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Characterization of Nanoparticles by Capillary Electrophoresis

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Technical Report

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Abstract

The report describes the use of capillary electrophoresis on gold, polymer and iron oxide nanoparticles. The analyte species were detected by UV/Vis or laser-induced fluorescence (LIF) spectroscopy. For analytes that possessed neither UV/vis absorbance nor LIF, indirect methods of detection were employed whereby a signal-generating molecular species was added to the running buffer. Nanoparticles from a variety of commercial sources and from custom syntheses were studied. The work presents calculations and determinations of physico-chemical characteristics of Nanoparticles such as electrophoretic mobility, size distribution, and charge/size ratio. It is also the first report of capillary electrophoresis of nanoparticles using indirect detection. The report demonstrates that capillary electrophoresis is a valuable tool in nanoparticle research.

Résumé

Ce rapport décrit la caractérisation de nanoparticules d'or, de polymère et d'oxyde de fer par électrophorèse capillaire. Les analytes ont été détectés par des méthodes spectroscopiques, soit UV/Vis, soit fluorescence induite par laser (FIL). Dans les cas où l'analyte n'était sensible ni à l'UV/Vis, ni au FIL, des témoins indirects ont été employés. Les nanoparticules étaient de provenance commerciale et de nos laboratoires (NRC: IMI). Nous résumons ici nos calculs et la détermination de propriétés physico-chimiques: mobilité électrophorétique, distribution de taille, et ratio masse/charge. A notre connaissance, ce rapport présente une première démonstration de la détection indirecte de nanoparticules par électrophorèse capillaire avec témoins. Le rapport démontre l'utilité de l'électrophorèse capillaire dans la caractérisation complète de nanoparticules en solution.

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Executive summary

Characterization of Nanoparticles by Capillary Electrophoresis

T. Tang; W.E. Lee; A.B. Jemere; N.S. Cameron; T. Veres; DRDC Suffield TR 2008-216; Defence R&D Canada – Suffield; December 2008.

Introduction or background: The work in this report was carried out under CRTI project # 03-0005RD. DRDC Suffield was a research partner in the project that was lead by Industrial Materials Institute (National Research Council Canada Boucherville QC). One of the roles of DRDC Suffield in the project was to provide research and analytical support in areas of nanoparticles and in design of micro-sized biological detection systems. To this end the physico-chemical properties of a variety of nanoparticles were determined using capillary electrophoresis .

Results: Analyses of nanoparticles composed of polymer, gold or iron oxide were carried out to determine electrophoretic mobility, size distribution and charge/size ratio.

Significance: The work demonstrated that capillary electrophoresis is a valuable tool in nanoparticles research. The physico-chemical properties of nanoparticles have critical effects on their behaviour in bio-analytical devices. Thus nanoparticles used in such devices must be subject to quality control and quality assurance. For a technology to advance from the experimental stage to the technology-demonstration model, chemical methods, reagents and other components need to be standardized. Analyses presented in this report have pointed the way to using capillary electrophoresis in routine methods for quality control/quality assurance of nanoparticles.

Future plans: CRTI project #03-0005RD was completed in 2007. Subsequent and future work on a technology demonstration for a microfluidic biological detection system is funded through a CRTI technology demonstration project. DRDC Suffield continues as a partner in the project (CRTI #06-0187TD) providing bio-analytical chemistry and microfluidic system design.

Sommaire

Caractérisation des nanoparticules par électrophorèse capillaire

T. Tang; W.E. Lee; A.B. Jemere; N.S. Cameron; T. Veres; RDDC Suffield TR 2008-216; R et D pour la Défense Canada – Suffield; décembre 2008.

Introduction ou contexte : Les travaux présentés dans le rapport ont été réalisés dans le cadre du projet de l'IRTC n° 03-0005RD. RDDC Suffield était l'un des partenaires de recherche de ce projet dirigé par l'Institut des matériaux industriels (Conseil national de recherche Canada à Boucherville, QC). Un des rôles de RDDC Suffield était de fournir du soutien en recherche et analyse dans certains domaines de nanoparticules (NP) et dans la conception de micro-systèmes de détection biologiques. À cette fin, on a déterminé les propriétés physico-chimiques de diverses nanoparticules par électrophorèse capillaire.

Résultats : Des nanoparticules composées de polymères, d'or ou d'oxyde de fer ont analysées pour déterminer leur mobilité électrophorétique, leur distribution selon la taille, et leur ratio charge/taille.

Importance : Les travaux ont démontré que l'électrophorèse capillaire est un outil précieux dans la recherche sur les nanoparticules. Les propriétés physico-chimiques des NP ont des effets critiques sur leur comportement dans des appareils bio-analytiques. Les NP utilisées dans ce genre d'appareils doivent donc faire l'objet d'une procédure de contrôle de la qualité et d'assurance de la qualité (cq/qa). Pour qu'une technologie puisse passer de l'étape expérimentale à un modèle de démonstration de la technologie, les méthodes chimiques, les réactifs et les autres composants doivent être normalisés. Les analyses présentées dans le rapport ouvrent la voie à l'utilisation de l'électrophorèse capillaire dans les méthodes régulières de cq/qa des NP.

Perspectives : Le projet de l'IRTC n° 03-0005RD a été achevé en 2007. Des travaux subséquents et futurs au chapitre de la démonstration de la technologie de systèmes de détection biologiques microfluidiques sont financés par l'intermédiaire d'un projet de démonstration technologique de l'IRTC. RDDC Suffield continue de contribuer au projet (IRTC n° 06-0187TD), en fournissant des travaux de chimie bio-analytique et de conception de systèmes microfluidiques.

