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CEE 772: Instrumental Methods in Environmental Analysis

Lecture #11

Sample Preparation: Basics and Physical Methods (cont.)

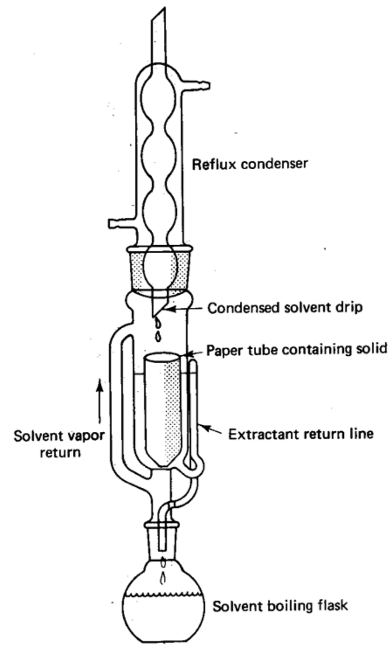
(Skoog, nothing)

(Harris, Chapt. 28)
(817-839)

Pretreatments to LLE

- Acidify to <2
  - 8040: Phenols
- No pH adjustment
  - 8060: Phthalate Esters
  - 8100: PAHs
- Neutralize
  - 8080: Oganochlorine pesticides & PCBs
  - 8090: nitroaromatics & cyclic ketones
  - 8140: organophosphorus pesticides
- Alkaline (>11) then acidify (<2)
  - 8250 & 8270: GC/MS for Semivolatiles
- Drying is sometimes necessary
  - 10 cm column of anhydrous Na_2SO_4
  - Addition of powdered anhydrous $MgSO_4$

Soxhlet Ext



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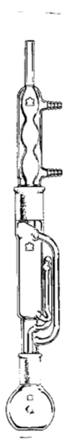
Soxhlet Extraction



- Good for soils, sediments & sludges
- Used with thimbles
  - Or glass wool plugs
- Typical extraction solvents
  - Toluene/methanol (10:1)
  - Acetone/hexane (1:1)
  - Methylene chloride

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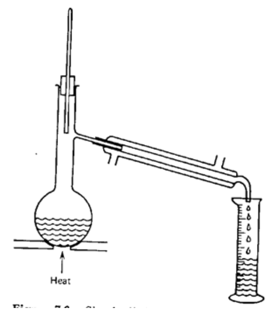
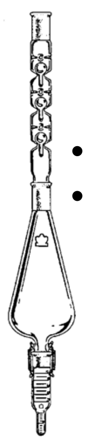
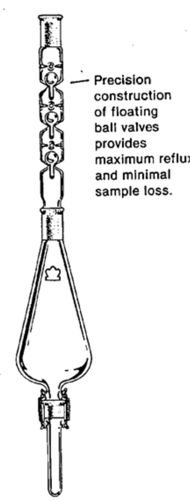
Distillation Theory





Kuderna Danish Concentrators

- Ideal for concentrating ether extracts
- Common procedures
  - Use with heated water bath
  - Boiling chips
  - Good to pre-wet snyder column by adding 1 mL of solvent to top
  - Balls should “chatter”, but chambers should not be flooded
    - May take 10-20 minutes
  - Stop at apparent volume of ~1 mL
  - Exchange solvent may be added at this time & concentration continued



Rotary Evaporation

- Rotary evaporators (also called "rotavaps" in lab slang) are used to remove solvents from reaction mixtures and can accommodate volumes as large as 3 liters.
- A typical rotary evaporator has a heatable water bath to keep the solvent from freezing during the evaporation process. The solvent is removed under vacuum, is trapped by a condenser and is collected for easy reuse or disposal. Most labs use a simple water aspirator vacuum on their rotavaps, so a rotavap can not be used for air and water-sensitive materials unless special precautions are taken.

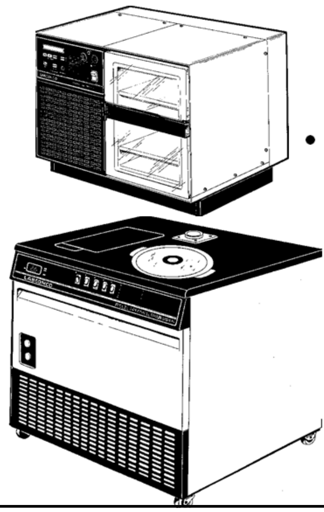
Use of Rotavaps

1. Empty and then replace the solvent collection flask on the unit.
2. Place your flask on the rotary evaporator.
3. Use the speed control to rotate the flask. A typical rotavap uses a variable speed sparkless induction motor that spins at 0- 220 rpm and provides high constant torque.
4. Turn on the aspirator vacuum. On most models, the vacuum on/off control is managed by turning a stopcock at the top of the condenser .
5. Lower your flask into the water bath. On most models, a convenient handle (with height locking mechanism) moves the entire condenser/motor/flask assembly up and down. You can also adjust the tilt of the condenser assembly. Be sure not to put the flask into a water bath that exceeds the boiling point of your solvent!! For small amounts of common solvents you don't need to turn on the bath heater.
6. The solvent should start collecting on the condenser and drip into the receiving flask. Some solvents (such as ether or methylene chloride) are so volatile that they will also evaporate from the receiving flask and be discharged down the drain. To prevent this you can place a cooling bath on the receiver or (on some models) use a dry ice condenser.
7. Once all your solvent has evaporated, release the vacuum, raise the flask out of the water bath and turn off the rotation. Remove your flask.

