# Comparison of Nanoparticle Size and Electrophoretic Mobility Measurements Using a Carbon-Nanotube-Based Coulter Counter, Dynamic Light Scattering, Transmission Electron Microscopy, and Phase Analysis Light Scattering

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The precision and accuracy of measurements of the diameter and electrophoretic mobility ($\mu$) of polymeric nanoparticles is compared using four different analytical approaches: carbon-nanotube-based Coulter counting, dynamic light scattering (DLS), transmission electron microscopy (TEM), and phase analysis light scattering (PALS). Carbon-nanotube-based Coulter counters (CNCCs) use a 132 nm diameter channel to simultaneously determine the diameter (28–90 nm) and $\mu$ value for individual nanoparticles. These measurements are made without calibration of the CNCC and without labeling the sample. Moreover, because CNCCs measure the properties of individual particles, they provide true averages and polydispersities that are not convoluted into the intrinsic instrumental response function of the CNCC. CNCCs can be used to measure the size of individual nanoparticles dispersed in aqueous solutions, which contrasts with the TEM-measured size of individual dehydrated particles and the ensemble size averages of dispersed particles provided by DLS. CNCCs provide more precise values of $\mu$ than PALS.

## Introduction

Here, we compare the precision and accuracy of measurements of the size and electrophoretic mobility ($\mu$) of polymeric nanoparticles using four different analytical approaches: carbon-nanotube-based Coulter counting (Scheme 1a), dynamic light scattering (DLS), transmission electron microscopy (TEM), and phase analysis light scattering (PALS).[1,2] The results indicate that carbon-nanotube-based Coulter counters (CNCCs) simultaneously provide accurate and precise information about the size and electrophoretic mobility of dispersed particles ranging in diameter from 28 to 90 nm. Neither calibration of the CNCC nor labeling of the sample is required. Moreover, because CNCCs measure the properties of individual particles, they provide true averages and polydispersities that are not convoluted into the intrinsic instrumental response function of the device. CNCCs can be used to measure the size of individual nanoparticles dispersed in aqueous solutions, which contrasts with the TEM-measured size of individual dehydrated particles and the ensemble size averages of dispersed particles provided by DLS. CNCCs provide more precise values of $\mu$ than PALS.

We previously reported that Coulter counters based on multiwall carbon nanotubes (MWNTs) (channel diameter, 132 nm; channel length, ~1 $\mu$m) provide a reliable means for simultaneously determining the diameter ($d_s$) and electrokinetic surface charge ($Q$) of individual, polymeric nanoparticles having diameters ranging from 28 to 90

**Scheme 1**
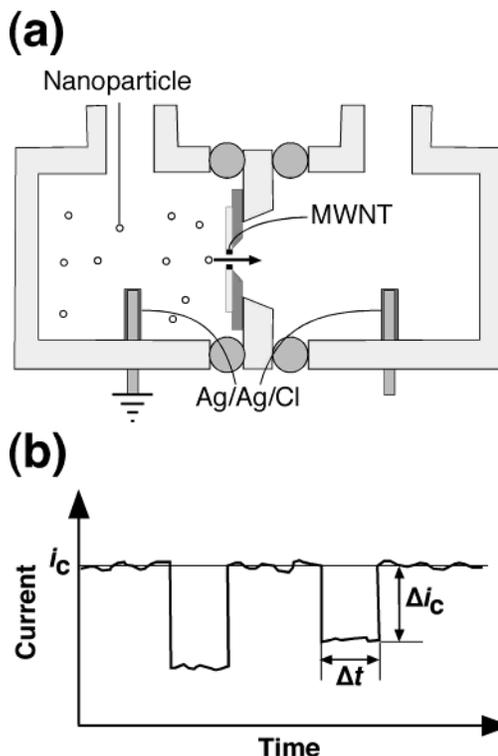


nm.[1,2] CNCC measurements also provide the concentration of such particles. This information derives from the change in channel resistance resulting from the transport of single nanoparticles through the MWNT channel.[3,4] The $d_s$ and

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$Q$ (or $\mu$) values for each nanoparticle can be obtained without calibration of the CNCC from the height and width of each resistive-pulse signal (Scheme 1b). By obtaining data for many individual particles, we also showed that CNCCs can be used to measure the intrinsic distribution of $d_s$ and $Q$ (or $\mu$) for a collection of polymeric nanoparticles.

