Center of Physico-Chemical Methods of Research and Analysis

Modern methods for sample preparation prior to analysis

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Sample preparation

- ✓ It takes 60% of the time, labor and money costs in the analysis
- ✓ Introduces additional errors

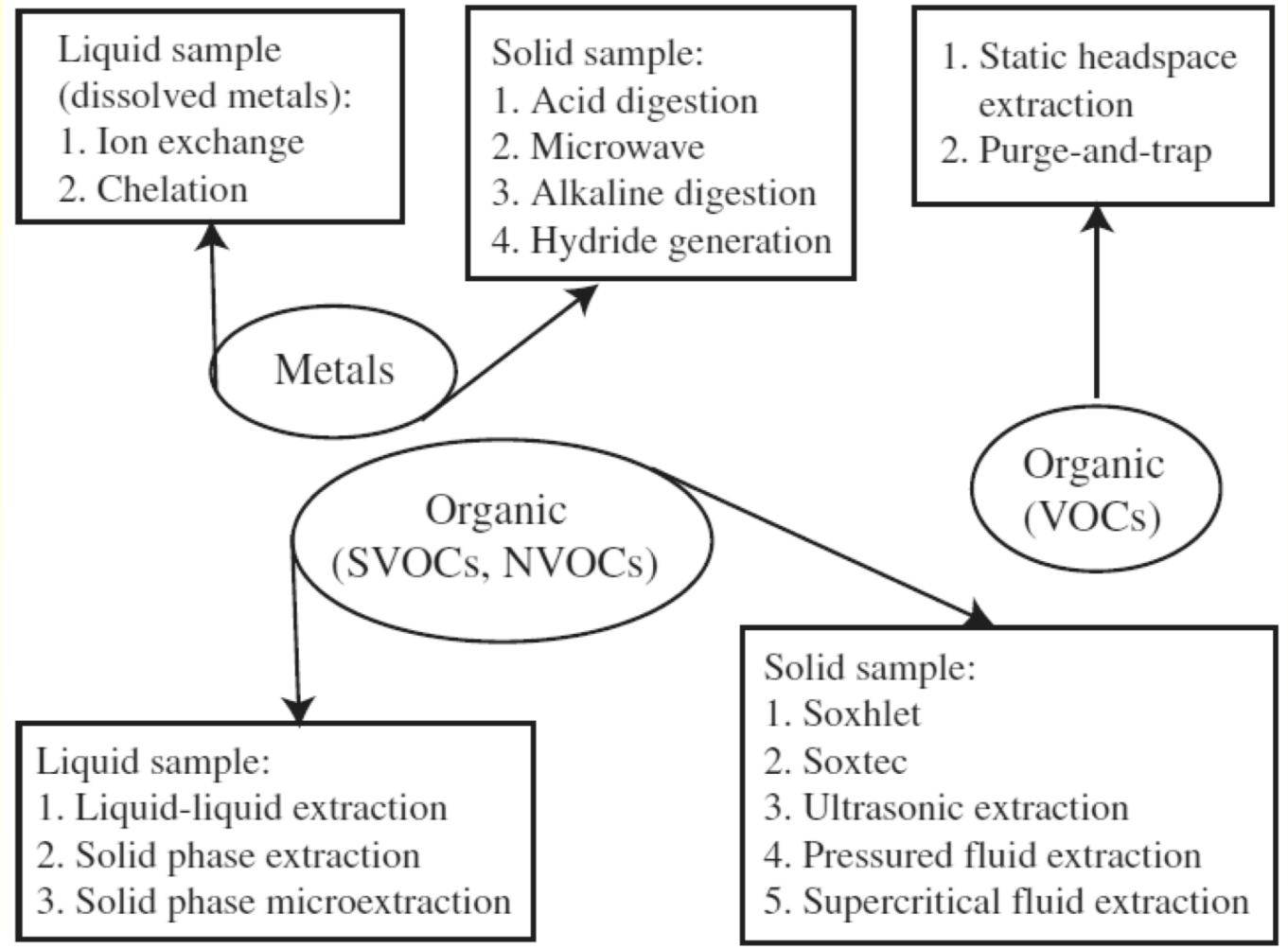
Purpose

- Separate analytes from each other
- To increase concentration of analyte

Type of sample preparation

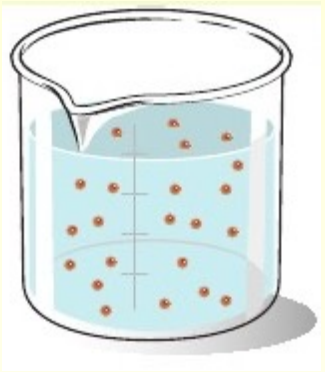
- Dilution, concentration of analyte
- Centrifugation
- Filtration
- Extraction
  - Liquid-liquid extraction
  - Solid phase extraction
  - Vapor phase extraction
  - Purge and Trap method
  - Solid phase microextraction

