ELECTRONIC SUPPLEMENTARY INFORMATION:

Quantitative separation of polystyrene nanoparticles in environmental matrices with picogram detection limits using capillary electrophoresis

Michael A. Caprise[a], Ana C. Quevedo[b], Kathryn R. Riley[a]*

[a]*Department of Chemistry & Biochemistry, Swarthmore College, Swarthmore, PA 19081, USA*

[b]*Department of Chemical Engineering, McGill University, Montreal, Quebec H3A0C5, Canada*

*To whom correspondence should be addressed; email: kriley1@swarthmore.edu tel: +1-610-690-3904

CONTENTS:

S1. Chemicals and reagents

Sodium bicarbonate (≥ 99.5%) and hydrochloric acid (37% in water) were purchased from Millipore Sigma (Burlington, MA). Sodium hydroxide pellets (> 97%) were purchased from Fisher Scientific (Hampton, NH). Polystyrene particles (PSPs) were purchased from Polysciences, Inc. (Warrington, PA). Bare (nonfunctionalized) PSPs were purchased with varying diameters: 50 nm, 100 nm, or 200 nm (herein, PS-50, PS-100, and PS-200, respectively). In addition, 100 nm PSPs were purchased with varying surface functionalities: amino, bare, or carboxyl (herein, PS-NH_2, PS-100, and PS-COOH, respectively). Hydrous aluminum silicate (ASP® 600 kaolin clay) with an average nominal diameter of 600 nm was obtained from KaMin Performance Minerals, LLC (Macon, GA). Suwanee River Humic Acid (Standard III) was purchased from the International Humic Substances Society (St. Paul, MN). All buffers and sample dilutions were prepared in Millipore water (≥18.2 MΩ.cm at 25°C, < 2.5 μg L^{-1} total organic carbon).

S2. Sample preparation

Sodium bicarbonate buffer was used for CE experiments and PSP characterization experiments. The optimized buffer to separate PSPs based on size was 10 mM sodium bicarbonate, pH 8.5 and the optimized buffer to separate PSPs based on surface functionalization was 15 mM sodium bicarbonate, pH 8.5. Both buffers were prepared by dissolving the appropriate mass of sodium bicarbonate powder in Millipore water and the pH was adjusted to 8.5 through dropwise addition of 0.1M NaOH.

All PSPs were dialyzed prior to use to remove excess surfactants and initiators remaining after the synthesis.[1,2] Spectrum™ Spectra/Por™ 7 Membrane Tubing with a MWCO between 1-50 kDa was used (Fisher Scientific). Samples were placed in the dialysis tubing, secured with clips, and placed into a 2L beaker of Millipore water with gentle stirring. The water was exchanged every 3h for the first 12h and then once every 12h thereafter. Dialysis was continued for a total of 5 days. Following dialysis, the samples were transferred to a clean centrifuge tube and stored in the dark at 4°C. The success of the dialysis procedure was determined by measuring the residual sodium ion concentration (see Section S3) and the concentrations of PSPs following dialysis were determined (see Section S4).

A clay suspension with an initial concentration of ≈1 g L^{-1} was prepared in a 50 mL centrifuge tube and bath sonicated for 15 min to disperse the clay. Then, the clay suspension was

centrifuged at 74×*g* for 10 min to remove larger particles. The supernatant was decanted, and the pellet was lyophilized to determine the mass of clay removed during centrifugation. The final concentration of clay was determined to be 178 mg L^{-1} based on the initial mass of clay weighed and the mass of clay removed. The clay suspension was bath sonicated for 5 min before each use.

A solution of SRHA was prepared to a final concentration of 500 mg L^{-1} in Millipore water. The solution was bath sonicated for 5 min to dissolve. The solution was bath sonicated for 5 min before each use.

S3. Inductively coupled plasma optical emission spectroscopy (ICP-OES)

After dialysis, ICP-OES (iCAP 6000 Series, Thermo Scientific) was used to determine the residual sodium ion concentration in the nanoparticle stocks. First, PSPs were bath sonicated for 10 minutes and immediately diluted 200-fold with 4% trace metal analysis HNO_3 (T0030905, Thermo Scientific) for a final volume of 10 mL. Then, the diluted suspensions were filtered using 0.22 µm syringe filters (PES, 25mm, VWR) to avoid blocking the ICP-OES sample introduction tubes due to nanoplastic aggregation. Filtered samples were placed in 15 mL autosampler tubes (17 x 100 mm, SCP Science) and set for ICP-OES analysis.

Sodium standard (Plasma CAL, Na, SCP Science) was prepared at concentrations of 0.1, 1, and 10 µg mL^{-1} in 4% HNO_3 to calibrate the ICP-OES. RQC-2 solution (Plasma CAL, SCP Science) was used as an external standard control. Three individual replicates per sample were analyzed.

S4. Nanoparticle tracking analysis (NTA)

Nanoparticle Tracking Analysis (NTA, NanoSight LM12, Malvern) was used to assess the number of particles in the dialyzed PSPs (particles mL^{-1}). PSPs were sonicated for 10 minutes using a bath sonicator (5510R-DTH, Branson). Then, particles (PS-50, PS-100, PS-NH_2, and PS-COOH) were diluted 1000-fold with Millipore water (≥18.2 MΩ.cm), while PS-200 was diluted 200-fold with Millipore water. Each sample was vortexed prior to NTA analysis.

