**8 lesson.**

**Gas chromatography** is a physicochemical method of separation of substances based on the distribution of the components of the analyzed mixture between two phases that are immiscible and move relative to each other, where the gas (carrier gas) acts as the mobile phase, and the solid sorbent or liquid acts as the stationary phase, deposited on an inert solid carrier or the inner walls of the column.

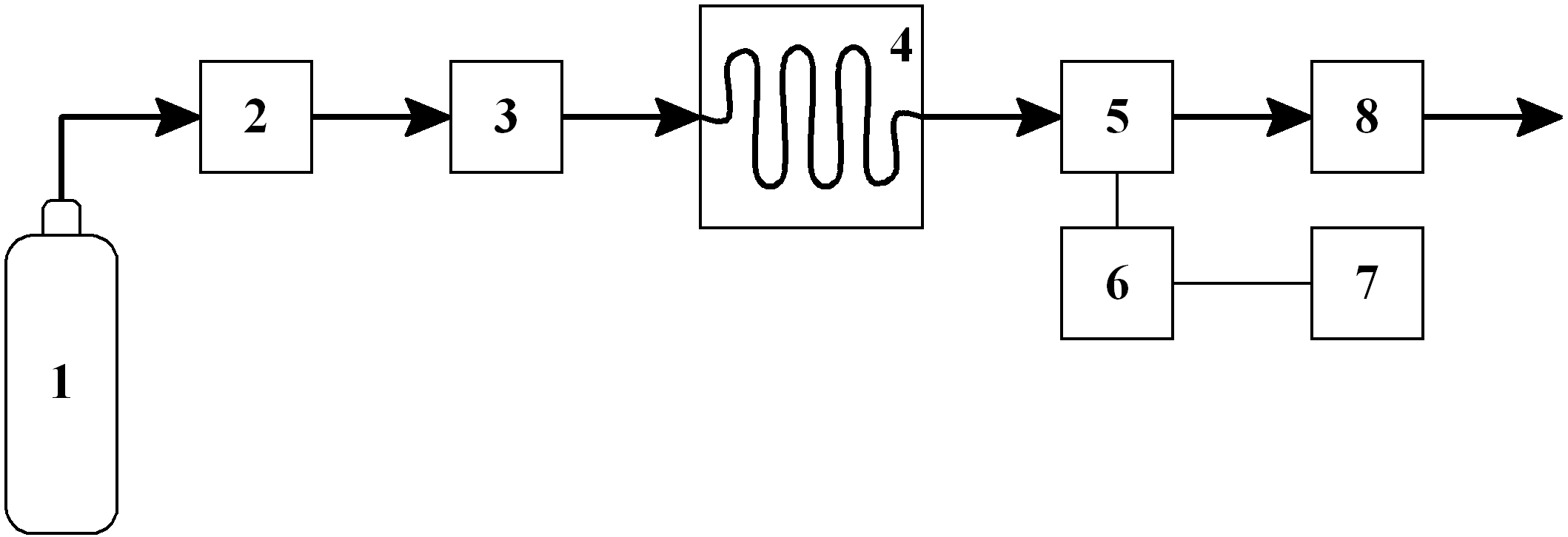
Depending on the type of stationary phase used, gas chromatography is divided into gas-adsorption (in foreign scientific literature, it is commonly referred to as gas-solid-phase) and gas-liquid chromatography. In the first case, the stationary phase is a solid carrier (silica gel, coal, aluminum oxide), in the second case, it is a liquid deposited on the surface of an inert carrier.

Gas-liquid chromatography - separation of a gas mixture due to different solubility of the sample components in a liquid or different stability of the resulting complexes. The stationary phase is a liquid deposited on an inert carrier, the mobile phase is a gas.

Separation is based on differences in volatility and solubility (or adsorbability) of the components of the mixture being separated.

This method can be used to analyze gaseous, liquid and solid substances with a molecular weight of less than 400, which must meet certain requirements, the main of which are volatility, thermal stability, inertness, and ease of preparation. As a rule, organic substances fully satisfy these requirements; therefore, gas chromatography is widely used as a serial method for the analysis of organic compounds.