Selection of method

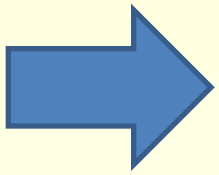


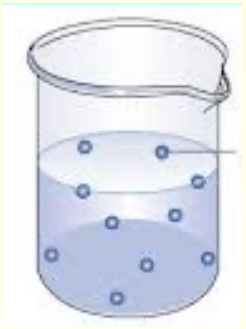
Types of sample preparation

- Dilution, concentration of sample

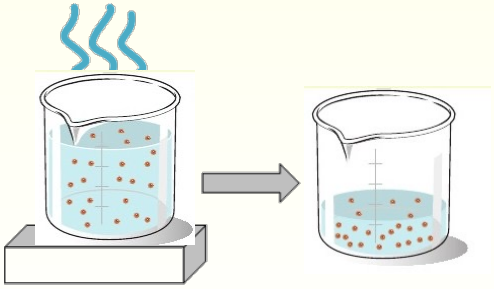


Dillution





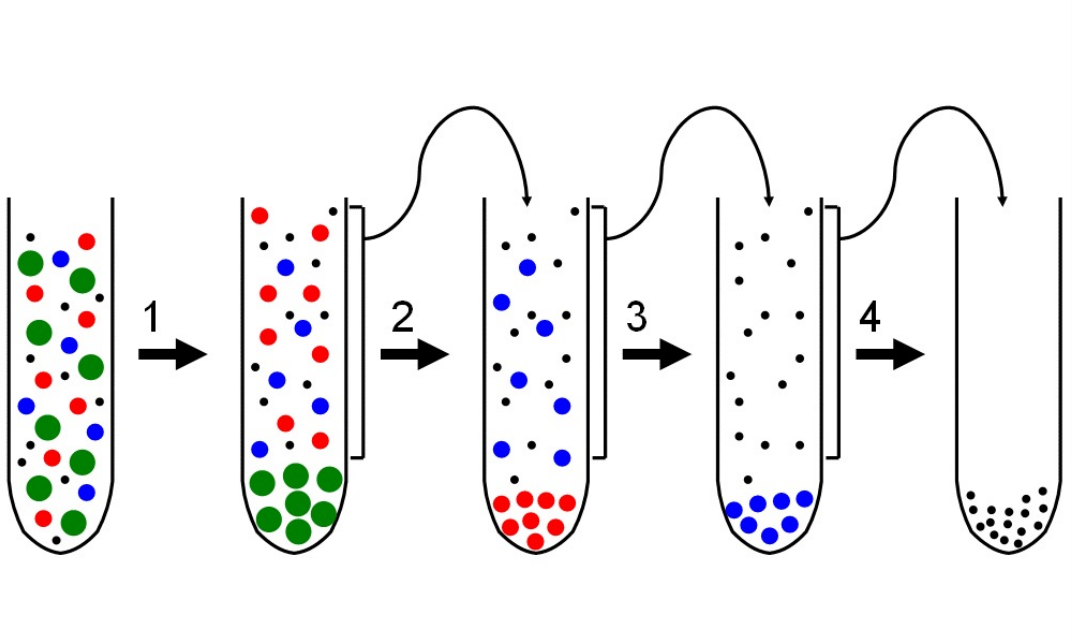
Concentration



$$C_1V_1=C_2V_2$$

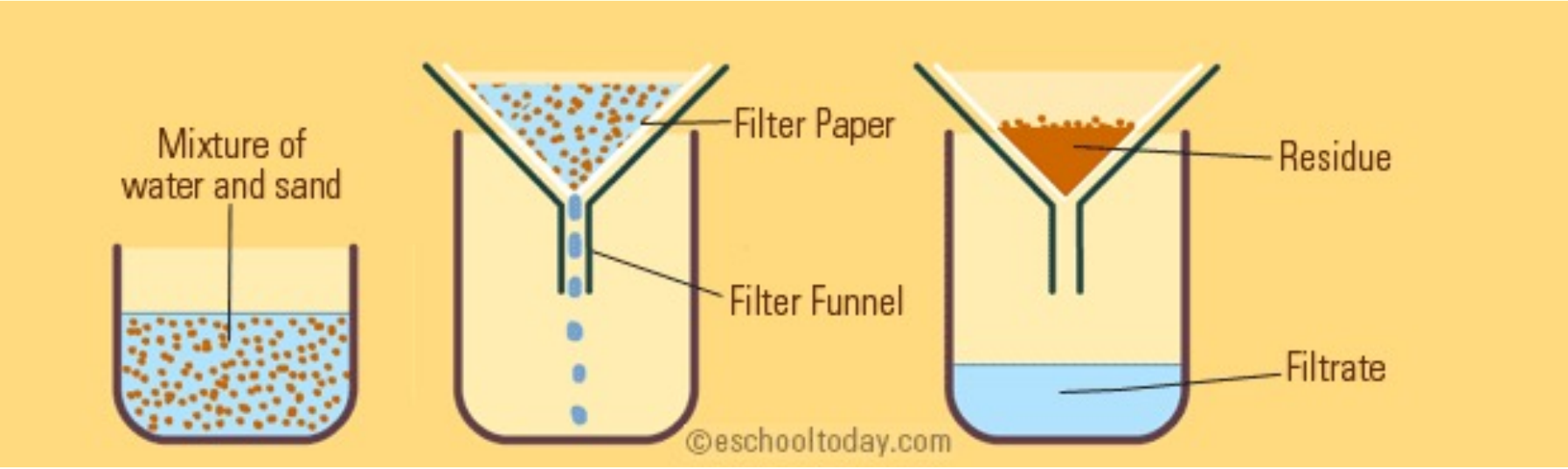
Types of sample preparation

- Centrifugation



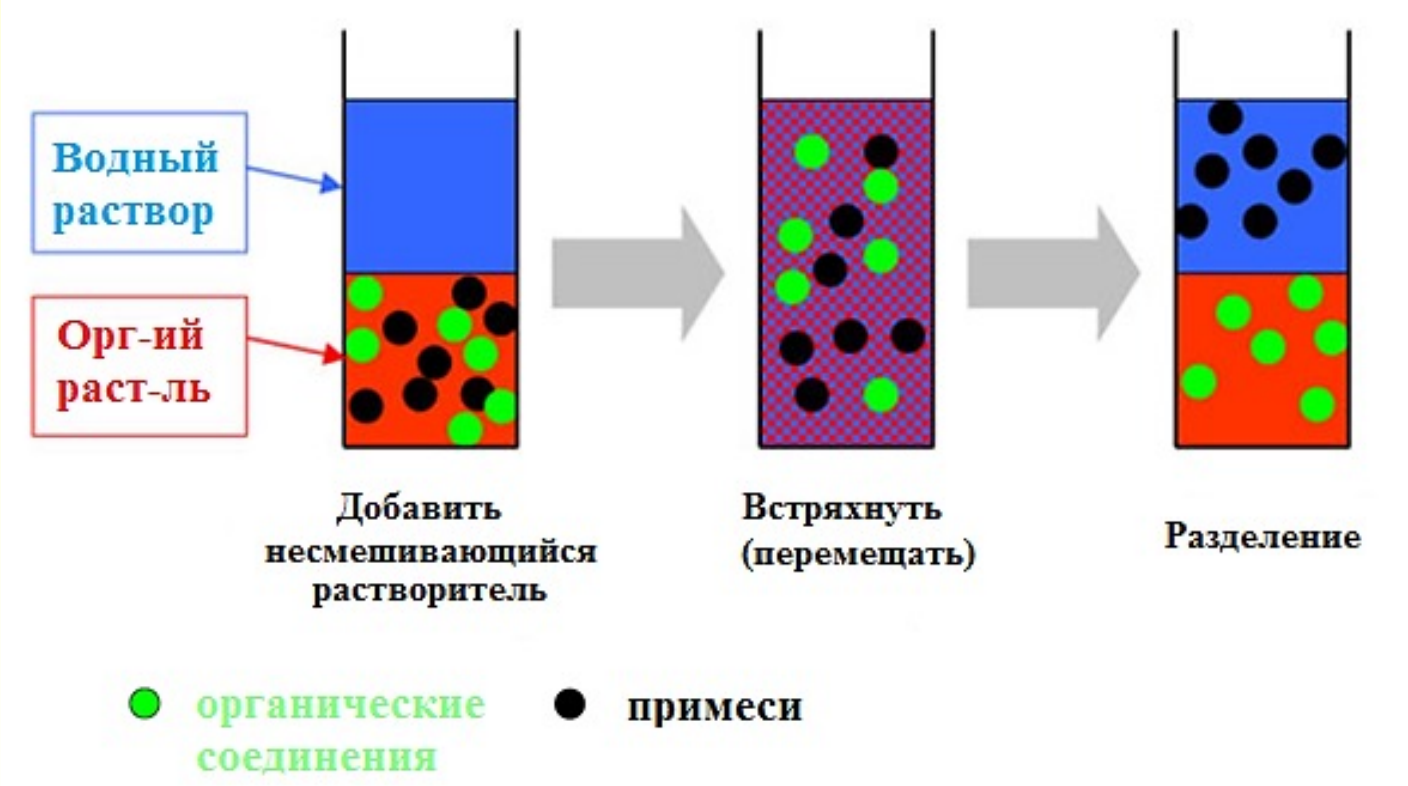
Types of sample preparation

- Filtration



Liquid-liquid extraction

A method of separating and concentrating substances based on their different distribution between two immiscible liquid phases, usually between water and an immiscible organic solvent



Liquid-liquid extraction

- A common type of sample preparation
- For non-volatile organic compounds
- The division of the sample between two miscible phases
- The extraction efficiency depends on the solubility of immiscible solvents

Theory

Distribution coefficient

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

$[A]_{org}$ – equilibrium concentrations of compounds in the organic phase;

$[A]_{aq}$ – equilibrium concentrations of compounds in the aqueous phase

How to choose a solvent

- Immiscible with water
- Relatively low boiling point
- High solubility of organic compounds
- Non-toxic, affordable, inexpensive

Typical solvents in L/L extraction

❑ Aqueous solvents

- Clean water
- Acid solution
- Alkaline solution
- The combination of solutions

❑ Organic solvent

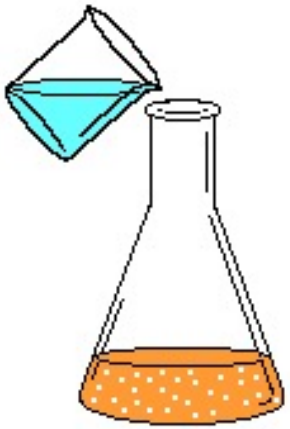
- Diethyl ether
- Methylene chloride
- Chloroform
- Ethyl acetate
- Toluene and xylenes
- Aliphatic ketones

Example

Sugar is more soluble in water than in oil.

