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Journal of Chromatography A, 1161 (2007) 314–321

JOURNAL OF CHROMATOGRAPHY A

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# On-line enhancement and separation of nanoparticles using capillary electrophoresis

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Received 19 January 2007; received in revised form 22 May 2007; accepted 23 May 2007

**Abstract**

We describe a rapid, simple, and highly efficient capillary electrophoresis (CE)-based method for the analysis of nanoparticles (NPs). In this study, we used the reversed electrode polarity stacking mode (REPSM) of CE to assess the feasibility of enhancing the detection of Au NPs and Au/Ag NPs, optimizing parameters such as the length of time for which the REPSM was applied, the concentrations of the buffer and the sodium dodecylsulfate (SDS) surfactant, and the pH. Under the optimized on-line enhancement conditions [buffer: SDS (40 mM) and 3-cyclohexylamino-1-propanesulfonic acid (CAPS; 10 mM) at pH 10.0; applied voltage: 20 kV; REPSM applied for 24 s], the detection limits of the Au NPs and Au/Ag NPs increased by ca. 30- and 140-fold, respectively. In addition, when the NPs were subjected to on-line enhancement and separation by CE using diode array detection (DAD), this approach allowed chemical characterization of the NP species. Our results suggest that such CE analyses will be useful for accelerating the rates of fabrication and characterization of future nanomaterials.

*Keywords:* On-line enhancement; Separation; Nanoparticles; Capillary electrophoresis

## 1. Introduction

Nanometer-sized metal materials are attracting a great deal of attention in various fields of physics, chemistry, and biology [1] because their diameters dramatically affect their physicochemical properties [2]. During the past few years, many methods have been introduced for synthesizing Au and Ag NPs, with one of the major goals being the manufacture of NPs for sensing applications [3]. For example, combining surface-enhanced Raman spectroscopy (SERS) with NPs allow chemical analyses to be performed at very low concentrations—sometimes with single-molecule sensitivity [4,5]. A prerequisite for SERS analysis through Raman signal enhancement is adsorption of the compound under investigation onto the surfaces of nanosized Au and Ag particles [4,5]; the sensitivity of analysis depends on the size and surface roughness of the metallic NPs [6]. Although Au NPs have been applied extensively to sensing technologies because of their readily controllable size distributions, long-term stability, and biocompatibility with antibodies and antigens [7] and DNA and RNA [8], their enhancement factor is lower than that of Ag NPs [9,10]. For this reason, many attempts at preparing core/shell Au/Ag NPs through the deposition of Ag onto preformed Au NPs have been undertaken to obtain more highly homogeneous Ag NPs exhibiting greater enhancing efficiency over that of Au NPs alone [5,11]. The fabrication of Au/Ag core/shell NPs is currently attracting a great deal of interest, as is the study of how their SERS phenomena relate to their sizes. Therefore, manipulation of the crystal growth process and rapid determination of the sizes of the final Au/Ag core/shell NPs are two critical features that must be optimized when employing these materials in SERS applications.

Presently, characterization of the size distribution of solution-grown NPs is performed almost exclusively using dynamic light scattering (DLS) [12] and electron microscopy (EM) techniques [13]. Although DLS can provide rapid measurement

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doi:10.1016/j.chroma.2007.05.072

of the sizes of NP products, it is difficult to infer the properties of an ensemble of NPs, such as their physical properties (e.g., absorbance). Likewise, transmission electron microscopy (TEM) is employed widely to measure the sizes of metal particles, but it is a time-consuming technique that has high operating costs and does not include a separation process. Although we are by no means advocating abandoning DLS and TEM approaches to qualitative NP analyses, we hope to demonstrate herein that separation approaches can be used to analyze NPs' sizes while simultaneously measuring their physical properties (e.g., absorbance). Such analyses can be performed on a single sample using a small volume and with a short analysis time.