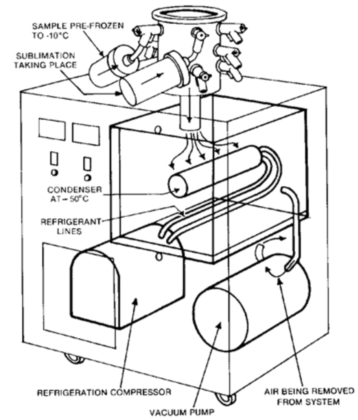
Rotavap tips

- Always use distilled water in your heating bath. Otherwise, scale will build up in the bath and coat the thermistor and heating coils. This is very difficult to remove and reduces the efficiency of the bath. In addition, regular tap water will promote the growth of spectacularly disgusting algae colonies, particularly during the summer months.
- To reduce the amount of evaporation from your water bath, simply add some small plastic balls to the water bath. This reduces the surface area for evaporation and therefore the rate at which the water level drops.
- The ground glass joint holding your flask does not need to be greased, but on rare occasions it (or the bump bulb) may get "frozen". Some companies sell special joint clips that can free frozen joints simply by screwing them in one direction.

Freeze Dryers in EVE



- Manifold Dryer
  - Labconco Freeze Dryer 8
    - 8 liter
    - Marcus 5c
- Tray Dryer
  - Labconco FreeZone 6
    - 6 liter
    - Marston 24



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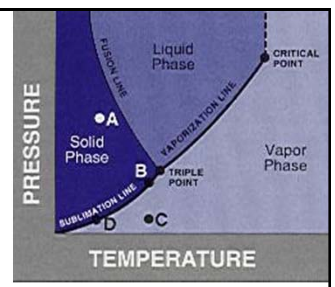
Freeze Drying



- Three stages
  - Pre-freezing at atmospheric pressure (A)
    - Slow cooling produces large crystals, which improves sublimation
    - Fast cooling better preserves biological samples
  - Primary Drying
    - Pressure is lowered in sample compartment (C) to 0.06 mBar or below
    - Sample pressure drops (B) and sample warms leading to removal of ice by sublimation
    - Rate depends on difference between vapor pressure of the product (B) compared to the vapor pressure of the condenser (D)
      - Condenser is usually 20 C cooler than product (e.g., -50 to –80C)

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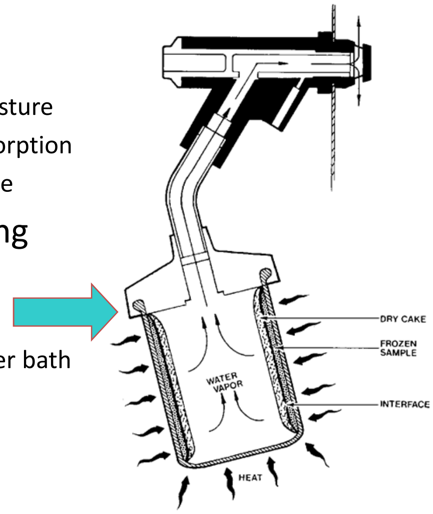
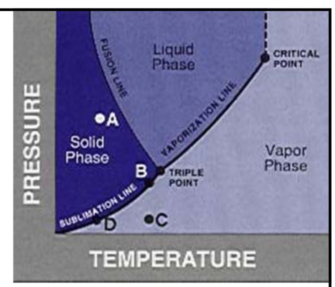
Freeze Drying II

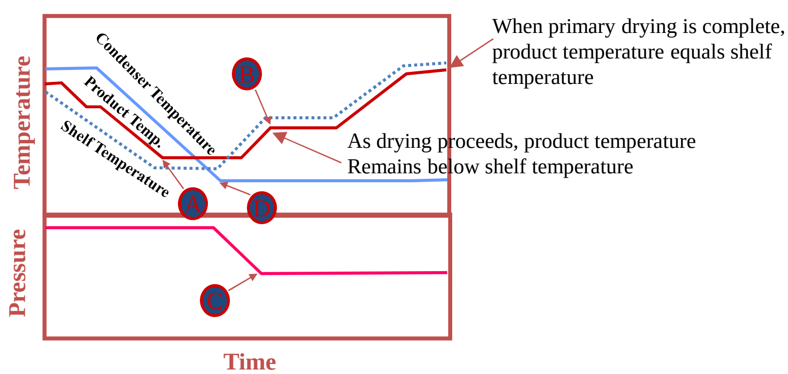
- Secondary Drying
  - Removal of last 5-10% of moisture
  - Higher temp: isothermal desorption
  - 30-50% of primary drying time
- Source of heat during drying
  - Ambient (room) heat
    - Manifold drying (Model 8)
    - May be increased with a water bath
  - Thermal conductor shelf
    - Tray drying (FreeZone 6)



