Marine Organic Geochemistry

Analytical Methods – I.

Extraction and separation methods.

Reading list

Selected Reading (Sample processing and lipid analysis)

- Bidigare R.R. (1991) Analysis of chlorophylls and carotenoids. In *Marine Particles: Analysis and Characterization*. Geophysical Monograph 63. 119-123.
- Wakeham S.G. and Volkman J.K. (1991) Sampling and analysis of lipids in marine particulate matter. In *Marine Particles: Analysis and Characterization*. Geophysical Monograph 63. 171-179.
- Nordback J., Lundberg E. and Christie W.W. (1998) Separation of lipid classes from marine particulate matter by HPLC on a polyvinyl alcohol-bonded stationary phase using dual-channel evaporative light scattering detection. *Mar. Chem*. 60, 165-175.

Background Reading

- Jennings W. (1978) *Gas Chromatography with Glass Capillary Columns*. Academic press
- Snyder L.R., Kirkland J.J., Glajch J.L. (1997) *Practical HPLC method development*. 2nd Edition. Wiley.
- Christie W.W. (1982) *Lipid Analysis*. 2nd Edition. Pergamon.
- Ikan R. and Cramer B. (1987) Organic chemistry: Compound detection. *Encyclopedia of Physical Science and Technology* **10**, 43-69

Practical considerations regarding sample storage, preparation, and safety (an organic geochemical perspective)

Sample storage

- Many organic compounds are biologically or chemically labile.
- Susceptibility to:
  - Enzymatic hydrolysis (e.g., DNA, Phospholipid Fatty Acids - PLFA)
  - Photolysis (e.g., certain PAH - anthracene).
  - Oxidation (e.g. polyunsaturated lipids)
- Ideally, samples should be kept frozen, in the dark and under N_2.
  - < -80°C : minimal enzymatic activity - optimal.
  - < -30°C: a good compromise.
  - 4°C (refridgeration): OK for short periods of time
  - room temp – ok once sample has been dried (for many compounds).
  - **total lipid extracts are more stable than purified compounds/fractions*

Sample preparation

- Drying of samples will deactivate enzymes, but can lead to oxidation of selected compounds.

Options:

- air-dry (40°C): OK for non-volatile, chemically inert compounds (*may promote microbial activity*)
- freeze-dry under vacuum: good for semi-volatile compounds
- extract wet: good for thermally labile, and highly polar compounds

Practical considerations

Potential Contamination Sources

Materials compatible with organic geochemical analysis:

- Glass
- Teflon
- Aluminum foil (combusted, UV irradiated, or solvent-rinsed)
- Stainless steel

Materials to avoid (contaminants):

- Parafilm (hydrocarbons)
- Polystyrene (dissolves in many common solvents)
- Plasticizers (phthalates) (e.g., Tygon tubing)
- Silicone grease (silicones)
- Finger grease (fatty acids) (*you can detect lipids in 1/100th of a fingerprint by GC!*)

Cleaning of reagents/labware

- Combustion @ 450°C > 3hr (pyrex, quartz, aluminum)
- Sulfuric acid based oxidizing solutions “Nochromix”
- (thermally “fragile” glassware or glassware for or volumetric measurement, teflon)
- Certain Detergents
- Solvent rinsing (DCM, MeOH)

Practical considerations

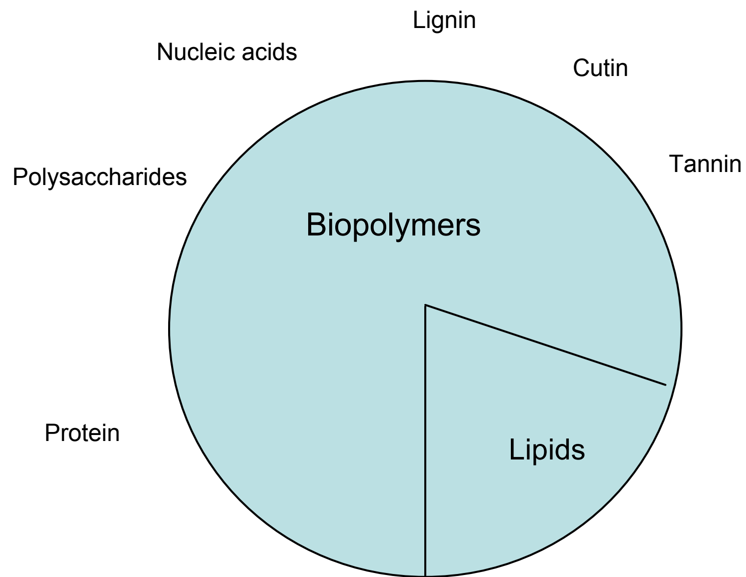
Common solvents/reagents

- Dichloromethane/methylene chloride (DCM)
- Methanol (MeOH)
- Hexane (Hex)

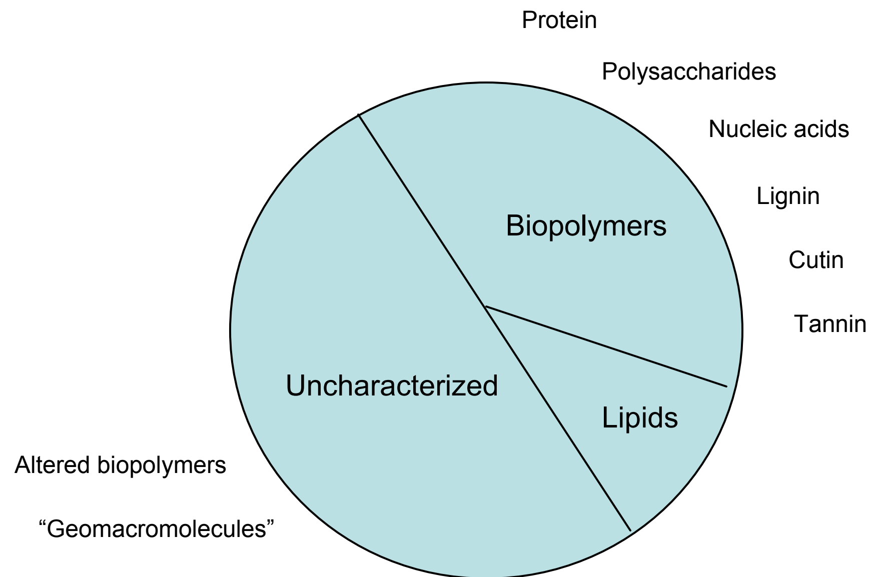
Safety Issues

- Many of solvent and reagents commonly used are toxic, carcinogenic, flammable, or exhibit the potential to form unstable products (e.g. peroxides) during storage (e.g. diethyl ether) that may lead to spontaneous combustion/explosions.
- Always work with solvents/reagents in fume hood
- Nitrile gloves offer good protection against <u>most</u> solvents
- Safety glasses

Building blocks of natural organic matter



Biochemicals



Sedimentary organic matter

Modes of Sample Analysis

Bulk measurements

- Optical assessement (identification of spores, pollen, algae, higher plant debris etc.)
- TOC (%)
- Elemental analysis (C,H,N,O,S)
- Isotopic composition ($\delta^{13}C$, $\delta^{15}N$, δD, $\delta^{34}S$, $\Delta^{14}C$) of bulk organic matter.

Advantages:

- Potentially all inclusive
- Rapid, "easy" measurements, quick
- No fractionation of the sample (except removal of inorganic species)

Disadvantages

- Low information content
- Insensitive to subtle (yet often important) chemical variations

Modes of Sample Analysis

Molecular-level measurements

Advantages:

- High biological information content based on molecular structure.
- Often can simultaneously isolate and quantify more than one molecular type.
- Ability to assign isotopic compositions to biomolecules from specific sources.

Disadvantages:

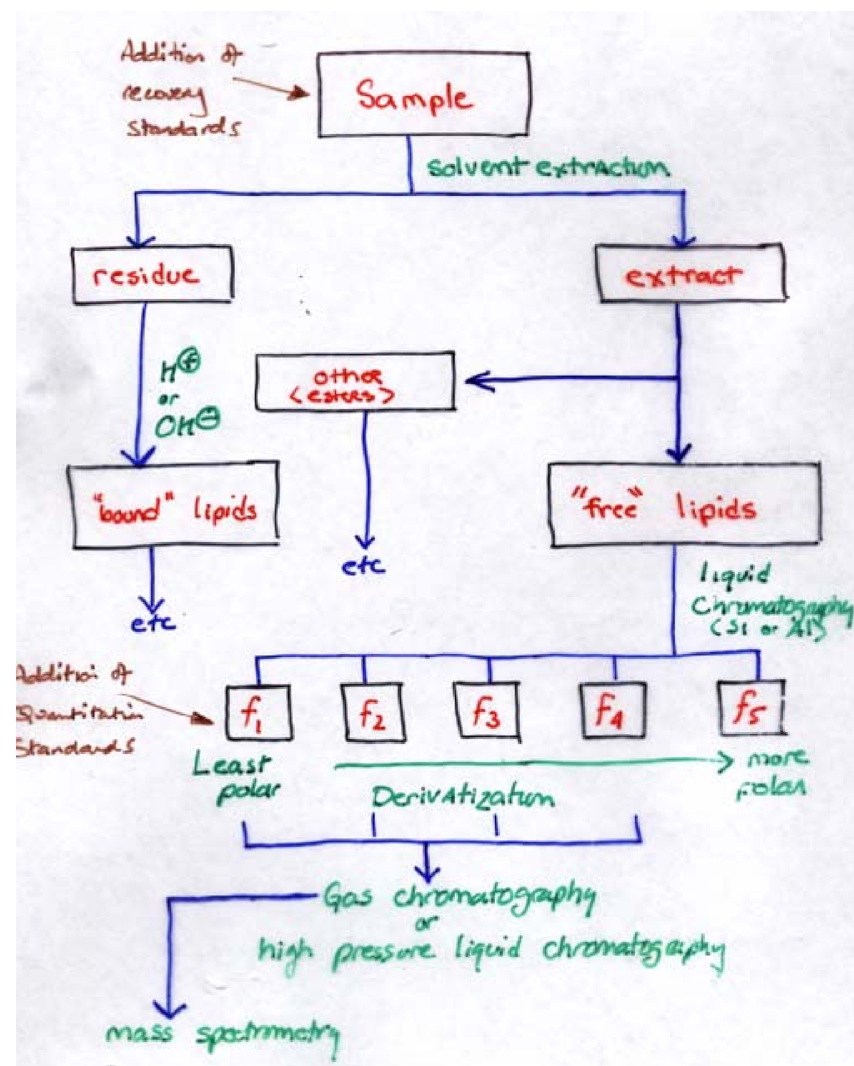
- Slow, and often procedurally difficult - multistage isolations.
- Intrinsically selective and biased.
- Very complex mixtures encountered - often can't identify all compounds isolated.
- Accurate quantification challenging.