In addition to MWNTs, Coulter counter channels have also been fabricated from materials such as glass, sapphire,[4] polycarbonate,[5,6] and poly(dimethylsiloxane) (PDMS).[7,8] Very recently, 3−10 nm diameter nanopores prepared within a $Si_3N_4$ substrate were fabricated by ion-milling and used to count individual, stretched DNA molecules.[9,10] However, it is difficult to obtain quantitative information about $\mu$ using channels prepared from materials such as these because their surfaces carry a permanent electrostatic charge. This complicates data interpretation because the effects of electrophoresis and electroosmosis on charged nanoparticles transversing such channels are inseparable.[11,12] In contrast, streaming potential measurements have shown that the surface of MWNT channels are uncharged, indicating that electroosmosis is negligible. This greatly simplifies data interpretation.[1,2] Porous membrane proteins, which have diameters on the order of 2 nm, inserted into fluid lipid bilayers have also been used to prepare Coulter counters, and such devices have been used to detect individual metal ions, small organic molecules, and stretched, single-stranded DNA.[4,13] However, transport of particles through these types of channels is poorly understood, and in addition, the channels themselves are not very stable. In contrast, the stability and well-defined chemical properties of carbon nanotubes suggest that Coulter counters prepared using these materials may offer some advantages for real analytical applications.

In this paper, we fully characterize the precision and accuracy of CNCC measurements and compare these results to conventional methods used for characterizing nanoparticles. These include particle size determination by transmission electron microscopy (TEM) and dynamic light scattering (DLS) and electrophoretic mobility determination using phase analysis light scattering (PALS). TEM provides information about the size and shape of individual nanoparticles dried on a substrate under high vacuum.[14] Dynamic light scattering (DLS) measures diffusion in particle dispersions, which can be interpreted using the Stokes−Einstein equation to yield an ensemble average hydrodynamic particle diameter.[14] There is often a discrepancy between TEM and DLS measurements that is attributed to factors associated with the high vacuum conditions of TEM and the hydrodynamic and electrokinetic effects operative in DLS measurements.[15,16] PALS involves detection of the frequency shift of scattered light, arising from the Doppler effect, caused by electrophoretic particle motion in an alternating current (ac) electric field,

and this information can be used to obtain $\mu$.[17,18] In contrast to these other methods, CNCCs directly measure the size and electrophoretic mobility of individual nanoparticles.

## Experimental Section

**Chemicals and Materials.** All solutions were prepared with 18.2 MΩ·cm (Milli-Q, Millipore) water that was filtered through a 0.2 $\mu$m membrane filter (Fisher Scientific, Pittsburgh, PA). KCl (EM Sciences, Gibbstown, NJ), Triton X-100 (Sigma Chemical, St. Louis, MO), and $KH_2PO_4$ and $K_2HPO_4$ (Mallinckrodt Chemical, Paris, KY) were of reagent grade quality or better and used without further purification. Polymeric nanoparticles having a low (IDC-PS, polystyrene, 57 ± 6 nm diameter, 120 −COOH functional groups per particle; from Interfacial Dynamics, Portland, OR) and high (Bangs-PS/PA, copolymer of polystyrene/ poly(acrylic acid), 60 ± 10 nm diameter, 24 220 −COOH functional groups per particle; from Bangs Laboratories, Fishers, IN) acid surface density were used as received. The manufacturers indicate that the particle size and number density of acid groups were determined by TEM and conductometric titration, respectively.

**Coulter Counting Measurements.** Single channels prepared using a MWNT (Applied Sciences, Cedarville, OH)[19,20] were fabricated according to our previous reports.[1,2] Briefly, a membrane containing a short section of a MWNT (the channel) contained within an epoxy membrane was mounted on a PDMS/ glass support and sealed with a silicone O-ring between two half cells made of polycarbonate (Scheme 1a). The cell was placed in a Faraday cage on a vibration isolation table (model AS-130, TMC, Peabody, MA). Current was measured in voltage-clamp mode with an integrated data acquisition system (Axopatch 200B and Digipack 1200, Axon Instruments, Foster City, CA). Ag/ AgCl electrodes were used in both half cells. Prior to carrying out Coulter counting measurements, the diameter of the MWNT channel ($d_c$) was measured by TEM and a cyclic voltammogram of the channel was obtained in a solution containing 0.1 M KCl, 0.01 M $KH_2PO_4$−$K_2HPO_4$ (pH 7.3), and 0.1% (w/v) Triton X-100 to determine its length ($l_c$).[1,2,21] Coulter counting measurements were made by replacing the electrolyte solution in the half cell held at ground potential with a solution containing polymeric nanoparticles ($5 \times 10^{11}$ particles/mL, 0.1 M KCl, 0.01 M $KH_2$-$PO_4$−$K_2HPO_4$ buffer, and 0.1% (w/v) Triton X-100). The data shown in this paper were obtained with MWNT channels having a diameter of 132 nm and a length ranging from 0.94 to 1.21 $\mu$m. The membrane potential ($E_M$) is defined as the voltage of the electrode in the receiving chamber against the ground electrode in the feed chamber. Current-versus-time data were collected and processed as we have previously reported.[2]