Immediately, 1 mL of each of the diluted PSPs was loaded into a 1 mL syringe (Luer lock syringe, LM1, Air-Tite products) and flushed into the NTA chamber. The focus and alignment of the microscope were manually adjusted to ensure optimum imaging position. As soon as the particles

were visualized, a 60-second video was recorded and analyzed using the automatic video processing settings of the NTA software (version 2.3). The equipment's temperature was equilibrated at 24°C for all analyses. Each sample was prepared fresh and 4 replicates per nanoparticle stock were analyzed.

S5. Dynamic light scattering (DLS) and zeta potential measurements

A Malvern Zetasizer Nano ZS dynamic light scattering (DLS) instrument (Malvern Panalytical, Ltd.) was used to measure the hydrodynamic diameter (d_H), polydispersity index (PDI), and ζ potential (ζ) of the PSPs. Prior to use, the 10 mM and 15 mM bicarbonate buffers were twice filtered using a 0.20 µm nylon syringe filter. Then, PS-50, PS-100, and PS-200 were diluted 1:100 in 10 mM bicarbonate buffer and vortexed prior to analysis, while PS-NH_2, PS-100, and PS-COOH were diluted 1:100 in 15 mM bicarbonate buffer and vortexed prior to analysis. The clay and SRHA suspensions were diluted to a final concentration of 100 mg L^{-1} in each buffer and vortexed prior to analysis. All samples were prepared in triplicate. Cuvettes were rinsed with the filtered buffer to remove any dust before the samples were added. Samples were equilibrated at 25ºC for 120s and 5 measurements with 11 sub-runs per measurement were collected for each replicate sample. Zeta potential measurements were conducted using a Pd dip cell and by applying the Smoluchowski equation. Samples were equilibrated at 25 ºC for 120 s and 5 measurements with 50 sub-runs per measurement were collected for each replicate sample.

S6. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra were collected using Nicolet iS5 FTIR Spectrometer with a iD5 ATR diamond insert (Thermo Fisher Scientific, Waltham, MA, USA). Spectra were collected from 4000 to 500 cm^{-1} (resolution of 0.500 cm^{-1}, 16 scans averaged). Prior to each use, the ATR crystal and sample holder were cleaned with methanol and allowed to dry. A 2 µL drop of Millipore water was placed on the crystal and allowed to dry to collect a background spectrum. Then, a 2 µL drop of each PSP was pipetted onto the crystal and allowed to dry. The ATR crystal and sample holder were cleaned with methanol and allowed to dry between each sample.

S7. X-ray photoelectron spectroscopy (XPS)

Nanoplastic stocks were dried for XPS analysis. First 300 µL of each stock was placed into 1.5 mL tubes (05-408-129, Fisher Scientific) and dried at 60 °C (Isotemp®, Fisher Scientific) for 24 h.

Dried samples were placed onto copper foil tape (12.70 mm x 16.5 m, 3M™) and mounted onto a sample bar. XPS analysis was performed using a Kα X-ray photoelectron spectrometer source with a flood gun in a 10^{-8} mbar vacuum (Nexsa G2, Thermo Scientific). Parameters were set as follows: pass energy of 200 eV, energy step size of 1.000 eV, start binding energy of 10.000 eV, and end binding energy of 1350.000 eV. For each sample, three points were set and analyzed for carbon, oxygen, and nitrogen. Spectra were processed using Thermo Avantage Data System software.

S8. Capillary electrophoresis (CE) measurements

Capillary electrophoresis measurements were conducted using a Sciex P/ACE MDQ Plus capillary electrophoresis system (Framingham, MA). A fused silica capillary with an inner diameter of 75 µm, a total length of 60.2 cm, and effective length of 50.0 cm was used for all separations. UV detection occurred at 214 nm. Unless otherwise stated, samples were injected hydrodynamically at 0.4 psi for 5 sec (18 nL injection volume) and the applied separation voltage was 25 kV (applied field ≈ 415 V cm^{-1}). At the beginning of each day the capillary was flushed successively for 10 min with Millipore water, 10 min with 1M NaOH, 10 min with Millipore water, and 20 min with separation buffer. Between each run, the capillary was flushed for 1 min with Millipore water and 2 min with separation buffer. The separation buffer and the solutions used for rinsing the capillary were filtered with a 0.2 µm nylon syringe filter prior to use.

A working stock solution of each PSP mixture (PS-50, PS-100, and PS-200 or PS-NH_2, PS-100, and PS-COOH) was prepared by diluting the stock PSPs (Table S1) 1:20 in the separation buffer. Then, calibration standards of each mixture were prepared by diluting the working stock solution to a final dilution of 1:50, 1:100, 1:200, 1:500, and 1:1000 relative to the original stock solution (Table S1). All dilutions were prepared in the appropriate separation buffer (10 mM bicarbonate buffer for the size separation and 15 mM bicarbonate buffer for the surface functionality separation). All calibration curves were prepared in triplicate and each calibration standard was subjected to triplicate CE analysis to obtain reliable parameters for quantitative analysis.

