Modern sample preparation methods for organic analytes

Structure of lecture

- Liquid-liquid extraction
- Solid-liquid extraction
- Solid-phase extraction
- Headspace extraction
- Purge and Trap extraction
- Solid-phase microextraction

Liquid-liquid extraction

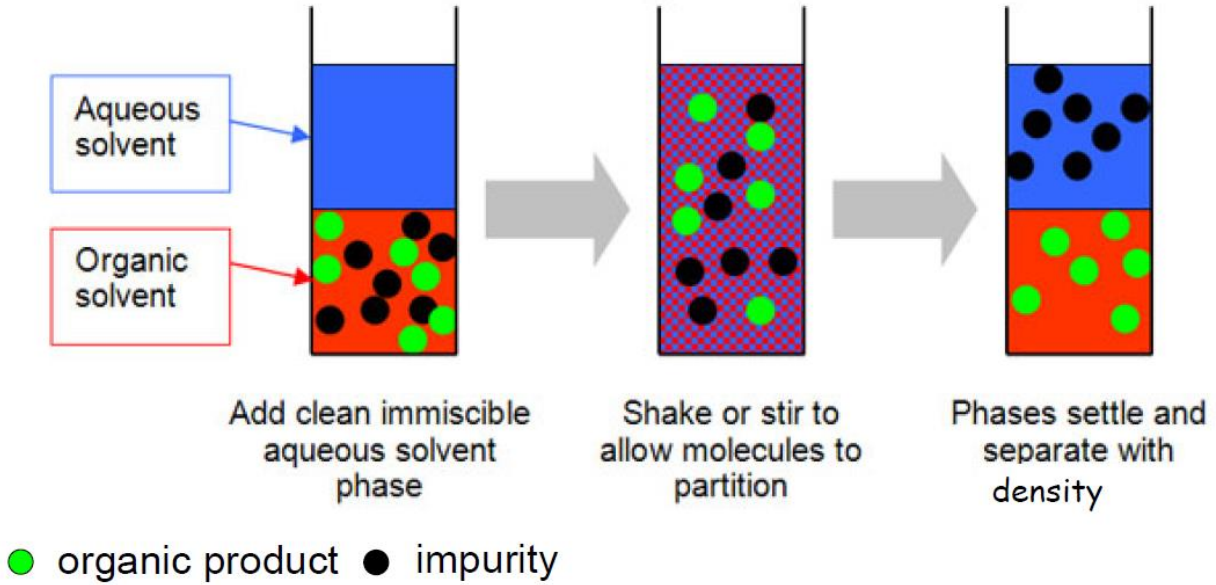
LLE

Liquid-liquid extraction

Liquids (solvents) must be immiscible

Extraction efficiency depends on differential solubility

the two immiscible solvents



Solvent Extraction - LLE

- Popular technique
- For non-semi volatile organic compound
- Partitioning the sample between two immiscible phases
  - Aqueous phase: Sample matrix
  - Organic phase: Organic solvent
- Like dissolves like

A (aq)  A(org)

Basic Theory

- Distribution Coefficient (K_d)

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

$[A]_{org}$ – concentration in organic solvent
$[A]_{aq}$ - concentration in water

- K_d is constant at a particular temperature

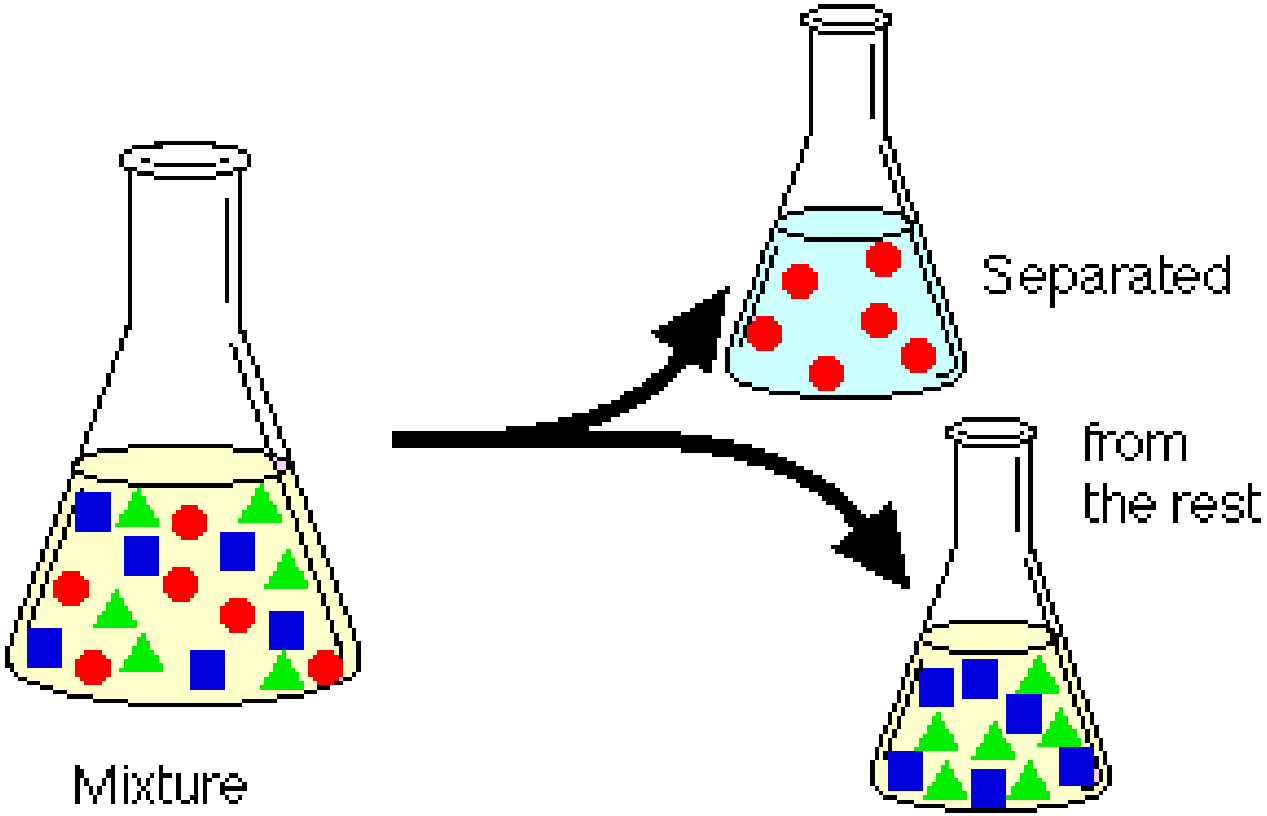
Properties of extraction solvents:

- high solubility of the organic compound
- immiscible with water
- relatively low boiling point
- nontoxic, nonreactive, available, inexpensive

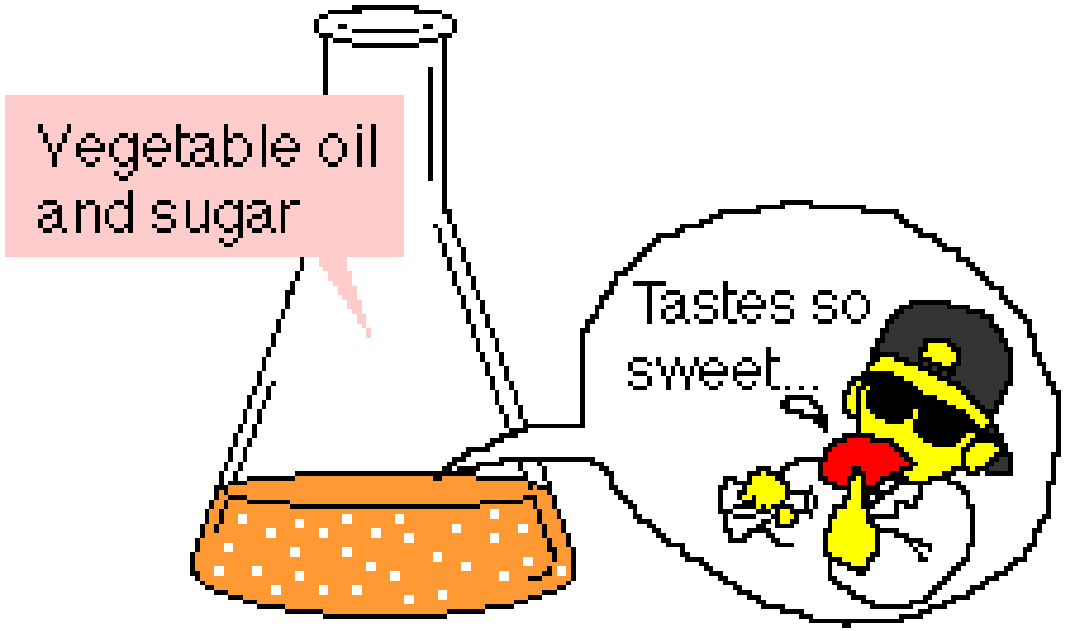
Typical Solvents used in LLE

- Aqueous solvent
  - Pure water
  - Acidic solution
  - Basic solution
  - High salt (Salting out)
  - Complexing agents (ion-pairing, chelating and chiral agents)
  - Combination of two or more above
- Water immiscible organic solvent
  - Diethyl ether
  - Methylene chloride
  - Chloroform
  - Ethyl acetate
  - Aliphatic ketones (C6+)
  - Aliphatic alcohol (C6+)
  - Toluene and xylenes
  - Combination of two or more above

Liquid-liquid extraction is a useful method to separate components (compounds) of a mixture

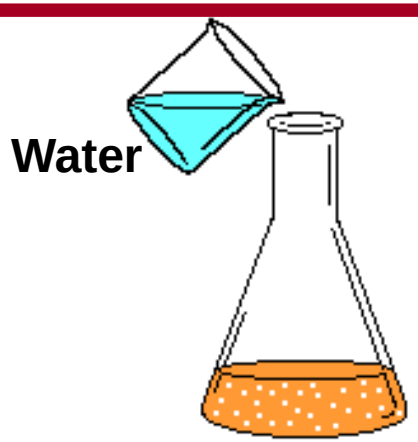


A mixture of **sugar** in **vegetable oil** (it tastes sweet!) and you want to **separate the sugar from the oil**.



