**Lesson 1**

**"CHROMATOGRAPHY AND CHROMATOGRAPHIC INSTRUMENTS"**

 The use of various physical and physicochemical properties of a substance for analytical purposes underlies the physicochemical methods of analysis. Physico-chemical methods of analysis are called, based on the measurement of the physico-chemical and physical properties of a given substance. Together with the physical methods of analysis, they are called instrumental, because. they require the use of instruments and measuring devices.

 Physicochemical methods of analysis are based on a chemical reaction or a physicochemical process.

 A characteristic feature of physical methods is that they measure physical parameters without first conducting a chemical reaction.

 All analytical methods have much in common: the composition of a substance, its structure and quantity is determined by its properties. The properties of a substance are recorded using instruments.

The main task of the device is to convert chemical information into a form convenient for the operator to observe, which is carried out using a transducer. Here the electrical signal is amplified and transmitted to the reader.

 The choice of the best method of analysis is dictated by many considerations and is a difficult task. The criteria for evaluation and selection of analysis methods are their metrological characteristics:

- reproducibility

- limit of detection (sensitivity)

- upper and lower limits of determined contents

A revolution in instrumental methods took place in the 1930s. This is due to the rapid development of electronics at that time.

Classification of physical and chemical methods

 Depending on the measured characteristics, the following groups of physicochemical methods are distinguished:

1. Optical (spectral), based on measuring the optical properties of the analyzed systems (on the interaction of substances with an electromagnetic field). They allow you to determine the structure, geometry and polarity of molecules, bond lengths, as well as the amount of substance by the intensity of the bands in the spectrum.

2. Electrochemical, based on the measurement of electrochemical properties. Allows analysis of electrolyte solutions.

3. Physical and chemical methods of separation and concentration (chromatography, ion exchange, dialysis, electrophoresis).

4. Radiometric, based on the measurement of the radioactivity of the objects under study.

5. Mass spectrometric, based on the ionization of atoms and molecules of the substance under study, followed by the separation of the resulting ions in space and the determination of their masses. They allow to determine the composition and structure of molecules, the energy of toning, as well as the characteristics of reversible processes.

Physicochemical methods of analysis have the following advantages:

1) selectivity: some methods allow you to simultaneously determine dozens of components that make up the system under study;

2) rapidity - high speed of analysis;

3) detection limit is lower than that of chemical methods. Physico-chemical methods can be used to analyze when the content of the component is 10-4 - 10-5% mass, chemical methods - 10-1 - 10-2% mass;

4) physicochemical methods make it possible to work with undisturbed samples, so they are widely used in biology and medicine.

In 1903 M.S. Tsvet was the first to set out the principles of chromatography (Greek “chromo” - color, “grapho” - I write) and created a method for separating pigments from green plants.

The chromatographic method allows the separation and analysis of complex mixtures. The separation of substances occurs due to the different adsorbability of the components of the mixture.

Chromatography is a dynamic process that occurs in a system of two immiscible phases, one of which is mobile and the other is immobile. The mobile phase can be either a gas or a liquid, and the stationary phase can be a solid or a thin film of a liquid adsorbed on a solid.

Chromatography is a method of separating complex mixtures based on the distribution of substances between two phases, one of which is stationary, and the other is a stream moving through a stationary phase (mobile).

Chromatography is based on the repeated repetition of the acts of sorption and desorption of substances as they move in the flow of the mobile phase along the stationary sorbent.

Any sorption mechanism can be used for the chromatographic separation of mixtures of substances.

Mobile phase - liquid or gas;

The stationary phase is a solid carrier, adsorbed solid or solution.

Stages of development of chromatography:

1903 Discovery of chromatography (Tsvet M.S.)

1938 Thin-layer or planar chromatography (Izmailov N.A., Shraiberg M.S.)

1941 Liquid Partition Chromatography (Martin A.D.P., Synge R.L.M.)

1952 Gas partition chromatography (Martin A.D.P., James A.)

1956 Capillary gas chromatography (Golay M.)

1975 Ion Chromatography (Small H., Stevens T.S., Bauman W.W.)

1990+ Chromatomass Spectrometry

Basic chromatographic concepts:

• Stationary (stationary) phase - eluent, solid carrier (coated);

• The mobile phase is a liquid or gas flowing through the stationary phase, sometimes under pressure.

• Sorption - the concentration of one of the substances in one of the phases.

• Adsorption - the absorption of a substance on the surface of a solid or liquid body.

• Absorption - absorption of gases, vapors or dissolved substances in the entire volume of the solid or liquid phase. Sorbents are solid substances or liquids that selectively absorb (sorb) gases, vapors or dissolved substances from the environment.

• Elution - expulsion of the substance by the turbidity of washing with the corresponding solvent (eluent).

Classification of chromatographic methods

Classification of chromatographic methods according to the state of aggregation of phases

Gas chromatography

o Mobile phase - inert gas (carrier gas)

o Temperature has a big influence

o For chromatography of volatile substances and gases

1. gas-solid-phase (gas-adsorption)

2. gas-liquid

Liquid chromatography

o PF - liquid

o Suitable for chromatography of polar substances and macromolecules

1. liquid-liquid

2. liquid-solid phase

3. liquid-gel

Classification of chromatographic methods according to the separation mechanism (by the nature of the elementary act)

1. Adsorption - based on different adsorption of substances on the surface of the sorbent

2. Distribution - based on different solubility (absorption) of substances in PF and NF

3. Ion exchange - based on different ion exchange ability

4. Chelated - based on different ability to form chelate complexes

5. Gel-filtration (exclusive, gel-penetrating) - based on different ability to penetrate into the pores of the carrier. Substances are separated by size, substances with a higher molecular weight come out first from the column, since they are larger and do not linger in the pores.

6. Chemichromatography - based on different reactivity. The rate of advancement of the reaction product through the NF is proportional to the equilibrium constant of the reaction.

7. Affinity - based on different biospecificity of the analyte and ligand. Substances with high affinity for ligands (molecules covalently bound to NF) are retained, while the rest are "washed away" by the mobile phase.

Classification of chromatographic methods according to the method of moving sorbates along the sorbent layer

Developing (eluent) chromatography

The chromatographed mixture is divided in the column into separate zones separated by sections of the PF. Suitable for separating multicomponent mixtures.

Cons: Requires a lot of solvent

Frontal chromatography

Advantages:

o effective method

o requires a small amount of solvent

Cons: only one component is clean

They will be used in installations to reduce water hardness, in respirators, in industrial filters.

displacement chromatography

Not a pure solvent is used, but a substance (displacement) with a high sorption capacity.

Advantages:

o high performance

o requires a small amount of solvent

o no blurring of zones

o the speed is constant and equal to the speed of the displacer

Disadvantages: long duration of the chromatographic process

Classification of chromatographic methods according to the method of carrying out

1. Speaker

2. Planar

2.1 paper

2.2 thin layer

Classification of chromatographic methods according to goals and objectives

1. Analytical chromatography - obtaining information (qualitative and quantitative analysis)

2. Preparative chromatography - separation and purification of substances

3. Industrial chromatography - automated control of emissions

Chromatographic picture of the separation

1. Integral (practically not used)

2. Differential

**Basic principles of chromatographic separation.**

**Colo-night chromatography**

Consider the external chromatogram of two substances. The X-axis represents the chromatography time or the volume of the effluent, and the Y-axis represents the analytical signal.



Rice. Differential chromatogram: 1 - zero line; 2 - peak of the non-sorbing component; 3, 4 – peaks of determined components; tR is the retention time; h is the peak height; μ - peak width

1. The height of the output curve (peak) h is the perpendicular dropped from the maximum of the peak to the zero line. The zero line is a part of the chromatogram obtained by registering the detector signal during the exit of the pure mobile phase from the column.

2. Peak width μ - a segment cut off on the zero line by tangents to the curve at inflection points, or the distance between points of the peak contour at the middle of the height μ0.5.

3. The sorption capacity of the stationary phase with respect to the substances to be separated is characterized by the retention time tR. The retention time tR is the time elapsed from the moment the sample was injected into the column until the maximum peak of the substance was released, i.e. this is the residence time of a substance in the mobile and stationary phases. This is a very important value, since if the separation conditions (mobile phase flow rate, pressure, temperature, composition of the mobile and stationary phases) are constant, then the retention time is strictly reproducible and is a characteristic of the substance, therefore it can be used to identify substances.

4. The holding volume VR is an equally important characteristic: VR = F ∙ tR, where F is the volumetric flow rate. The symbols tR0 and VR0 denote the retention time and volume of the non-sorbing component.

5. Separation of two neighboring peaks is characterized by resolution. The resolution of the peaks depends on their sharpness (bandwidth) and on the distance between the peaks (band separation). The sharpness of the peaks depends on the efficiency of the column, and the distance between the maxima is determined by its selectivity.

Column efficiency refers to the production of narrow peaks, i.e. restriction of blurring (expansion) of bands. In an efficient column, the smearing of the bands is small and the peaks are narrow. The distance between the peak maxima is determined by the selectivity of the column, i.e., the selectivity of the sorbent and the differences in the thermodynamic properties of the chromatographed substances in relation to the chromatographic system.

The selectivity of the column depends on the constants and distribution coefficients of the components of the mixture and the coefficients of column capacity. At low values of the coefficients, the components are weakly retained by the column, and poor separation is observed. With large values of the coefficients, the separation increases, but the separation time also increases.

Quantitative analysis is carried out by measuring the height or area of the peak, since these parameters are proportional to the concentration of the substance or its amount in the chromatographic zone.

The peak height is used only when the retention time is short (the peak is sharp) and the peak shape is not distorted (the peak height varies linearly). Therefore, the peak area is used more often.

Several methods are used to calculate chromatograms:

1) normalization (method of internal normalization);

2) external standard (calibration chart);

3) internal standard.

Sedimentary chromatography

Qualitative Analysis

If the zones of the chromatogram are colored, then the qualitative composition of the analyzed mixture is judged by their number, color and location. If the chromatogram is colorless, then use a developer solution that forms colored compounds with separable ions.

Quantitative Analysis

The dependence of the height of the chromatogram zone on the concentration of the substance is used.