Water does not mix (dissolve) with oil

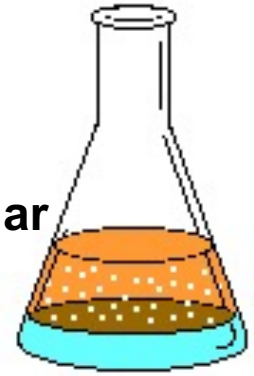
Water



Aqueous phase the lower layer

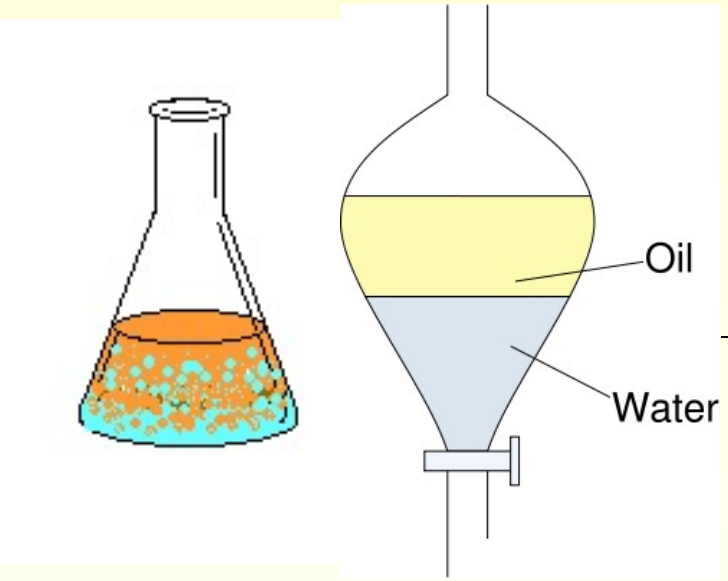
The oil phase is the upper

Oil and sugar

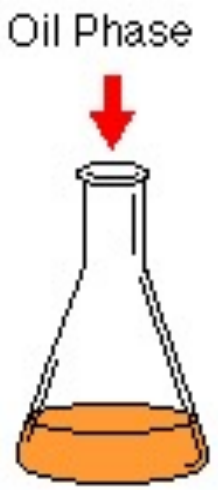


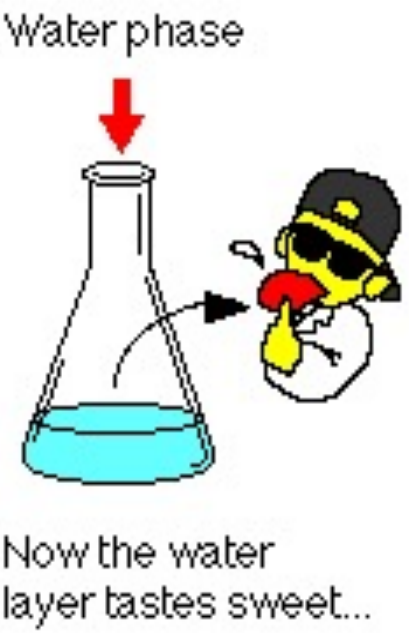
Вода

Extraction of sugar from oil with water

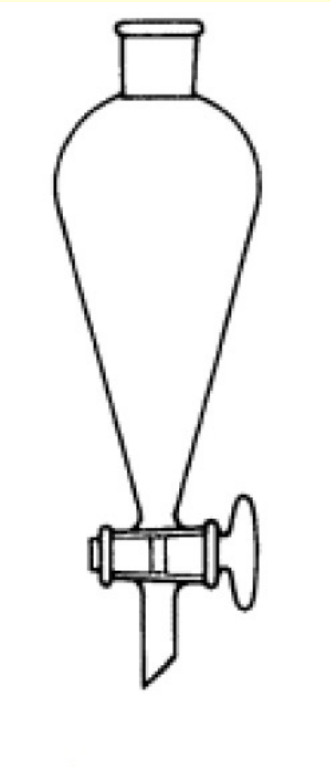


Water + Oil + sugar

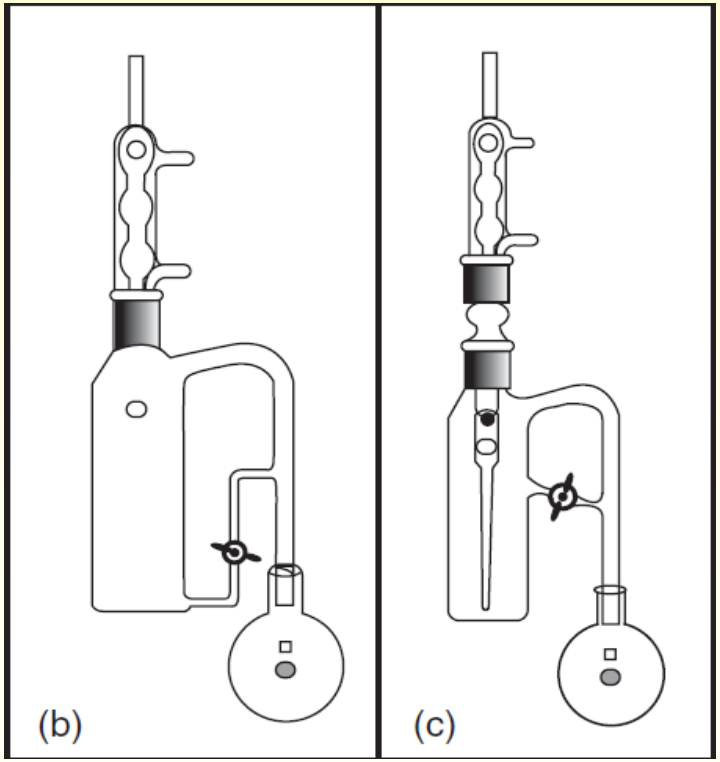




Devices for L/L extraction



Separating funnel



Continuous extractor designed for heavier/lighter water solvents

Increasing the efficiency of L/L extraction

- Addition of salt
- pH
- Proper choice of solvent
- Sequential extraction (several portions of extractant for procedure)

The advantages of the L/L extraction

☺ Quick and easy

☺ Relatively low solvent use in the extraction of hydrophobic compounds

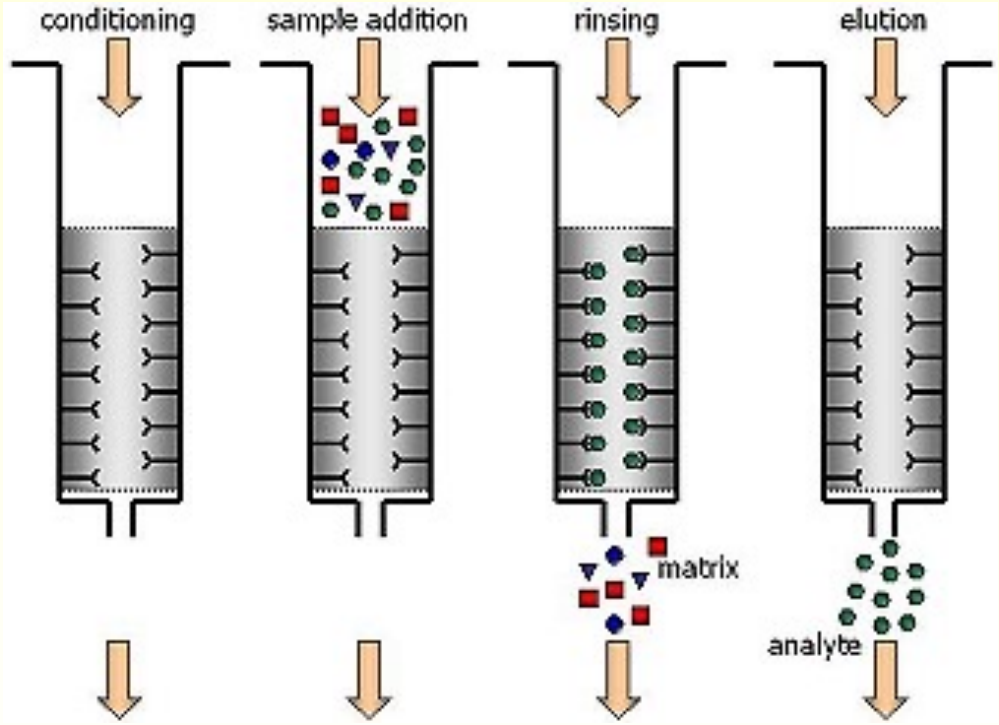
☺ High extraction efficiency for analytes with low K_d using sequential or continuous extraction

Disadvantages of the L/L extraction

- Loss of volatile compounds
- Difficult to achieve 100% extraction efficiency
- Use and disposal of more toxic organic solvents
- Emulsion formation (difficulty in separating emulsions)
- Bulky glass equipment
- Laborious process, difficult to automate
- Pre-concentration of the sample is often required

Solid-phase extraction (SPE)

A rapid sample preparation method in which a sorbent is used to concentrate and separate the target component or components followed by elution (washout) with a suitable solvent



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.

Main objectives (SPE)

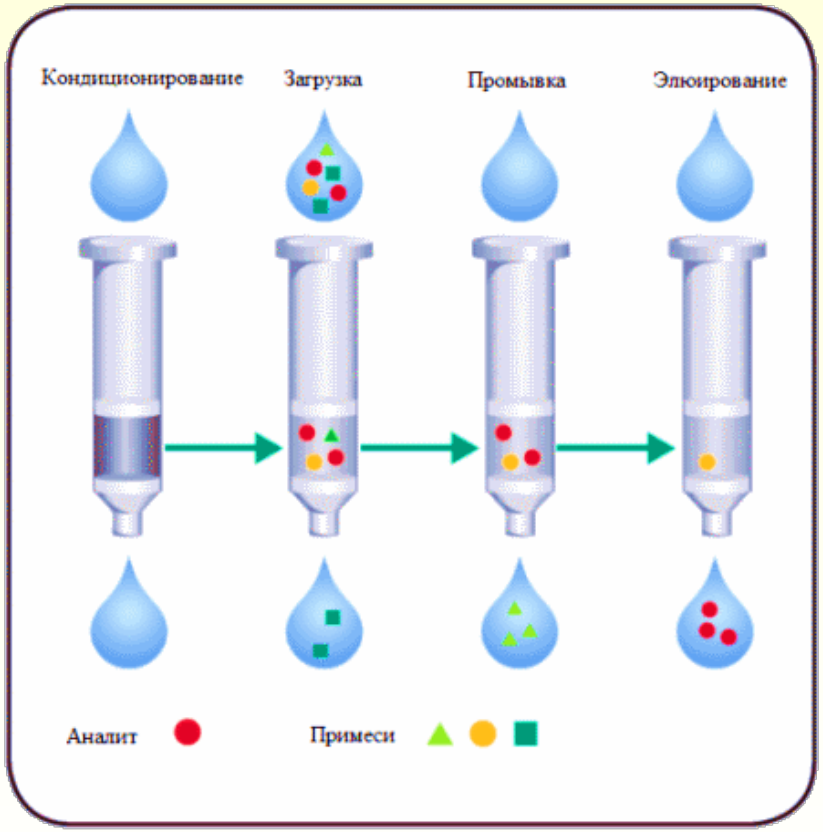
- purification of the sample from undesirable impurities
- concentration of sample components
- transfer of sample components to another matrix.

