**Lesson 5**

**Column chromatography method.**

Separation of solid mixtures and liquid-liquid mixtures.

Column chromatography is a method for the preparative separation of mixtures of liquid or solid substances, based on the different affinity of the substances to be separated for the stationary (sorbent) and mobile (eluent) phases. As a rule, the better the substance is sorbed by the stationary phase, the slower the substance leaves the column.

Column chromatography is the single most important method for separating mixtures of liquid or solid organic substances on a preparative scale (from a few milligrams to tens of grams). Usually separation is carried out by liquid adsorption chromatography (physical nature is the same as in the case of TLC), which is effective for most non-ionic compounds.

The separation is carried out on a column, which is a glass tube filled with a sorbent (usually silica gel), which plays the role of a porous layer through which the mobile phase flows (eluting solvent, for example, hexane, chloroform).

The mixture to be separated is fed into the upper part of the column, where it is sorbed by the stationary phase, and then the eluent is continuously fed through the column. Each component of the mixture is carried down the column by the mobile phase (eluent) at a rate that depends on the affinity for the sorbent (it can be estimated from the Rf value of the TLC method). Ideally, the mixture is separated into a number of individual components (bands) which slowly move down the column and are eventually collected in a receiver. After the solvent is distilled off, pure substances are isolated from the corresponding receivers.

The eluent for column chromatography is selected using the thin layer chromatography method. The mixture is chromatographed in several eluents, and the one with the larger spot spacing is used for column chromatography. As a rule, less polar solvents allow more efficient separation of mixtures by chromatography. By increasing polarity and the ability to "wash" substances from the surface of silica gel (eluting ability), solvents are arranged in the following row:

cyclohexane < heptane < pentane < carbon tetrachloride < benzene < chloroform < diethyl ether < ethyl acetate < acetone < ethanol < methanol < water < acetic acid.

Application area

- Separation of mixtures of liquid or solid substances differing in Rf (or tR).

-Separation of the target substance from non-sorbable impurities.

1. Eluent.

eluent requirements.

1. The released substances should not interact with the eluent or be destroyed in its presence.

2. The eluent may be either an individual solvent or a mixture of several solvents. Solvents must be easily removed after separation of substances (therefore, dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) are not suitable due to the high boiling point).

3. The eluent is selected in such a way that on the sorbent, the Rf of the products differs by at least 0.15, and the spots come out with an Rf of no more than 0.5-0.6 after one run of the chromatogram.

4. If under the action of solvents of different polarity: polar (methanol, possibly with the addition of acetic acid or triethylamine) and non-polar (hexane, pentane)) the substance does not move from the start or moves with the front, you should switch to another sorbent

The choice of eluent begins with the least polar solvents, such as n-heptane, n-hexane, n-pentane, cyclohexane.

Addition of eluent.

The eluent is added either directly or using a dropping (separating) funnel. During chromatography, the sorbent layer must never dry out, otherwise it may crack, leading to a decrease in separating power.

2. Chromatographic column and amount of sorbent.

It is a glass tube, one of the ends of which has a porous nozzle or is plugged with a piece of cotton wool so that the sorbent does not spill out. The length of the column depends on the Rf of the substances to be separated. The smaller the difference in Rf, the longer the column (sorbent layer).

3. Sorbent.

It is selected based on the properties of the mixture to be separated.

sorbent requirements.

1. Substances to be separated should not be destroyed in the presence of a sorbent.

2. If under the action of solvents of different polarity (polar (methanol, possibly with the addition of acetic acid or triethylamine) and non-polar (hexane, pentane)) the substance does not move from the start or moves with the front, you should switch to another sorbent (from a polar sorbent to a non-polar and vice versa).

One of the most common sorbents used in column chromatography is silica gel. The use of methanol and ethanol as the mobile phase reduces the activity of the silica gel. Aluminum oxide for chromatography is basic, neutral and acidic. In column chromatography, basic (pH 9.0-10.0) and neutral alumina are usually used. When using alumina, acetone and ethyl acetate cannot be used as eluents. The properties of magnesia (magnesium oxide) are similar to those of alumina, but magnesia is more effective in separating unsaturated compounds. Activated charcoal is suitable for the separation of carbohydrates, peptides, amino acids. It selectively sorbs aromatic hydrocarbons.

