1

GC Instruments

- Fairly simple instrumentation

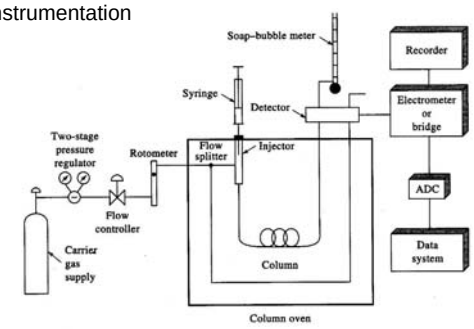


Figure 27-1 Schematic of a gas chromatograph.

- Maintaining constant average pressure is important!
  - Pressure controls flow rate
  - T influences retention (k')
  - Flow rate monitoring
  - Changing flow rate changes chromatogram (B/u)
    - Sometimes use Retention Volume (V_g)

2

GC Instruments - Columns

- Two general classes: Packed and Open Tubular
  - three components: column, support, coating
- **Packed Columns**
  - Column materials: glass, metal (stainless), Teflon
    - few meters in length
    - few mm in diameter (i.d.)
  - Support Materials:
    - small particles, uniform (spherical) shape, porous, inert
      - Typically 100-300 μm diameter
    - Most common: diatomaceous earth
      - also polymeric materials
- **Capillary (Open Tubular) Columns**
  - Column materials
    - Small diameter (typically <500 μm)
    - metal, plastic, glass (FRAGILE)
    - More recently: Fused Silica - robust, flexible
  - Support methods:
    - Support-Coated OT:
    - Wall-Coated OT

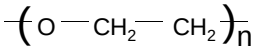
3

GC Instruments – Columns

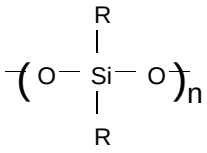
Stationary Phase Materials

- **Gas-Liquid Chromatography (GLC)**
- Characteristics:
  - appropriate chemical nature ("like dissolves like")
  - low volatility
  - thermal stability
  - chemical inertness

- Typical coatings (< 1 to several μm thickness):
  - Polyethylene Glycol (PEG, Carbowax)



  - Poly(dialkyl)silane:



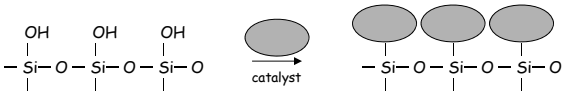
  - Vary R groups to vary retention characteristics

4

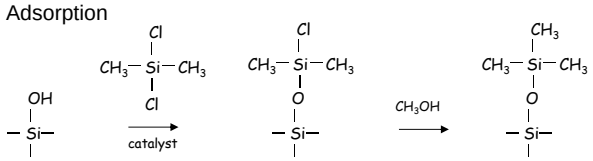
GC Instruments - Columns

- Lifetime of column is limited by:
  - adhesion of liquid coating to stationary phase (bleeding)
  - irreversible adsorption of contaminants to column

- Minimizing bleeding
  - Cross-linking: on-column reaction
  - "Bonding": Utilize surface chemistry of column (or packing)



- Minimizing Adsorption

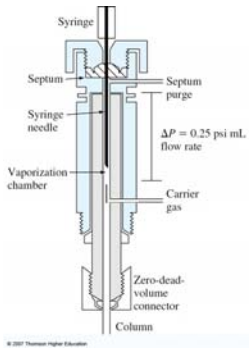


- **Gas-solid Chromatography (GSC):**
  - Typically porous solid adsorbed to walls (OT) or a porous solid support
    - molecular sieves
    - Porous polymers (beads or coatings)

GC Instruments - Sample Introduction

- Want small plug
  - Can doom the separation from the start!
- Most common: Direct injection (microflash vaporizer)
  - Inject and vaporize simultaneously
    - Need appropriate temperature
  - Ideally low dead volume
  - Fairly low maintenance
- More precise: Injection Loop
- Headspace and Purge-and-Trap methods
- SPME

http://people.whitman.edu/~dunnivfm/C_MS_Ebook/CH2/Figures/Fig_2_3_SPME.jpg



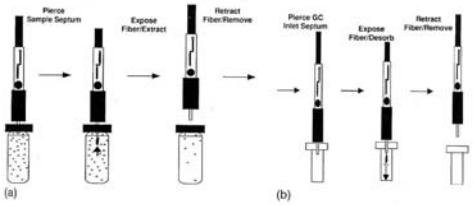


Figure 12.1 Diagrams of SPME (a) extraction sampling, and (b) GC despoption injection. (Used with permission from Supelco, Inc.)