The main stages of SPE

- Conditioning
- Equilibration
- The sample application
- Washing
- Eluting

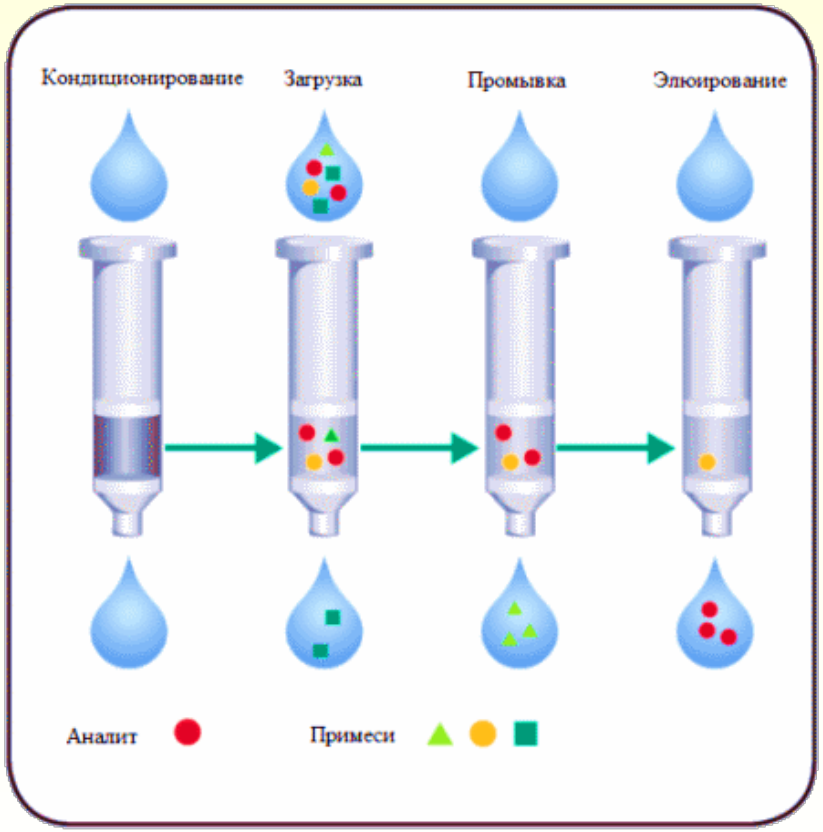
Conditioning

SPE is performed using the cartridges filled with a dry sorbent. Conditioning is required to activate the sorbent.



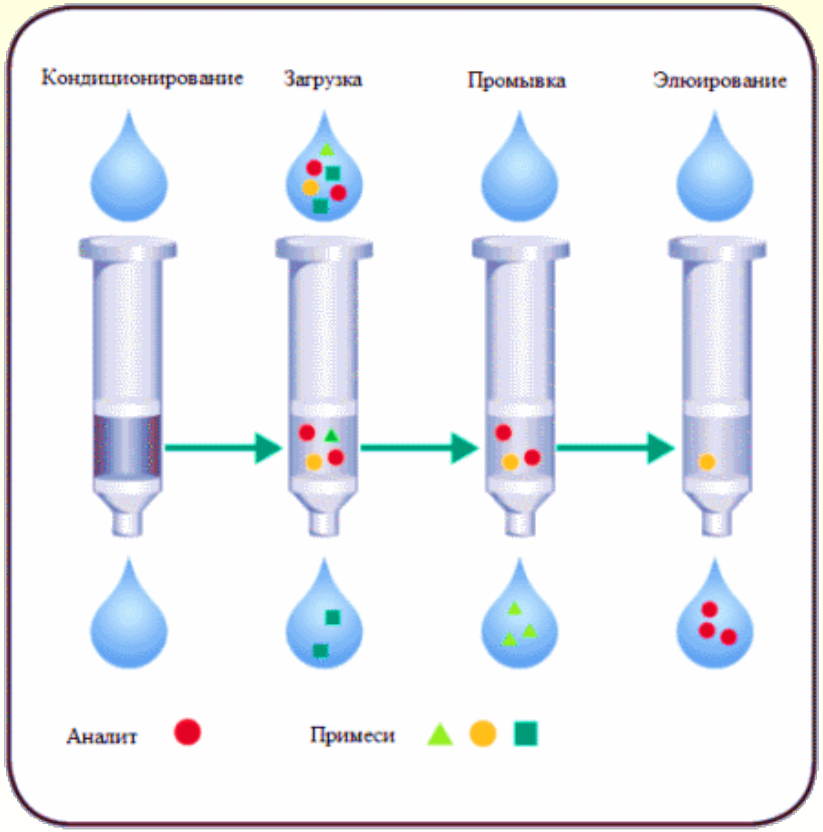
Loading of sample

The aim is to quantitatively retain the analyte on the SPE cartridge while the matrix impurities are removed. The sample is introduced into the SPE column and it passes through the sorbent.



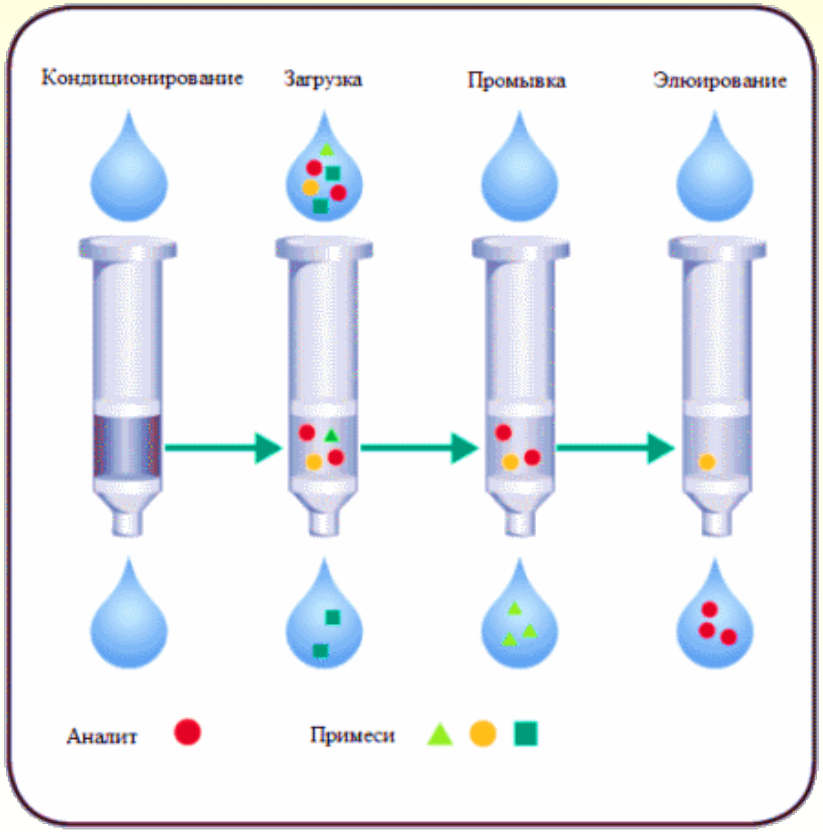
Washing

After the analyzed substance(s) is retained by the sorbent, the sorbent is washed from impurities.

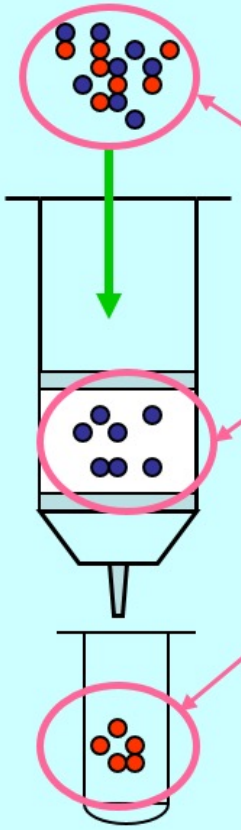


Eluting

Quantitative elution of the analyte. This can be done in one or more steps to collect different analytes into different test tubes.

