

Fundamentals of Sample Preparation for Environmental Analysis

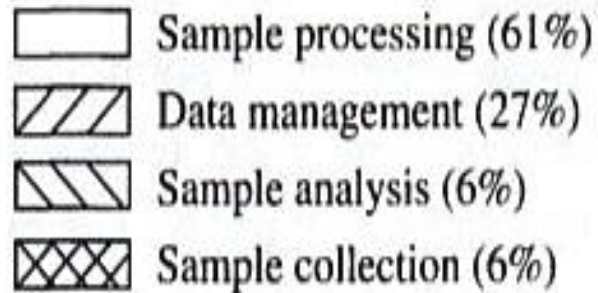
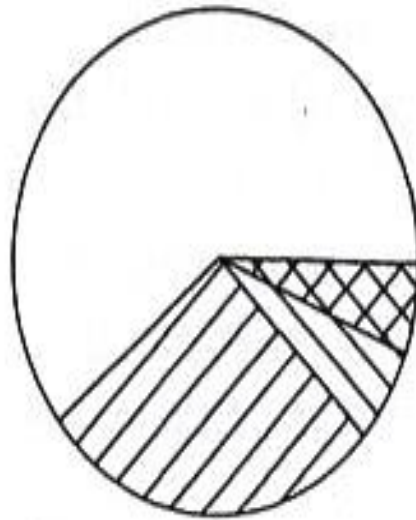
Contents

- Overview of Sample Preparation
- Sample Preparation for Metal Analysis
- Extraction for SVOC and Non-VOC from Liquid or Solid Samples
- Post-Extraction Clean-up of Organic Compounds
- Derivatization of Non-VOC for Gas Phase Analysis
- Sample Preparation for VOC, Air and Stack Gas Emission

Purpose Of Sample Preparation

- Very rarely can environmental samples be directly injected into an instrument without pretreatment
- Less Labor-intensive and time-consuming
- **Main categories:**
 - Digestion for metals
 - Extraction and post-extraction for SVOC's
 - Derivatization for non-VOCs
 - Preparation for VOC and air samples

- Sample preparation may not be needed, e.g.
 - Drinking water quality analysis
- May be very time consuming, e.g.
 - Chromatographic analysis



Purpose Of Sample Preparation

- Purpose may be one or a combination of the following:
 - (i) *To homogenize sample or remove moisture:* air-drying or freeze-drying, homogenization, grinding, and sieving
 - Assures that the subsample taken for analysis is representative
 - (ii) *To increase/decrease analyte concentration:* pre-concentration is needed for almost all trace analysis, dilution is used for the analysis of highly contaminated samples so the concentration falls within the calibration range

Purpose Of Sample Preparation

(iii) *To remove interfering chemicals*: major issue for trace organic compounds

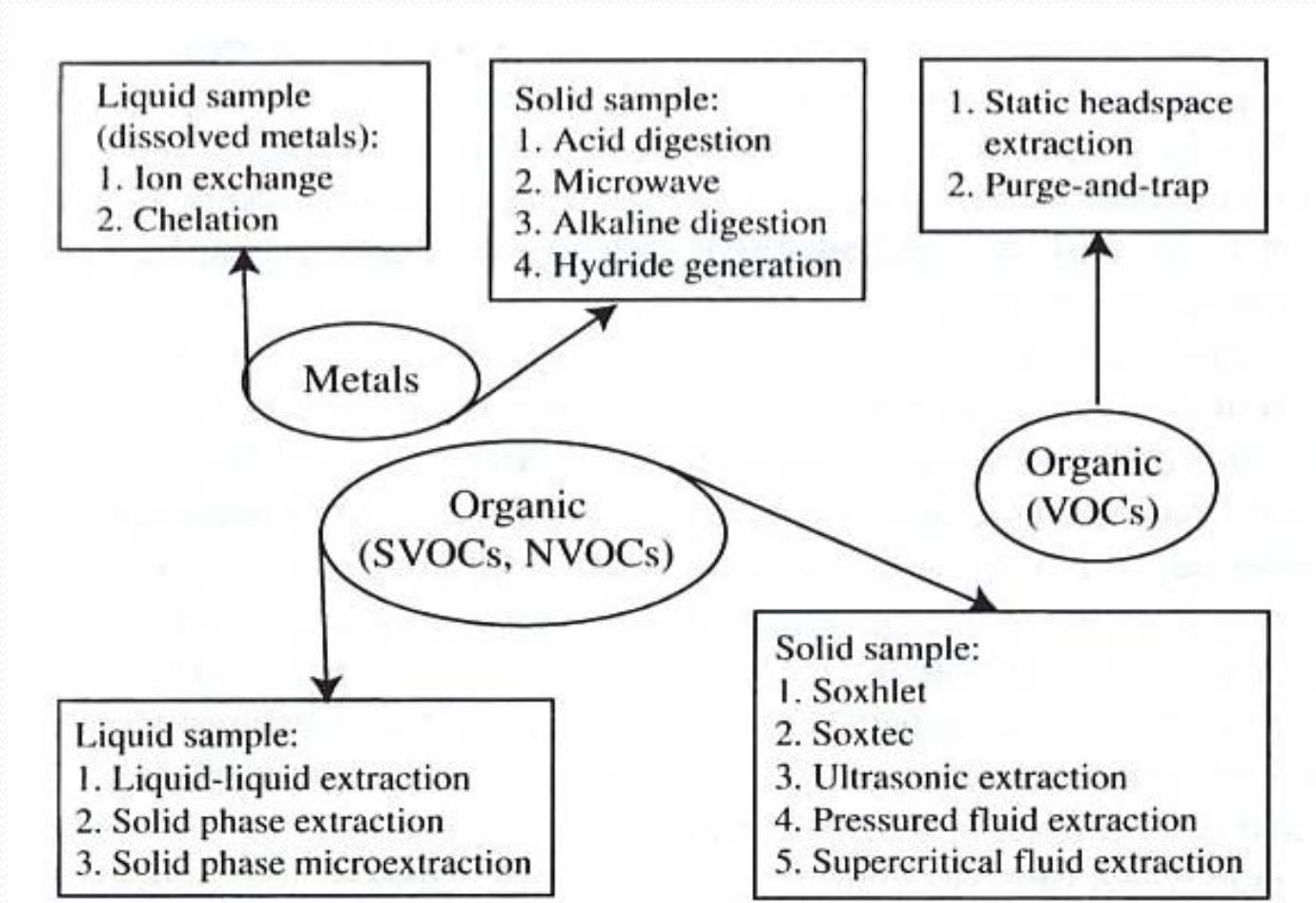
(iv) *To change sample phase*: sample phase may be needed to be changed to fit the instrument


(v) *To liberate analyte from sample matrix*: analyte species may be needed to be liberated from sample matrix

(vi) *To modify chemical structure*: chemical derivatization is used to increase or decrease volatility for HPLC or GC analysis

Types of Sample Preparation

- Types of needed sample preparation depend on the sample matrix, chemical properties, instrument used





Sample Preparation for Metal Analysis

Various Forms of Metals and Preparation Methods

Aqueous samples:

- ***Dissolved metals*** – hydrated ions, inorganic/organic complexes, colloidal dispersions
 - Operationally defined as dissolved metals in an unacidified sample that pass through a 0.45 μm filter
- ***Suspended metals*** – chemically bonded to Fe-Mn oxides, Ca oxides, or sorbed to organic matter
 - Operationally defined as metals in an acidified sample retained by a 0.45 μm membrane filter
- ***Total metals***
 - Equivalent to operationally defined by the metals determined in an unfiltered sample after acid digestion (total = dissolved +suspended)

