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Determination of the Mobility of Amine- and Carboxy-Terminated Fluospheres and Quantum Dots by Capillary Electrophoresis

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Abstract

The pharmacokinetics of nanoparticle (NP) theranostics can, in principle, be predicted based on NP size and zeta potential. Zeta potentials are typically measured using bench top zetasizer instruments, which calculate zeta potential based on mobility data collected from solutions in a small sample cell. However, correlations between zeta potentials measured by zetasizer instruments and those calculated from mobilities determined by instruments designed for capillary electrophoresis may not be direct. To that end, mobilities of a variety of NPs were determined by a capillary electrophoresis and used to calculate zeta potentials based on Henry's equation. The calculated zeta potentials were then compared to zeta potentials measured directly from a zetasizer. It was found that absolute values of the two methods differed, but the relative zeta potential trends per particle type were similar. These trends were demonstrated by data that showed that the zeta potentials measured using a zetasizer correlated highly with zeta potentials determined by capillary electrophoresis.

Key Words: nanoparticles, predictive pharmacokinetics, nanoparticle zeta potential, capillary electrophoresis, nanoparticle mobility, polystyrene nanospheres, quantum dots

Introduction

Nanoparticles (NPs) are increasingly used in medical applications such as drug delivery, imaging, and bio-diagnostics, particularly for cancer.¹⁻⁴ Such nanotherapeutic approaches exploit the enhanced permeability and retention (EPR) effect whereby NPs accumulate in angiogenic tissue surrounding growing tumors due to the “leaky” nature of the newly forming blood vessels.⁵⁻⁷ Since predictive pharmacokinetic models for sequestration of NPs into angiogenic tissues are unavailable, much of the NP delivery vehicle development has been empirical. NP surface functionalization, zeta potential, effective charge, and size all play a role to determine NP fate in biological systems.⁸⁻¹² Specifically, we have recently found that zeta potential and size play a role in NP uptake and accumulation rates in cells and blood vessels of the chorioallantoic membrane of the chicken embryo and zebrafish embryo.¹³ The relationship with size was compelling as we found that uptake rates depend on the footprint area of the particles. With respect to zeta potential, we could only determine a cutoff for immediate uptake and continue to look into the relationship between uptake and zeta potential. For research on the interactions of nanomaterials with biological systems, it is crucial to use quantitative data that predictively relates size and zeta potential of NPs to their bioaccumulation behaviour.

Capillary electrophoresis (CE) is an established tool for the characterization of NPs and many groups have reported separation of a wide variety of NPs such as liposomes, polystyrene nanospheres, and gold NPs.¹⁴⁻¹⁹ Furthermore, CE is a technique that allows the calculation of zeta potential based on electrophoretic mobility, while taking size into consideration. It uses high voltages to generate electrophoretic

movement of buffer and charged species in narrow capillaries and separate them based on zeta potential and size. Electrophoretic mobility is determined from the migration time (Equation 1):

$$\mu = \frac{L_t L_d}{V t} \quad \text{Equation 1}$$

where μ is the mobility, L_t is the total length of the capillary, L_d is the length of the capillary to the detector, V is the applied voltage, and t is the migration time.

From the electrophoretic mobility, zeta potential can be calculated using an approximated analytic formula proposed by Ohshima²⁰.

Researchers often measure zeta potential using commercial instruments, which measure electrophoretic mobility across the short distance in a small capillary cell with electrodes on either side. Conversely, the same measurement can be taken with a system designed for capillary electrophoresis that has longer, thinner capillaries that are seemingly more conducive to separating particles based on size and zeta potential. To our knowledge, there has been no systematic study comparing zeta potentials measured using CE with those using light scattering.

Therefore, in this study, CE with laser-induced fluorescence (LIF) detection was used to determine the mobility of FluoSpheres (FS) and Quantum Dots (QDs) that we have previously studied in biological systems¹³ in order to calculate NP surface zeta potential. Zeta potentials of the same NPs were measured using a Malvern Nano ZS.

Measurements of both instruments were compared to determine similarities and differences of data and to determine the best method for reliable characterization of NPs.