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Introduction

Nanoparticles have many applications in defence sciences, especially in chemistry and bioanalysis [1-2]. During the past decade many methods have been reported to synthesize metal nanoparticles (Au, Ag, Fe, etc.) with one of the major goals being the manufacturing of nanoparticles for sensor development [3,4]. In separation science, significant advances have been made in electrophoresis and microchip separations employing nanoparticles [5]. Such advances with nanoparticles include their use as stationary phases for liquid chromatography [6], gas chromatography [7], and capillary electrochromatography [8], as well as roles as run buffer additives to enhance electrophoresis [9]. Separation, characterization and quantification are important issues in the development of applications for submicron particles. The size is a significant factor affecting the physical and chemical properties of nanoparticles. Similarly electronic charge and charge-to-size also have critical effects on such properties. The commonly used methods to determine the size distribution of nanoparticles involve transmission electron microscopy (TEM), scanning electron microscopy (SEM), and size-exclusion chromatography [10]. These methods, however, have inherent problems such as degradation of samples and irreversible adsorption of surface-active nanoparticles. EM methods are expensive and time consuming and do not provide separation or charge-to-mass data. Chromatography techniques have inherent limitations with respect to sample degradation and irreversible adsorption of active-surface nanoparticles. More rapid and robust analysis methods are of great interest and utility.

Capillary electrophoresis (CE), also referred to as capillary zone electrophoresis, is a well established powerful technique for the separation of charged macromolecules according to their size-to-charge ratio. CE is not limited to the separation of discrete molecules but is able to separate charged aggregate states of matter such as micelles [11, 12] and non-bonded aggregates such as nucleic acid hybrids [13], paired conjugates of biotin-streptavidin [14] as well as bio-particles [15]. CE offers a number of advantages, including short analysis time, small sample mass and volume requirements, and the convenience of autosampling. The use of CE for the study of nanoparticles is relatively new although introductory reports have been published on a variety of inorganic oxide, latex, polystyrene, silicate, gold and silver nanoparticles [16-20].

The principle of CE separation is the differential electrophoretic mobilities of the charged analytes in an electric field [11]. In this work we use capillary zone electrophoresis, wherein a plug (i.e., a small volume or zone) of analyte sample solution is injected into the end of an open free-flowing buffer-filled capillary. Application of high voltage to the buffer reservoirs that contain the ends of the capillary causes an electroosmotic flow (EOF) of buffer (including the analyte plug) from the anode to cathode (negative terminal). As the sample plug travels the length of the capillary, electrophoresis occurs in the sample zone. Positively charged species (cations) are attracted to the negative electrode as they travel through the capillary, and hence run faster than neutral species. Conversely, negatively charged species (anions) are repelled as they travel through the capillary and run slower. In the CE experiment, analytes are separated according to the electrophoretic mobility which in turn is proportional to the charge-to-mass ratio [11a].

Several analyte detection modes were available for this work. The most common mode of detection in CE is absorption of ultraviolet/visible light wherein the absorbance (or transmittance) of an analyte species is measured as it passes an optical detector. Another often-used detection

method is laser-induced fluorescence (LIF). For this method a narrow laser beam (usually argon-ion 488 nm) is focused on the detection area of the capillary to excite fluorescence in the analyte species. Direct detection of analyte occurs when the experimental signal, either absorption of light or fluorescence emission, derives from the analyte species themselves. In cases where the analyte species have no measurable absorption or fluorescence, indirect detection methods can be used, whereby a signal-generating marker molecule is added to the background electrolyte (also referred to as running buffer). Displacement of the marker by the analyte species in the running buffer results in a decrease (or dip) in the signal in the electropherogram as the analyte molecules pass through the detection volume. In this work we employed both direct and indirect detection.

The purpose of the work in this report was to demonstrate the analytical potential and utility of CE in NP research. For this research we obtained nanoparticles from several commercial sources and through custom syntheses. nanoparticles composed of polystyrene and gold have generated recent interest and have been used in molecular biology [21] and diagnostics [22]. We studied these classes of nanoparticles in order to determine whether CE is a useful technique for characterizing nanoparticles and establish a foundation of knowledge for applying CE to new types of nanoparticles.

Experimental Section

Chemicals and reagents

Tris (hydroxymethyl)aminomethane (Tris) and thiourea were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). 2-(N-Morpholino)ethane sulphonic acid (MES) was purchased from ICN Biomedical Inc. (Aurora, OH). BODIPY 493/503 (4,4-difluoro-1,3,5,7,8-penta methyl-4-bora-3a,4a-diaza-s-indacene), and fluorescein were purchased from Invitrogen Molecular Probes (Eugene, OR), and were prepared as a 0.1 mM stock solution in HPLC grade methanol (Fisher, Fair Lawn, NJ) and in ultra pure water, respectively. 55 nm and 210 nm polystyrene nanoparticles, labeled with Dragon green (interior) and FITC (exterior), respectively, were purchased from Bangs Laboratories (Fishers, IN, USA). The 55 nm particles were obtained in DI water that contained 0.1% Tween 20 and the 210 nm particles were obtained in DI water that contained 0.5% HEPES and 0.1% Tween 20. Polystyrene-coated, activated Dynal beads (Invitrogen) were covalently labeled with Lucifer Yellow CH (Invitrogen) according to methods provided by Invitrogen. Beads were washed after modification and stored at 4 °C until used in CE analysis. Magnetic nanoparticles of $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$ (the symbol “@” denotes “decorated with”, i.e. iron oxide particles (Fe_3O_4) decorated with NMe_4OH ; “Me” denotes methyl group, CH_3) were prepared according to methods of Berger et al. [23]. Transmission electron microscopy (TEM) indicated particles of approximately 8 (± 2) and 18 (± 3) nm (data not shown). Silica coated-iron oxide core particles ($\text{Fe}_3\text{O}_4@\text{SiO}_2$) were produced according to methods of Stober et al. [24, 25]. TEM indicated particle size of approximately 50 (± 7) nm diameter (data not shown). Buffers were prepared using ultra pure water prepared with a deionizing system (Millipore Canada, Mississauga, ON) from distilled water, and filtered through a nylon syringe filter (0.2 μm pore size, Nalgene, Rochester, NY) prior to use. Gold nanoparticles were purchased from Sigma Aldrich Canada as colloidal gold 5 nm 0.01% as HAuCl_4 and 20 nm 0.01% as HAuCl_4 .