The main instrument for this research method is a gas chromatograph.



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| --- |
| *Схема газового хроматографа* |
| 1 — источник газа-носителя (подвижной фазы) 2 — регулятор расхода газа носителя 3 — устройство ввода пробы 4 — хроматографическая колонка в [термостате](https://ru.wikipedia.org/wiki/%D0%A2%D0%B5%D1%80%D0%BC%D0%BE%D1%81%D1%82%D0%B0%D1%82) 5 — детектор 6 — электронный усилитель 7 — регистрирующий прибор (самописец, [компьютер](https://ru.wikipedia.org/wiki/%D0%9A%D0%BE%D0%BC%D0%BF%D1%8C%D1%8E%D1%82%D0%B5%D1%80)) 8 — расходомер |

Carrier gas source.

Most often, this is a 40-liter cylinder with compressed or liquefied gas, which is usually under high pressure (up to 150 atmospheres), by means of a reducer, the outlet pressure is reduced to the operating pressure of the chromatograph (usually chromatographs operate at a pressure of 4 to 10 atmospheres). Most often, helium is used in chromatography, less often argon and nitrogen, and even more rarely hydrogen and other gases.

In the case of using hydrogen or nitrogen as a carrier gas, hydrogen or nitrogen generators, respectively, can serve as gas sources in addition to cylinders.

Color marking of cylinders containing various gases.

|  |  |  |
| --- | --- | --- |
| **аз** | **Окраска баллона** | **Цвет надписи с названием газа** |
| [Азот](https://ru.wikipedia.org/wiki/%D0%90%D0%B7%D0%BE%D1%82) | Чёрный | Жёлтый |
| [Водород](https://ru.wikipedia.org/wiki/%D0%92%D0%BE%D0%B4%D0%BE%D1%80%D0%BE%D0%B4) | Тёмно-зелёный | Красный |
| [Гелий](https://ru.wikipedia.org/wiki/%D0%93%D0%B5%D0%BB%D0%B8%D0%B9) | Коричневый | Белый |
| [Аргон](https://ru.wikipedia.org/wiki/%D0%90%D1%80%D0%B3%D0%BE%D0%BD) (техн.) | Чёрный | Синий |
| [Аргон](https://ru.wikipedia.org/wiki/%D0%90%D1%80%D0%B3%D0%BE%D0%BD) (чист.) | Серый | Зелёный |
| [Кислород](https://ru.wikipedia.org/wiki/%D0%9A%D0%B8%D1%81%D0%BB%D0%BE%D1%80%D0%BE%D0%B4) | Голубой | Чёрный |
| Горючие газы | Красный | Белый |

Gas flow regulator. The purpose of this component of the gas chromatograph is to control the gas flow in the system, as well as maintain the required gas pressure at the system inlet. Typically, a reducer or throttle is used as a gas flow regulator.

Sample injection device. Designed to supply a sample of the analyzed mixture to the chromatographic column.

In the event that the chromatograph is intended for the analysis of liquid samples, the sample injection device is combined with an evaporator.

The sample is introduced into the evaporator using a microsyringe by piercing the elastic seal. The evaporator is usually heated to a temperature 50°C higher than the column itself. Injection volume from 0.1 to several microliters

In the case of gaseous samples, the sample can be injected in 2 ways:

1. The sample is introduced into the evaporator using a gas syringe (a special gas-sealed chromatographic syringe for injecting gaseous samples into the evaporator, usually with a volume of 1 ml) by piercing the elastic gasket.

2. Inclusion in the gas circuit of a "gas cock" instead of or before the evaporator. The gas valve has 2 positions: "sampling" and "analysis". In the “sampling” position, the carrier gas enters directly into the column, at the same time, the loop is connected at one end to the sampling fitting, and at the other end is connected to the sample discharge fitting (atmosphere). When the gas cock is turned to the "analysis" mode, the gas flows are switched: now the carrier gas enters the column through the sampling loop (usually 1 or 2 ml loops are used), thus introducing the sample into the column, at the same time the sampling fitting is connected with the atmosphere bypassing the sampling loop.