Experimental Methods

Theoretical Considerations:

The most widely used equation for the calculation of zeta potential from mobility for colloidal particles in an electrolyte was introduced by Smoluchowski in 1921²¹.

$$\mu = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta} \quad \text{Equation 2}$$

where ε_r is the relative electric permittivity of the dispersion medium, ε_0 is the permittivity of a vacuum, ζ is the zeta potential, and η is the dynamic viscosity of the continuum.

This equation is only valid for high ionic strength electrolytes that form Debye thicknesses much narrower than their hydrodynamic radii, a , or very large particles ($ka \gg 1$), where κ is the inverse Debye thickness. The effective thickness of the ion cloud is important in mobility calculations and is determined by the ionic strength, which is characterized by the inverse of the Debye thickness, κ ²¹:

$$\kappa = \left(\frac{e^2 N_A \sum z_i^2 c_i}{\varepsilon_r \varepsilon_0 k T} \right)^{1/2} \quad \text{Equation 3}$$

where e is the elementary electric charge, N_A is Avogadro's number, z_i and c_i are the charge number and molar concentration, respectively, of the i^{th} electrolyte component, k is the Boltzmann constant, and T is the absolute temperature.

For small particles, where $ka \ll 1$, Hückel introduced the following equation (1924)²¹:

$$\mu = \frac{2 \varepsilon_r \varepsilon_0 \zeta}{3 \eta} \quad \text{Equation 4}$$

Equation 4 is a great approximation for small particles; however, it is important to note that both Equations 3 and 4 do not take size into consideration. Therefore, both of these equations denote that it is impossible to separate spherical particles based on size when zeta potential remains the same. In practice, this is not the case and therefore

Equations 3 and 4 cannot be used reliably to calculate zeta potentials from mobilities of NPs of various sizes. Therefore, in 1931, Henry introduced a theory to calculate zeta potential from mobility for a wide range of κa values²²:

$$\mu = \frac{2\varepsilon_r\varepsilon_0\zeta}{3\eta} f(\kappa a) \quad \text{Equation 5}$$

This equation can be approximated with a negligible error by a formula published by Ohshima²³:

$$\mu = \frac{2\varepsilon_r\varepsilon_0\zeta}{3\eta} \left[f_1(\kappa a) - \left(\frac{2e\zeta}{kT} \right)^2 f_3(\kappa a) - \frac{m_+ + m_-}{2} \left(\frac{ze\zeta}{kT} \right)^2 f_4(\kappa a) \right] \quad \text{Equation 6}$$

Here, f_1 , f_3 , and f_4 are functions based on κa defined by:

$$f_1(\kappa a) = 1 + \frac{1}{2 \left(1 + \frac{2.5}{[\kappa a(1+2e^{-\kappa a})]^3} \right)} \quad \text{Equation 7}$$

$$f_3(\kappa a) = \frac{\kappa a(\kappa a + 1.3e^{-0.18\kappa a} + 2.5)}{2(\kappa a + 1.2e^{-7.4\kappa a} + 4.8)^3} \quad \text{Equation 8}$$

$$f_4(\kappa a) = \frac{9\kappa a(\kappa a + 5.2e^{-3.9\kappa a} + 5.6)}{8(\kappa a + 1.55e^{-0.32\kappa a} + 6.02)^3} \quad \text{Equation 9}$$

The dimensionless coefficients, m_+ and m_- , are used to characterize ionic drags of cations and ions:

$$m_{\pm} = \frac{2\varepsilon_r\varepsilon_0 k T N_A}{3\eta z \Lambda_{\pm}^0} \quad \text{Equation 10}$$

where $\Lambda_{\pm}^0$ are limiting conductances of cations and anions in the electrolyte.

After using the above Equations 5-10, the surface charge density, σ , and the effective charge, q , can then be calculated according to the following expression developed by Ohshima²⁰:

$$\sigma = \frac{q}{4\pi a^2} = \frac{2\varepsilon_r\varepsilon_0 k k T}{e} \sinh\left(\frac{y}{2}\right) \left[1 + \frac{1}{\kappa a} \left(\frac{2}{\cosh^2\left(\frac{y}{4}\right)} \right) + \frac{1}{(\kappa a)^2} \left(\frac{8 \ln\left(\cosh\left(\frac{y}{4}\right)\right)}{\sinh^2\left(\frac{y}{2}\right)} \right) \right]^2 \quad \text{Equation 11}$$

where $y = \frac{e\zeta}{kT}$. This equation assumes a spherical geometry of the NPs and uniform

distribution of charge on the NP surface.

