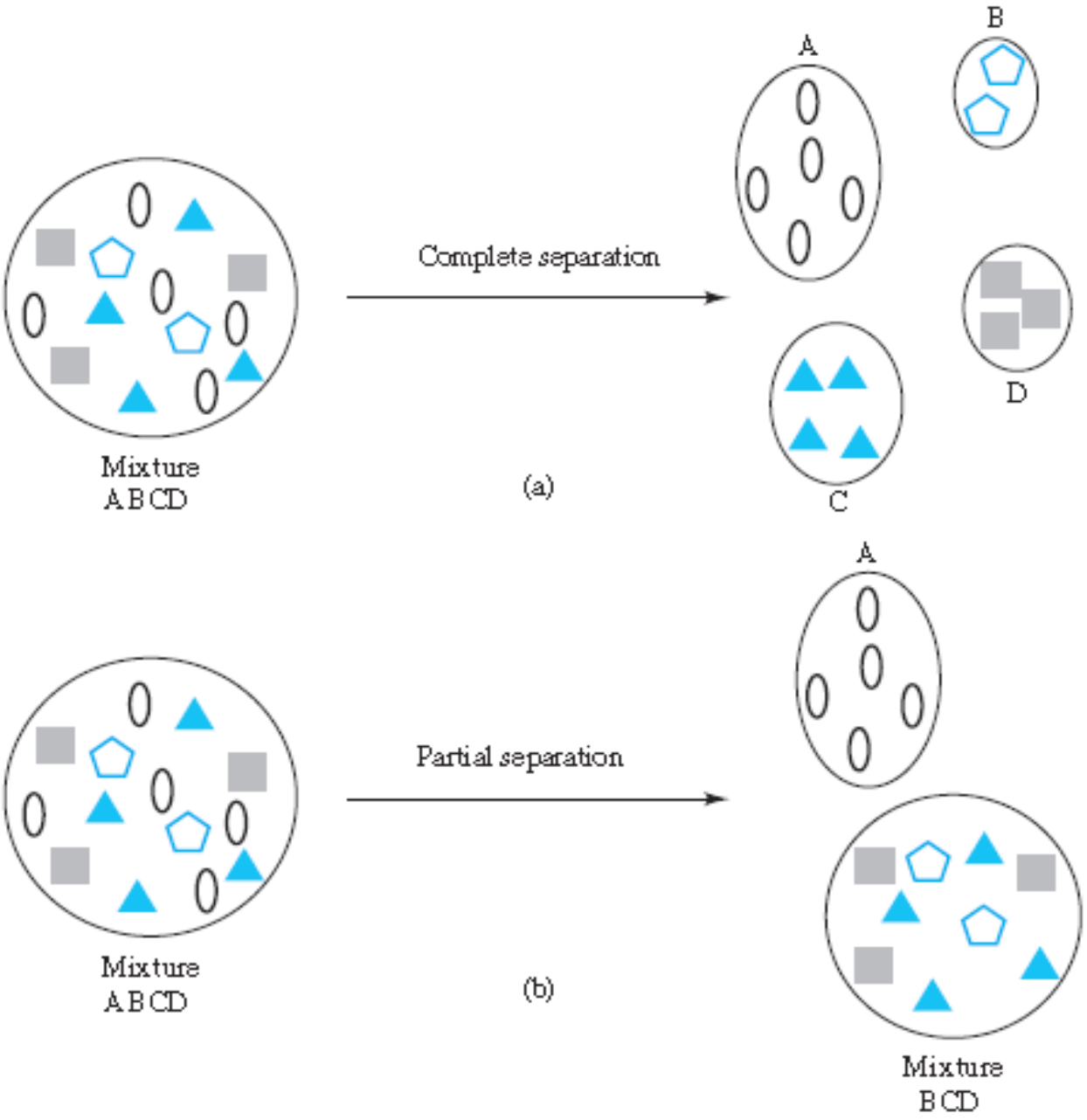
Analytical chemistry

Lecture №10

Methods of separation and concentration

Introduction to Analytical Separations



Separation principles

In (a), a mixture of four components is completely separated so that each component occupies a different spatial region. In (b), a partial separation is shown. In the partial separation, species A is isolated from the remaining mixture of B, C, and D.

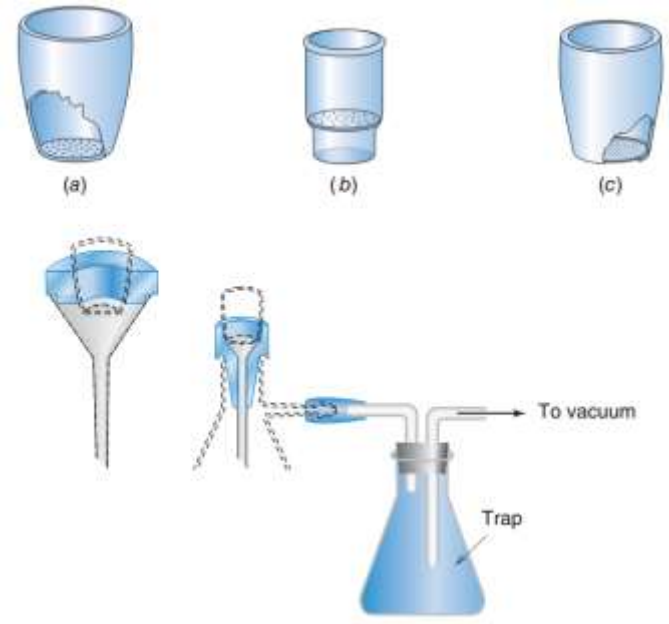
Classification of Separation Techniques

Basis of Separation	Separation Technique
size	filtration
	dialysis
	size-exclusion chromatography
mass and density	centrifugation
complex formation	masking
change in physical state	distillation
	sublimation
	recrystallization
change in chemical state	precipitation
	ion exchange
	electrodeposition
	volatilization
partitioning between phases	extraction
	chromatography

A substance that affects an analytical signal or the background is called an interference or an interferent. Several methods can be used to deal with interferences in analytical procedures. Separations isolate the analyte from potentially interfering constituents. In addition, techniques such as matrix modification, masking*, dilution, and saturation are* often used to offset the effects of interferents*.*

1. Separation based on size

The simplest physical property that can be exploited in a separation is size. The separation is accomplished using a porous medium through which only the analyte or interferent can pass. **Filtration**, in which gravity, suction, or pressure is used to pass a sample through a porous filter is the most commonly encountered separation technique based on size. Particulate interferents can be separated from dissolved analytes by filtration, using a filter whose pore size retains the interferent. This separation technique is important in the analysis of many natural waters, for which the presence of suspended solids may interfere in the analysis. Filtration also can be used to isolate analytes present as solid particulates from dissolved ions in the sample matrix. For example, this is a necessary step in gravimetry, in which the analyte is isolated as a precipitate.