Characterization of Solvent-Extractable Organic Matter

Typical Procedure for Isolation and Characterization of Lipids

Key steps

- a. Extraction of Soluble Components with Solvents (total lipid extract, TLE).
- b. Saponification (hydrolysis of ester-bound lipids, e.g. wax esters, phospholipids)
- c. Separation of neutral compounds (alcohols and hydrocarbons) from acids
- d. Acidification of carboxylic acid salts (convert to protonated forms)
- e. Isolation of protonated acids using apolar solvent
- f. Purification (column or thin-layer chromatography)
- g. Derivatization (acids and alcohols)
- h. Chromatographic separation of individual compounds.
- i. Identification and quantification of compounds.



Lipid Extraction Methods

Soxhlet extraction

- Recycling distillation of solvent(s) followed by condensation, percolation through sample and return to distillation flask

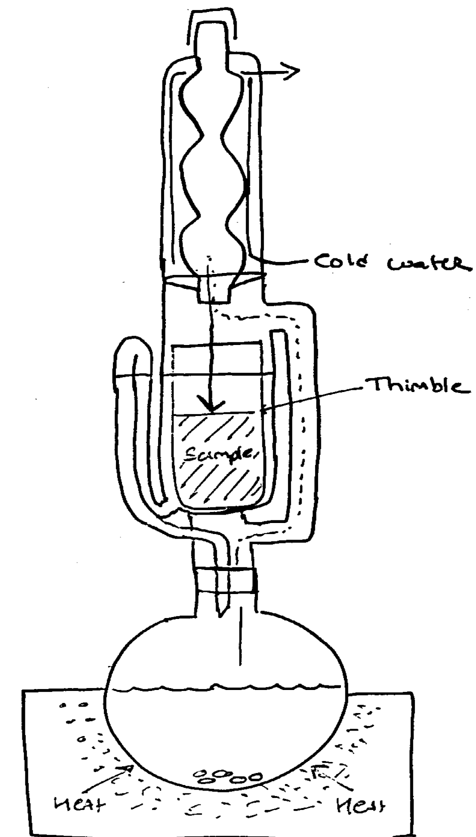
Advantages:

- Mechanically gentle process yet quite efficient extraction
- Can perform elemental sulfur removal simultaneously (activated Cu)

Disadvantages:

- Lengthy process (>24 hrs)
- Require azeotropic mixtures* to ensure efficient extraction.

**Definition of an azeotrope – A liquid mixture of two or more substances that retains the same composition in the vapor state as in the liquid state when distilled or partially evaporated.*



Lipid Extraction Methods

Ultrasonic extraction

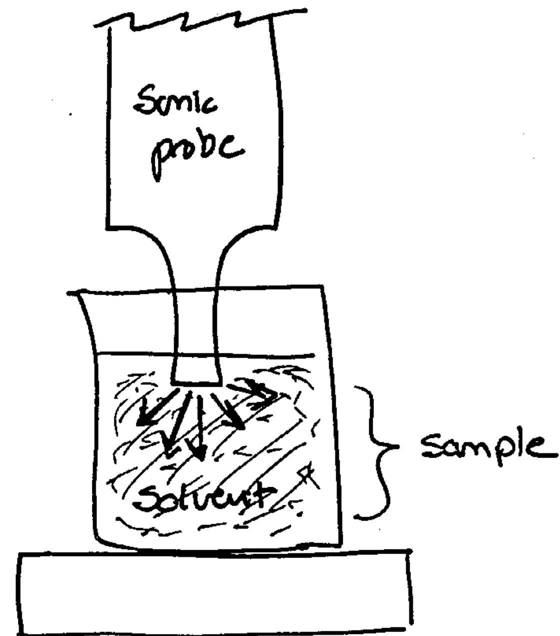
- Vibrational disruption of samples

Advantages:

- Cold extraction - good for thermally labile compounds
- Quick (a few hours)
- A sequence of solvents may be used

Disadvantages:

- Physically violent process - may cause breakdown of macromolecules and clays.
- Labor intensive.



Lipid Extraction Methods

Supercritical Fluid Extraction (SFE)

- Flow through of supercritical CO_2 through sample.

Advantages:

- Ideal (in theory) - Supercritical fluid has the solvating power of a liquid (will readily dissolve compounds) and viscosity of a gas (will fully permeate the sample).
- Quick (<1 hr).
- Does not use large amounts of solvent to extract samples (environmentally sound).
- Good for extraction of non-volatile components.

Disadvantages:

- Requires expensive equipment.
- In practice it is difficult to extract a wide range of polarities.
- Use of "modifiers" (e.g. Methanol) with CO_2 partially but not completely overcomes this problem.

Lipid Extraction Methods

Accelerated Solvent Extraction (ASE)

- Extraction of samples are elevated temperature and pressure
- (e.g., 100°C, 1000 psi).

Advantages:

- Quick (ca. 15 min. / sample).
- Low solvent consumption.
- Solvent systems similar to Soxhlet extraction, so easy to optimize.
- Automated (>20 samples can be processed as a batch).

Disadvantages:

- May not be appropriate for thermally labile compounds.
- Expensive.

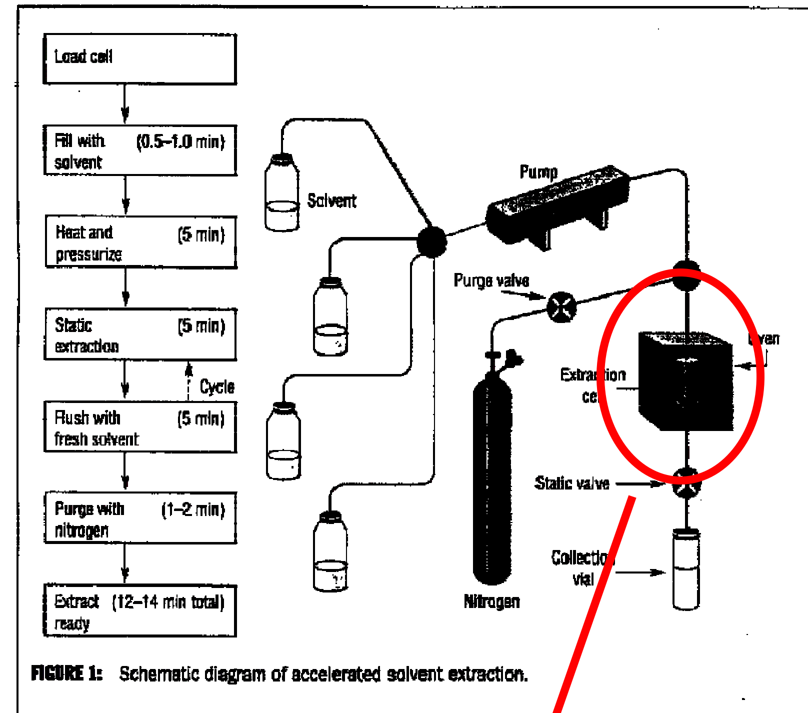
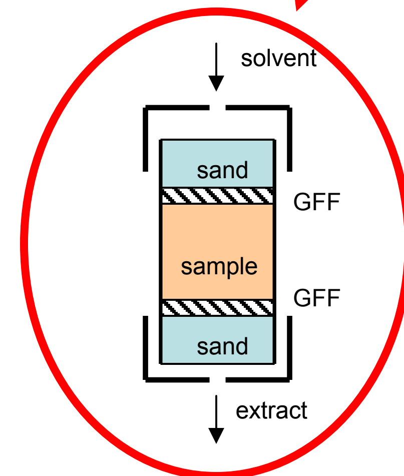


FIGURE 1: Schematic diagram of accelerated solvent extraction.



Chromatographic Separation, Purification and Analysis

Definition of Chromatography : "The resolution of material on two phases using chromatographic apparatus"

Nomenclature:

Technique

- GC: Gas Chromatography*
- CC: Column (gravity chromatography)#
- TLC: Thin Layer Chromatography#
- HPLC: High Performance/Pressure Liquid Chromatography*

**Typically (but not exclusively) used as analytical tools (molecular level separation)*

#Typically used as screening or semi-preparative tools (compound class separation)

Chromatographic Separation, Purification and Analysis

Mobile phase:

- developer (TLC)
- eluent (CC)
- carrier gas (GC)

Stationary phase:

- adsorption chromatography: surface active solid material
- partition chromatography: fixed liquid (e.g, film coated on solid support)

Solute:

- A mixture of substances to be separated

Chromatogra<u>ph</u>:

- Instrument used for chromatographic separation

Chromatogra<u>m</u>

- Trace showing separation achieved in a chromatographic analysis

Principles of chromatography

Chromatography uses two phases one of which is stationary and the other mobile. Chromatography is based upon use of different physical properties to induce separation of compounds or compound classes:

- solubility
- adsorption
- volatility
- size

Polarity

- Polarity is a relative term applied to solvents, solutes and adsorbents

Compound(s)	Properties	Polarity
water	strong dipole strong H-bonding	very polar
alcohols, esters, ketones	weaker H-bonding	intermediate polarity
aromatic hydrocarbons	no dipole, but polarisable electron cloud	less polar
aliphatic hydrocarbons		least polar/apolar

Principles of chromatography

Adsorbent	Solute type	Polarity
Alumina (Al_2O_3)	basic/neutral	polar
Silica (SiO_2)	acid/neutral	polar
Charcoal		non-polar/apolar

In general:

- (1) The polarity of organic compounds increases with an increase in the # of functional groups (e.g. sugars - polar; *n*-alkanes - apolar).
- (2) The polarity decreases with increasing molecular weight
- (3) Partition and adsorption chromatography both primarily depend on polarity differences between solutes. Partition depends on solubility in two liquids, but also on molecular size. Therefore members of a homologous series (e.g. n-alkanes) are best separated by partition chromatography.
- (4) Partition chromatography has more resolving power than adsorption chromatography

Chromatography Types

Mobile phase	Stationary phase	Chromatography type
Gas	Gas	Not important
Gas	Liquid	Partition (GLC, abbrev. GC)
Gas	Solid	Adsorption (packed column GC)
Liquid	Liquid (film on a solid support)	Partition (RP-HPLC)
Liquid	Solid	Adsorption (TLC, CC, NP-HPLC)
Solid	Solid	Not important

Column (Gravity) Chromatography (CC)

Advantages:

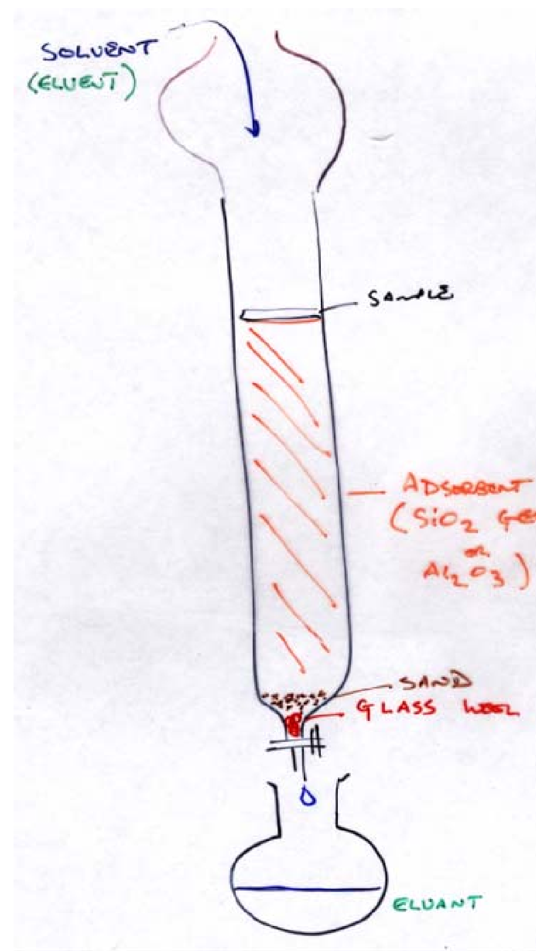
- Can perform large scale separations (several hundreds of milligrams of solute)

Disadvantages:

- Lower resolution than TLC or HPLC (see below)
- Separation is performed "blind"

Methodology:

- The stationary phase is gravity packed into a glass column plugged with glass wool.
- The sample is applied to the top of the column followed by the eluent(s)
- The separated compound(s) are collected after they have passed through the column (eluant).



Column (Gravity) Chromatography (CC)

Elution techniques:

1. Frontal analysis

- Continual addition of two components (A+B) to column.
- Only is A is retrieved (B is adsorbed).
- Limited by capacity of the adsorbent to retain B

2. Displacement analysis/development

- A+B introduced onto the column followed by a more polar eluent.
- Induces sequential elution of A , A+B and B

  *(*1&2 are used in Solid Phase Extraction (SPE) methods)*

3. Elution development (most popular)

- Sequential addition of solvents increasing in polarity
- Weak eleunt (apolar)-> weak + strong -> strong eluent (polar)
- This is done by addition of strong to weak eluent over time.
- Gradient elution is a modification of above used in HPLC

Factors limiting column efficiency

Column Dimensions

- Size of column determined by the difficulty of separation and amount of solute
- For adsorption chromatography usually adsorbent : solute = 30:1 to 100:1
- For partition chromatography usually adsorbent : solute = 50:1 to 500:1
- Column efficiency usually increases with length/width ratio

Particle Size

- Smaller the particle size (i.e., > surface area) = better separation.
- CC mesh size typically 60-100 μm
- TLC mesh size 5 or 10 μm
- HPLC mesh size 3 μm

Pressure

- The smaller the particle mesh size the more pressure is required in solvent delivery
- Small particle sizes used in HPLC require pressures up to 5000 p.s.i.

Time

- The length of time required to afford separation

Flow rate

- Typically 0.3 - 3 column volumes per hour is optimal

Thin-Layer Chromatography (TLC)

Advantages:

- Higher resolution than CC due to smaller particle size and larger surface area.
- More reproducible (although sensitive to ambient temperature/humidity)
- Can assess separation at end of development by spraying with fluorescent dye and visualizing under UV irradiation. Can run standards in separate "lanes" to check separation.

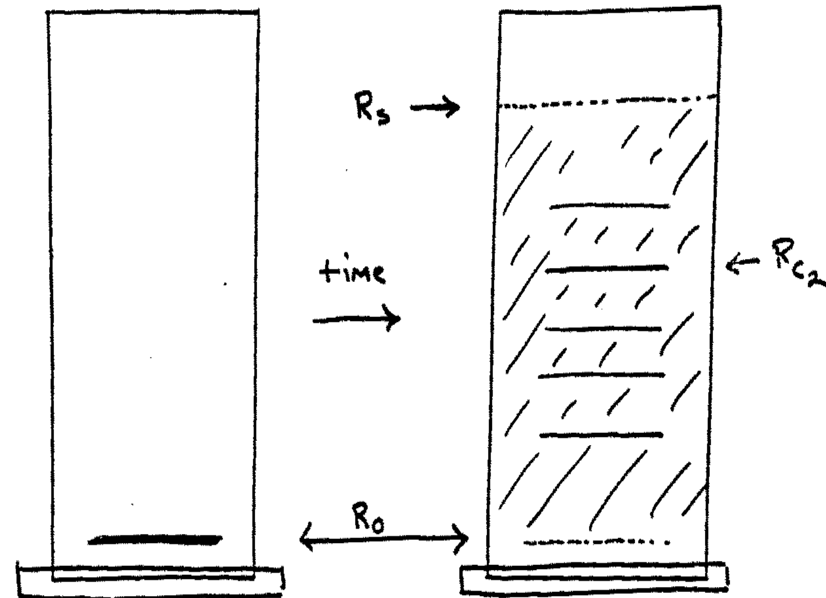
Disadvantages:

- Volatility losses due to exposure of sample on surface
- Difficult to perform quantitative recovery of sample (requires scraping off of bands)
- Limited sample capacity (<20 mg)

Thin-Layer Chromatography (TLC)

Methodology:

- The adsorbent (e.g. silica gel) is deposited as a thin layer (0.1 - 2mm) on a flat supporting surface (usually glass). The adsorbent is held with a binding agent (e.g. $CaSO_4$.xH2O)
- Silica plates are "activated" by removal of water from active sites (e.g. 120°C, 1hr)
- The sample mixture (in a suitable solvent) is applied to the plate in a series of spots or a line and the plate is "developed" in a solvent of choice to afford a chromatographic separation.
- By using mixtures of solvents can modify separation to optimize for particular compounds of interest.
- "Iatroscan" - rapid version of TLC with Flame Ionization Detection



$$R_f = \frac{(R_{c_2} - R_o)}{(R_s - R_o)}$$

Retention data

- Rf = distance of compound from origin/distance of solvent from origin

2-Dimensional TLC

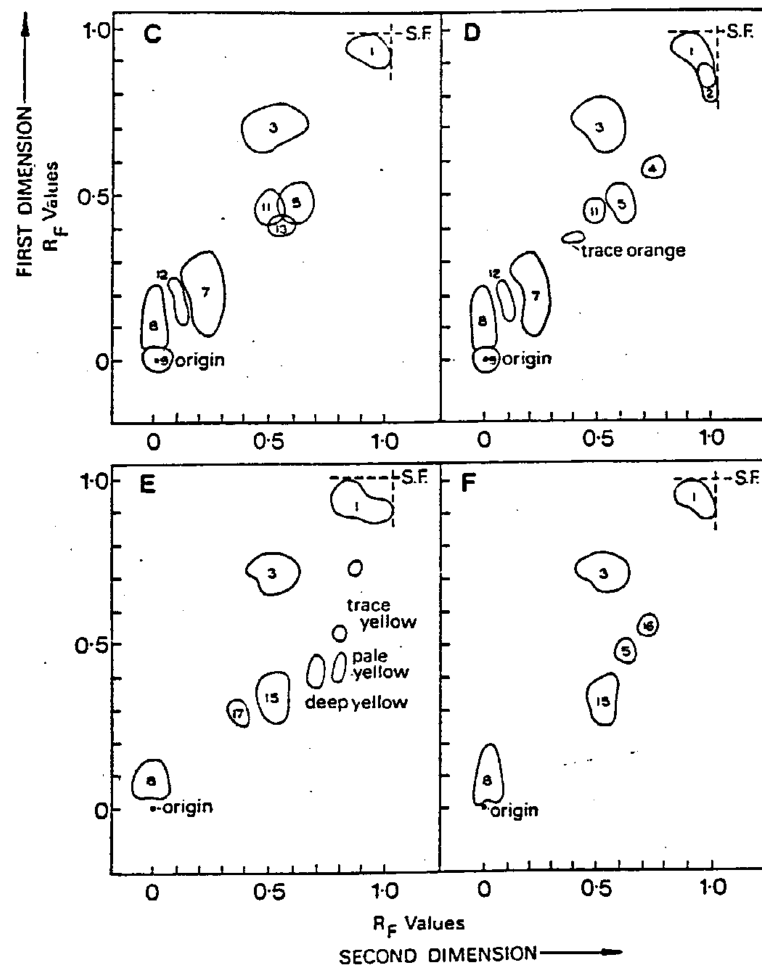
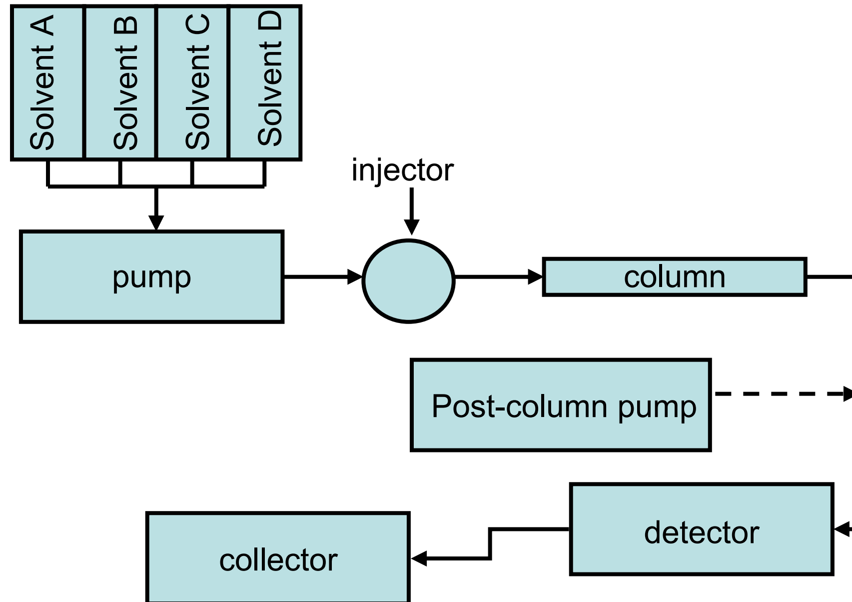


FIG. 1. Sucrose thin layer chromatograms of selected dinoflagellates. *A. Gyrodinium resplendens*; *B. Amphidinium carterae* (PY32); *C. Cachonina niei*; *D. Amphidinium carterae* (PY1); *E. Exuviella* sp.; *F. Peridinium foliaceum*. Fraction numbers and pigment identification as in TABLE 3. Solvent systems, first dimension, 1.3% n-propanol in ligroine (63–75°); second dimension 40% chloroform in ligroine (63–75°).