Tray Dryers

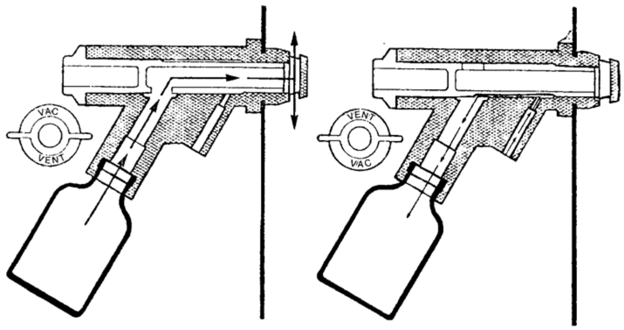


- Typical Sublimation Cycle



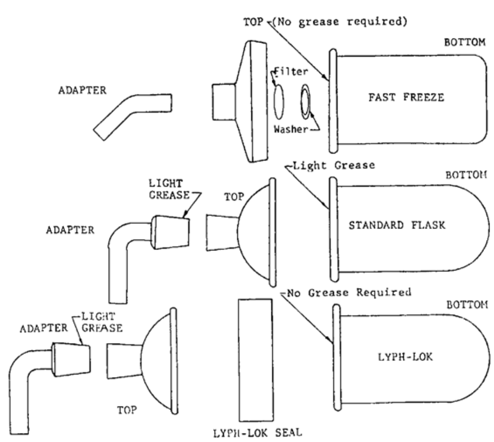
Manifold Dryers

- External valve



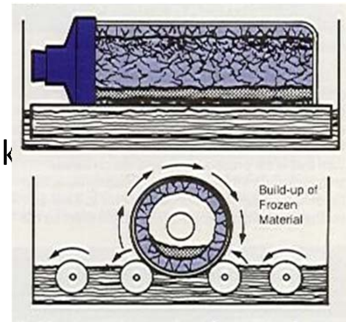
Manifold Dryers

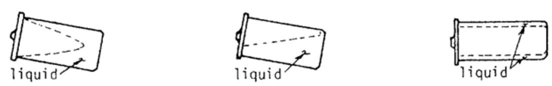
- Flasks



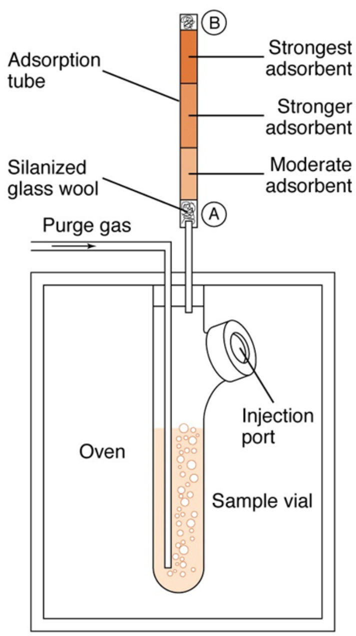
Manifold Dryers

- Pre-freezing
  - Either shell freeze or angle flask to avoid breakage
    - Water expands when frozen





Purge and Trap



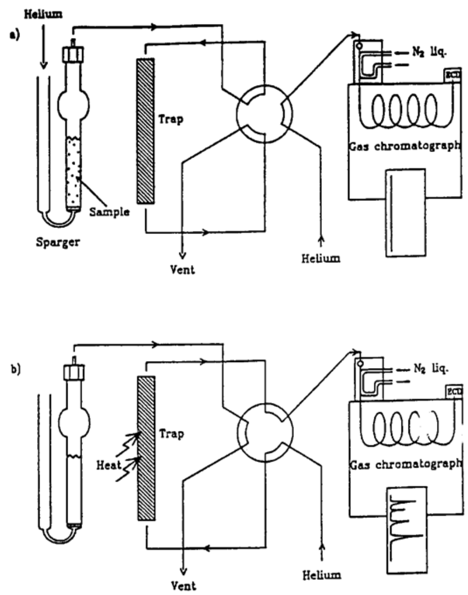
The purge and trap method is used specifically to remove and concentrate volatile analytes from liquids or solids. The goal in this case is to concentrate 100% of the analyte

Purge gas is swept through the heated sample and volatilized components go with it into a “trap”. The trap contains particles made of adsorbent compounds that the analytes adsorb onto.

After trapping the analytes for a specified procedure time, the flow through the trap is reversed and analytes desorb back off of the trap and into the injection port of the GC.

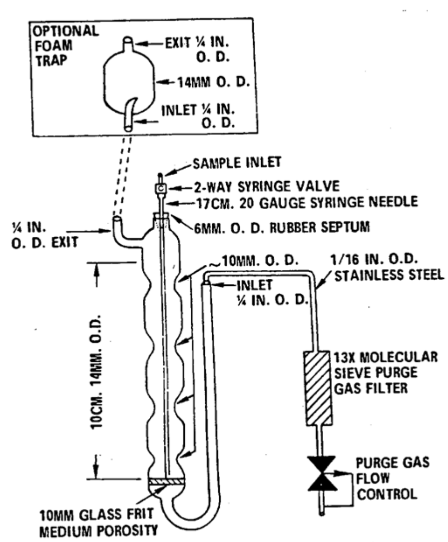
Purg

- A. purge & simultaneous trap
- B. thermal desorption

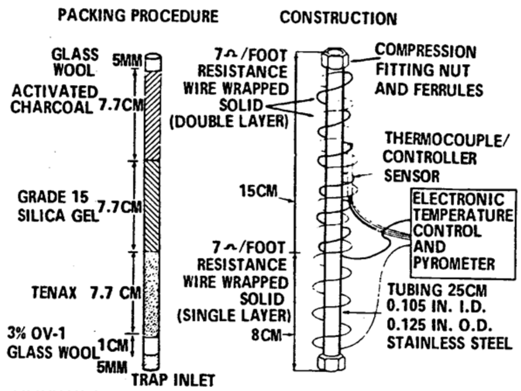


P&T

- EPA purging vessel design
  - 5 mL sample
  - Room temp.
  - Use N_2 or He
  - 20-40 mL/min
  - 10-15 min