**Transmission Electron Microscope (TEM) Measurements.** Polystyrene nanoparticle samples for TEM measurements were prepared by placing a drop (1 $\mu$L) of an aqueous nanoparticle solution ($4 \times 10^{11}$ particles/mL) on a Cu TEM grid coated with carbon film (Electron Microscopy Sciences, Fort Washington, PA) and allowing water to evaporate in air. TEM measurements were performed with a JEOL JEM-2010 TEM (Tokyo, Japan) calibrated against a grating-replica size standard (Electron Microscopy Sciences).

**Dynamic Light Scattering (DLS) and Phase Analysis Light Scattering (PALS) Measurements.** DLS and PALS measurements of the average $d_s$ and $\mu$ values were obtained using a ZetaPALS instrument (Brookhaven Instruments, Holtsville, NY) at 25 °C. Sample solutions contained $5 \times 10^{11}$ particles/ mL, 0.1 M KCl, 0.01 M $KH_2PO_4$−$K_2HPO_4$ (pH 7.3), and 0.1% (w/v) Triton X-100. A scattering angle of 90° was used for the DLS measurements of particle size, and the results are reported as the average and standard deviation from more than five DLS

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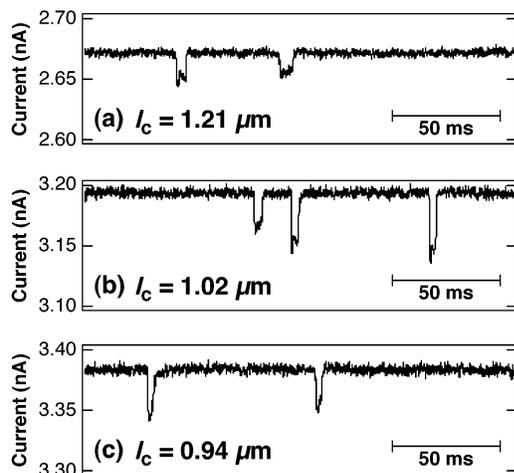
**Figure 1.** Plots of current vs time obtained at $E_M = +0.20$ V using a solution containing nominally $5 \times 10^{11}$ IDC-PS particles/mL, 0.1 M KCl, 10 mM $KH_2PO_4-K_2HPO_4$ buffer (pH 7.3), and 0.1% (w/v) Triton X-100. The diameter of the MWNT channel is 132 nm for all the four channels, but the length is (a) 1.21 $\mu$m (membrane no. 1), (b) 1.02 $\mu$m (membrane no. 2), and (c) 0.94 $\mu$m (membrane no. 4). The membrane numbers refer to Table 1.

measurements. For PALS, $\mu$ values were fitted to the data obtained from 35 measurement cycles using an applied electric field of 1.5−2.5 V/cm and a frequency of 2 Hz.[18] At higher electric fields, the Pd electrodes in the PALS apparatus turned black and did not yield reliable $\mu$ data for a reference sample (ZR3, Brookhaven Instruments). The $\mu$ values reported here are averages and standard deviations obtained from more than 100 data points obtained from more than five separately prepared solutions having the same nominal composition.

## Results and Discussion

**Reproducibiliy of Nanoparticle Size and Electrophoretic Mobility Data Obtained Using CNCCs.** We have previously shown that CNCCs provide a means for simultaneously determining the size and electrophoretic mobility of individual nanoparticles.[2] Here, we first discuss the reproducibility of data obtained with different MWNT channels prepared from the same MWNT ($d_c = 132$ nm), and we then compare these results with other sizing and mobility-measurement techniques. The MWNT channels each had a slightly different length ($l_c$), which is a consequence of the inherent variations arising from the microtoming procedure used to slice the membranes from a monolithic block.[1] The length of each channel was determined by cyclic voltammetry (CV).[1,2,21] The diameter and electrophoretic mobility of nanoparticles determined using CNCCs are expressed as $d_{s,CNCC}$ and $\mu_{CNCC}$, respectively.

