantly neutral nanoparticles along with a limited fraction of singly charged particles. It is estimated that ~1% of all airborne analyte molecules become singly charged.[6,16,17] The charged particles entering the macroIMS at an aerosol flow rate of 1.1 L/min are swept into a laminar flow of air or argon (sheath gas flow typically 6 L/min) and also experience a radial electric field orthogonal to the gas flow. The resulting aerosol-to-sheath volumetric flow ratio of 5.45 is similar to that of 7.5/1.5 = 5 shown by Chen et al. [18] to provide slightly poorer resolution compared to that achieved at ratios of 30.0/1.0 and 30.0/1.5. In our study, higher ratios (i.e., higher Ar sheath flow rates) were not used in order to keep operational costs of the system as low as possible. Charged particles are classified based on their electrophoretic mobility in the sheath gas under these conditions. As the macroIMS voltage is scanned (−10 to −3000 V), particles of increasing size have a stable trajectory, allowing them to exit the macroIMS and enter the CPC where they are counted.

The coupling with the ICPMS was achieved by removing the impact bead spray chamber and the connector tube used to connect the spray chamber to the back of the torch. A quartz female ball joint connector was used instead to couple the macroIMS outlet rubber transfer tube directly to the back of the ICP torch. The ICPMS was connected in parallel with the CPC as shown in Figure 1.

The commercial version of the nES-macroIMS-CPC system operates with air as the sheath gas; i.e., the flowmeters on the macroIMS control unit are calibrated for air flows. Thus, when using Ar as the sheath gas, as is necessary when coupling to the ICPMS (discussed in detail in Results and Discussion), alternative means of measuring and regulating the Ar flow are used. Switching gases necessitates bypassing the air-calibrated macroIMS control unit and introducing the Ar sheath gas directly into the macroIMS. The Ar gas is regulated using a dual-stage pressure regulator equipped with a flowmeter. However, because of the low precision of this flowmeter, cross-calibration of particle size in air and in Ar is required. The cross-calibration procedure involves sizing particles in air and subsequently in Ar. Air-to-Ar cross-calibration was carried out using particles formed by electrospraying solutions containing various concentrations of CsI (5−2000 μg/mL). These particles ranged in size from 3.5 to 16 nm. Typical cross-calibrations were conducted by using 10 different particle sizes. The linear relationship determined between size in air and size in Ar is used to correct for the size of all other nanoparticles determined in Ar. Cross-calibration is carried out at the beginning and end of each working day. Typical parameters of the linear relationship between particles in Ar and in air are as follows: $y_{[\text{EMD in air}]} = 0.8 + 0.68x_{[\text{EMD in Ar}]}$, $r = 0.998$, $S_{y/x} = 0{,}25$, $S_b = 0.015$, and $S_a = 0.2$. All subsequent EMDs determined using the Ar-based nES-macroIMS-ICPMS are corrected for using such a cross-calibration equation.

Analyte solutions were sprayed for ~2 min prior to acquisition. The spray current, typically 320 nA, was stable during experiments