Various Forms of Metals and Preparation Methods

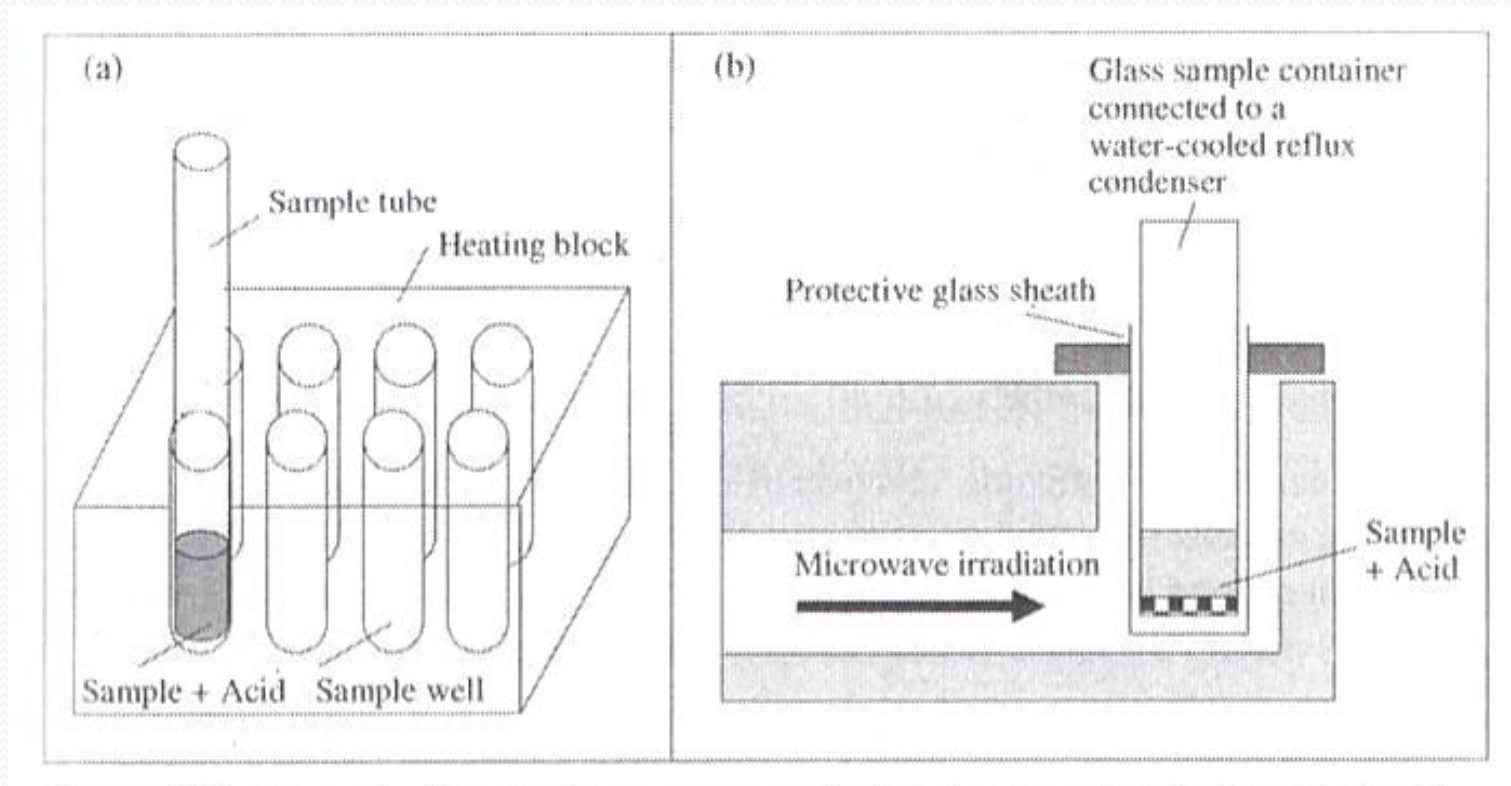
Solid samples:

- Metals in solid samples also have various forms (**speciation**)
- Species analysis mainly for toxicity/bioavailability assessments

Various Forms of Metals and Preparation Methods

Solid samples:

- *Total metal analysis* – acid digestion via hot-plate digestion or microwave-assisted



Principles of Acid Digestion and Selection of Acid

- Hotplate under ventilation hood (acid proof)
- Required to liberate analyte from sample matrix
- Different methods use different amounts of different acids
- Selection of acids should be based on instrument used



Principles of Acid Digestion and Selection of Acid

- Selection of acids should be based on:

(i) instrument used:

e.g. GFAA (Graphite Furnace Atomic Absorption) – do not use HCl since Cl-interferes

(ii) Sample matrix

(a) for clean samples or easily oxidizable materials use HNO_3

(b) for readily oxidizable organic matter, HNO_3 -HCl or HNO_3 - H_2SO_4

(c) for difficult to-oxidize organic matter, HNO_3 - HClO_4

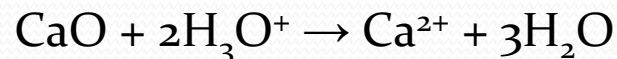
(d) for silicate materials, HNO_3 -HF

Why is HNO_3 acid of choice?

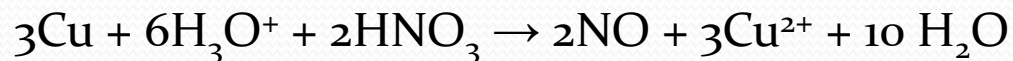
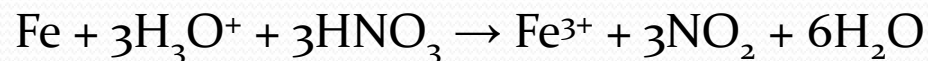
- Answer !

(i) HNO_3 acts as both acid and oxidizing agent

As acid it dissolves inorganic oxides



As oxidizing agent HNO_3 can oxidize zero valence inorganic metals and non-metals into ions



(ii) HNO_3 does not form any insoluble compounds with metals and nonmetals, whereas H_2SO_4 and HCl do

Alkaline Digestion and Other Extraction Methods

- Specific digestion/extraction procedures required for Cr, Hg, As, and Se
- Speciation is more important than total metal content

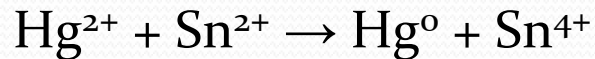
1. Chromium (Cr)

- Toxicity and mobility dependent on oxidation state, hexavalent Cr^{6+} , trivalent Cr^{3+}
- Cr^{6+} known carcinogen
- US-EPA method 3061A requires alkaline digestion using 0.28 M Na_2CO_3 /0.5 M NaOH heated at 90-95 °C for 60 min
- Extracts/dissolves Cr^{6+} from soluble, adsorbed, and precipitated forms of Cr compounds
- pH must be adjusted to avoid reduction of Cr^{6+} or oxidation of Cr^{3+}

Alkaline Digestion and Other Extraction Methods

2. Mercury (Hg)

- Nonessential element, all forms of inorganic and organic mercury are toxic
- Total Hg is measured using H_2SO_4 - HNO_3 with oxidizing agents KMnO_4 - $\text{K}_2\text{S}_2\text{O}_8$ (potassium permanganate-potassium persulfate)
- Acid digested sample containing Hg^{2+} is reacted with SnCl_2 to produce free Hg



- Hg is unique from analytical viewpoint, only metal which is volatile at room temperature
- Purged directly from samples by specific atomic absorption technique called cold vapor atomic absorption spectroscopy

Alkaline Digestion and Other Extraction Methods

3. Arsenic (As) and Selenium (Se)

Species of As and Se

- Both As and Se are metalloids
- Nonessential for plants, As is essential to some animals, Se to most
- Inorganic As has two oxidation states: arsenate, AsO_4^{3-} (As^{5+}) and arsenite, AsO_3^{3-} (As^{3+})
- Predominate As(5+) species in water is H_2AsO_4^- at pH 3-7 and HAsO_4^{2-} at pH 7-11
- Inorganic Se exists predominantly as selenate, SeO_4^{2-} (Se^{6+}) and selenite, SeO_3^{2-} (Se^{4+})
- Other forms include Se^{2-} , HSe^- , and Se^0

Alkaline Digestion and Other Extraction Methods

3. Arsenic (As) and Selenium (Se)

Digestion for total As and Se

- *Total recoverable As/Se:* H_2SO_4 - HNO_3 - HClO_4 digestion destroys organics and particulates in wastewater or solid samples (does not convert all organics)
- *Total As/Se:* H_2SO_4 - $\text{K}_2\text{S}_2\text{O}_8$ for converting organic As to As(V) and organic Se to Se^{6+} in water samples

