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# Identification and Accurate Size Characterization of Nanoparticles in Complex Media**

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***Abstract:*** *We have developed a new method for the identification and accurate size characterization of nanoparticles (NPs) in complex media based on capillary electrokinetic (CE) separation coupled to inductively coupled plasma mass spectrometry (ICP-MS). Through mass scanning and Gaussian fitting of electropherogram peaks, we can obtain multidimensional information on chemical compositions, size distributions, and ionic species of multiple NPs in a single run. The results are more accurate than those obtained by using conventional methods. This method provides a powerful tool for investigating polydisperse NP systems and rapid screening of NP-containing products.*

Nanoparticles (NPs) are becoming increasingly common in various consumer products.[1] However, a large obstacle is the lack of proper methods for characterizing and detecting of NPs in complex media. As a result, the standardization of NP applications in consumer products has been quite poor.[2] Currently available methods for the characterization of NPs mainly include microscopic and spectroscopic techniques.[3] Microscopic techniques, such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM), can provide visual size and shape information about the NPs, but suffer from imaging artifacts introduced during sample preparation and poor statistical accuracy.[4] Spectroscopic techniques, such as dynamic light scattering (DLS), are used to determine the average diameter of particles in suspensions.[5] Chromatographic techniques, such as liquid chromatography (LC) and field flow fractionation (FFF), have shown some potentials in separation of NPs.[6] However, the identification of chemical compositions and discrimination of different types of NPs and their relative ionic forms are not possible by using these techniques. Moreover, most of the currently available methods are not applicable in complex media because of matrix interference, and thus require complicated sample preparation procedures.

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Herein we report a method for the simultaneous identification and size characterization of NPs in complex media by combining the powerful separation ability of capillary electrophoresis (CE) with the excellent qualitative analysis ability of inductively coupled plasma mass spectrometry (ICP-MS). Polydisperse NPs are separated on-line from the sample matrix by CE and the chemical species of multiple NPs are identified by ICP-MS (see Figure 1). Notably, the CE peak

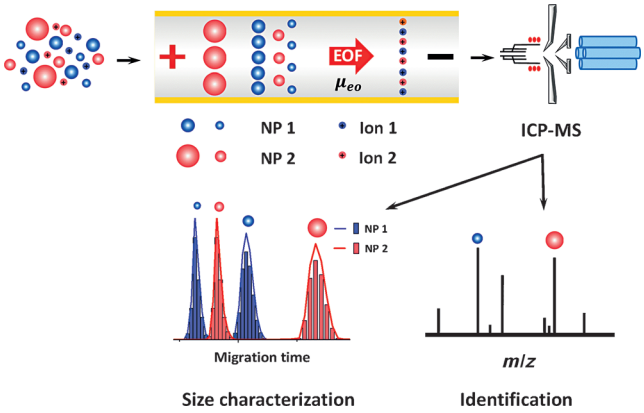


***Figure 1.*** Simultaneous identification and size characterization of NPs in complex media by CE-ICP-MS. EOF = electroosmotic flow.

shape reflects the size distributions, and accurate sizes of the NPs can be obtained by the Gaussian fitting of the peaks. The proposed strategy allows both chemical species recognition and size distribution characterization of multiple NPs in a single run without any complicated sample preparation procedures.

To demonstrate this concept, we firstly used silver nanoparticles (AgNPs) as model NPs. For spherical NPs that are negatively charged, their electrophoretic mobility ($\mu_{ep}$) is given by:

$$\mu_{ep} = \frac{q}{6\pi\eta r} \quad (1)$$

$$\mu_{ep} = \frac{q}{6\pi\eta r} \propto \frac{4\pi r^2}{6\pi\eta r} = Kr \quad (2)$$

where $q$ represents the net charge of particle, $\eta$ is the viscosity of the surrounding medium, and $r$ is the radius of the spherical NP. In Equation (1), the net charge of a particle in the buffer solution ($q$) is proportional to the surface area of NPs ($4\pi r^2$).[7] Therefore, the electrophoretic mobility of a NP could be given by Equation (2), where $K$ is a constant of proportion-

  

ality that is related to the viscosity of the medium.[8] Equation (2) indicates that the electrophoretic mobility ($\mu_{ep}$) is proportional to the radius of the NPs ($r$).[8,9] In the case of AgNPs, the negatively charged NPs move oppositely to the electroosmotic mobility ($\mu_{eo}$). Therefore, the apparent mobility ($\mu_{ap}$) of the NPs is calculated by:

$$\mu_{ap} = \mu_{eo} - \mu_{ep} = \mu_{eo} - Kr = \frac{L}{tE} \quad (3)$$

where $L$ is the length of the capillary, $t$ is the apparent migration time of the NPs, and $E$ is the electric field strength. The term $\mu_{eo}$ is a constant of proportionality that is related to the dielectric constant of the medium, the zeta potential at the capillary–buffer interface and the viscosity of the medium. Equation (3) therefore suggests a linear relationship between the radius ($r$) and the reciprocal migration time ($1/t$) of the NPs. On the other hand, the positively charged silver ion species moves at same direction of the $\mu_{eo}$ and therefore their apparent mobility ($\mu_{ap}$) is given by:

$$\mu_{ap} = \mu_{eo} - \mu_{ep} \quad (4)$$

According to Equations (3) and (4), AgNPs and silver ion species can be separated in a single run based on their different electrophoretic mobilities.