S9. Data analysis

OriginPro® 2023b (OriginLab Corporation) was used for data analysis and visualization. Each replicate calibration curve was plotted individually, and the limit of quantitation (LOQ) was calculated as $\frac{10\sigma}{m}$, where σ is the standard deviation in the y-intercept and *m* is the slope calculated

from the linear regression line. The LOQ values from the triplicate calibration curves of each PSP were averaged and the standard deviation was determined. In addition, the quantified mass of PSPs was estimated using the LOQ (in particles mL^{-1}), the CE injection volume (18 nL), V_{inj}, the volume of each PSP based on the hydrodynamic diameter determined by DLS, $V_{sphere,\ r=\frac{d_h}{2}}$, and the density of polystyrene (1.0 g cm^{-3}), d_{PS}:

$$\frac{LOQ \times V_{inj} \times d_{PS}}{V_{sphere,\ r=\frac{d_h}{2}}}$$

NTA analysis of PSPs:

Table S1. PSP stock concentrations determined by NTA

Sample	Concentration ($\times 10^{11}$ particles mL^{-1})
PS-50	2.33 ± 0.58
PS-100	1.12 ± 0.09
PS-200	0.36 ± 0.08
PS-NH_2	1.21 ± 0.35
PS-COOH	1.27 ± 0.15

DLS and zeta potential analysis of PSPs with different sizes:

Table S2. Characterization of PS-50, PS-100, and PS-200 in 10 mM bicarbonate buffer

Sample	d_H (nm)	PDI	ζ (mV)
PS-50	61.6 ± 0.6	0.014 ± 0.011	-51 ± 1
PS-100	102 ± 1	0.013 ± 0.007	-60 ± 5
PS-200	226 ± 2	0.020 ± 0.012	-82 ± 7

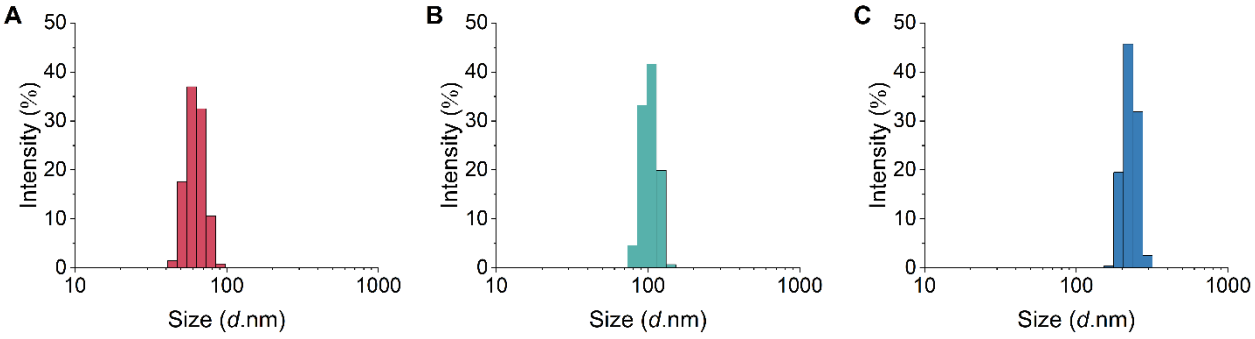

Figure S1. DLS histograms of **(A)** PS-50, **(B)** PS-100, and **(C)** PS-200 measured using DLS. Samples were prepared in triplicate and diluted 100-fold in 10 mM bicarbonate buffer, pH 8.5. All other conditions are as reported in Section S5. Histograms represent the average of three replicate samples.

ATR-FTIR analysis of PSPs:

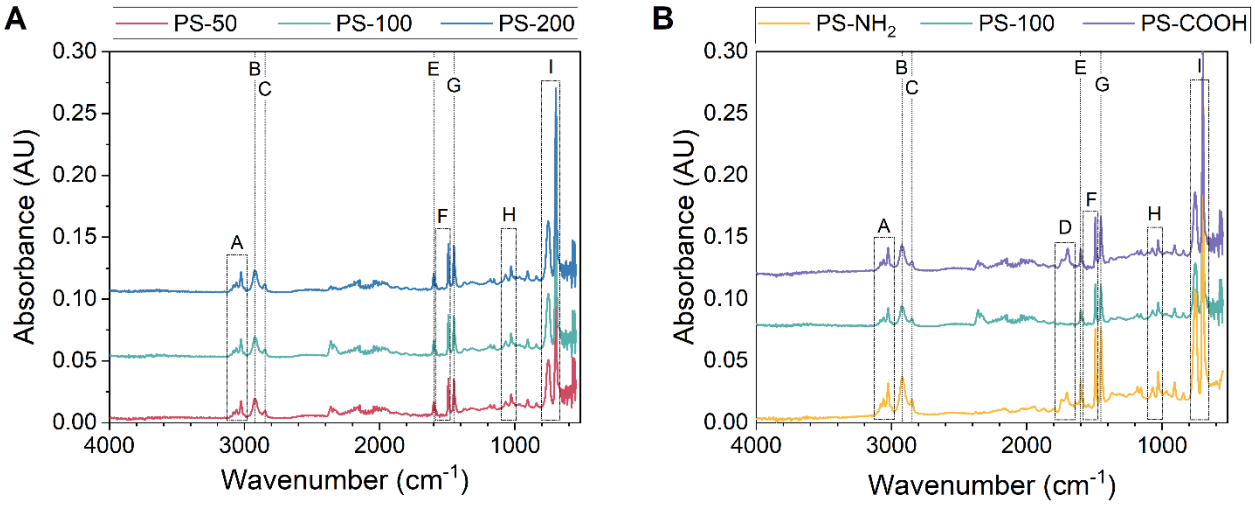

Figure S2. ATR-FTIR spectra of PSPs with **(A)** different sizes (PS-50, PS-100, PS-200) or **(B)** different surface functionalities (PS-NH_2, PS-100, PS-COOH). Spectra were collected using the conditions reported in Section S6.

Table S3. Peak assignments from ATR-FTIR analysis[3,4]

Peak label	Wavenumber (cm^{-1})	Assignment
A	3002 - 3103	C–H aromatic stretching vibration
B	2925	C–H asymmetric stretching vibration of CH_2
C	2850	C–H symmetric stretching vibration of CH_2
D	1696 - 1738	COOH stretching vibration
E	1601	C–C stretching frequency of ring in plane
F	1583, 1493	C–H stretching vibration of ring in plane
G	1452	C–H deformation of CH_2
H	1069, 1028	C–H bending vibration of ring in plane
I	754, 697	C–H out-of-plane bending vibration of ring

XPS analysis of PSPs:

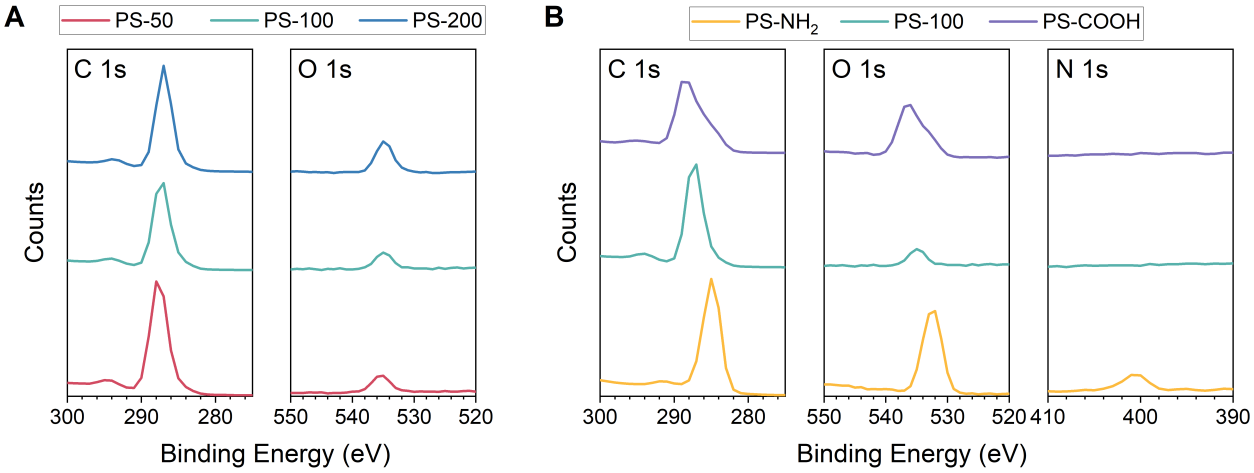

Figure S3. Representative X-ray photoelectron spectra of PSPs with **(A)** different sizes (PS-50, PS-100, PS-200) or **(B)** different surface functionalities (PS-NH_2, PS-100, PS-COOH). In (A) spectra are provided for the C 1s and O 1s regions. In (B) spectra are provided for the C 1s, O 1s, and N 1s regions. Spectra were collected using the conditions reported in Section S7.

Table S4. XPS elemental analysis of PSPs

Sample	Atomic %		
	C 1s	O 1s	N 1s[a]
PS-50	98.48 ± 0.03	1.5 ± 0.1	
PS-100	98.2 ± 0.1	1.67 ± 0.03	
PS-200	97.2 ± 0.2	2.4 ± 0.2	
PS-NH_2[b]	91.6 ± 0.1	6.5 ± 0.2	1.9 ± 0.2
PS-COOH	93.6 ± 0.3	6.1 ± 0.1	

[a] A blank entry indicates that no signal was observed in the N 1s region.

[b] The lower C:O ratio observed for PS-NH_2 is presumed to be a result of the synthetic precursor used to functionalize the particles in the proprietary synthetic scheme used by the manufacturer.