Alkaline Digestion and Other Extraction Methods

3. Arsenic (As) and Selenium (Se)

Measurement of As Species

- *Arsenite* (AsO_3^{3-}): reduced by aqueous sodium borohydride (NaBH_4) to arsine AsH_3 at pH 6, AsH_3 measured by FAAS
- *Arsenate* (AsO_4^{3-}): sample from above is acidified with HCl and NaBH_4 , AsH_3 produced corresponds to amount of arsenate present
- *Total inorganic arsenic*: sample is reduced at pH 1 under above conditions

- AsH_3 is measured via atomic absorption

Alkaline Digestion and Other Extraction Methods

3. Arsenic (As) and Selenium (Se)

Measurement of Se Species

- *Persulfate ($S_2O_8^{2-}$) digestion*: small amount of potassium persulfate is added to a mixture of sample and HCl to remove interferences from reducing agents and to oxidize organic Se compounds. Used for most natural waters.
- *Alkaline hydrogen peroxide digestion*: H_2O_2 digestion required for organics Se compounds. Removes interfering reducing agents and oxidizes organic Se to Se^{6+} . Used for unfiltered water samples containing particulate Se.
- *Permanganate digestion*: $KMnO_4$ oxidizes Se and removes interfering organic compounds. Used for heavily contaminated water samples.

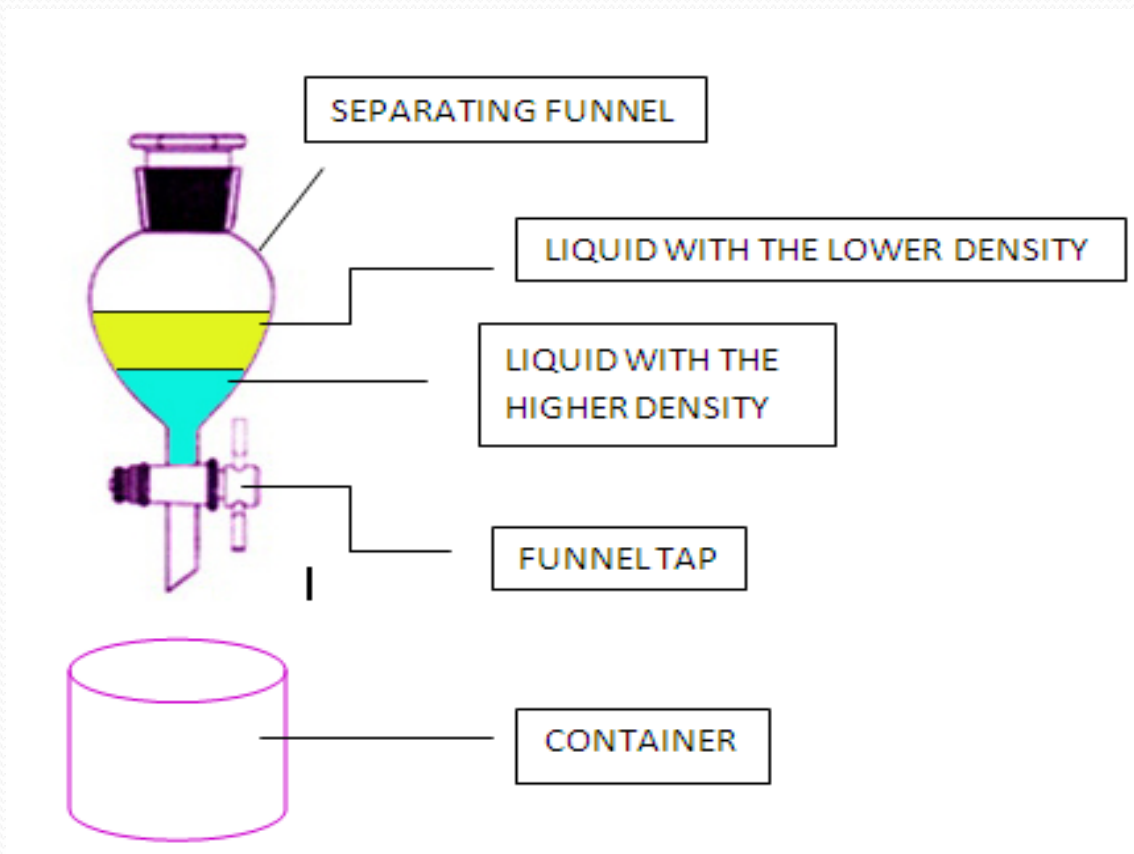


Extraction for SVOC and Non-VOC from Liquid or Solid Samples

- Extraction is the primary sample preparation method for organics
- Used for SVOCs and nonvolatile compounds (due to open vessels)
- Methods:
 - Liquid-Liquid extraction (LLE)
 - Solid Phase Extraction (SPE)
 - Solid Phase Micro Extraction (SPME)
 - Soxhlet extraction
 - Ultrasonic extraction
 - Pressurized Fluid Extraction (PFE)
 - Supercritical Fluid Extraction (SFE)

Separatory Funnel and Continuous Liquid-Liquid Extraction (LLE)

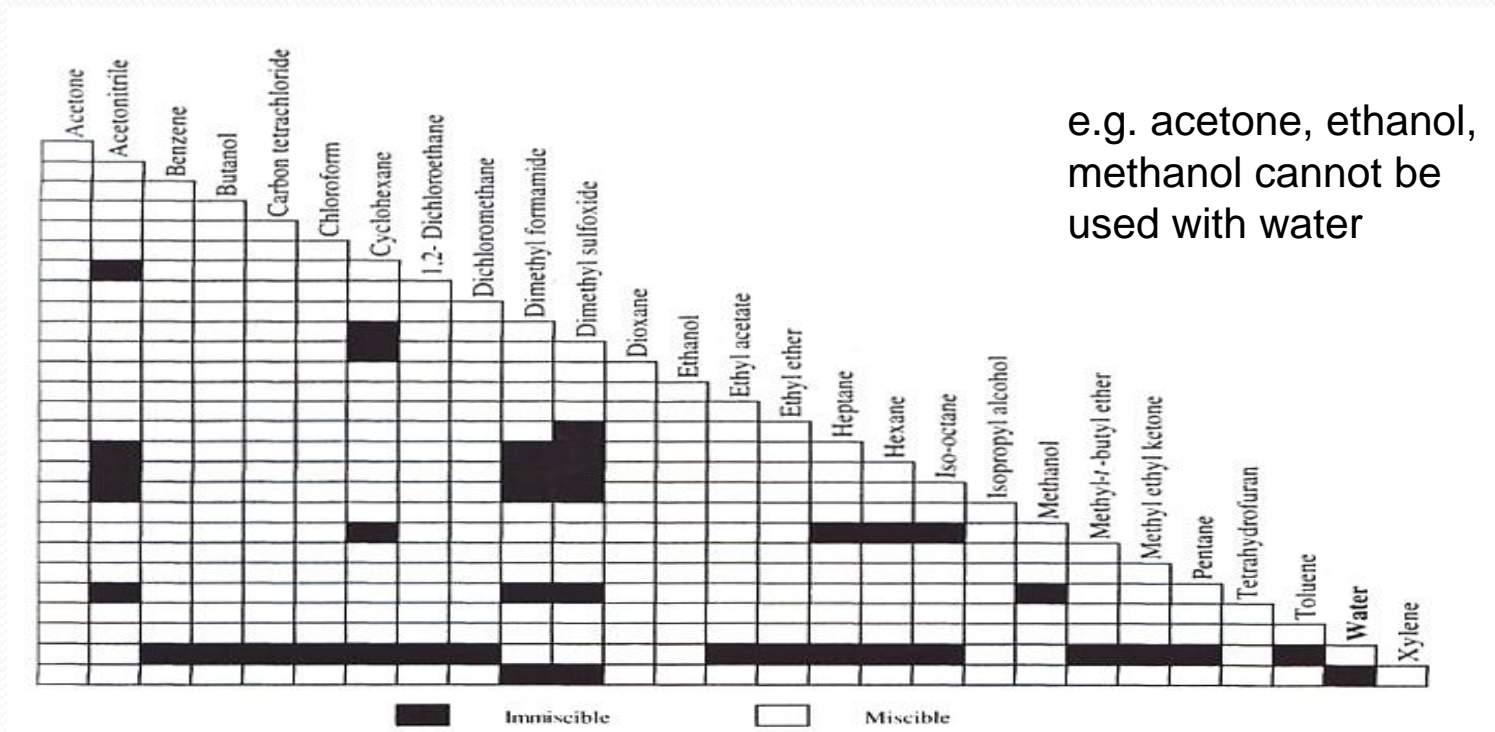
- Partitioning of analytes between water phase and organic phase