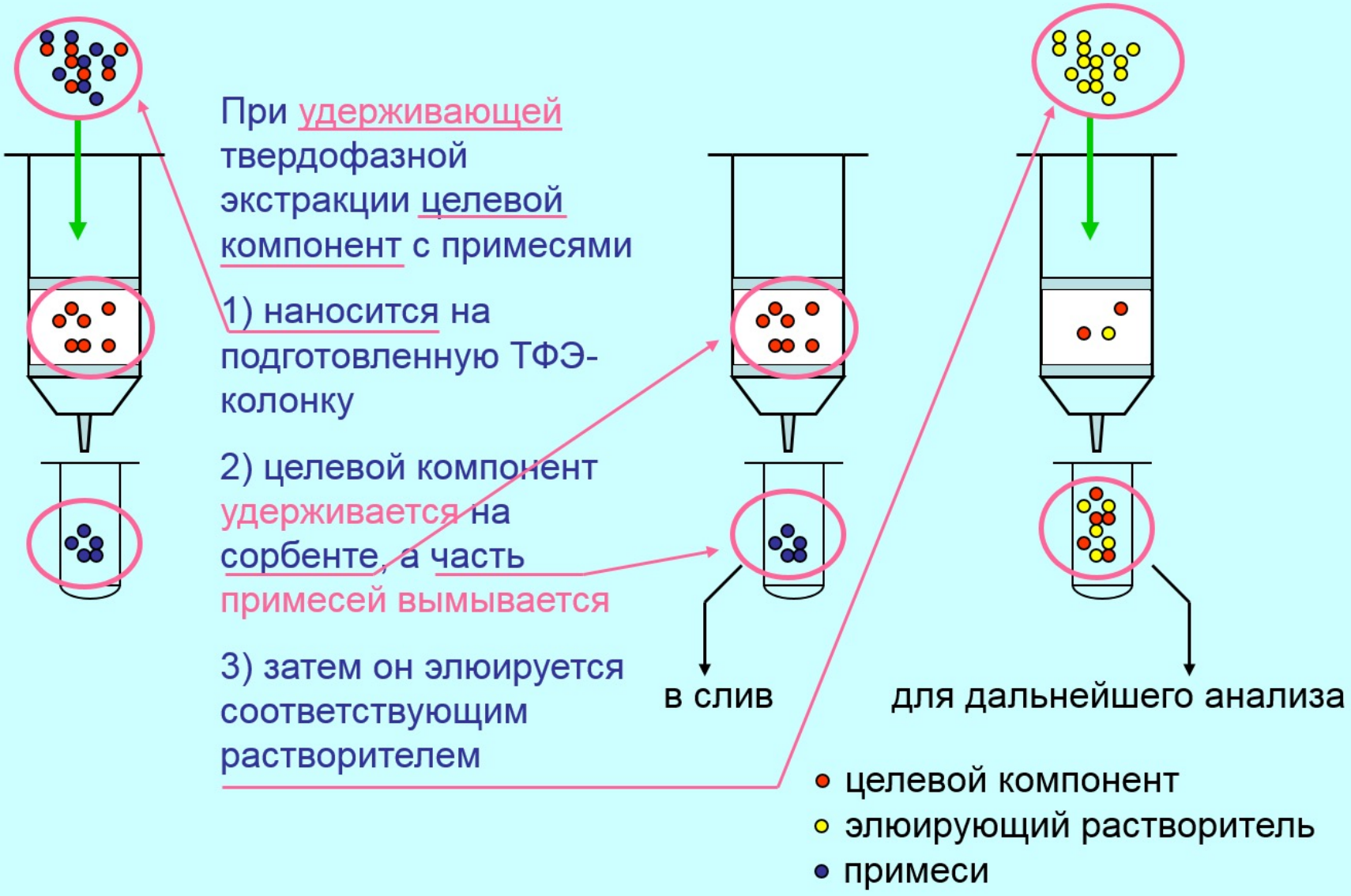


Все методы ТФЭ по принципу удерживания следует разделять на два типа: удерживающая и неудерживающая твердофазная экстракция



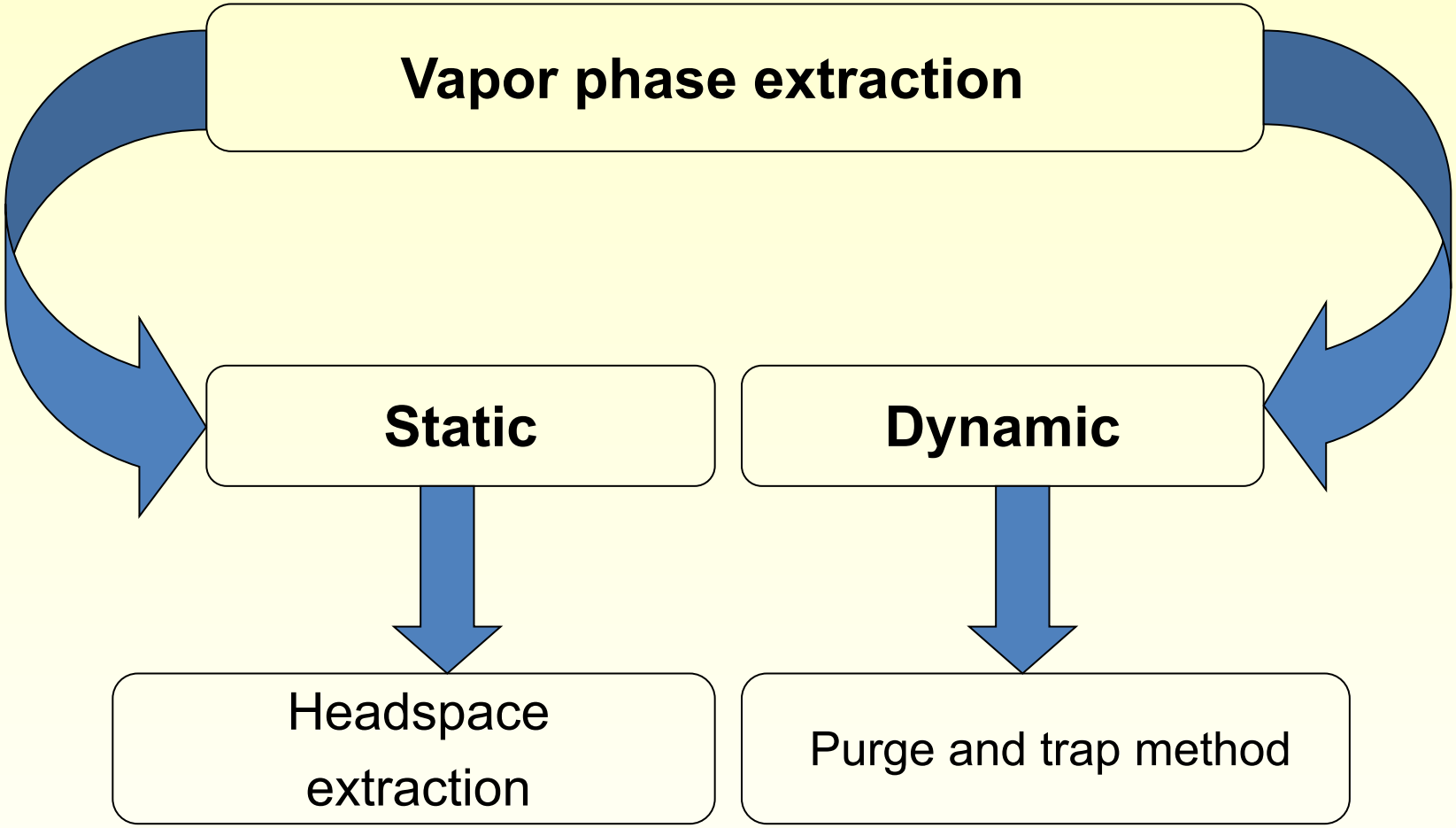
При неудерживающей твердофазной экстракции целевой компонент с примесями наносится на подготовленную ТФЭ-колонку. После этого, в процессе элюирования на сорбенте удерживается часть примесей, а целевой анализируемый компонент отправляется в емкость для сбора экстракта.