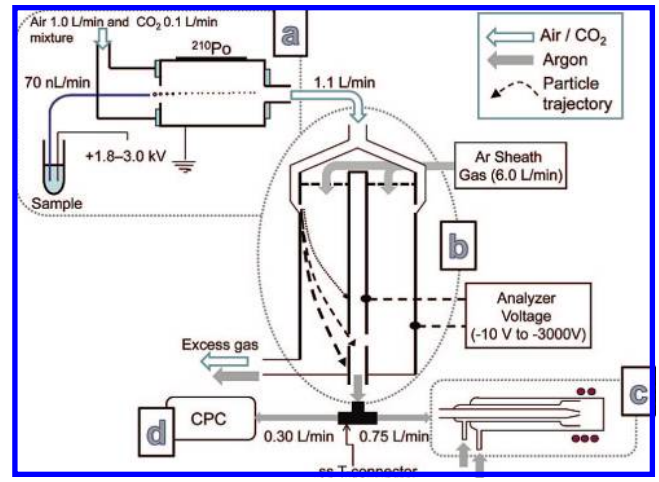

Figure 1. Configuration of the nanoelectrospray (a) macro-ion mobility spectrometer (b) coupled online with ICPMS (c) and CPC (d), showing gas flows and rates. An air/CO_2 mixture (1.1 L/min) was used in the nES source, whereas Ar was used as the sheath gas (6.0 L/min) in the macroIMS for separating and sizing the analyte nanoparticles and for their transfer into the ICPMS. A stainless steel T-connector was used to split the macroIMS outlet to the ICPMS (0.75 L/min) and the CPC (0.30 L/min). Silicon conductive tubing (5 mm i.d., 10 mm o.d.) of various lengths was used to make all connections between the nES and the macroIMS (36 cm), between the macroIMS and the stainless steel T-connector (13 cm), and from the T-connector to the CPC (54 cm) and also from the T-connector to the back of the ICPMS torch (21 cm). The silicon conductive tubing was fitted directly to the back of a standard ICPMS torch via a ball joint glass connector.

(RSD = 3.5%). Between samples, the fused-silica capillary was washed with buffer solution. Also, when required, 0.1 M KOH was run through the capillary for 5 min to remove protein residues, followed by buffer solution. The ICPMS was operated under dry plasma conditions. Signal optimization of the nES-macroIMS-ICPMS was carried out by introducing inorganic nanoparticles continuously into the ICPMS. This is easily achieved by setting the macroIMS voltage to a specific value in order to continuously allow particles of a certain size to enter into the ICPMS.

Because the IMS control unit was only connected electronically with the CPC detector, and not the ICPMS, it was necessary to manually initiate each system separately for data acquisition. As software for both systems were on the same computer, a manual and sequential start was achieved with less than 1-s time difference, thus not affecting the acquired electropherograms. Data files of response versus time and response versus EMD were recorded with the CPC detector, while response versus time was recorded with the ICPMS. All data files were exported and processed using spreadsheet software.

Calculating Relative Molecular Mass. Assuming the biomolecule to be approximately spherical and knowing its density, it is possible to calculate its relative molecular mass (M_r) from eq 1, derived from the combination of eqs 2 and 3:[2]

$$M_r = \frac{\rho(\text{EMD})^3 \pi N_o}{6} \quad (1)$$

$$\rho = \frac{M_r}{VN_o} \quad (2)$$

and

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$$V = \frac{(\mathrm{EMD})^3 \pi}{6} \quad (3)$$

where ρ is biomolecule density (a much used literature value for globular protein density is 0.58 ± 0.05 g/cm^3 [3,5]), V is volume of the biomolecule, and N_o is Avogadro's number.

RESULTS AND DISCUSSION

Coupling of nES-macroIMS Online with ICPMS. The argon plasma used in all commercially available ICPMS instruments is difficult to ignite and problematic to sustain in the presence of even moderate levels of air. As a result, the direct coupling of an air-based nES-macroIMS system to an Ar-based ICPMS entails some technical challenges. Even though the macroIMS can operate efficiently with a variety of gases, including argon, electrospray is not as flexible with respect to gas compatibility. In fact, it is extremely difficult to achieve stable ES conditions (cone jet mode) in an Ar atmosphere. The reason for this is the occurrence of electrical breakdown of Ar when voltages of ~2−5 kV are applied to the electrospray source. This incompatibility has rarely been reported in the literature,[19] possibly because few attempts have been made to conduct electrospray in an Ar atmosphere. Our attempts to achieve stable nES in an Ar gas failed as corona discharge occurred; i.e., high currents ranging from 500 to 800 nA were observed in the source, prior to the onset of electrospray. As a result no further attempts were made to achieve stable nES conditions in an Ar gas environment. It should, be mentioned, however, that it may be possible to achieve stable electrospray in Ar by adding large amounts of a high electron affinity gas such as SF_6 or even higher amounts of CO_2 than the 0.1 L/min supplied to the nES in this study.