1. Separation based on size

Dialysis as method of separation in which ansemipermeable membrane is used to separate the analyte and interferent. Dialysis membranes are usually constructed from cellulose, with pore sizes of 1–5 nm. The sample is placed inside a bag or tube constructed from the membrane. The dialysis membrane and sample are then placed in a container filled with a solution whose composition differs from that of the sample. If the concentration of a particular species is not the same on the two sides of the membrane, the resulting concentration gradient provides a driving force for its diffusion across the membrane. Although small particles may freely pass through the membrane, larger particles are unable to pass. Dialysis is frequently used to purify proteins, hormones, and enzymes. During kidney dialysis, metabolic waste products, such as urea, uric acid, and creatinine, are removed from blood by passing it over a dialysis membrane.

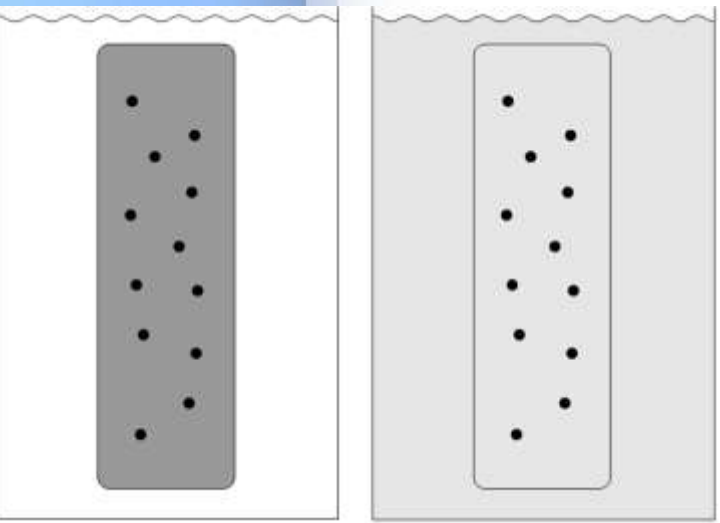


Illustration of a dialysis membrane in action.
In (a) the sample solution is placed in the dialysis tube and submerged in the solvent.
(b) Smaller particles pass through the membrane, but larger particles remain within the dialysis tube.

1. Separation based on size

Size-exclusion chromatography, which also is called ***gel permeation or molecularexclusion chromatography***. In this technique a column is packed with small, approximately 10-μm, porous particles of cross-linked dextrin or polyacrylamide. The pore size of the particles is controlled by the degree of cross-linking, with greater cross-linking resulting in smaller pore sizes. The sample to be separated is placed into a stream of solvent that is pumped through the column at a fixed flow rate. Particles too large to enter the pores are not retained and pass through the column at the same rate as the solvent. Those particles capable of entering into the pore structure take longer to pass through the column. Smaller particles, which penetrate more deeply into the pore structure, take the longest time to pass through the column. Size-exclusion chromatography is widely used in the analysis of polymers and in biochemistry, where it is used for the separation of proteins.

2. Separations Based on Mass or Density

If there is a difference in the mass or density of the analyte and interferent, then a separation using ***centrifugation*** may be possible. The sample, as a suspension, is placed in a centrifuge tube and spun at a high angular velocity (high numbers of revolutions per minute, rpm). Particles experiencing a greater centrifugal force have faster sedimentation rates and are preferentially pulled toward the bottom of the centrifuge tube. For particles of equal density the separation is based on mass, with heavier particles having greater sedimentation rates. When the particles are of equal mass, those with the highest density have the greatest sedimentation rate. Centrifugation is of particular importance as a separation technique in biochemistry.

Components	Centrifugal Force ($\times g$)	Time (min)
eukaryotic cell	1000	5
cell membranes, nuclei	4000	10
mitochondria, bacterial cells	15,000	20
lysosomes, bacterial membranes	30,000	30
ribosomes	100,000	180

3. Separations Based on Complexation Reactions (Masking)

Masking **-** a pseudo-separation method in which a species is prevented from participating in a chemical reaction by binding it with a masking agent in an unreactive complex.

Masking Agent	Species Which Can Be Masked
CN^-	Ag, Au, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pd, Pt, Zn
SCN^-	Ag, Cd, Co, Cu, Fe, Ni, Pd, Pt, Zn
NH_3	Ag, Co, Cu, Fe, Pd, Pt
F^-	Al, Co, Cr, Mg, Mn, Sn, Zn
$S_2O_3^{2-}$	Au, Cd, Co, Cu, Fe, Pb, Pd, Pt, Sb
tartrate	Al, Ba, Bi, Ca, Ce, Co, Cr, Cu, Fe, Hg, Mn, Pb, Pd, Pt, Sb, Sn, Zn
oxalate	Al, Fe, Mg, Mn, Sn
thioglycolic acid	Cu, Fe, Sn

? Suggest a masking agent for the analysis of Al in the presence of Fe. Repeat for the analysis of Fe when Al is an interferent.

To find a suitable masking agent, we look for a species that binds with the interferent but does not bind with the analyte. Oxalate, for example, is an inappropriate choice because it binds with both Al and Fe. From Table we find that thioglycolic acid is a selective masking agent for Fe in the presence of Al and that F^- is a selective masking agent for Al in the presence of Fe.

4. Separations Based on a Change of State

Since an analyte and interferent are usually in the same phase, a separation often can be effected by inducing a change in one of their physical or chemical states. Changes in physical state that have been exploited for the purpose of a separation include liquid-to-gas and solid-to-gas phase transitions. Changes in chemical state involve one or more chemical reactions.

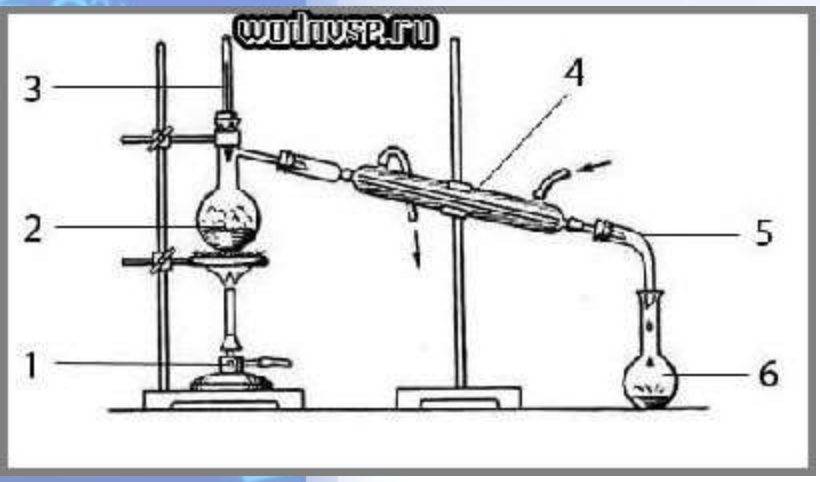
Changes in physical state when the analyte and interferent are miscible liquids, a separation based on ***distillation*** may be possible if their boiling points are significantly different. Distillation is widely used to separate volatile analytes from nonvolatile interferents. A common example is the separation of nitrogen analytes from many other species by converting the nitrogen to ammonia, which is then distilled from basic solution.