Chromatographic columns.

A column is a vessel whose length is much greater than the diameter. For gas chromatography, two types of columns are used - capillary and packed. Packed columns have an outer diameter of 2 to 4 mm and a length of 1 meter to 4 meters. The inner diameter of capillary columns (ID - inner diameter) is 0.15-0.53 mm, and the length is 15-100 m. The material for the manufacture of columns is glass, stainless steel, copper, sometimes fluoroplastic. Recently, capillary columns made of fused silica with a stationary phase deposited inside have become most widespread. The length of such columns can reach hundreds and even thousands of meters, although columns with a length of 30-60 m are more often used.

It is extremely important to densely fill the columns with the stationary phase, as well as to ensure that the column temperature is constant throughout the entire chromatography process. The accuracy of maintaining the temperature should be 0.05-0.1 °C. Thermostats are used to precisely control and maintain the temperature.

Detectors.

The detectors are designed for continuous measurement of the concentration of substances at the outlet of the chromatographic column. The principle of operation of the detector should be based on measuring a property of the analytical component that the mobile phase does not have.

The following types of detectors are used in gas chromatography:

• flame ionization detector

• thermal conductivity detector (katharometer)

• electronic capture detector

• flame photometric detector

• thermionic detector

• photoionization detector

• mass spectrometer

• FT-IR spectrometer

Gas-solid-phase chromatography is a type of gas chromatography. Another name is gas adsorption chromatography.

Gas-solid-phase chromatography is a method for separating volatile components, in which the mobile phase (eluent) is a flow of an inert carrier gas (hydrogen, helium, nitrogen, argon, carbon dioxide), and the stationary phase is particles of a solid body (adsorbents with a high specific surface area (10 -1000 m2g-1) - active carbons, silica gels, porous glass, aluminum oxide). The carrier gas does not react with the stationary phase and the substances to be separated. The distribution of substances between the stationary and mobile phases is determined by the processes of sorption-desorption

It is used for the analysis and preparative separation of gas and liquid mixtures, as well as volatile solids in the course of physical and chemical studies. For analysis, liquids and solids are vaporized in an evaporator heated to a high temperature. In the case of the analysis of solid non-volatile or thermally unstable substances, gaseous products of their thermal decomposition are analyzed (pyrolysis, pyrolytic chromatography) or mixtures are preliminarily modified to obtain volatile and thermally stable derivatives (for example, using trimethylsilyl chloride)

Gas-solid-phase chromatography is used for the qualitative and quantitative analysis of mixtures and individual substances. For quantitative analysis, either an external standard is used, i.e. calibrate the device with a known amount of a substance, or an internal standard, i.e. add to the mixture a known amount of a substance of a similar nature with a close retention time.

Gas adsorption chromatography is rarely used in pharmaceutical analysis. GLC is more widely used because it is more versatile. The advantage of this method is the ability to vary the properties of the liquid phase over a wide range and thereby achieve the separation of even substances with very similar properties.

The essence of the GLC method lies in the fact that the analyzed substances in a vapor state with a carrier gas flow pass through a column with a stationary liquid phase deposited on a solid carrier. As one moves along the column, the substances are constantly redistributed between the phases due to repeated repetition of sorption-desorption processes and are separated due to the difference in distribution coefficients. Then the separated substances are eluted from the chromatographic column by the flow of carrier gas, registered by the detector and fixed on the chromatogram in the form of peaks. The first to elute from the column is a substance with a low partition coefficient, that is, less soluble in the liquid phase and more volatile at a given temperature. A compound with a high partition coefficient, i.e. more soluble and less volatile, is retained by the column and migrates more slowly. The substances leaving the column, together with the carrier gas, enter, as noted above, into the detector, which reacts to a change in some physical property of the gas mixture passing through it.