CE Analysis

CE-LIF studies were performed using a Beckman 5010 CE instrument (Beckman Coulter Inc., Fullerton, CA) equipped with an argon ion laser operated at 488 nm excitation and 520 nm emission. UV-Absorbance measurements were made at 214 nm. Samples were run on a 37 cm or 47 cm long and 50 μm i.d. fused silica capillary from PolyMicro Technologies (Phoenix, Az, USA) at column temperature of 25 °C. Prior to sample run, the capillary was conditioned with 0.1 M NaOH for 10 minutes, water for 5 minutes followed by equilibration with the separation buffer for 30 minutes. In between runs, the capillary was conditioned with 0.1 M NaOH for 2 minutes followed by the run buffer for 4 minutes. Sample was pressure injected for 5 sec at 0.5 psi. The separation voltages and buffer conditions are indicated in the figure captions. Peak heights and peak areas were calculated and plotted as required. CE-LIF studies of LY-labeled beads (data in Figure 3) were performed on a Beckman Coulter Proteome Lab PA 800 instrument installed with 50 μm fused silica capillary, 47 cm in length, 40 cm to the detector.

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Results and Discussion

Capillary electrophoresis of polymeric nanoparticles by direct LIF detection

Laser-induced fluorescence (LIF) detection is ideally suited for CE. Sensitivity approaching single molecule level has been achieved [26]. However, direct LIF detection requires the presence of a fluorophore as an integral part of the analyte. Here, commercial-sourced fluorescent polystyrene beads were used in the research to gain understanding of the behavior of nanoparticles in CE. Commercial nanoparticles provided a practical and expedient sample material for investigation since they were of known size and distribution and had fluorophores covalently linked or embedded in the particles. The results of polystyrene nanoparticles in CE with LIF detection are shown in Figure 1a. The electropherograms have sharp narrow peaks and low background, indicative of small size distribution and low leakage for fluorophore. The migration times (t_{mig}) were 2.773 min for 55 nm and 3.921 min for 210 nm particles when run under the same conditions.

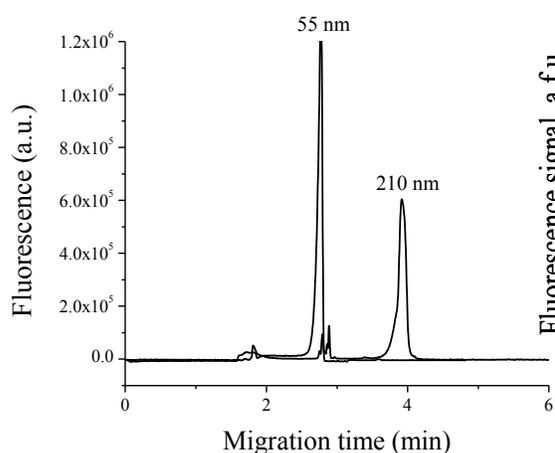


Figure 1a: Electropherograms of 55 nm polymeric nanoparticles labeled with Dragon green (1.094×10^{14} particles/mL) and 210 nm polymeric nanoparticles labeled with fluorescein (1.965×10^{12}) particles/mL). Separation was effected by applying 14 kV. The separation buffer was 50 mM Tris, pH 9.0.

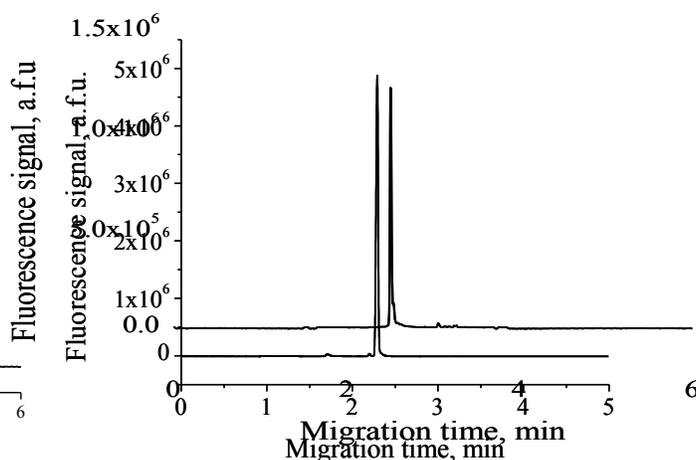


Figure 2b: Capillary electropherograms of fluorescein; $t_{\text{mig}} = 2.513$ min (top) and BODIPY, $t_{\text{mig}} = 2.292$ min (bottom), neutral marker for Figure 1a;

Comparison of t_{mig} for nanoparticles to that of the neutral EOF marker, BODIPY, (2.292 min, Figure 1b) indicated that the nanoparticles were negatively charged. In Figure 1a (210 nm sample) it can be seen from the electropherogram that the sample contained species other than 210 nm particles. The small peak at 1.815 min is indicative of a positively charged species; the small doublet at 2.76 and 2.83 min indicative of negatively charged. In this study these minor peaks were not identified. Neither of the minor peaks co-migrated with the negatively charged fluorescein dye ($t_{\text{mig}} = 2.513$ min, Figure 1b). In Figure 1a (55 nm sample) the very slight rise in the baseline at 1.8 min could be attributable to the same positively charged species present in the 210 nm sample. These results provide a convenient method to evaluate the samples of nanoparticles. The data shows that the 55 nm sample was relatively homogeneous with respect to negatively charged fluorescent nanoparticles whereas the 210 nm sample contained non-identified (to date) charged species.

The reproducibility of t_{mig} was measured for the samples of nanoparticles. Over 5 consecutive runs the relative standard deviation (rsd) was calculated to be 0.9% and 1.2% for 55 and 210 nm particles, respectively. The band shapes of the major peaks in Figure 1a show minor amounts of “fronting” and low-to-negligible “tailing”. Such band-shape features are expected in CE experiments where true electrophoresis separation is occurring.