Figure 1 shows resistive-pulse signals for IDC-PS nanoparticles obtained using three different MWNT channels at a membrane potential ($E_M$) of +0.20 V. The signals appear only at positive $E_M$ values, indicating that the negatively charged nanoparticles pass through the channels by electrophoresis.[1,2] Consistent with intuition, the widths of these signals ($\Delta t$ values) are larger for longer channels (Table 1). The current pulses obtained from all the channels are approximately square, but they vary slightly in shape. TEM images of the MWNT from which the individual channels were fabricated indicate that the inner wall of the tube is uniform and very smooth. We infer, therefore, that the slight variation in signal shape reflects differences in the ends of the tubes that arise from the microtoming procedure used during their preparation.[1,2]

**Table 1. Diameter, Transport Time, Electrophoretic Mobility, and Electrokinetic Surface Charge for IDC-PS and Bangs-PS/PA Determined from Five Different CNCCs[a]**

| membrane no. | $l_c$ ($\mu$m) | $N^b$ | $d_s{}^c$ (nm) | $\Delta t^c$ (ms) | $\mu^c$ ($\mu$m·cm/V·s) |
|---|---|---|---|---|---|
| | | | IDC-PS | | |
| 1 | 1.21 | 114 | $60 \pm 6$ | $6.1 \pm 2.1$ | $-0.15 \pm 0.03$ |
| 2 | 1.02 | 145 | $61 \pm 8$ | $3.5 \pm 0.6$ | $-0.19 \pm 0.03$ |
| 3 | 0.96 | 161 | $57 \pm 7$ | $3.0 \pm 0.5$ | $-0.19 \pm 0.03$ |
| 4 | 0.94 | 180 | $57 \pm 8$ | $3.4 \pm 0.7$ | $-0.17 \pm 0.05$ |
| | | | Bangs-PS/PA | | |
| 1 | 1.21 | 19 | $61 \pm 4$ | $0.8 \pm 0.2$ | $-1.08 \pm 0.16$ |
| 4 | 0.94 | 290 | $59 \pm 5$ | $0.4 \pm 0.1$ | $-1.26 \pm 0.14$ |
| 5 | 1.20 | 500 | $54 \pm 4$ | $0.7 \pm 0.1$ | $-1.20 \pm 0.16$ |

[a] Obtained at $E_M = +0.20$ V in a nanoparticle solution ($5 \times 10^{11}$ particles/mL) containing 0.1 M KCl, 10 mM $KH_2PO_4-K_2HPO_4$ buffer (pH 7.3), and 0.1% Triton X-100. The absence of data for IDC-PS (no. 5) and for Bangs-PS (nos. 2 and 3) is due to the irreversible blocking of the MWNT channels during the measurements. [b] Total number of current pulses measured. The total data acquisition time is different for each measurement. [c] Average $\pm$ standard deviation.

The height ($\Delta i_c$) and width ($\Delta t$) of the current pulses provide information about the volume of single particles, which can be used to obtain the diameter ($d_{s,CNCC}$) of spherical particles and their electrophoretic mobility ($\mu_{CNCC}$), respectively (eqs 1 and 2).[2]

$$\frac{\Delta i_c}{i_c} = S(d_c,\, d_s) \frac{d_{s,CNCC}{}^3}{l_c' d_c{}^2} \tag{1}$$

$$\mu_{CNCC} = \left(\frac{l_c'}{\Delta t}\right)\left(\frac{E_M}{l_c'}\right)^{-1} = \frac{l_c'{}^2}{E_M \Delta t} \tag{2}$$

Here, $l_c'$ is the channel length, after correction for end effects ($l_c' = l_c + 0.8 d_c$),[5] and $S(d_c,\, d_s)$ is a correction factor that depends on the relative values of $d_c$ and $d_s$.[5] In Table 1, the averages and standard deviations for the $d_{s,CNCC}$ and $\mu_{CNCC}$ values for IDC-PS and Bangs-PS/PA particles obtained at $E_M = +0.20$ V are summarized for five different MWNT channels having the same diameter ($d_c = 132$ nm) but different lengths ($l_c = 0.94-1.21$ $\mu$m). Similar results were obtained at different $E_M$ values (+0.04 to +0.50 V) for the same channels.[2] The similarity of the $d_{s,CNCC}$ values obtained for channels having different lengths indicates that the interiors of the channels are uniform and that their diameters are not affected by the method used to slice them from the monolith.[1,2]

For both IDC-PS and Bangs-PS/PA, the $d_{s,CNCC}$ values shown in Table 1 are very similar to the TEM data provided by the manufacturers: $57 \pm 6$ and $60 \pm 10$ nm for IDC-PS and Bangs-PS/PA, respectively. These results indicate that, once the diameter of a MWNT is accurately determined by TEM, multiple channels prepared from a single MWNT provide accurate particle diameters without calibration of the CNCC. Note that ~300 Coulter counter membranes can be prepared from a single 400 $\mu$m long MWNT. This point will be expanded upon later in the context of particle diameters determined by TEM and DLS.

