Chromatographic separation procedures: gas chromatography with Cobra4 (Item No.: P3031760)

Curricular Relevance

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Overview

Short description

Principle

Chromatographic procedures allow a separation of substance mixtures with the aid of a stationary separating phase and a mobile phase. In gas chromatography the mobile phase is a gas. The mobile phase, to which the mixture to be separated is added, transports the substance mixture through the separation column at a constant flow rate. Interactions occur between the mobile phase and the stationary phase. The establishment of equilibria between the stationary phase and the different substances (distribution equilibria, adsorption-desorption equilibria) results in different migration rates of the individual components. At the end of the column there is a detector in the form of a thermal conductivity cell, which can detect the different substances on the basis of their differing thermal conductivities. The detector signal is recorded as a function of time.

The different thermal conductivities of the carrier gas and the substances cause temperature alterations in the electrically heated temperature sensor, which is located in a Wheatstone bridge circuit. The resulting electrical signal is recorded by a plotter as a function of time (chromatogram).
Safety instructions

Fig. 1: Experimental setup.

Disposal
The organic substances have to be collected in a correspondingly labelled container and passed to safe waste disposal.

Acetone
H220: Extremely flammable gas.
H225: Highly flammable liquid and vapour.
H319: Causes serious eye irritation.
H336: May cause drowsiness or dizziness.
EUH066: Repeated exposure may cause skin dryness or cracking.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233: Keep container tightly closed.
P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.

Ethyl alcohol, absolute
H220: Extremely flammable liquid and vapour.
H319: Causes serious eye irritation.
P210: Keep away from heat/sparks/open flames/hot surfaces – No smoking.
P280: Wear protective gloves/protective clothing/eye protection/face protection.

Ethyl acetate
H220: Extremely flammable gas.
H225: Highly flammable liquid and vapour.
H319: Causes serious eye irritation.
H336: May cause drowsiness or dizziness.
EUH066: Repeated exposure may cause skin dryness or cracking.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P240: Ground/bond container and receiving equipment.
P305+351+338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do –
continue rinsing.

Chromosorb, PAW, 80/100 mesh
H319: Causes serious eye irritation.
H335: May cause respiratory irritation.
H373: May cause damage to organs through prolonged or repeated exposure.
P261: Avoid breathing dust/fumes/gas/mist/vapours/spray.

Helium
P280: Wear protective gloves/protective clothing/eye protection/face protection.

n-butane
H220: Extremely flammable gas
H280: Contains gas under pressure; may explode if heated.
P210: Keep away from heat/sparks/open flames/hot surfaces – No smoking.
P220: Keep/Store away from clothing/…/combustible materials.
P377: Leaking gas fire – do not extinguish unless leak can be stopped safely.
P381: Eliminate all ignition sources if safe to do so.
P403: Store in a well ventilated place.

iso-butane
H220: Extremely flammable gas
H280: Contains gas under pressure; may explode if heated.
P210: Keep away from heat/sparks/open flames/hot surfaces – No smoking.
P220: Keep/Store away from clothing/…/combustible materials.
P377: Leaking gas fire – do not extinguish unless leak can be stopped safely.
P381: Eliminate all ignition sources if safe to do so.
P403: Store in a well ventilated place.

Equipment

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Tasks

1. Determine the retention times of different gases and perform a chromatographic material separation of a mixture of butane gases.

2. Separate and identify the components of a two-component mixture consisting of ethanol and ethyl acetate chromatographically.

Setup and procedure

Setup
Set up the experiment as shown in Fig. 1 and Fig. 2.

Prepare the stationary phase for the gas separating column by pouring acetone onto 10 g of Chromosorb in a round bottom flask until a thin film of liquid overlays the Chromosorb. Add a solution of 1.11 g of dinonylphthalate in about 10 ml of acetone and shake the mixture vigorously for several minutes. Place the flask in a dish containing warm water and use a water jet pump to reduce the pressure in the flask so much, that the acetone starts to boil gently.

