

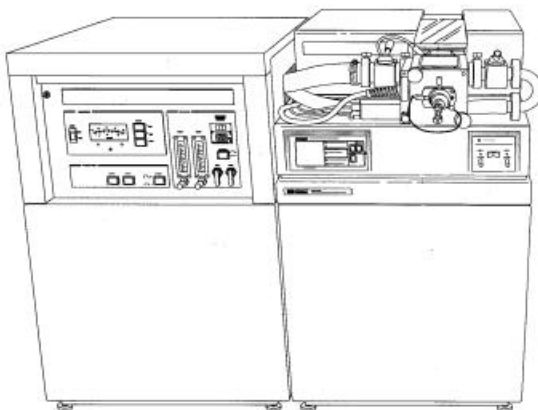
Electrospray Ionization Mass Spectrometry

Jessica Gilman
Courtney Mashburn

17 September 2002
Chemistry 5181



“Many users tend to view ESI as a ‘Black Box,’ because sources of instability, background, interference, competition, and suppression are not always understood.”





Outline

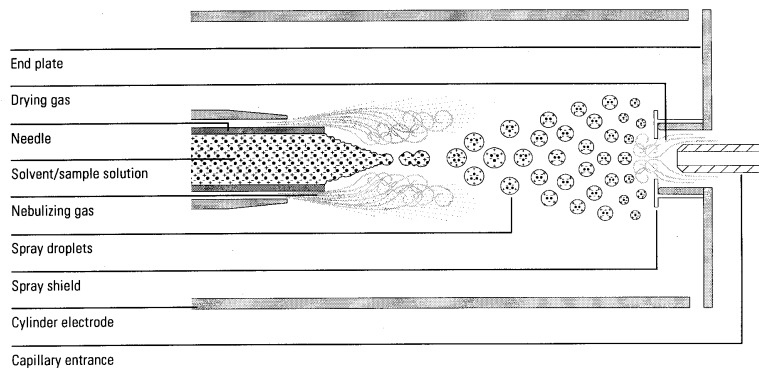
- Introduction
- Ionization Process
- Introduction of Ions into MS
- Operational Conditions and Parameters
- Solvent and Analyte Characteristics
- Sensitivity and Detection Limits
- Tandem Techniques
- Summary



Introduction

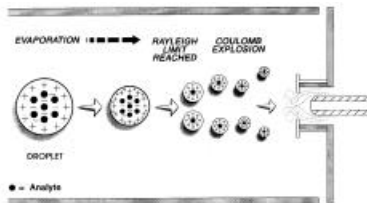
- ESI allows for large, non-volatile molecules to be analyzed directly from the liquid phase
- Used for:
 - Mass determination of biomolecules
 - Analysis and sequencing of proteins and oligonucleotides
 - Analyzing drugs, pesticides, and carbohydrates
 - Long chain fatty acids

Electrospray Ionization Chamber

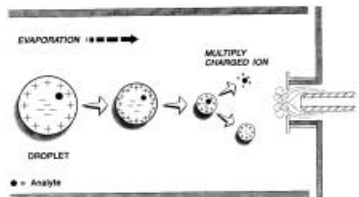


- API-Electrospray occurs at 760 torr
- Products = neutrals, ions, and clusters of ions

Ionization Mechanisms



- Coulomb Fission :
 - Assumes that the increased charge density, due to solvent evaporation, causes large droplets to divide into smaller droplets eventually leading to single ions.



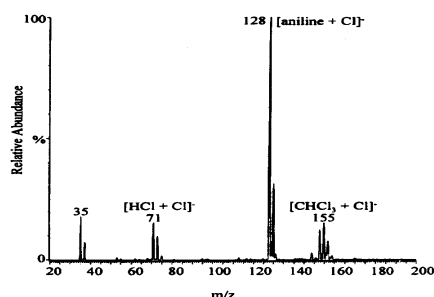
- Ion Evaporation:
 - Assumes the increased charge density that results from solvent evaporation causes Coulombic repulsion to overcome the liquid's surface tension, resulting in a release of ions from droplet surfaces

Charging the Analyte



• Charge Separation

- Gas-phase ions are formed when the droplets from the Taylor Cone evaporate and the ions carrying excess charge are released into the gas-phase.



