Chromatography

Chromatography is a physical-chemical method of separation and analysis of mixtures by adsorption methods in dynamic conditions.

It is based on different distribution of mixture components between two phases: mobile and immobile.

The immobile (stationary) phase can be liquid or solid. It can be a finely ground sorbent or water fixed by a sorbent or paper fiber.

The mobile phase is a flow of liquid or a gas which is transferred together with the components of the mixture through the immobile phase (sorbent).

When the mixture of substances passes through the adsorbent layer, acts of adsorption-desorption take place constantly.

Any substance in a mobile phase interacts with new sections of adsorbent or sorbent (it is adsorbed or it is sorbated) but under the influence of the mobile phase it is desorbed.

Classification of chromatographic methods

Numerous chromatographic methods can be classified according to the following principles:

1. the state of aggregation of mobile and immobile phases,
2. the mechanism of interaction sorbent-sorbate,
3. technique of carrying out chromatography,
4. its aims.

4. According to the purpose of chromatography (its aim) it can be divided into:

a) analytical (qualitative and quantitative analyses),

b) preparatory (for obtaining substances in pure state, for concentration and isolation of trace contaminants),

c) industrial chromatography.

1. According to the aggregative state of mobile phase chromatographic methods are divided into:

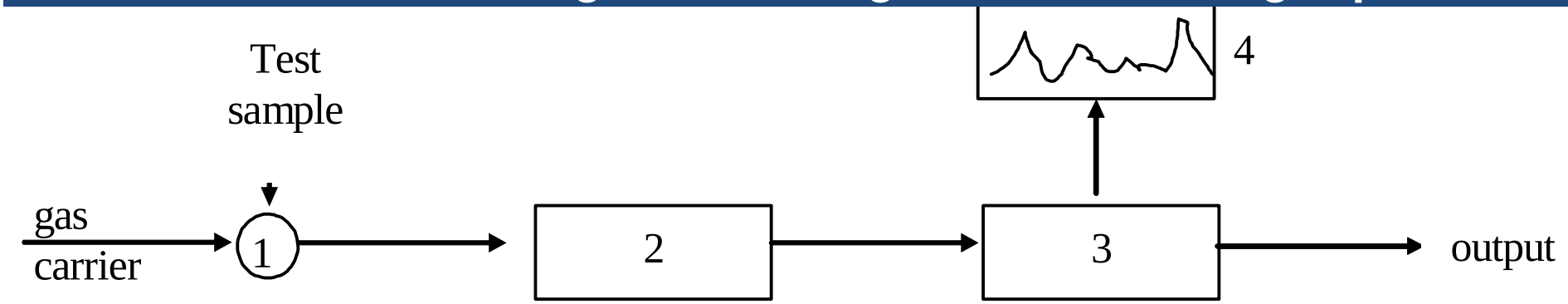
a) gaseous,

b) liquid ones.

Gas chromatography used for separation, analysis and investigation of substances and their mixtures which turn into vaporous state without decomposition. According to the aggregative state of immobile phase it can be gas-solid phase and gas-liquid phase chromatography.

In gas chromatography such inert gases as helium, argon, nitrogen and less often hydrogen and carbon dioxide are used as the mobile phase (gas-carrier).

Schematic diagram of a gas chromatograph



1. It is the device for the input of a test sample into a chromatographic column (dispensing apparatus);
2. is a chromatographic column;
3. is a detector (analyzing system); 4. is the recorder.

The flow of carrier gas is constantly sent to the chromatographic column and after that to the detector. This device constantly measures the component concentration at the output and transforms it into an electrical signal registered by the potentiometer. At the recorder chart we can see the output curve called a chromatogram.

3. According to the technique of carrying out we can single out:

a) column,

b) planar chromatography.

In planar chromatography the separation is carried out on some special paper (paper chromatography) or in a thin layer of a sorbent (thin layer chromatography – TLC).

At TLC the immobile solid phase is applied at a plate made of glass, aluminum foil or a polymer film. As a sorbent it’s possible to use silica gel, aluminum oxide, starch, cellulose and other substances with high adsorption ability.

A liquid phase (a solvent or a system of solvents) acts as a mobile phase. Under the influence of capillary forces the solvent moves up along the sorbent layer transferring the components of this mixture at different rate, which explains their separation.

2. According to the mechanism of interaction of a sorbent and a sorbate we can single out:

- adsorption,
- distributive,
- ion exchange,
- exclusion,
- affine chromatography.

Regardless of the distribution mechanism all chromatographic methods are based on the differences in the degree of distribution of mixture components between the mobile and immobile phases.