- целевой компонент
- примеси

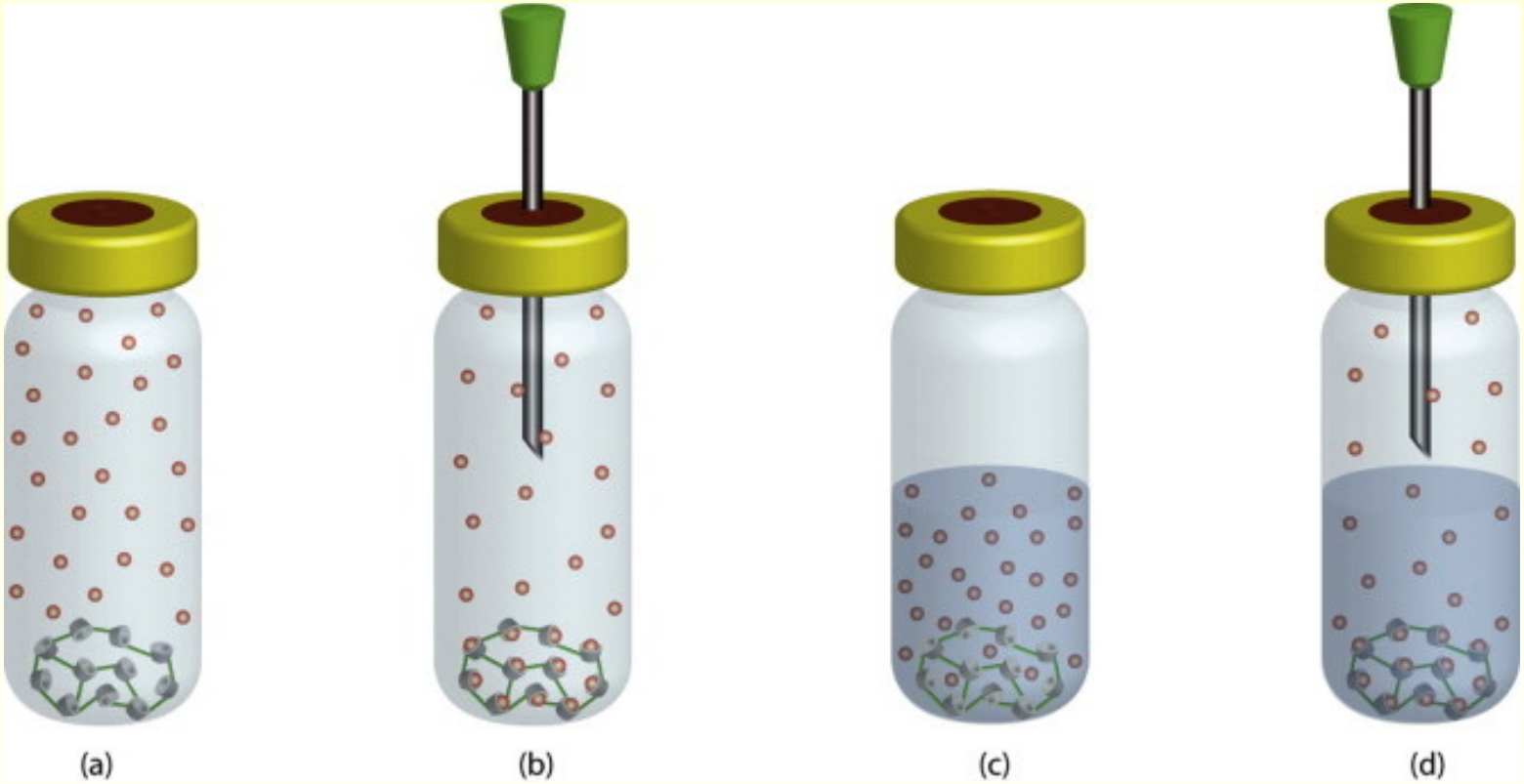


Advantages of SPE in comparison with L/L extraction

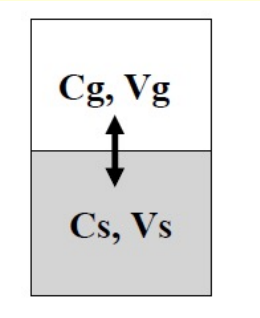
- Selectivity and specificity
- A better separation
- High quantitative recovery of the test sample(> 75%)
- Excellent reproducibility
- Ease of handling
- Possibility of optimization
- Savings of expensive solvents



Headspace extraction



Headspace extraction



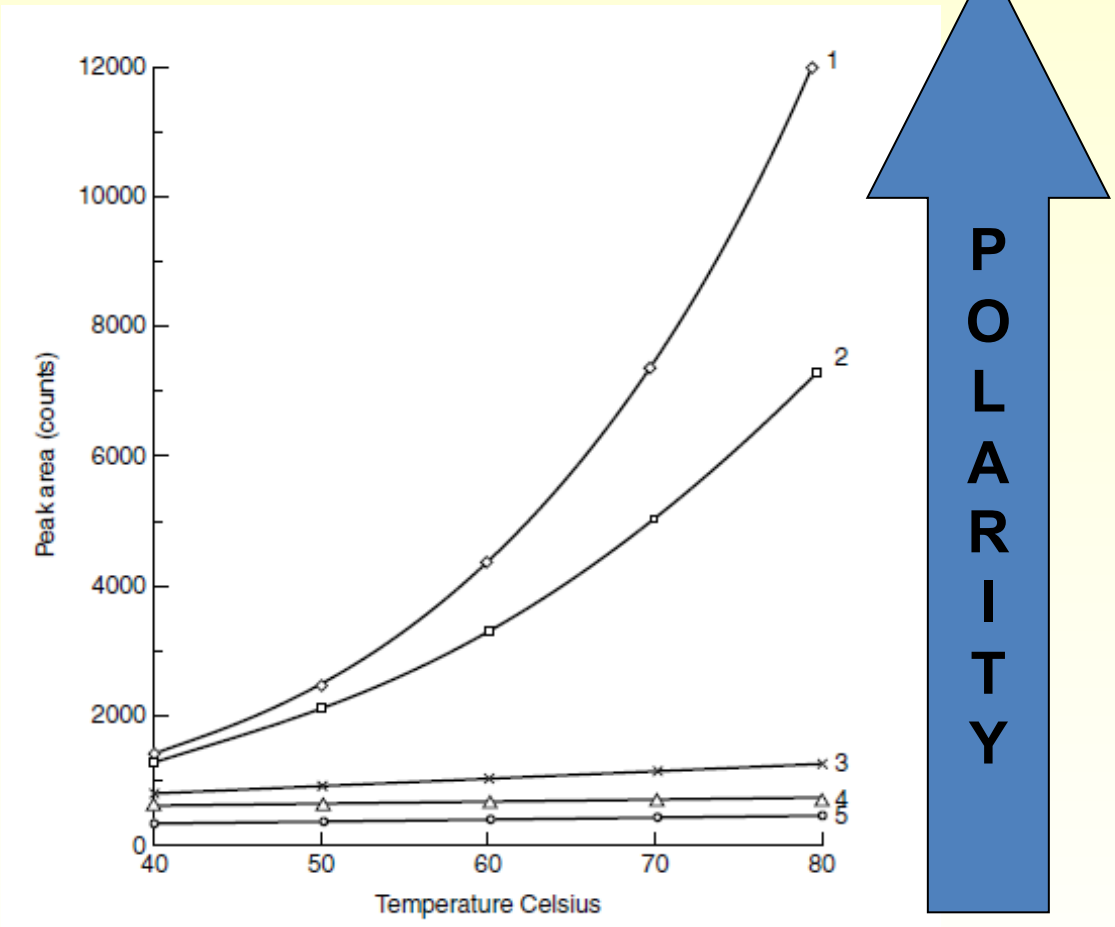
Distribution coefficient (K) = Cs/Cg

Phase ratio (β) = Vg/Vs

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

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

Temperature influence



1 – Ethanol;

2 – Methyl-ethyl ketone;

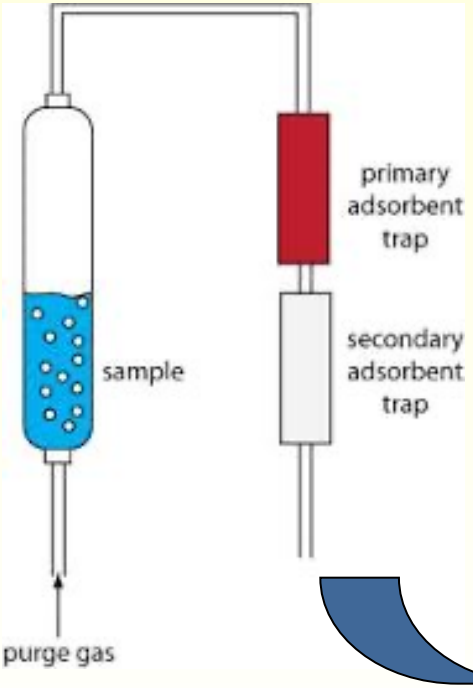
3 – толуол;

4 – n-hexane;