To overcome the gas incompatibility between nES and ICPMS, an alternative approach was adopted. The nanoparticles generated in the nES are driven to the macroIMS in a stream of air/CO_2. However, the macroIMS is operated using Ar as the sheath gas instead of the air sheath that is normally used (Figure 1). In the macroIMS, the charged nanoparticles undergo transfer from the air/CO_2 mixture into the Ar sheath flow as they experience an attractive electrostatic force from the internal cylinder of the macroIMS. A portion of the Ar sheath gas is subsequently introduced into the ICPMS. This approach for coupling a macroIMS to an ICPMS was first demonstrated by Myojo et al.[20] However, the major difference between the system they developed and the one we describe in the present study is that they used a pneumatic nebulizer (Myojo et al. refer to it as a glass nebulizer) for nanoparticle generation with a separate radioactive source (^{241}Am) for ionization, instead of the nES source used in the present study. A pneumatic nebulizer creates droplets that are several orders of magnitude larger (μm diameter) than those produced by the nES source (nm diameter). The Myojo et al. approach also requires that the resulting nanoparticles are independently ionized (^{241}Am source) prior to classification in the macroIMS. Only a single application of that technique has been reported; i.e., Pb aggregates, ranging from 30 to 140 nm, originating from solutions containing elevated concentrations of $Pb(NO_3)_2$ were detected. On the other hand, as described in detail in the introduction of this paper, nES with macroIMS has been shown to afford accurate sizing data for nanometer-sized biomolecule nanoparticles (proteins and protein complexes), and thus, this unique combination of ionization source and separating capability when coupled to an ICPMS constitutes a novel hyphenated technique for biomolecule analysis. This is to the best of our knowledge the first report of the coupling of a nES-based macroIMS system with ICPMS.

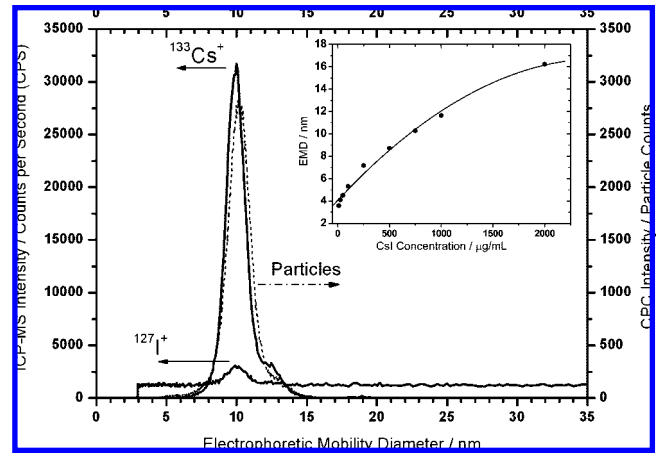

Figure 2. nES-macroIMS gas electropherograms for CsI nanoparticles recorded using parallel CPC and ICPMS detection. Inset shows the relationship between CsI concentration in the electrosprayed solution and the resulting nanoparticle electrophoretic mobility diameter.

Generating, Sizing, and Detecting Inorganic Nanoparticles. To demonstrate the successful coupling of the nES-IMS online with ICPMS, a series of solutions containing cesium iodide were analyzed. A typical macroIMS gas electropherogram resulting from the analysis of such solutions, i.e., containing 750 μg/mL CsI, with parallel ICPMS and CPC detection is shown in Figure 2. The peaks observed by using ICPMS and CPC detection have similar profiles and electrophoretic migration times, and thus, both detectors give the same EMD for the particles analyzed. It is obvious that the main advantage of the ICPMS, over the CPC detector, is its capability to provide element composition information as it detects specific isotopes, i.e. $^{133}Cs^+$ and $^{127}I^+$ in this case.

Similar agreement of the determined EMDs using both detectors was observed for all other CsI solutions analyzed. The relationship between CsI concentration and detected nanoparticle size is shown in the inset of Figure 2. These data clearly demonstrate that electrospraying solutions containing increasing concentrations of CsI results in the formation and thus detection of nanoparticles of increasing size, according to the following equation: $y = 4.068 + 0.00991x - 1.93701 \times 10^{-6}x^2$, R^2=0.991, std dev = 0.46, n = 9, and $P < 0.0001$. These nanoparticles are believed to be formed as a result of the nES process. They are then sized in the macroIMS, and their elemental composition is determined using the ICPMS, whereas the CPC detector only provides particle numbers. These data also suggest that the size of the generated nanoparticles can be controlled by electrospraying solutions containing appropriate salt concentrations.