To recover the acetone, insert a security flask between the round bottom flask and the water jet pump. During the evaporation of acetone, frequently shake the round bottom flask. When the acetone has evaporated, empty the acetone collected in the security flask into the waste container for combustible organic material. Use the water jet pump to exert suction on the round bottom flask, held at about 70°C (water bath), for about another 2 hours. Subsequently it is important that the separating medium for the gas chromatographic column is completely dry and contains no remaining traces of solvent. If necessary, dry it further in a drying oven at about 100°C. Fill the prepared separating medium into the separating column using a funnel and a water jet pump. To do this, use a thin rod to push a quartz glass wool plug in the separating column connector through which the carrier gas used as mobile phase will later flow into the column (8 mm glass nipple), until it is at the start of the separating column, and push it a little so that it is quite firmly positioned. Following this, connect the glass nipple to the water jet pump and suck air through the column. Now hold the column with one hand and slant it so that a funnel can be held in the GL 18 threaded glass connector. Fill the prepared stationary phase in portions into this funnel, from which it will be immediately sucked into the column by the flow of air. It is recommended that the filling of the stationary phase is supported by slight, careful tapping on the glass tube with the free hand. It must be ensured, that the column is evenly filled. When the column has been filled, close the end through which the stationary phase has been filled in with a quartz glass wool plug, as previously done for the other end. Do not press the two plugs in too firmly, however, otherwise their resistance to the flow of carrier gas will be too high when the column is later put into operation. On the other hand, they must be firm enough to prevent carrier gas from pressurizing stationary phase out of the column.

To set up the gas chromatograph fit the gas separation column into the glass jacket and mount this combination with two clamps vertically on the left support rod (cf. Fig. 2). Then connect a circulating thermostat, which is in a plastic vessel with a volume of about 6 litres (bath for thermostat), to the glass jacket via a rubber tubing so that the water input passes through a glass tube fitted to the lower tubular glass sleeve [8] into the glass jacket. The outflow occurs via the hose connection tube (hose olive) [4] at the rounded head of the separation column.

Fit a thermometer into the upper tubular glass sleeve of the glass jacket [7] as an additional temperature control. Before the set-up is further assembled, it is appropriate to turn on the water circulation (from the thermostat to the gas chromatograph and back) to test the system and concurrently to fill the glass jacket and the rounded glass head with water (bubble-free). While doing so, check for leaks in the water circulation system and if necessary eliminate them. If this partial setup functions properly, set-up the remainder of the experimental apparatus in the following sequence.

Connect the pressure tubing (vacuum tubing) coming from the gas source (helium cylinder) to the glass hose connection (hose olive) [1]. Since this is under pressure throughout the experiment, secure it on both ends with hose clips. Fit the measuring probe [5] (sealing ring: GL 18/8 mm) into the connecting cap on the tubular glass sleeve [2] and secure it with a clamp.

Connect the measuring probe via the lateral tubular glass sleeve [6] and a piece of rubber tubing with a soap bubble flow meter, in which 1 to 2 ml of soap solution has been poured. It is possible to occasionally add a small amount of soap solution to the gas flow via the rubber cap during the experiment. The gas bubble created in this way makes it possible to observe and measure the flow rate.
Connect the measuring probe to the control unit for the gas chromatograph, which serves both to provide power and to calibrate the measuring probe, with its coaxial cable.

Combine the Cobra4 Sensor Unit Chemistry and with the Cobra4 Wireless-Link.
Attach them to the retort stand with the holders for Cobra4 and right angle clamps.
Connect the Cobra4 Sensor Unit Chemistry to the output socket of the control unit using two connecting cables and the adapter.
Fit a rubber cap onto the injection port of the glass separating column as septum. Switch on the thermostat with circulating pump. Room temperature is sufficient for the separation of gases, but higher temperatures are generally required for the separation of liquid mixtures.
When the required temperature has been attained, allow carrier gas (helium) to flow slowly through the separating column. Adjust the flow rate with the pressure reducing valve on the helium cylinder (while continually measuring with the soap bubble flow meter) to about 30 ml gas per minute. Make measurements with the flow rate with the soap bubble flow meter, attached as in Fig. 1, as follows: Repeatedly press the rubber cap with the 2 ml of soap solution to position some soap bubbles in front of the side glass nozzle. The entering flow of gas carries the bubbles up the tube. Measure the time taken for such a bubble to pass from the 0 ml mark to the 5 ml or 10 ml mark with a stopwatch. Calculate the flow rate per minute from this value. For example, when a soap bubble reaches the 10 ml mark in 15 seconds, then the gas flow in the gas separating column is 40 ml per minute.
The stock of the gases that is to be examined by gas chromatography is best stored in the small gasometers of the gas bar (see Fig. 3). Fill these as follows: Fill the Erlenmeyer flasks with water. Connect the right-angled tube to the valve of the pressurized gas container with a length of tubing. To connect the tubing to the butane burner, screw off the upper part of the burner and fix the tubing over the thread on the burner base. Open the valve to allow the gas concerned (n-butane, iso-butane, camping gas) to flow into the Erlenmeyer flask, where it displaces the water into the plastic funnel. After filling, close the outflow tube with a rubber cap. To prevent gas from being forced out of the flask by the water pressure on it, previously close the upper opening of the plastic funnel with a rubber stopper. This stopper must later be removed.