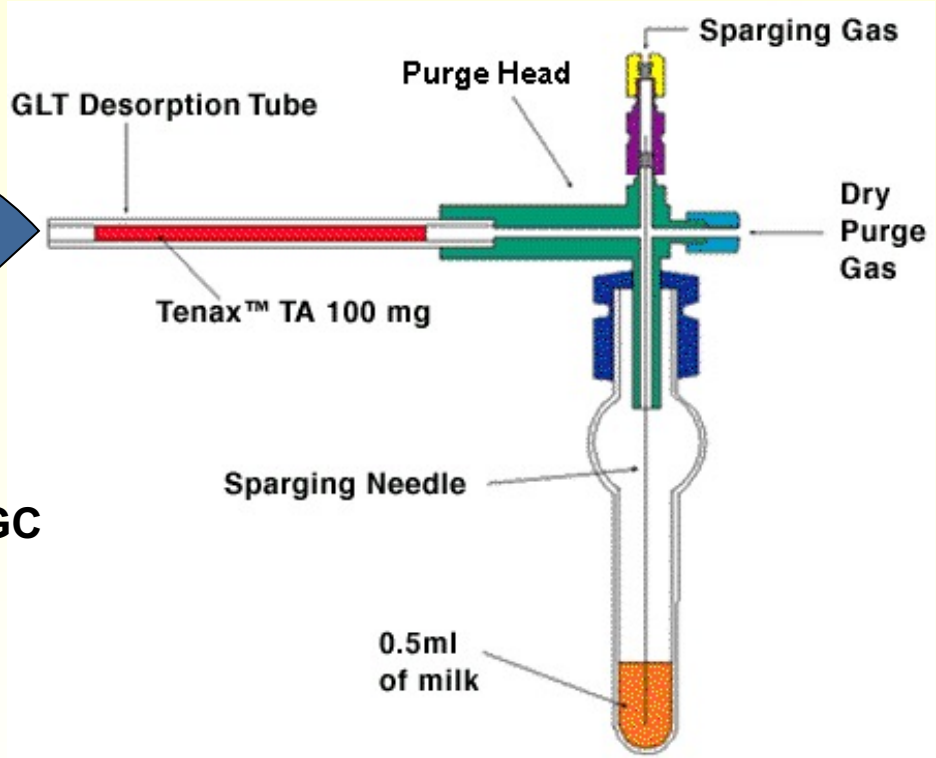
5 - tetrachloroethylene

Purge and trap method

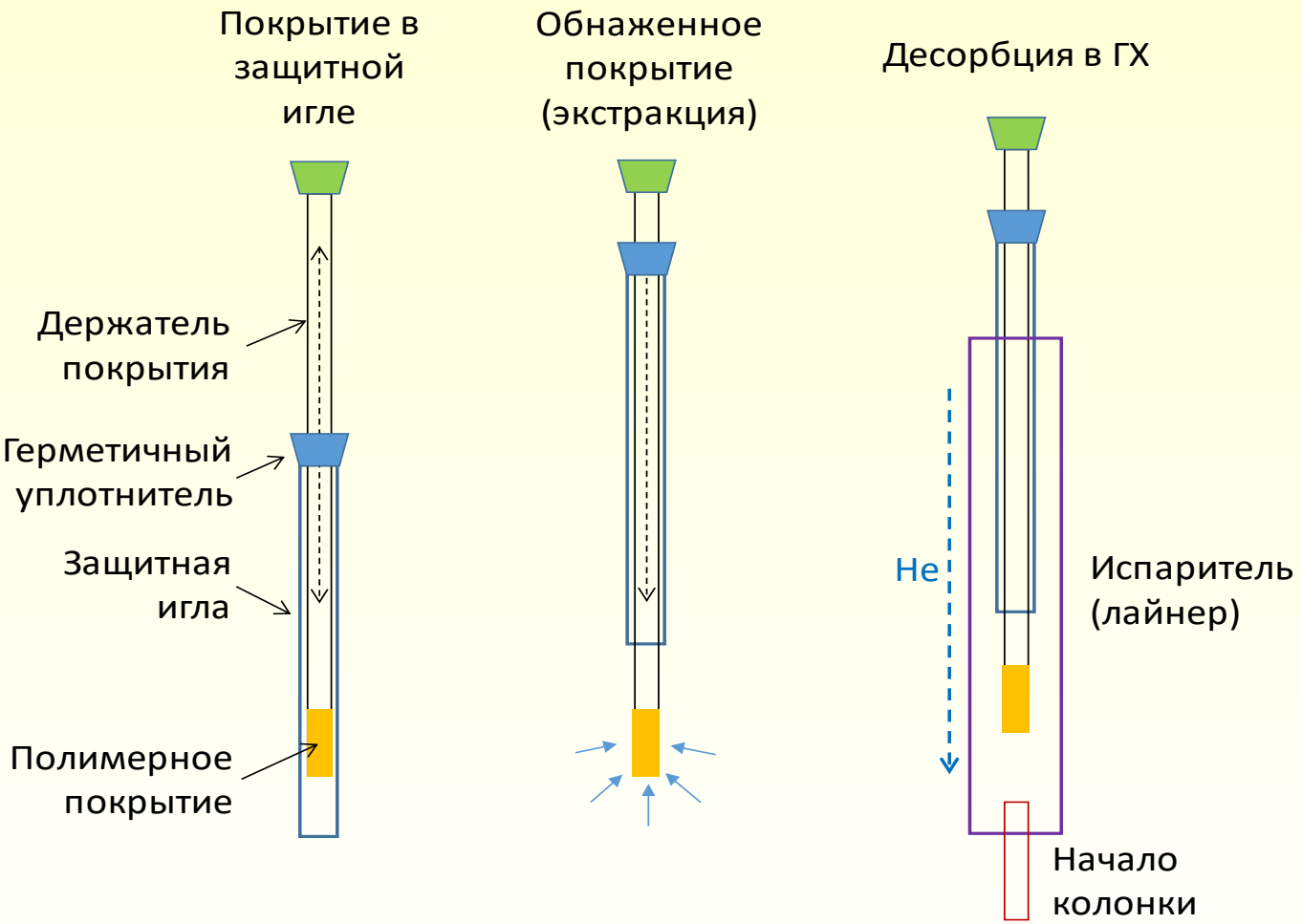
Method of separation of volatile organic compounds from the matrix by gas extraction (inert) over a sample (liquid, solid)