Regarding the relative performance of the two detectors, it seems that even though the CPC is a nonspecific detector it

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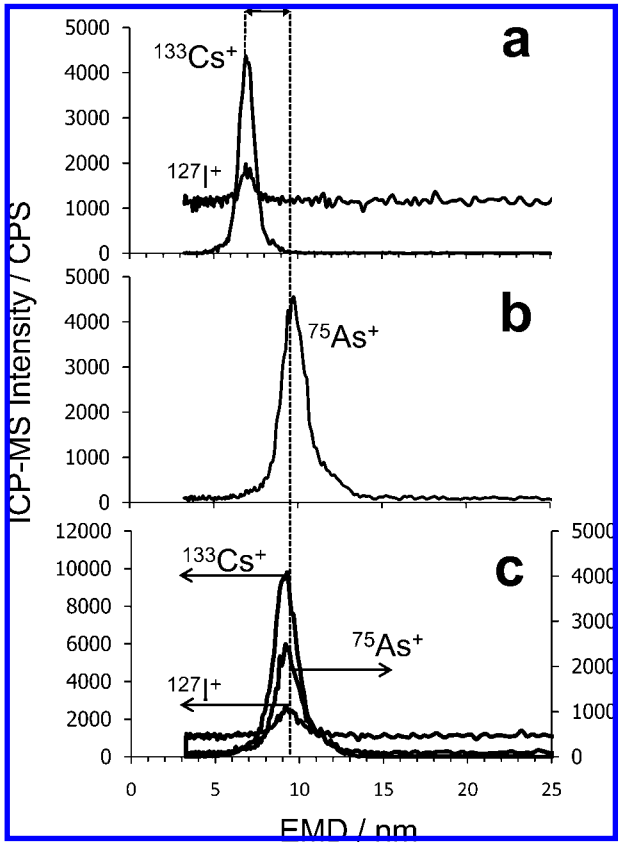

Figure 3. nES-macroIMS gas electropherograms obtained from the analysis of solutions containing (a) CsI (250 μg/mL), (b) dimethylarsinic acid (500 μg/mL), and (c) a mixture of CsI (250 μg/mL) and dimethylarsinic acid (500 μg/mL). All gas electropherograms were obtained using ICPMS detection for the isotopes $^{133}Cs^+$, $^{127}I^+$, and $^{75}As^+$.

exhibits higher signal-to-noise ratios compared to the ICPMS even for easily ionized elements such as Cs and, thus, gives lower limits of detection.

Formation of nanoparticles was also observed when electrospraying organometalloid substances such as dimethylarsinic acid. When electrospraying a solution containing 500 μg/mL dimethylarsinic acid, As-containing nanoparticles having an EMD of 9.6 nm are detected (Figure 3b). Solutions containing 250 μg/mL CsI generated Cs-containing nanoparticles having an EMD of ~6.9 nm (Figure 3a). Whereas from a solution containing a mixture of 500 μg/mL dimethylarsinic acid and 250 μg/mL CsI, monodisperse nanoparticles were observed (Figure 3c) having the same size as the dimethylarsinic acid originating nanoparticles (Figure 3b). To rationalize this observation, it is speculated that Cs^+ forms adducts with dimethylarsinic acid nanoparticles during electrospray. In fact it is well-known that other cations of the same group, i.e., sodium and potassium, act in a similar way with several types of compounds (M) affording $[M + Na]^+$, $[M + K]^+$, or both during electrospray mass spectrometry.[21] The main advantage that the ICPMS brings to this type of analysis is that it provides important element composition information, i.e., that the nanoparticles formed contain As, Cs, and I, something the CPC is unable to provide. From these data it is also important to note that the addition of Cs^+ to the dimethylarsinic acid particles did not affect the size of the dimethylarsinic nanoparticle. This suggests the possibility of using Cs^+ to tag larger molecules such as proteins, protein complexes, and even DNA molecules in order to size them using the nanoES-macroIMS-ICPMS system developed here.

It should be mentioned that the exact nature of the generated nanoparticles is not yet known. However, it is well documented that because metal halides are nonvolatile solutes, during the electrospray process their concentrations in charged droplets progressively increase as solvent evaporation occurs, and thus, they tend to give cluster ions such as $M_{n+1}X_n^+$ and $M_nX_{n+1}^-$.[22–24]