Equations 6-10 are used to calculate zeta potentials from the mobilities of the NPs studied in this work.

Chemicals:

QDs (Qdot® 525 ITK™ Amino (PEG) and Qdot® 525 ITK™ Carboxyl-terminated Quantum Dots) and FS (polystyrene nanospheres: FluoSpheres® carboxylate-modified (580/605) in 0.02 µm, 0.04 µm, 0.1 µm, and 0.2 µm sizes and FluoSpheres® amine, 0.2 µm) were obtained from Life Technologies and diluted in 10 mM phosphate buffered saline (PBS) at a pH of 7.4. FS were sonicated for 5 min immediately before separation.

Table 1 lists the particles injected and their approximate molar concentrations.

Table 1. Concentration of FluoSphere and Quantum Dot suspensions injected into capillaries.

Particle	Particle Concentration
	Molar Concentration (mol/L)
Carboxy:	
20 nm QD	8.00×10^{-7}
20 nm FS	7.55×10^{-6}
40 nm FS	2.36×10^{-6}
100 nm FS	6.04×10^{-8}
200 nm FS	7.55×10^{-9}
Amine:	
20 nm QD	8.00×10^{-7}
200 nm FS	7.55×10^{-9}

Instrumentation:

Capillary Electrophoresis system PA 800 Plus System (Beckman Coulter, Mississauga, Ontario) equipped with a 488 nm laser induced fluorescence with a 520 nm emission filter (Beckman Coulter) and a 600nm emission filter (Chroma, Bellows Falls, Vermont)

was used along with 32 Karat software to determine electrophoretic mobilities.

Uncoated fused-silica capillaries of 50 μm I.D., 150 μm O.D., with a total length of 40.2 cm, and an effective length of 30.0 cm were used. All separations were performed under positive polarity at a constant applied voltage of 20 kV at 25°C. For each run, capillaries were first rinsed with 0.1 M NaOH for 2 min at 30 psi, and then with PBS for 5 min at 20 psi. The sample was injected for 3.0 s at 0.5 psi and the separation voltage (20 kV) was applied. All NPs were excited with a 488 nm laser. FS were detected with the 600 nm detection filter and the QDs were detected with the 520 nm emission filter. Vials were refilled with fresh buffer every 3 runs.

NPs' hydrodynamic radii in buffer solution were characterized via dynamic light scattering (Malvern Nano ZS). Zeta potentials were also measured using the Malvern Nano ZS.

Results and Discussion

The object of the present work was to determine the relationship of NP zeta potentials measured by a Zetasizer (ZS) with those calculated using mobility data obtained from capillary electrophoresis.

Figure 1. Electropherograms of A) 20 nm carboxy FS, B) 20 nm carboxy FS with longer time between sonication and injection than A), C) 40 nm carboxy FS, D) 100 nm carboxy FS, E) 200 nm carboxy FS, F) 200 nm amine FS. G) 20 nm carboxy QDs, H) 20 nm amine QDs

Typical electropherograms obtained from the CE system are shown in Figure 1 for various sizes and surface modifications of NPs. Figure 1A and 1B display the electropherograms of two sequential data collection runs, respectively, for the 20 nm carboxy-terminated FS particles. It is important to note there is an increased number of features at longer migration times to the right of the main peak in Figure 1B. These

peaks possibly indicate aggregation of the particles at a longer time after the sonication step used in the particles' preparation. If true, these data suggest that capillary electrophoresis could be a useful tool in studying stability, or aggregation, of NPs in various buffers, in addition to mobilities.

Figure 1C-1H give electropherograms of the other particle samples, showing particle migration times, which are listed in Table 2, along with their electrophoretic mobilities. Particle migration times were used to calculate apparent mobilities from Equation 1.

The apparent mobilities were then used to calculate actual electrophoretic mobilities by subtracting out the effects of electroosmotic flow (Equation 12).

$$\mu_{ep} = \mu_{app} - \mu_{EOF} \quad \text{Equation 12}$$

where μ_{ep} is the electrophoretic mobility, μ_{app} is the apparent mobility (calculated directly from the migration time, and μ_{EOF} is the mobility of the electroosmotic flow. The electroosmotic mobility was determined to be $0.00067 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$. using mesityl oxide as the EOF marker.