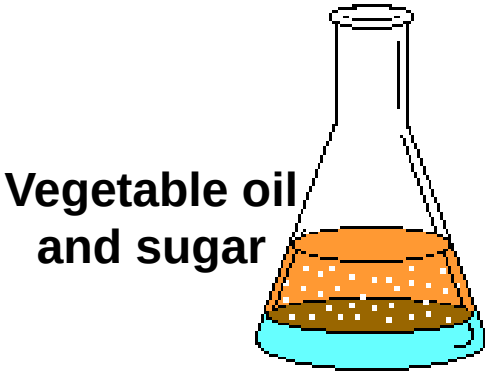
Sugar is much more soluble in water than in vegetable oil.

Water is ***immiscible*** (=not soluble) with oil.



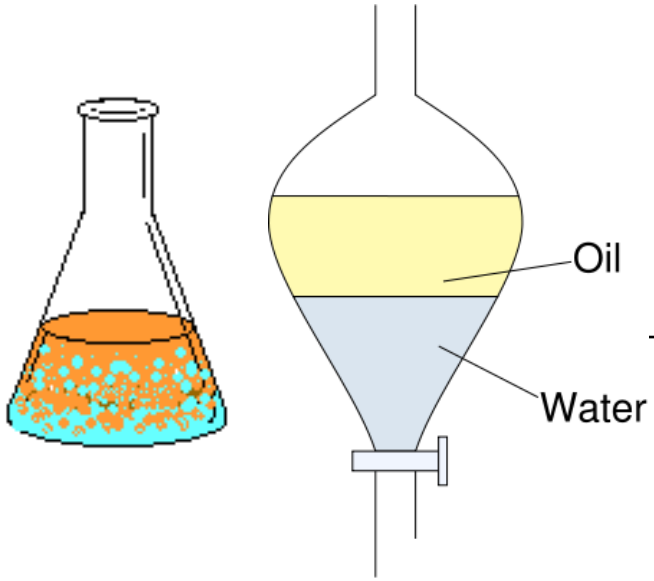
Vegetable oil and sugar

The **water phase is the bottom layer** and the **oil phase is the top layer**, because **water is *denser*** than oil.

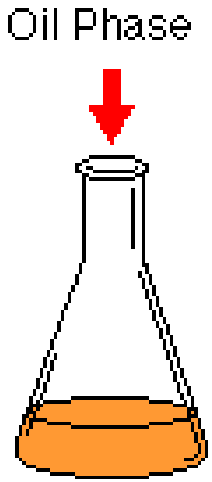


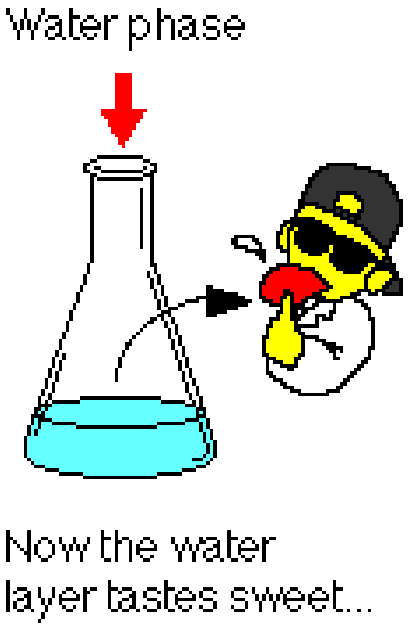
Water

Extraction of sugar from the oil with water



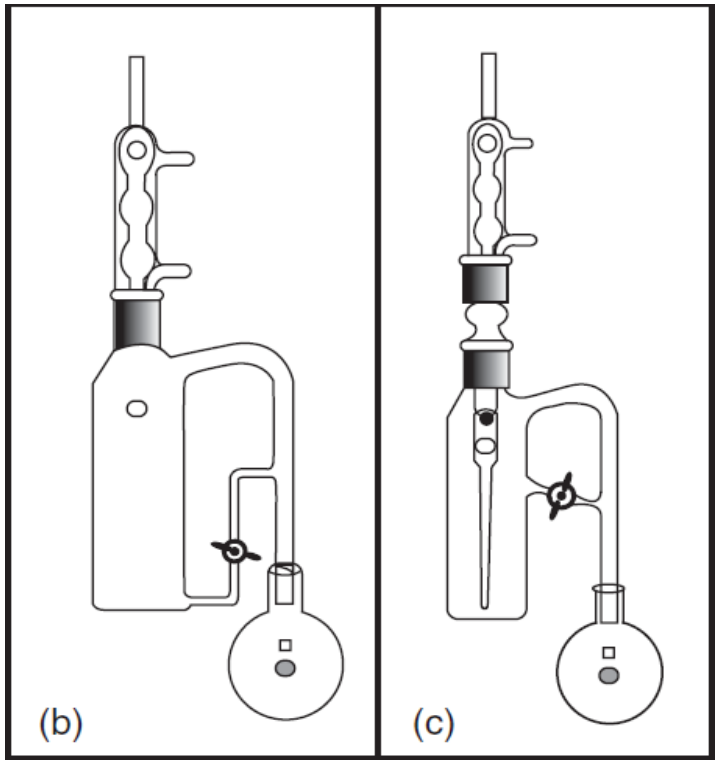
Water + Vegetable oil + sugar





Liquid–liquid extraction apparatus

Separating funnel



Continuous LLE apparatus designed for heavier-than-water or lighter-than-water extracting solvents

Enhancing LLE efficiency

Salting out

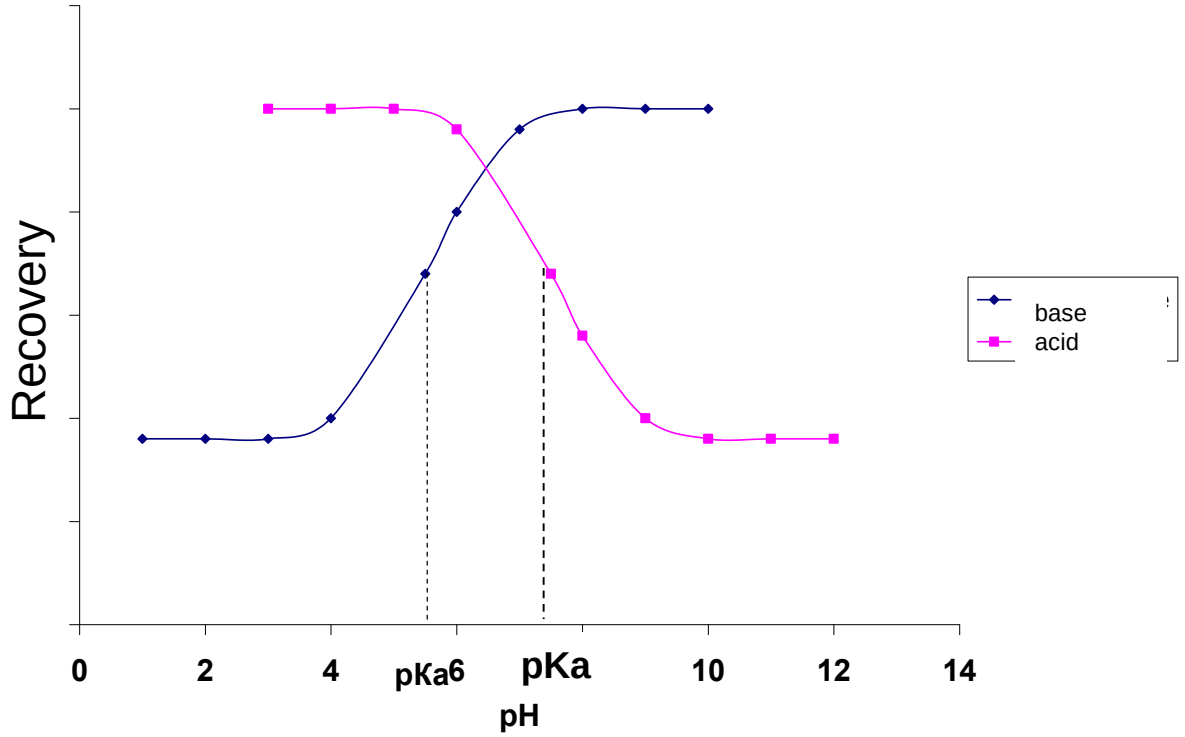
pH

Appropriate solvent selection

Consecutive extraction (by several portions of extractant)

pH effect of LLE recovery

$$HA \rightleftharpoons H^+ + A^- \qquad BH^+ \rightleftharpoons H^+ + B$$



Liquid–liquid extraction advantages

☺ Simple and fast operation

☺ Relatively low solvent usage when extracting hydrophobic compounds

☺ Excellent extraction efficiency can be achieved for low K_d analytes using consequent or continuous extractions

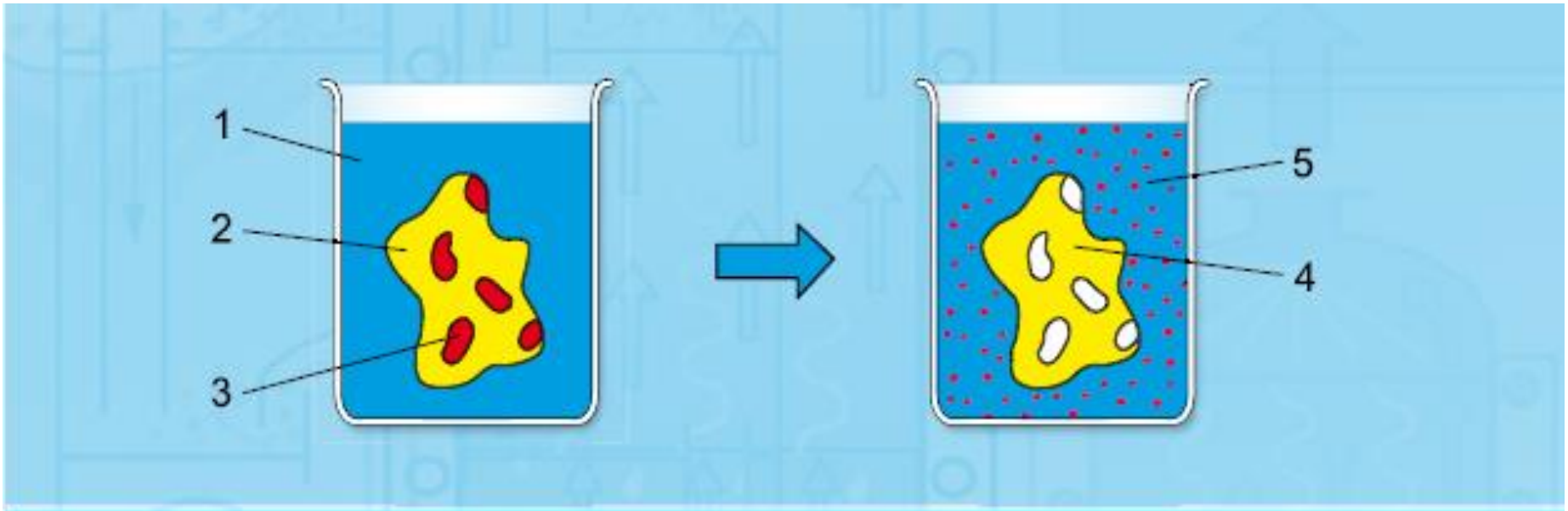
Liquid–liquid extraction disadvantages

- Very volatile compounds can be lost
- Difficult to achieve 100% extraction
- Use and disposal of large volumes of toxic organic solvents
- Formation of emulsion; hard to break emulsion
- Cumbersome glassware
- Labor-intensive process
- Sample pre-concentration is often required
- Not easily automated

Solid-liquid extraction
SLE
(Leaching)

Solid-liquid extraction

Solid-liquid extraction (leaching) is the process of transfer of a solute or solutes from a solid to liquid solvent



Schematic extraction – before extraction (left) and after extraction (right):
1 solvent, 2 extraction material (solid carrier phase with transition component), 3 transition component,
4 depleted solid carrier phase, 5 solvent with dissolved transition component

SLE always involves two steps

1. Contact of solvent with solid and transfer of solutes to solvent;

2. Separation of the solution from the residual solid

These two steps may be conducted in separate equipment or in the same piece of equipment.