High Performance Liquid Chromatography (HPLC)

System Components:

- Solvent Reservoir(s)
- Pump
- Injection loop
- Column(s)
- Detector(s)



Modes of operation

- Isocratic operation: single solvent system
- Gradient elution: binary, ternary or quaternary gradient system

Chromatography Type

- Normal phase: mobile phase <u>less</u> polar than stationary phase
- Reverse phase: mobile phase <u>more</u> polar than stationary phase

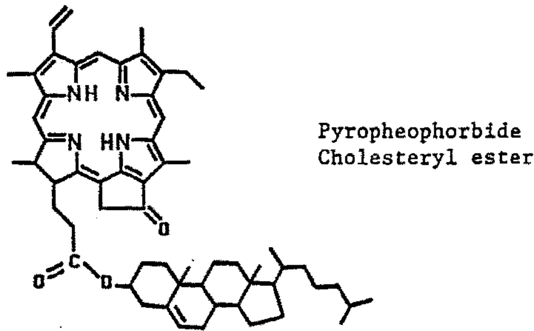
High Performance Liquid Chromatography (HPLC)

Detectors

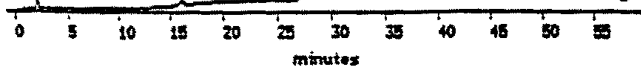
- UV/Vis
- Photodiode array
- Fluorescence
- Refractive Index
- Chemiluminescence
- Evaporative Light Scattering (ELSD)
- Mass spectrometer (MS)

HPLC separation of pigments

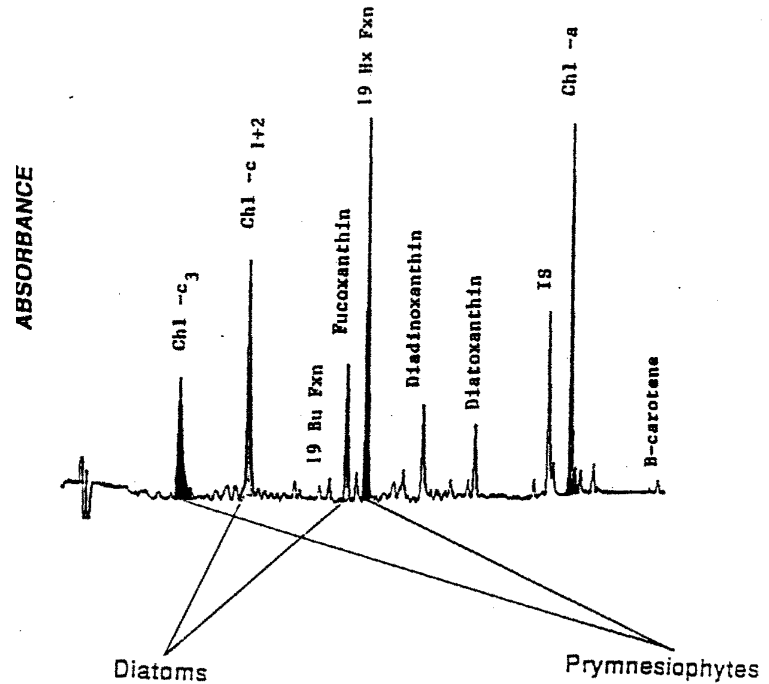
Chlorophyll alteration products
(RP-HPLC, MeOH/acetone)



HPLC of PCEs
in Black Sea
sediments.
After King and
Repeta (1991).



Carotenoids
(RP-HPLC)



(NB these compounds are too non-volatile for analysis by GC)

NP-HPLC-ELSD of marine particulate lipid classes

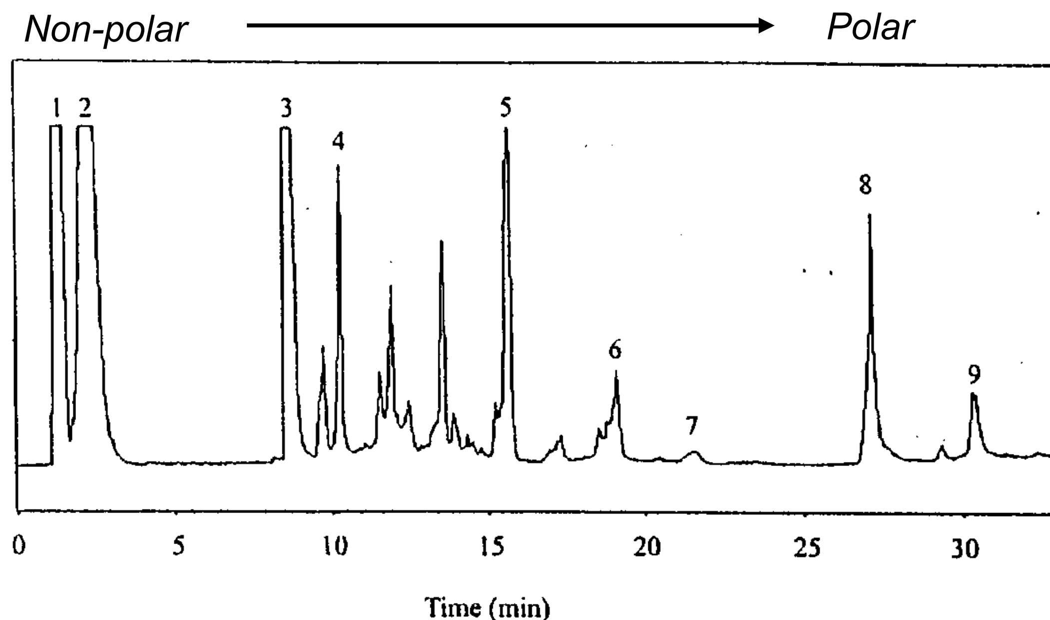


Fig. 5. Separation of lipid classes extracted from seawater spring-bloom particulate material (0.7–100 μm). 1 = nonpolar lipids like hydrocarbons, sterol esters and wax ester; 2 = triacylglycerols; 3 = sterols; 4 = chlorophyll a; 5 = monogalactosyldiacylglycerols; 6 = digalactosyldiacylglycerols; 7 = sulphoquinovosyldiacyl-glycerols; 8 = phosphatidyglycerols; 9 = phosphatidylcholine.

Gel Permeation Chromatography (GPC) or Size Exclusion Chromatography (SEC)

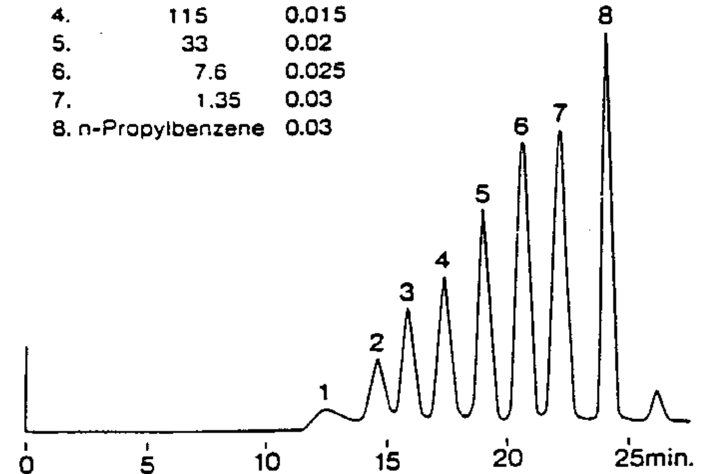
Principle

- Separates solutes according to molecular size
- Use columns packed with gels of different nominal pore sizes
- Molecules larger than this pore size are excluded from pores and therefore move with solvent and elute first . Smaller molecules follow a more tortuous route in and out of the pores and elute later.

Methodology:

- Similar instrumental set-up to HPLC
- Use only isocratic solvent elution

Polystyrene standards

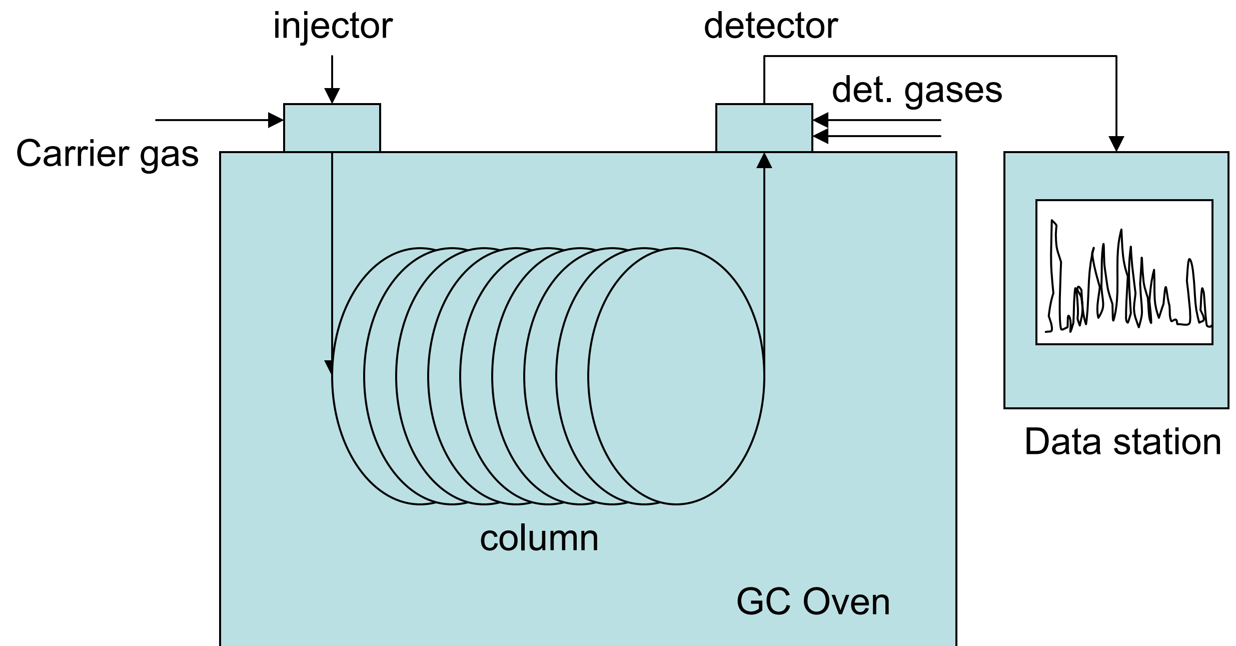


Column : Shodex GPC KF-80M × 2
Eluent : THF
Flow rate : 1.0ml/min.
Detector : UV(254nm)
Column temp.: Ambient

