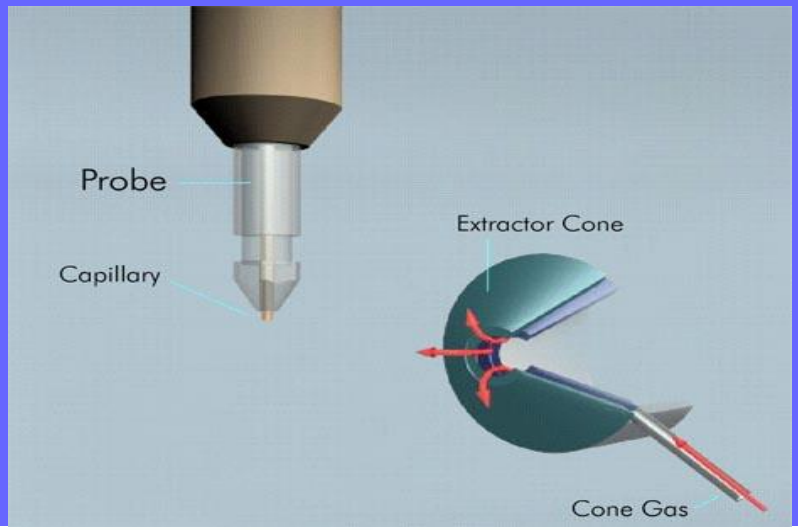
Chemistry 4631

Instrumental Analysis

Lecture: LC-MS



Mass Spectrometry (MS)

Most analytes separated by HPLC are thermally stable and non-volatile (liquids) (unlike in GC) – so not ionized easily by EI or CI techniques.

MS must be at 10^{-6} torr

Mass Spectrometry (MS)

Instrumentation

Detectors

Liquid Chromatography / Mass spectrometry

- Advantages:
- More definitive identifications
- Wide range of analytes can be studied
- Sensitivity (pg)

Mass Spectrometry (MS)

Instrumentation

Detectors

LC-MS

- **Problems for LC-MS combination:**
- **HPLC mobile phase – liquid w/ water or organics**
- **MS must be at 10^{-6} torr**
- **Most analytes separated by HPLC are thermally stable and non-volatile (unlike in GC) – so not ionized easily by EI or CI techniques**

Mass Spectrometry (MS)

Instrumentation

Detectors - LC-MS

- Ideal Interface:
- Has no reduction in chromatographic performance
- No chemical modifications
- High sample transfer
- Reliable and reproducible

Mass Spectrometry (MS)

