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JMS Letters

Dear Sir,

Preparative Mass Spectrometry with Electrospray Ionization

In the early 1940s, E. O. Lawrence developed a mass spectrometry-based separation approach to enrich radioactive uranium ^{235}U from the natural isotopic distribution of uranium.[1–3] This method used Calutron[3] mass spectrometers to separate ions according to their mass-to-charge ratio (m/z), and once separated, the ions were collected. As part of the Manhattan Project, Lawrence applied this preparative mass spectrometry approach to the purification of radioactive ^{235}U, which was then used to construct the first nuclear weapon. Since those early experiments more efficient (non-mass spectrometric) means of generating and separating uranium isotopes have been established, yet separating compounds based on mass is certainly an intriguing idea which has not lost its appeal. The question of whether modern mass spectrometry can be further developed as a practical separation and collection device has been addressed here with electrospray ionization mass spectrometry. In the experiments described, a monodisperse polymer was generated from a synthetically derived polydisperse version of the polymer. This simple example served to illustrate some of the advantages and disadvantages of using electrospray mass spectrometry as a preparative tool for molecular separation and purification.

The science of mass spectrometry has undergone dramatic changes in the past decade, expanding its utility as the mass range and sensitivity of the instrumentation have increased. More specifically, electrospray ionization[4] mass spectrometry (ESI-MS) has been applied in many diverse ways extending far beyond routine molecular mass determination, especially in the chemical and biochemical sciences.[5] One area where ESI-MS has been found to be interesting and useful is in the study of non-covalent interactions. For instance, it was recently demonstrated[6] that non-enveloped viruses (which are non-covalent complexes containing proteins and genetic material) can be non-destructively introduced into the gas phase using electrospray ionization, and can then be electrostatically directed through a mass spectrometer while retaining native structure and viability. The collection of the virus after mass selection (albeit crude)[7] suggests that electrospray ionization mass spectrometry can also be used as a preparative tool.

In order to test further whether electrosprayed ions could be collected following mass separation, a quadrupole mass spectrometer (Perkin-Elmer SCIEX API100) was used to separate gas-phase ions. Once the ions had been generated and separated they were collected on a vacuum grease-coated brass plate designed as a collector with an orifice on the surface to allow some ions to pass through and be detected by an electron multiplier detector. Thus, this design permitted the simultaneous collection and detection of the ions (Fig. 1). The preparative mass spectrometric experiments were performed on a polydisperse polymer, polypropylene glycol (PPG), to create a monodisperse version of the polymer. Once the polymer had been collected it was extracted with an aqueous solvent system containing 0.1% trifluoroacetic acid and transferred on to a sample analysis plate of a matrix-assisted laser desorption/ionization (MALDI) mass spectrometer and analyzed. The MALDI data for the polymer before and after electrospray preparative mass spectrometry are shown in Fig. 1.

By estimates based on the MALDI signal of the PPG polymer at a range of standard concentrations, the efficiency of the electrospray preparative mass spectrometric experiments was approximately 10^{-7} with an error estimated at an order of magnitude. Therefore, for every 10^7 molecules in solution that were sent into the electrospray mass spectrometer, one molecule was collected. While this represents a significant limitation for ESI-MS as a preparative tool, it also has potential to be improved. Just as the Calutron was scaled up for high throughput, and its low efficiency was compensated for by removing sample from the side of the analyzer for reintroduction into the instrument, unused electrosprayed sample can also be collected and returned to the source for ESI. At normal liquid chromatography/mass spectrometry flow-rates of 0.5 ml min^{-1}, only a small portion of the analyte is ionized, leaving a significant amount of material to be recycled and delivered back to the ESI source. Other, more efficient electrospray alternatives exist such as nanoelectrospray,[8] which offers higher efficiencies ($\sim 10^{-3}$–10^{-4}), but does so at the cost

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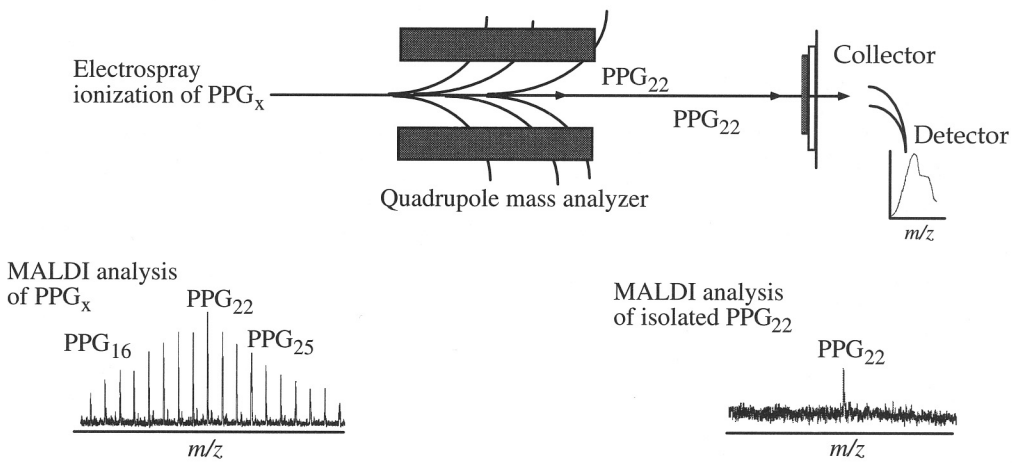

Figure 1. The preparative mass spectrometry experiments were performed with a Perkin-Elmer SCIEX API100 electrospray ionization single-quadrupole mass analyzer. The 22mer of PPG was separated from the polydisperse polymer and collected on a brass plate coated with vacuum grease. The brass plate contained an orifice which allowed some ions to pass to the electron multiplier detector, thus allowing for simultaneous detection and collection. The PPG sample was examined by MALDI-MS before and after separation. The samples were introduced into the mass spectrometer at a rate of 4.0 μl min^{-1}, the declustering potential was maintained at 70 V and the electrospray voltage was typically maintained at 4000 V.

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of using very low solution volumes (typically the analysis of 1 μl is performed over the course of 10 min).

One example of the utility of large-scale preparative mass spectrometry is its potential in generating monodisperse synthetic polymers. Generating enough truly monodisperse polymer could provide a useful system for polymer experimental studies since most theoretical models are based on experimental data from polydisperse systems. In another example, the application of electrospray with preparative mass spectrometry also offers a means of initiating unique chemical reactions since ions can be generated, selected for and reacted upon through their deposition on a surface coated with other molecules, similar to the work of Cooks and co-workers[9,10] and Busch and co-workers.[11]

Overall, ESI with preparative mass spectrometry offers the ability to generate intact molecular ions and to then separate and collect these ions based on mass, the primary advantage being that it allows for separations that may be difficult or impossible otherwise.

Yours,

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