Optimization of CE separation conditions:

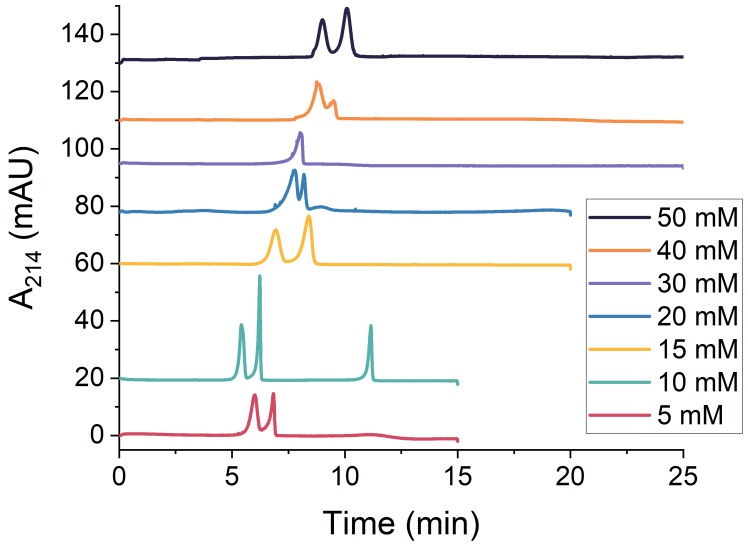

Figure S4. Representative CE electropherograms comparing the effect of the bicarbonate buffer concentration on the separation of PS-50, PS-100, and PS-200. All analyses were recorded using a buffer pH of 8.5 and a separation voltage of 25 kV. The sample mixture was prepared by diluting all PSPs 200-fold in the bicarbonate buffer. All other conditions are as reported in Section S8.

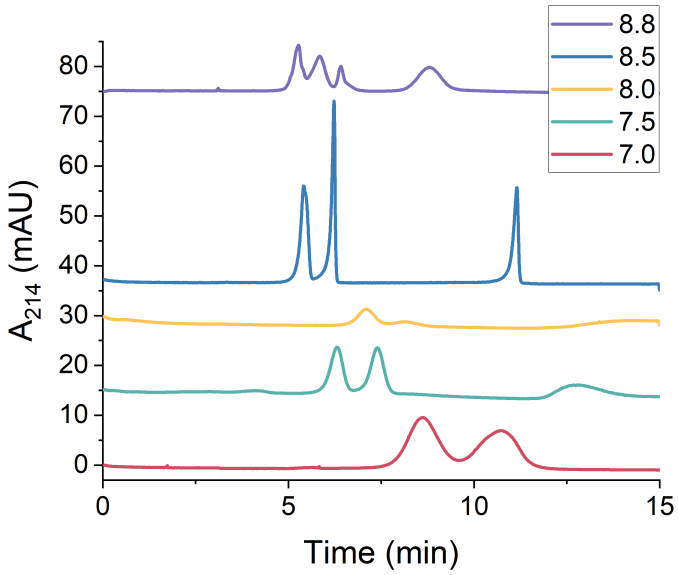

Figure S5. Representative CE electropherograms comparing the effect of the bicarbonate buffer pH on the separation of PS-50, PS-100, and PS-200. All analyses were recorded using a buffer concentration of 10 mM and a separation voltage of 25 kV. The sample mixture was prepared by diluting all PSPs 200-fold in the bicarbonate buffer. All other conditions are as reported in Section S8.

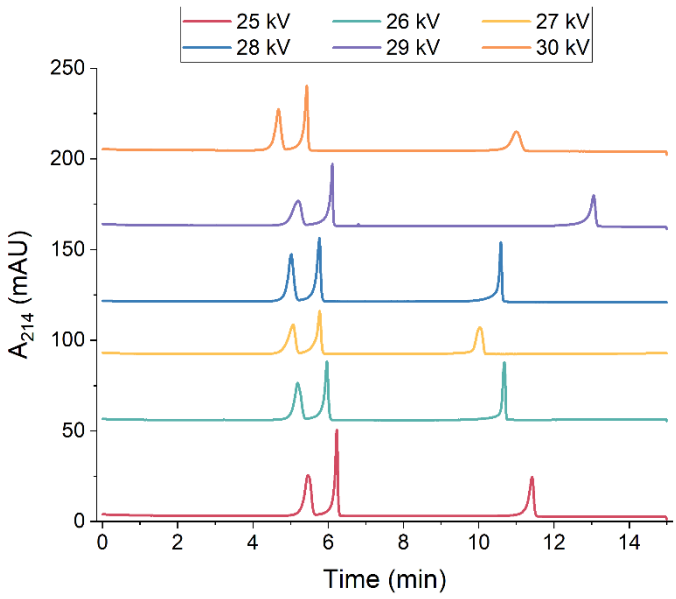

Figure S6. Representative CE electropherograms comparing the effect of the separation voltage on the separation of PS-50, PS-100, and PS-200. All analyses were recorded using 10 mM bicarbonate buffer with a pH of 8.5. The sample mixture was prepared by diluting all PSPs 200-fold in the bicarbonate buffer. All other conditions are as reported in Section S8.