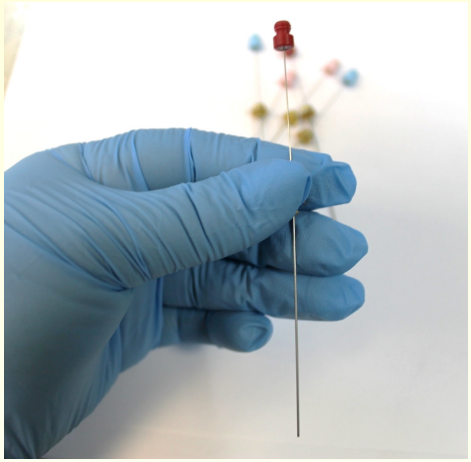
Analysis on GC



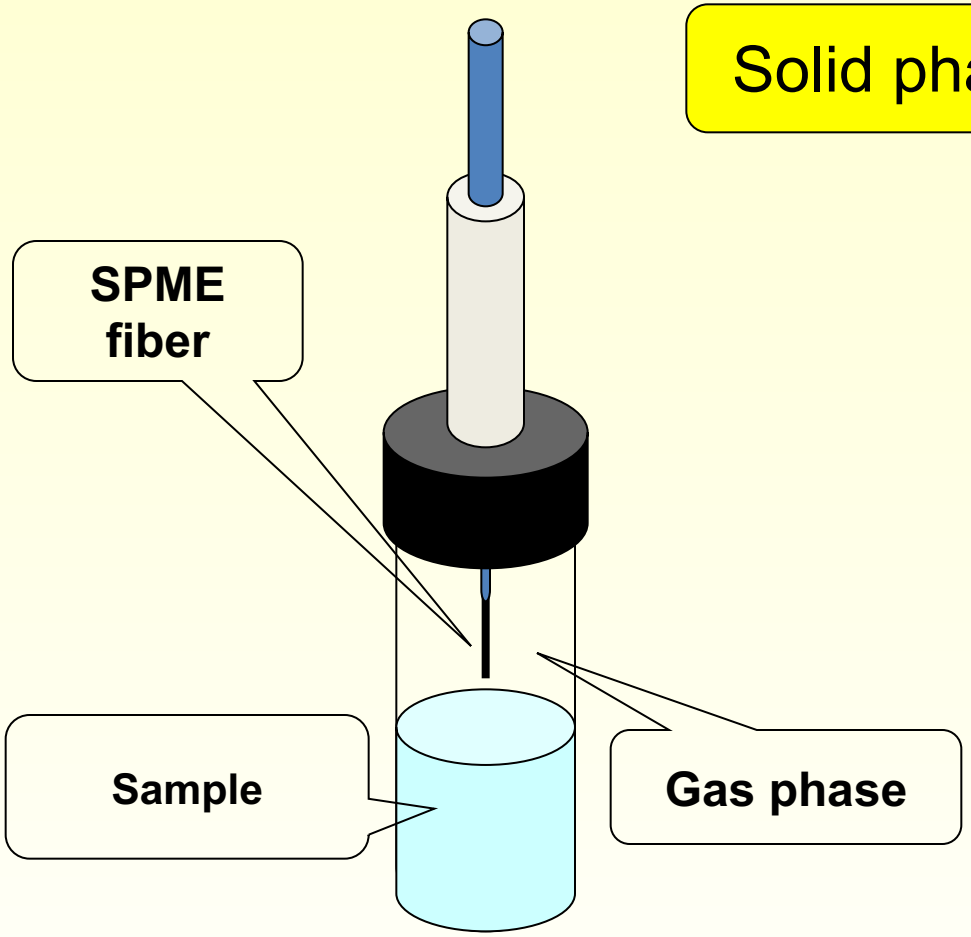
Solid phase microextraction

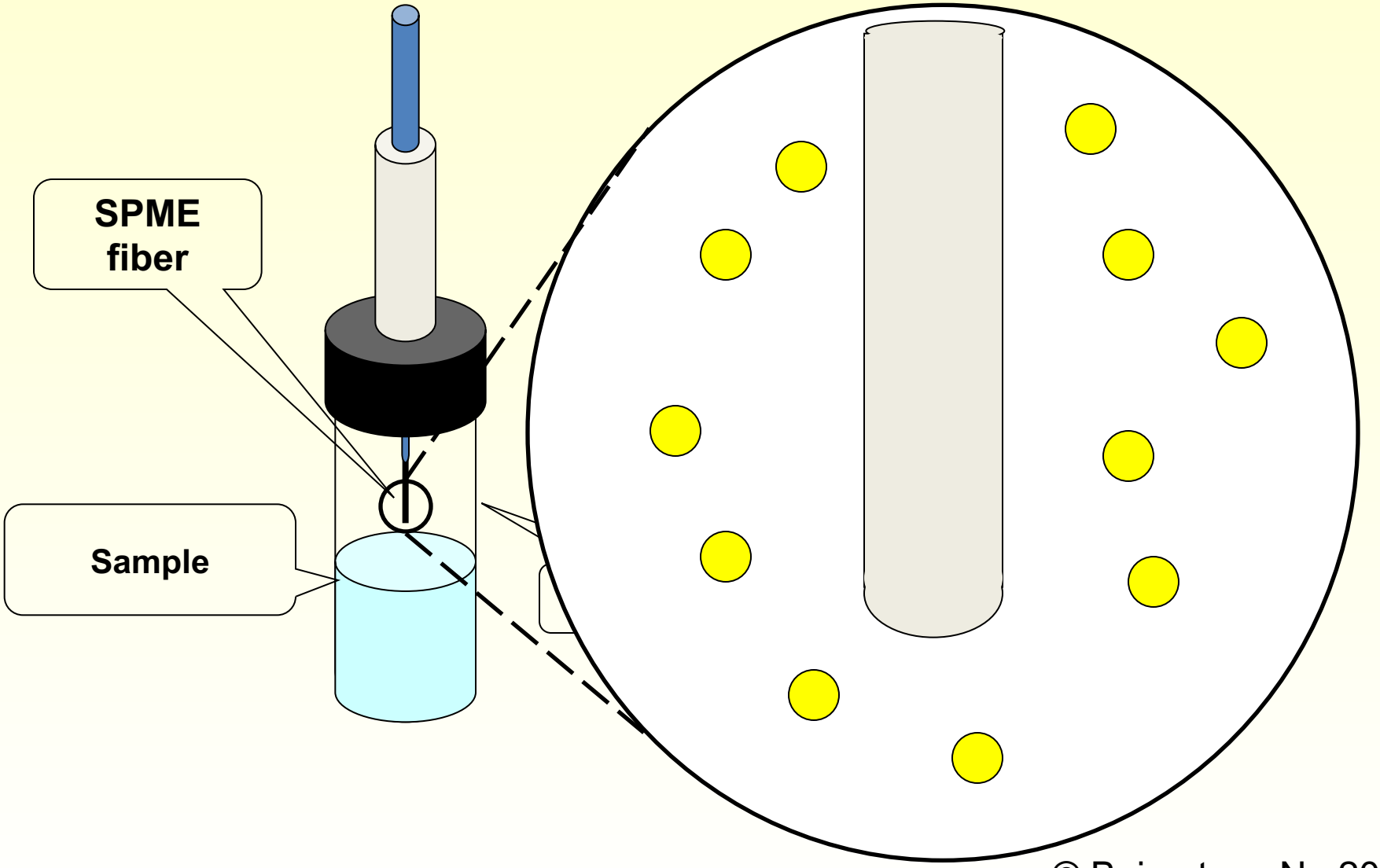


Solid phase microextraction

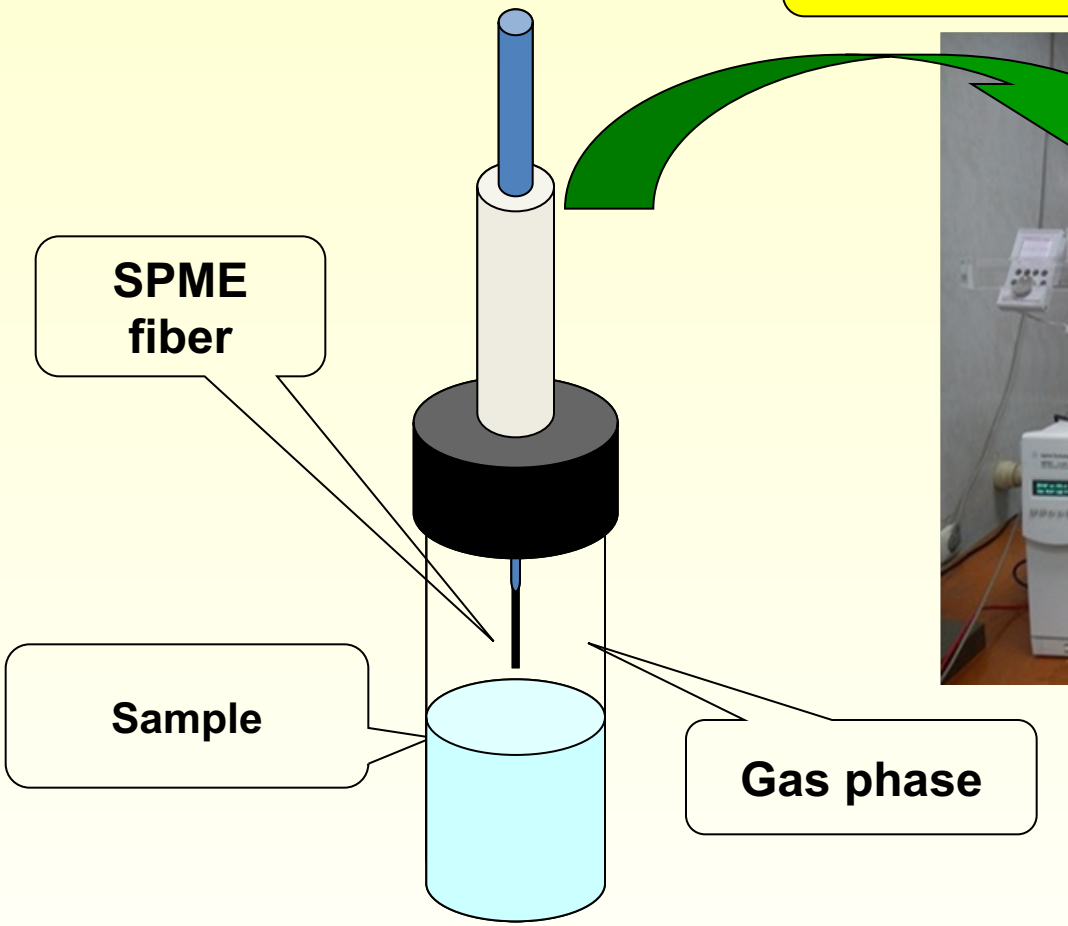


Solid phase microextraction (SPME)





Solid phase microextraction (SPME)



Analysis on GC/MS

The identification and quantification

The benefits of SPME

- Easy to use method;
- No need for toxic organic solvents;
- High concentration of non-polar compounds on the micro-coating;
- High final sensitivity of the method;
- Fully automated;
- Simple micro-coating regeneration;
- Low cost per 1 analysis.

Disadvantages of SPME

- The complexity of the calibration.
- Cost of fibers ($150 for 1 fiber).
- Competition between analysts.

Thank you for your attention!

Задачи

- К 1 л раствора HNO_3 с концентрацией 0,4 моль/л добавили 200 мл воды. Какова концентрация получившегося раствора?

Задачи

- Образец воды (V=50 мл), содержащий фенол был экстрагирован 10 мл метиленом хлористым. Коэффициент распределения для фенол между водой и метиленом хлористым составил 8,7. Экстракт был проанализирован методом ГХ/МС и концентрация фенола составила 22 мкг/л. Рассчитайте концентрацию фенола в образце воды и степень извлечения фенола из образца воды.

Задачи

- Образец воды (V=50 мл), содержащий фенол был пропущен через картридж для ТФЭ. Аналит был элюирован 2 мл метиленом хлористым. Степень извлечения составила 100%. К экстракту было добавлено 50 мкл додекана и метилен хлористый был полностью упарен. Концентрация фенола в экстракте составила 230 мкг/л. Рассчитайте концентрацию фенола в образце воды.

Задачи

- Образец воды (V=50 мл), содержащий фенол был пропущен через картридж для ТФЭ. Аналит был элюирован 2 мл метиленом хлористым. Степень извлечения составила 100%. К экстракту было добавлено 50 мкл бромофенола (С=25 мкг/мл). Анализ экстракта методом ГХ/МС показало соотношение аналита и внутреннего стандарта равной 5,6. Рассчитайте концентрацию фенола в образце воды.