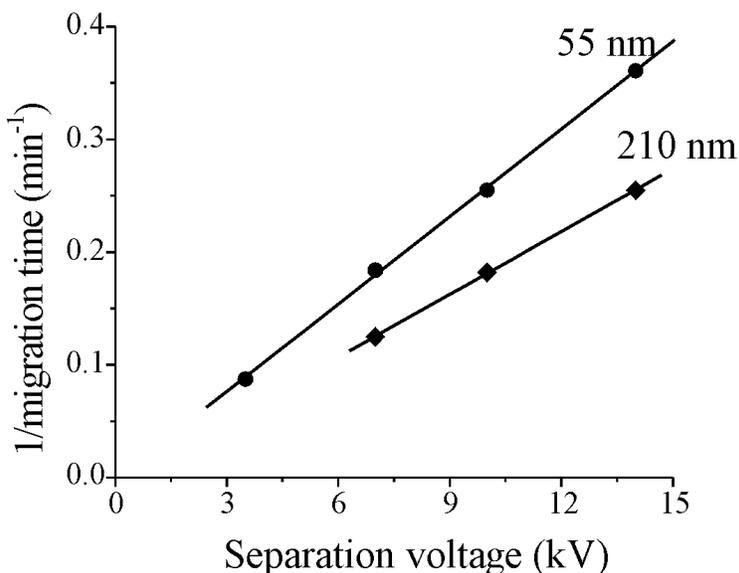


Figure 3: Effect of separation voltage on the migration times of the polymeric nanoparticles. Other than voltage all experimental conditions were as in Figure 1a.

The NP samples were subjected to CE over a range of high voltage to further assess the electrodynamic behavior. Theory predicts, for free flowing species in electrophoresis, the relationship [11b]

$$\mu_{ap} = L_d \times L_t / (V \times t_{mig}) \quad (1)$$

where μ_{ap} is the apparent electrophoretic mobility, L_d is the length to the detector, L_t is the total length of the capillary, V is the applied voltage across the capillary, t_{mig} is the measured migration time. Rearranging,

$$1/t_{mig} = (\mu_{ap} \times V) / (L_d \times L_t) \quad (2)$$

Thus the plot of $1/t_{mig}$ versus V is predicted to be linear and, as shown in Figure 2, is indeed the case for the fluorescent nanoparticles. The slope of the plot is

$$\mu_{ap} / (L_d \times L_t) \quad (3)$$

Since L_d and L_t are known from the experimental set-up, calculation of μ_{ap} can be made. The apparent electrophoretic mobility, μ_{ap} , is the sum of the EOF mobility, μ_{eof} , and the electrophoretic mobility, μ_{ep} . The value of μ_{eof} can be determined from the neutral marker. Thus rearranging this relationship we can write

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

From the experiment we calculated electrophoretic mobilities, μ_{ep} , to be $-6.0 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ min}^{-1}$ (55 nm) and $-1.44 \times 10^{-2} \text{ cm}^2 \text{ V}^{-1} \text{ min}^{-1}$ (210 nm). The ratio of electrophoretic mobilities is

$$\mu_{ep210} / \mu_{ep55} = (-1.44 \times 10^{-2} \text{ cm}^2 \text{ V}^{-1} \text{ min}^{-1}) / (-6.0 \times 10^{-3} \text{ cm}^2 \text{ V}^{-1} \text{ min}^{-1}) = 2.4$$

Since the particles were negatively charged, their mobility was counter to the electroosmotic flow and hence the negative values. The experiments associated with Figures 1 and 2 allow us to state with confidence that the polystyrene nanoparticles separate by CE and behave according to electrodynamic theory of free flowing particles. Debye-Huckel-Henry theory of electrophoretic mobility of a charged molecular species is given by

$$\mu_{ep} = q / 6\eta\pi r \quad (5)$$

where q is the charge of the particle, η is the viscosity of the solution and r is the Stokes' radius [11a]. Rearranging

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

Thus,

$$q_{210} / q_{55} = \mu_{ep210} / \mu_{ep55} \times (r_{210}/r_{55}) \quad (7)$$

where the ratio, $\mu_{ep210} / \mu_{ep55} = 2.4$, was determined above from experiment and Stokes' radius, r , is approximated as half the nominal diameters (105 and 27.5 nm). Substituting in equation 7, the ratio of charge on the particles is

$$q_{210} / q_{55} = 2.4 \times (105/27.5) = 9.1$$

If the surface of the particles contained a constant amount of charge per unit area, then the ratio of charge would be dependent on the ratio of surface area, where $A = 4\pi r^2$. This assumption would predict

$$q_{210} / q_{55} = A_{210} / A_{55} = (105/27.5)^2 = 14.6$$

a ratio of 14.6 compared to a ratio based on experiment of 9.1. This analysis does not consider true Stokes (hydrodynamic) radii (larger than the nominal radii used) or the effect of dielectric friction [27]. In addition the surface chemistries of the two polystyrene NP samples were not identical. The fluorescent dye in the 55 nm beads was embedded in the polystyrene matrix whereas in the 210 nm beads the dye was covalently attached to the polystyrene surface. The calculations in equations 7 and 8 are presented as illustrative examples of CE in NP research. The experimental approach contained in Figures 1 and 2 can provide a rich source of physico-chemical knowledge.

Capillary electrophoresis of fluorescently-labeled particles by direct LIF detection

The analytical method used above for commercial polymeric nanoparticles was employed for investigating a sample of custom-modified fluorescent particles. The particles were polystyrene-coated iron oxide-core (1000 nm diameter) and were rendered fluorescent through covalent attachment of the dye Lucifer Yellow-CH (LY). Figure 3 (top panel) shows an electropherogram of a sample of LY-labeled beads. Compared to the fluorescein-labeled particles (Figure 1a), the electropherogram has a large number of peaks indicating that the sample contained many species. Based on the t_{mig} of the neutral marker, BODIPY, (data not shown) the beads and other species were negatively charged. The electropherogram of the supernatant (Figure 3 middle panel) possesses some of the features of that of the beads although the overall t_{mig} values in the bead-containing sample (top panel) were greater by about 0.2-0.5 min (i.e., the migration was slower). The main difference is the fewer number of peaks in the supernatant sample over the time range of 7-9 min. The electropherogram of supernatant resembles that of free LY (Figure 3 bottom panel). Since the beads were previously washed after modification with LY-CH, the CE data suggest that LY dye, initially covalently bound to beads, was released during storage.