GC Instruments - Sample Introduction

- Split/Splitless Injection
- Most common for capillary columns
  - Avoids overloading
- Split ratio is controllable by adjusting carrier gas flow through split vent.

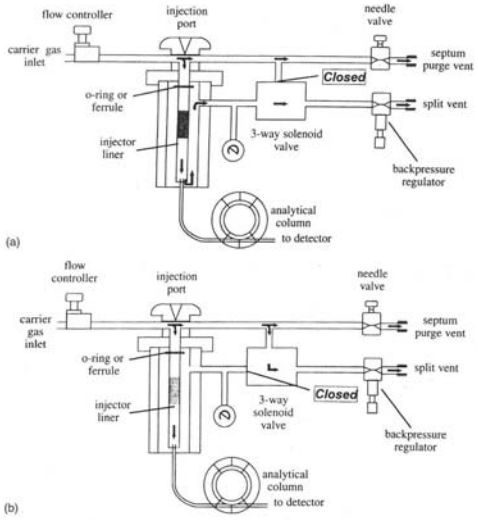


Figure 12.2 Diagram of GC flows for split and splitless injections: (a) split mode; (b) splitless mode. (Adapted with permission of Restek Inc.)

7

GC Instruments - Detectors

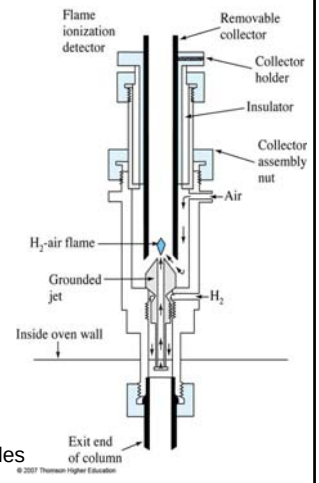
- Characteristics of a “good” detector
  - Sensitivity appropriate to sample
  - Large linear dynamic range
  - Useful at a range of temperatures
  - Rapid response time
  - Easy to use (idiot proof?)
  - Stable, Predictable response
  - Nondestructive (probably least important)

8

GC Instruments – Detectors

Flame Ionization Detector (FID)

- Column effluent is passed through a H_2-Air flame
  - Produces ions and electrons
- Charged particles are accelerated by voltage applied between jet and collector
  - results in current (pA)
- Number of ions depends on number of reduced (methylene) carbons in molecule
  - one molecule of ethane gives twice the signal of one molecule of methane
  - less sensitive for non-hydrocarbon groups
  - insensitive to H_2O, CO_2, SO_2 and other noncombustibles
- High sensitivity, good LDR (10^7) , low noise, destructive



GC Instruments - Detectors

Thermal Conductivity Detector (TCD):

- Element is electrically heated at constant power
  - Temperature depends on thermal conductivity of surrounding gas
- Measure conductivity (resistance) with respect to a "reference"
- Hydrogen and helium carrier gas provide best sensitivity
  - most thermally conductive
  - Organics are less so
  - when analyte comes off, filament temperature goes up, resistance goes down
- Poorer sensitivity than FID, but more universal
- Large LDR (10^5), non-destructive

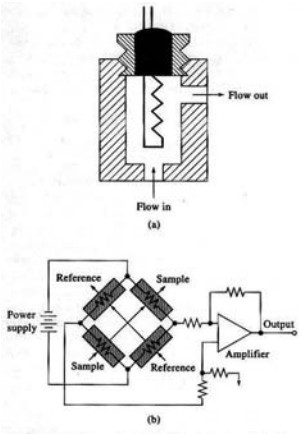


Figure 27-7 Schematic of (a) a thermal conductivity detector cell, and (b) an arrangement of two sample detector cells and two reference detector cells. *(From J. V. Hinshaw, LC-GC, 1990, 8, 298. With permission.)*

GC Instruments – Detectors

Electron Capture Detector (ECD):

- Carrier gas (and analyte) passes over β-emitter, resulting in ionization and e^- production
- Produces current between electrodes
- In the presence of other compounds (especially halogens, etc.) electrons are captured, causing decrease in current

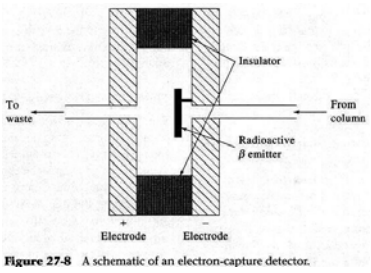


Figure 27-8 A schematic of an electron-capture detector.

