

electric field maximum is demonstrated to be a linear function of the voltage applied to filter plate **40**. For example, an applied filter voltage of 15 V results in an electric field of about  $E_{max}=45$  V/cm. According to experimental data discussed previously in reference to FIG. **4c**, a DC offset of 15 V at filter plate **40** corresponds to a low mass cut off at about  $m/z$  200. The mobility coefficient  $K$ , estimated using equation [1] through [2], is then determined to be  $K=0.07$   $m^2/V$  s, typical for conditions of 2 Torr  $N_2$  buffer gas. Substituting the estimated mobility value of  $0.07$   $m^2/V$  s into equation [2], a cross section  $\sigma=120$   $\text{\AA}^2$  is obtained which agrees with those reported for ions of similar  $m/z$ . In the instant case, the effective upper limit of low-mass filtering provided by filter plate **40** is an  $m/z$  of about 500. At filter voltages that produce a cut-off greater than  $\sim m/z$  500, some attenuation of higher- $m/z$  species is observed. High  $m/z$  ions experience reduced RF focusing and are ejected in the radial direction when potential of filter plate **40** is increased above 20 V, corroborating observations of low-mass filtering below about  $m/z$  less than or equal to about 500 obtained experimentally. The effective cut-off limit can be increased by increasing RF voltage (the effective potential) of ion funnel **10** which decreases the attenuation. However, overall transmission efficiency of funnel **10** may be reduced in some cases as a consequence. Example 4 presents an example of this phenomenon, shown for analysis of a polyethylene glycol solution.

#### Example 4

Example 4 describes attenuation effects observed in mass spectra of higher-massed peaks above an  $m/z$  of 500 with and without use of filter plate **40**.

A polyethylene glycol (M.W.=1450) stock solution was prepared by dissolving  $\sim 15$  mg PEG (Sigma-Aldrich, St. Louis, Mo., USA) and  $\sim 15$  mg ammonium acetate (Fisher Scientific, Hampton, N.H., USA) into 10 mL of 50:50 acetonitrile/water and 0.2% by volume formic acid (Sigma-Aldrich, St. Louis, Mo., USA). A PEG solution for ESI infusion was then prepared from the stock by performing a  $100\times$  dilution in 0.02M ammonium acetate in 50:50 acetonitrile/water and 0.2% formic acid. Mass spectra were acquired for the diluted PEG solution using the ESI/ion funnel source described in Example 1.

FIG. **6a** shows a mass spectrum obtained from analysis of the PEG solution at an RF peak-to-peak voltage of 80 ( $V_{p-p}$ ) without use of mass filtering. Peaks spanning the entire mass window were detected. FIGS. **6b-6f** show spectra from analysis of the PEG solution acquired in conjunction with use of filter plate **40** at an applied voltage of 24.0 V and a mass filter cut-off of  $m/z$  1100. Spectra were acquired by incrementally reducing RF peak-to-peak voltage ( $V_{p-p}$ ) over the range from about  $100$   $V_{p-p}$  (FIG. **6b**) to  $60$   $V_{p-p}$  (FIG. **6f**) in 10 V decrements. FIGS. **6b-6e** show gradually increasing peak attenuation with each decrement in voltage. In FIG. **6f**, at  $60$   $V_{p-p}$ , attenuation is sufficiently large to effectively prohibit detection of any PEG peaks. Increasing RF voltage results in an increased effective potential in ion funnel **10** that greatly reduces attenuation by prohibiting radial ion loss in the mass filtering region of ion funnel **10**.

#### Example 5

Example 5 details use and evaluation of low-mass filtering in conjunction with use of filter plate **40** for a liquid chromatography-mass spectrometry (LC-MS) analysis of a Bovine Serum Albumin (BSA) tryptic digest.

A tryptic digest of BSA (Sigma-Aldrich, St. Louis, Mo., USA) was made by denaturing the BSA protein in urea and thiourea, and reducing it with dithiothreitol followed by a  $10\times$  dilution in 100 mM ammonium bicarbonate. Digestion was then performed with trypsin (Promega, Madison, Wis., USA) in a 1:50 ratio of enzyme to protein. The digest was cleaned using a  $C_{18}$  SPE column from Supelco (Bellefonte, Pa., USA) and then concentrated down to 0.1 mg/mL.

The ESI/ion funnel configuration described in Example 1 and in reference to FIG. **2b** was employed. Mesh plate **42** was biased with a DC power supply **63** used to float the ground of picoammeter **64** (Keithley Model 6485, Cleveland, Ohio, USA). Picoammeter **64** was used to detect ion current impacting mesh **44** and plate **42**. The LC-MS analyses of the BSA tryptic digest were performed using an Agilent 1100 series capillary LC system. Samples were analyzed by loading 800 ng of a BSA tryptic digest solution onto a  $150$   $\mu\text{m}$  i.d. $\times 25$  cm long reverse-phase packed capillary column with  $5$ - $\mu\text{m}$  diameter  $C_{18}$  particles (Jupiter, Phenomenex, Schlieren, Switzerland). Samples were separated at a constant flow rate of 2  $\mu\text{L}/\text{min}$  and a linear gradient was used to elute the peptides, using 0.2% acetic acid and 0.05% TFA in water (solvent A) and 90% acetonitrile with 0.1% TFA (solvent B).

Ion current measurements were collected by biasing mesh plate **42** in ion funnel **10** to  $-10$  V and detecting ion current values with picoammeter **64**. In order to record ion current during the LC-MS experiments, in-house developed software was used to receive and store the current measurements from picoammeter **64** in one second intervals.

The BSA tryptic digest was separated via capillary LC and analyzed with a single quadrupole mass spectrometer, as described in Example 1, with and without mass filtering. In order to provide a control data set, filter **40** was initially set at  $+5$  V which did not create a potential energy barrier and did not block any low-mass ions and detecting the resulting ion current once every second with picoammeter **64**, FIG. **7a**. Space charge related to low-mass ("background") species during the LC-MS analysis of the protein digest was detected, and the ability of mass filter **40** to block this background space charge was tested under the same conditions as the control LC-MS experiment except with filter plate **40** biased to a voltage which produced a mass filter **40** with a cut-off at  $m/z$  **200** and detecting the resulting ion current once every second with picoammeter **64**, FIG. **7b**.

During LC-MS analysis, a large portion of total ion current exiting ESI source **25** was from unwanted, background species, which unnecessarily increased the space charge in the mass spectrometer. As shown in FIG. **7a**, without use of filter **40** and associated filtering, ion current measured during peptide elution ranged from about 130 to 230 pA. The ion current background was about 90 pA, representing from 40 to 70% of the total current. In FIG. **7b**, use of filter **40** and associated filtering during peptide elution resulted in an ion current of from 40 to 140 pA, with a contribution from background species of about 5 pA, a drop of from 40% to 70% to about 4% to 13% of the total ion current. Use of filter plate **40** and low-mass filtering assured that a large majority of ions entering the mass spectrometer from the ESI source were actual tryptic peptides and not unwanted background species that would unnecessarily add to space charge.

In sum, a significant portion of ions exiting funnel **10** are of no interest. Use of filter plate **40** removes low-mass background species, lowering space-charge effects which can hamper the ability of MS to perform. Space-charge shields the potentials the MS uses. Removal of space-charge effects by use of filter plate **40** (either with pre-analysis or real-time adjustments) thus improves instrument performance, and is