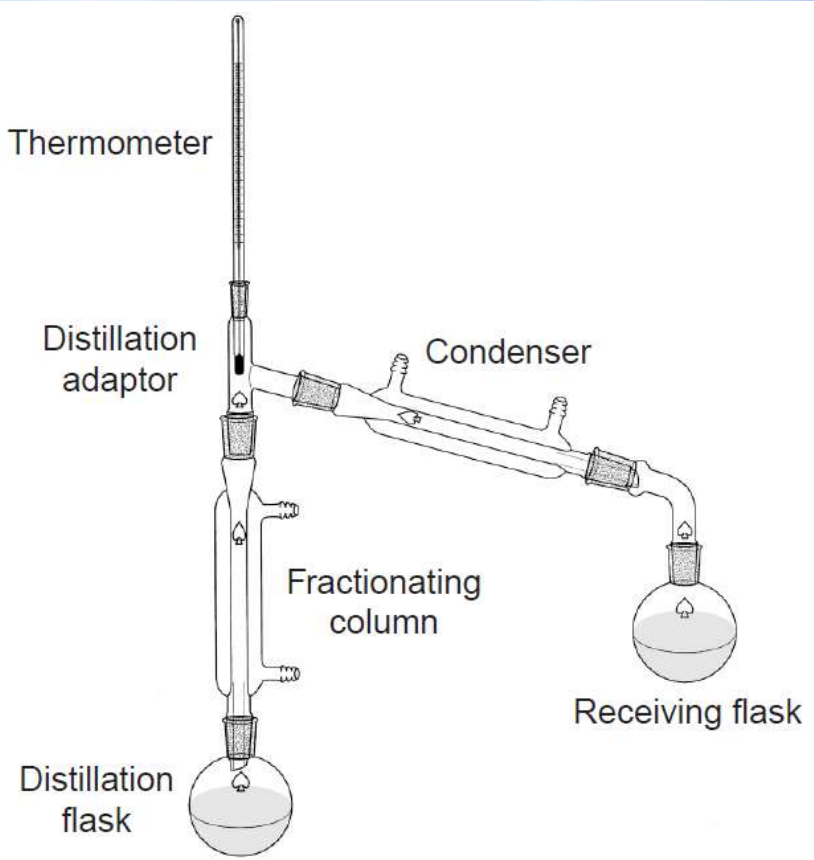
There are many types of distillation.

- **Vacuum distillation** is used for compounds that have very high boiling points.
- **Molecular distillation** occurs at very low pressure (,0.01 torr) such that the lowest possible temperature is used with the least damage to the distillate.
- **Pervaporation** is a method for separating mixtures by partial volatilization through a nonporous membrane.
- **Flash evaporation**

When the sample is a solid, a separation of the analyte and interferent by ***sublimation*** may be possible. The sample is heated at a temperature and pressure below its triple point where the solid vaporizes without passing through the liquid state. The vapor is then condensed to recover the purified solid. A good example of the use of sublimation is in the isolation of amino acids from fossil mollusk shells and deep-sea sediments.

4. Separations Based on a Change of State





Typical equipment for a
(a) simple distillation; and a
(b) fractional distillation.

Another approach for purifying solids is ***recrystallization***. The solid is dissolved in a minimum volume of solvent, for which the analyte's solubility is significant when the solvent is hot, and minimal when the solvent is cold.

Separation by *Precipitation*

Separations by precipitation require large solubility differences between the analyte and potential interferents.

Separations Based on Control of Acidity

Reagent	Species Forming Precipitates	Species Not Precipitated
Hot concd HNO_3	Oxides of W(VI), Ta(V), Nb(V), Si(IV), Sn(IV), Sb(V)	Most other metal ions
NH_3/NH_4Cl buffer	Fe(III), Cr(III), Al(III)	Alkali and alkaline earths, Mn(II), Cu(II), Zn(II), Ni(II), Co(II)
$HOAc/NH_4OAc$ buffer	Fe(III), Cr(III), Al(III)	Cd(II), Co(II), Cu(II), Fe(II) Mg(II), Sn(II), Zn(II)
$NaOH/Na_2O_2$	Fe(III), most +2 ions, rare earths	Zn(II), Al(III), Cr(VI), V(V), U(VI)

Precipitation of Sulfides

Elements	Conditions of Precipitation*	Conditions for No Precipitation*
Hg(II), Cu(II), Ag(I)	1, 2, 3, 4	
As(V), As(III), Sb(V), Sb(III)	1, 2, 3	4
Bi(III), Cd(II), Pb(II), Sn(II)	2, 3, 4	1
Sn(IV)	2, 3	1, 4
Zn(II), Co(II), Ni(II)	3, 4	1, 2
Fe(II), Mn(II)	4	1, 2, 3

*1 = 3 M HCl; 2 = 0.3 M HCl; 3 = buffered to pH 6 with acetate; 4 = buffered to pH 9 with $NH_3/(NH_4)_2S$.