After the Cobra4 Wireless-Link has been switched on, the sensor is automatically recognized. An ID number (01) is allocated to the sensor, which is indicated in the displays of the Cobra4 Wireless-Link.
Call up the “measure” programme.
Boot the experiment “Chromatographic separations procedures: gas chromatography with Cobra4” (experiment > open experiment). The measurement parameters for this experiment are loaded now.

Procedure

1. The separation and identification of butane isomers
The separation of a mixture of butane isomers is carried out at room temperature. The circulating thermostat is to be switched on, but not to be used to heat the water (set the thermostat to 20°C).
Switch on the control unit some minutes before starting measurement. Press the coarse balancing button on the control unit, wait a few moments, then adjust the fine balancing rotary knob so that the value measured for the base line is approximately at 0 V. After three minutes, check the display. If the measured values have changed a lot, again press the coarse balancing button.
(but first turn the fine balancing rotary knob back to the middle position!).

Use the valve of the helium cylinder and the soap bubble flow meter (with cap containing 1 to 2 ml of detergent solution) to adjust a flow rate of about 30 ml helium/min (10 ml/20 sec). Press the cap as above to appropriately produce and position bubbles.

To demonstrate the gas chromatographic separation, mix n-butane and iso-butane in a 1 ml syringe with attached cannula, by successively drawing 0.5 ml of each substance from the appropriate gasometer into the syringe.

Start data collection with the PC by pressing

then carefully insert the cannula of the syringe deeply through the septum and quickly press the plunger of the syringe. Draw the syringe rapidly out of the rubber cap. If possible, practice this procedure several times, to ensure an even and rapid introduction of the sample. Note the starting point of the separation – the time of injection – the computer now takes over the recording. When the measurement is finished, terminate it with

After each measurement, send all data to “measure” (see Fig. 4) and save the data (File > Save measurement as).

To identify and assign the individual peaks, prepare another mixture of the two gases, but this time first with 0.4 ml of iso-butane with 0.6 ml of n-butane, then with 0.6 ml of iso-butane with 0.4 ml of n-butane. Carry out a gas chromatographic separation on each and compare the results with the initial chromatogram (1:1 mixture).

A gas mixture from the butane burner cartridge (cigarette lighter gas) can be separated in the same way. Additional mixtures of cartridge gas and the two isomers n- and iso-butane can also be injected into the column for separation.

Change the rubber cap used as septum after a few injections, as it will leak after several of them. Stop the flow of gas before changing it.

2. The separation of a mixture of ethanol and ethyl acetate

Use two 1 ml syringes to fill 1 ml each of ethanol and ethyl acetate into a snap-cap vial. This mixture can be stored in the vial for quite some time for later usage.

Carry out this separation at 80°C. Set the thermostat to this temperature and wait until the thermocouple in the glass jacket shows a constant temperature of 80°C. The further procedure for the chromatographic separation is almost exactly as that described above.

Draw 5 µl of the mixture, bubble-free, into the 10 µl syringe. Start measurement by pressing

then carefully insert the cannula of the syringe deeply through the septum, using the free hand to guide it, and quickly press the plunger. Draw the syringe rapidly out of the rubber cap (but take care, the cannula can easily snap). When the measurement is finished, terminate it with

After each measurement, send all data to “measure” (see Fig. 4) and save the data (File > Save measurement as).

For certain identification of the ester, inject a further mixture made by first drawing 4 µl of the original mixture into the microliter syringe and then a further 2 µl of ethyl acetate.