- Most commonly used for halogenated organics (insecticides, etc.), small LDR (10^2)

Other Detectors:

- Atomic Emission
  - Microwave induced plasma, grating monochromator, diode array detector
- Mass Spectrometry Detection
- Thermionic Detector
  - Sensitive to phosphorous and nitrogen

LC Techniques

- LC covers a wide range of analytes and interactions
  - Very similar instrument components
  - All have liquid mobile phase
    - solution of analyte in a solvent
    - equilibrium occurs between solvent in mobile phase and solid stationary phase

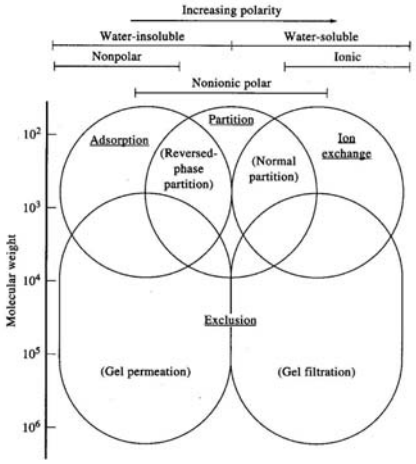


Figure 28-1 Applications of liquid chromatography. *(From D. L. Saunders, in* Chromatography, *3rd ed., E. Heftmann, Ed., p. 81. New York: Van Nostrand Reinhold, 1975. With permission.)*

LC Instruments

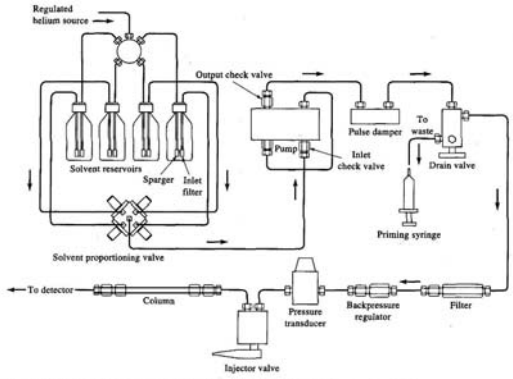


Figure 28-4 Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

- Solvent purification - Filtering/Degassing
- Complexity of pumping system depends on sample requirements
  - Isocratic elution
  - Gradient Elution

13

LC Columns

- Generally stainless steel a few mm in diameter and 10-30 cm long
- Packed with two types of stationary phase support
  - Pellicular particles
  - Porous particles
- Column life is extended with the use of guard columns
  - sacrificial
  - packed similarly to analytical column

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Bandshapes in LC

- Subject to the same general broadening considerations as any chromatography technique
  - Multipaths
  - Longitudinal
  - Mass Transfer
- Particle size plays a major role
- Extracolumn Broadening
- Sample Size Effects

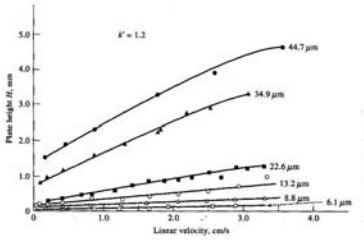
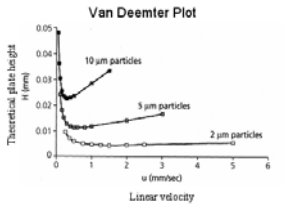


Figure 28-2 Effect of particle size of packing and flow rate upon plate height H in liquid chromatography. Column dimensions: 30 cm × 2.4 mm. Solute: N,N-diethyl-*m*-aminoazobenzene. Mobile phase: mixture of hexane, methylene chloride, isopropyl alcohol. *(From R. E. Majors, J. Chromatogr. Sci., 1973, 11, 92. With permission.)*

"New" Column Options

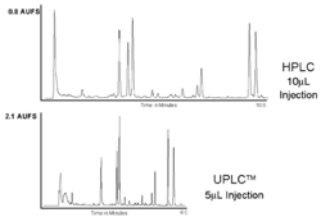
- UPLC: Ultra Performance Liquid Chromatography