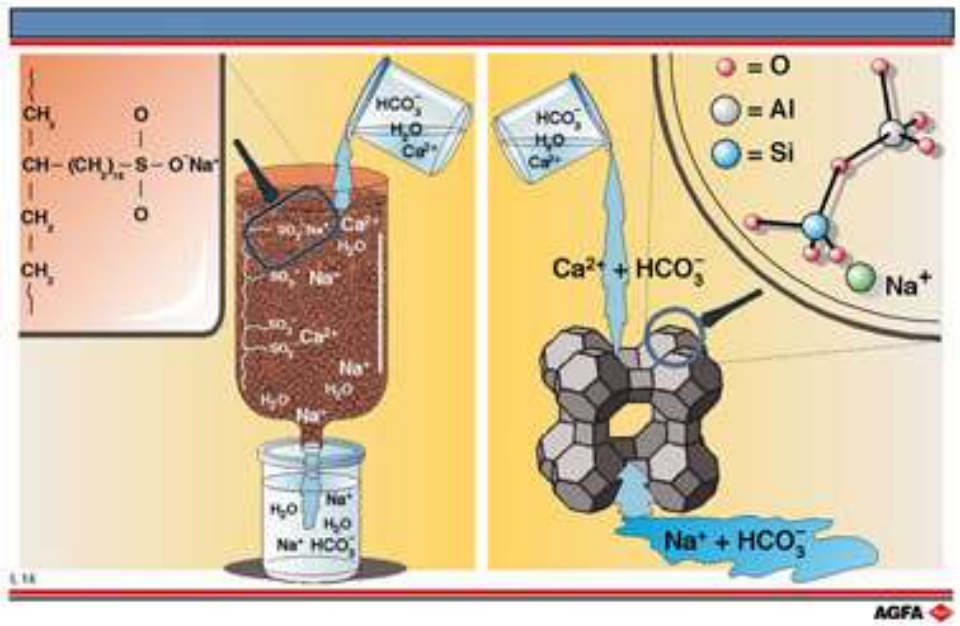
Separations by Organic Precipitants

dimethylglyoxime,
8-hydroxyquinoline

Electrolytic Precipitation

Separating Ions by Ion Exchange

In the ion-exchange process, ions held on an ion-exchange resin are exchanged for ions in a solution brought into contact with the resin.



Ion exchange is a process by which ions held on a porous, essentially insoluble solid are exchanged for ions in a solution that is brought in contact with the solid. The ionexchange properties of clays and zeolites have been recognized. Synthetic ion-exchange resins were first produced in the mid-1930s and have since found widespread application in water softening, water deionization, solution purification, and ion separation.

Synthetic ion-exchange resins are high-molecular-mass polymers that contain large numbers of an ionic functional group per molecule.

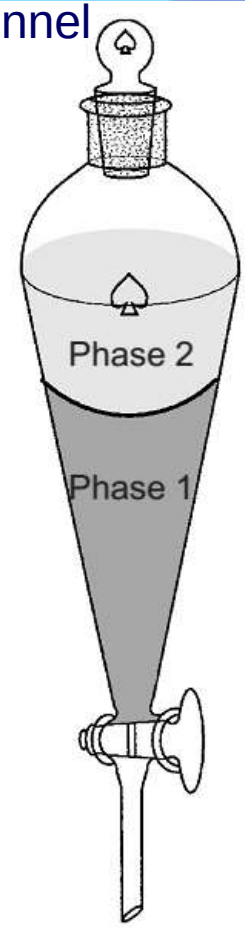
5. Separations Based on a Partitioning Between Phases

Separation by Extraction

“Like dissolves like.”

Solvent and solid-phase extraction are two techniques for separating mixtures of substances, either by selective transfer between two immiscible liquid phases or between a liquid and a solid phase.

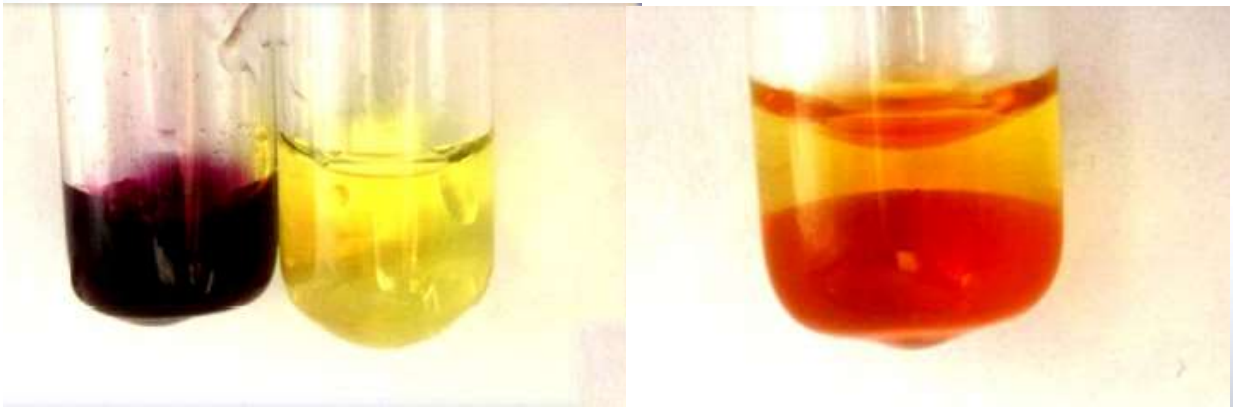
Separatory funnel



Solvent extraction - Procedures are based on the extraction of nonpolar, uncharged species from an aqueous solution into an immiscible organic solvent, or the extraction of polar or ionized species into an aqueous solution from an organic solvent.

The two liquids are placed in the separatory funnel and shaken to increase the surface area between the phases. When the extraction is complete, the liquids are allowed to separate, with the denser phase settling to the bottom of the separatory funnel.

$$2MnO_4^- + 10Br^- + 16H^+ \leftrightarrow 5Br_2 + 2Mn^{2+} + 8H_2O$$



Nernst Distribution or Partition Law

$$K_d = \frac{[A]_{org}}{[A]_{aq}}$$

distribution or partition coefficient

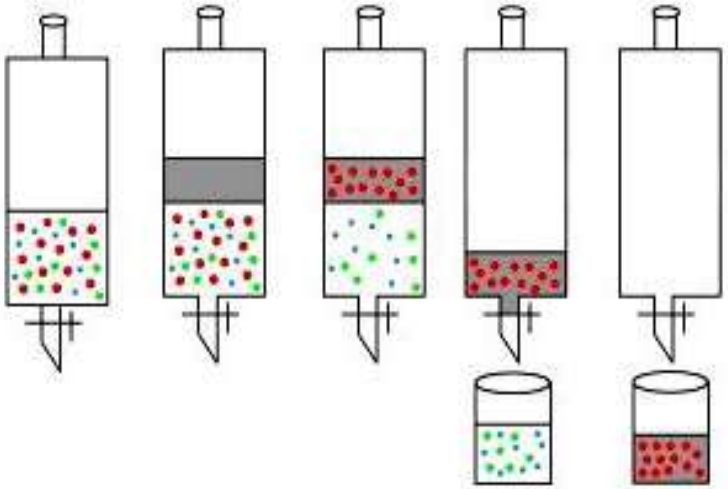
$$D = \frac{(C_s)_{org}}{(C_s)_{aq}}$$

distribution or partition ratio

$$\%E = \frac{100D}{D + (V_a/V_o)}$$

the percentage of a solute extracted

If $V_a = V_o$, then



$$\%E = \frac{100D}{D + 1}$$

Extraction will be quantitative (99.9%) for *D values of 1000.*

Extraction efficiency is defined as the fraction or percentage of a substance that can be extracted in one or more steps. Selectivity is the degree to which a substance can be separated from others in a mixture.