Sizing of Proteins. Because of the relatively low sensitivity of the ICPMS instrument used in this study (10−20 million counts/s (cps) per mg/L of analyte) it was not possible to directly determine the metal, metalloid, or halogen content of proteins while simultaneously determining their size. The latter was not possible because when spraying concentrated protein solutions by nES artifact protein dimers, trimers, etc., are highly likely to form. This is because of the high probability of each nES droplet containing more than one protein molecule when analyzing concentrated protein solutions. Under optimum conditions, one analyte molecule per droplet volume is required. Our attempts to determine an iodine-containing protein, i.e., thyroglobulin, by monitoring for $^{127}I^+$ were only successful when analyzing solutions containing ≥1000 μg/mL protein. Unfortunately, at such elevated concentrations artifact aggregates seem to form, thus giving elevated EMD for thyroglobulin (26 nm compared to 15 nm determined for the low-concentration thyroglobulin solutions) (Figure 4). As a result, the calculated M_r is 3.2 MDa, compared to 623 kDa corresponding to the 15-nm EMD obtained for protein concentrations between 1 and 50 μg/mL (Figure 4c) and the 660 kDa already reported for this dimeric protein.[6] This seems to indicate extensive formation of nonspecific aggregates of thyroglobulin, with the main aggregate consisting of five of these protein dimers. The nES-IMS-ICPMS technique was also used to detect the presence of iron in ferritin; however, this was only possible at protein concentrations of ≥1000 μg/mL. As expected, such elevated concentrations resulted in the formation of artifact aggregates of ferritin and thus the protein's size could not be determined, as was the case when analyzing elevated concentrations of thyroglobulin.

Enhancement of the system's sensitivity is anticipated by increasing the flow rate of the nES (i.e., increase the amount of analyte entering the ICPMS per unit time). This can be achieved by using capillaries with larger i.d.; however, this will also enlarge the generated droplets and thus promote the formation of artifact aggregates. A more substantial improvement is probably obtainable by increasing the amount of singly charged ions that is introduced to the macroIMS. This may be achieved by reionizing the particles, after they exit the nES and charge reduction chamber, by using an atmospheric pressure chemical ionization chamber. However, it is expected that an ICPMS that is more sensitive by ~2 orders of magnitude would allow for more accurate sizing of proteins and other biomolecules by directly monitoring

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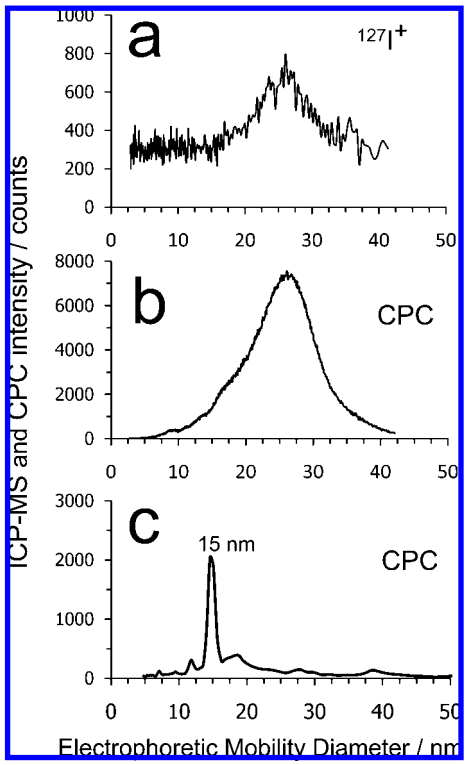

Figure 4. nES-macroIMS gas electropherograms of solutions containing thyroglobulin: (a) ICPMS detection for $^{127}I^+$, (b) CPC detection of 4 mg/mL thyroglobulin solutions, corresponding to 40 μg of iodide/mL, and (c) CPC detection of a solution containing 5 μg/mL thyroglobulin.