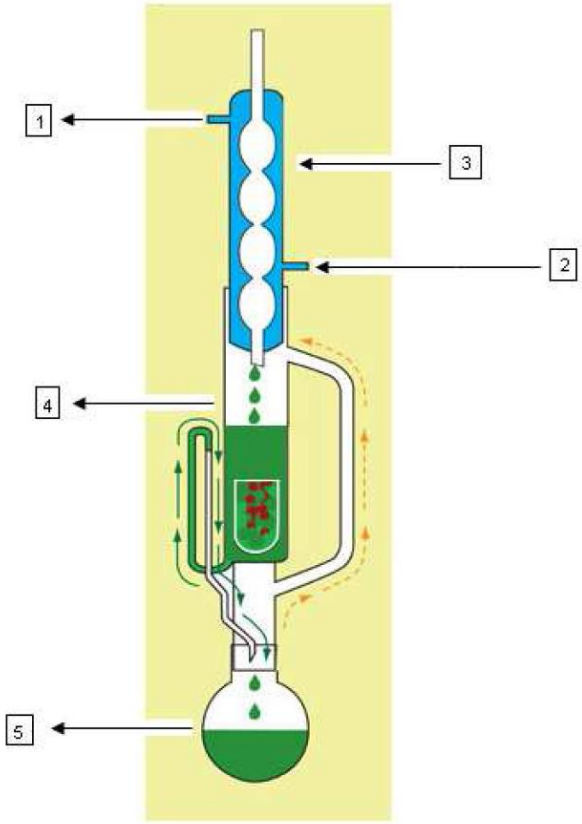
Leaching process can be considered in three parts:

1. Diffusion of the solvent through the pores of the solid.

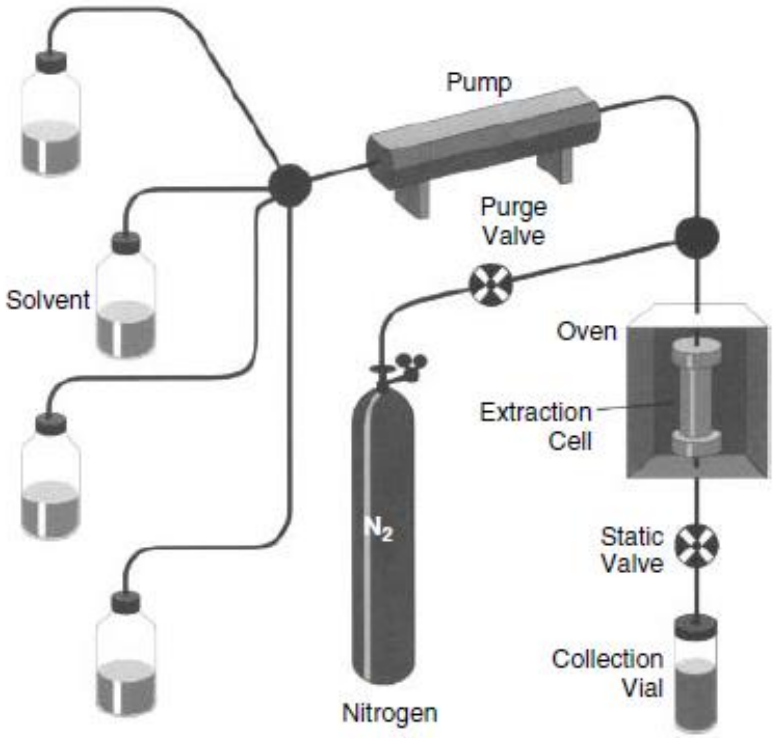
2. The diffused solvent dissolves the solutes (i.e. transfer the solute to the liquid phase).

3. Transfer of the solution from porous solid to the main bulk of the solution.

Soxhlet apparatus and Accelerated solvent extractor



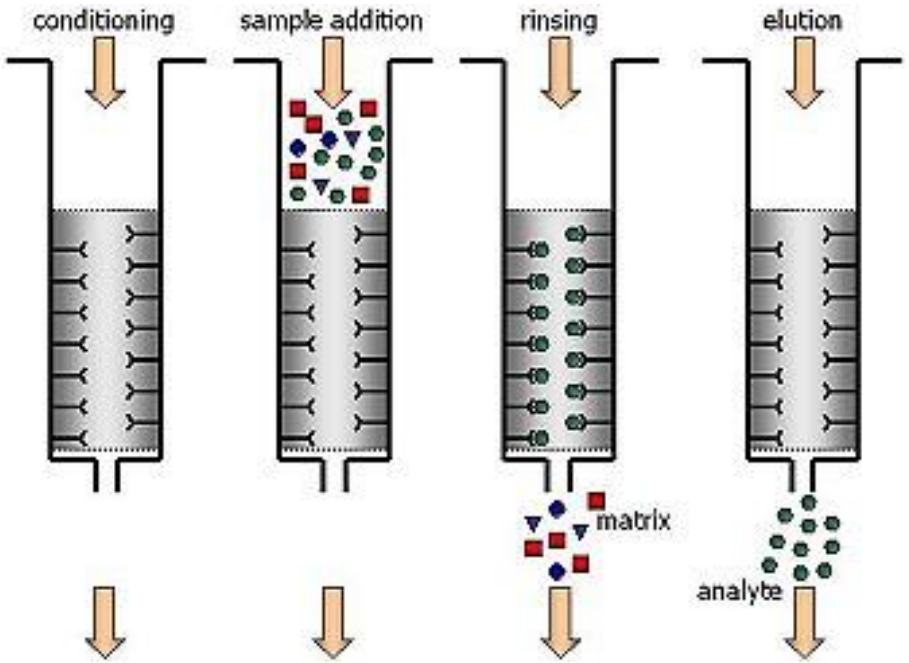
1. Water Outlet
2. Water Inlet
3. Soxhlet condenser
4. Soxhlet extractor
(lower d = 1.5 cm, upper d = 4.3 cm)
5. Round bottom flask



Solid-phase extraction (SPE)

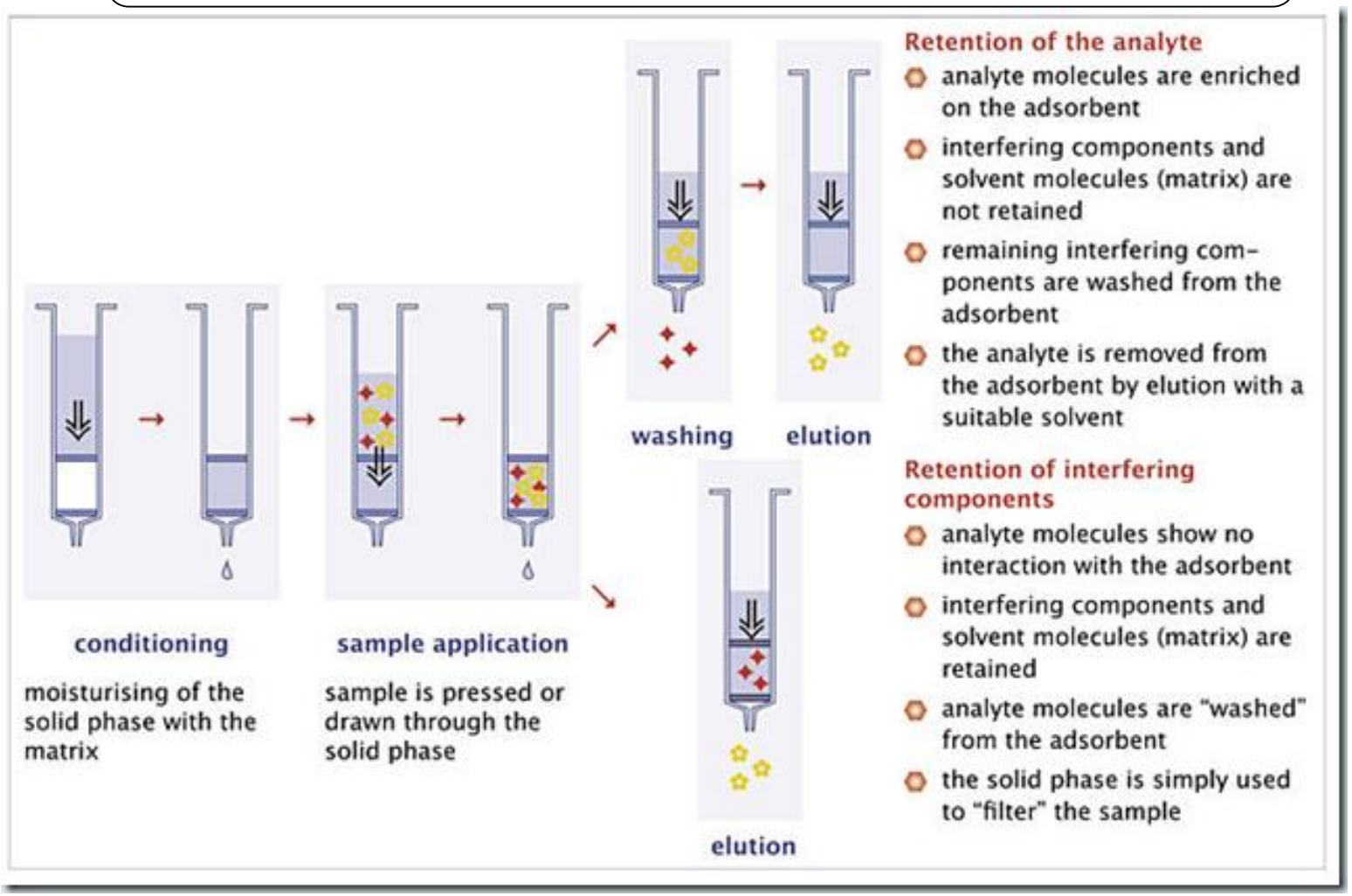
Solid-phase extraction

Solid-phase extraction refers to the nonequilibrium, exhaustive removal of chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent recovery of selected constituents by elution from the sorbent