4. Fraction receivers.

To collect fractions, you can use both ordinary flat-bottomed flasks and test tubes. When the substances to be separated are colored, you can see how they exit the column. Each substance is collected in a separate receiver. If the substances come out as a mixture, the mixture is collected separately. If the substances to be separated are not colored, fractions of a certain volume are collected, which depends on the size of the column and on the degree of separation of substances (Rf).

5. Foot.

The column is fixed on a tripod with a foot.

The metal tab must NOT come into contact with the glass to avoid cracking the flask during distillation. To do this, rubber gaskets are placed between the flask and the foot.

Recommendations for

Modern methods of column chromatography make it possible, perhaps, to separate mixtures of any composition. To do this, it is necessary to carefully select for each component of the mixture: eluent and sorbent.

1. Before carrying out column chromatography, it is necessary to select the eluent and sorbent using thin layer chromatography. For effective purification of the separated component of the mixture, the Rf value should be ~0.5. Impurities should differ in Rf by at least 0.15.

2. The eluent for column chromatography should be less polar than for TLC analysis.

3. For efficient separation of a mixture of several substances, gradient elution can be used, i.e. (using silica gel chromatography as an example), start eluting with non-polar solvents (pentane, hexane), then gradually increase the polarity of the mixture (mixtures: hexane/ethyl acetate from 20:1 to 1:5) and finally move on to highly polar solvents and mixtures ( methanol, methanol/triethylamine mixtures 20:1).

4. Methods for filling the column with a sorbent:

1.Dry method. The column is filled with sorbent. After that, the substance is immediately applied and elution begins. A big disadvantage of this method is the frequent cracking of the sorbent layer, which results in poor separation. This method is suitable for flash chromatography, i.e. separation of the target substance from impurities with Rf ~ 0.

2. Eluent then sorbent. The column is filled up to half the length with the eluent, then the sorbent is poured in portions, making sure that it is completely wetted. The disadvantage of this method is that if the sorbent is small enough, it sticks together in lumps and falls to the bottom of the column in the form of balls. The method is often used when working with coarse silica gel.

3. Sorbent in the form of a suspension in the eluent. The required amount of dry sorbent is poured into the column (to determine the required amount), it is poured from the column into a beaker. Pour the sorbent with eluent and mix for uniform wetting. After some time, the suspension is shaken and poured onto the column using a chemical funnel. The remains of the sorbent are washed off the walls of the beaker with an eluent.

5. Ways of applying the substance to the column:

1.Dry method. Substances are applied individually - solid (simply poured onto the column) or liquid (transferred to the column with a pipette).

2. In solution. The substance is dissolved in the minimum amount of eluent or less polar solvent than the eluent and applied with a pipette. The solvent must be less polar than the eluent, otherwise the material will be drawn from the column along with this solvent. The narrower the solution layer with the substance, the better the separation.

3. In the form of a solid mixture with a sorbent. The substance is mixed with the sorbent with the addition of a small amount of solvent. The solvent is removed on a rotary evaporator. Dry sorbent with the substance is poured onto the column.

6. Apply the substance to the column (liquid or in solution) along the walls of the column so as not to erode the sorbent layer. The more evenly the substance is applied, the better its separation.

7. After applying the substance, the top layer of the sorbent on the column can be covered with cotton wool or filter paper so that when the eluent is added, the sorbent layer does not wash out. The smoother the sorbent layer, the better the separation of substances.

8. Elution under pressure. In principle, in most cases the elution is carried out at atmospheric pressure. Since the faster the elution rate, the worse the separation. However, when fine-grained sorbents are used, elution at excess pressure is indispensable due to the extremely low rate of the process. In these cases, Bunsen flasks (A) are used, in which chromatographic columns are placed and connected to a vacuum pump (most suitable for flash chromatography on a Schott filter) or pressurized with a bulb (B).

9. During chromatography, the sorbent layer should never dry out, otherwise it may crack, leading to a decrease in separating power.

10. The substance must be soluble in the eluent used, otherwise it will crystallize in the column, which impairs separation. If crystallization did occur, you can:

1. change the eluent to one that dissolves the resulting substance;

2. add a solvent close in polarity to the eluent, which dissolves the resulting substance;

3. slightly heat the sorbent layer to a temperature below the boiling point of the eluent.