CE has emerged recently as one of the most powerful separation techniques [14]. It can be used to separate – and sometimes analyze – a variety of differently sized materials, including inorganic Au and Ag particles [15–18]. The separation mechanism relies upon the electrophoretic mobility, which is a function of the zeta potential, defined by the net charge present within the layer of liquid enveloping the particle surface (the so-called "surface of shear") [19]. The resulting net charge is usually smaller than the particle surface charge determined, for instance, through titration [19]. Thus, the size-dependent separation cannot be accounted for on the basis of the zeta potential, because that value depends on the net charge density, which is the same for chemically homogeneous particles of different sizes [20]. The size-dependent separation is likely to arise from the particular conditions present during of CE, viz., the use of high electric fields and high-ionic-strength electrolyte buffers, which can lead to the pronounced relaxation effect [19–23]. The relaxation effect is described by a dimensionless parameter $\kappa a$, where $a$ is the particle radius and $\kappa^{-1}$ is the thickness of the electric double layer. Deformation of the electric double layer around the particle – the degree of which differs depending on the size of the particles and buffer ionic strength – results in size-dependent electrophoretic migration of the particles; as a result, CE can be used to separate the particles based on their sizes [16].

Although CE is a powerful technique that is simple to perform, provides rapid results, has high efficiency and resolution, and involves low sample consumption, the benefits provided by the high number of theoretical plates obtained with CE can be overshadowed by the low sensitivity of UV detection systems [24]. Because of the small dimensions of CE capillaries [typical inside diameters (I.D.) and lengths are 25–100 μm and 35–80 cm, respectively], only very small sample volumes can be loaded into them. Additionally, for most common optical detection techniques, CE suffers from a drastically reduced path length relative to, for example, liquid chromatography (LC). To preconcentrate samples and to increase the amount of sample that can be loaded into the column without degrading the separation, several on-line sample enhancement techniques have been developed for various electrophoretic modes [25–27], including the reversed electrode polarity stacking mode (REPSM) [28,29]. The REPSM introduces the sample into the capillary hydrodynamically, a stacking voltage is applied at negative polarity to preconcentrate the analytes at the interface between the sample zone and the background electrolyte, and then the sample matrix is pumped from the capillary under electroosmotic flow (EOF). Thus, using the REPSM technique allows the concentrated analytes to be separated and detected effectively.

In this study, we investigated whether REPSM could be used for on-line enhancement in the detection of NPs. We examined the effects that several electrophoresis parameters had on the on-line enhancement, including the time for which the REPSM was applied, the concentrations of the buffer and SDS surfactant, and the pH. If the detection ability of CE for NPs could be enhanced, then this approach would provide a simple means of characterizing the sizes of NPs at trace levels and might accelerate the process of analyzing future nanomaterials. In this preliminary study, we focused on the ability of CE to enhance the separation of mixtures of Au NPs and Au/Ag core/shell NPs. An additional attractive feature is that the optical spectra of NPs, which depend on both their sizes and compositions, are readily obtained when using a CE system equipped with an on-line DAD system. Thus, a single sample of NPs can be analyzed within a rapid analysis time to characterize their size- and/or species-dependent properties.

## 2. Experimental

### 2.1. Apparatus

A JEOL (Tokyo, Japan) JSM-6500F scanning electron microscopy (SEM) system was used to characterize the sizes of the Au NPs and the Au/Ag core/shell NPs. The particle sizes of the samples were determined through two-dimensional grain analysis after digitizing the SEM images. All separations were performed using an Agilent CE system (Waldbronn, Germany). Uncoated fused-silica capillaries (I.D.: 75 μm; length: 33.5 cm) were obtained from Polymicro Technologies (Phoenix, AZ, USA). A detection window was fabricated ca. 8.5 cm from the capillary outlet. In the on-line enhancement studies, sample injection was performed at a pressure of 50 mbar for 50 s. Under such injection conditions, the injection plug was estimated to fill 40% of the capillary column. In the conventional process, sample injection was performed at a pressure of 50 mbar for 2 s. The following equation was used to calculate the sensitivity enhancement factor (SEF) of the REPSM in terms of the relative peak heights:

$$\mathrm{SEF} = \frac{\text{Peak height}_{\text{obtained with REPSM}}}{\text{Peak height}_{\text{obtained with conventional injection}}} \quad (1)$$

The values of pH of the electrolytes were measured using an Orion 420A pH meter (Boston, MA, USA). Prior to use, the capillary was pretreated by flushing sequentially, for 3 min each time, with 1 M NaOH, 0.1 M NaOH, water, and buffer. The capillary was also rinsed with running buffer for 3 min between each run. All CE experiments were performed at a temperature of 25 °C.