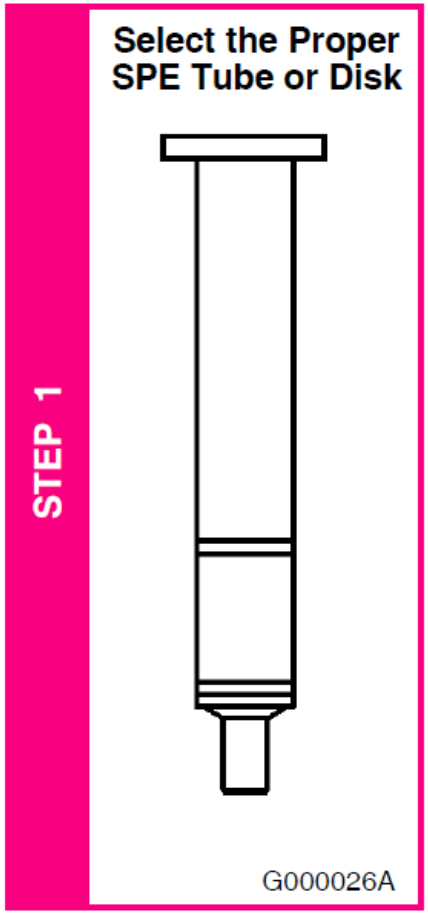


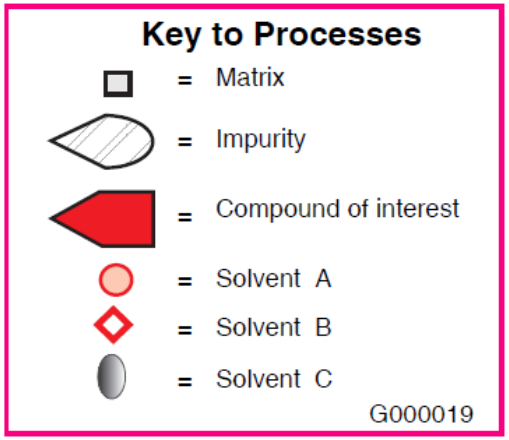
M.J.M. Wells, Essential guides to method development in solid--phase extraction, in I.D. Wilson, E.R. Adlard, M. Cooke, and C.F. Poole, eds., Encyclopedia of Separation Science, Vol. 10, Academic Press, London, 2000, pp. 4636--4643.

5 Typical steps of SPE



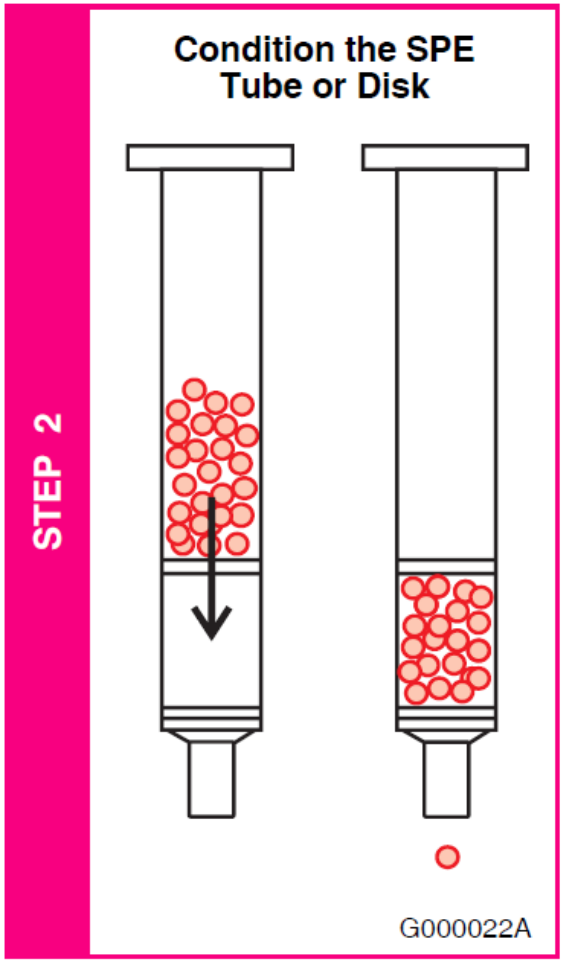
The 1st step of SPE

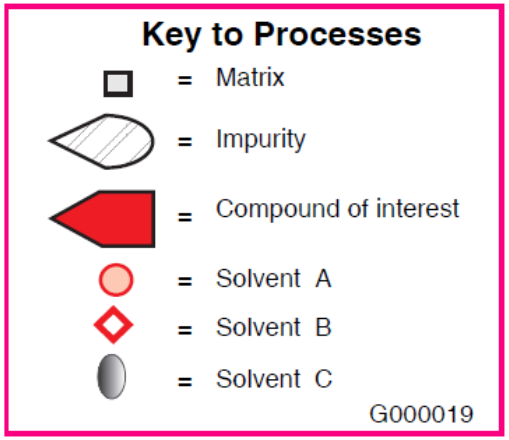




SUPELCO
Bulletin 910

The 2nd step of SPE





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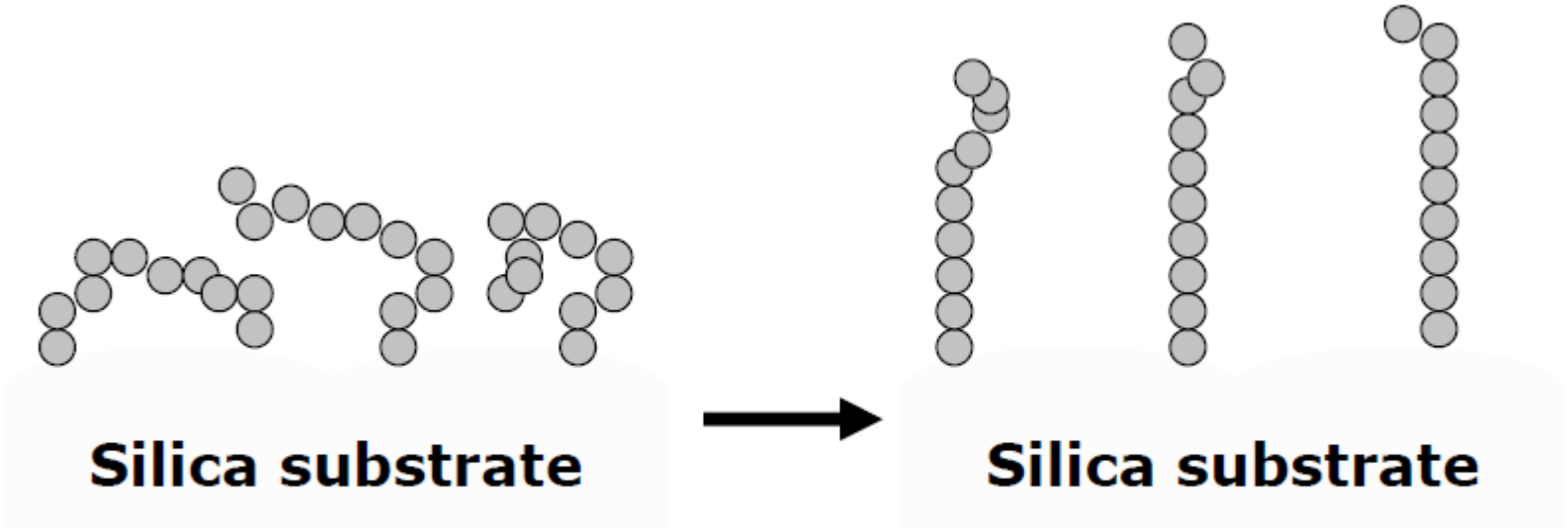
Conditioning

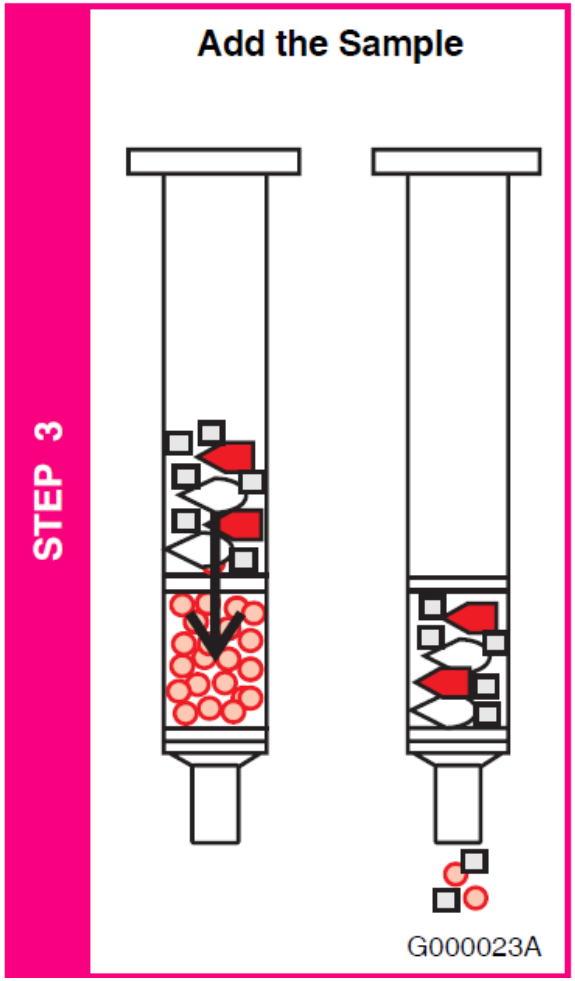
- Make the sorbent compatible with sample solution for close contact in small channels