Separatory Funnel and Continuous Liquid-Liquid Extraction (LLE)

Solvent density and Immiscibility:

- Density difference is necessary
- Immiscibility of phases is necessary



Separatory Funnel and Continuous Liquid-Liquid Extraction (LLE)

Analyte Volatility:

- If analyte is volatile L-L extraction cannot be used
- Volatility can be compared based on Henry's Law constants (H)
 - Highly volatile: $H > 10^{-3}$ atm.m³/mol
 - Volatile: $10^{-3} < H < 10^{-5}$ atm.m³/mol
 - Semivolatile: $10^{-5} < H < 3 \times 10^{-7}$ atm.m³/mol
 - Nonvolatile: $H < 3 \times 10^{-7}$ atm.m³/mol

Separatory Funnel and Continuous Liquid-Liquid Extraction (LLE)

- Analyte Partition Coefficient:

Defined as

$$D = C_s/C_w$$

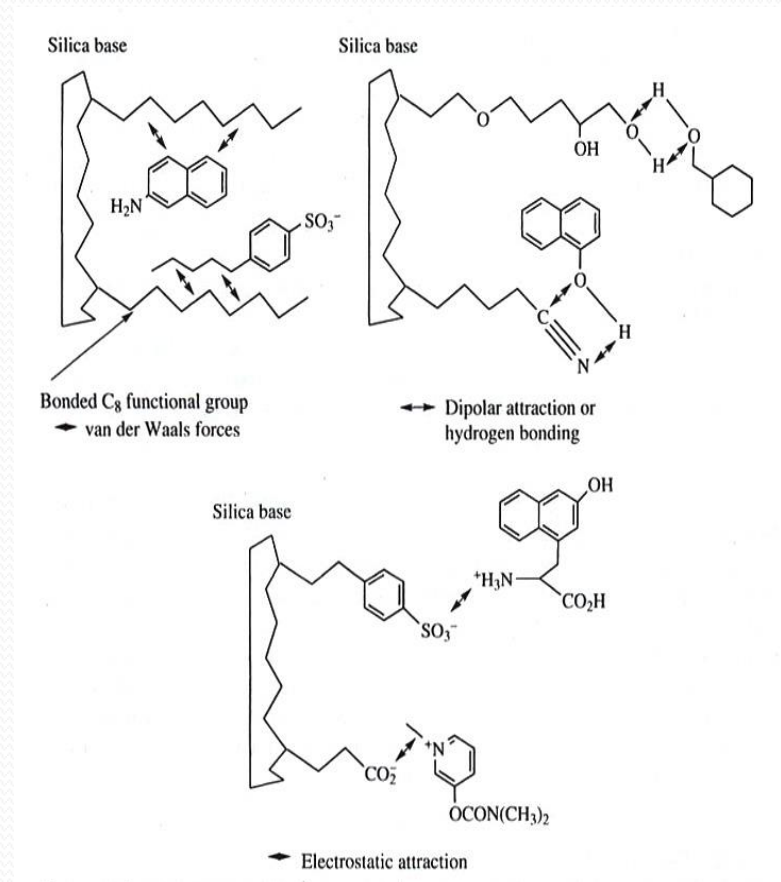
- Where C_s = equilibrium concentration in solvent
- C_w = equilibrium concentration in water
- Extraction efficiency is independent of initial analyte concentration, but dependent on D and water--solvent volume ratio V_w/V_s
- E increases with increasing D and decreasing V_w/V_s

Solid Phase Extraction (SPE)

- SPE retains analyte from a flowing liquid sample on solid sorbent, analyte is recovered via elution from the sorbent
- Phase types:
 - Reverse phase
 - Normal phase
 - Ion exchange
 - Adsorption

Solid Phase Extraction (SPE)

- Nonpolar:
 - Reverse phase C₁₈ (octadecyl bonded silica) and C₈ (octyl bonded silica) are most commonly used for hydrophobic analytes
- Polar:
 - Normal phase SPE uses cyanopropyl bonded, diol bonded, or amino propyl bonded silica (used for polar analytes such as cationic compounds and organic acids)
- Electrostatic:
 - Ionic Exchange SPE is based on electrostatics uses quaternary amine, sulfonic acid, or carboxylic acid bonded silica
- Adsorption type SPE uses unmodified materials such as alumina, Florosil, resins



Solid Phase Extraction (SPE)

- SPE uses a cartridge or disc
- May be performed a one time use syringe or a multiple cartridge unit

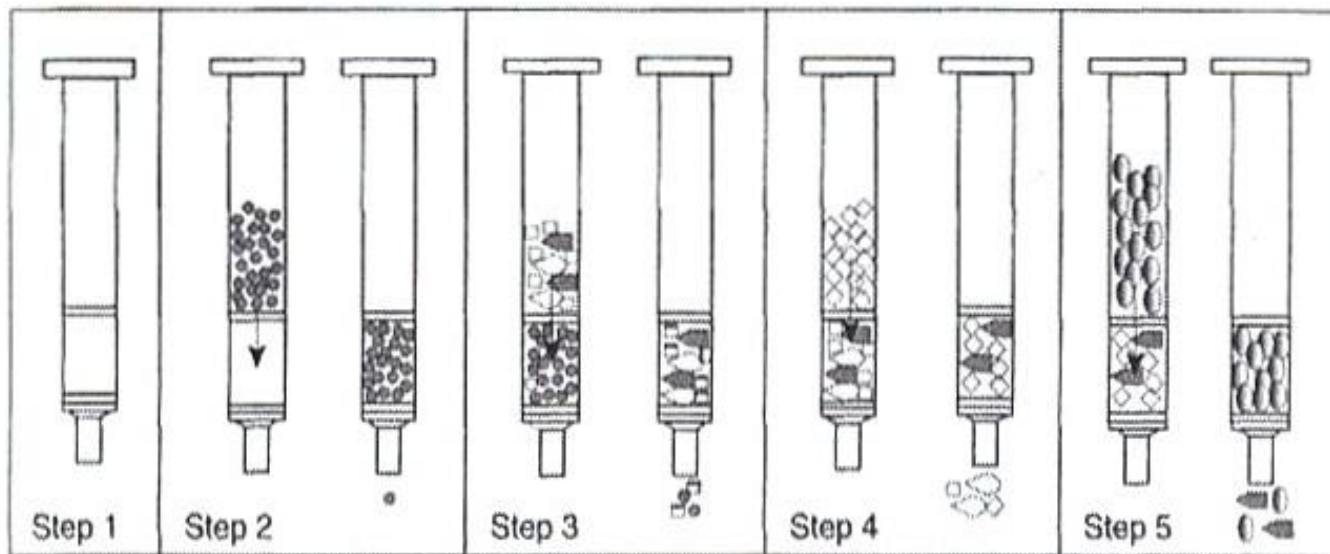


Figure 7.7 Five steps of solid phase extraction: (a) Select the proper SPE tube or disk from various commercially available SPEs, (b) Condition the SPE tube or disk, (c) Add the sample; (d) Wash the packing; (e) Elute the compounds of interest (Courtesy of Supelco)

Solid Phase Extraction (SPE)

- SPE uses a cartridge or disc
- May be performed a one time use syringe or a multiple cartridge unit