Table S5. %RSD of CE migration times for PS-50, PS-100, and PS-200 as a function of the separation voltage

Separation Voltage (kV)	50 nm		100 nm		200 nm	
	Migration Time (min)	%RSD	Migration Time (min)	%RSD	Migration Time (min)	%RSD
25	5.45	0.6	6.26	0.8	11.33	1.3
26	5.25	1.7	6.00	0.9	10.48	1.8
27	5.00	0.3	5.73	0.5	10.96	3.8
28	5.03	1.0	5.83	2.1	10.48	4.8
29	5.42	4.2	6.42	4.2	13.70	4.2
30	4.66	0.7	5.42	1.0	11.04	2.7

Discussion: Under all voltage conditions PS-200 had the poorest reproducibility in migration time and did not behave predictably in response to changing the electric field (Fig S6). Inconsistent migration times can result from fluctuations in electroosmotic flow, temperature, current, or the pH and ionic strength of the buffer.[5] Since PS-200 had the slowest migration time, it would be more significantly influenced by these factors. The migration time

reproducibility generally appeared to worsen with increasing separation voltage (Table S5), so it is likely that fluctuations in the current played the most significant role.

Peak Identification for the CE Separation of PSPs with different sizes:

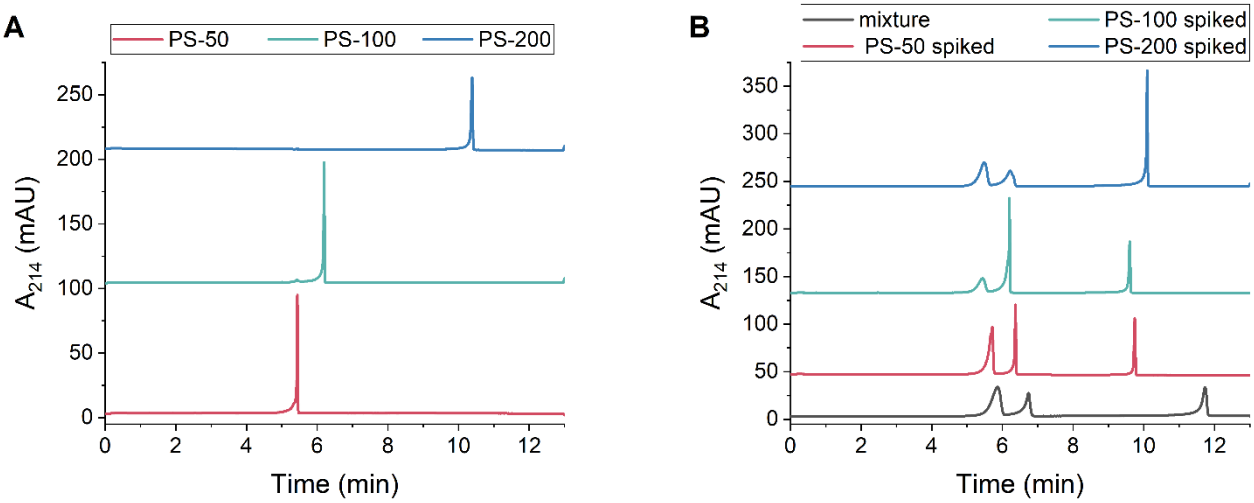

Figure S7. Representative CE electropherograms demonstrating the confirmation of peak identities for the separation of PS-50, PS-100, and PS-200 through **(A)** individual injections of each PSP and **(B)** spiking experiments. In (A) each PSP was individually diluted 200-fold in the separation buffer. In (B) the mixture was prepared by diluting all three PSPs 200-fold in the separation buffer. Then, each PSP was systematically spiked into the mixture by doubling its concentration (representing an overall 100-fold dilution). All samples were prepared in 10 mM bicarbonate buffer, pH 8.5 and all other conditions are as reported in Section S8.

DLS and zeta potential analysis of PSPs with different surface functionality:

Table S6. Characterization of PS-NH_2, PS-100, and PS-COOH in 15 mM bicarbonate buffer

Sample	d_H (nm)	PDI	ζ (mV)
PS-NH_2	114 ± 1	0.016 ± 0.013	-49 ± 2
PS-100	102 ± 1	0.018 ± 0.012	-56 ± 8
PS-COOH	99 ± 1	0.011 ± 0.006	-54 ± 5