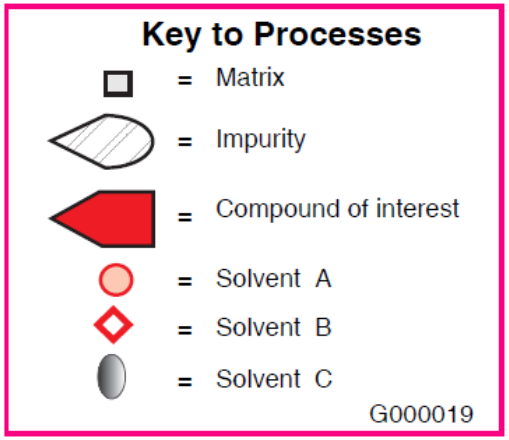
- The sorbent should not be dry at any stage



C-18 activated with n-hexane

The 3rd step of SPE





SUPELCO
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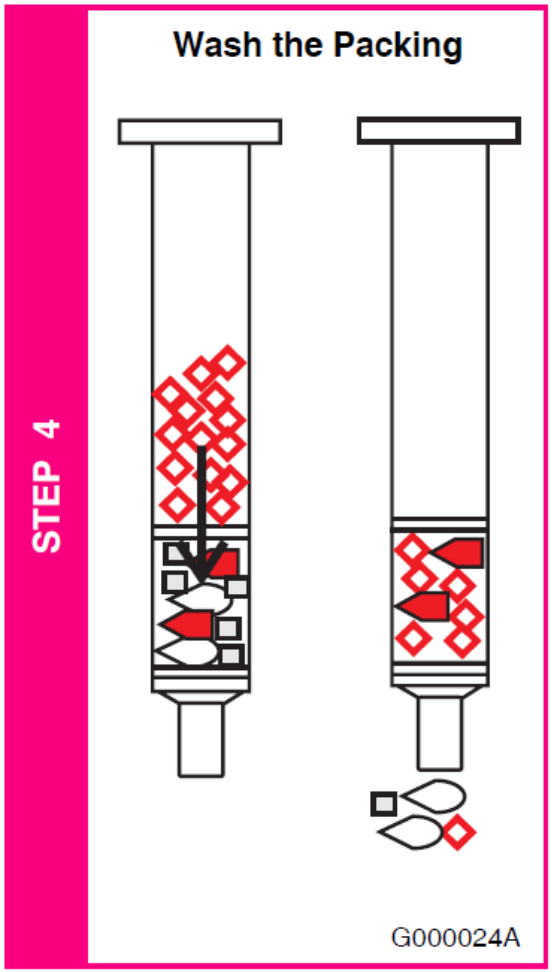
Loading/Adsorption

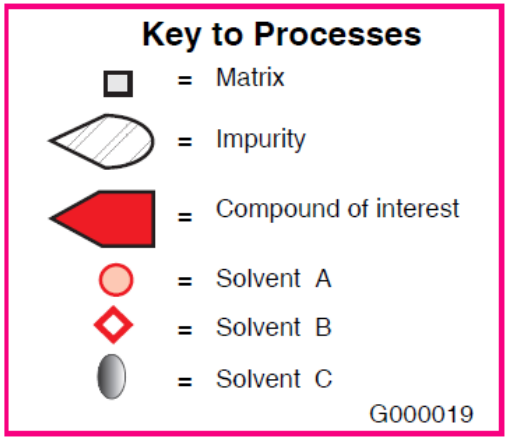
- Gentle vacuum, or pump

- At reasonable rate, depend on column dimension, particle size
  - Small particles, more efficient, permit faster flow rate

- The sorbent should not be allowed to go dry at any point
  - Air in the column prevent efficient interfacial contact between liquid and solid phase

The 4th step of SPE





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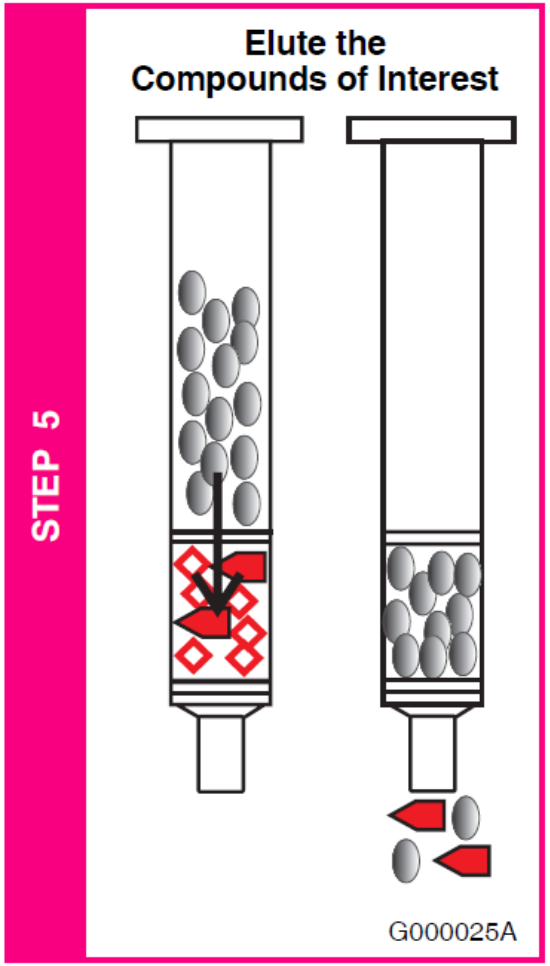
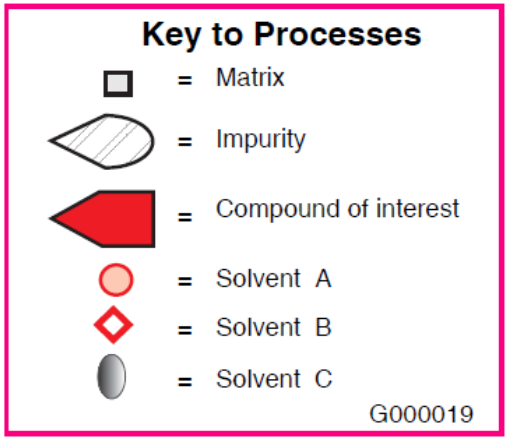
Washing

- Remove interferences coadsorbed from the SPE column
- The wash solution must not be too strong to partially eluted the analyte of interest

The 5th step of SPE





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Elution

- Eluting solvent should be strong enough to completely removed adsorbed analytes from the sorbent as small a volume as possible (small k)

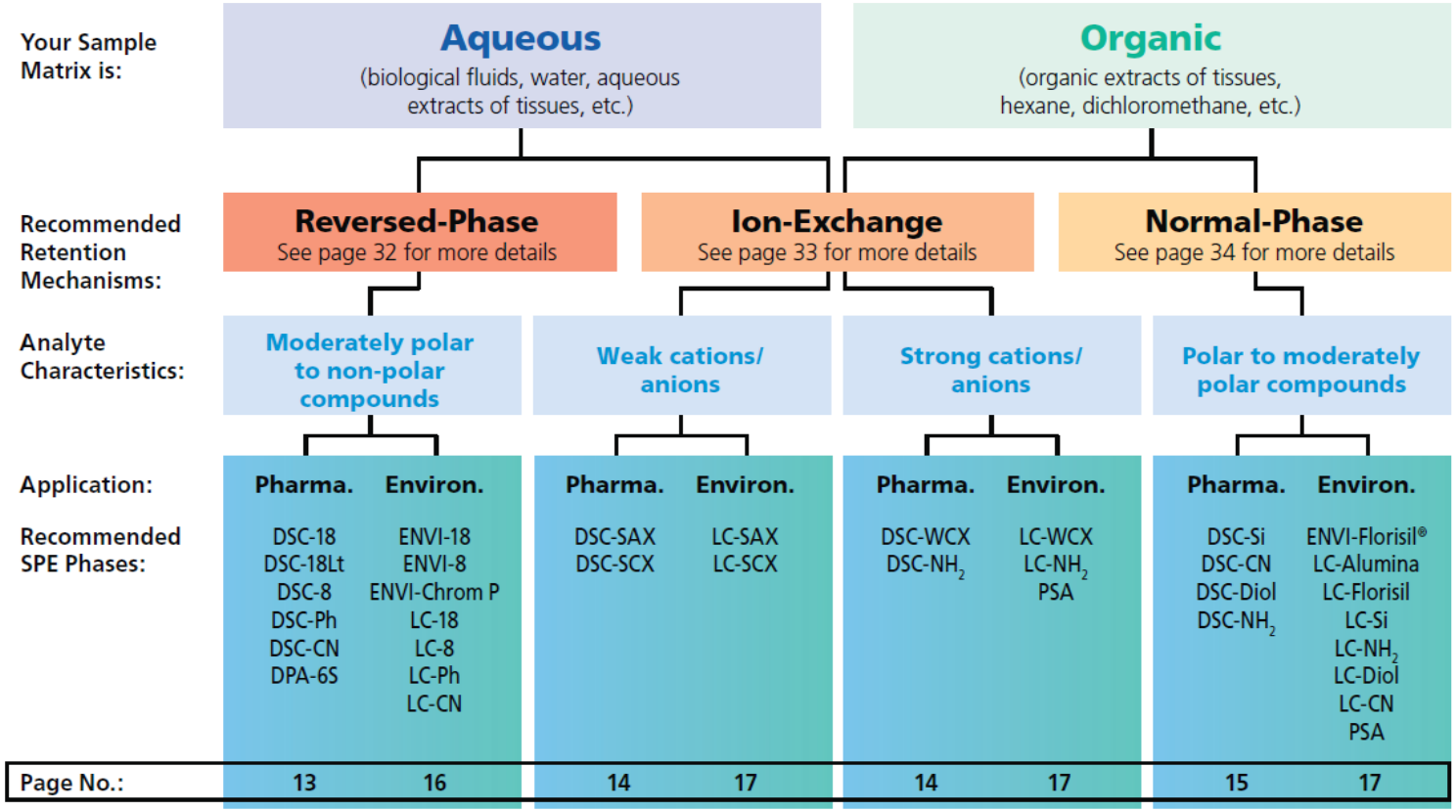
- Compatible with the analytical method

- Free from impurity

- Low cost and non toxic

SPE Phase Selection

SPE Phase Selection Quick Look-Up Guide



Supelco SPE Specialty Phases:

Phase Description	Field/Applic.	Page	Description
HybridSPE-Precipitation	Ph	8	Combines the simplicity of protein precipitation with the selectivity of SPE for the targeted removal of proteins andphospholipids in biological samples
Supel-Select HLB	Ph, G, E	9	Hydrophilic modified styrene based polymer for the broad range extraction of diverse analytes from aqueous

sigma-aldrich.com/spe

Solid–phase extraction advantages

☺ High recoveries of the analyte

☺ Concentartion of analyte

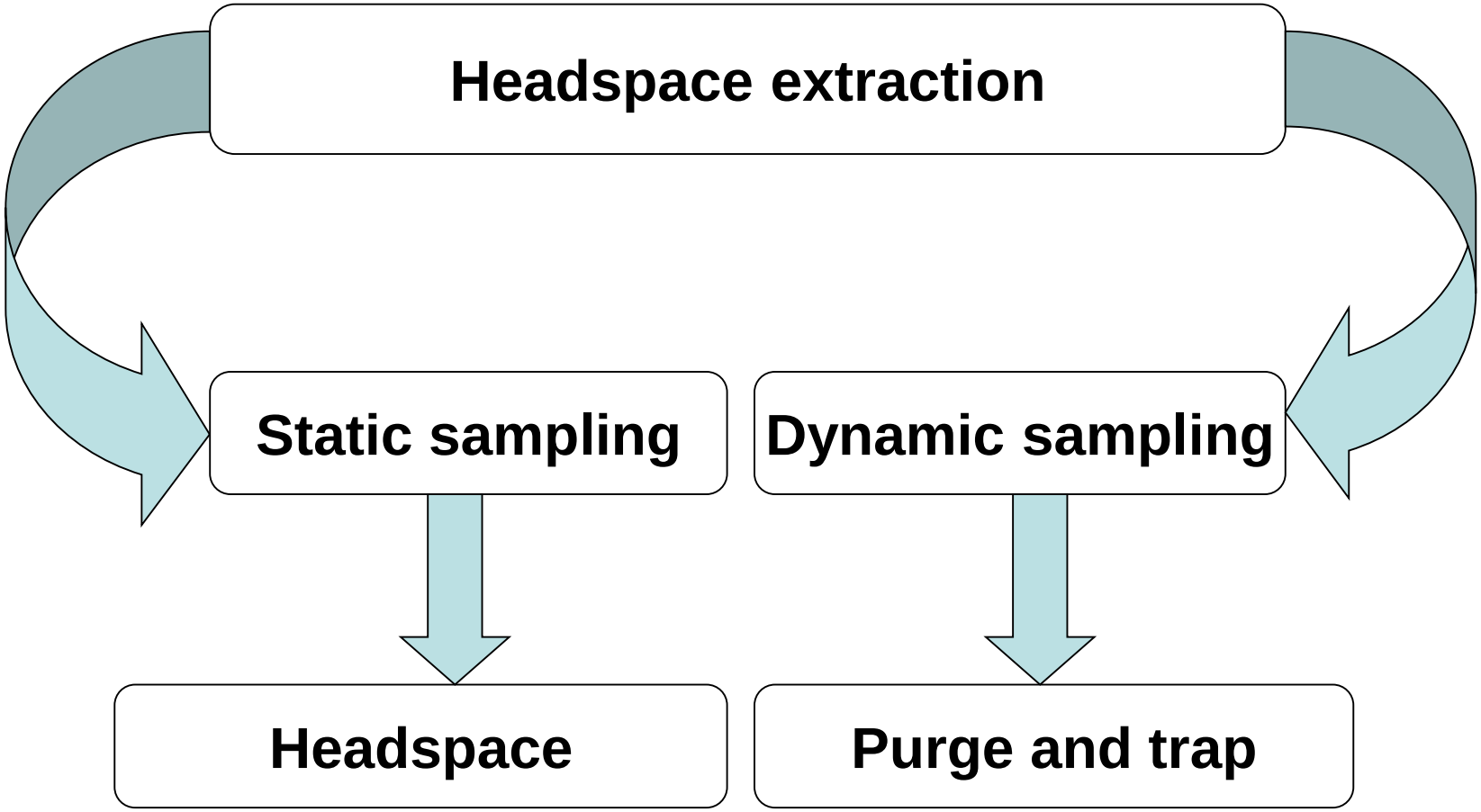
☺ Highly purified extracts

☺ Ability to simultaneously extract analytes of wide polarity range

☺ Ease of automation

☺ Compability with instrumental analysis reduction in organic solvent

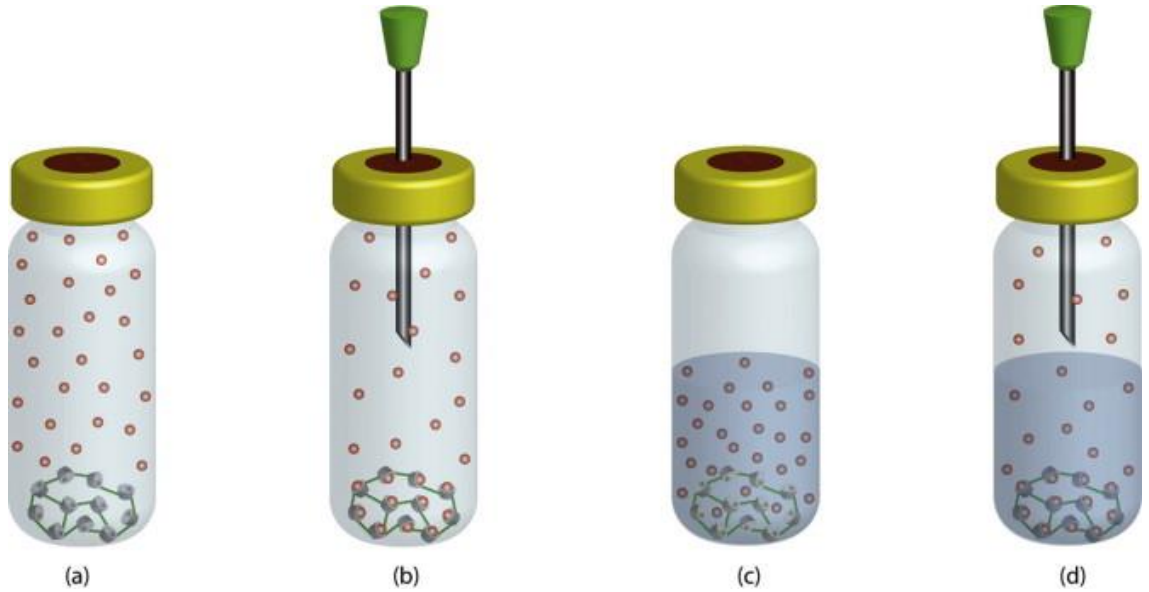
Headspace extraction



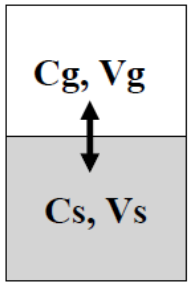
Gas extraction methods

Sample preparation method	Principle of technique	Comments
Headspace sampling	A solid or liquid sample is placed in a closed glass vial until equilibrium. Analytes partition themselves between a gas phase and a solid or liquid phase; gas phase is sampled and injected into a GC	For determining trace concentrations of VOCs in samples that are difficult to handle by conventional GC. Increasing temperature, salting out, adjusting pH, would shift equilibrium of analytes from the matrix
Purge & trap (dynamic headspace)	A solid or liquid sample is placed in a closed container, VOCs are continually purged by an inert gas and subsequently trapped by SPE sorbent and then thermal desorbed into GC (Thermal desorption)	For determining trace concentrations of VOCs in samples and for analytes that have unfavorable partition coefficient in static headspace sampling

Headspace extraction



Basic of Headspace



Partition Coefficient (K) = Cs/Cg

Phase Ratio (β) = Vg/Vs

Cs=concentration of analyte in sample
Cg=concentration of analyte in gas phase
Vs=volume of sample
Vg=volume of gas phase

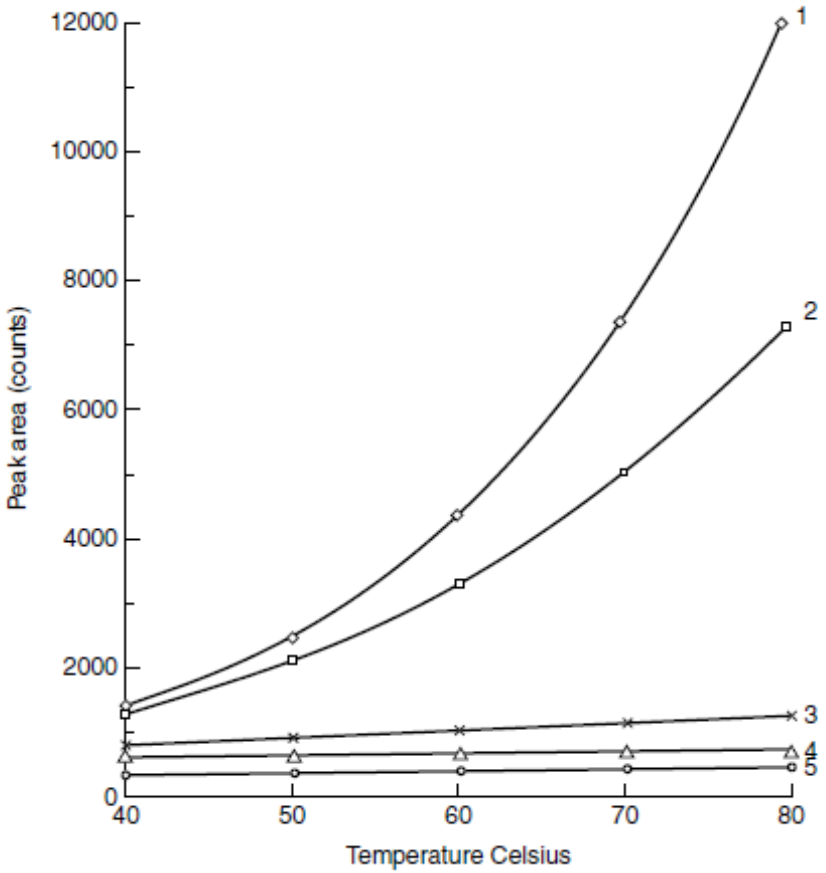
$$C_g = \frac{C_o}{K + \beta}$$

* K and β are important variables in headspace analysis.