Electrophoretic mobilities determined using different channels ($\mu_{CNCC}$ values) are also similar in magnitude (Table 1). The $\zeta$ potentials of IDC-PS and Bangs-PS/PA nanoparticles, calculated from $\mu_{CNCC}$ values using the Smoluchowski equation,[22] are about $-2.5 \pm 0.3$ and $-17$

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**Table 2. Averages and Standard Deviations of the Diameter of the Polymeric Nanoparticles Obtained Using Carbon-Nanotube-Based Coulter Counting, TEM, and DLS**

| method | $d_s$ of IDC-PS (nm) | $d_s$ of Bangs-PS/PA (nm) |
|---|---|---|
| *Determined from Individual Particle Diameters[a]* | | |
| CNCC[b] | 57 ± 8 | 59 ± 5 |
| TEM | | |
|   manufacturers' data | 57 ± 6 | 60 ± 10 |
|   our results | 61 ± 9 | 57 ± 12 |
| *Determined from Multiple Individual Measurements[c]* | | |
| CNCC[d] | 59 ± 2 | 58 ± 3 |
| DLS[e] | 57 ± 1 | 72 ± 1 |

[a] The values are the average and standard deviation of the diameter of individual nanoparticles. [b] Measured using membrane no. 4 (Table 1 and ref 2). [c] These values are the averages and standard deviations obtained from several separate ensemble measurements. [d] Calculated from the average diameters of 16–500 nanoparticles obtained using membrane nos. 3 and 4 (Table 1) for IDC-PS and Bangs-PS/PA, respectively. [e] Hydrodynamic diameter obtained using greater than or equal to five measurements.

± 1 mV, respectively. For IDC-PS, the $\mu_{CNCC}$ values agree with the calculated value ($-0.18 \mu$m·cm·V$^{-1}$·s$^{-1}$), assuming that the surface charge is the same as the number of surface COOH groups reported by the manufacturer based on an exhaustive titration. This result suggests that most of the COOH groups on the particle surface are deprotonated and that nearly all counterions are located outside the spherical shell defined by the hydrodynamic shear plane. That is, the immobile COO$^-$ groups experience little or no Coulomb screening from counterions.[2] This interpretation is further supported by an almost identical $\mu_{CNCC}$ value at a higher pH (pH 8.4)[23] and by the apparent acid dissociation constant of IDC-PS (p$K_{a,app}$ = 5.8) determined by Coulter counting measurements at different pHs.[23] For Bangs-PS/PA, $\mu_{CNCC}$ obtains a limiting value of $-1.20 \pm 0.24 \mu$m·cm·V$^{-1}$·s$^{-1}$ in the pH range 6.4–9.2,[23] but this is much smaller than the calculated $\mu$ value ($-32 \mu$m·cm·V$^{-1}$·s$^{-1}$) using the assumption that all surface COOH groups are deprotonated and contribute to the surface charge. This discrepancy indicates the presence of a significant number of counterions within the hydrodynamic plane of shear.[14] Specifically, our results indicate that 96% of the negative charge residing on the COO$^-$ groups is screened by counterions. This hypothesis is reasonable and consistent with literature results for surfaces having a similar charge-group density.[14] Later, we will confirm the accuracy of $\mu_{CNCC}$ by comparing it to $\mu$ obtained using the PALS technique ($\mu_{PALS}$).

**Characteristics of CNCCs as Tools for Determining Nanoparticle Size.** The diameter of individual nanoparticles in an aqueous solution can be calculated from the ratio of signal height to background current (eq 1) determined using CNCCs. We compared the diameter of nanoparticles obtained using CNCCs ($d_{s,CNCC}$) to those obtained using TEM ($d_{s,TEM}$) and DLS ($d_{s,DLS}$). In both the CNCC and TEM methods, each particle is sized individually. For each of these methods, Table 2 reports the averages and standard deviations of the particle diameters from an analysis of ~100 individual particle measurements. The standard deviation in these experiments is a true measure of the polydispersity of the nanoparticles.