Gas Chromatography (GC)

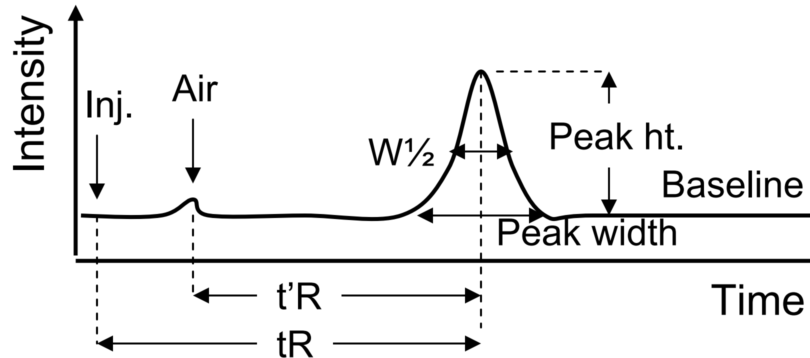
System Components

- Gas supplies
  - carrier gas
  - detector gases
- Injector
- Column
- Oven
- Detector



Basic theory of Gas Chromatography

- A gas chromatogram usually represents a plot of detector response versus time (or volume of carrier gas)



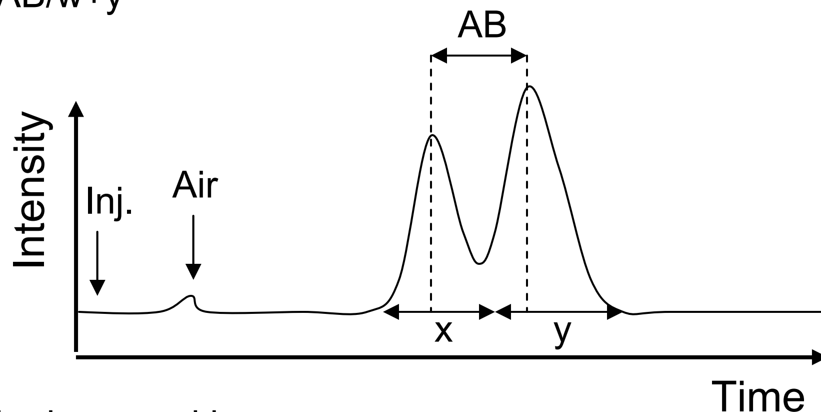
Definitions

- *Baseline*: The response of the detector to zero solute
- *Peak width*: obtained by drawing tangents to the peak and measuring at the baseline
- *Peak height*: from baseline to top of peak
- *Width at half height* - self explanatory
- *Retention time (uncorrected, tR)*: Time from injection to peak max.
- *Retention time (corrected, t'R)*: Time from the air peak to peak max.
- *Retention volume, VR* = tR x flow rate
- *Relative retention time (α)* = retention time of peak relative to another peak (usually a standard)

Chromatographic resolution

- *Relative retention time (α)* = retention time of peak relative to another peak (usually a standard)
- If α = 1 then A and B are inseparable
- If α >> 1 then still might not get good separation if peaks are v. broad

- RESOLUTION is the critical factor
- Resolution = 2xAB/w+y

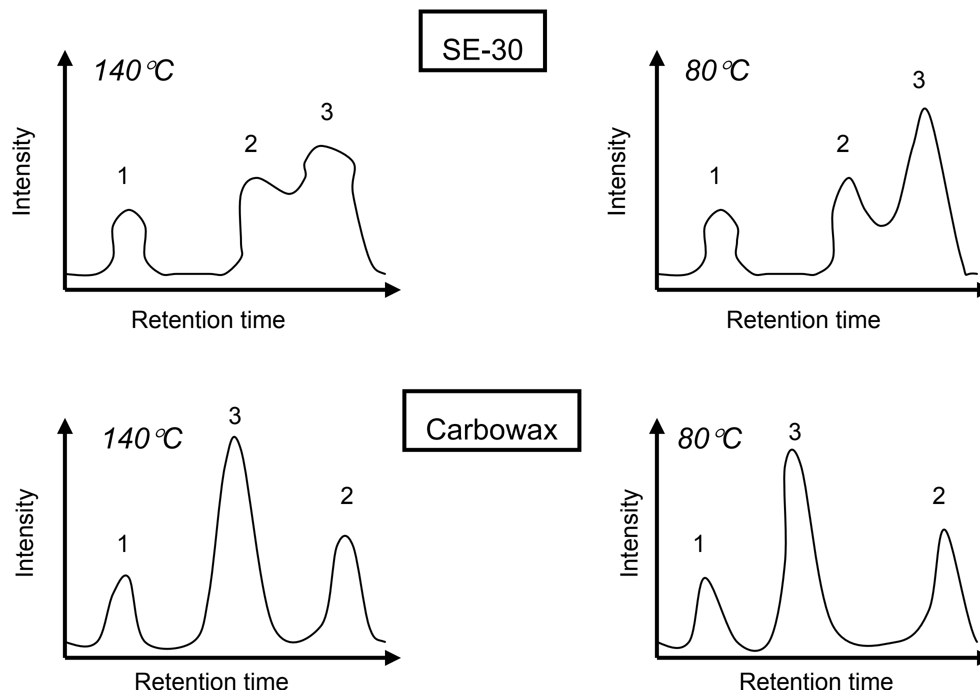


- Resolution can be improved by:
- (a) increasing the efficiency of the liquid phase (solvent)
- (b) improving the efficiency of the column (increases # of theoretical plates)

Improving Gas Chromatographic resolution

(a) Increase solvent efficiency

- Increased temperature reduces efficiency by reducing time spent by solute in liquid phase
- Use lower temps
  - more liquid phase interaction
  - more separation
  - longer time
- Change liquid phase
- e.g. SE-30 vs carbowax



The increased efficiency in the first solute is due to boiling point differences
The peak order in the second solute is changed by differing solubilities

Improving GC resolution

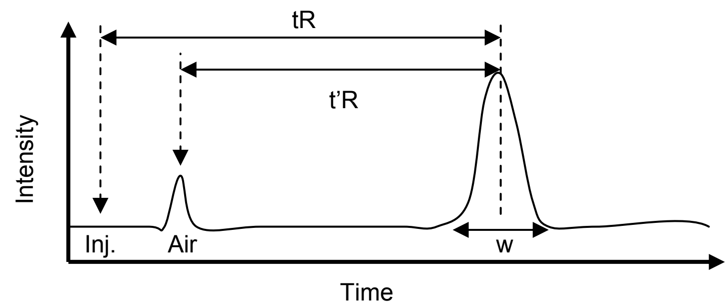
(b) Column efficiency

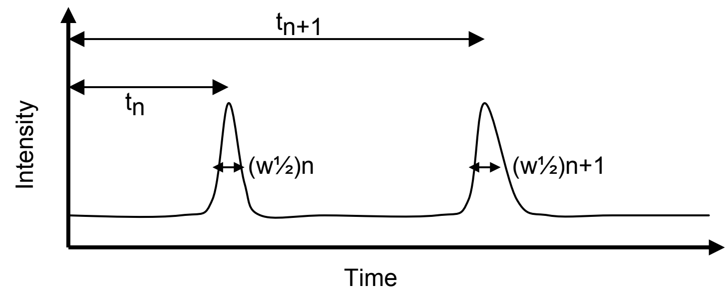
Key parameter:

- HETP = Height Equivalent to the Theoretical Plates

Definition of plate

- Plate = length of the column required for the solute to come into equilibrium in the distribution between gas and liquid phase.
- Number of theoretical plates of a column, $n = 16 \times tR^2/w^2$
- Number of effective theoretical plates, $n_{eff} = 16 \times t'R^2/w^2$
- HETP = l/n (l = length of column (cm)
- Require column height (length) to be small relative to the # plates
- Separation number (Trennzahl value) Tz = the number of completely separated peaks that can be obtained between 2 homologous n-alkanes
- $Tz = [(t_{n+1} - t_n)/((w½)_n + (w½)_{n+1})]-1$
- Good columns give Tz values up to 40





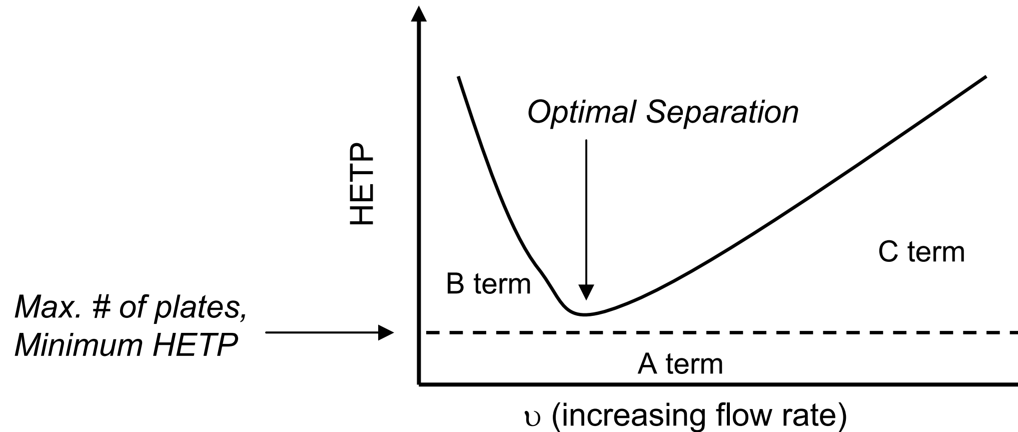
Improving GC resolution

Column Efficiency (cont'd)

- 3 main factors infuence column efficiency (band broadening)
- These are known as the A,B and C terms in the *Van Deemter* equation:

$$HETP = A + B/\upsilon + C\,\upsilon \text{ where } \upsilon = \text{linear gas velocity}$$

- Therefore υ works in 2 opposing ways, both working to make HETP large (detrimental) and small (beneficial).