Table 2. Nanoparticle migration times and electrophoretic mobilities

Nanoparticle	Migration Time (min)	$\mu_{ep} \times 10^{-5} \text{ +/- std dev}(\text{cm}^2\text{V}^{-1}\text{s}^{-1})$
20 nm Amine QD	3.65	-6.5 +/- 3.4
200 nm Amine FS	5.10	-10.2 +/- 1.3
20 nm Carboxy QD	3.25	-35.8 +/- 1.1
20 nm Carboxy FS	4.09	-36.0 +/- 1.1
40 nm Carboxy FS	1.77	-47.3 +/- 1.1
100 nm Carboxy FS	3.22	-41.6 +/- 1.8
200 nm Carboxy FS	1.66	-42.2 +/- 2.8

The electropherograms in Figure 1 demonstrate a large peak width variation between different types of particles. More specifically, the elution peaks are comparatively much sharper in Figures 1G and 1H, which are the electropherograms for

the carboxy-, and amine-functionalized QDs, respectively. This led us to examine if size distributions could be the cause of this phenomenon. Figure 2 shows the peak width comparisons of DLS size and electropherogram elution time data. There appears to be little correlation between peak width of the size data (normalized by dividing peak width by average size) and the CE data, which leads us to believe that another factor, or a variety of factors, such as zeta potential distributions may be causing the differences in elution time peak width (Figure 3).

Figure 2. Comparison of nanoparticle dynamic light scattering size (normalized by dividing peak width by average particle size) and capillary electrophoresis peak widths. Error depicts standard deviation of all measurements taken.

Figure 3. A) CE peak width versus zeta potential measured by Zetasizer and B) Zeta potential peak width (from Zetasizer measurement) versus CE peak width.

Figure 3A displays the relationship of CE peak width with zeta potentials measured by the Zetasizer and shows that the CE peak width decreases with increasing zeta potential. This may be a result of particles with higher zeta potentials travelling more quickly to the detection window. Particles with lower zeta potentials take more time to travel the same distance and therefore have more time to spread out owing to Brownian diffusion, hence the larger peak width. This shows that zeta potential may have a larger effect on mobility than the size does as no correlation was observed between CE peak width and NP hydrodynamic radius (Figure 2). Figure 3B shows that there is a correlation between the zeta potential peak width and the CE peak width. This is expected since the zeta potential measurements are based on the mobility measurements taken by the Zetasizer.

Figure 4 compares the electrophoretic mobilities of the NPs studied. The mobilities of the particles studied here under the aforementioned conditions are similar

to mobilities of similar type particles found in previous studies. For example, Jones and Ballou found mobilities for 80 nm carboxy-modified polystyrene nanospheres to be approximately $-5 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ at a similar pH,¹⁵ while Vanifatova et al also measured similar mobilities for unmodified polystyrene spheres.²⁴

As can be seen in Figure 4, the amine-modified particles are the lowest of this group, while the carboxy-modified particles are much higher. This is intuitive since the carboxy particles will be more affected by the applied voltage. Interestingly, the 20 nm amine-modified QDs have a very similar mobility to the 200 nm amine FS. This is likely a result of the combination of the differences in surface zeta potential and size between the two types of particles, since mobility is complexly related to both zeta potential and hydrodynamic radius as per Henry's equation (Equation 5).

Figure 4. Comparison of nanoparticle mobility based on surface modification and diameter. Error bars depict standard deviation of all trials.

From Figure 4, one observes that mobility gradually becomes more negative with an increase in size for most of the carboxy-modified NPs. However, the 40 nm FS have

a much more negative mobility in comparison, which lies outside of this observed trend. It may be possible that for some reason the 40 nm FS are less colloiddally stable than other sizes and therefore are formulated with higher charge density on the surface to keep them from aggregating.

Table 3. Nanoparticle mobilities, estimated charge, and zeta potentials.