- Particle size impacts separation efficiency
  - Surface area and broadening considerations
  - Generally, as flow rate increases, efficiency becomes poorer
    - Less of an impact as particles get smaller



http://hitachi-hta.com/sites/default/files/icu-van_deemter_plog_0.jpg

- Smaller particles have several advantages…and challenges
  + Better separations with shorter columns
  + Potential for shorter separation times
  + Smaller sample and solvent needs
  - Higher pressures (can be ~15,000 psi!)
  - Less sample to detect
  - High demands on particle fabrication



http://www.americanlaboratory.com/914-Application-Notes/19157-New-Frontiers-in-Chromatography/

Separation Schemes: Combinations of Mobile Phase/Analyte/Stationary Phase

Partition Chromatography

- Separation results from intermolecular interactions between analyte and mobile/stationary phase
  - Hydrophobic, dipole-dipole, H-bonding, ionic…

- Typically use bonded-phase packing
  - Derivatized silica
  - Hydrolyze silica in HCl
  - React silanol groups with derivatizing agents
  - Remove unreacted silanol by endcapping
    - prevents unwanted adsorption/interaction

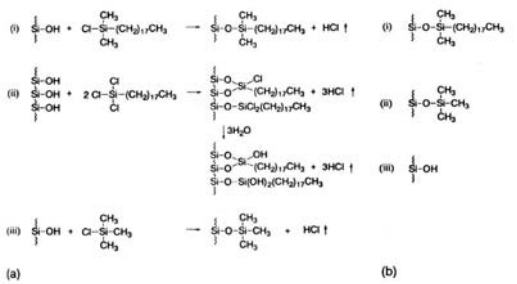
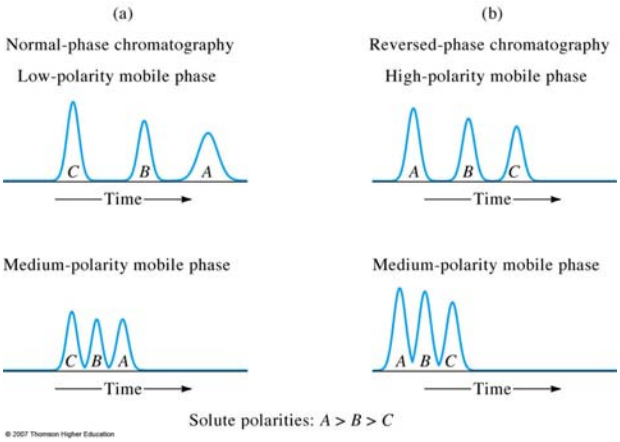


Figure 13.1 Types of ODS-silica HPLC stationary phases: (a) i, synthesis of monomeric C18; ii, synthesis of polymeric C18; iii, endcapping process; and (b) i, monomeric C18 ligand; ii, encapped silanol; and iii, residual silanol. (Cazes, used with permission.)

Separation Schemes

Normal Phase versus Reverse Phase

- Controls elution order and (in part) separation quality
- Normal Phase: *Polar* stationary phase, *less polar* mobile phase
- Reverse Phase: *Nonpolar* stationary phase, *more polar* mobile phase



Separation Schemes

- Most modern separations are done using reverse-phase column
  - spherical particles coated with alkane chains
    - varying chain lengths changes polarity
    - Behaves much like a "liquid" hydrocarbon coating

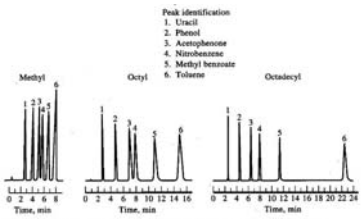


Figure 28-15 Effect of chain length on performance of reversed-phase siloxane columns packed with 5-μm particles. Mobile phase: 50/50 methanol/water. Flow rate: 1.0 mL/min.

- Possible to manipulate the quality of the separation (N, k', α) by changing solvent composition OR by modifying analyte characteristics
  - Derivatization
    - change polarity
    - change sensitivity
    - change selectivity
  - Ion-Pairing
    - Ion-pair is retained on column
    - Anions - Tetraalkylammonium salts
- Chiral Separations: "Chiral" stationary phase

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Separation Schemes

Adsorption Chromatography:

- Direct interaction between analyte and solid stationary phase (silica, alumina)
- Normal phase-like separation
  - Nonpolar mobile phase

Size-Exclusion Chromatography:

- Separation is a result of "trapping" of molecules in the pores of the packing material
  - Very large molecules can't get into the pores - unretained
  - Very small molecules get hung up in to pores for a long time - most retained - longest retention time
- Separation is based exclusively on size (shape)
  - No physical interaction occurs (ideally)
  - Use "inert" stationary phases
    - silica, polymer beads
- Pore size determines range of analytes that can be separated
  - If two different analytes are too large to fit in the pores, they will co-elute
    - *Exclusion limit*
  - If two analytes are small enough to freely move into the pores, they will also co-elute
    - *Permeation limit*
- Result is rapid separation, long column life, but need range of sizes (molecular weights)

20

Separation Schemes

Ion Exchange Chromatography:

- Use ionic stationary phase
  - ions separated on the basis of their tendency to displace counterions adsorbed on stationary phase
    - Depends on charge, hydration, "solubility"…
- Anionic sulfonated styrene/divinylbenzene stationary phases: Typically H^+ is counterion
  - used for cation separation
- Cationic stationary phases are usually quaternary amines

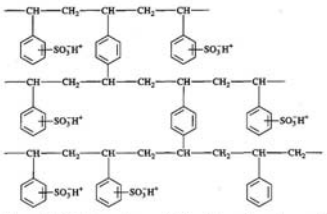


Figure 28-21 Structure of a cross-linked polystyrene ion-exchange resin. Similar resins are used in which the $-SO_3^-H^+$ group is replaced by $-COO^-H^+$, $-NH_3^+OH^-$, and $-N(CH_3)_3^+OH^-$ groups.

Detection in ion chromatography

- **Conductivity** seems like a good idea!
  - BUT large background
  - Minimize background by using suppressor column
    - post-separation process
    - Converts eluent (not analyte) ions into neutrals
      - typically an acid-base reaction…results in low background conductivity
    - Cation Separations: $H^+ + Cl^- + Resin^+OH^- \rightarrow Resin^+Cl^- + H_2O$
    - Anion Separation: $HCO_3^- + Na^+ + Resin^-H^+ \rightarrow Resin^-Na^+ + H_2CO_3$
- Other modes work, too
  - Photometric
    - Direct or Indirect

LC Pumps

- Pump Requirements:
  - High pressure
  - Pulse Free
  - Variable flow rates
    - ~0.1 to 10 mL/min
  - Reproducible flow rates
  - Stable components
- Most common: Reciprocating Pump
  - Pulsed, but high pressure capability

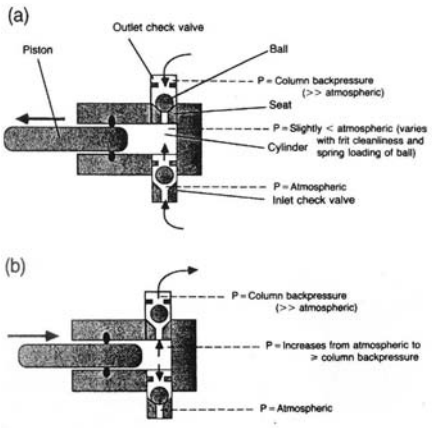
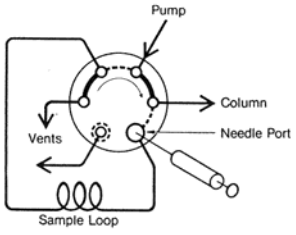


Figure 13.5 Operation of piston and check valve reciprocating pump: (a) suction stroke and (b) exhaust stroke. (Katz et al., used with permission.)

- Others:
  - Syringe (displacement) pumps
    - (+) Pulse free, (-) low volume, (-) low pressure, (-) single solvent
  - Pneumatic Pumps
    - (+) Pulse free, (-) no gradient capability, (-) low pressure

Sample Introduction

- Injection Loop…Autosampler
  - Reproducibility!



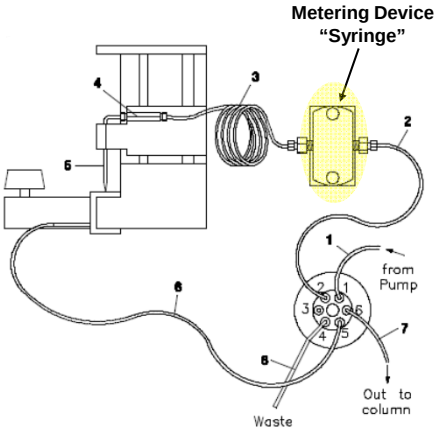


Figure 13.6 (a) Diagram of operation and (b) photo of two-position rotary injection valve. (Katz et al., used with permission.)