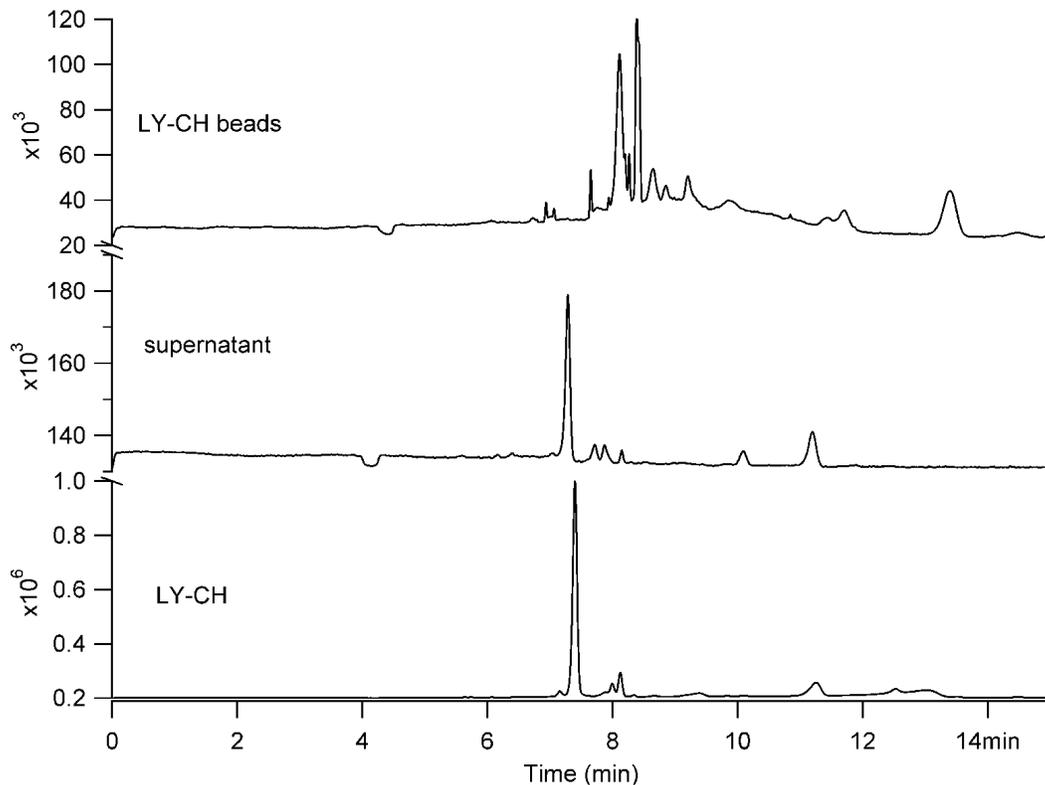


Figure 4: CE LIF of polystyrene-coated iron oxide-core beads (1000 nm diameter), rendered fluorescent through covalent attachment of Lucifer Yellow-CH (LY) dye (top panel); supernatant of LY-bead preparation (middle); LY free dye (bottom).

Figure 4 presents electropherograms of freshly prepared LY run consecutively over one hour. These electropherograms were obtained on a different CE instrument (Beckman 5010) than those in Figure 3 and with slightly different running buffer (50 mM Tris buffer, pH 9.0) thus the electropherograms in Figures 4 are similar but not identical to that of Figure 3 (bottom panel). Negatively charged LY dye in solution shows two major peaks (Figure 4) that have migration times of 5.876 ± 0.047 min and 6.622 ± 0.051 min ($n = 4$). Although the migration times of the two major peaks were highly reproducible (within $\pm 1\%$), the intensity of the peak at 5.88 min decreased in successive runs by about 75% during the time elapsed (ca. 1 h) from run 1 to run 4. The inset of Figure 4 shows a semi-logarithmic plot of peak height versus time. The first order decay constant was calculated to be $3.1 \times 10^{-2} \text{ min}^{-1}$. The other peaks in the 6-min interval appear to be relatively constant. The electropherogram of LY dye Run 4 (Figure 4) shows resemblance to that of LY dye in Figure 3. Given the instability of LY dye in solution it is not surprising that the labeled beads showed instability. From this analysis we conclude that the custom-modified preparation was not as homogeneous or stable as the commercial-sourced fluorescent beads.