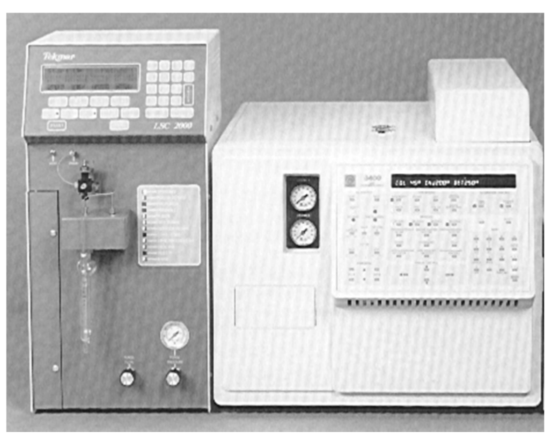
- Example Trap
  - Desorb @ 180 C
  - 20-60 mL/min backflush
  - 1.5-4 min
  - Tenax
    - 2,6-diphenylene oxide polymer
  - OV-1
    - Methyl silicone polymer
- Used for EPA methods#
  - 8010: Halogenated VOCs
  - 8015: Nonhalogenated VOCs (high temp): Ether, Ethanol, MEK
  - 8020: Aromatic VOCs (BTEX, chlorobenzenes)
  - 8030: (high temp) Acrolein, Acetonitrile, Acrylonitrile



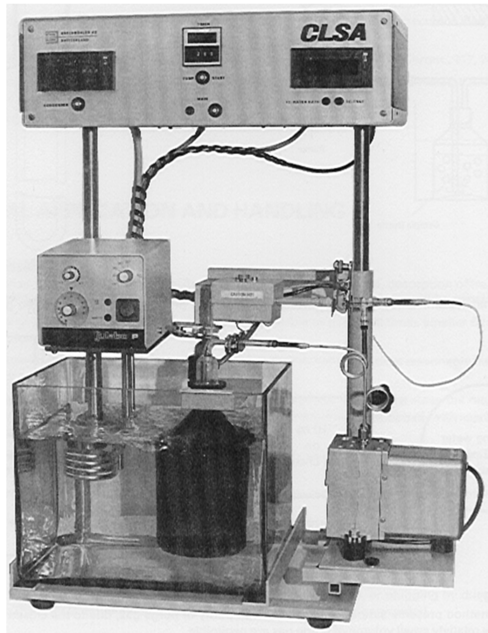
From CFR Title 40

Purge and Trap

- Commercial Unit
  - Varian LSC-2000

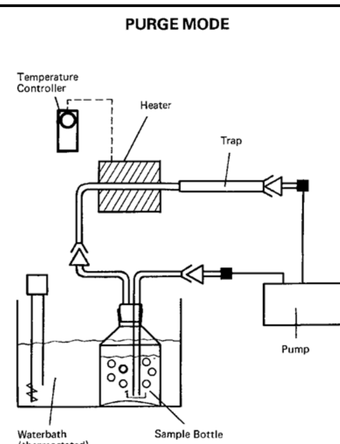


Closed Loop Stipping

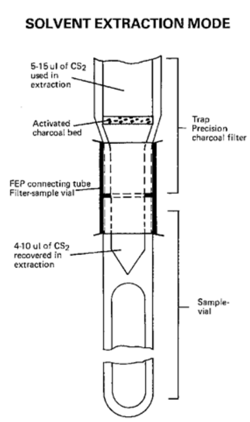


- Brechbuhler unit
  - Marston 5
- High concentration factor
  - 1 liter of sample down to 0.010 mL extract

CLSA



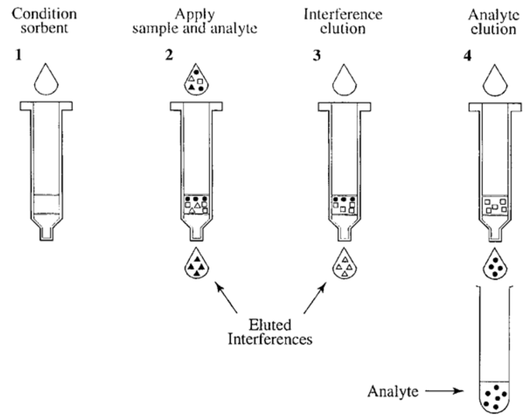
- Purge and Adsorb on 1.5 mg activated carbon



- Extract into carbon disulfide

Solid Phase Extraction

- Steps
  - 1. Addition of solvent to solvate the functional groups
  - 2. Adsorption of s step): 1 mL to 1 L
  - 3. Sample clean-u
  - 4. Elution



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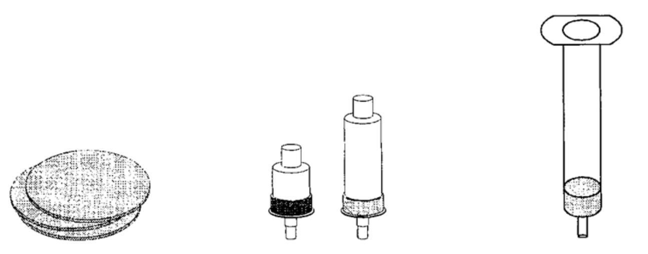
SPE cont.