The detector signal is converted into an electrical signal, which, after amplification, is transmitted to a recording device, such as a potentiometer. The dependence of the detector signal on time is called a chromatogram. Such a chromatogram has the following form. A single substance appears on the chromatogram as a peak.



Chromatographic peak and its parameters:

A - sample injection;

AB is the retention time;

СDE – chromatographic peak;

AC - baseline;

h is the peak height;

2 -1 (0.5) – chromatographic peak width at half its height

As can be seen from the figure, the chromatogram has a baseline. It corresponds to the period of time during which the detector registers the signal only from the mobile phase. A chromatographic peak is a curve that describes a gradual increase in the concentration of a substance at the outlet of a column and its gradual decrease. The main parameters of the peak are the retention time, the height and width of the peak at half its height, and the area of the peak. The time at which a peak appears on a chromatogram is called the retention time.

Analysis by GLC is carried out using a special device - a gas chromatograph.

The main unit of the chromatograph is the gas chromatographic column. It is a straight, spiral or U-shaped tube made of stainless steel or glass with an internal diameter of 2 to 4 mm. The most commonly used columns are 1–5 meters long, filled with a solid carrier with a deposited liquid phase. Such columns are called packed columns.



Gas chromatography columns

Modern instruments use capillary columns in which the stationary liquid phase is deposited on the inner surface of the capillaries. Capillary columns are made of quartz or metal and are shaped like a spiral. The inner diameter of such columns is 0.2–0.5 mm. Due to the absence of a solid carrier, the gas flow rate is significantly increased. This, in turn, leads to a reduction in the duration of the analysis. The high gas flow rate allows the use of huge column lengths from 5 to 100 meters. As a result, the efficiency and resolution of the columns are significantly increased. While packed columns can be used to separate mixtures containing no more than 10–20 components, their number in capillary columns can reach several hundred.

Various liquids are used as the stationary phase in GLC. According to their polarity, they are divided into three categories:

- non-polar (squalene is a high molecular weight saturated hydrocarbon; apiesons are complex mixtures of hydrocarbons; polyalkylsiloxanes are silicone polymers);

- moderately polar (esters of dibasic or tribasic acids: sebacic, phthalic, citric, phosphoric);

- strongly polar (polyethylene glycols, polyesters - esters of dibasic acids and dihydric alcohols, for example, neopentyl glycol succinate).

The liquids used must meet such requirements as low volatility at the operating temperature of the column, thermal stability, chemical inertness, low viscosity, and the ability to dissolve chromatographed substances.

The liquid phase is deposited in the form of a thin film on a solid carrier, which is most often used as diatomites, consisting mainly of silicon dioxide. They are produced under the name chromosorb, spherochrome, celite, etc. Carriers are also made from polymers (Teflon, polystyrene) or the smallest glass beads. Such carriers are used for the analysis of highly polar or very aggressive substances. An important characteristic of the carrier is the particle size. The optimal particle size is from 125 to 150 microns.

Solid carriers should be chemically inert, catalytically inactive, mechanically strong, thermally stable and should not have adsorption activity.

The analyzed sample passes through the separating column in the form of gas or vapor. Therefore, temperature, as a process parameter, plays a greater role in gas chromatography than in other chromatographic methods. With an increase in temperature, the analysis time is reduced, since the process of desorption of substances is accelerated and, accordingly, their retention time on the column decreases. On the other hand, an increase in temperature can cause decomposition of thermolabile compounds. Thus, the column temperature must be optimal. It is determined primarily by the volatility of the sample and can reach 350 ° C. The operating temperature of the column must be constant, since the retention time of substances changes with its fluctuations. Temperature constancy is ensured by thermostatic devices.

For the analysis of mixtures with a wide range of boiling points of the components, gas chromatography with temperature programming is used, when the temperature of the column is periodically or continuously increased during separation. Temperature programming allows you to get well-defined component peaks and reduce analysis time. The flame ionization detector (FID) is more commonly used as a detector due to its high sensitivity to most organic compounds.

Other types of detectors are also used - a thermal conductivity detector (katharometer), thermionic, electron-capture, mass spectrometric, etc.