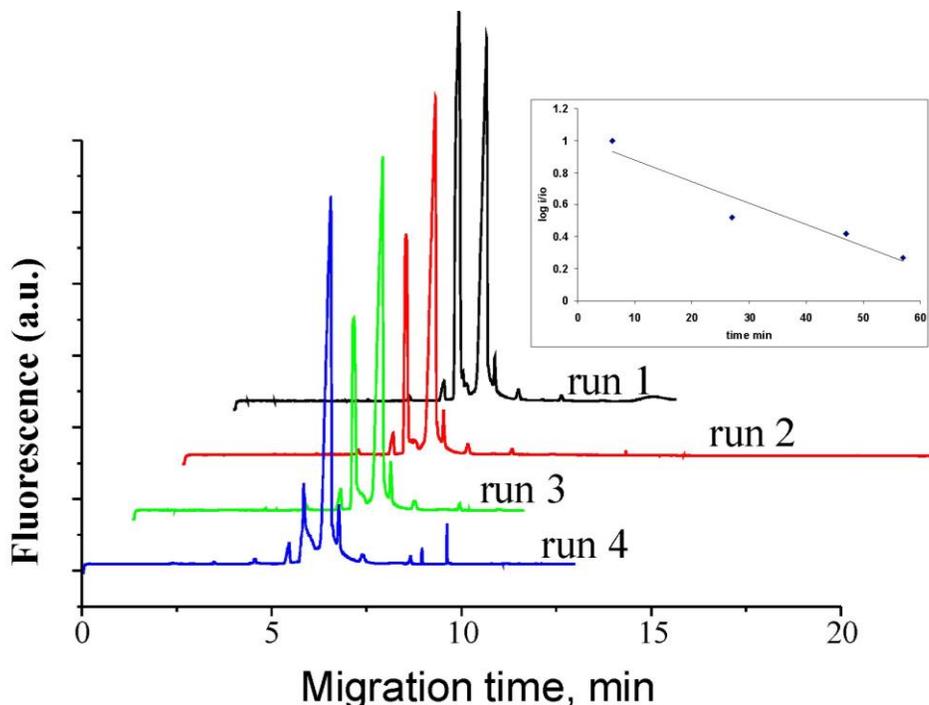


Figure 5: Electropherograms of freshly prepared Lucifer Yellow CH run consecutively over one hour on Beckman 5010 CE instrument using running buffer of 50 mM Tris, pH 9.0. CE was performed using 37 cm long, 50 μm i.d. capillary. Sample was injected for 3 sec by pressure and separation was effected by applying 7 kV. The X and Y axis were off-set for easy visualization. Inset shows a semi-logarithmic plot of peak heights (I/I_0) versus time of peak at 5.9 min. The time values on the decay plot were determined by summing the migration time for each run and total time for preceding electrophoresis runs and wash runs. The first order decay constant was calculated to be $3.1 \times 10^{-2} \text{ min}^{-1}$ from linear regression ($y = -0.0134x + 1.0117$ $R^2 = 0.9212$)

Capillary electrophoresis of gold nanoparticles by direct UV detection

Fluorescent emission from Au nanoparticles has been reported by several groups [16, 28, 29] but the literature on spectroscopic properties of gold (Au) nanoparticles is not entirely clear. Au nanoparticles have an intense rose/pink color indicating absorption in the 500-550 nm range of the visible spectrum. They also have strong absorption in the 200-230 nm range of the UV spectrum [29]. For UV detection herein 214 nm was selected. The electropherogram of 5 nm Au nanoparticles alone shows a single sharp peak at 9 min (Figure 5a). The electropherogram of a mixture of commercial-sourced 5 and 20 nm Au nanoparticles (Figure 5b) shows near-baseline separation of the peaks at 9.44 and 11.06 min, respectively.

We can use the results of Figure 5b to construct an electrophoretic ruler to determine particle distribution of the Au nanoparticles in each sample. From peak-to-peak (5-to-20 nm particles) migration time increased by 1.62 min (11.06 - 9.44 min) or 0.108 min/nm. The band widths (half-width at half-height) were 0.105 and 0.135 min for 5 and 20 nm nanoparticles respectively, after correction for plug injection and diffusion. These half-width values of the 5 and 20 nm peaks represent 0.97 and 1.25 nm, respectively. In terms of mass distribution, roughly 70% of the Au mass for each sample was contained in particles of 5 (± 0.97) and 20 (± 1.25) nm. The calculation is based on the fact that the UV absorbance is proportional to the mass of Au-NP in the sample [29] and the variation in t_{mig} is proportional to particle size [20]. The estimates for distribution of mass in the samples are consistent with technical data provided by the commercial supplier of gold nanoparticles.

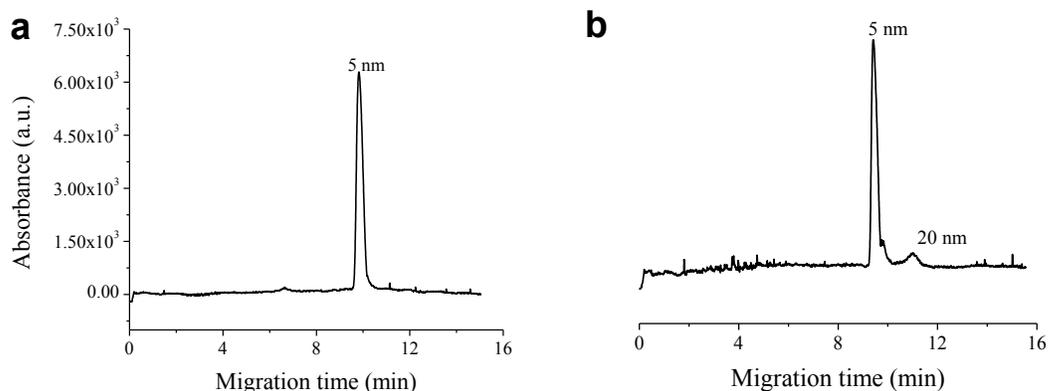


Figure 6: Electropherograms of (a) 5 nm Au nanoparticles and (b) a mixture of 5 nm and 20 nm nanoparticles. CE was performed using a 47 cm long, 75 μm i.d. capillary. Sample was injected for 5 sec by pressure and separation was effected using 15 kV. The running buffer was 50 mM Tricine, pH 8.5, buffer and detection was made at 214 nm.

Capillary electrophoresis of iron oxide nanoparticles by indirect LIF detection

In cases where the analyte has no appreciable absorption or fluorescence it is possible to use indirect detection by measuring the effect that the analyte has on a more readily detectible signaling molecule. By adding a signaling molecule to the running buffer and monitoring the effect of the analyte on the signaling molecule, the analyte can be detected indirectly. A negative peak indicates a decrease in the concentration of the signaling molecule, and similarly a positive peak indicates an increase. During CE, the analyte molecules can physically displace a portion of the signaling molecules from the separation buffer and a corresponding decrease in intensity (or dip) is observed. The mechanism of displacement may be due to conservation of volume (that is, dilution) and/or conservation of charge [11c]. For conservation of charge, an example would be the displacement of anionic signaling molecules by anionic analyte molecules so that the overall electrostatic charge in the volume element surrounding the analyte remains constant.