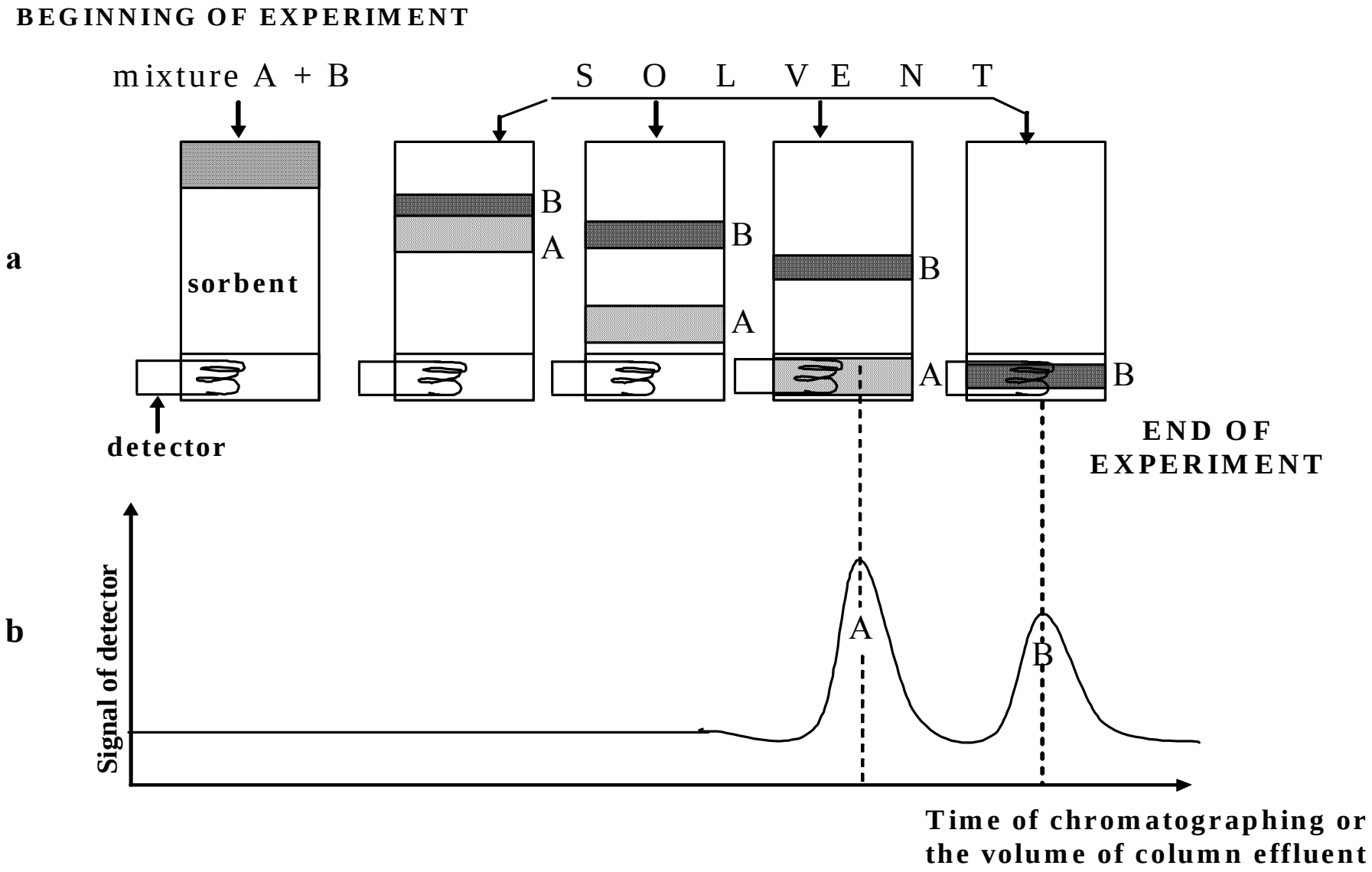
The distribution degree of substances can be quantitatively described by
the coefficient of distribution K:

$$K = \frac{C_s}{C_m},$$

where C_s, C_m are the concentrations of a substance in immobile (stationary) and mobile phases respectively.

To separate substances in the column in practice we can often use elution chromatography method. The mixture of substance A and substance B is loaded into the upper part of the column. While a pure solvent is passed through the column, the substances are washed away from it. This process is called elution, which gave its name to elution chromatography.

Scheme of elution chromatography of a two-component mixture.



- The mixture components A and B are distributed between two phases: solid sorbent (immobile phase) and liquid solvent (mobile phase) according to their distribution coefficients:

$$K(\mathrm{A}) = \frac{C_{\mathrm{s}}(\mathrm{A})}{C_{\mathrm{m}}(\mathrm{A})}$$

$$K(\mathrm{B}) = \frac{C_{\mathrm{s}}(\mathrm{B})}{C_{\mathrm{m}}(\mathrm{B})}$$

Molecules of A and B substances are rearranged between the mobile and immobile phases. The moving rate of the substance depends on its duration of stay in the mobile phase and its ability to be adsorbed in the immobile phase. At the output the first is the least sorbated component A and only then appears component B. At the output the column can be joined to the detector reacting at the concentration change of the given substances.