$$(q_{org})_1 = \frac{(\text{moles org})_1}{(\text{moles org})_0} = 1 - (q_{aq})_1 = \frac{DV_{org}}{DV_{org} + V_{aq}}$$

multiple extractions

$$\text{Fraction remaining } (Q_{aq})_n = \left(\frac{V_{aq}}{DV_{org} + V_{aq}} \right)^n$$

Extraction of organic acids and bases

extraction of benzoic acid from an aqueous solution into either.

$$K_D = \frac{[HBz]_e}{[HBz]_a} \qquad \boxed{D = \frac{[HBz]_e}{[HBz]_a + [Bz^-]_a}} \qquad [Bz^-]_a = \frac{K_a[HBz]_a}{[H^+]_a}$$

$$K_a = \frac{[H^+]_a[Bz^-]_a}{[HBz]_a} \qquad [HBz]_e = K_D[HBz]_a$$

$$D = \frac{K_D[HBz]_a}{[HBz]_a + K_a[HBz]_a/[H^+]_a}$$

$$\boxed{D = \frac{K_D}{1 + K_a/[H^+]_a}}$$

? A solute, S, has a K_D between water and chloroform of 5.00. A 50.00-mL sample of a 0.050 M aqueous solution of the solute is extracted with 15.00 mL of chloroform. (a) What is the extraction efficiency for this separation? (b) What is the solute's final concentration in each phase? (c) What volume of chloroform is needed to extract 99.9% of the solute?

For a simple liquid–liquid extraction, the distribution ratio, D, and the partition coefficient, K_D, are identical.

(a) The fraction of solute remaining in the aqueous phase after the extraction

$$(q_{aq})_1 = \frac{50.00\ \text{mL}}{(5.00)(15.00\ \text{mL}) + 50.00\ \text{mL}} = 0.400$$

The fraction of solute present in the organic phase is, therefore, 0.600. Extraction efficiency is the percentage of solute successfully transferred from its initial phase to the extracting phase. The extraction efficiency is, therefore, 60.0%.

(b) The moles of solute present in the aqueous phase before the extraction is

$$(\text{Moles aq})_0 = [S_{aq}]_0 \times V_{aq} = \frac{0.050\ \text{mol}}{\text{L}} \times 0.05000\ \text{L} = 0.0025\ \text{mol}$$

Since 40.0% of the solute remains in the aqueous phase, and 60.0% has been extracted into the organic phase, the moles of solute in the two phases after extraction are

$$(\text{Moles aq})_1 = (\text{moles aq})_0 \times (q_{aq})_1 = 0.0025\ \text{mol} \times (0.400) = 0.0010\ \text{mol}$$

$$(\text{Moles org})_1 = (\text{moles aq})_0 - (\text{moles aq})_1 = 0.0025\ \text{mol} - 0.0010\ \text{mol} = 0.0015\ \text{mol}$$

$$[S_{aq}]_1 = \frac{(\text{moles aq})_1}{V_{aq}} = \frac{0.0010\ \text{mol}}{0.05000\ \text{L}} = 0.020\ \text{M}$$

The solute's concentration in each phase

$$[S_{org}]_1 = \frac{(\text{moles org})_1}{V_{org}} = \frac{0.0015\ \text{mol}}{0.01500\ \text{L}} = 0.10\ \text{M}$$

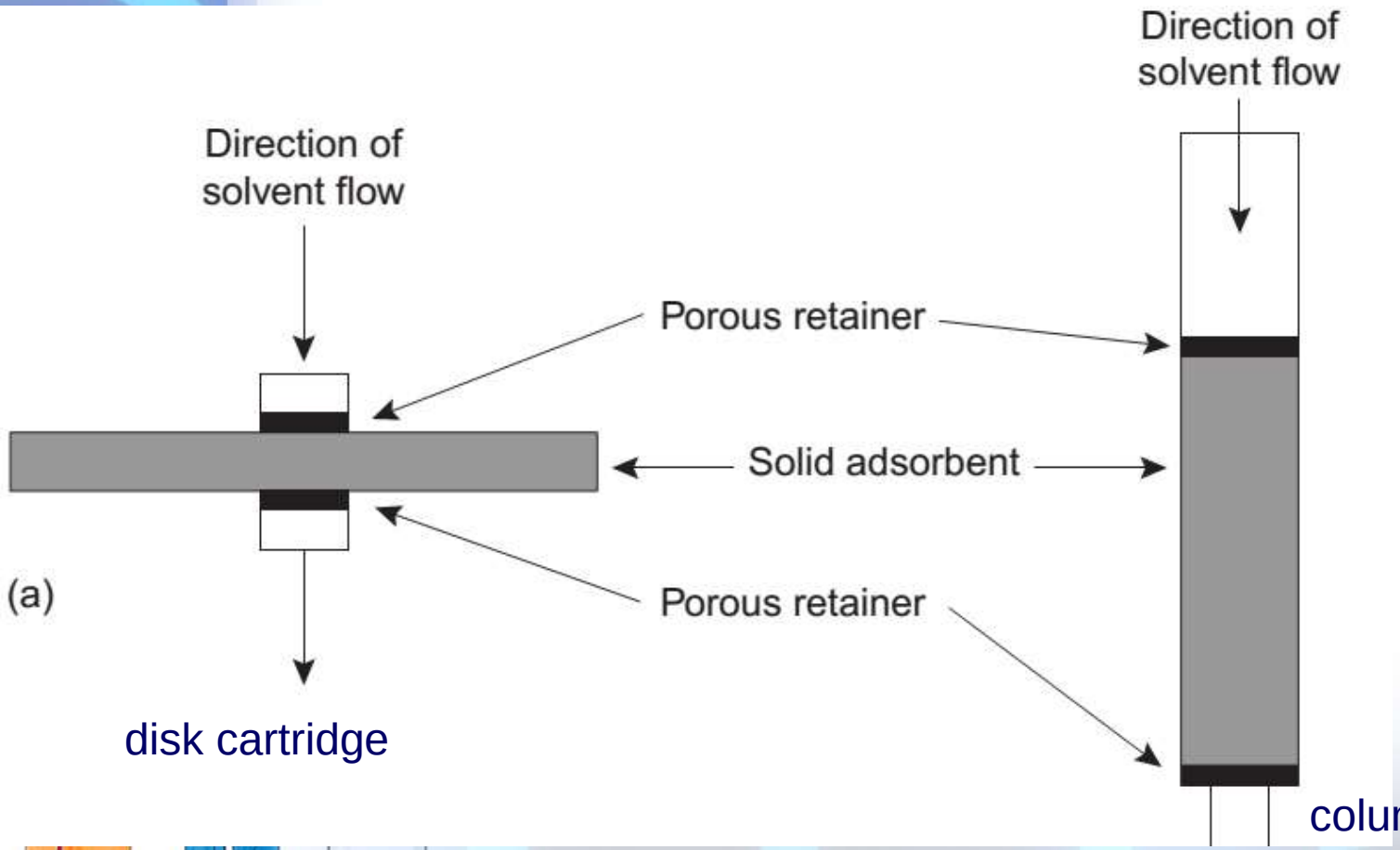
c) To extract 99.9% of the solute $(q_{aq})_1$ must be 0.001.