HP 1050 Autosampler

LC Detectors

- Same demands as GC detectors
  - sensitivity, universally applicable, etc.
- No (truly) universal detectors exist!
  - Bulk Property vs Solute Property:

Absorbance Detectors

- Small volume cells
  - but high concentration!
- Why Z-shaped?
- Often double-beam
- D_2 or filament sources
- Filter or monochromator-based
- Single or multi-channel detectors
- UV-Vis most common, but IR is also used

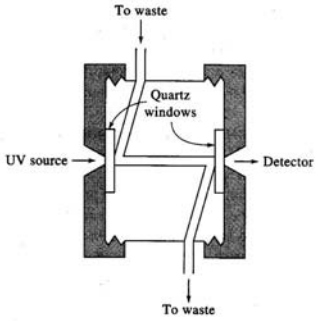


Figure 28-9 Ultraviolet detector cell for HPLC.

LC Detectors

Fluorescence Detectors

- Similar optics, but collect light at 90° to excitation.
- Not universally applicable directly, but can be "tweaked"
  - Chemical Derivatization
    - Pre- vs. Post- column derivatization.
  - Indirect Detection

Electrochemical Detectors

- Most common type is amperometric detector
  - Potential is applied to working electrode to drive redox process
  - Measured current is related to concentration
- Applicable to a wide range of compounds and organic functional groups
- Sensitive, simple, cheap?
- Susceptible to electrode fouling

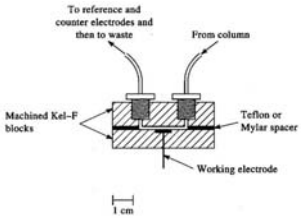


Figure 28-13 Amperometric thin-layer detector cell for HPLC.

LC Detectors

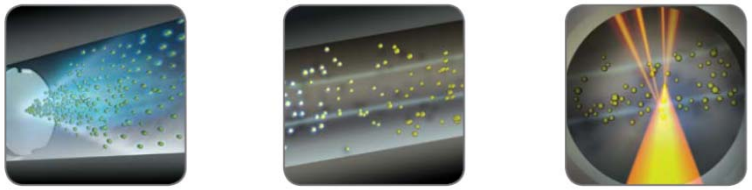
Refractive Index Detectors

- Monitors refractive index of the solution as it exits the column
- Anything that changes the refractive index (like an analyte) will result in a peak
- More universal than most LC detectors (bulk property detector)
- BUT not as sensitive, highly susceptible to temperature fluctuation

Evaporative Light Scattering Detectors

- As sample leaves column, it is nebulized and solvent evaporated to produce small particles of sample.
- Laser light is scattered off the particles.
  - More analyte → More particles → More scatter
- Fairly universal and more sensitive than RI!

Charged Aerosol Detection?!?

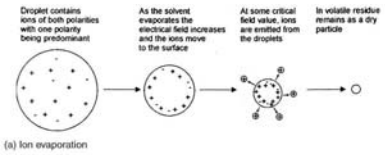


elsd.com

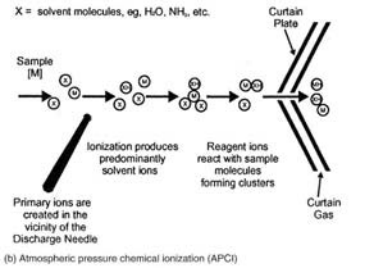
LC Detectors

Mass Spectrometric Detectors:

- Interfacing challenges



(a) Ion evaporation



(b) Atmospheric pressure chemical ionization (APCI)

Figure 13.15 LC-MS interface ionization mechanism diagrams. (a) coulomb explosion in ESI droplets and (b) reactions leading to molecular ion in APCI. (Adapted with permission from Applied Biosystems/MDS Sciex.)

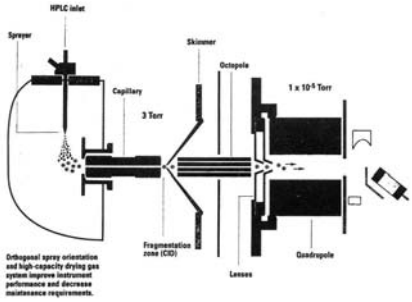


Figure 13.16 Diagram of orthogonal electrospray LC-MS interface. (Adapted with permission from Agilent Inc.)