Headspace Sampling

- Gas tight syringe
- Autosampler

Effect of temperature for water samples



POLARITY

1 – ethanol;

2 – methyl ethyl ketone;

3 – toluene;

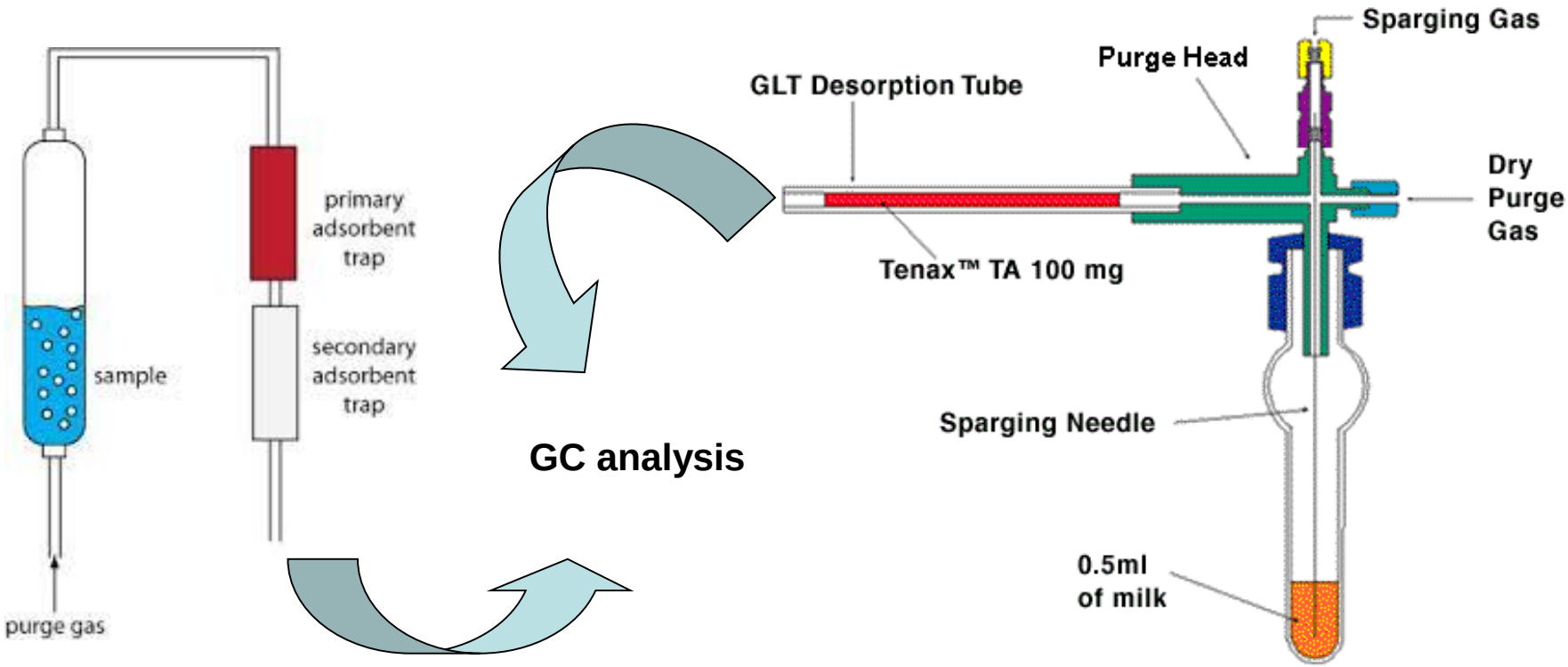
4 – n-hexane;

5 - tetrachloroethylene

Purge and trap

Purge and trap

Continuous method of gas extraction and separates volatile sample constituents from the matrix by a continuous flow of an inert gas above a solid or liquid sample



Solid phase microextraction (SPME)

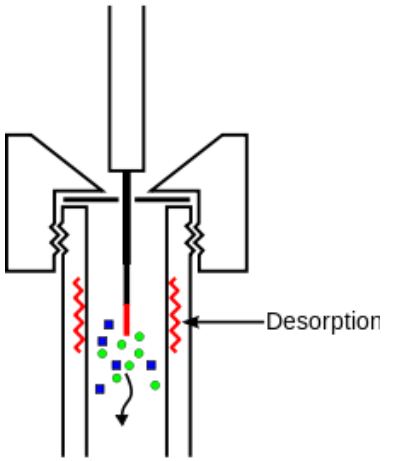
Solid Phase Microextraction

An innovative, solvent-free sample prep technology fast, economical, and versatile.

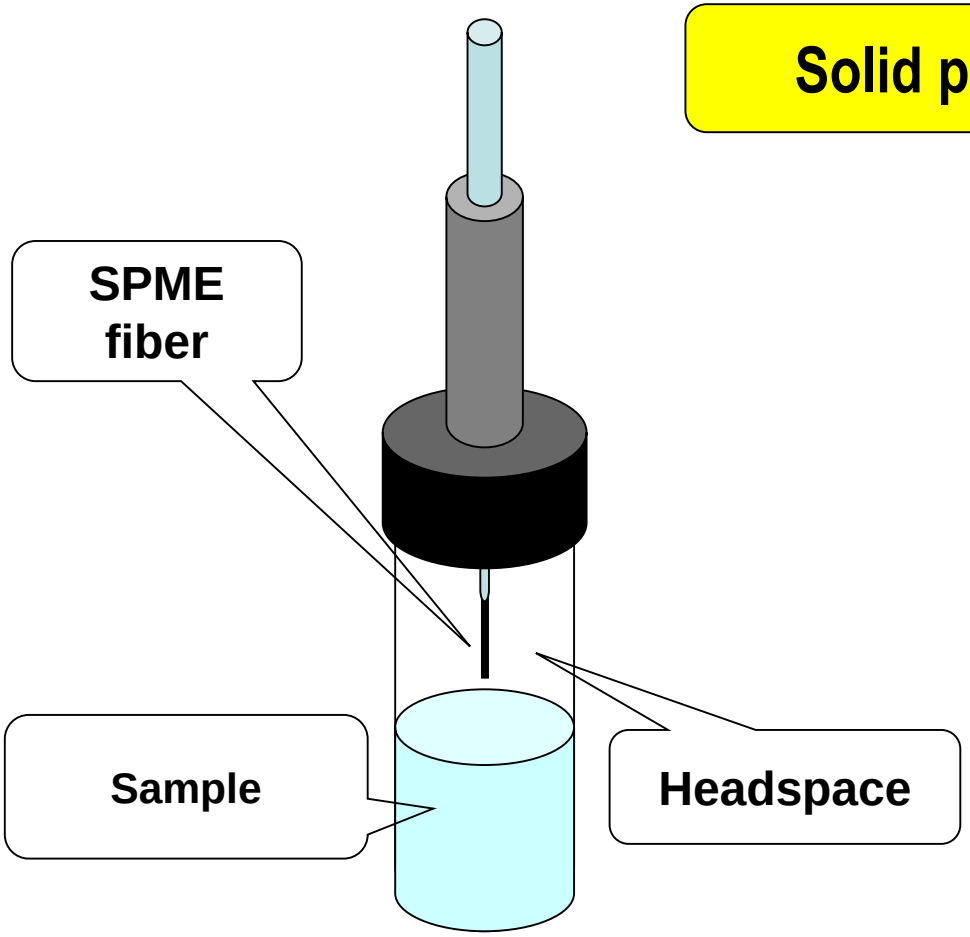
SPME uses a fiber coated with a liquid (polymer), a solid (sorbent).

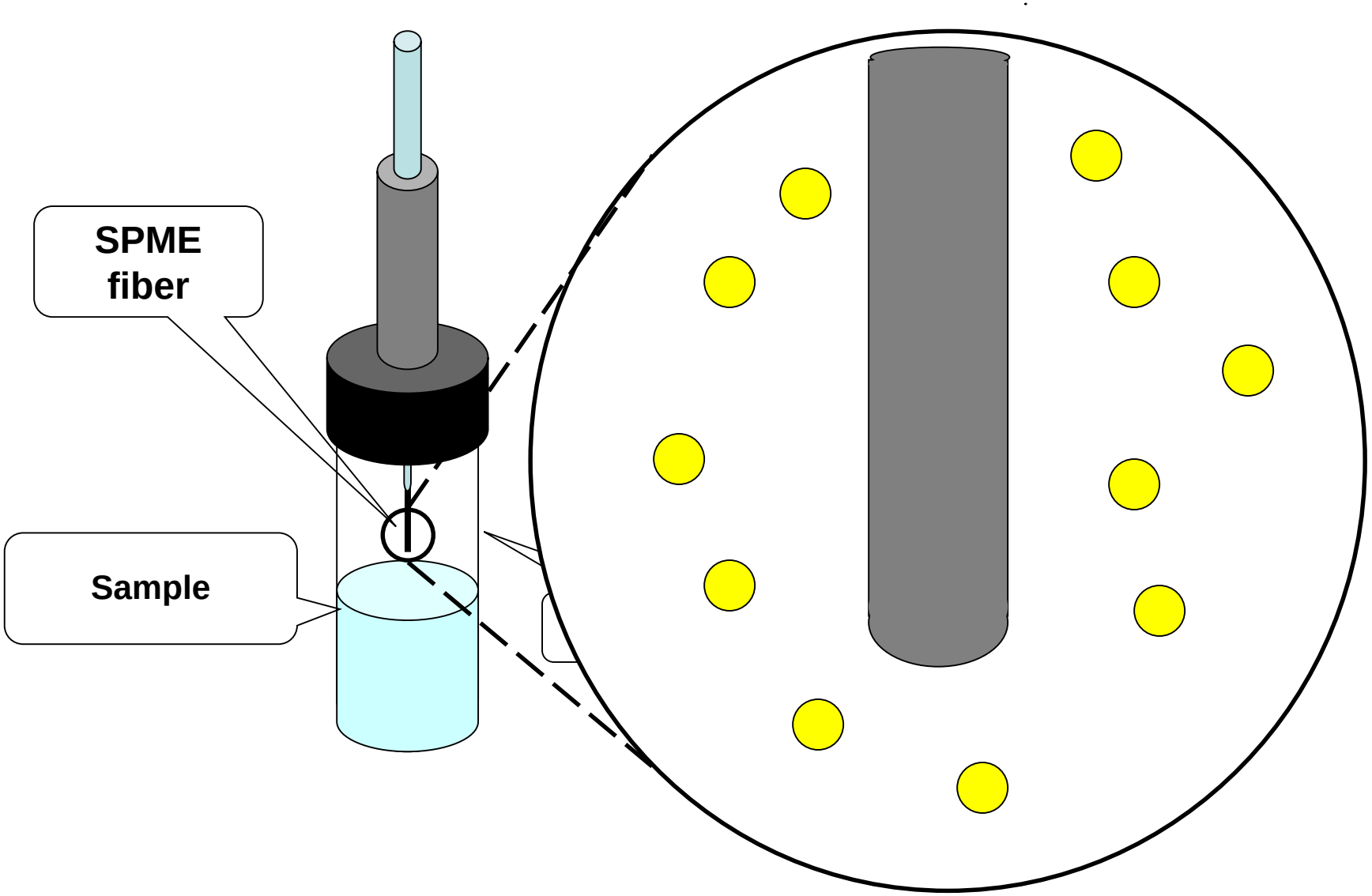
The fiber coating removes the compounds from sample by absorption in headspace or with direct injection to sample

The SPME fiber is then inserted directly into the CG for desorption and analysis.