Theory and evaluation

The chromatographic separation of substance mixtures occurs between a stationary phase, with which the chromatographic column is filled, and the mobile phase, which moves with constant velocity through the separation column. In gas chromatography the mobile phase is a gas: it is termed the carrier gas. For material separation, distinct separation functions can
be used (e.g. adsorption, distribution, ion exchange, gel permeation).

Gas chromatography is most frequently employed as distribution chromatography. In this case the stationary phase is composed of stationary phase consisting of a solid substrate on whose surface a thin liquid film with the separation phase proper is located. Under isothermic, isobaric conditions Nernst’s law of distribution is valid for the establishment of a distribution equilibrium

\[ K = \frac{a^I_A}{a^II_A} \]  

where

- \( K \): Nernst distribution coefficient
- \( a^I_A \): Activity of material A in phase I
- \( a^II_A \): Activity of the material A in phase II

When a substance migrates through the separation column, a great number of equilibria are established in accordance with the principle of multiple or Craig distribution. Substances, whose activity in the stationary phase is great, are transported slowly through the separation column, whereas substances which are poorly soluble in the stationary phase pass rapidly through the column.

Analogously to distillation, the separation power of a column is described by the number of theoretical trays. The larger the number of theoretical trays of a column, the better its separation power. The number of theoretical trays \( N \) can be determined as the quotient of the length of the column \( L \) and the height equivalent to theoretical trays \( H \).

\[ N = \frac{L}{H} \]

where

- \( N \): Number of theoretical trays
- \( L \): Column length
- \( H \): Height equivalent to theoretical trays

The height equivalent to theoretical trays can be directly calculated from the chromatogram. Fig. 5 shows the characteristic quantities which can be read from a chromatogram. The signal intensity is plotted as a function of time. The mobile time (dead time) is the time which a gas that is not retained by the column requires to pass through the column.

From the chromatogram, the height equivalent to theoretical trays can be determined. It is a measure of the separation power of the column.
The retention time is used for the identification of the substance (qualitative information). This time is characteristic for a specific substance at constant conditions.

The area under the respective substance peak provides the quantitative information. For needle peaks, the peak height can be used directly for quantitative determination.

\[ H = \frac{n^2 L}{16 t^2} \]  

(3)

\[ I \] Signal intensity
\[ t_0 \] Start time
\[ t_m \] Mobile time (dead time)
\[ t_{RA} \] Retention time of substance A
\[ t_{RB} \] Retention time of substance B
\[ b_A \] Basal width of substance A
\[ b_B \] Basal width of substance B

Data and results
The results differ greatly for different separation columns. Under the conditions given above, the following results were obtained:

1. The separation of butane isomers
The measurements for this part of the experiment have been carried out at a separating column temperature of 22 °C. The position “1” in the following chromatograms shows the moment of gas injections. Peak “2” is caused by traces of air injected in with the sample.

Fig. 6 and Fig. 7 show the chromatograms of pure \( n \)- and \( iso \)-butane.

Fig. 6: Chromatogram of pure \( n \)-butane.
Peak No. 4 in Fig. 6 represents $n$-butane while peak No. 3 in Fig. 7 shows iso-butane. Peak No. 3 in Fig. 6 is caused by some hydrocarbons which are part of the sold gas. Thus the sample of the “pure” $n$-butane is contaminated.

The two isomers of butane can be separated by gas chromatography, as is shown by the gas chromatograms (Figs. 8-10).
When the chromatogram from a 1:1 mixture of the isomers (Fig. 8) is compared to those in which one of the gases was in excess (Figs. 9 and 10), it can be seen that there are changes in the relative heights of the peaks. The peak with increasing intensity relative to the 1:1 mixture can be assigned to the isomer that was present in excess, and the peak with decreasing intensity relative to the 1:1 mixture can be assigned to the isomer that was present to a lower amount. It can so be demonstrated, that in this separation, iso-butane (peak 3) leaves the separating column before n-butane (peak 4) (Figs. 9 and 10).

The separation of the gas mixture from the burner cartridge results in a chromatogram with five peaks (Fig. 11). The first of these can again be assigned to air (peak 2). A comparison with the chromatograms from Figures 7 to 9 allows peak 4 to be assigned to iso-butane and peak 5 to n-butane. Peak 3 is due to propane, which is also present in burner gas.