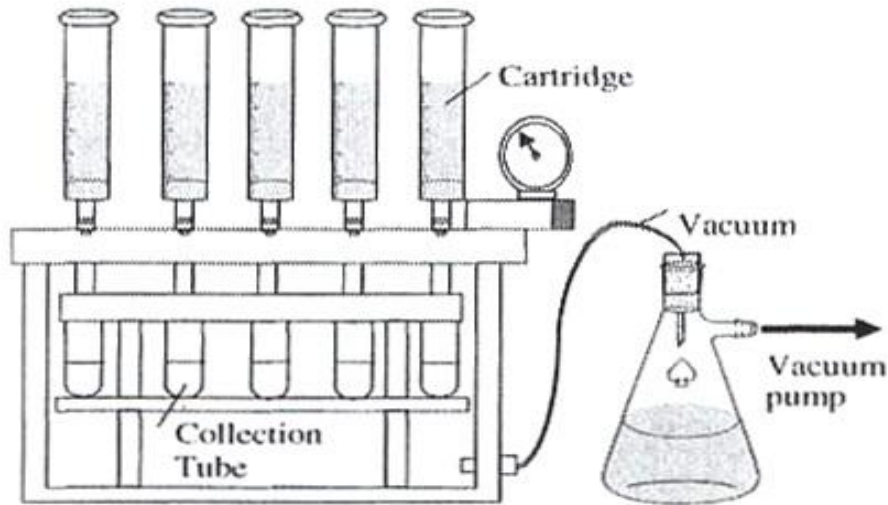


Figure 7.8 Vacuum manifold for solid phase extraction (SPE) of multiple cartridge units

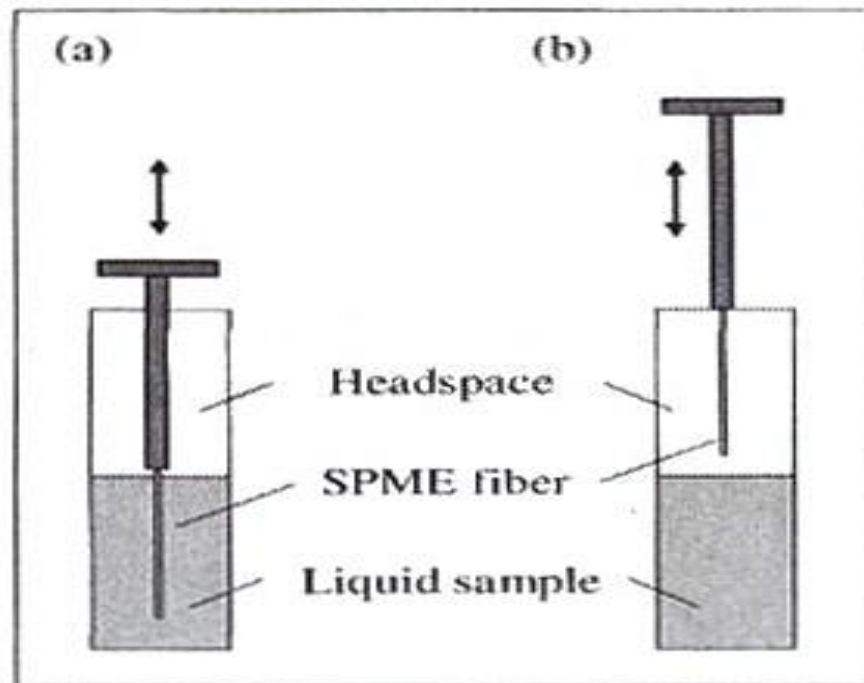
(c) Automation is possible for SPE, resulting in reduced analytical cost, time, and labor.

Solid Phase Microextraction (SPME)

- A 5 step process
- Advantages over L-L include: smaller volume solvent used, no emulsions, automation results in reduced time and cost

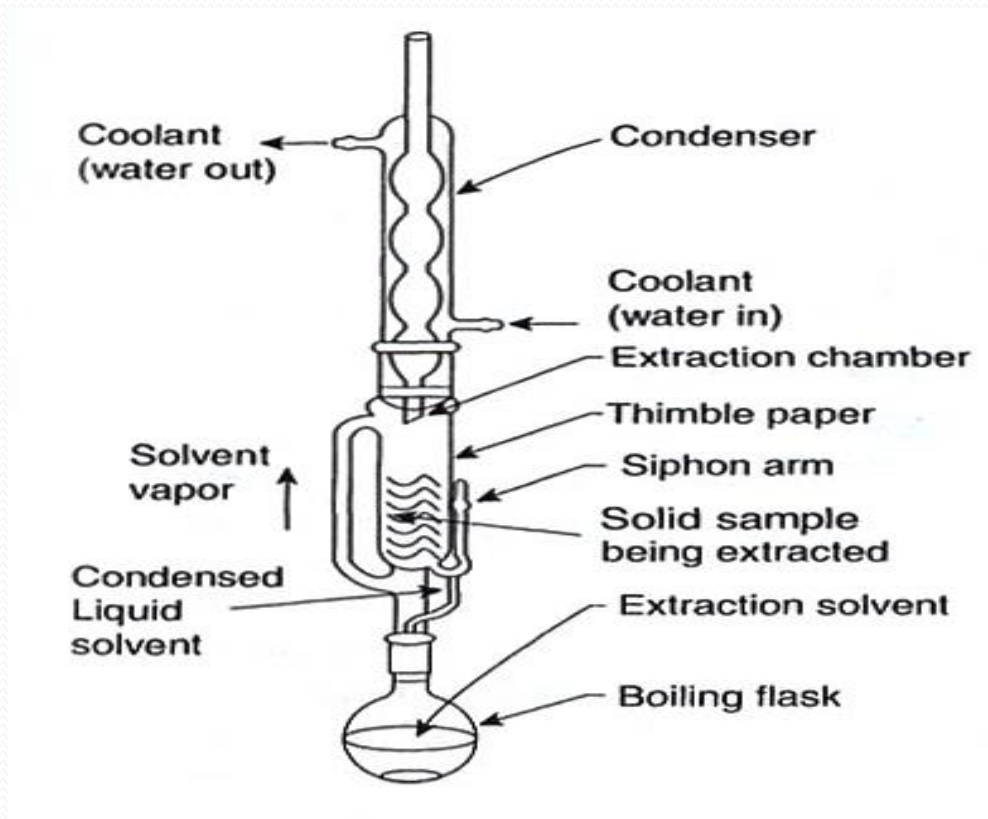
Solid Phase Microextraction (SPME)

- Based on a solvent-free sorption-desorption process
- SPME is a fused silica fiber coated with solid adsorbent
- Organic analytes absorb to the fiber, released by heating in GC port
- Combines extraction, concentration, and injection in one process



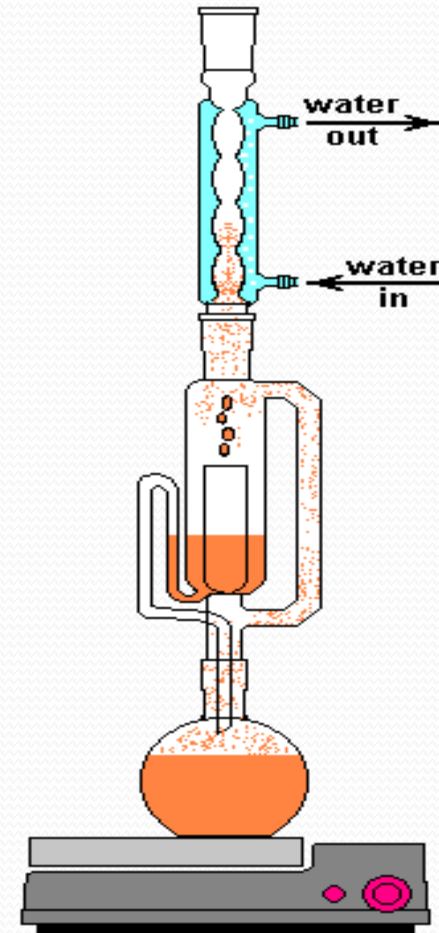
Soxhlet and Automatic Soxhlet Extraction

- Extraction of SVOCs from solids (soil, sludge, sediment)



Soxhlet and Automatic Soxhlet Extraction

- Dry solid sample is placed in a permeable cellulose 'thimble'
- Extraction solvent in the flask is heated to boiling
- Vapors rise through the outer chamber and into the condenser
- Condense and drip down onto the extracting sample
- Extraction chamber with sample fills until it empties through the siphon arm into the flask below
- Continues until solution in Soxhlet chamber is same color as solvent