• Adduct Formation

- Polar molecules that do not have acidic or basic groups can be charged through formation of adducts with various ions.
- Negative ion ESI: form adducts with Cl⁻ ions
- Positive ion ESI: form adducts with Na⁺, Li⁺, NH₄⁺, or other cationic species
- Problem: High [salt] causes background interference.

Charging the Analyte, Cont'd



• Gas-Phase Reactions:

- Ionized at atmospheric pressure.
- Generally through gas-phase proton transfer.
- Proton goes to species with higher gas-phase proton affinity
- Analyte must have higher proton affinity than the solvent.

• Electrochem Redox Rxns:

- “The continuous flow of charge from the metallic contact to the sample solution must occur via an electrochemical reaction at that contact.”
- Positive ion ESI: oxidation
- Negative ion ESI: reduction

Pneumatically-Assisted ESI

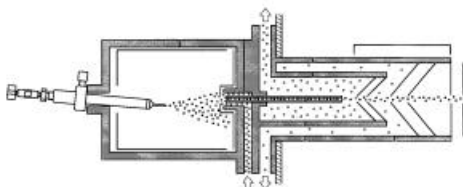


- Must separate ions from neutrals and establish complete desolvation.

- Use a neutral sheath gas
 - Aids in droplet formation and desolvation.

- Off-Axis Positioning

- Max amount of desolvated analyte
- Selects against un-evaporated droplets



Introducing Ions into the MS

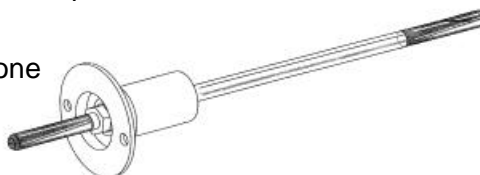


- Use a dry N₂ “curtain” gas:
 - Charged species penetrate the curtain because they are electrostatically attracted toward the orifice by an electric field gradient.
- Use a heated metal capillary interface
 - Aids in desolvation and declustering of ions from neutrals.

ESI Operating Conditions



- ESI operation depends on the ability to balance many variables simultaneously
 - Self-adjusted flow rate of sample
 - Self-adjusted voltage between power supply and contact with solution
- Capillary tube parameters:
 - Non-conductive: fused silica tubing
 - Conductive: metalized glass capillaries
 - Inner diameter = flow rate
 - Outer diameter = Taylor cone



Instrumental Parameters



- Stable and effective ESI spray conditions:
 - Taylor cone has constant shape
 - Constant stream of droplets from Taylor cone
 - Nebulizing gas and solution flow rates
 - Applied voltage, viscosity, and dielectric constant
 - Distance between spray capillary and counter electrode
- Methanol or pneumatic assistance is required
 - High water content means high surface tension
 - Higher voltages must be applied

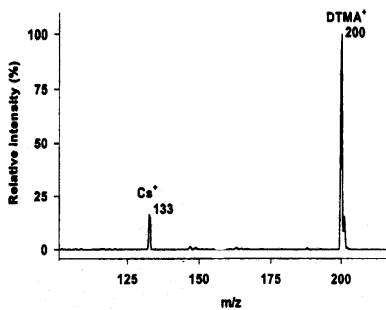


Analyte Characteristics

- Surface-active analytes have a higher response
 - They follow charge during fissioning process
- ESI response is directly related to:
 - Nonpolar surface area
 - ΔG transfer from NP to P solutions
 - Reverse-phase HPLC retention time
 - Difference between analyte pH and solvent pH
- If low response, change the molecular structure
 - Derivatation can make the analyte more easily charged or can increase the surface activity



Surface Activity



- Equimolar amounts and all other variables equal
 - Surface-inactive = Cs⁺
 - Surface-active = DTMA⁺
- For quantitative work, calibration curves, etc. must be employed
 - Intensity of peak is not solely related to conc.