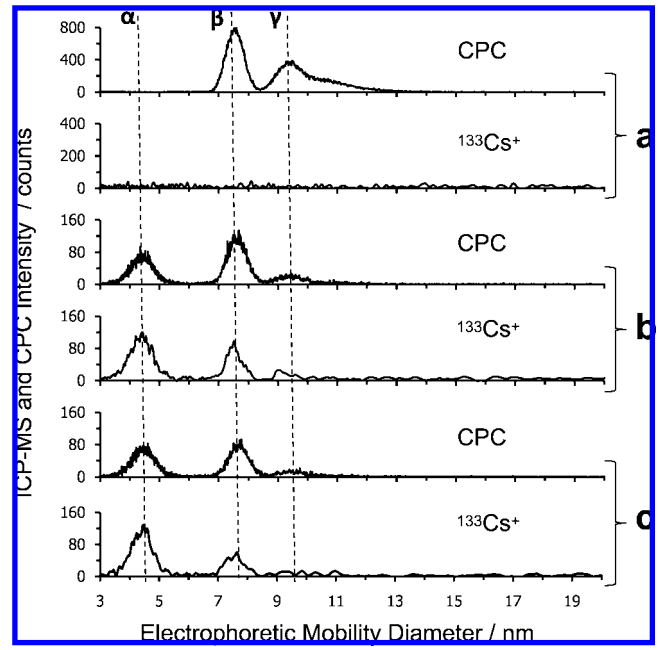

Figure 5. nES-macroIMS gas electropherograms with CPC detection and ICPMS detection of isotope $^{133}Cs^+$ of the following: (a) 5 μg/mL transferrin solution (no Cs^+ added); (b) 5 μg/mL transferrin and 50 μg/mL CsI containing solution; and (c) 2.5 μg/mL transferrin and 50 μg/mL CsI solution. The observed peaks are identified as peak α corresponding to CsI-containing nanoparticles, peak β resulting from transferrin monomer, and peak γ as corresponding to the transferrin dimer.

their metal, metalloid, or halogen content. Such extremely sensitive ICPMS instruments (1000−2000 million counts/s per mg/L of analyte), are currently commercially available. However, it remains to be seen if they can provide the required limits of detection for such an application. Even though their sensitivity is ~2 orders of magnitude greater, it is not clear if their corresponding background levels, observed under the dry plasma conditions used for the nES-macroIMS-ICPMS system, will be sufficiently low in order to allow for the required low limits of detection to be achieved.

In view of this difficulty, alternative approaches for sizing proteins and protein complexes using nES-macroIMS-ICPMS were explored. One such approach involves the use of Cs^+ to tag or metalate the proteins for subsequent ICPMS detection and thus for their size measurement to be possible. A typical example is presented in Figure 5, in which case transferrin was analyzed. Initially, no Cs^+ was added to the transferrin (5 μg/mL) solution and thus the protein monomer (EMD 7.5 nm; peak β) and its dimer (EMD 9.3 nm; peak γ) were only observed with the CPC detector (Figure 5a). In a second experiment, CsI (50 μg/mL) was added to the 5 μg/mL transferrin solution and subsequently analyzed using the nES-macroIMS-CPC/ICPMS system. The resulting macroIMS gas electropherograms from the analysis of this solution using both detectors are shown in Figure 5b. Peaks β and γ corresponding to the transferrin monomer and dimer, respectively, were observed with both detectors. The ICPMS detector in this case reveals protein−Cs adduct formation, as well as the fact that the presence of Cs^+ (ionic diameter 0.338 nm)[25] did not cause an increase in the protein's EMD. The latter is also confirmed with CPC detection. In the presence of CsI, both detectors reveal an additional early peak (peak α). The ICPMS verified this to contain Cs and I. As expected from our earlier experiments involving the analysis of CsI solutions, peak α is a result of CsI containing nanoparticles. Figure 5c shows ICPMS and CPC eletropherograms resulting from the analysis of 2.5 μg/mL transferrin solution doped with Cs^+. For a 2-min analysis, which is a typical macroIMS scan time, plus a 2-min uptake time, this is equivalent to detecting 4−5 fmol of protein with the ICPMS, with a total consumption of 8−10 fmol of protein.

Using a density value of 0.58 ± 0.05 g/cm^3,[3,5] the relative molecular mass, calculated using eq 1 (see Experimental Section), for transferrin is 78 ± 7 kDa. This value is in agreement with the 78.4-kDa value already reported.[6]

Even though this approach affords high sensitivity and very good reproducibility, it also has some pronounced limitations. The main limitation is the requirement for use of an optimum amount of Cs salt for detecting the protein in order to avoid creating Cs nanoparticles of the same or similar size, which would cause peak overlapping in the macroIMS gas electropherograms. This is especially critical and likely to occur for small proteins with EMD of <5 nm. In Figure 5, it was observed (compare Figure 5a with b) that the presence of CsI caused a 4−5-fold suppression of CPC detection of transferrin, which is most likely a result of suppression occurring during the electrospray process.