Improving GC resolution

A Term - Eddy diffusion (multiple path term)

- Caused by different paths that solute takes along column.
- Not affected by υ (flow rate)
- N.B. This only applies for packed columns.
- Minimize by using:
  - (i) small particle size
  - (ii) regular packing

B Term - Molecular (longitudinal) diffusion

- Due to longitudinal diffusion of the solute in the column
- i.e. get peak spreading at low flow rates
- Minimize by using:
  - (i) increased υ (flow rate)
  - (ii) increase molecular weight of gas

Improving GC resolution

C Term - resistance to mass transfer

- Describes the difficulty of transferring a solute into and out of the liquid phase. This term should be minimized to induce rapid transfer between phases.
- A function of:
  - thickness of stationary phase (film thickness)
  - film viscosity
  - amount of stationary phase in the column
- Minimize by using:
  - (i) Thin film columns
  - (ii) low viscosity phases
  - (iii) lower flow rate to allow equilibrium

Gas Chromatography using multicapillary columns

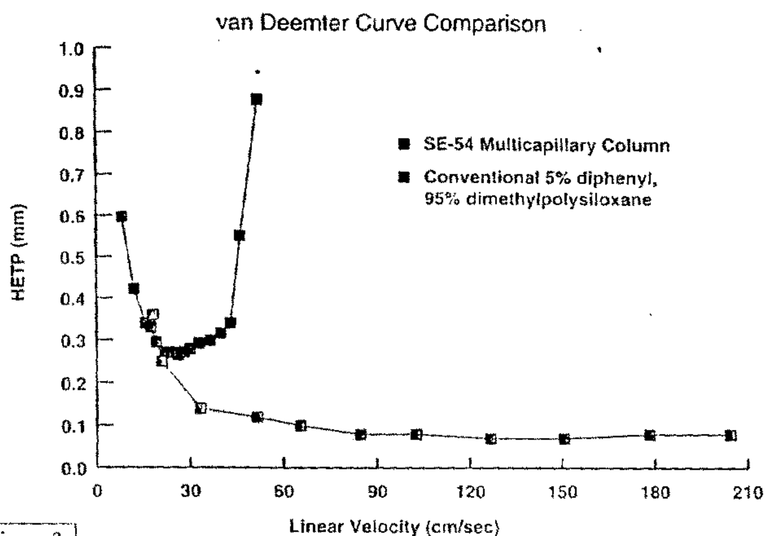


Figure 2

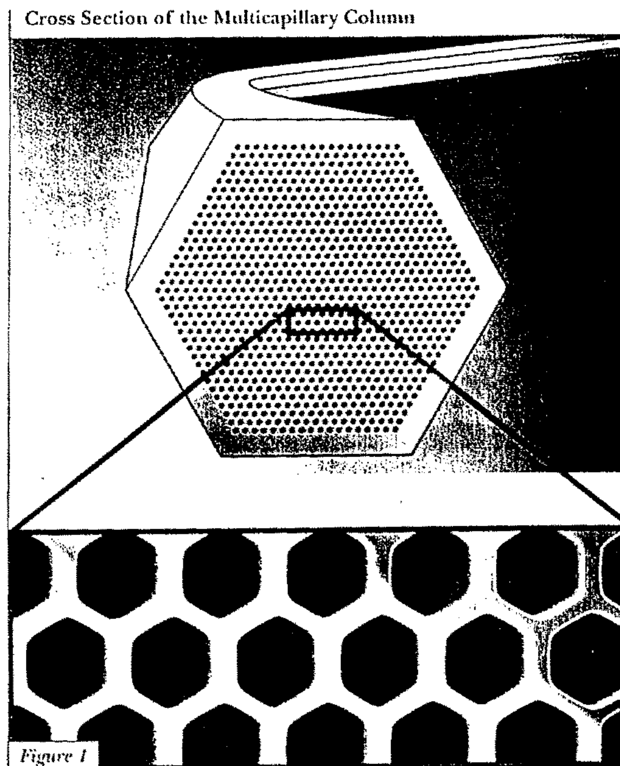
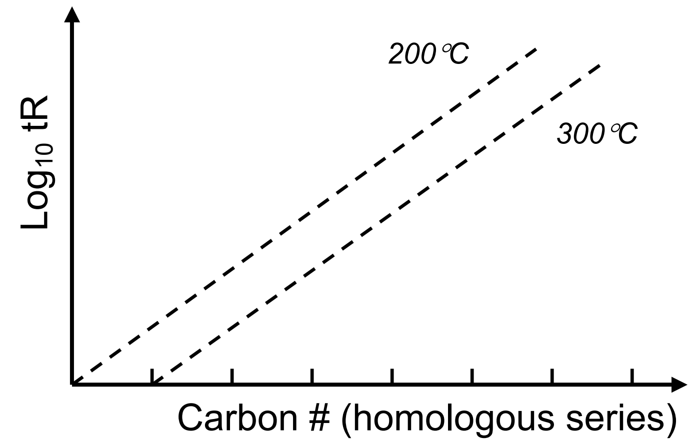


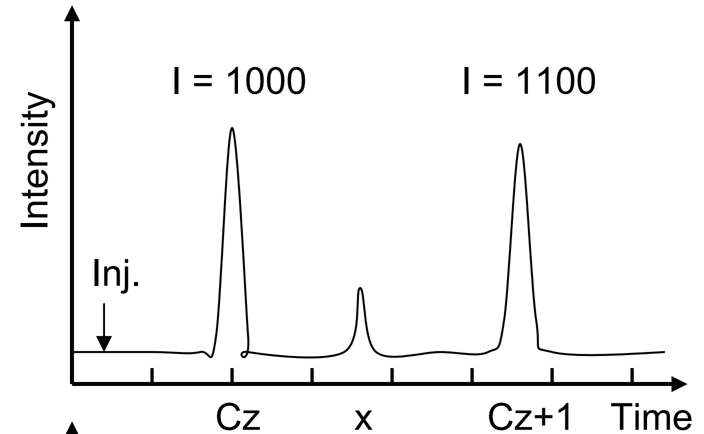
Figure 1

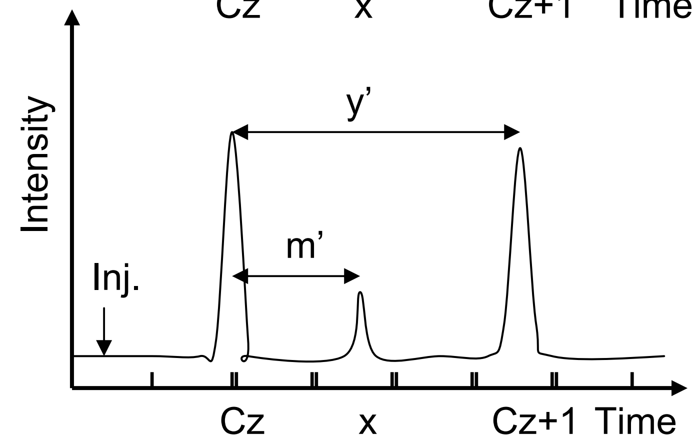
Retention Data

- Relationship between $\log_{10}$ tR vs Carbon #
- Isothermal run

- Kovat's Retention Index (Kovat's Indices, I).
- Based on linear relationship between $\log_{10}$ tR vs Carbon #.
- I of any alkane on any liquid phase = 100 x Carbon # of alkane.
- This index system can be used for compound identification.
- $Ix = 100z + 100[(\log_{10}t'Rx - \log_{10}t'Rz)/(\log_{10}t'Rz+1 - \log_{10}t'Rz)$.
- For fatty acids, a different expression is often used, the Equivalent Chain Length (ECL).
- All the above is for ISOTHERMAL chromatography.
- However most analyses are done on a LINEAR TEMPERATURE PROGRAM.
- Ix(program) = 100z + 100 m'/y'.







GC Column Characteristics

Column Types

Packed columns

- Typical dimensions: 5'-20' long x 1/16-1/4" diameter
- Total effective plates: ca. 5,000
- Not used so much today, except for gas-solid chromatography (GSC)
- Good for resolving gases and light hydrocarbons (e.g. methane, ethene, ethane etc.)
- Adsorbents in GSC: molecular sieve, silica gel, charcoal, alumina

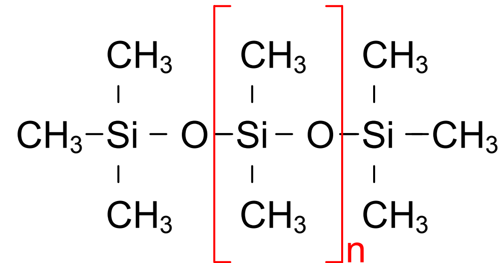
Capillary columns

- Typical dimensions: 10 m (30') – 100 m (300') long x 0.1, 0.25, 0.32 or 0.53 mm i.d.
- Total effective plates: up to 90,000
- Support coated open tubular (SCOT) columns (walls of column coated with silica with liquid phase)
- Wall coated open tubular (WCOT) **most commonly used today*

- Capillary columns Initially made of stainless steel
- Made of glass or, more recently, polyimide-externally coated fused silica
- Polyimide provides additional strength and flexibility.

GC Column Characteristics

- **Liquid phase**
- *Requirements:*
- Non-volatile over chromatographic temperature range
- Thermally stable
- Must not chemically react with solute
- Should exhibit appropriate solubility characteristics for solutes
- Liquid phases are designated as “polar” or “non-polar”
- *Non-polar phases ("boiling point" phases)*
- <u>Silicones</u>
- e.g. methylsilicone



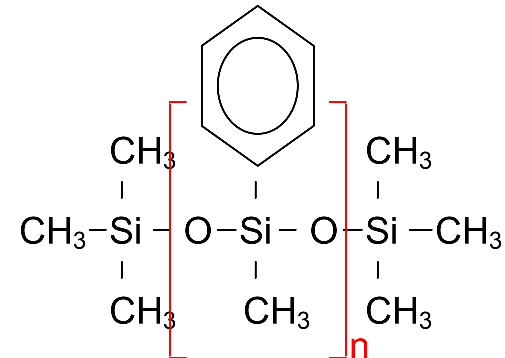
- where n=40,000-55,000 (mol wt. 3-4 × 106)
- Commercial names: OV-1, SE-30, HP-1, DB-1, BP-1
- This is the most popular apolar phase

GC Column Characteristics

Polar phases

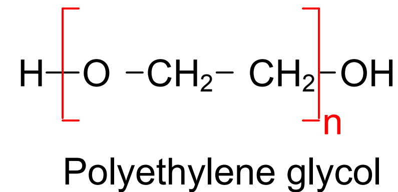
A. Silicones

- 5% phenyl (SE-52, DB-5, HP-5)
- 5% phenyl, 1% vinyl (SE-54)
- 50% phenyl (OV-17)
- 75% phenyl (OV-25)