Instrumentation

LC-MS

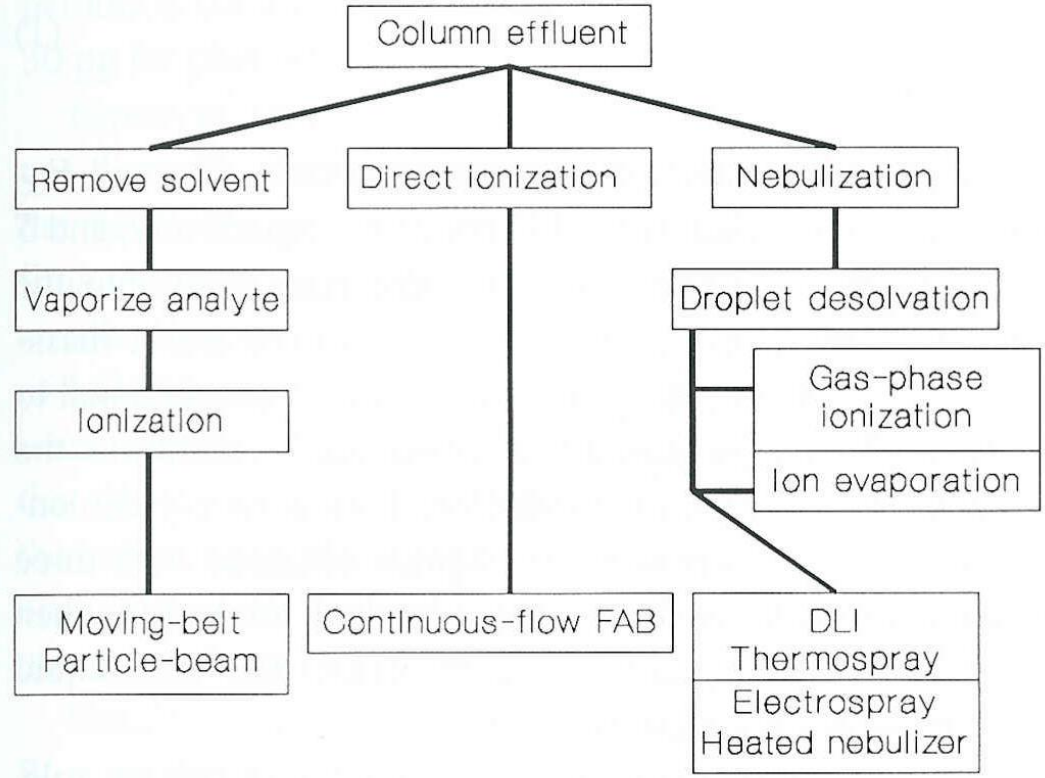


Fig. 1.5. Three general strategies to LC-MS interfacing.

Mass Spectrometry (MS)

Instrumentation

Ion source

- Moving-belt 1977
- Direct-liquid-introduction 1980
- Thermospray 1983
- Frit FAB/continuous-flow FAB 1985/1986
- Atmospheric-pressure chemical ionization 1986
- Particle-beam 1988
- Electrospray 1988

Mass Spectrometry (MS)

Thermospray 1983

- **The thermospray setup overcame many of the problems encountered with the moving-belt and direct-liquid-introduction interfaces and with the advent of this, LC–MS became a routine analytical tool in a large number of laboratories.**

- **It was also the first interface in which ionization is effected directly from solution within the interface itself, i.e. the mass spectrometer was not used to produce ions from the analyte simply to separate them according to their m/z ratios.**

Mass Spectrometry (MS)

Instrumentation

Ion source

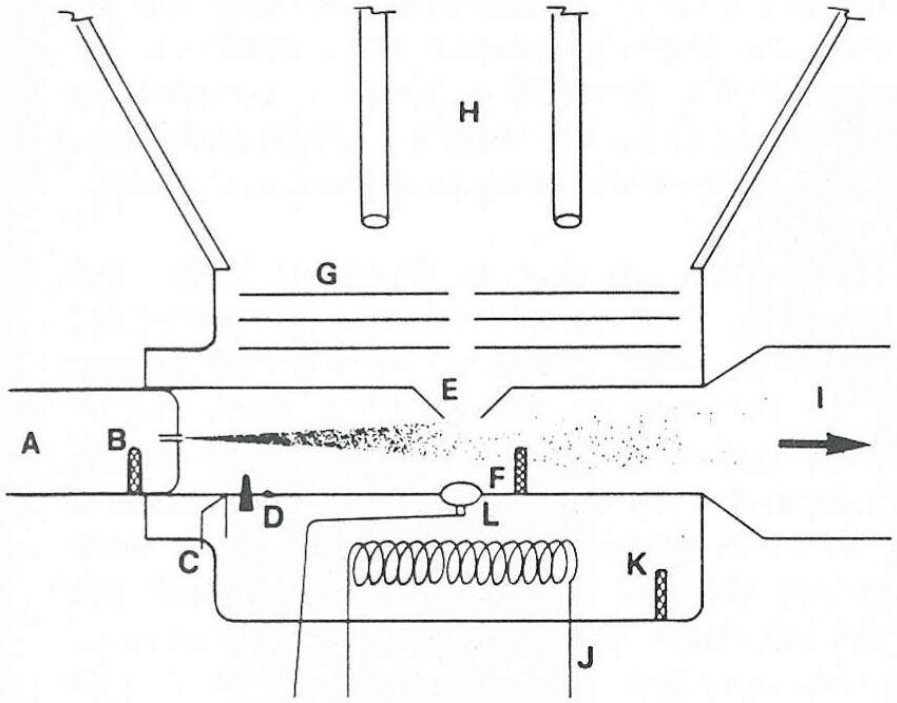
Thermospray

Ionization comprises the following four stages:

- the formation of droplets from the HPLC eluate
- charging of these droplets
- desolvation of the droplets
- the formation of ions from the analyte

Mass Spectrometry (MS)

Instrumentation



Schematic of Thermospray Interface

A Direct heated vaporizer
B Vaporizer thermocouple
C Filament*
D Discharge electrode*
E Ion exit cone
F Aerosol thermocouple
G Lenses
H Quadrupole assembly
I Liquid nitrogen trap and forepump
J Source block heater
K Source thermocouple
L Repeller

*Dependent on model and manufacture

Figure 4. Schematic of a thermospray LC/MS interface. [From reference 11]

Mass Spectrometry (MS)

Thermospray

A thermospray system consists of

- **a heated capillary through which the LC eluate flows, the temperature of this capillary is controlled to bring about around 95% vaporization of the liquid.**
- **the vapour produced acts as a nebulizing ‘gas’ and aids the break-up of the liquid stream into droplets.**
- **the droplets undergo desolvation as they traverse a heated region of the interface and ions are formed from analytes contained in the liquid stream by means of ion–molecule reactions.**

Mass Spectrometry (MS)

Thermospray

A thermospray system consists of

- **the ions formed are then directed though a sampling cone at 90◦ to the direction of vapour flow.**

- **downstream of the end of the capillary is usually a filament and/or a discharge electrode, which provided secondary methods of ionization, while opposite or slightly downstream of the sampling cone is a repeller or retarding electrode.**

Mass Spectrometry (MS)

Thermospray – Disadvantages

- The presence of a buffer is usually needed so must be present in the HPLC mobile phase.
- Decomposition of some thermally labile analytes occurs.
- Thermospray is not suitable for high-molecular-weight (>1000 Da) analytes.
- The reproducibility of analytical results is affected by a number of experimental parameters and is sometimes difficult to control.
- The formation of adducts may confuse the assignment of molecular weight.

Mass Spectrometry (MS)

Thermospray – Disadvantages

- **Usually little structural information is immediately available and repeller induced fragmentation or MS–MS is required.**

- **Thermospray generates a significant amount of solvent-associated chemical noise at low mass which makes this region unusable for analytical purposes.**

Since the ions generated from the analyte are molecular species, this is not usually a problem but must be considered when spectra are being interpreted.

Mass Spectrometry (MS)

Thermospray – Advantages

- It is easier to use than other available alternatives.
- Can operate under a wide range of HPLC conditions, regular HPLC flow rates and mobile phases containing high percentages of water.
- Can allow unequivocal determination of molecular weight as thermospray spectra usually contain ions simply from molecular species with little fragmentation being observed.

Mass Spectrometry (MS)

Thermospray – Advantages

- The addition of a discharge electrode and a filament to the thermospray source widens the range of compounds that may be studied and HPLC solvents that may be accommodated.
- Since the sample is ionized directly from solution it is protected from heat and many thermally labile analytes may be studied with little or no degradation.
- The sensitivity is compound-dependent but generally high sensitivity is possible by using one of the ionization methods available (thermospray, filament and discharge).

Mass Spectrometry (MS)

Instrumentation

Ion source

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- Particle-beam 1988
- Electrospray 1988

Mass Spectrometry (MS)

Instrumentation

Atmospheric-pressure chemical ionization 1986

- Although work demonstrating APCI was published in parallel with that demonstrating ESI, APCI was not widely adopted until ESI was commercialized, which occurred in the wake of Fenn's work.