$$V_{org} = \frac{V_{aq} - (q_{aq})_1 V_{aq}}{(q_{aq})_1 D} = \frac{50.00\ \text{mL} - (0.001)(50.00\ \text{mL})}{(0.001)(5.00)} = 9990\ \text{mL}$$

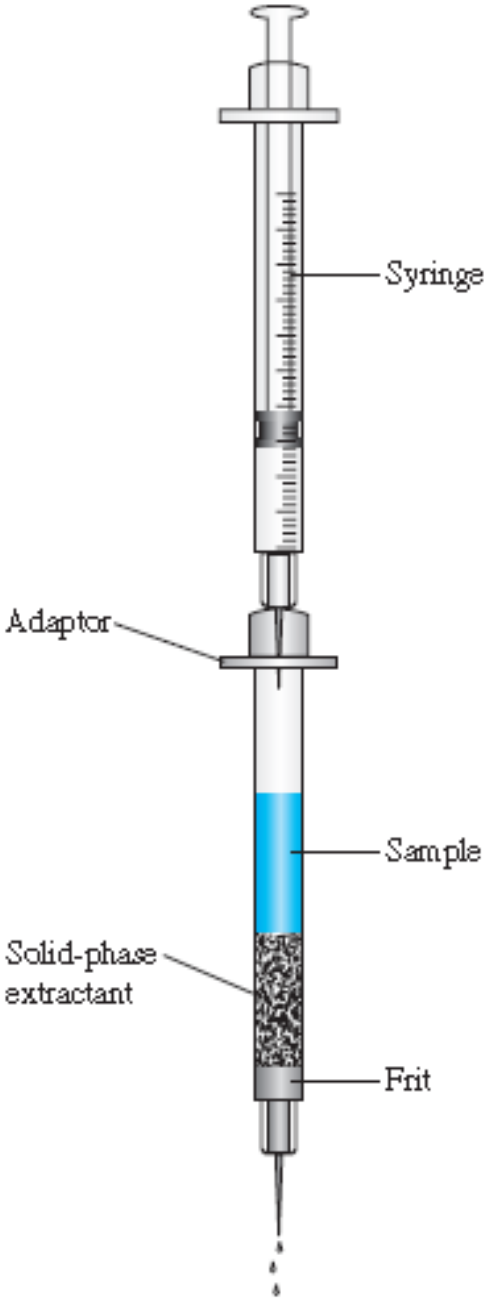
Clearly, a single extraction is not reasonable under these conditions.

Solid-Phase Extractions In a solid-phase extraction the sample is passed through a cartridge containing solid particulates that serve as the adsorbent material. For liquid samples the solid adsorbent is isolated in either a disk cartridge or a column. The choice of adsorbent is determined by the properties of the species being retained and the matrix in which it is found.

Solid-phase extraction is a technique in which hydrophobic functional groups are bonded to solid particle surfaces and act as the extracting phase. They reduce the need for large volumes of organic solvents.



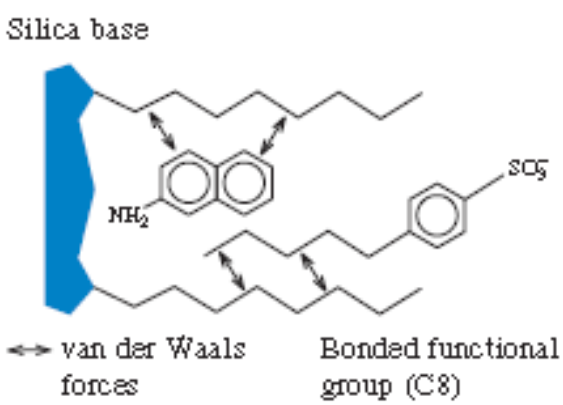
Solid-Phase Extraction

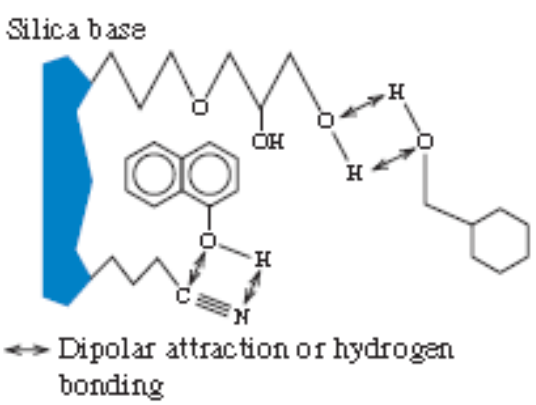


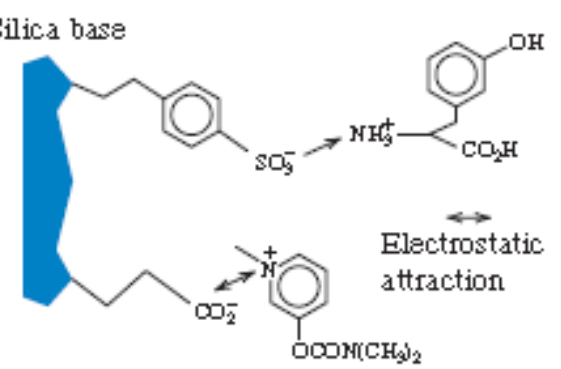
Solid-phase extraction performed in a small cartridge. The sample is placed in the cartridge and pressure is applied via a syringe plunger. Alternatively, a vacuum can be used to pull the sample through the extracting agent.

A hydrophobic organic compound is coated or chemically bonded to powdered silica to form the solid extracting phase. The compounds can be nonpolar, moderately polar, or polar. For example, an octadecyl (C18) bonded silica (ODS) is a common packing. The functional groups bonded to the packing attract hydrophobic compounds in the sample by van der Waals interactions and extract them from the aqueous solution.

Organic molecules are then extracted from the sample and concentrated in the solid phase. They can later be displaced from the solid phase by a solvent such as methanol. By extracting the desired components from a large volume of water and then flushing them out with a small volume of solvent, the components can be concentrated.

