Fundamentals of Electrospray

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Electrospray basics literature:


Figure 1. Aerosol formation by ES. (a) Simple ES; (b) ES with sheath flow; (c) ES with pneumatic assistance; (d) ES with ultrasonic assistance.

Electrospray capillary (+3 kV)

Taylor cone

Adapted from:
D.P.H. Smith, IEEE Trans (1986) 6, 496
Figure 7. Free-jet expansion of gas and ions into vacuum; (A) basic principle; (B) arrangement with skimmer penetrating into the silent zone; (C) skimmer located more distant than the mach disk.

Schematic of Typical Electrospray Process

Figure 2. Schematic illustration comparing the basic instrumental components of (a) a controlled-current electrolytic (CCE) cell and (b) an electrospray (ES) ion source.

Figure 3. Schematic illustration, based on the operation of an ES ion source as a CCE cell, showing the expected interdependence of the potential at the electrode/solution interface, $E_{E/S}$, in the ES capillary as a function of the ES current, $i_{ES}$, and as a function of the composition of the electroactive species in the solution. Solid line: three electroactive species, viz., A, B, and C, with electrode potentials $E_{A+/A} < E_{B+/B} < E_{C+/C}$, respectively, are present in the solution at equal concentration. Dashed line: only the electroactive species C is present in the solution.

Figure 1. Schematic representation of the Charged Residue Model (CRM) and the Ion Desorption Model (IDM) for ion formation. The upper series depicts a sequence of evaporation fission steps that by the CRM leads to ultimate droplets containing only one solute molecule. The lower series shows an intermediate stage at which a droplet's surface charge density is below the Rayleigh stability limit but high enough to provide a surface field sufficiently intense to desorb a solute molecule as an ion in accord with the IDM.

FIG. 6. Closeup views of two droplets in the act of bursting. Mean flow direction: top to bottom.

Multiply Charged Ions from Small Proteins:

Figure 7. Representative electrospray mass spectra for protein samples in acidified mixtures of water, methanol, and isopropanol. The adduct ions were protons. Solution concentration varied from 5 to 19 μmol/L and injection was at 8 μL/min. Each spectrum resulted from a single 30-s scan of the indicated mass range. The charged species were proton.


- Each spectrum contains a sequence of peaks representing intact molecules of different charge states. Adjacent peaks differ by one charge.

- Each additional charge is provided by an additional proton (or other small cation).

- "Compact" molecules do not acquire/retain as many charges as "stretched out" molecules.

- Multiple charging increases the nominal mass range of the mass analyzer by a factor equaling the maximum number of charges per ion.
Horse heart myoglobin (mol. wt. 16,951)

Calculation of molecular weight of unknown:

Choose two adjacent peaks in 'envelope'

higher m/z peak
\[\frac{m}{z} = \frac{m + z (1.0079)}{z}\]

lower m/z peak
\[\frac{m}{z} = \frac{m + (z + 1) (1.0079)}{z + 1}\]
Figure 1. Positive ion ESI mass spectra of $10^{-5}$ M bovine cytochrome c obtained with different acetic acid concentrations in aqueous protein solutions. Solution conditions: (a) 4% acetic acid (pH = 2.6); (b) 0.2% acetic acid (pH = 3.0); and (c) no acid (pH = 5.2). The labels on the peaks, $n^+$, indicate the number of protons, $n$, attached to the protein molecule. The observation of two distinct distributions of charge states was correlated to a change in protein conformation from a folded (higher pH) to an unfolded (lower pH) state. Reprinted with permission from S. K. Chowdhury et al., J. Amer. Chem. Soc., 1990, 112, 9012–9013. © 1990 American Chemical Society.
Figure 7. Apparent gas-phase basicity as a function of charge state of cytochrome c ions, measured (●); calculated, linear ($\epsilon_r = 1.0$, △; best fit $\epsilon_r = 2.0$, ○); intrinsic, ▲; calculated, X-ray crystal structure ($\epsilon_r = 2.0$, ◆); calculated, α-helix ($\epsilon_r = 4.1$, □). The dashed line indicates GB of methanol (174.1 kcal/mol) and the dash-dot line indicates GB of water (159.0 kcal/mol).