The correctness of this assignment can be demonstrated by carrying out separate gas chromatographic separations, each with burner gas mixed with a small amount of one of the pure gases (Figs. 12 and 13). Propane was not used here, but can in principle also be subjected to such a separation.
A further peak is found in the chromatograms shown in Figures 11 to 13 (peak 6). This must result from small amounts of 1.3-butadiene, as, according to the label on the gas cylinder, this is contained in the mixture of gases in camping gas.

The experimentally determined results can be explained as follows. The isomers of butane differ in their boiling points. Iso-butane is an almost spherical molecule with weak inter-molecular forces and has a boiling point of -11.7 °C, whereas n-butane is a linear molecule and so is exposed to great adhesion forces, which result the much higher boiling point of -0.5 °C. Iso-butane therefore leaves the separating column before n-butane.

The chromatographic separations of the gas mixtures shown here were carried out under the following conditions:

Column temperature: 22 °C
Carrier gas flow rate: 30 ml/min, helium
Total amount injected: 1 ml gas/gas mixture

2. The separation of a mixture of ethanol and ethyl acetate

The separation of a mixture of ethanol and ethyl acetate (1:1 by volume) supplies, as expected, a chromatogram with two peaks (Fig. 14).
Fig. 14: Chromatogram for a mixture of 0.5 µl ethanol and 0.5 µl ethyl acetate.

Peak 1 in this chromatogram must be assigned to ethanol and peak 2 to the ester. This can be simply demonstrated by carrying out a second separation using 4 µl of the mixture, and drawing 2 µl of the ester into it in the microliter syringe. The chromatogram from this separation shows a distinctly larger relative size of peak 2 compared to peak 1 (Fig. 15), which leads to the conclusion that peak 2 is correctly assigned to the ester.

Fig. 15: Chromatogram for a mixture of 4 µl of a 1:1 mixture of ethanol and ethyl acetate and 0.2 µl of pure ethyl acetate.

The chromatographic separation of the mixture of ethanol and ethyl acetate was carried out under the following conditions:

Column temperature: 80 °C
Carrier gas flow rate: 30 ml/min, helium

Composition of the mixtures analysed:
Fig. 13: 5 µl mixture (ethanol/ester = 1/1)
Fig. 14: 4 µl mixture + 2 µl ester
If gas chromatographic separations took place strictly according to the height of the boiling points, then a substance with a lower boiling point would have to be eluted from the column before a substance with a higher boiling point. As we see in this example, however, ethyl acetate clearly leaves the column after ethanol, although it has a boiling point that is 1.44 K below that of ethanol (boiling points, ethyl acetate 77.06 °C, ethanol 78.5 °C).

Therefore gas chromatography allows separating mixtures into their individual components which cannot be separated by distillation, such as azeotropic mixtures or mixtures of substances whose boiling points are very similar. The quality of the separation can be distinctly improved by alterations in the conditions (separating medium, temperature, carrier gas flow rate, etc.).

**Remarks**

The use of gas chromatographs to separate mixtures of gases is a well proven method in analytical work. They can separate even extremely small portions of mixtures into their components, for them to be recognised by suitable detectors. In the experiments described here, a gas chromatograph is used that can be easily assembled from the individual parts, and so has the particular benefit, that the construction and functioning of all the essential parts of a gas chromatograph can be made understandable to the students. The detection (display, recognition) is achieved here by means of a measuring probe based on an NTS resistor. It recognises the separated gas fractions by their different heat conductances. The separating column used in this gas chromatograph is filled with dinonylphthalate on Chromosorb as separating medium (stationary phase), according to the directions given above. Although this separating column could be used alone to separate mixtures, its insertion in the glass jacket is always recommended, so that it can be kept at constant temperature by the circulating thermostat.

This is important, as the positions of the peaks of the substances (retention times) are strongly dependent on the temperature, among other factors, and fluctuating temperatures lead to very unsteady base lines. Helium can be used as carrier gas (mobile phase) in this gas chromatograph. The helium flow rate can be adjusted and monitored by use of a very simple soap bubble flow meter.

The composition of the gas in the cartridge for the burner (camping gas or cigarette lighter gas) is subject to fluctuation. There may be no propane in it, or it may only consist of n -butane. The composition of the cartridge available should therefore be tested before using the gas for a demonstration experiment.

This is not the place to discuss the theory of gas chromatography. This experiment is intended merely to vividly demonstrate its principles. More exact and extensive descriptions of these principles are to be found in the appropriate technical literature.