Electrospray Ionization (ESI)
Electrospray is produced by applying a strong electric field to a liquid passing through a capillary tube with a weak flux.

Desolvation by gas flow (N\textsubscript{2}) or gentle capillary heating (100-300 deg C).

Ions are mostly preformed in solution before desorption.

It is good for both small and large molecules.

Produces mostly multiply protonated ions.

Very low energy transfer process.
Looking inside Electrospray

Charged Residue Model (CRM) proposed by Dolc

Both are currently accepted.

Ion Evaporation Model (IEM) proposed by Iribarne and Thompson.
Ion Desolvation in ESI
Why is ESI so popular?

1. Proteins can be ionized without denaturization: non covalent, receptor-ligand complexes remain intact.
2. Working directly from a dilute soln: 0.001-10mM, very good for catalyst systems (active species is found under such conditions).
3. Any polar solvent (H₂O, ACN, THF etc) suitable.
4. Flow rates of nano to ul per min: direct sampling possible.
Interpreting ESI Spectra

- The Y axis is labeled relative intensity.
- The X axis is mass divided by charge, m/z.
- C: the "base peak"
- D: spectrum will have a certain number of counts associated with the tallest peak in the spectrum. This number can be used to gauge the concentration of the analyte.

For warning: the count number is relative and can be adjusted with the multiplier gain and strictly speaking cannot be related to concentration without an internal standard. Counts will also be affected by spray needle and over all source maintenance.
NOTE

- In positive ion mode, the number of charged species normally observed in an electrospray spectrum is reflected in the number of basic sites on a molecule that can be protonated at low pH.
In positive ion mode the analyte is sprayed at low pH to encourage positive ion formation.

In negative ion mode the analysis is normally carried out well above a molecule’s isoelectric point to deprotonate the molecule.
Nature of ESI Spectra

Detecting large molecules

Multiple charged, protonated ions:

\([M+H]^+\)
\([M+2H]^{2+}\)
\([M+3H]^{3+}\)
\([M+4H]^{4+}\) etc.
Isotopic peaks are spaced by 1 a.m.u. (the difference between $^{12}$C and $^{13}$C).

On the m/z scale of a mass spectrum, the m/z spacing of two contiguous isotopic peaks will correspond to $\frac{1}{z}$.

In mathematical terms: m/z spacing = $\frac{1}{z}$ and $z = \frac{1}{\text{m/z spacing}}$.

Examples: 

a. (m/z spacing) = 1, therefore $z = \frac{1}{1} = 1$ 

b. (m/z spacing) = 0.2, therefore $z = \frac{1}{0.2} = 5$
Interpretation of ESI

- For large molecular weight biomolecules, the measured mass is the average mass and that the peak envelope extends over many individual masses.
Myoglobin: 16000 Da

- A protein with a mass of 10 kDa, will have a peak envelope that is approximately 20 mass units wide (counting all isotope containing peaks with intensities greater than 1% of the most abundant peak).
ESI Pros and Cons

1. Ionizes very fragile biomolecules, and even non-covalent complexes can be detected with no dissociation
2. Large biomolecules (MW > 70,000) can be analyzed in a small m/z range (< 2000 m/z)
3. Can be easily interfaced with HPLC and CE

1. Very sensitive to salts (buffers), incompatible with some solvents
2. Not useful for non-polar compounds (rescued by APCI)
3. Spectra of mixtures get too complex (front-end separation may become crucial)