Each peak on the chromatogram corresponds to the definite substance.

Elements of the Kinetic Theory of Elution Chromatography

Let's take:

L is the column length;

t_m is the time of solvent transfer through the column;

t is the time of substance transfer;

n_m is the movement rate of the solvent;

n is the movement rate of the substance.

$$\nu_m = \frac{L}{t_m}$$ and $$\nu = \frac{L}{t}$$

The ratio of the movement rate of the substance to the movement rate of the solvent is called the retention index R.

$$\frac{v}{v_m} = \frac{L/t}{L/t_m} = \frac{t_m}{t} = R$$

The higher is the movement rate of the substance, the greater is the value R of it in the mobile phase. The retention index characterizes the time fraction of the substance presence or a part of the substance in the mobile phase.

> R, > n , > n of the substance in the mobile phase.

R is the time fraction spent by the substance or a part of the substance in the mobile phase.

(1 – R) is the time fraction spent by the substance or a part of the substance in the immobile phase.

At the dynamic equilibrium the ratio of time intervals spent by the substance in each of the phases will be equal to the ratio of substance amounts in both phases (n=CV):

$$\frac{R}{1 - R} = \frac{C_m V_m}{C_s V_s}$$

C_s, C_m are the molar concentrations of the substance in the immobile and mobile phases respectively;

V_s, V_m are the volumes of immobile and mobile phases respectively.

Any process of substance distribution between two phases is characterized by the distribution coefficient

$K=C_s / C_m$; $C_s=KC_m$.

Carrying out some mathematical transformations we obtain the following for the retention index:

$$R = \frac{V_m}{KV_s + V_m}$$

The equation connects the part of the substance in the mobile phase with the distribution coefficient of the substance and the volumes of both phases.

The less is the distribution coefficient K, the higher is the rate of the substance moving along the column as its retention index R in the mobile phase is greater.

!!! >R, >V, <K

!!! >K, <V, <R

As we can see at the scheme of elution chromatography of a two-component mixture substance B is retained in the column stronger than substance A, therefore

$$K(B) > K(A),$$

$$R(B) < R(A).$$

substance B is more related to the immobile, stationary phase (sorbent)

substance A is more related to the mobile phase (solvent)

This example can be considered a typical example of molecular-adsorption or simple adsorption chromatography.

Adsorption Chromatography

It is based on different adsorption of substances by a solid adsorbent.

Polar sorbents are $Al_2O_3 \times H_2O$, $SiO_2 \times H_2O$, starch, cellulose.

Non-polar sorbents are activated carbon, graphitized [carbon] black.

As a solvent it's possible to use polar solvent (water) or non-polar organic solvent (alcohols, benzene, hexane, ester and ethers).

Adsorption of different substances from the solutions depends on the nature of the sorbent, separated substances and the solvent.

Ion Exchange Chromatography

The base of it is the ion exchange adsorption carried out with the help of adsorbents which are called ionites. Ionites are solid substances containing functional groups the ions of which are able to exchange ions for ions in the solution. Ionites are almost insoluble in water and organic solvents.

Ionite structure

1 – matrix – space skeleton;

2 – functional ionized groups;

3 – counterions connected elecrtostatically with the ionized groups.

Counterions can be substituted by other ions with the same charge.

Classification of ionites.

Depending on the charge of exchanging ions ionites can be divided into:

1. Cationites: $R–An^-H^+$, where R– cationite matrix, An^- – ionized groups of cationite, H^+– counterions connected to the ionized groups electrostatically.

Cationite exchanges it's H^+-cations on the cations from the electrolytic solution. The ion exchange process occurring stoichiometrically.

$$2R–An^-H^+ + Ca^{2+} \leftrightarrow (R–An^-)_2Ca^{2+}+2H^+$$

2. Anionites: $R–Kt^+OH^-$, where R–anionite matrix, Kt^+ – ionized groups of anionite, OH^- – counterions connected to the ionized groups electrostatically.

Anionite exchanges it's OH^- -anions on the anions from the electrolytic solution. The ion exchange process occurring stoichiometrically.

$$R – Kt^+OH^- + Cl^- \leftrightarrow R – Kt^+Cl^- + OH^-$$

3. Amphoteric ionites which contain simultaneously acid and base ionized groups mentioned earlier.

The adsorption ability of electrolyte ions on the ionite depends on their charge.