For DLS, each experiment measures the size of an ensemble of particles (~10$^{11}$), and fitting of the data

**Scheme 2**
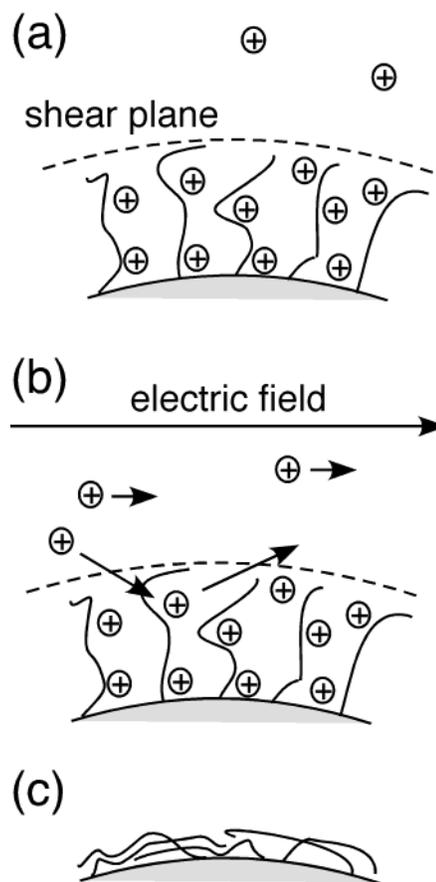


provides the measured sample average and polydispersity. In Table 2, the average and standard deviation of the diameters measured by DLS are reported. These values were calculated using the average values from many independent DLS ensemble measurements. Because this value is an average of many separate ensemble measurements, the standard deviation in this case mainly reflects the reproducibility of sample preparation and measurement, rather than the true polydispersity of the nanoparticles. To compare $d_{s,CNCC}$ and $d_{s,DLS}$ directly, the bottom half of Table 2 also shows the average and standard deviation of the average particle diameter obtained using the different MWNT-based Coulter counters shown in Table 1. The standard deviation of $d_{s,CNCC}$ in this case primarily reflects the reproducibility of sample preparation and measurement.

Table 2 shows that for both types of nanoparticles the averages and standard deviations of $d_{s,CNCC}$ and $d_{s,TEM}$ are quite similar. For IDC-PS, the average of $d_{s,CNCC}$ determined using different counters is very similar to that of $d_{s,DLS}$. The coincidence of $d_{s,CNCC}$, $d_{s,TEM}$, and $d_{s,DLS}$ for IDC-PS demonstrates that the accuracy and precision of the CNCC method is comparable to that of the other methods. In contrast, the average $d_{s,DLS}$ value for Bangs-PS/PA is significantly larger than the average for either $d_{s,CNCC}$ or $d_{s,TEM}$. This result might arise from swelling of the polystyrene/poly(acrylic acid) copolymer particle initiated by electrostatic repulsion between poly(acrylic acid) chains at neutral and basic pHs (Scheme 2a).[24] The swelled polymeric surface layer would then be hydrodynamically impermeable during Brownian diffusion, resulting in a larger $d_{s,DLS}$ value.[16,25] Note, however, that such a swelled

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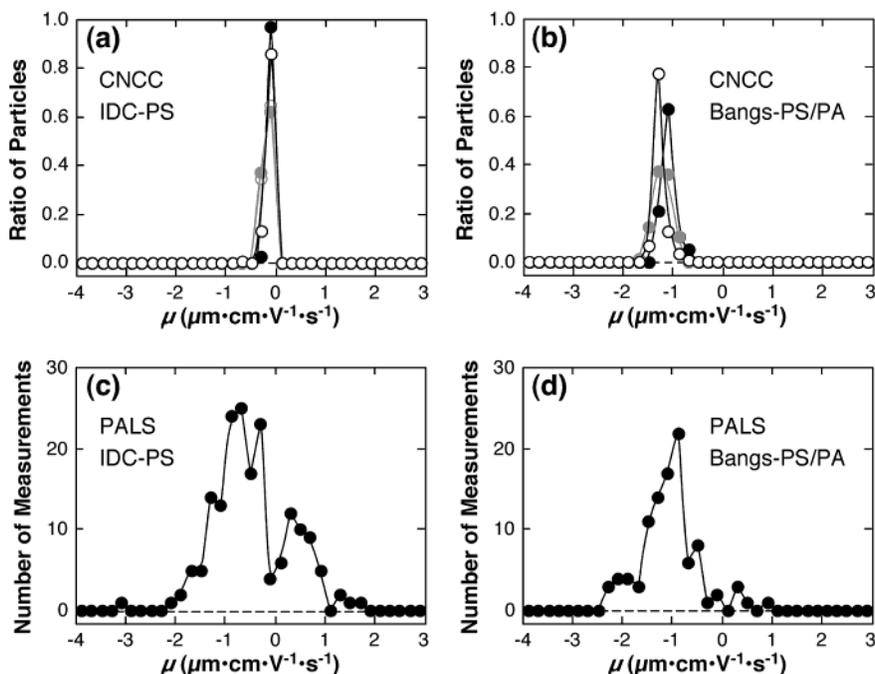
**Figure 2.** Distribution of electrophoretic mobility ($\mu$) for IDC-PS and Bangs-PS/PA nanoparticles. The data in parts a and b were obtained using the CNCC method, and the data points are color coded according to the membrane number used (see Table 1): membrane no. 1, closed black circles; membrane no. 2, open gray circles; membrane no. 3, closed gray circles; membrane no. 4, open black circles; membrane no. 5, closed gray circles. All membranes were prepared from the same MWNT. The data shown in parts c and d were obtained using the PALS method. All data were measured in solutions containing nominally $5 \times 10^{11}$ IDC-PS or Bangs-PS/PA particles/mL, 0.1 M KCl, 10 mM $KH_2PO_4-K_2HPO_4$ buffer (pH 7.3), and 0.1% (w/v) Triton X-100.