Nanoparticle	$\mu \times 10^{-5} \pm \text{std dev (cm}^2\text{V}^{-1}\text{s}^{-1}\text{)}$	ζ , calculated from mobility $\pm \text{std dev (mV)}$	ζ , measured by zetasizer $\pm \text{std dev (mV)}$
20nm Amine QD	-7 $\pm$ 3	-14 $\pm$ 7	-22 $\pm$ 4
200nm Amine FS	-10 $\pm$ 1	-18 $\pm$ 2	24 $\pm$ 5
20nm Carboxy QD	-36 $\pm$ 1	-78 $\pm$ 2	-43 $\pm$ 8
20nm Carboxy FS	-36 $\pm$ 1	-79 $\pm$ 3	-60 $\pm$ 15
40nm Carboxy FS	-47 $\pm$ 1	-121 $\pm$ 3	-70 $\pm$ 16
100nm Carboxy FS	-42 $\pm$ 2	-101 $\pm$ 4	-58 $\pm$ 10
200nm Carboxy FS	-42 $\pm$ 3	-93 $\pm$ 6	-55 $\pm$ 7

For ease of comparison, Table 3 gives NP mobilities as compared to their zeta potentials both calculated from the mobilities and measured by the Zetasizer. According to the Zetasizer measurements, the amine-terminated FS have the most positive zeta potentials ($\zeta = 24 \text{ mV}$), while the rest of the NPs have negative zeta potentials with the least negative being the amine-terminated QD ($\zeta = -22.1 \text{ mV}$). This gap in zeta potential is intriguing since the mobilities of both of the amine-terminated particles are quite similar ($7 \times 10^{-5} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ and $10 \times 10^{-5} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, respectively). The carboxy-modified QDs and FS all have much more negative zeta potentials and significantly higher mobilities than the amine-terminated particles. There seems to be no trend in the zeta potentials with size, but the mobilities do increase slightly with size. It is interesting to note that the 40 nm FS (indicated by 20 nm radius in Table 3) have an apparent greater negative zeta potential than other particles and as previously discussed, the mobility was also

observed to be higher (Figure 4). Other than the large difference observed in the measured zeta potentials of the amine-terminated particles, the zeta potentials calculated from the mobilities are quantitatively different, but the trend in zeta potentials is quite similar and correlate with one another based on the different particles (Figure 5). It is important to note that although the exact values of the zeta potentials from the two methods are different, the slope of the line in Figure 5 is 1.1. This indicates that although the absolute numbers are different, there is a direct correlation of the relative zeta potentials calculated from the mobilities using Henry's equation and those measured by the Zetasizer. Absolute differences may be a result of difference in calibration or assumptions made of parameters in either the CE or Zetasizer. This leads to show that when measuring zeta potentials using a zetasizer, relative values may be more indicative of trends rather than absolutes.

Figure 5. Zeta potential calculated from CE mobility versus zeta potential measured by Zetasizer. Slope of line = 1.1, $R = 0.88$.

Conclusions

The results obtained here show the potential of capillary electrophoresis to further quantify the behaviour of NPs based on surface zeta potential, functionalization, and size and compares zeta potential results of CE to those from a zetasizer. Capillary electrophoresis gives the advantage of obtaining highly accurate results based on the length and diameter of the capillaries used, and multiple data sets are easily obtained based on small injection volumes. Obtaining direct measurements from a zetasizer is advantageous as it is quick and easy (can be done in less than 5 minutes) and most zetasizers have the ability to determine hydrodynamic radii of small particles, making it practical for characterizing nanoparticles in multiple ways. Due to the shorter distance between electrodes and the higher diameter tubes, the measurements may not be as accurate as those obtained from capillary electrophoresis, but it was shown here that the relative zeta potentials measured by the zetasizer do correlate well with the zeta potentials calculated from mobility obtained by CE. Therefore, zetasizer measurements should suffice for most uses in the lab as long as researchers use caution when viewing zeta potentials obtained from zetasizer instruments and take relative measurements into consideration rather than absolute values. When more accurate measurements are needed, CE mobility measurements must be employed.

CE may also be a very useful tool in determining NP stability (via aggregation) in various buffers and biological media as it can identify specific aggregates through separation whereas zetasizing provides averages. It would also be an invaluable tool in further correlating mobilities with biological NP uptake rates to determine if NP mobility can lead to predictive pharmacokinetics of NPs in the blood stream.

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