Mass Spectrometry (MS)

Instrumentation

- Atmospheric-pressure chemical ionization (APCI) is another of the techniques in which the stream of liquid is dispersed into small droplets, in this case by the combination of heat and a nebulizing gas.
- As such, APCI shares many common features with thermospray.
- The differences between the techniques are the methods used for droplet generation and the mechanism of subsequent ion formation.

Mass Spectrometry (MS)

Atmospheric-pressure chemical ionization

- 1st generation

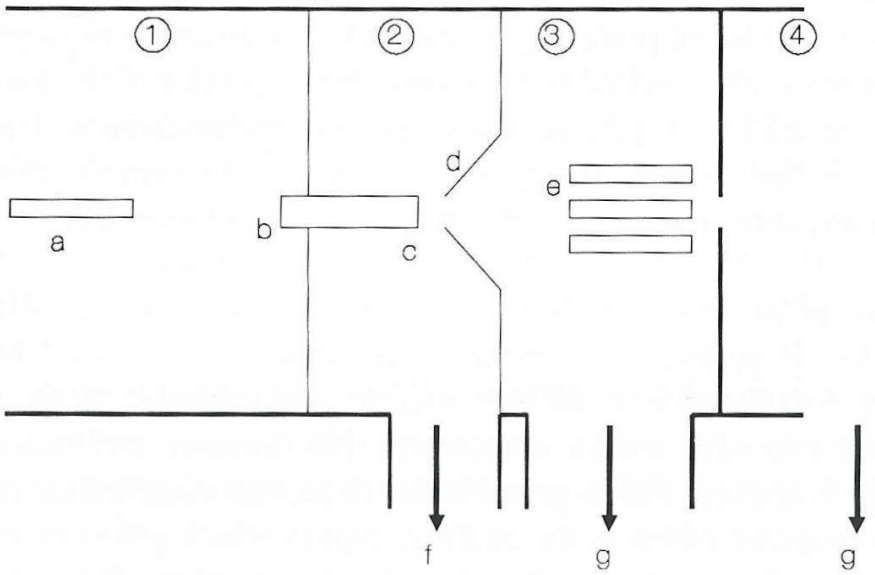

Fig. 1.15. Schematic drawing of the atmospheric-pressure ion source as a molecular beam apparatus. (1) Atmospheric-pressure ionization source region, (2) first pumping stage, (3) second pumping region, and (4) mass analyzer region. (a) Transfer tube from LC (either electrospray needle assembly or APCI heated nebulizer), (b) sampling orifice, (c) nozzle, (d) skimmer, (e) quadrupole, hexapole, or octapole ion collection and focusing device, (f) high through-put mechanical pump, and (g) turbomolecular pumps.

Mass Spectrometry (MS)

Atmospheric-pressure chemical ionization

- The liquid is sprayed through a heated (250-400 ^{o}C) vaporizer at atmospheric pressure.
- The heat vaporizes the liquid, and the solvent molecules are ionized by electrons discharged from a corona needle.
- The solvent ions transfer charge to the analyte molecules through chemical reactions (chemical ionization).
- The analyte ions then pass through a capillary sampling orifice into the mass analyzer.

Mass Spectrometry (MS)