- Three formats: disks, cartridges & syringe barrel



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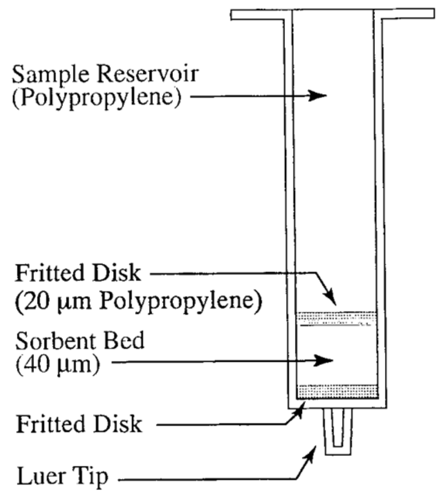
SPE cont.

- Cut-away of a syringe barrel



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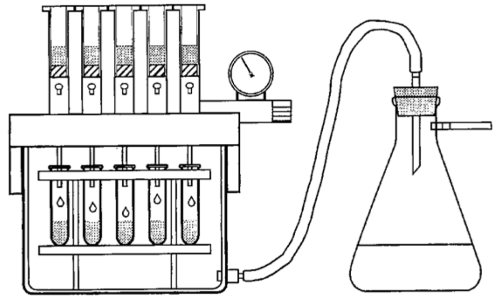
SPE cont.

- Syringe barrel method with vacuum manifold



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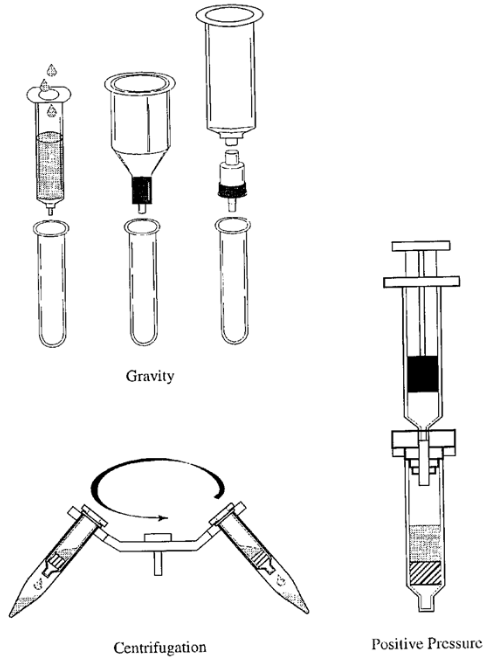
SPI

- Other methods of sample application to syringe barrels



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SP

- Disk methods

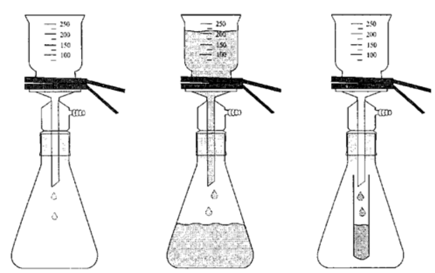

Figure 11.5. Procedure for using Empore disk, SPEC-47, Novo-Clean disk, or the Speedisk for analysis of water samples using a reversed-phase sorbent.

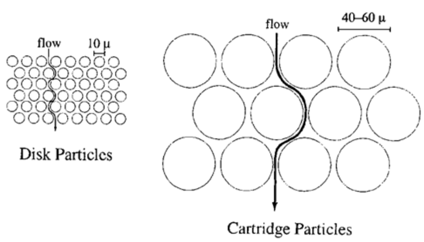

Figure 11.7. Relative difference in particle size between a disk and standard SPE cartridge.

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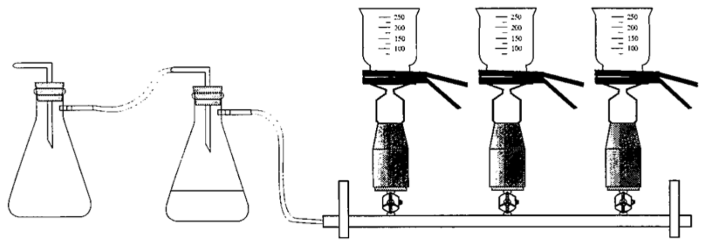
SPE cont.

- Manifold assembly for disk extractions



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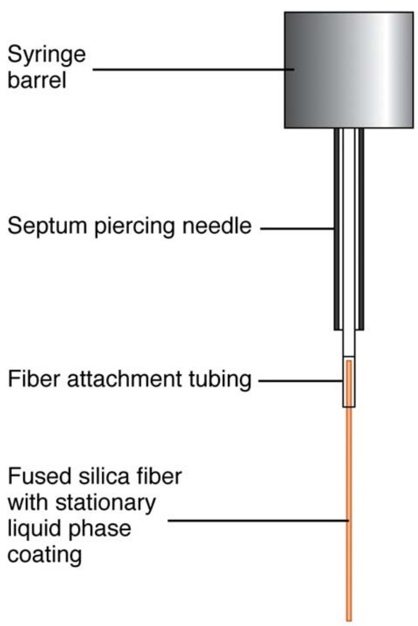
SPME