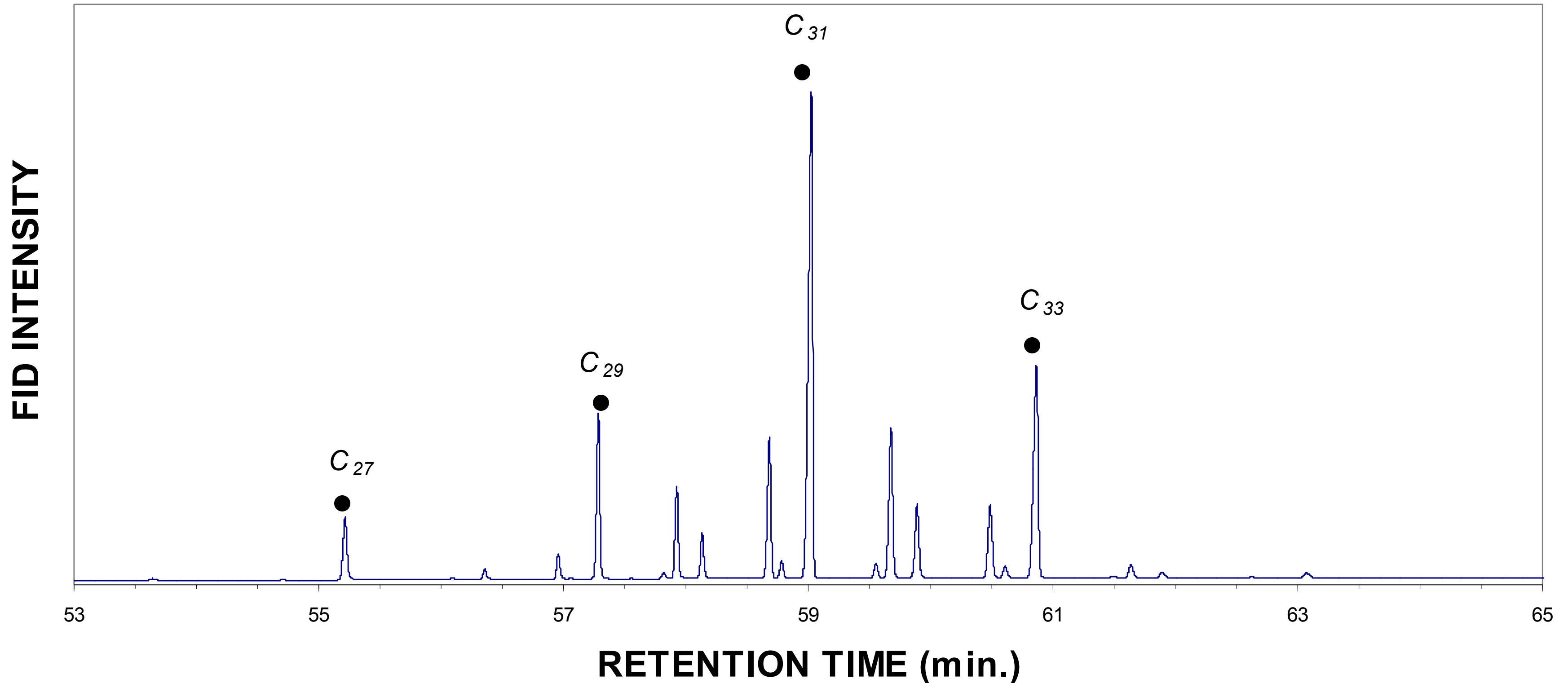
B. Carbowax

- 2 common polar liquid phases
- Carbowax 6000 (Mw = 6000)
- Carbowax 20M (Mw = 20,000)



Polyethylene glycol

Example gas chromatogram of waxes (alkane fraction) from Tobacco leaves



This figure shows a typical gas chromatography trace of a hydrocarbon (alkane) fraction extracted and purified from a higher plant leaf sample. Note the predominance of long-chain ($>C_{24}$) odd-carbon-numbered *n*-alkanes (marked with circles) that is highly characteristic of higher plant leaf waxes. The chain-length distribution of these compounds is indicative of growth temperature.

Gas Chromatography conditions

- **Carrier Gas**
- Must be inert
  - hydrogen, helium, nitrogen, argon
  - Hydrogen is generally favored for most applications
  - Helium is the usually the best alternative (esp. for GC/MS)
  - Constant pressure vs constant flow
- **Injectors**
- Four main types:
  - Split
  - Split/splitless
  - PTV
  - On-column
- **Temperature Control**
- Two modes of operation
  - isothermal (analogous to isocratic HPLC)
  - temperature program
- Typical temperature program 50°C - 320°C @ 4°C/min. (≥ C_{40})
- Maximum temperature limited by stability of stationary phase.
- New columns can attain temperatures of 450°C (≥ C_{90})

GC Detectors

Thermal conductivity detector (TCD)

- advantages:
    - simple
    - universal sensitivity (H_2, CO_2)
- disadvantages
    - low sensitivity (10^{-10}g/s)
    - narrow linear range (10^4)

Flame Ionization detector (FID)

- Advantages:
    - detects anything that burns
    - high sensitivity (10^{-12}g/s)
    - broad linear range (10^7)
- Disadvantages
    - will not detect some materials (e.g. CO_2)

GC Detectors

Electron Capture Detector (ECD)

Responds to halides, NO_2

- Advantages:
  - Very high sensitivity for some compound types (10^{-14}g/s)
  - Very selective
- Disadvantages:
  - Very selective (highly variable response factors)
  - Poor linearity

Flame Photometric Detector (FPD)

Element selective detection of Sulfur and Phosphorus

- Advantages:
  - High selectivity for S and P
  - High sensitivity (10^{-11} to 10^{-12} g/s for S and P, respectively)
- Disadvantages
  - Non-linear (quadratic) detector response
  - Quenching at high concentrations

GC Detectors

Atomic Emission Detector (AED)

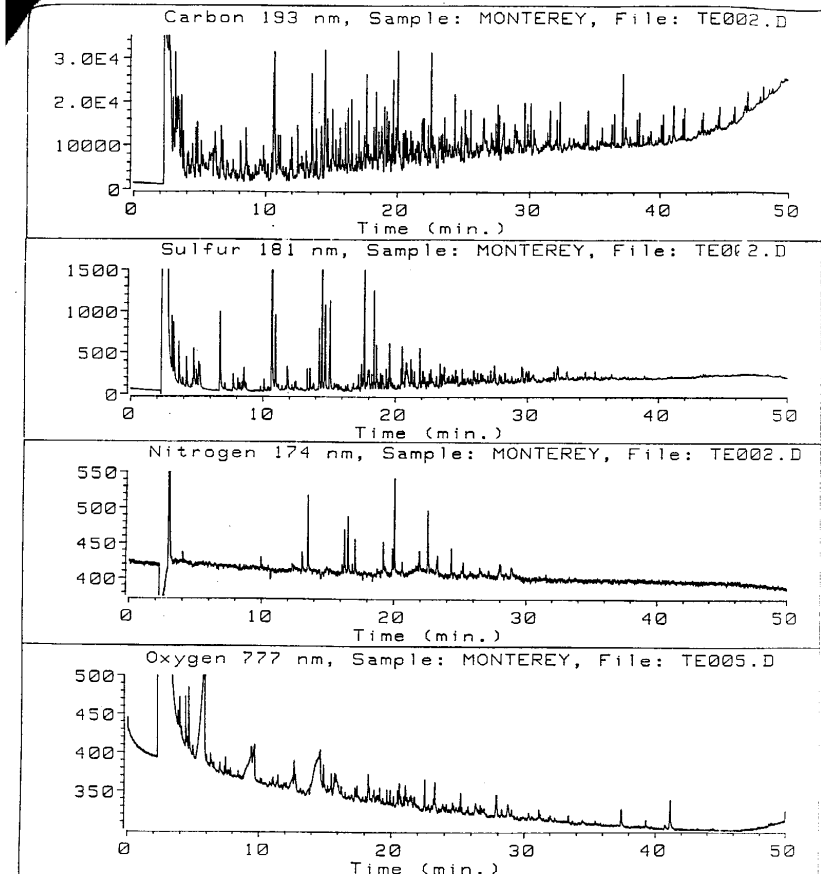
- "GC-ICP"
- Advantages:
- Will detect emission lines from a wide variety of elements (presently up to 4 in any run)
- High selectivity
- Good linear response
- Sensitivity generally as good as dedicated detectors
- Disadvantages:
- Very expensive (ca. $100,000)
- Costly to operate and maintain

Infrared Detector (IR)

Mass Spectrometer (MS)

- More to follow……

Pyrolysis-GC-AED of Miocene sediment



Gas chromatography

Quantitation Methods

- Usually better to measure peak area rather than peak height
- Detector responses vary depending on compound type
- Determination of response factors
- Internal standards
- External standards

Other chromatographic methods

- Preparative Capillary Gas Chromatography (PCGC)
- Comprehensive 2-dimensional GC (GCxGC)
- (Capillary) Electrophoresis (CE)
- Ion Exchange Chromatography (IEC)
- Supercritical Fluid Chromatography (SFC)

Other Methods of Sample Sub-fractionation

- Molecular sieves
- Urea/thiourea adducts
- Derivatization
- Ion exchange chromatography

Derivatization

- Organic compounds with readily exchangeable hydrogens (acids, alcohols) generally exhibit poor chromatographic behavior on apolar GC stationary phases. Derivatization serves to "protect" polar group.

Advantages

Provides improved chromatography (better sensitivity and quantification).

Prevents GC column degradation.

Disadvantages

- Derivatives often unstable
- Cleavage of derivative group during MS analysis can dominate mass spectrum (good & bad)
- Additional carbon introduces error in isotopic measurements from GC-irMS.