QUALITATIVE ANALYSIS OF SUBSTANCES BY GLC

The time from the moment the sample is injected into the column until the registration of the peak maximum is called the retention time. The retention time depends on the nature of the substance, so it can serve as its qualitative characteristic. However, in addition to the nature of the substance, the retention time is affected by various experimental parameters, such as fluctuations in the column temperature, pressure and velocity of the carrier gas, column packing density, mass of the stationary liquid phase, and others. As a result, the identification of the substances of the analyzed sample is carried out either by comparison with standard samples (RS) or by the method of relative retentions.

The method of witness substances consists in the fact that under the same conditions the analyzed sample and CO are sequentially chromatographed. The coincidence of the retention times of the components of the analyzed sample and CO is proof of their identity.

In the absence of standard samples, the identification of components is carried out by the method of relative retentions. Relative retention does not depend much on the parameters of the column, except for temperature, so this value is more or less constant and better reproduced in contrast to the absolute value of the retention time. To determine the relative retention, the analyzed sample is chromatographed, to which a certain amount of a reference substance, called an internal standard, has been added. As an internal standard, a foreign compound is used that is absent in the analyzed sample and is well separated from all other components of the sample. After chromatography of the mixture of the analyzed sample and the reference substance, the relative retention () is calculated using the formula:

τ = tR/ tRср

where tR and tRav are the retention times of the analyte and reference substance, respectively.

In some cases, the reference substance is one of the studied substances of the mixture. The retention time of such a substance is taken as unity. In relation to it, the retention parameters of the remaining substances are expressed. For example, the relative retention time of the second and third components with respect to the first, taken as a reference substance, is determined as follows:

τ2 = tR2/ tR1 τ3 = tR3/ tR1

**QUANTITATIVE ANALYSIS OF SUBSTANCES BY GLC**

Since the area of the chromatographic peak is directly proportional to the concentration of the substance, quantitative analysis is carried out by the area of the peak. Peak areas on a chromatogram are determined using a planimeter or by multiplying the peak height by its half-width. In modern instruments, peak areas are calculated using an electronic integrator, and a personal computer with installed software is used to process chromatographic information. There are several methods for quantification:

– area normalization method;

– method of absolute calibration;

– internal standard method;

– method of comparison with a standard sample.

The simplest method is the area normalization method. It is based on the assumption that all sample components exit the column and are detected by the detector. Then the sum of the areas of all peaks is taken as 100%:  Sn = 100%. The content of the i-th component in% is calculated by the formula:

Х = Si x 100% / ∑ Sn

The absolute calibration method is based on the use of a linear relationship between the peak area and the mass of the substance. CO solutions of various concentrations are prepared, chromatograms are taken, and a graph of the dependence of the peak area on the CO concentration is plotted. The calibration graph is a straight line passing through the origin. Then the area of the peak on the chromatogram of the test sample (Sx) is measured and its concentration (Cx) is determined from the graph.



Calibration graph

Less time-consuming and most accurate is the method of comparison with a standard sample (RS). The accuracy of the method is 2–3%. The test solution of the substance and its CO solution are prepared, chromatographed alternately and the peak areas are measured. Since the peak areas are directly proportional to the concentrations of substances, a system of equations is obtained:

*Scо = Ccо*

*Sx = Cx*

Find the ratio of the areas of the peaks of the standard and test samples, which, as can be seen from the equation, is directly proportional to the ratio of their concentrations.

*Scо / Sx = Ccо/ Cx*

The content of the analyte is calculated from the equation:

*Cx = Sx x Ccо/ Scо*

If it is necessary to improve the accuracy of the analysis, then the internal standard method is used. An internal standard is a foreign compound that is not present in the analyzed mixture, but is structurally similar to the compound being determined.

Advantages of the GLC method

– speed of analysis (10–30 min.);

– clarity of separation of components;

– high sensitivity of the method, depending on the detector used (if FID, then the sensitivity reaches 10–9 g);

– a high degree of automation of the separation and processing of the information received.