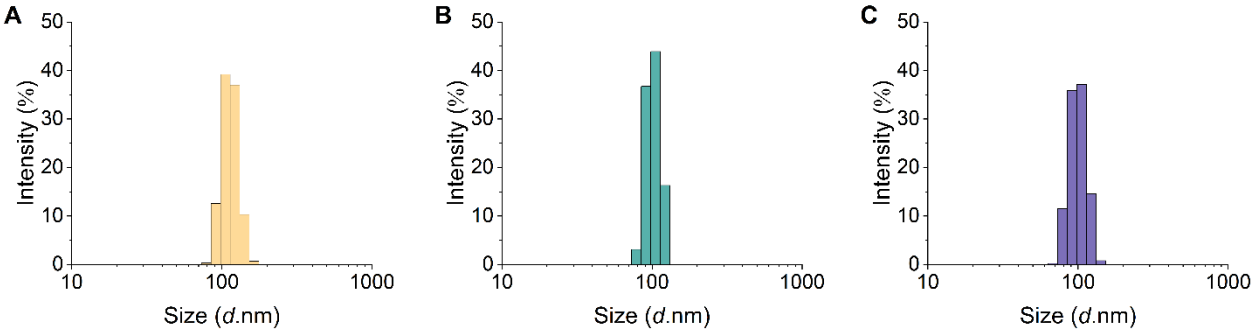

Figure S8. DLS histograms of **(A)** PS-NH_2, **(B)** PS-100, and **(C)** PS-COOH measured using DLS. Samples were prepared in triplicate and diluted 100-fold in 15 mM bicarbonate buffer, pH 8.5. All other conditions are as reported in Section S5. Histograms represent the average of three replicate samples.

Peak Identification for the CE Separation of PSPs with different surface functionality:

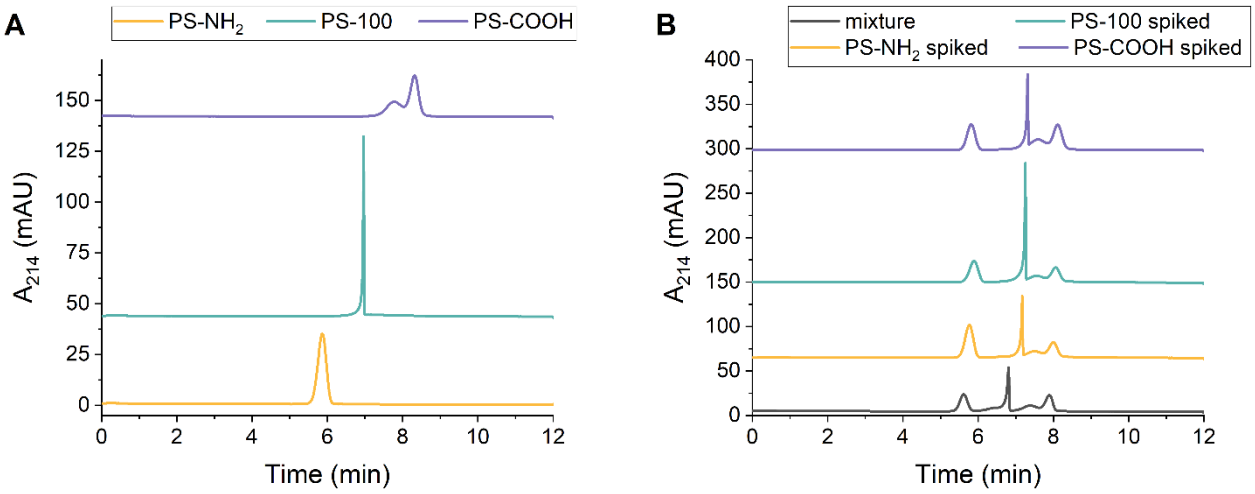

Figure S9. Representative CE electropherograms demonstrating the confirmation of peak identities for the separation of PS-NH_2, PS-100, and PS-COOH through **(A)** individual injections of each PSP and **(B)** spiking experiments. In (A) each PSP was individually diluted 200-fold in the separation buffer. In (B) the mixture was prepared by diluting all three PSPs 200-fold in the separation buffer. Then, each PSP was systematically spiked into the mixture by doubling its concentration (representing an overall 100-fold dilution). All samples were prepared in 15 mM bicarbonate buffer, pH 8.5 and all other conditions are as reported in Section S8.

Calibration curves of PSPs in environmental matrices:

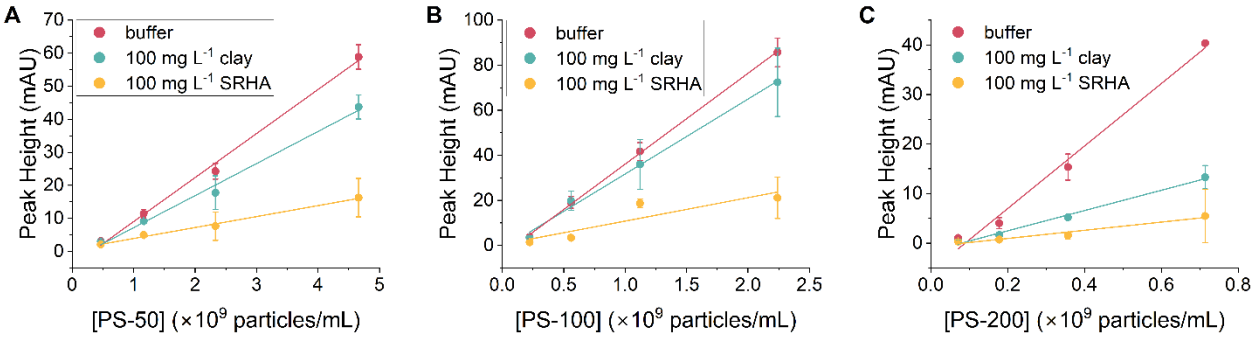

Figure S10. Representative calibration curves of **(A)** PS-50, **(B)** PS-100, and **(C)** PS-200 in bicarbonate buffer, 100 mg L^{-1} clay, and 100 mg L^{-1} SRHA. All analyses were recorded using 10 mM bicarbonate buffer with a pH of 8.5. All other conditions are as reported in Sections S8 and S9.