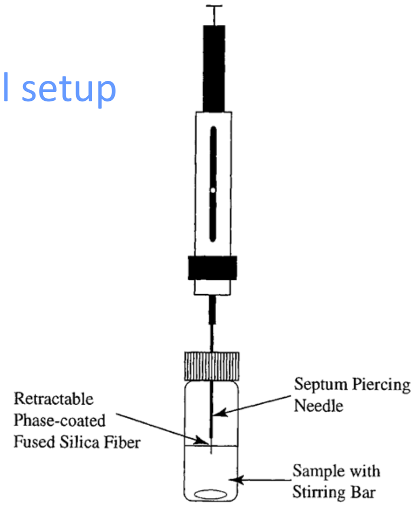
SPME is used to extract from liquid, air, or sludge without using any solvents. A silica fiber coated with a stationary phase for a GC is attached to a syringe. The fiber is exposed to sample for a certain time to allow the phase to become saturated with analyte.

After sampling, the fiber is retracted into the syringe and the syringe gets injected into the inlet of the GC. SPME does not remove all of the analyte because it is an equilibrium reaction. It usually can obtain 30-50% of the molecules.

Because the binding to the stationary phase of the fiber is a partitioning reaction, there is an equilibrium involved. Equilibration time for analytes must be obtained using calibration experiments.

SPME

- Schematic of a typical setup



From: Solid Phase Extraction by Thurman & Mills

Table I. Characteristics of Polymeric Adsorbents Used for Water Analyses

Common Name of Adsorbent	*Type*	*Area (m^2/g)*	*Pore Size (Å)*	*Method Refs.*	*Supplier*
Chromosorb 102	styrene-DVB	350	90	357	Johns-Manville
Chromosorb 105	polyaromatic	650	500	68, 353	Johns-Manville
Chromosorb 106	polystyrene	750	—	68, 353	Johns-Manville
Ostion SP-1	styrene-DVB	350	85	109, 124	Laboratory Instr.
Synachrom	ethylvinylbenzene-DVB	570	45	425	Laboratory Instr.
XAD-1	styrene-DVB	100	200	76, 78	Rohm and Haas
XAD-2	styrene-DVB	350	90	78	Rohm and Haas
XAD-4	styrene-DVB	780	50	112, 386	Rohm and Haas
Porapak Q[a]	ethylvinylbenzene-DVB	735	70	68, 337	Waters Associates
XAD-7	methyl methacrylate	450	80	114, 191	Rohm and Haas
XAD-8	methyl methacrylate	140	250	329	Rohm and Haas
Spheron MD	methacrylate-DVB	320	—	427	Laboratory Instr.
Spheron SE	methacrylate-styrene	70	—	417	Laboratory Instr.
Tenax	diphenylphenylene oxide	20	720	345, 346	Applied Science
Polyurethane[b]	ester-open pore	0.6	—	87	varied
Polyurethane[b]	amide ester-foam	0.02	—	7, 83	varied
Polypropylene	propylene	—	—	415	varied
Polyethylene	ethylene	—	—	412, 414	varied
A-24[c]	anion exchange	28	—	449	Rohm and Haas
Teflon	tetrafluoroethylene	—	—	375, 377	varied
Chromosorb T	tetrafluoroethylene	5	—	378	Johns-Manville
Fluoropak 80	tetrafluoroethylene	3	—	378	Fluorocarbon Company
Unspecified[d]	coated supports	low	—	394	varied
Unspecified[e]	bonded phases	—[f]	—	68, 480	varied

NOTE: DVB denotes divinylbenzene.
[a] Other Porapaks (P to T) have surface areas from 300 to 700 m^2/g.
[b] Generally unspecified; many different types are available.
[c] Single example of many different kinds.
[d] Many possibilities are available.
[e] Most are porous silica with C_4 to C_{18} alkyl bonded groups.
[f] A wide range is available from about 100 to 600 m^2/g.

From: Junk, 1987

- Common sorbents used for SPE

Sorbent	Structure	Typical Loading
	Reversed Phase	
Octadecyl (C-18)	—$(CH_2)_{17}CH_3$	17%C
Octyl (C-8)	—$(CH_2)_7CH_3$	14%C
Ethyl (C-2)	—CH_2-CH_3	4.8%C
Cyclohexyl	—CH_2CH_2-cyclohexyl	12%C
Phenyl	—$CH_2CH_2CH_2$-Phenyl	10.6%
Graphitized carbon	Aromatic carbon throughout	
Copolymers	Styrene–divinylbenzene	
	Normal Phase	
Cyano (CN)	—$(CH_2)_3CN$	10.5%C, 2.4%N
Amino (NH_2)	—$(CH_2)_3NH_2$	6.4%C, 2.2%N
Diol (COHCOH)	—$(CH_2)_3OCH_2CH(OH)CH_2(OH)$	8.6%C
Silica gel	—SiOH	—
Florisil	Mg_2SiO_3	—
Alumina	Al_2O_3	—
	Ion Exchangers	
Amino (NH_2)	—$(CH_2)_3NH_2$	1.6 meq/g
Quaternary amine	—$(CH_2)_3N^+(CH_3)_3$	0.7 meq/g
Carboxylic acid	—$(CH_2)_2COOH$	0.4 meq/g
Aromatic sulfonic acid	—$(CH_2)_3$-Phenyl-SO_3H	1.0 meq/g
	Size Exclusion	
Wide pore hydrophobic (Butyl)	—$(CH_2)_3CH_3$	5.9%C
Wide pore ion exchangers	—COOH	12.2%C