Two custom-synthesized sample iron-core nanoparticles, $\text{Fe}_3\text{O}_4@\text{SiO}_2$, and $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$, did not demonstrate any inherent absorption or fluorescence in preliminary direct detection CE measurements (data not shown). In subsequent indirect CE experiments we used anionic (negatively charged) fluorescein as the signaling molecule for indirect LIF detection. The choice of fluorescein was based on conventional practice of CE LIF, in which fluorescein (or one of its analogues) is a commonly used fluorescent probe owing to its advantageous spectroscopic properties and compatibility with the argon-ion laser.

In the present CE LIF experiments, the instrumentation (Beckman PACE 5010) automatically assigns the baseline as zero whether direct or indirect detection was used. For direct detection, CE LIF electropherograms show increases in signal, i.e., peaks (e.g., Figures 1 and 3) and for indirect detection, decrease in signal, i.e., dips. An example of indirect LIF detection is shown by the electropherogram in Figure 6. The separation buffer was MES (12 mM, pH 6.6) containing fluorescein (5 μM) as the signaling molecule. The sample was the same MES buffer without fluorescein.

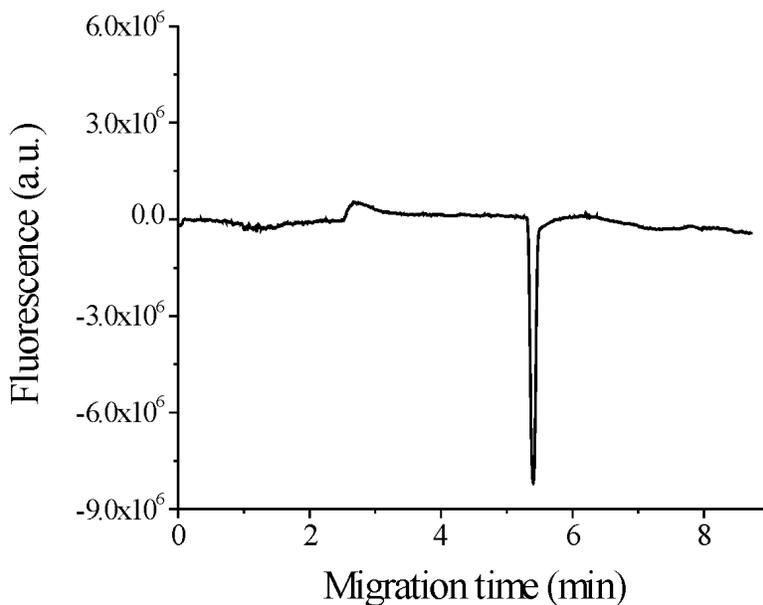


Figure 7: Electropherogram obtained by indirect LIF detection of a sample MES buffer (12 mM, pH 6.6) using separation buffer of MES (12 mM, pH 6.6) containing 5 μM fluorescein as signaling molecule. Sample was injected into a 47 cm long (50 μm i.d., 360 μm o.d.) capillary by pressure for 5 sec. Separation was effected by applying 7 kV.

Figure 7 shows an indirect CE LIF electropherogram of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles. The sharp peak at 1.88 min indicates that the overall distribution of the electrophoretic mobility of the nanoparticles is narrow. Thus, the particle size and charge are relatively uniform. However instead of the expected dip, we observed a positive peak. Upon this result, direct CE LIF experiments were repeated to re-confirm that the particles themselves were not responsible for the peak that appears in indirect mode (data not shown). Comparison of t_{mig} of neutral BODIPY (3.27 min, data not shown) to that of the iron core nanoparticles in the same running buffer suggested that the particles were positively charged since the t_{mig} of the particles was less than that of the neutral molecule. This data is consistent with synthesis methods that produce core-shell particles of relatively uniform size (50 nm in diameter in this case) and positively charged [25]. Based on the notion that the particles are positively charged (which is consistent with t_{mig} and synthetic methods) we postulate that the peaks are a result of conservation of charge. The same forces that cause displacement of the anionic signaling molecules by anionic analyte (as discussed above) and result in dips would cause enrichment of the anionic signal-molecules in the zone to where the positively charged nanoparticles migrated. Positive peaks in indirect CE have been reported for studies of hydrated cations [30] and for amines [31]. These cited results are consistent with the present work in that the observed positive peaks were attributable to conservation of charge. This report is the first to observe positive peaks in indirect CE LIF analysis of nanoparticles. Figure 8 shows a CE indirect LIF of the second sample of iron oxide nanoparticles, $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$. The electropherogram containing two peaks of t_{mig} 2.048 and 3.040 min suggests two sizes of positively charged particles since t_{mig} values are less than neutral BODIPY. This data is also consistent with the synthetic processes [23] and with electron microscopy data (not shown) indicating two sizes of particles diameters approximately 8 (± 2) and 18 (± 3) nm.

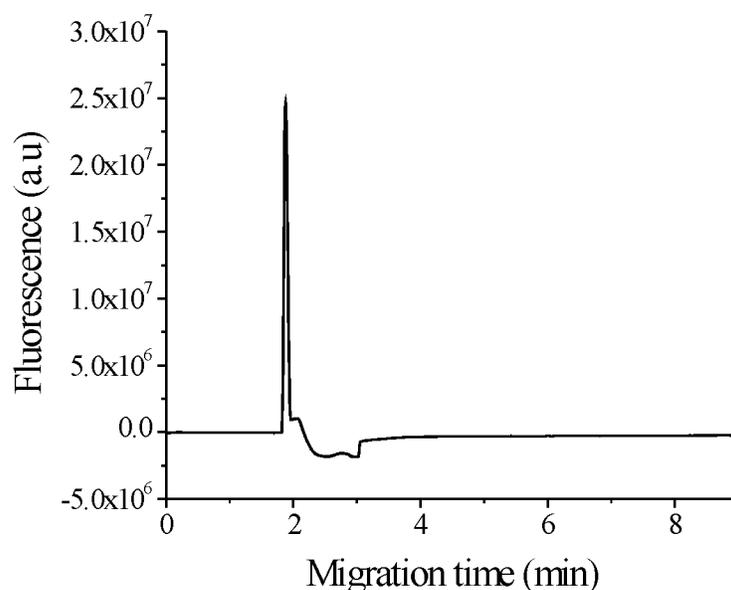


Figure 8: Electropherogram of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanoparticles by indirect LIF detection. Sample was vortexed for 30 sec 4 minutes prior to injection. All experimental conditions are as in Figure 6.