We tested this principle with commercially available AgNPs (citrate-coated). The CE separation was carried in a tris-borate buffer system (tris = tris(hydroxymethyl)aminomethane). Three kinds of AgNPs (nominal diameters 10 nm, 20 nm, and 40 nm) were characterized by using CE-ICP-MS. The results showed a good linear relationship ($R^2 = 0.998$) between the reciprocal migration time and NP size (in the 10–40 nm range). The migration time increased with the increase of the radius of NPs and followed a size-dependent migration mechanism. Thus, the migration time could be translated into the diameter of NPs and the curve in the electropherogram expressed the size distribution of the NPs. The actual size distribution of the AgNPs were obtained by using the Gaussian fitting (see Figure S1 in the Supporting Information).

As shown in Figure 2a,c,e the AgNP size distribution profiles obtained from CE-ICP-MS are generally consistent with those obtained from TEM. The peak shape of 40 nm AgNPs is consistent with the histogram obtained from TEM data (Figure 2e), thus indicating a reliable and accurate characterization result. The amount of AgNPs (nominal diameter of 10 nm) in the small diameter range (0–5 nm) derived from TEM studies is less than that derived from the CE-ICP-MS method (Figure 2a). This difference can be attributed to limitations of the TEM technique itself, as the small NPs could not be clearly recognized and identified in the TEM image. In contrast, the present method uses ICP-MS with high sensitivity and specificity for detection, thus avoiding the above-mentioned problem. This point is very important, because the number of small NPs in a polydisperse NP system is normally large and microscopic methods such as TEM may omit this size range of NPs, thus making the results inaccurate. On the other hand, in a polydisperse system, NPs

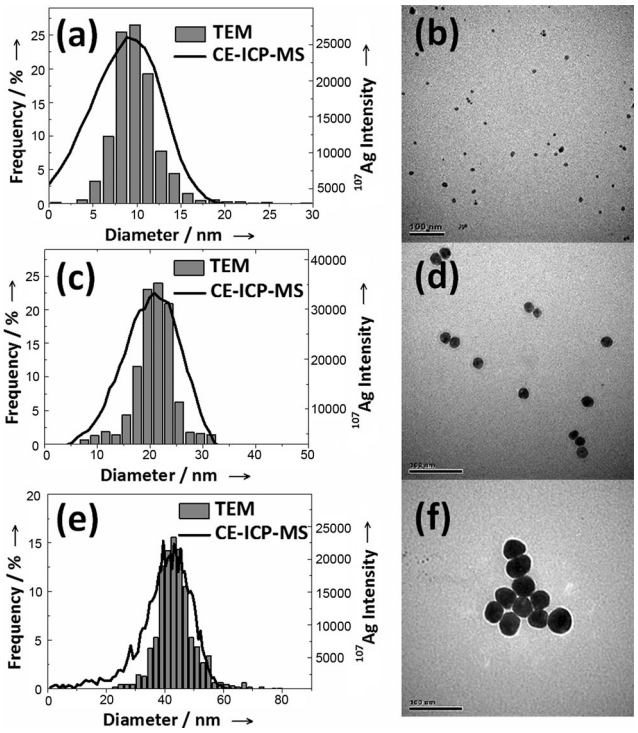


***Figure 2.*** Comparison of particle size distributions derived from CE-ICP-MS and TEM. In (a), (c), and (e), the lines represent the peaks obtained from CE-ICP-MS, and the histograms are the statistical results from the TEM measurements. (b), (d), and (f) are the corresponding TEM images for nanoparticles with nominal sizes of 10 nm, 20 nm, and 40 nm, respectively. Scale bars = 100 nm.

may dissolve to form smaller ones or agglomerate to larger ones. Our method shows a characterization ability covering wide particle size ranges, thus providing more statistically accurate results than microscopic methods. Furthermore, the sample pretreatment in microscopic methods may cause dehydration artifacts. In the case of CE-ICP-MS, NPs dispersed in running buffer solutions can maintain their original morphology, thus avoiding any artifacts during sample preparation. This is a prominent advantage provided by CE over other chromatographic methods for separation of NPs.[6] Another advantage of CE is that it can be easily tailored for different types of NPs by optimizing the buffer composition to obtain symmetric Gaussian peaks. Those advantages make the CE-ICP-MS method highly suitable for characterizing polydisperse NP systems. It should be noted that capillary zone electrophoresis may only work for charged NPs; for neutral or uncharged NPs, other separation modes such as micellar electrokinetic chromatography (i.e., adding surfactants in the running buffer) may be required.