Soxhlet and Automatic Soxhlet Extraction

- Time consuming (6-48 hr)
- Large solvent usage requires concentration step to evaporate solvent



Soxhlet and Automatic Soxhlet Extraction

- EPA uses approved Soxtec device (automated)
- Faster due to contact between boiling solvent and sample, solvent drips through sample (1 hr)
- Second stage sample is lifted above solvent (1 hr)
- Third stage concentrates to 1-2 mL via evaporation (20 min)

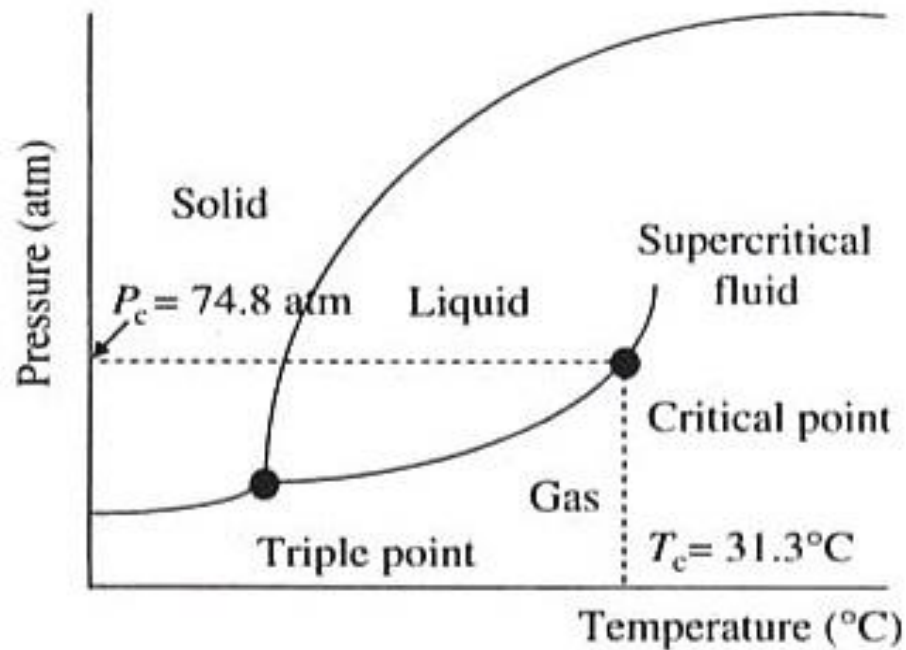
Ultrasonic Extraction

- A solid sample is mixed with anhydrous sodium sulfate (to absorb water)
- Extracted 3 times with a solvent mixture (1:1 acetone: CH_2Cl_2 ; 1:1 acetone hexane)
- Uses 300 W ultrasonic disruptor horn
- Fast procedure but extraction efficiency is low



Supercritical Fluid Extraction (SFE)

- Extracting solvent is CO₂ in supercritical fluid state (SCF)
- SCF is defined as a substance above its critical temperature and pressure (highest temp. and pressure at which substance is a vapor and liquid in equilibrium)



Supercritical Fluid Extraction (SFE)

- CO₂ in SCF state has physical and thermal properties in-between pure liquid and gas forms
- Gas-like high mass transfer coefficient, liquid-like high solvent property
- High diffusivity of SCF allows it to penetrate porous solids

Post-Extraction Clean-Up of Organic Compounds

- “Cleanup” removes interfering chemicals from samples
- Critical for analysis of trace analytes
- Analytes are masked by high background organics

Theories and Operation Principles of Clean-up Methods

- Several methods available:
 - Alumina Column: porous granular aluminum oxide, packed into a column with water absorbing substance
Used to separate analytes from interfering compounds of a different chemical polarity
 - Florisil Column: activated form of magnesium silicate with basic properties, used for clean-up of pesticide residues and other chlorinated hydrocarbons, for separation of nitrogen compounds from hydrocarbons, and separation of aromatics from aliphatic-aromatic mixtures
 - Silica Gel Column: silica with weak acidic amorphous silicon oxide, forms strong H-bonds to polar materials leading to analyte decomposition

• *Theories and Operation Principles of Clean-up Methods*

- Gel Permeation Chromatography (GPC): size-based separation using hydrophobic gel, selectively passes large macromolecules (e.g. proteins, phospholipids, resins, lignins, fulvic, humic acids) whilst retaining smaller molecules, methylene chloride is then used to displace the analytes
- Acid-Base Partition Clean-up: liquid-liquid clean-up to separate acid analytes from base/neutral analytes, uses pH adjustment in a liquid-liquid extraction
- Sulfur Clean-Up: removed by agitation with powdered Cu, mercury, or tetrabutyl ammonium sulfite
- Sulfuric Acid/Permanganate Clean-up: removes most interferences except PCBs, chlorinated benzenes, and chlorinated naphthalenes, cannot be used for other analytes as it will destroy them

Recommended Clean-up Method for Selected Compounds

Table 7.2 Selection of cleanup methods and their environmental applications

Purpose of cleanup	Cleanup type (mechanism)	Method no.
Analysis of phthalate esters (neutral alumina) and nitrosamines (basic alumina)	Alumina (adsorption)	EPA 3610
Separation of petroleum wastes into aliphatic, aromatic, and polar fractions (neutral alumina)	Alumina (adsorption)	EPA 3611
Analysis of phthalate esters, nitrosamines, organochlorine pesticides, PCBs, chlorinated hydrocarbons, organophosphorus pesticides	Florisil (adsorption)	EPA 3620
Analysis of PAHs, PCBs, and derivatized phenol	Silica gel (adsorption)	EPA 3630
Analysis of phenols, phthalate esters, nitrosamines, organochlorine pesticides, PCBs, nitroaromatics, cyclic ketones, PAHs, chlorinated hydrocarbons, organophosphorus pesticides, priority pollutant SVOCs	Gel-permeation (size-separation)	EPA 3640
Analysis of phenols, priority pollutant semivolatile	Acid-base partition (partitioning)	EPA 3650
Analysis of organochlorine pesticides, PCBs, priority pollutant SVOCs	Sulfur clean-up (oxidation-reduction)	EPA 3660
Analysis of PCBs, chlorinated benzenes and chlorinated naphthalenes	H ₂ SO ₄ -permanganate (oxidation-reduction)	EPA 3665



Derivatization of Non-VOC Gas Phase Analysis

- Many compounds of interest (particularly high MW compounds with polar functional groups) are difficult to analyze
- Derivatization transforms a chemical into a derivative
- Used for the following:
 - (a) increase volatility and decrease polarity
 - (b) increase thermal stability
 - (c) increase detection response
 - (d) improve separation

- **Common methods:**

- *Silylation*: replaces active hydrogens with trimethylsilyl (TMS) group [-Si(CH₃)₃]. Silyl derivatives are more volatile and thermally stable
- *Acylation*: adds an acyl group (RCO-), converts compounds with active hydrogens into esters, thioesters and amides. Reduces polarity of amino, hydroxyl, and thio groups
- *Alkylation*: reduces polarity by replacing active hydrogens with an alkyl group (e.g. CH₃, C₂H₅). Acidic hydrogens in carboxylic acids and phenols form esters, ethers, and amide
- *Esterification*: acid reacts with alcohol to form an ester with a lower bpt.



Sample Preparation for VOC, Air and Stack Gases

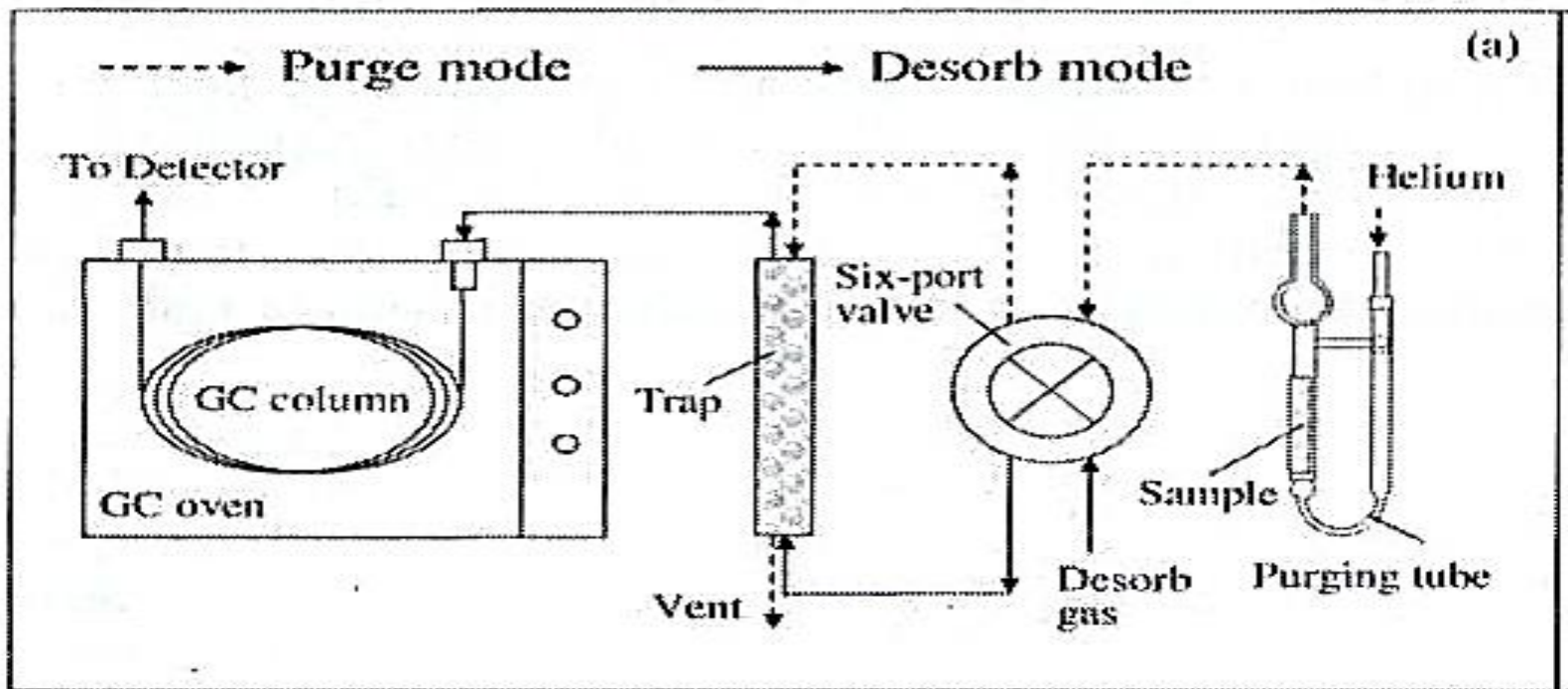
- Previous extraction methods do not apply to VOCs

Sample preparation methods for volatile organic compounds

Matrix	Extraction type	Analytes
Solids	Automated headspace	Volatile organics
Aqueous	Purge-and-trap	Volatile organics
Aqueous	Azeotropic distillation	Polar volatile organics
Aqueous and solids	Vacuum distillation	Nonpolar and polar volatile organics
Solids, organic solvents, oily waste	Closed system purge- and-trap	Volatile organics

Purge-and-Trap (*Dynamic Headspace Extraction*)

- P&T sample is purged with an inert gas (N_2 or He)
- Volatile materials are trapped in a sorbent
- Trap is designed for rapid heating so that it can be desorbed directly into GC
- Designed for compounds bpt. $< 200\text{ }^\circ\text{C}$ and insoluble/slightly soluble in water

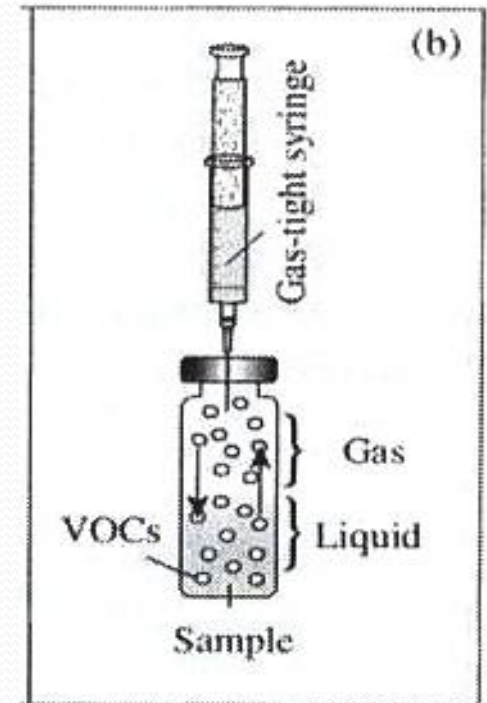


Static Headspace Extraction (SHE)

- Two phases in equilibrium in a sealed vial
- Gas phase is the headspace
- Sample phase may be solid or liquid
- Headspace equilibrates with sample phase via diffusion
- Partition coefficient:

$$K = C_g/C_s$$

- Where
- C_g = concentration of analyte in gas phase,
- C_s = concentration of analyte in sample phase



Azeotropic and Vacuum Distillation

- **Azeotrope** is a mixture of two or more liquids whose proportions cannot be altered by simple **distillation**.
- Each **azeotrope** has a characteristic **boiling point**.
- If the boiling point of an **azeotrope** is either less than the boiling point temperatures of any of its constituents (a **positive** azeotrope)
- if the boiling point is greater than the boiling point of any of its constituents (a **negative** azeotrope)

Azeotropic and Vacuum Distillation

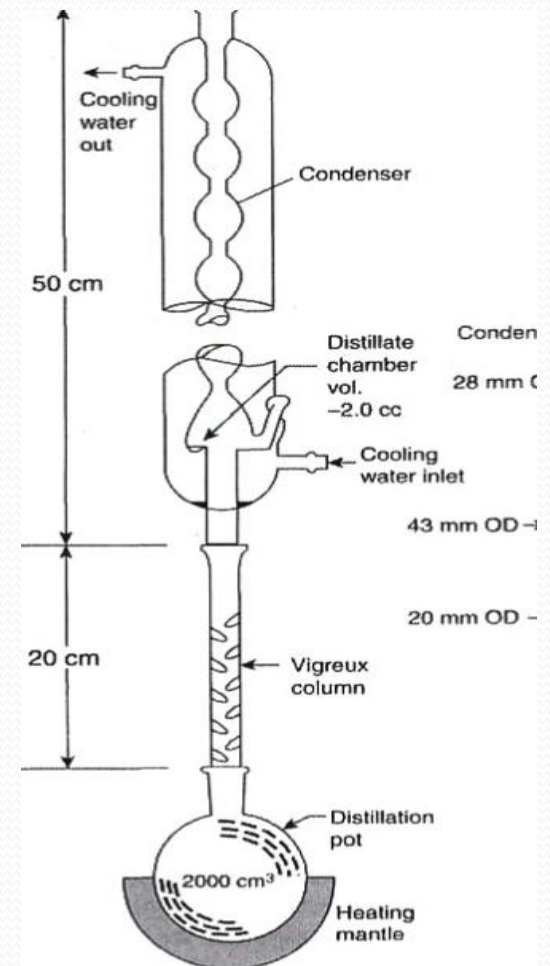
- **Azeotropic distillation** usually refers to the specific technique of adding another component to generate a new, **lower-boiling** azeotrope that is heterogeneous (e.g. producing two, immiscible liquid phases), such as the example below with the addition of benzene to water and ethanol.
- Designed for nonpurgable, water soluble (polar), and volatile organic compounds

Table 7.3 Volatile and non-purgeable compounds

Acetone	Acetonitrile	Acrylonitrile
Allyl alcohol	1-Butanol	<i>t</i> -Butyl alcohol
Crotonaldehyde	1,4-Dioxane	Ethanol
Ethyl acetate	Ethylene oxide	Isobutyl alcohol
Methanol	Methyl ethyl ketone	Methyl isobutyl ketone
<i>n</i> -Nitroso-di- <i>n</i> -butylamine	Paraldehyde	2-Pentanone
2-Picoline	1-Propanol	2-Propanol
Propionitrile	Pyridine	<i>o</i> -Toluidine

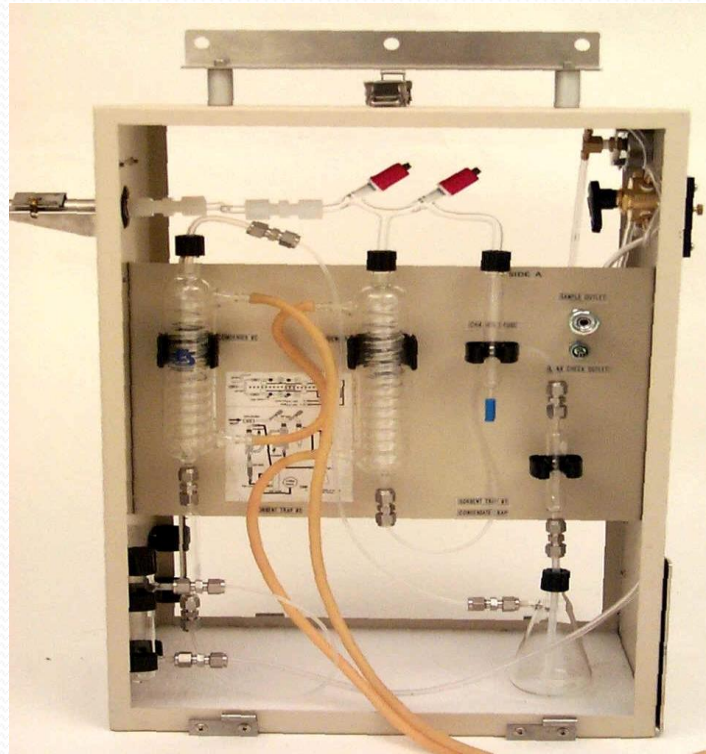
Azeotropic and Vacuum Distillation

- Azeotrope is a liquid mixture of two or more substances that behaves like a single substance
- Boils at single temperature, vapors have constant composition
- Azeotropic distillation uses ability of selected compounds to form binary azeotropes with water in order to separate the compound from a matrix
- Vacuum distillation can be used for VOCs with bpt. $< 180\text{ }^{\circ}\text{C}$, insoluble/slightly soluble
- For example BTEX compounds (benzene, toluene, ethylbenzene, xylenes)



Volatile Organic Sampling Train (VOST)

- VOST used to collect volatile principal organic hazardous constituents from gas effluents of hazardous waste incinerators
- Consists of sorbent cartridge (Tenax), impinger for condensate removal, water-cooled gas condenser, a second sorbent cartridge + charcoal, silica gel drying tube, calibrated rotameter, pump, and a dry gas meter



Comparison and Selection of Organic Extraction Methods

- Many methods appear to have time and cost advantages over L-L extraction (liquid samples) and Soxhlet extraction (solids samples)
- Extraction efficiencies are lower for these new methods

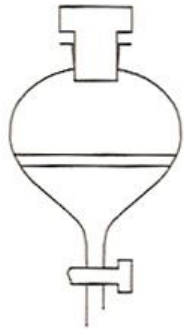
Table 7.1 Comparison of sample preparation techniques for SVOCs and VOCs

Extraction method	Application	Cost	Extraction time	Solvent usage	Simplicity	EPA method
Purge & trap	VOCs (L/S)	High	30 min	None	No	5030, 5035
Headspace	VOCs (L/S)	Low	30 min	None	Yes	3810, 5021
LLE	SVOCs, NVOCs (L)	Low	1 h	500 mL	Yes	3510/3520
SPE	SVOCs, NVOCs (L)	Medium	30 min	100 mL	Yes	3535
SPME	VOCs, SVOCs, NVOCs (L)	Low	30 min	None	Yes	None
Soxhlet/ Soxtec	SVOCs, NVOCs (S)	Low	4–24 h	250/50 mL	Yes	3540/3541
Ultrasonic	SVOCs, NVOCs (S)	Medium	10 min	200 mL	Yes	3550
SFE	SVOCs, NVOCs (S/L)	High	30 min	20 mL	No	3560/3561

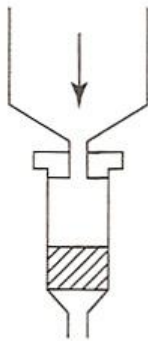


Test

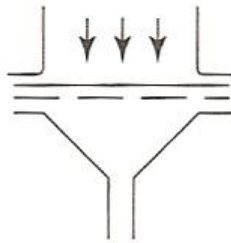
(a)



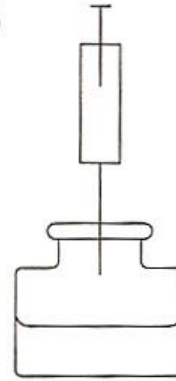
(b)



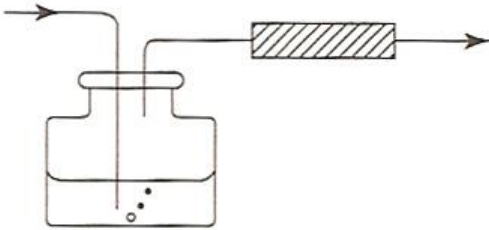
(c)



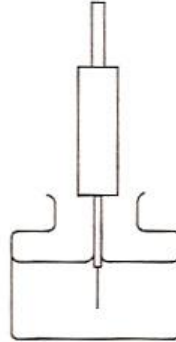
(d)



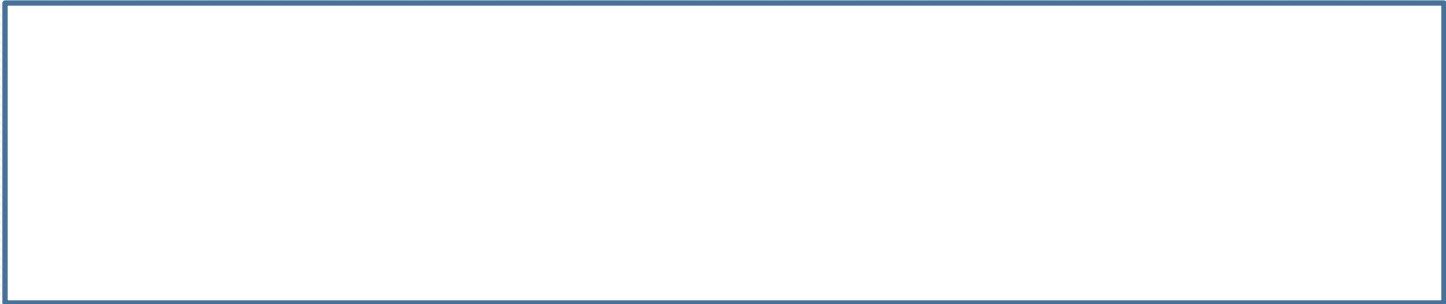
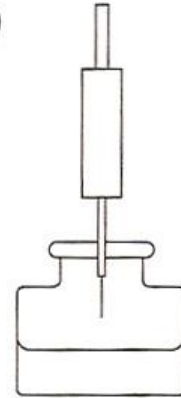
(e)



(f)



(g)



Questions

1. Explain briefly: (a) why HNO_3 , rather than other acids is most commonly used for acid digestion? (b) why HF digestion is used for samples containing silicates? (c) why or why not acid-preserving samples is appropriate for dissolved metals analyses?
2. Explain why speciation analysis is particularly important for Cr, As, and Se? What are the major valence (oxidation number) for each of these three elements? Describe their impact with regard to plant, and animal/humans.
3. Explain: (a) why a chemical with a Henry's law constant (H) of $10^{-4} \text{ atm}\cdot\text{m}^3/\text{mol}$ cannot be separated using a separation funnel L-L extraction?
4. Explain: (a) why solid phase extraction (SPE) can only be used for liquid samples? (b) why anhydrous sodium sulfite is added during solid sample extraction? (c) why SPME can be used for both liquid and solid samples?
5. Discuss the advantage of: (a) microwave assisted acid digestion over hot plate digestion.



END