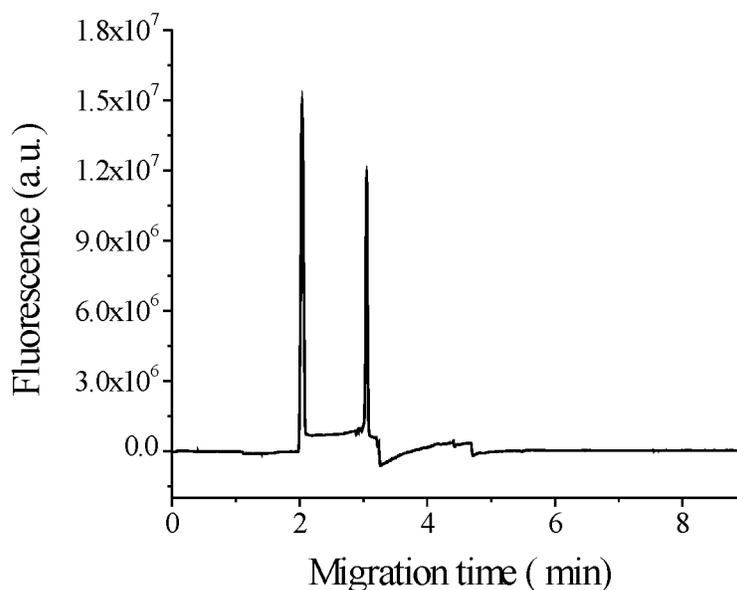


Figure 9: Electropherogram of $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$ by indirect LIF detection. Sample was vortexed prior to injection. All experimental conditions are as in Figure 6.

When the $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$ sample was repetitively injected without capillary wash between runs, the migration times of the peaks increased approximately 2-4 min with each injection suggesting that the EOF of the capillary was reduced due to surface modification of the capillary wall by the NMe_4^+ (or other cationic species) present in the sample. It is well known that cations adsorb on the walls of the negatively charged capillary surfaces due to electrostatic attraction and cause decreases in cathodic EOF [11d]. When the capillary was washed with 0.1 M NaOH for 2 min followed by conditioning with the fluorescein-containing buffer for 4 minutes in between runs, the EOF was regenerated and the migration times of the peaks were recovered supporting the above suggestion. When successive injections of $\text{Fe}_3\text{O}_4@\text{NMe}_4\text{OH}$ were re-run with 2 min wash of 0.1 M NaOH followed by 4 min buffer conditioning between sample injections, very stable migration times were recorded: 2.038 ± 0.061 min (%RSD = 1.39, n=4) and 3.034 ± 0.089 min (%RSD = 2.93, n=4) (data not shown). For these iron oxide nanoparticle preparations, CE indirect LIF provided a useful method of characterization.

Conclusions

The premise of the research herein was that capillary electrophoresis is a valuable analytical method for the study of nanoparticles. The experiments were conducted as part of a larger project (CRTI #03-0005RD) wherein the authors provided research support in analytical chemistry plus several other areas. The report describes the success of capillary electrophoresis as an analytical method in nanoparticle research. We show that the direct detection modes of CE can be used to study a variety of nanomaterials. We show how CE can be used as an electrophoretic ruler to determine size distribution of gold nanoparticles. For nanoparticles that possess no inherent fluorescence we used indirect LIF detection to provide physical characterization of the material. This latter method gave unexpected, though reproducible results, namely appearance of peaks in the electropherograms where dips were expected. We postulate an explanation for the phenomenon to be conservation of charge in the analyte zone but readily concede that this area warrants more work outside the scope of CRTI project. With each class of NP studied, new and useful scientific knowledge that provides value to our project collaborators and to the scientific community was obtained. The study of the electro-dynamic properties of nanoparticles by CE is a new pursuit. Our contribution to this area has raised as many questions as it answered. The importance of this first scientific report on nanoparticles from our laboratory was in establishing a framework of analytical methods that can be applied to future investigations.

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The report describes the use of capillary electrophoresis (CE) on gold, polymer and iron oxide nanoparticles (nanoparticles). The analyte species were detected by UV/Vis or laser-induced fluorescence (LIF) spectroscopy. For analytes that possessed neither UV/vis absorbance nor LIF, indirect methods of detection were employed whereby a signal-generating molecular species was added to the running buffer. Nanoparticles from a variety of commercial sources and from custom syntheses were studied. The work presents calculations and determinations of physico-chemical characteristics of nanoparticles such as electrophoretic mobility, size distribution, and mass/charge ratio. It is also the first report of capillary electrophoresis of nanoparticles using indirect detection. The report demonstrates that CE is a valuable tool in NP research.

Ce rapport décrit la caractérisation de nanoparticules d'or, de polymère et d'oxyde de fer par électrophorèse capillaire. Les analytes ont été détectés par des méthodes spectroscopiques, soit UV/Vis, soit fluorescence induite par laser (FIL). Dans les cas où l'analyte n'était sensible ni à l'UV/Vis, ni au FIL, des témoins indirects ont été employés. Les nanoparticules étaient de provenance commerciale et de nos laboratoires (NRC: IMI). Nous résumons ici nos calculs et la détermination de propriétés physico-chimiques: mobilité électrophorétique, distribution de taille, et ratio masse/charge. A notre connaissance, ce rapport présente une première démonstration de la détection indirecte de nanoparticules par électrophorèse capillaire avec témoins. Le rapport démontre l'utilité de l' électrophorèse capillaire dans la caractérisation complète de nanoparticules en solution.

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capillary electrophoresis; nanoparticles