The average AgNP sizes derived from three different techniques are compared in Table 1. The results obtained from CE-ICP-MS were consistent with those obtained from TEM, and showed little deviation from those obtained from DLS. A possible reason is that the diameters obtained from DLS are the apparent diameters of dynamic hydrated particles, which are often slightly larger than real ones.[10] Some artifact sources, such as dust, may also result in

  

*Table 1:* Size distributions of AgNPs [nm] obtained by using different techniques.

| Technique | Sample form | Nominal AgNP diameter [nm] | | |
|---|---|---|---|---|
| | | 10 | 20 | 40 |
| DLS | liquid suspension | 12.8 | 26.7 | 48.2 |
| TEM | dry, deposited on substrate | 9.5 ± 2.1 | 21.1 ± 2.8 | 42.9 ± 4.6 |
| CE-ICP-MS | liquid suspension | 8.2 ± 4.3 | 20.2 ± 5.7 | 42.0 ± 7.2 |

interference with the DLS results. Our method can distinguish the dust particles and NPs by ICP-MS, thus eliminating false positive results.

In addition to size characterization, the CE-ICP-MS method could distinguish the NPs and related ionic species. A mixture of silver ion species and three kinds of AgNPs (nominal diameters of 10 nm, 20 nm, and 40 nm) were simultaneously analyzed by using CE-ICP-MS. As shown in Figure 3a, the silver ion species as well as different-sized AgNPs were satisfactorily separated and detected in a single run. As shown in Figure 3b, a linear relationship existed between the diameter and the reciprocal migration time of the AgNPs. The size characterization results of AgNPs from CE-ICP-MS were consistent with the results from TEM (see Figure 3c), thus demonstrating the CE-ICP-MS method as an accurate method for characterization of polydisperse NP systems.

To test the universality of this method, we also applied it to gold nanoparticles (AuNPs), another widely used type of NPs. A mixture of different sized AgNPs and AuNPs (10 and 20 nm AuNPs, 10, 20, and 40 nm AgNPs, citrate coating) as well as gold and silver ion species were analyzed simultaneously. We found that identification and size characterization of AgNPs and AuNPs could be achieved in a single run (Figure S2 in the Supporting Information). The different types of NPs could be recognized by mass scanning in ICP-MS and characterized in different mass channels. Thus, the specificity and multielemental detection ability of ICP-MS allows us to characterize mixtures of multiple NPs.

To demonstrate the applicability of the present method in complex media, we used it in the rapid screening of NPs in consumer products. Taking AgNPs as an example, the method showed excellent reproducibility and high sensitivity, with detection limits in the submicrogram-per-liter range (see Table S1 in the Supporting Information). AgNPs are widely used in consumer and medical products as a broad-spectrum antimicrobial agent.[11] We analyzed two kinds of consumer products, an antiseptic kitchen cleansing spray and a nanosilver antibiotic lotion for gynecological care that apparently contains AgNPs. Silver ion species were found at a concentration of 623.8 $\mu g L^{-1}$ in the antiseptic kitchen cleansing spray (Figure S3). However, AgNPs could not be detected. In the antibiotic lotion, two sizes of AgNPs ((7.8 ± 4.8) nm and (33.5 ± 2.6) nm) were detected and the results were successfully verified by using TEM (Figure 4). Thus, these results demonstrated that this method can be used for rapid screen-

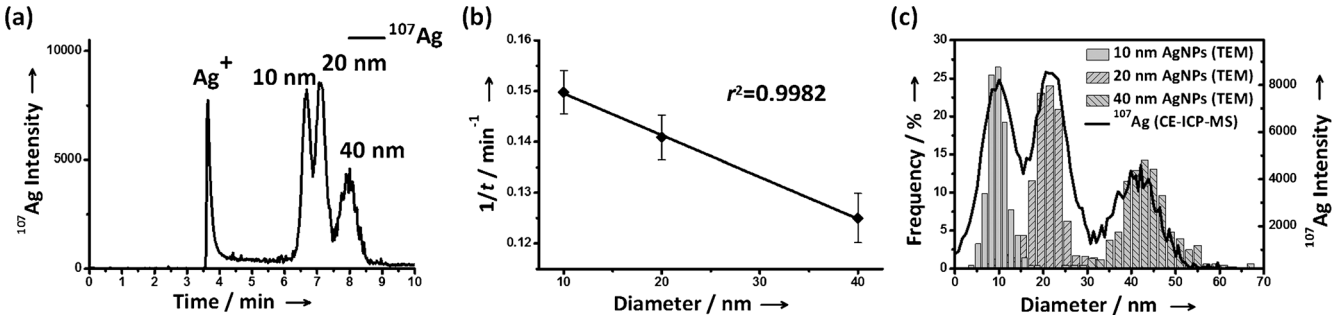


*Figure 3.* Typical electropherogram of a mixture of $Ag^+$, 10 nm, 20 nm, and 40 nm AgNPs (a); relationship between the reciprocal migration time and the diameter of AgNPs (b); and size distributions generated by CE-ICP-MS and TEM (c).