From: Solid Phase Extraction by Thurman & Mills

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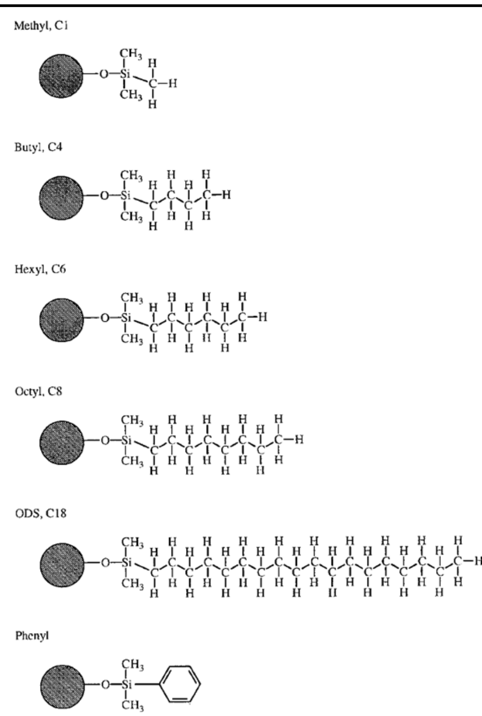
SPE

- Bonded phases



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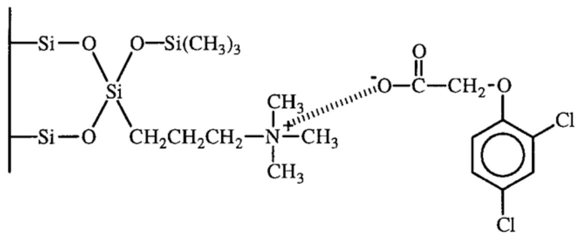
SPE cont.

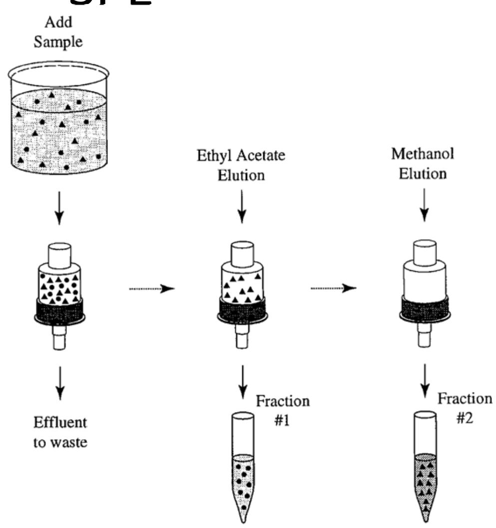
- Ion exchange mechanism for 2,4-D



From: Solid Phase Extraction by Thurman & Mills

SPE

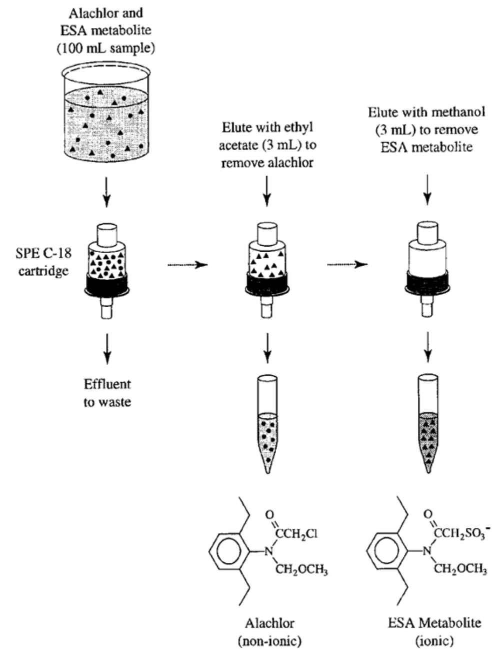
- Method for elutic of reverse phase SPE using ethyl acetate and methanol



From: Solid Phase Extraction by Thurman & Mills

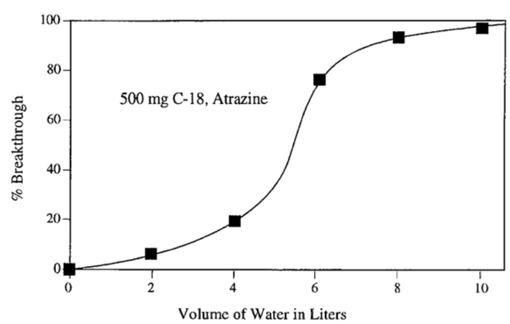
SPE

- Selective elution of alachlor and its sulfonic acid metabolite with ethyl acetate and methanol



From: Solid Phase Extraction by Thurman & Mills

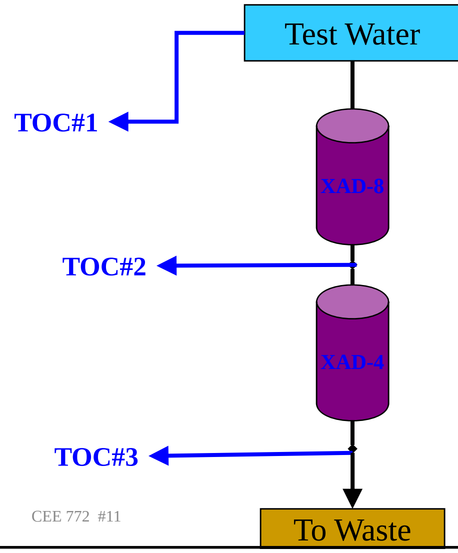
- Breakthrough curve
  - Atrazine on C18