It is also worth noting that a linear relationship was observed between protein concentration and the $^{133}Cs^+$ signal detected by

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ICPMS for solutions containing constant CsI concentrations, i.e., 50 μg/mL, and transferrin concentrations from 5 to 50 μg/mL, as detected from the resulting monomer peak (peak β in Figure 5): $y_{[133Cs+intensity]} = 121 + 10.0x_{[transferrin\ concentration]}$, $R = 0.9994$, $S_a = 7$, $S_b = 0.2$, $S_{y/x} = 8.6$, and $n = 4$. For the transferrin dimer peak (peak γ in Figure 5) linearity was observed for up to 100 μg/mL of transferrin: $y_{[133Cs+intensity]} = 0.2 + 6.2x_{[transferrin\ concentration]}$, $R = 0.9985$, $S_a = 8.0$, $S_b = 0.2$, $S_{y/x} = 14.4$, and $n = 6$. It seems that linearity no longer holds when Cs is depleted at the high protein concentrations. However, the linear range observed is a useful range for potential protein quantification using this technique.

Sizing DNA Molecules. Sizing of large DNA complexes was also attempted using the Cs tagging approach. In this case, a solution containing 10 μg/mL DNA and 50 μg/mL CsI was analyzed. The resulting size for this macromolecule as expressed by its EMD was determined to be 14.2 nm (figure provided as Supporting Information). Assuming a density of 0.7 g.cm^{-3}, as reported by Mouradian et al.[14] for double-stranded DNA, the calculated relative molecular mass is 631 kDa. It should be noted that Mouradian et al. determined density values for DNA of up to 300 kDa. It is thus possible that density values for larger molecules may differ. This value is substantially lower than the reported 1.3 MDa (~2000 bp) for which no information of the technique used was provided.[26] It is evident that more research is required in order to optimize the system for accurate mass determination of large DNA molecules. It is also of interest to note that our findings indicate that no size increase was observed for the DNA when forming Cs adducts at these concentrations (data not shown). The analysis of DNA, desalted by means of size exclusion chromatography, gave peaks with the same EMD as the nondesalted DNA.

It is anticipated that a high-sensitivity or collision cell or even a high-resolution ICPMS will allow for the direct detection of the phosphorus present in DNA, thus making the nES-macroIMS analysis much more specific and attractive for mixture analysis.

System Stability. The stability of the nES-macroIMS-ICPMS signal intensity was determined by separately evaluating the potential contribution of each of the system's components. For comparison purposes, CPC stability was monitored in parallel to ICPMS stability. More specifically, the stability of the nES source was evaluated by analyzing a 750 μg/mL CsI solution while the macroIMS was manually set to continuously (for 40 min) allow monodisperse particles to go through to the ICPMS. In this case, signal stability for Cs^+ and I^+ was determined to be 4 and 6% RSD, respectively. Most of the signal change was attributed to typical ICPMS signal drift. Replicate analysis of a 25 μg/mL CsI solution (solution loaded once into the nES source) gave RSD = 2.0%, for $n = 5$ (for CPC detection RSD = 1.4%). Multiple loadings ($n = 5$) of the same CsI solution resulted in RSD = 0.7% for the ICPMS signal and RSD = 0.8% for the CPC. Similar precisions were obtained for a solution containing 25 μg/mL CsI and 50 μg/mL hemoglobin.

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CONCLUSIONS

The development of a hyphenated nES-macroIMS-ICPMS system, suitable for detecting metals, metalloids, and halogens in nanoparticles and for sizing proteins, DNA, and inorganic nanoparticles has been demonstrated. Even though this report only describes the preliminary evaluation and exploration of the many possibilities afforded by this hyphenated combination of analytical systems, it seems obvious that it is promising for a variety of other application areas. These potentially include the direct (without Cs metalation) detection and sizing of metal, metalloid, and halogen containing biomolecules, as well as for DNA and RNA by monitoring their phosphorus content. It is also anticipated to become an invaluable tool for investigating interactions of metal/metalloid species with various large biomolecules. However, to achieve such advances, it is imperative to obtain higher detection sensitivity from the ICPMS. This is now realistic, as commercial ICPMS instruments with sensitivity of 1000−2000 million counts/s per μg/mL of element are available. Of course low background levels are also of great concern, and thus, this will need to be evaluated before substantial progress in these suggested areas can be further realized. These are all areas in which our research efforts are currently focused.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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