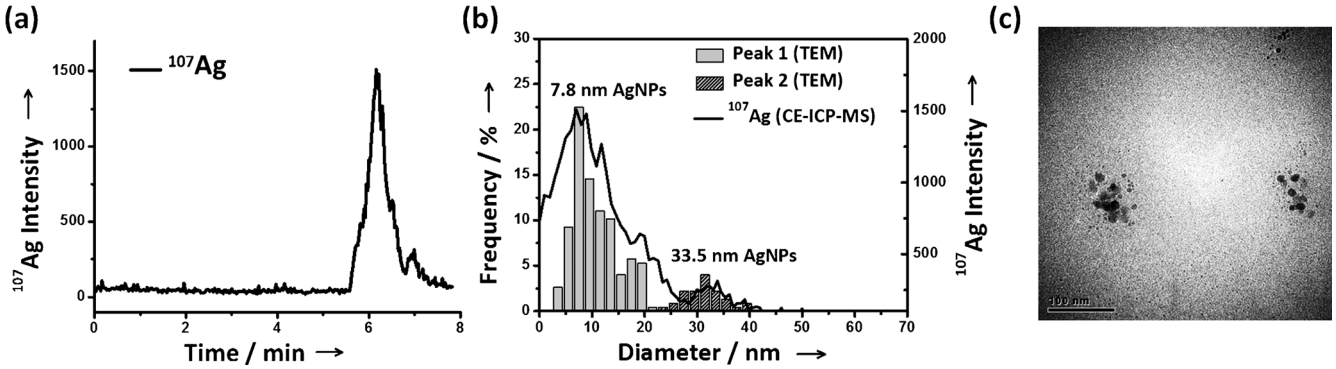


*Figure 4.* Electropherogram (a) and size distributions obtained from CE-ICP-MS and TEM measurements (b) of AgNPs in a commercial nanosilver antibiotic lotion for gynecological care, and the corresponding TEM image (c; scale bar = 100 nm).

ing of NP-containing products and for quality control of NPs in commercial products. We also tested this method in complex environmental samples, including river water and wastewater samples. Satisfactory recoveries of AgNPs were obtained (88.2–95.2%) with slight aggregation of AgNPs observed (see Table S3). The NP concentrations in the products or environmental samples are normally very low. Commonly used techniques, such as TEM and DLS, cannot be used to identify the NPs or the ionic species, while the CE-ICP-MS method provides a more sensitive tool for this purpose.

In summary, a new and versatile method based on capillary electrokinetic separation and ICP-MS was developed to provide a reliable platform for identification and size characterization of NPs in complex media. The CE-ICP-MS technique can provide multidimensional information of polydisperse NPs systems and exhibited significant advantages than existing methods. Firstly, we can obtain more accurate size-distribution information of NPs. Secondly, we can determine the chemical species of the NPs as well as related ionic species simultaneously in a single run. Finally, the method is applicable to the characterization and screening of NPs in complex media such as consumer products and environmental samples. Therefore, it provides a powerful tool for "nano" research and standardization.

## Experimental Section

The CE-ICP-MS system consisted of an Agilent HP$^{3D}$ CE system (Germany) and an Agilent 7500ce ICP-MS (USA). The interface for the coupling of CE with ICP-MS was a simple and highly efficient sprayer (G1607A, Agilent, USA) as described previously.[12] The CE separation was performed using a 60 cm length × 75 μm i.d. × 365 μm o.d. fused-silica capillary (Yongnian Optical Fiber Company, Hebei, China). The running buffer consisted of 10 mM tris, 10 mM $H_3BO_3$, and 10 mM $Na_2B_4O_7$ (pH 9.0). Other CE-ICP-MS parameters are given in Table S2. The samples were filtered through 0.22 μm nylon filter membranes, diluted by the running buffer solution, and then directly subjected to CE-ICP-MS analysis. The migration time of the CE-ICP-MS electropherogram was translated into the diameter of the NPs by normalizing using at least three NP standards with different nominal sizes. The average sizes of NPs and their standard deviations were obtained through the Gaussian fitting of the electropherogram peaks by using the OriginPro8 software (OriginLab, Northampton, MA).

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