Atmospheric-pressure chemical ionization

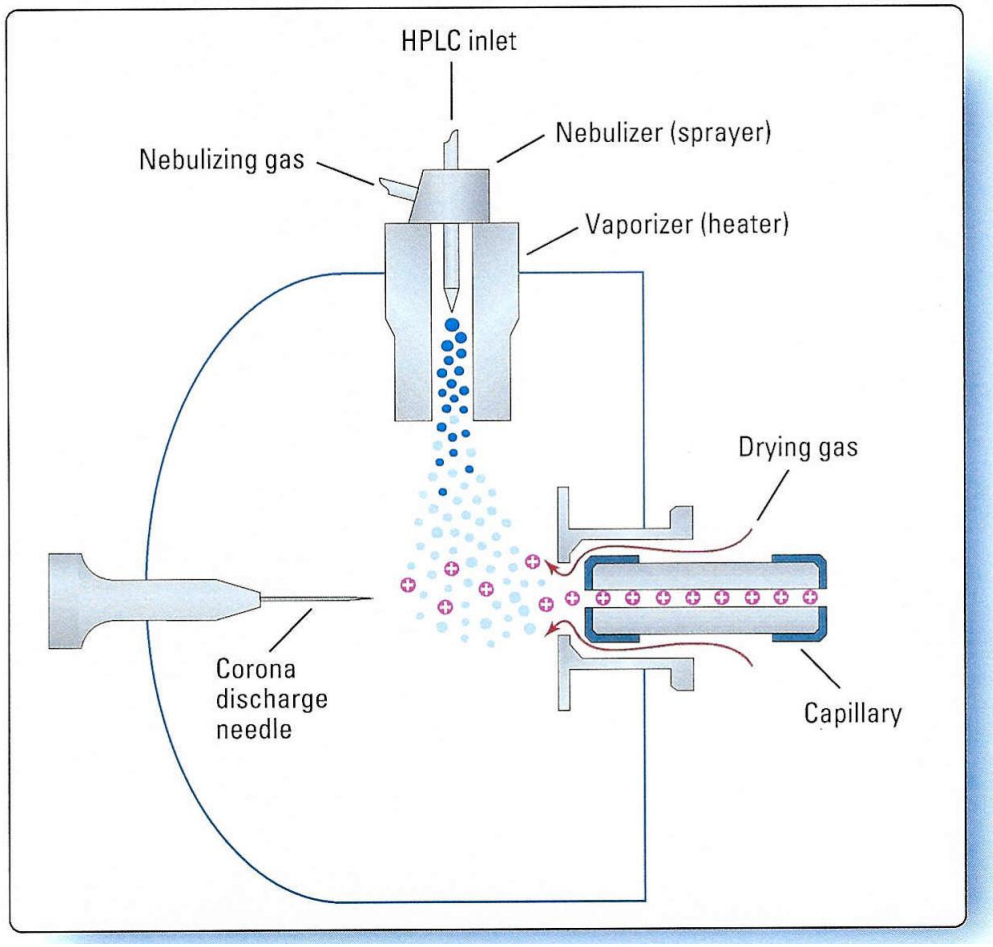

Figure 6. APCI ion source

Mass Spectrometry (MS)

APCI - Disadvantages

- APCI spectra can contain ions from adducts of the analyte with the HPLC mobile phase or organic modifiers, such as ammonium acetate, that may be present. The presence of ions such as $(M + NH_4)+$ and $(M + CH_3COO)-$ may hinder interpretation of the spectra obtained.
- Structural information is not usually available unless cone-voltage fragmentation or MS–MS is used.
- APCI is not able to function effectively at very low flow rates.
- APCI is not suitable for analytes that are charged in solution.

Mass Spectrometry (MS)

APCI – Advantages

- APCI produces ions from solution and compounds with a degree of thermal instability may be studied without their decomposition.
- APCI is best applied to compounds with low to moderately high polarities.
- APCI is a soft ionization technique which usually enables the molecular weight of the analyte under study to be determined.
- APCI is able to deal with flow rates up to 2 $mlmin^{-1}$ and is, consequently, directly compatible with 4.6 mm HPLC columns.
- APCI is more tolerant to the presence of buffers in the mobile phase stream than is ESI.
- APCI is more tolerant to changes in experimental conditions than ESI, including gradient elution.

Mass Spectrometry (MS)

Instrumentation

Ion source

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- Electrospray 1988

Mass Spectrometry (MS)

Instrumentation

Electrospray 1988

- A liquid, in which the analyte(s) of interest have been dissolved, is passed through a capillary (typically stainless steel), at atmospheric pressure, maintained at high voltage (3 to 4 kV).

- The liquid stream breaks up with the formation of highly charged droplets which are desolvated as they pass through the atmospheric-pressure region of the source towards a counter electrode.