Common derivatization methods:

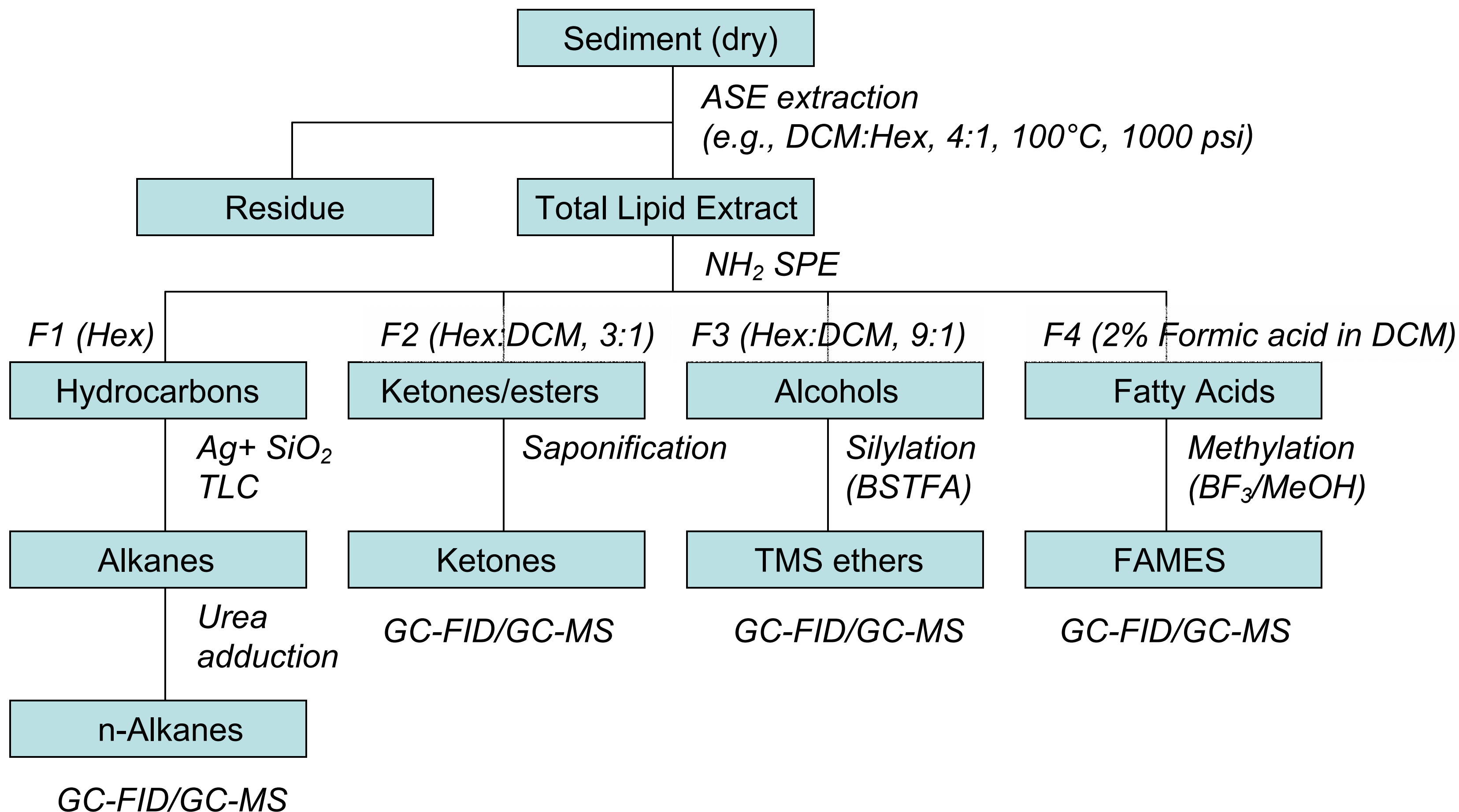
Fatty Acids

- Methylation (BF3/MeOH or MeOH/HCl) to yield Fatty Acid Methyl Esters (FAMES)
- Silylation (BSTFA) to yield trimethylsilyl (TMS) esters

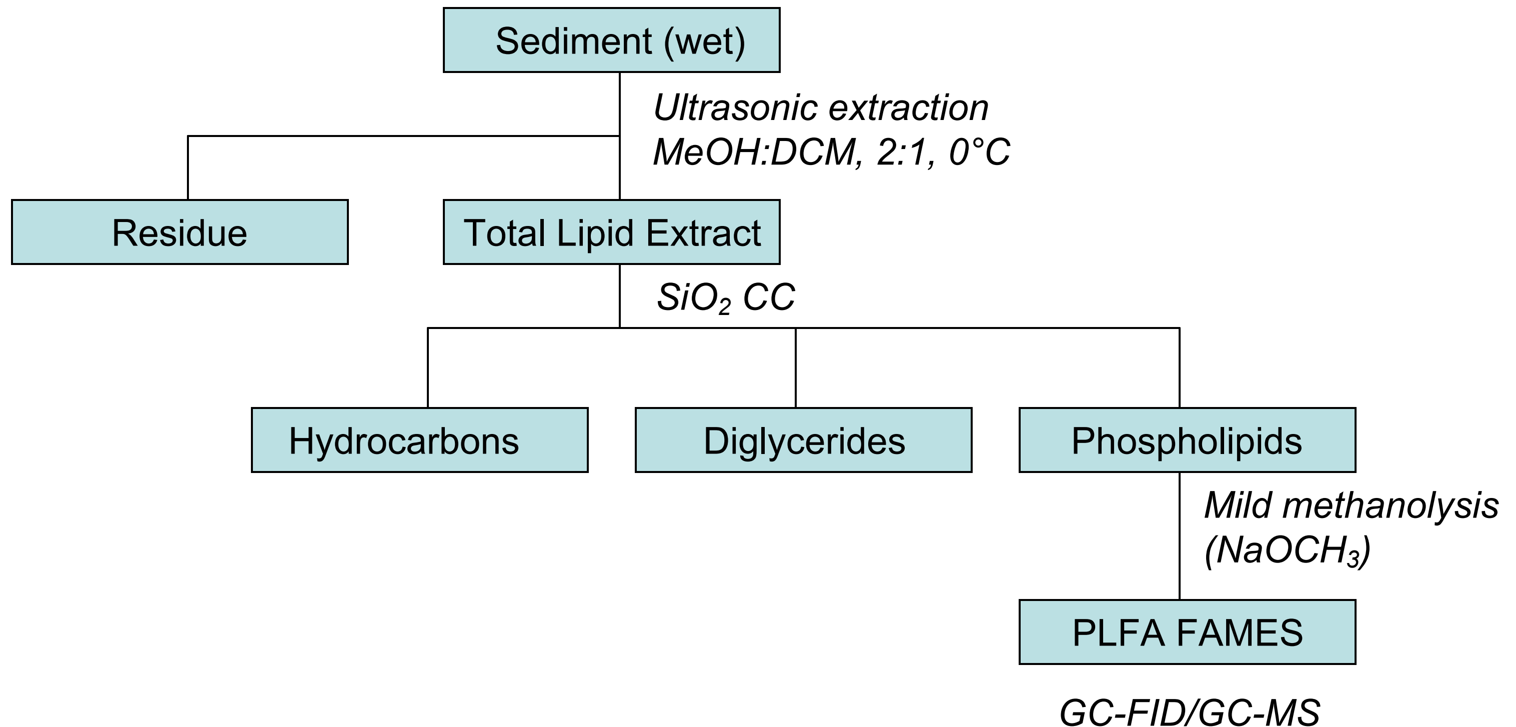
Alcohols (n-alkanols, sterols)

- Acylation (pyridine, acetic anhydride) to yield acetates.
- Silylation (BSTFA) to yield trimethylsilyl (TMS) ethers

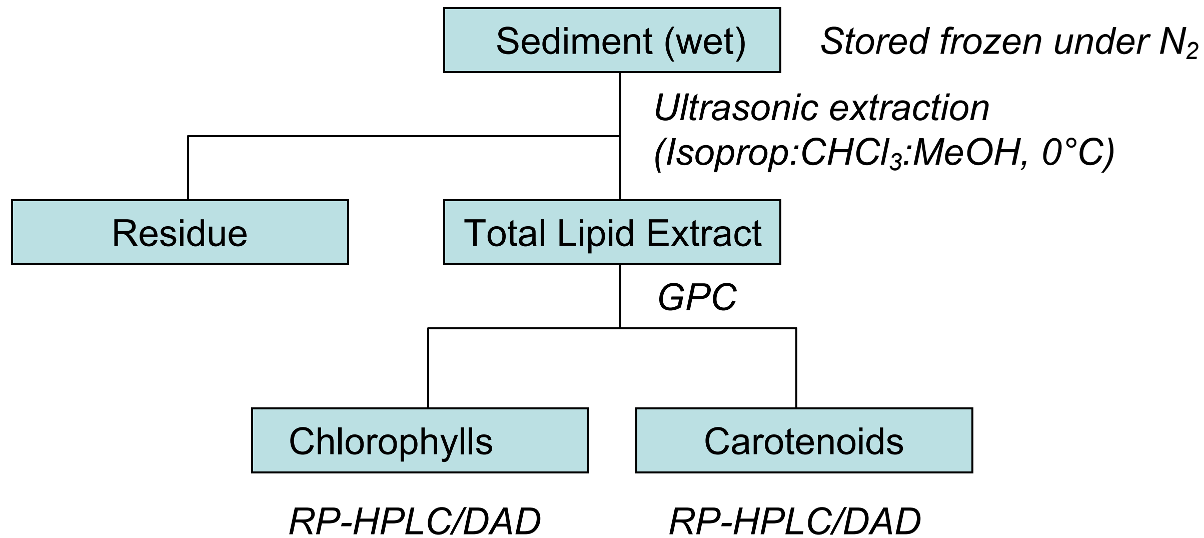
Recipe for Molecular Analysis of Lipids in Marine Particles & Sediments



Recipe for Analysis of Bacterial Signature Lipids (Phospholipid-derived Fatty Acids, PLFA)



Analysis of Pigments (chlorophylls, carotenoids)



Analysis of Polychlorinated Biphenyls (PCBs) in Sediments

Dry sediment (~1 g) is spiked with PCB-103 and PCB-198 and then extracted by pressurized fluid extraction (Hexane/Acetone; 1/1)
(to extract the PCBs from the sediment)

Reduce solvent volume to ~1 ml of hexane and react extract with ~1 ml of concentrated sulfuric acid
(to remove most other organic compounds)

Remove hexane layer from the acid and mix with activated copper (~1 g)
(to remove elemental sulfur)

Charge hexane extract to a fully activated silica gel column (15 cm long x 6 mm width) and elute with 12 ml of hexane
(to isolate a PCBs fraction)

Reduce volume to ~100 ul, spike with PCB-110, and analyze on either a GC-ECD or GC-MS
(to determine the concentration of each PCB congener)

Key points: 1) There are 209 possible PCB congeners and for simplicity, each congener is assigned numbers 1 to 209. However, there are only ~150 congeners present in the commercial mixtures (Aroclors). This has been beneficial as those remaining compounds are used as recovery and quantification standards. In this flowchart, PCB-103 (pentachlorobiphenyl), -198 (octachlorobiphenyl), and –110 (pentachlorobiphenyl) are used as standards. 2) The sulfuric acid treatment is particularly helpful in removing color and interfering compounds as PCBs do not react with sulfuric acid. 3) This method could also be used for analyzing tissue samples as well.