The greater is the ion charge, the greater is its adsorption ability: $K^+ < Ca^{2+} < Al^{3+} << Th^{4+}$

adsorption increase on cationite →

Among the ions with the same charge the maximum ion exchange ability is exhibited by those having a smaller radius in the solvated (hydrated) state:

$Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$

← increase in hydration

adsorption increase on cationite →

$Cl^- < Br^- < NO_3^- < I^- < CNS^-$

← increase in hydration

adsorption increase on anionite →

The most important characteristic of the ionite is the exchange capacity (EC).

Exchange capacity is the amount of exchanging ions in 1 gram of absolutely dry ionite or in 1 mL of swelled ionite.

[EC]=[(m)mol / g] or [(m)mol / mL]

The usage of ionites.

The deionization of water is carried out with the help of ionites. Water is firstly passed through the cationite filter and then through the anionite one.

At the cationite filter there is the adsorption of metal cations from water:

$$2R–An^-H^+ + CaCl_2 \leftrightarrow (R–An^-)_2Ca^{2+}+2HCl$$

Water containing chloride ions (Cl^-) goes through the anionite filter which changes hydroxide ions (OH^-) into chloride ions (Cl^-):

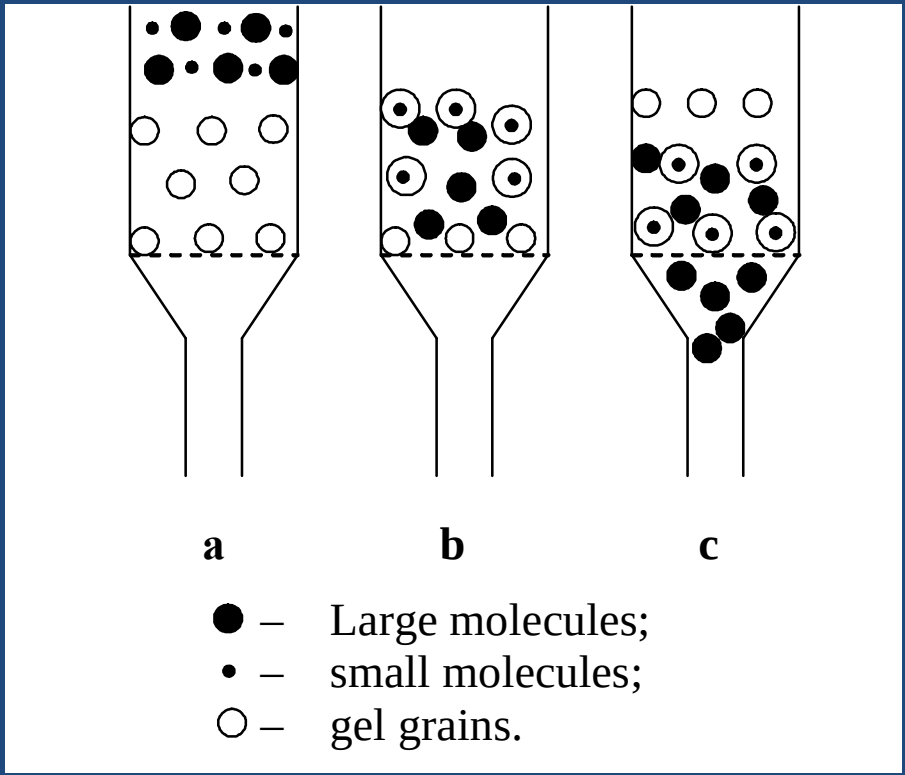
$$R–Kt^+OH^- + HCl \leftrightarrow R–Kt^+Cl^- +H_2O$$

In the result of it we obtain desalinized water.

Exclusion Chromatography, Gelfiltration.

At the base of exclusion chromatography there is a principle of division of a mixture of substances according to their molecular size and molecular mass.

The separation of the mixture is more effective with greater difference in the molecule size of these substances, i.e. in greater difference in the distribution coefficient K.



Gel grains contain pores of a certain size. Large molecules of the separated mixture of substances will be moving quickly along the column without penetrating inside the grains but smaller ones penetrating into all grain pores will be moving slower. When the column is washed away with a solvent the larger molecules will be the first at the output of the column.

Affine (Biospecific) Chromatography

It is a method of refining and separation of proteins based on their selective interaction with a ligand connected to the inert carrier (matrix) by a covalent bond.

As immobilized ligand it’s possible to use compounds the interaction of which with the separated substance is based on the biological function.

So, the enzyme is bonded with the substrate, antigen with antibody, hormone with its receptor.

Example

For obtaining pure antidiphtheritic toxin antibodies from blood serum its antigens are covalently bonded with the cellulose matrix and are placed in the column. Immune serum is passed through the column. Antibodies closely bond with antigens. Then the column is washed by the solution of 0,85% sodium chloride, all nonspecific proteins of blood serum remove . When the column is washed by phosphate citric buffer solution with pH 3,2 , pure antibodies are slivered.

The same method can be applied to obtain antiflu antibodies from blood serum.