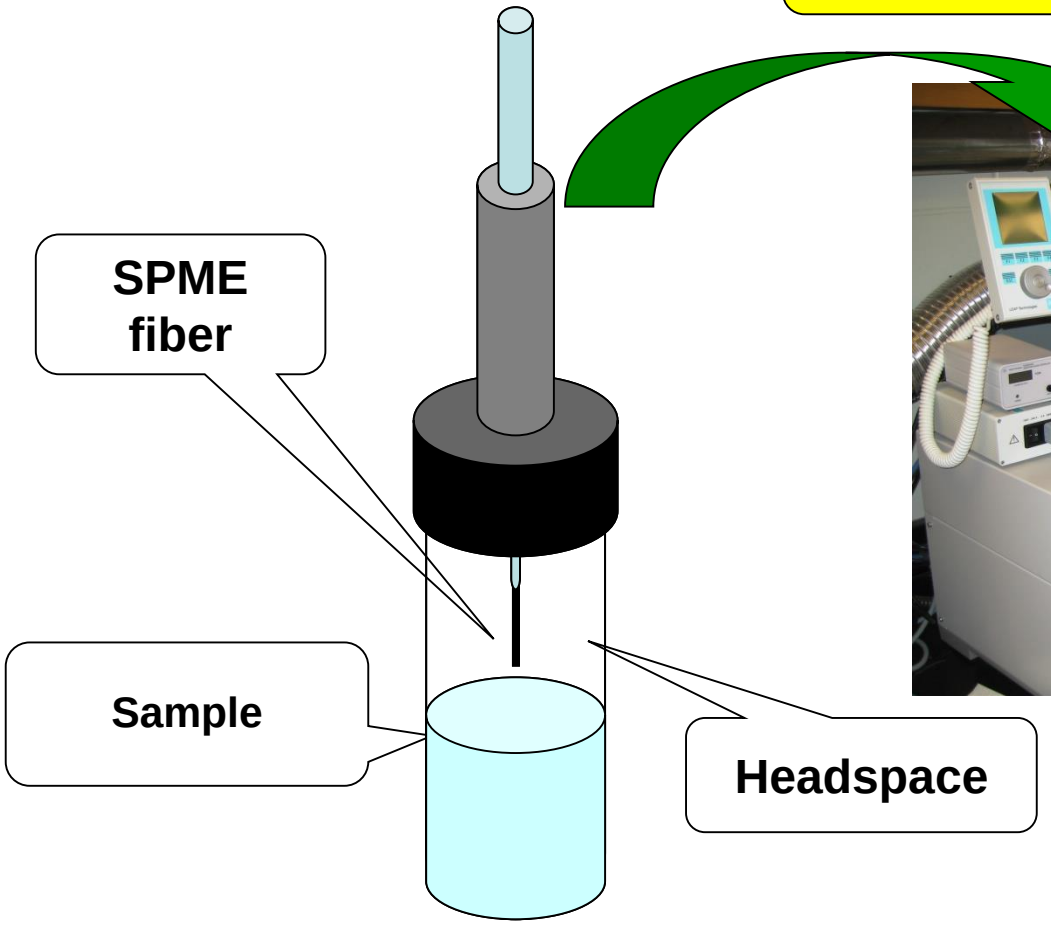


Solid phase microextraction (SPME)




SPME
fiber
Sample

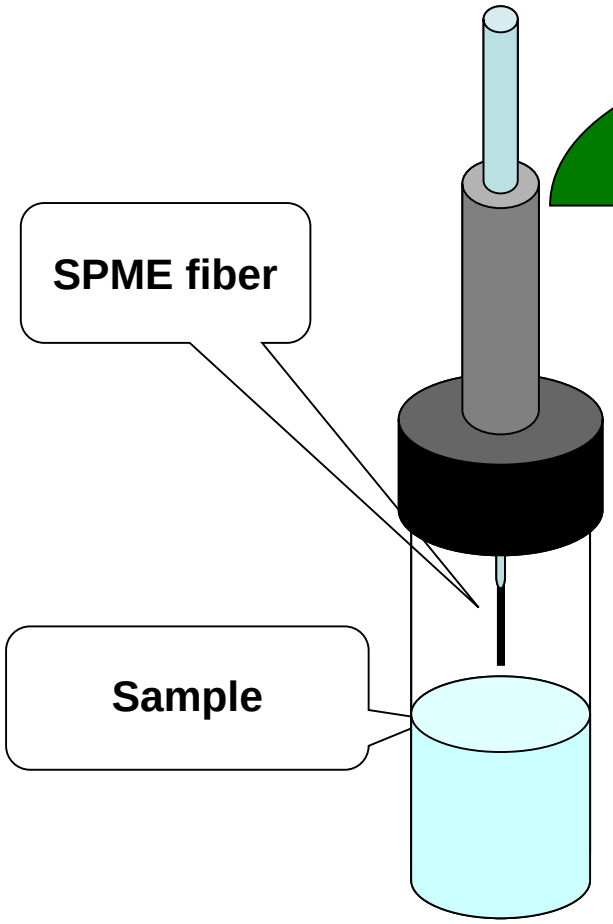
Solid phase microextraction (SPME)



GC/MS analysis

Identification and quantification of pollutants

EXPERIMENTAL



GC-MS analyses

Identification and quantification of pollutants

Solid Phase Microextraction advantages

☺ Simple and fast operation
☺ Solventless extraction
☺ High sensitivity and limit of determination

☺ All extracted analytes are transferred to the analytical instrument

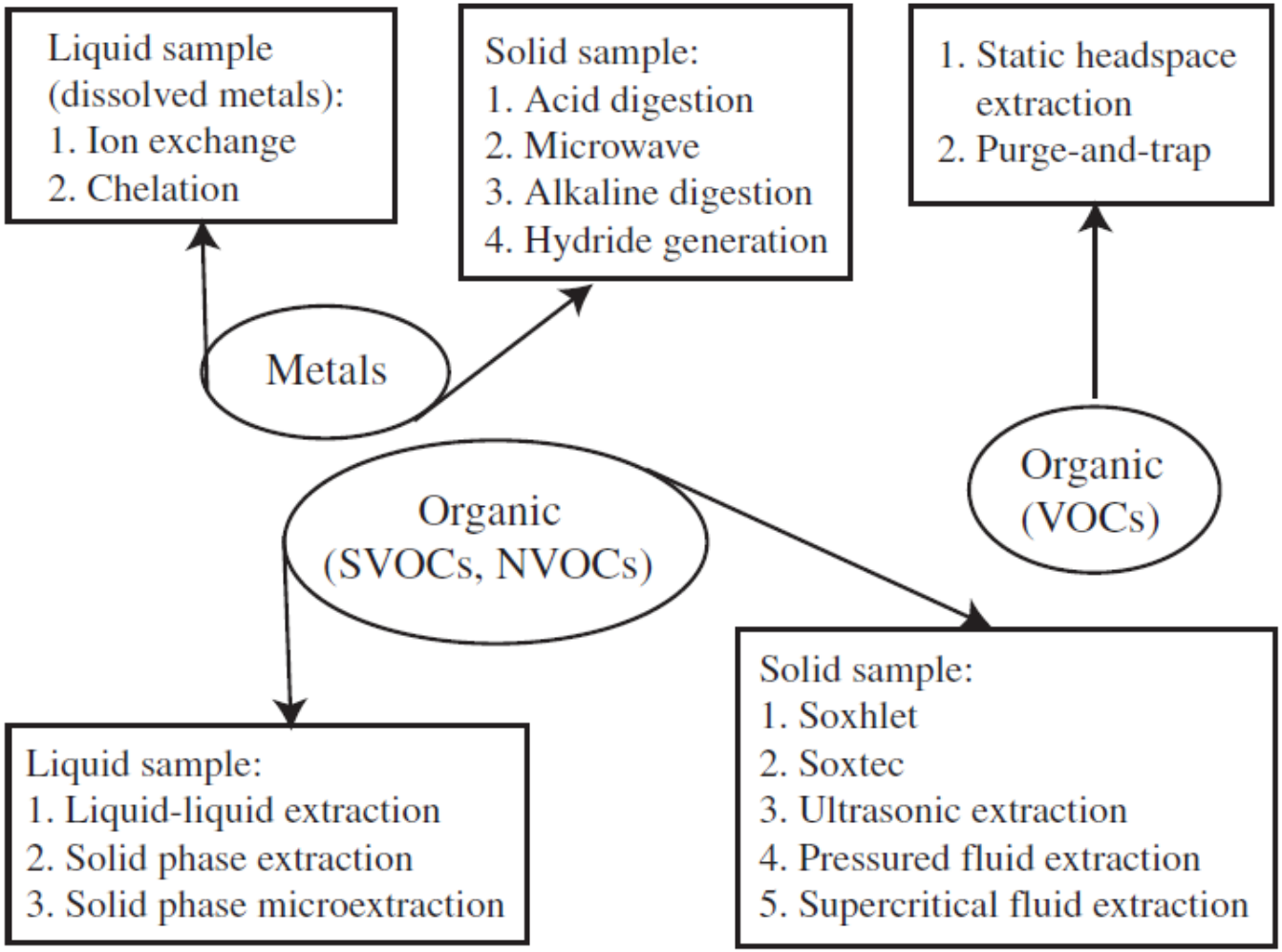
☺ Can sample directly into a sample or the headspace above sample

Solid Phase Microextraction disadvantages

- Expensive ($150 per fiber)

- Polymer coating is fragile, easily broken, and have limited lifetime

Method selection



Thank you!

Questions ?