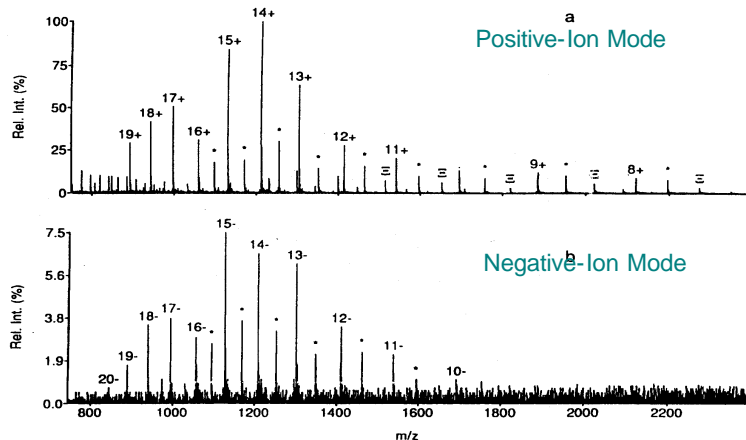


Solution Characteristics

- Variables that must be properly balanced:
 - Flow rate, applied voltage, conductivity, liquid surface tension
- Ideal solvent depends on application
 - Improves response and will not form clusters
- Positive ion mode: *50% MeOH or Acetonitrile*
- Negative ion mode: *Halogenated Solvents*



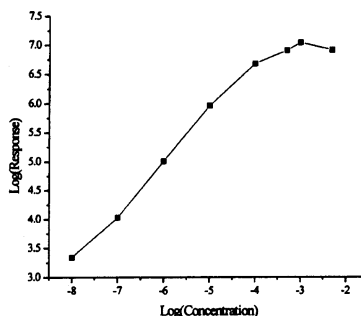
Typical ESI Spectrum



Ion Transmission and Sensitivity



- Methods to improve sensitivity:
 - Improving ion transfer through MS
 - Improve efficiency of charging ESI analytes
- The limiting factor in sensitivity with ESI is the transfer of ions through the MS.



Higher Sensitivity
is Better!!!!

Sources of Non-linearity



- **Background Interference:**
 - Chemical noise results from mass analysis of charged species other than the analyte
 - Heating droplets helps to drive off solvent and reduces interferences
- **Random Noise:**
 - Ubiquitous throughout MS spectrum
 - Possible Sources:
 - Ions reach detector without going through mass analysis
 - Passage of large, highly-charged clusters impacting the detector, creating a shower of fragments with various m/z ratios

Improving Detection Limits



- **To Lower Concentrations:**
 - Limited to concentrations above 10^{-8} M
 - Remove chemical noise if possible
 - Selected reaction monitoring
 - Increase in sensitivity
- **To Higher Concentrations:**
 - Limited to concentrations lower than 10^{-5} M
 - More efficient charging of gas-phase analyte
 - More effective evaporation techniques
 - When analyte concentration exceeds the excess charge, the ESI response should level off
 - Competition for a limited resource – Space and Excess Charge

ESI Coupled with LC or CE



- ESI is not a separation technique *per se*
- It can be used as a “detector” when coupled with separation techniques
- Separation protocols must be modified
 - Ideal LC/CE solvent may match ideal ESI solvent
 - Volatile buffers composed of weak acids and bases are used instead of non-volatiles which can cause signal suppression
 - Ion-pairing reagents must be eliminated



Summary

- Advantages
 - High mass samples
 - Non-volatile molecules
 - Large mass range (10^5 Da)
 - Low fragmentation
 - Determination of m.w.
 - Cheaper mass analyzers
 - Direct analysis of liquids
 - Compatible with various separation techniques
- Disadvantages
 - Limited transfer of ions through MS
 - Poor sensitivity
 - Low fragmentation
 - Sources of instability, interference, background, competition, and signal suppression are not well understood.
 - *Black Box!!!!*



References

- Cech, N, Enke C. 2001. Practical implications of some recent studies in electrospray ionization fundamentals. Mass Spectrometry Reviews 20:362-387.
- Hewlett Packard. 1994. API-electrospray LC/MS system. User's Guide.