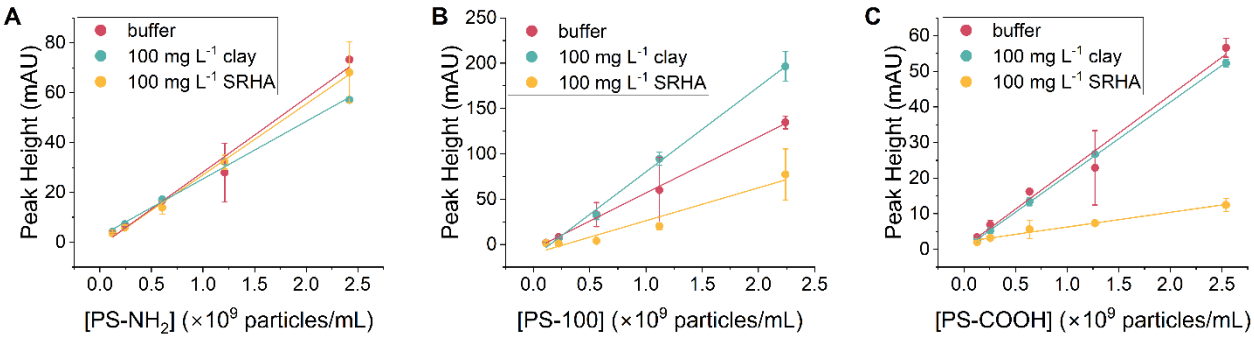

Figure S11. Representative calibration curves of **(A)** PS-NH_2, **(B)** PS-100, and **(C)** PS-COOH in bicarbonate buffer, 100 mg L^{-1} clay, and 100 mg L^{-1} SRHA. All analyses were recorded using 15 mM bicarbonate buffer with a pH of 8.5. All other conditions are as reported in Sections S8 and S9.

DLS and zeta potential analysis of clay and SRHA:

Table S7. Characterization of clay and SRHA in 10 mM and 15 mM bicarbonate buffers

[bicarbonate]	sample	d_H (nm)	PDI	ζ (mV)
10 mM	clay	400 ± 10	0.302 ± 0.031	-47 ± 3
	SRHA	400 ± 100	0.559 ± 0.062	-35 ± 1
15 mM	clay	390 ± 10	0.304 ± 0.027	-52 ± 5
	SRHA	370 ± 60	0.526 ± 0.056	-33 ± 7

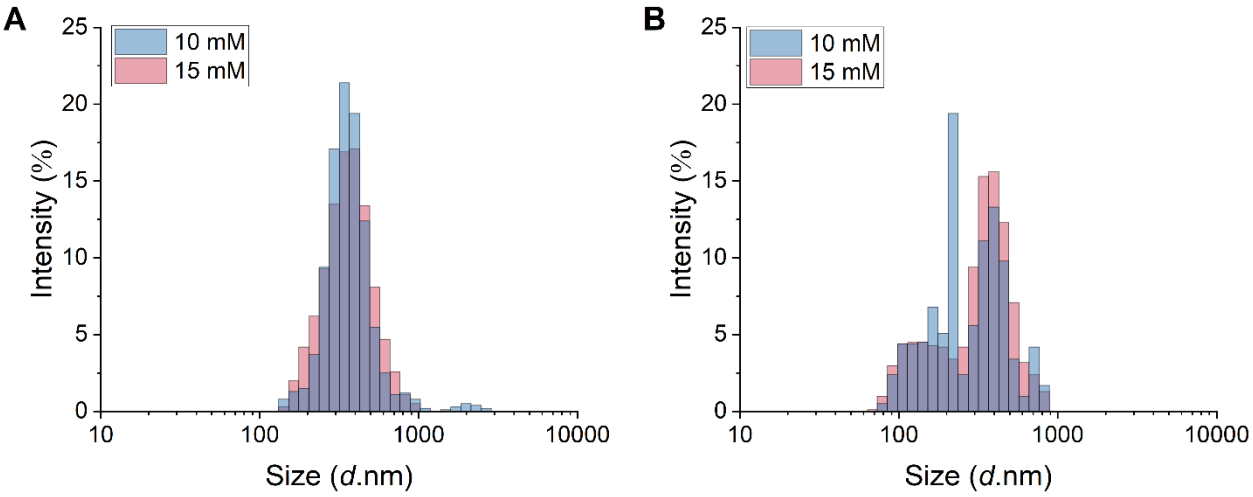

Figure S12. DLS histograms of **(A)** 100 mg L^{-1} clay and **(B)** 100 mg L^{-1} SRHA measured using DLS. Samples were prepared in triplicate and diluted in either 10 mM or 15 mM bicarbonate buffer, pH 8.5. All other conditions are as reported in Section S5. Histograms represent the average of three replicate samples.

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