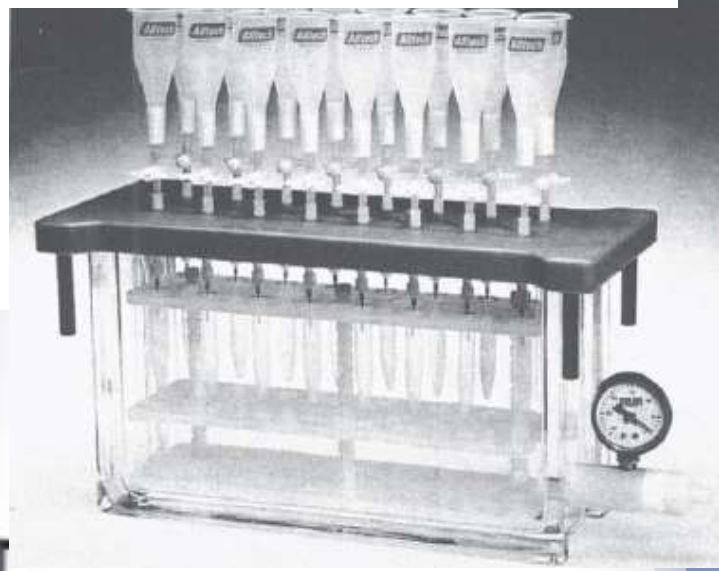






Solid-phase extractants utilizing nonpolar, polar, and electrostatic interactions.

The nature of the extracting phase, particularly the type of bonded functional group, can be varied to allow extraction of different classes of compounds.



16-Port vacuum manifold for use with solid-phase extraction tubes

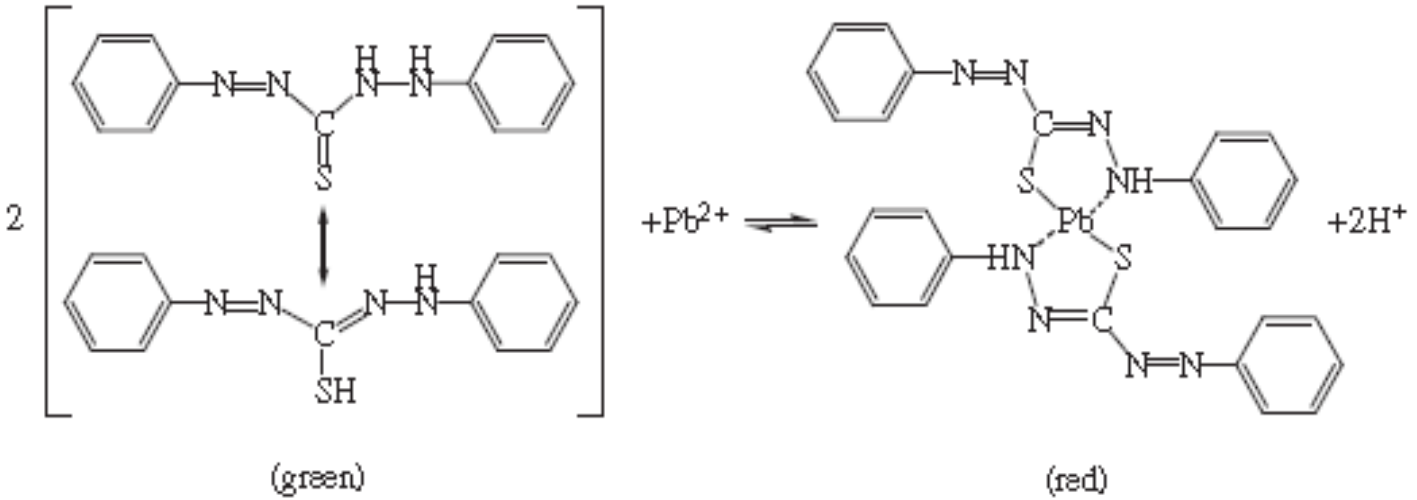
Selected Adsorbents for Solid-Phase Extraction of Liquid Samples

Adsorbent	Surface Structure	Properties and Uses
silica	—O—Si(HO)—O—Si(OH)—O—	• retains low-to-moderate polarity species from organic matrices • fat-soluble vitamins, steroids
alumina	—O—Al(HO)—O—Al(OH)—O—	• retains hydrophilic species from organic matrices
cyanopropyl	—C_3H_6CN	• retains wide variety of species from aqueous and organic matrices • pesticides, hydrophobic peptides
diol	—CH_2(OH)—CH_2(OH)—	• retains wide variety of species from aqueous and organic matrices • proteins, peptides, fungicides
octadecyl (C-18)	—$C_{18}H_{37}$	• retains hydrophobic species from aqueous matrices • caffeine, sedatives, polyaromatic hydrocarbons, carbohydrates, pesticides
octyl (C-8)	—C_8H_{17}	• similar to C-18
styrene divinylbenzene		• wide variety of organic species from aqueous matrices • polyaromatic hydrocarbons

Extraction of metals

1. Separating Metal Ions as Chelates

Many organic chelating agents are weak acids that react with metal ions to give uncharged complexes that are highly soluble in organic solvents such as ethers, hydrocarbons, ketones, and chlorinated species (including chloroform and carbon tetrachloride).



Diphenylthiocarbazone (dithizone), forms a chelate with lead ion

8-hydroxyquinoline (oxine)
acetylacetone (AcAc)

1-(2-pyridylazo)-2-naphthol (PAN)
sodium diethyldithiocarbamate (NaDDTC)

2. EXTRACTION OF ION-ASSOCIATION COMPLEXES

the metal ion is incorporated into a bulky molecule and then associates with another ion of the opposite charge to form an **ion pair, or the metal ion associates** with another ion of great size (organic-like)

Electrically neutral ion-association complexes consist of cationic (positively charged) and anionic (negatively charged) species that form an overall neutral aggregate extractable by an organic solvent.

The metal ion can be a cationic or an anionic complex, and can be an inorganic species such as $FeCl_4^-$, MnO_4^- or a chelated organic complex such as Fe(1,10-phenanthroline)$_3^{2+}$ or UO_2(oxine)$_3^-$. Suitable counter-ions of opposite charge to the metal-complex ions include $(C_4H_9)_4N^+$, ClO_4^- $(C_6H_5CH_2)_3NH^+$, $[(C_4H_9O)_3P{=}O]H^+$ and an oxonium ion such as $[(C_2H_5)_2O]_3H^+$.

Permanganate forms an ion pair with tetraphenylarsonium ion $[(C_6H_5)_4As^+, MnO_4^-]$, which makes it organic-like, and it is extracted into methylene chloride.

Reagent	Type of metal complex
8-Hydroxyquinoline (oxine) Di-alkyldithiocarbamates e.g. sodium diethyldithiocarbamate (NaDDTC)	Neutral metal chelate complexes, extractable into organic solvents. Intense color of many facilitates colorimetric determinations.
1,10-Phenanthroline (o-phen) 2,9-Dimethyl-1,10-phenanthroline (neocuproine) Ethylenediaminetetraacetic acid (EDTA)	Ion-association complexes. Metals as cationic or anionic chelated complexes extracted with suitable counter ion.
Oxonium systems: i.e. protons solvated with alkyl ethers, ketones, esters or alcohols	Ion-pairs with anionic metal halide or thiocyanate complexes. Chloride complexes extractable from strong HCl solutions.

1 Simple molecules. These involve the distribution of molecules such as iodine, bromine, fatty acids, and hydrocarbons between water and an organic solvent.

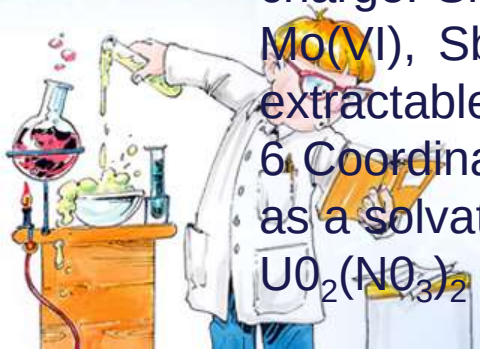
2 ***Pseudomolecular systems.*** These involve species that are partly ionized in water and are molecular in an organic solvent. Examples include weak acids such as phenols, dithizone, salicylic acid, cupferron, and many neutral metalchelate complexes.

3 ***Coordinately unsolvated salts.*** These involve large ions that would extensively disrupt the structure of the solvent water and whose hydration energies are small and hence can exist as ions in an organic solvent. Examples are Ph, As+, Ph, P+, and Bu, N+. These cations can be extracted as ions with large unsolvated.

4 ***Mineral acids.*** The tetrahydrated proton can be extracted into some basic organic solvents such as ethers and ketones. Large acid anions make the extraction more favorable. Thus HClO, is extracted more extensively than HCl.

5 ***Complex metal acids.*** The'best-known example is the extraction of $HFeCl$, into solvents such as ether. The anion is large and has low charge. Similarly, the halides of ions such as Ga(III), Au(III), In(III), Tl(III), Mo(VI), SbCV), As(III), Ge(IV), Hg(II), and NbO have been found to be extractable from strong-acid solution.

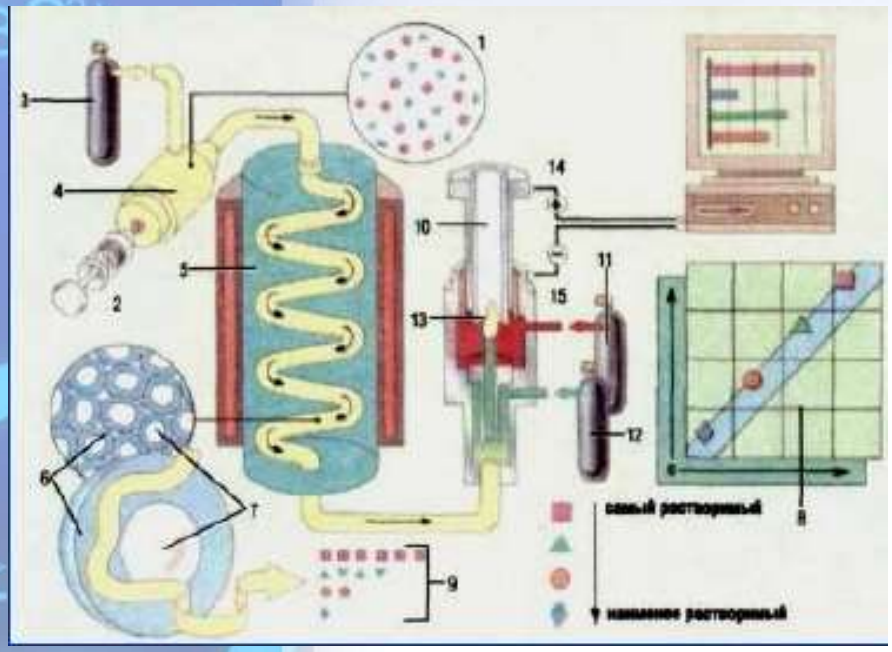
6 Coordinately solvated salts. Tri-n-butyl phosphate, for example, can act as a solvating group to enhance the extractability of metal nitrates such as $U0_2(N0_3)_2$ 2TBP.



When a substance is heated above its critical temperature and pressure, it forms a ***supercritical fluid*** whose properties are between those of a gas and a liquid. Supercritical fluids are better solvents than gases, making them a better reagent for extractions. In addition, the viscosity of a supercritical fluid is significantly less than that of a liquid solvent, allowing it to pass more readily through particulate samples.

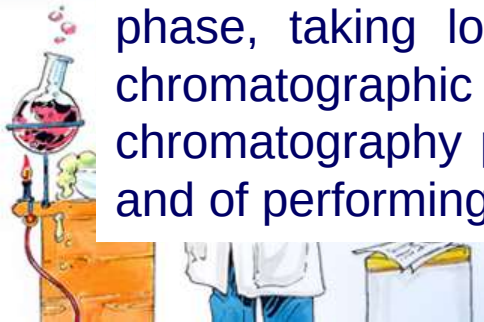
One example of a supercritical extraction is the determination of total petroleum hydrocarbons (TPHs) in soils, sediments, and sludges with supercritical CO_2. Approximately 3 g of sample is placed in a 10-mL stainless steel cartridge, and supercritical CO_2, at a pressure of 340 atm and a temperature of 80 °C, is passed through the cartridge for 30 min at flow rate of 1–2 mL/min. The petroleum hydrocarbons are collected by passing the effluent from the cartridge through 3 mL of tetrachloroethylene at room temperature. At this temperature the CO_2 reverts to the gas phase and is released to the atmosphere.

6. Chromatographic Separations



Chromatography is a technique in which the components of a mixture are separated based on differences in the rates at which they are carried through a fixed or **stationary phase** by a gaseous or liquid **mobile phase.**

Separations can also be accomplished by continuously passing one sample-free phase, called the mobile phase, over a second sample-free phase that remains fixed or stationary. The sample is then injected or placed into the mobile phase. As the sample's components move with the mobile phase, they partition themselves between the mobile and stationary phases. Those components having the largest partition coefficients are more likely to move into the stationary phase, taking longer to pass through the system. This is the basis of all chromatographic separation techniques. As currently practiced, modern chromatography provides a means both of separating analytes and interferents and of performing a qualitative or quantitative analysis of the analyte.



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