Mass Spectrometry (MS)

Instrumentation

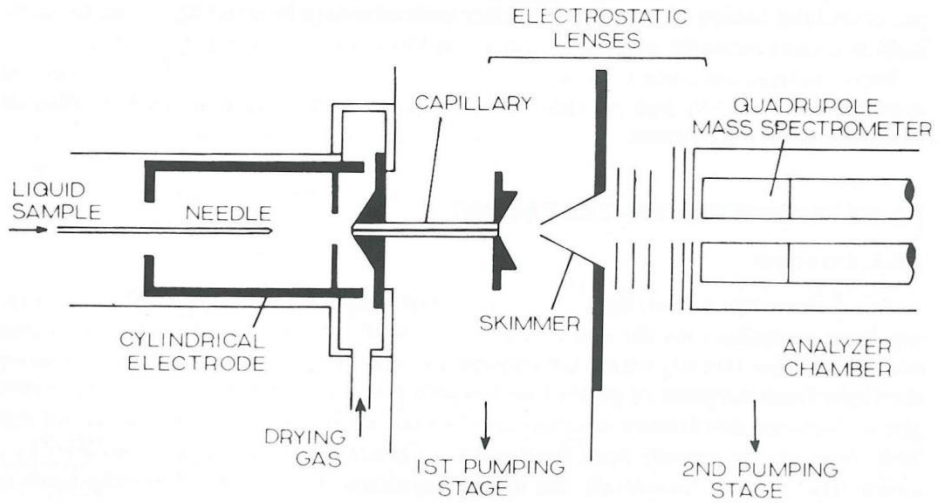
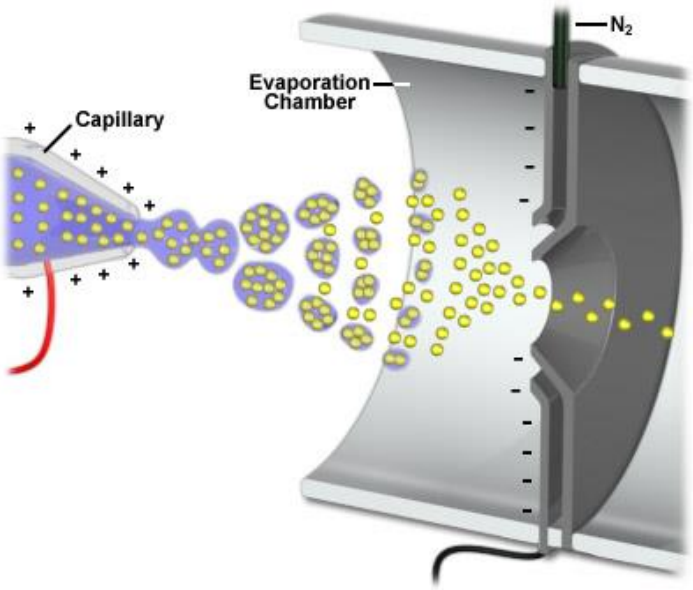


Fig. 1.13. Schematic diagram of the first-generation electrospray LC-MS interface as described by Whitehouse et al. [216]. Reproduced from Ref. [216] with permission. © 1985, American Chemical Society.



Mass Spectrometry (MS)