surface layer would not affect the change in the MWNT channel resistance because the counter detects only the effective volume of an insulating polymer mass and not the swelled acrylic acid shell through which a current can flow under a strong electric field (Scheme 2b).[26] This effect is very similar to an observation we have made for hydrogels that contain nanometer-scale pores. In the latter case, the polymer gel is practically impermeable against pressure-driven flow but highly permeable to ionic species in the presence of an external electric field.[27] That is, current easily moves through the swollen polymer, just as it does through the swollen shell of the polymeric particles discussed here. Note that this swelled layer will collapse under the high vacuum conditions used for TEM measurements (Scheme 2c). These arguments rationalize the data for Bangs-PS/PA shown in Table 2: the $d_{s,TEM}$ and $d_{s,CNCC}$ results are similar, but the $d_{s,DLS}$ value is significantly higher.

The above comparison indicates that carbon-nanotube-based Coulter counting, DLS, and TEM provide complementary information about particle size. For example, CNCCs provides information about the size distribution of the insulating portion of polymer particles in solution, while TEM provides the size distribution of dehydrated particles and DLS measurements yield an ensemble average of the particle size in solution.

**Characteristics of CNCCs as Tools for Determining the Electrophoretic Mobility of Nanoparticles.** CNCCs can be used to determine $\mu$ for individual nanoparticles in aqueous media from the signal width ($\Delta t$) once the channel diameter and length are known.[2] Here, we compare the accuracy and precision of measured $\mu_{CNCC}$ values with values of $\mu$ obtained using PALS ($\mu_{PALS}$).

Figure 2 shows the distributions of $\mu$ values for IDC-PS and Bangs-PS/PA particles using the CNCC and PALS methods under identical solution conditions. To be clear, the averages and standard deviations of particle mobilities in this section were calculated in a manner similar to the treatment of particle diameters in the previous section. That is, the average $\mu_{PALS}$ values were calculated by averaging the results of many PALS measurements where each measurement is made on an ensemble of particles. Because $\mu_{PALS}$ values are an average of many separate ensemble measurements, the standard deviation primarily reflects the reproducibility of sample preparation and measurement. In contrast, CNCC provides $\mu_{CNCC}$ values for individual particles, and such data permit us to obtain the true distribution in $\mu_{CNCC}$ values in addition to the measurement average and the standard deviation (Table 1).

In all the cases examined, the standard deviations for the $\mu_{CNCC}$ data are much smaller than those for the $\mu_{PALS}$ data (Figure 2). For example, for Bangs-PS/PA, the averages of $\mu_{CNCC}$ ($-1.19 \pm 0.08\ \mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$) and $\mu_{PALS}$ ($-1.09 \pm 0.56\ \mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$) are close, but the precision of the former technique is higher, as suggested by Figure 2. For IDC-PS, the average $\mu_{CNCC}$ value ($-0.18 \pm 0.02\ \mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$) was significantly smaller than that of $\mu_{PALS}$ ($-0.49 \pm 0.75\ \mu m \cdot cm \cdot V^{-1} \cdot s^{-1}$) but still within the rather large experimental error associated with PALS. The $\mu_{PALS}$ for IDC-PS varies from positive to negative (Figure 2c) and has a larger data variation than the $\mu_{PALS}$ of Bangs-PS/PA (Figure 2d). This is probably a reflection of the small surface charge on IDC-PS, which yields very low mobilities and correspondingly low precision in PALS.

