Gel electrophoresis gives some indication of the molecular weight of a protein based on its migration in a gel; however, the molecular weights determined from this method are not precise. In contrast, mass spectrometry can provide accurate and precise molecular weights that aid in identification of proteins. Initially, mass spectrometry experiments were limited to small molecules that could be readily volatilized into the gas phase and ionized before entering the mass analyzer. In the late 1980s, several ionization methods were developed and applied to biomolecules that permitted analytes from condensed phase samples, such as liquids and solids, to be volatilized and analyzed by mass spectrometry. Electrospray ionization (ESI) is one such technique.

ESI is a solution-phase “soft” ionization source which converts ions in solution to gaseous ions. The mechanism of ion formation in an ESI source is depicted schematically in Figure 1. Briefly, gaseous ions are formed when a solution containing the analytes of interest (from an LC column or from a syringe infusion pump) is sprayed through a stainless steel capillary to which a high voltage is applied, creating a fine mist of droplets which are charged on their surface. As solvent evaporates from the charged droplets, the charge density on their surface increases to a critical limit, at which point electrostatic repulsion causes the larger droplets to break up into smaller charged droplets. Finally, analyte ions are ejected into the gas phase by electrostatic repulsion, and these ions enter the mass analyzer for subsequent mass analysis. You can watch a video from the Johnson lab showing the formation of a stable electrospray as the voltage applied to the tip is increased.

For electrospray, the protein sample is prepared in a solution composed of water with acid and a low surface tension organic solvent, such as methanol. Relatively pure protein samples can be infused directly into the mass spectrometer using a syringe pump, or more complex protein and peptide mixtures may be separated by chromatography first and electrosprayed directly from the column.

Electrospray ion sources are compatible with many types of mass analyzers. For this application, we will consider the quadrupole ion trap as a mass analyzer. The quadrupole ion trap is composed of two end cap electrodes at the entrance and the exit of the trap and a ring electrode (shaped like a donut) in the middle. The voltages and AC frequencies of the applied potentials on the electrodes are varied to control the motion of ions in and through the trap. The ion trap mass analyzer is a small, relatively inexpensive mass analyzer that typically generates mass spectra with unit resolution ($\Delta m = 1$) or slightly better. This video shows the operating steps involved in generating a mass spectrum using an ion trap.
Video Question

1. Summarize what you saw in the video on the ion trap. What are the operating steps used to generate a mass spectrum using an ion trap mass analyzer?