Instrumentation

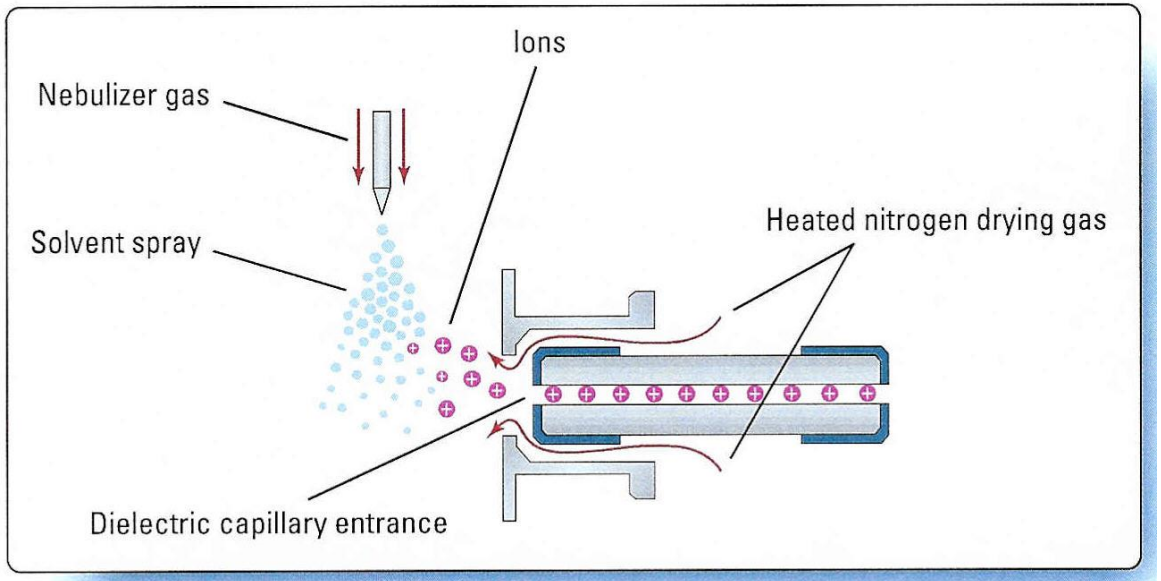


Figure 4. Electrospray ion source

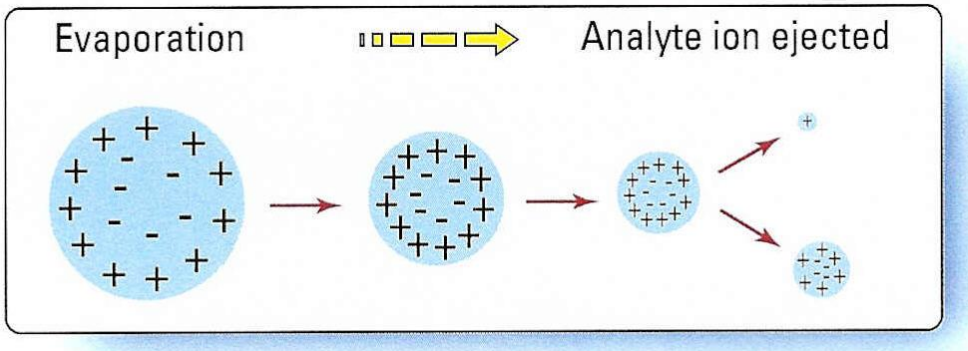


Figure 5. Desorption of ions from solution

Mass Spectrometry (MS)

Electrospray

- **Desolvation is assisted by a stream of a drying gas, usually nitrogen, being continually passed into the spraying region.**
- **As the droplets shrink, the charge concentration in the droplets increases. The repulsive force between ions with like charges exceeds the cohesive forces and ions are ejected (desorbed) into the gas phase.**
- **Analyte ions are obtained from these droplets which then pass through two differentially pumped regions into the source of the mass spectrometer.**

Mass Spectrometry (MS)

Electrospray

- **Since ionization takes place directly from solution, thermally labile molecules may be ionized without degradation.**
- **In contrast to most other ionization methods, the majority of ions produced by electrospray are multiply charged.**
- **Electrospray is useful for large biomolecules such as proteins, peptides, etc… while still able to analyze smaller molecules.**

Mass Spectrometry (MS)

Electrospray

In positive ionization mode:

a trace of formic acid is often added to aid protonation of the sample molecules.

In negative ionization mode:

a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules.

