flushed through the capillary for 3 min. The first monolayer of polymer (PDADMAC) was deposited by rinsing the polymer solution through the capillary for 20 min followed by a 5 min water rinse. All other polymer depositions were done with 5 min rinses followed by 5 min water rinses.

Multilayer coatings used for the protein separations and reproducibility studies comprised six and a half layered pairs (a layered pair generally comprises a layer of a cationic polyelectrolyte and a layer of anionic polyelectrolyte, also termed a "bilayer" in other studies), where the first 3.5 layer pairs were deposited with no salt present and the last three were deposited with 0.5 M NaCl present.

Nonporous silica particles of about 4.5 $\mu$m diameter were coated with polymer by the following method. The particles were suspended in a solution containing 10 mM polymer and 100 mM NaCl. The particles were settled out of suspension with the aid of a centrifuge. The polymer solution was decanted and deionized water was added. The particles were resuspended in the water for several minutes to rinse off any excess polymer. The particles were then centrifuged once again and the water removed. Using this procedure, alternating layers of poly (diallyldimethylammonium chloride) and poly (styrene sulfonate) were deposited onto the silica particles until 20 layers had been deposited. The outer layer comprised poly (styrene sulfonate), giving the particles a negative surface charge.

Particles coated with polyelectrolyte multilayers were suspended in water and pumped under pressure into fused silica capillaries with a sol gel frit at the outlet. The particles accumulated into a packed bed at the end of the capillary. Once the bed formed the particles adhered to each other. The bed was stable, and no secondary retaining frit was used.

The running electrolyte for electrophoresis experiments was phosphate buffer at various concentrations and from about pH 4.0 to 8.0. Electrolyte solutions were made by adding 20 mM solutions of phosphoric acid to 20 mM phosphate salt solutions until the proper pH was achieved. The capillary length was 37 cm, length to detector 30 cm, and the applied voltage was generally 15 kV. UV detection was performed at 254 or 214 nm. Injection of the sample was performed electrokinetically at about 5 kV for 5 sec, ca. 5 nL volume.

Acetone was used as a neutral electroosmotic flow marker, and 2-phenoxypropionic acid (2-PPA) and lidocaine as negative and positive markers, respectively. Standard deviation values are reported as ±1 $\sigma$. Electroosmotic mobility ($\mu_{eo}$) is used here to quantify the electroosmotic flow (EOF) and is given as the velocity of solvent flow per unit electric field strength ($cm^2\ V^{-1}\ s^{-1}$).

## Example 1

### Advantage of Multilayer over Single Layer

Separation of some representative basic proteins was performed using a capillary having a single layer of polyelectrolyte adsorbed and compared to the separation obtained by using a capillary coated with a multilayer comprising six and a half layer pairs of alternating PDADMAC/PSS layers. Materials and methods were as described, except that for this example electrophoresis was carried out at pH 4.0 in both types of layers. The single layer PDADMAC was deposited from 10 mM polymer solution in $3\times10^{-3}$ M NaOH. The multilayer was prepared as described above.

FIGS. **5** and **6** illustrate the single layer and multilayer separations, respectively. The first peak in each figure (unlabeled peak) represents 2-phenoxypropionic acid, which was used as a negative marker. The labeled peaks are as follows: 1) $\alpha$-chymotrypsinogen A; 2) ribonuclease; 3) cytochrome C; and 4) lysozyme. Peaks labeled with prime numbers represent impurities or degradation products. Comparing the results depicted in FIGS. **5** and **6**, it can be noted that the elution order did not change, and that peak shape is much more symmetrical and narrow for the separation on the multilayer, indicating greater separation efficiency.

A single adsorbed layer of positive polyelectrolyte proved reasonably effective in promoting reversed flow CZE separations of basic proteins, as shown in FIG. **5**. However, for separations at pH 6, the single layer column had to be "refreshed" by rinsing with polyelectrolyte in between runs. In addition, some tailing of the peaks was still observed even after rinsing.

Advantageously, capillaries coated with multilayers proved particularly effective in separating the proteins. FIG. **6** shows an electropherogram employing silica coated with 6.5 layer pairs of PDADMAC/PSS. Elution order and migration times are comparable to the results of the single layer PDADMAC column shown in FIG. **5**, however, the resolution has improved. In contrast, capillary zone electrophoresis failed to separate the molecules when performed in bare, uncoated capillaries due to irreversible adsorption of the analyte proteins to the passage wall.

## Example 2

### Separation at Near Physiological pH

Separation was performed using a capillary having a single layer of polyelectrolyte adsorbed and compared to the separation obtained by using a capillary coated with a multilayer comprising six and a half layer pairs of alternating PDADMAC/PSS layers. Materials and methods were as described, except that for this example electrophoresis was carried out at pH 6.0 in both types of layers. The single layer PDADMAC. was deposited from 10 mM polymer solution in $3\times10^{-3}$ M NaOH. The multilayer was prepared as previously described.

FIGS. **7** and **8** illustrate the single layer and multilayer separations, respectively. The first peak in each figure (unlabeled peak) represents 2-phenoxypropionic acid, which was used as a negative marker. The labeled peaks are as follows: 1) $\alpha$-chymotrypsinogen A; 2) ribonuclease; 3) cytochrome C; and 4) lysozyme. Peaks labeled with prime numbers represent impurities or degradation products.

Comparing the results illustrated, it can be noted that the elution order again did not change. The multilayer coating behaved as efficiently at pH 6.0 as at pH 4.0. It is well known that at close to neutral pH separation efficiency is expected to decrease, probably due to adsorption of analytes to uncovered silanol groups on the passage walls. Conversely, proteins tend to degrade at lower pH values, with the resulting degradation. products tending to clutter sensor readings, as noted above. The results illustrated in FIG. **8** for separation on the multilayer at pH 6.0 show that peak height and peak area for impurities are reduced, indicating fewer degradation products present in the sample analyzed. Protein separation and reproducibility of migration times were comparable to results obtained at pH 4.0. Analyte peak shape continues fairly symmetrical and narrow for the separation on the multilayer, whereas the single layer separation indicates less resolution of peaks.

## Example 3

### Separation of Neutral Molecules

Neutral molecules, which generally tend to migrate together, were used to test separation using a multilayer and