From: Solid Phase Extraction by Thurman & Mills

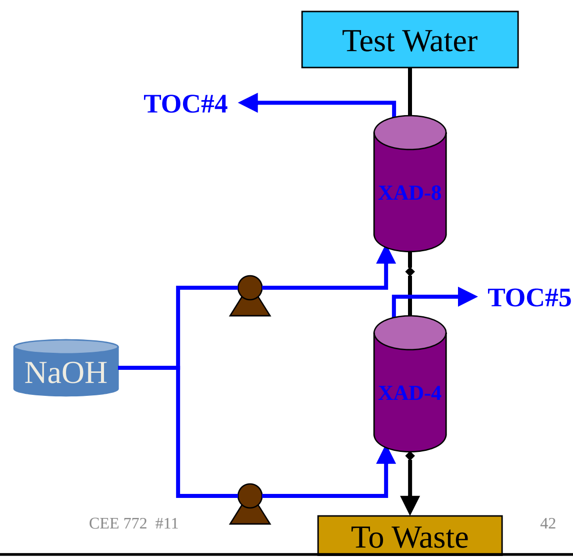
Simple Hydrophobicity Test

- Hydrophobic NOM
  - Retained on XAD-8
    - TOC#1-TOC#2
- Mesophilic NOM
  - Retained on XAD-4, but not on XAD-8
    - TOC#2-TOC#3
- Hydrophilic NOM
  - Not retained
    - TOC#3

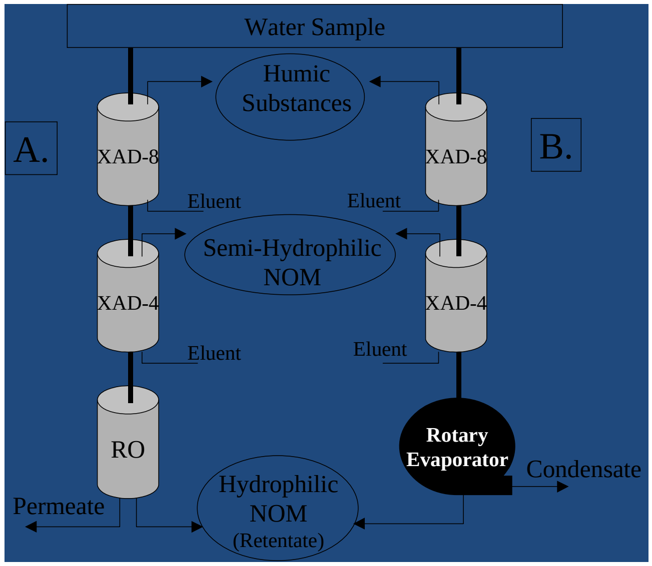


Isolation/Fractionation based on Hydrophobicity

- Back elution with NaOH
- Allows recovery of fractions and check of direct fractionation
- Desorbable hydrophobics = TOC#4
- Desorbable mesophilics = DOC #5



Fractionation based on Hydrophobicity II



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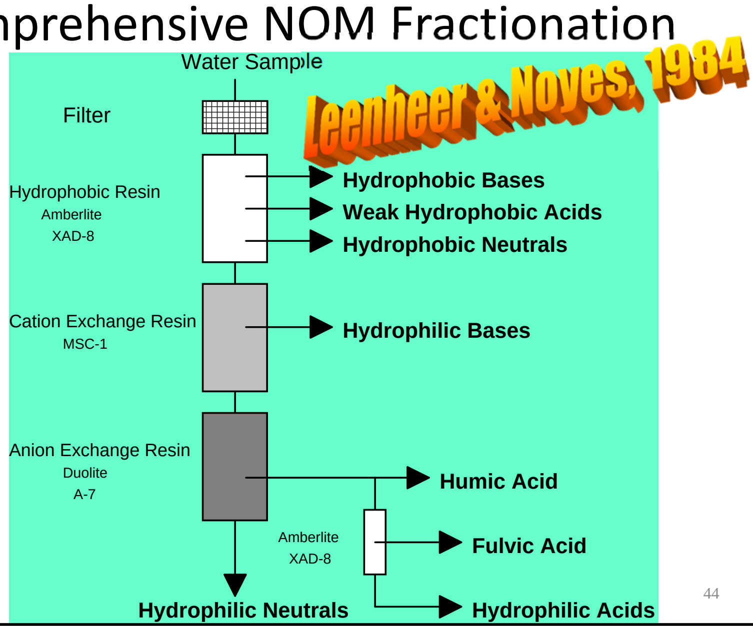
Comprehensive NOM Fractionation



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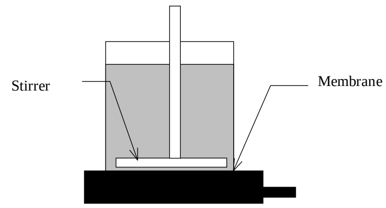
Molecular Size separations

- Ultrafiltration
  - series vs parallel
  - membrane calibration
- Size Exclusion Chromatography
  - HPSEC vs LC
- Others
  - FFF



- To next lecture