**FIGURE 5.** Intensity of solvent cluster response as a function of mole fraction methanol for various methanol/water mixtures. Even at very low mole fractions of methanol, the ESI response is predominantly that of methanol clusters, which have higher gas-phase PA than do water clusters [Reprinted from Amad et al., *J Mass Spectrom.*, 2000, 35:784–789 with permission of John Wiley & Sons, Ltd.].
Figure 3. Negative-ion ESI mass spectra of the disodium salt of cardiolipin dissolved in 10% chloroform and 90% (a) methanol, (b) dichloromethane, (c) 1,2-dichloroethane, (d) chloroform, and (e) carbon tetrachloride. The solvents are listed in order of decreasing polarity as measured by the dielectric constant. The relative abundances of doubly charged species progressively decrease with decreasing dielectric constant of the solvent. Reprinted by permission of Elsevier Science, Inc. from R. B. Cole and A. K. Harrata, J. Am. Soc. Mass Spectrom., 93, 4, 546–556. © 1993 American Society for Mass Spectrometry.
FIGURE 12. Typical ESI calibration curve generated on a TSQ 7000 triple quadrupole mass spectrometer. The analyte is the surface-active trimethyldecylammonium cation, dissolved in a solution of 50% water, 50% methanol, 0.5% acetic acid. Deviations in linearity are observed at the low end due to background interference, and at the high end due to saturation in ESI response.

**Figure 1.** Dependence of mass spectrometry signal (selected ion current) on initial solute concentration $n_i0$ for tetramethyl ($\square$), tetrabutyl (○), and tetraheptyl (▲) ammonium halides. The coordinate scales are logarithmic, but the lines have slopes of unity, so signal is directly proportional to $n_i0$. The concentration units along the abscissa are in moles per liter. The ordinate values are selected ion currents in arbitrary units.

FIGURE 7. Mass spectrum of an equimolar mixture of six tripeptides that have different C-terminal residues. ESI response increases in proceeding from G-G-G to G-G-F as the side chain on the C-terminal residue becomes increasingly nonpolar (see structures in boxes). The ESI response of the peptide G-G-Y is less than that of G-G-F due to the addition of the polar OH group on the phenyl ring [Reprinted from Cech & Enke, Anal Chem, 2000, 72:2717–2723 with permission of American Chemical Society].

FIGURE 8. Response as a function of experimental retention time for the same tripeptides shown in Figure 7. Isocratic analyses were performed in a solvent of 65:35 water/methanol, 0.5% acetic acid. Retention time and response were both calculated relative to G-G-G (responses of all other peptides were divided by that of G-G-G and the retention time of G-G-G was subtracted from the other retention times). The general trend in the data is that, as retention time increases, so does ESI response [Reprinted from Cech, Krone & Enke, Anal Chem, 2001, 73:208–213 with permission of American Chemical Society].
FIGURE 2. The process of solvent evaporation as the noncovalently bound complex is transferred from solution to the gas phase. Gentle desolvation conditions are generally required to maintain the intact gas-phase complex. For several protein systems, the ESI-MS data may be consistent with the solution-phase binding constants. Although some features of the solution structure may be preserved by the gas-phase ions, the stability of the gas-phase complex ion may not be reflected by the solution-phase binding constant.

Characteristics of Electrospray Ionization-MS:

- Analytes introduced in solution, highly suited for coupling to LC and CE

- Very low-energy ionization process
  Observe intact molecules
  Permits study of non-covalent interactions

- High sensitivity

- Multiple charging enables access to very high molecular weights

- Works best for compounds that contain polar functional groups
Disadvantages of electrospray-MS

- Presence of salts causes signal suppression
- Response/quantification affected by presence of other solutes
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