The $\mu_{PALS}$ values are less precise than those for $\mu_{CNCC}$ for both types of nanoparticles, and the $\mu_{PALS}$ value for IDC-PS is particularly imprecise. Fouling of the electrodes used for the PALS experiments, which can be caused by electrochemical reactions involving the electrolyte, the

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nanoparticles, and/or the surfactant, may at least in part explain this finding. In addition, such electrochemical reactions may induce a change in solution temperature. This temperature change might lead to unpredictable thermal convective drift in the phase difference spectra, which is known to deteriorate the precision of data.[17] Another possibility is that changes in the surface properties of the particles might arise due to contamination by byproducts electrogenerated during PALS measurements. All of these possibilities are given credence by the consistent observation that the solution used to make PALS measurements changed color (from transparent to brownish) under the high field conditions used to make the measurements (>3 V/cm, see the Experimental Section). This electrochemical reaction issue is not serious for CNCCs because the applied voltage is much smaller (0.5 V or less) and the current flowing between the two Ag/AgCl electrodes is also much smaller (<10 nA) as compared to those for the PALS measurements (typically 2.5 V and ~50 mA, respectively).

The higher precision of CNCCs also arises from its unique geometrical characteristics. Motion of a charged particle in an electric field includes both electrophoresis and diffusion, but interpretation of both the CNCC and PALS methods assumes that electrophoresis dominates Brownian diffusion.[18] This means that faster electrophoretic transport, relative to the rate of diffusional transport, leads to more reliable $\mu$ data. The length of the MWNT channels is ~1 $\mu$m, and therefore, the electric field applied for CNCC measurements is 2 kV/cm when $E_M$ = +0.20 V. This estimate assumes that nearly all of the field is dropped in the nanotube, but this is a good assumption. This electric field is 1000 times higher than that for the PALS measurements (~2 V/cm) and leads to a corresponding larger electrophoretic particle velocity. Moreover, it is known that the diffusion coefficient in a cylindrical channel is smaller than that in a bulk fluid. For example, the axial diffusion coefficient in a cylindrical channel is 0.2 times that in the bulk, due to hindered particle motion normal to the long axis of the channel, for a particle-to-channel diameter ratio of $d_s/d_c = 0.5$.[28] The decrease in the particle diffusion due to hydrodynamic confinement in the MWNT channel may also contribute to obtaining more precise $\mu_{CNCC}$ values in CNCC measurements.

## Summary and Conclusions

As discussed earlier, CNCCs provide a means for simultaneously determining the diameter (effective volume) and electrophoretic mobility of single nanoparticles from signal height and width measurements, respectively. Such measurements do not require calibration of the CNCC nor do they require that the sample be labeled.

Moreover, because CNCCs measure the properties of individual particles, they provide the true average and polydispersity distribution. The desirable features of CNCCs originate from the smooth uniform tubular structure of the MWNT, a negligible permanent charge on the interior surface of the MWNT, and the high electric field inside the channel.

TEM provides information about the size (and in some cases shape) distribution of particles, but the samples must be dehydrated and immobilized on a solid support. This can lead to structural distortions compared to the solvent-swollen state. DLS is useful for determining the size of an ensemble of dispersed nanoparticles, but curve fitting, which is prone to errors, is required to estimate the average and distribution of particle sizes.[14] Carbon-nanotube-based Coulter counting, DLS, and TEM provide different types of information about particle size, and in this sense, they are complementary.

CNCCs can be used to determine the electrophoretic mobility of dispersed particles more precisely than PALS. Furthermore, CNCCs provide the average and distribution of particle mobilities. In contrast, PALS provides only the ensemble average value. Although not discussed explicitly in this paper, the concentration of dispersed nanoparticles can also be determined from the average frequency at which they pass through the channel.[1,2]

In addition to its strengths, the CNCC method suffers some weaknesses. For example, it does not provide information about particle shape, accurate information on effective volume can be obtained only for insulating nanomaterials dispersed in aqueous solutions, and at this time only particles larger than 28 nm are detectable. Moreover, measurements are normally made in the presence of a relatively high concentration of supporting electrolyte. Finally, irreversible blocking of MWNT channels can be problematic. For example, 25% of MWNT channels prepared during the course of the work reported here worked well for CNCC measurements, but the remaining channels were blocked during the fabrication process.

In addition to sizing polymeric nanoparticles, we believe CNCCs will also be useful for detecting and characterizing biological materials such as viruses, proteins, and DNA under physiological conditions. Our present efforts are directed toward these targets.

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