4. do nothing, perhaps the substance will gradually dissolve in a large amount of eluent.

The success of column chromatography depends mainly on the correct choice of sorbent and mobile phase.

The disadvantages of adsorption column chromatography include the duration of elution.

Partition column chromatography resembles countercurrent extraction. It is based on the distribution of solutes between a mobile organic phase and an aqueous phase held by a solid carrier. The carrier must be inert with respect to the substances to be separated, but must retain the stationary liquid phase well. Commonly used carriers (silica gel, kieselguhr, cellulose) hold 0.5-1 mg of the liquid phase per 1 g of its own weight. Water is most often used as the stationary liquid phase. When separating substances that are highly soluble in organic solvents, the opposite is often done: a solid carrier is impregnated with an organic solvent, and water is used as the mobile liquid phase.

Partition column chromatography is useful for the preparative isolation of pure substances.

Separation of solid mixtures and liquid-liquid mixtures.

Methods for separating mixtures are the most important analytical operations necessary when the detection and quantitative determination of one element (substance) is interfered with by many other elements.

Separation of mixtures is the process of separating pure substances from mixtures. The products to be separated have different chemical and physical properties.

Individual substances can be isolated from mixtures. In mixtures, individual substances retain their properties. There are various ways to do this. The separation of mixtures into individual substances is based on the difference in the physical properties of the components that make them up.

Separation of heterogeneous mixtures.

Settling. The method is used to separate insoluble substances with different densities, such as river sand and coal. The mixture is dissolved in water and stirred. Sand, the density of which is greater than the density of coal, settles to the bottom, and coal floats to the surface of the water. Coal is removed from the surface of the water, after which water is carefully drained from the sediment, using a glass rod.

A mixture of two immiscible liquids, such as oil and water, can also be separated by settling. To do this, use a separating funnel with a tap at the end. The mixture to be separated is placed in the funnel and the boundary between the two liquids is waited for. In the upper layer there is a liquid with a lower density - oil, and in the lower - with a higher density - water. After that, the tap is opened, the water flows out, and oil remains in the separating funnel.

Decantation - separation of the solid phase of the suspension / suspension from the liquid phase, carried out by pouring the solution from the sediment after settling.

Filtration separates the precipitate from the solution. With the help of filtration, it is possible to purify water from impurities insoluble in it, for example, from sand, clay, chalk. In the laboratory, filter paper is used for filtration. The size of the pores in the filter freely passes the molecules of water and substances dissolved in it, but retains particles of undissolved substances. The filter is folded in four and inserted into a glass funnel. The glass funnel is then placed in a flask or beaker and the mixture to be separated is carefully filtered. The liquid that passes through the filter and is collected in a flask or beaker is called the filtrate.

Separation using a magnet is used to separate mixtures in which some substances have magnetic properties, such as iron, cobalt, nickel, while others do not exhibit magnetic properties, such as sulfur. Using a magnet, a mixture of iron and sulfur, iron and copper, iron and carbon (soot) can be separated.

Separation of homogeneous mixtures.

Evaporation is used to isolate soluble solids from solutions. To do this, the solution is gently heated in an evaporating porcelain cup. After the water has evaporated, solids remain at the bottom of the cup, which were contained in the solution. If evaporation is carried out slowly, then the substance dissolved in it can be isolated from the solution in the form of fairly large crystals. This method is called crystallization.

Sublimation (sublimation) and subsequent crystallization after cooling are used to purify substances with a low sublimation temperature, such as crystalline iodine.

When a solid mixture is heated, the necessary substance sublimates and takes on a gaseous state, then it is cooled. A distinctive feature of sublimation is that a substance subjected to sublimation passes from a solid state to a gas, bypassing the liquid stage.

To separate liquids or gases that mix with each other and have different boiling points, distillation or distillation is used. When the mixture is heated, the liquid components contained in it evaporate sequentially in order of increasing boiling points. The evaporating vapors are collected and condensed upon cooling. In this way, oil can be divided into fractions, water and acetone, water and alcohol can be separated. Distillation can also be used to purify water from the soluble salts it contains.

Natural water always contains dissolved salts, which can be removed by distillation. Water is heated, water vapor is collected and cooled to obtain distilled water. Distilled water is necessary for the preparation of solutions in the laboratory, for the manufacture of medicines. However, the use of distilled water in food is not recommended.