Mass Spectrometry (MS)

Electrospray – Disadvantages

- **Electrospray is not applicable to non-polar or low-polarity compounds.**
- **The mass spectrum produced from an analyte depends upon a number of factors and spectra obtained using different experimental conditions may therefore differ considerably in appearance.**
- **Suppression effects may be observed and the direct analysis of mixtures is not always possible. This has potential implications for co-eluting analytes in LC–MS.**

Mass Spectrometry (MS)

Electrospray – Disadvantages

- **Electrospray is a soft-ionization method producing intact molecular species and structural information is not usually available.**

- **Electrospray sources are capable of producing structural information from cone-voltage fragmentation but these spectra are not always easily interpretable. Experimentally, the best solution is to use a mass spectrometer capable of MS–MS operation but this has financial implications.**

Mass Spectrometry (MS)

Electrospray – Advantages

- **Ionization occurs directly from solution and consequently allows ionic and thermally labile compounds to be studied.**

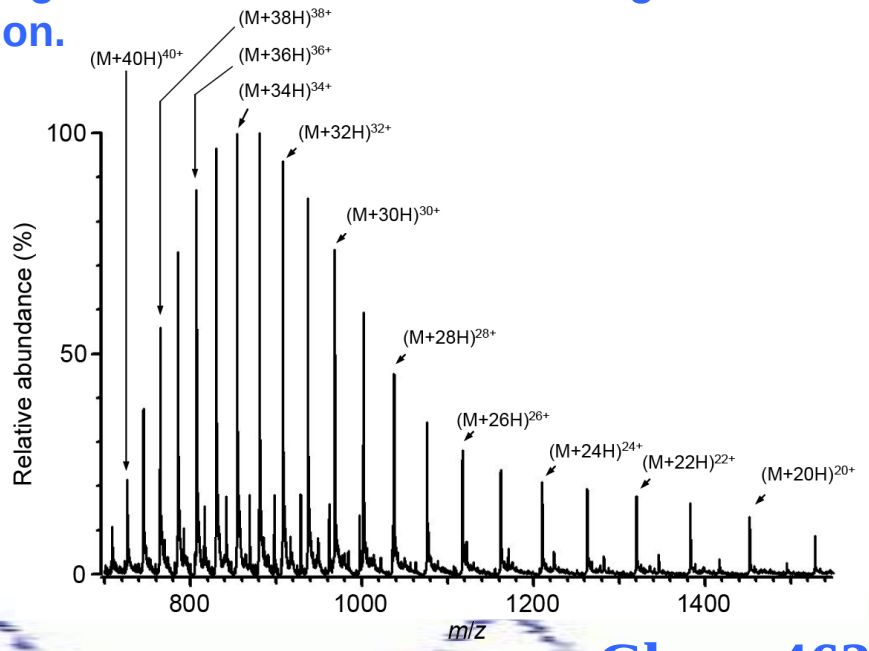
- **Mobile phase flow rates from nl min^{-1} to in excess of 1 ml min^{-1} can be used with appropriate hardware, thus allowing conventional and microbore columns to be employed.**

Mass Spectrometry (MS)

Electrospray – Advantages

- Electrospray ionization, in contrast to the majority of other ionization methods, produces predominantly multiply charged ions of the intact solute molecule. This effectively extends the mass range of the mass spectrometer and allows the study of molecules with molecular weights well outside its normal range.

- For high-molecular-weight materials, an electrospray spectrum provides a number of independent molecular weight determinations from a single spectrum and thus increased precision.



Mass Spectrometry (MS)

Ionization chemistry

For electrospray, formation of analyte ions in solution is essential to achieving good results.

Techniques to help ion formation include:

- **select more volatile buffers to reduce the buildup of salts in the ion source**
- **adjust solvent pH according to the polarity of ions desired and the pH of the sample**
- **use solvents that have low heats of vaporization and low surface tensions to enhance ion desorption**
- **make sure that gas-phase reactions do not neutralize ions through proton transfer or ion pair reactions**

Mass Spectrometry (MS)

Nanospray ionization - A low flow rate version of electrospray.

- **The flow rate of solute and solvent using this procedure is very low, 30 - 1000 nL/min, and so far less sample is consumed than with the standard electrospray ionization technique.**

- **A common application of this technique is for a protein digest mixture to be analyzed to generate a list of molecular masses for the components present, and then each component to be analyzed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques**