Physical chemistry lab courses for beginners

Experiment A14: Basics of gas chromatography

Important: Please bring a USB stick!

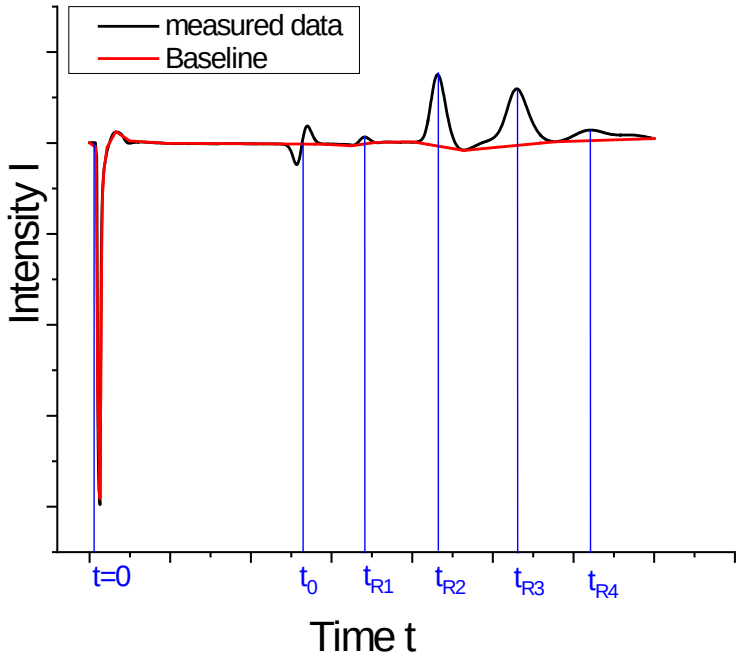
1. Introduction

Along with distillation and extraction, gas chromatography is one of the most important thermal separation processes and represents a sensitive method for identifying and analyzing the components of mixtures of substances. Gas chromatography is used, among other things, in the analysis of agricultural and meat products, in the analysis of pharmaceuticals, petroleum components and in environmental analysis. The enormous importance of chromatography in chemistry was made clear by the award of the 1952 Nobel Prize in Chemistry to two pioneers of partitions chromatography, Archer J. P. Martin and Richard L.M. Synge.

2. Theoretical background

The separation of individual components of a mixture takes place in a separation section (column) consisting of a stationary phase. The mixture as a component of a mobile phase (mixture in carrier gas) flows through the stationary phase. The interaction of the molecules of the mobile phase with the components of the column is molecule-specific and leads to different flow rates of the transported components. The molecules of the mixture oscillate back and forth between the phases and thus exhibit different residence times in the stationary phase. The extended residence times are characterized by thermodynamic equilibria between the gas and solid phases (adsorption/desorption) and thus depend on the temperature of the gas/solid interface. This fact is expressed by measurably different delays in the transport of the individual components of the mixture. This delay is called chromatographic retention. The different retention times in the stationary phase are determined by the free Gibbs enthalpy of the delay process (adsorption/desorption). The time required for the molecules to cross the separation distance is called residence time. Two components of a mixture are therefore separable if they have different gross retention times, which conversely means that they differ in their interaction with the stationary phase. Their separability is described by the so-called separation factor Δ ($\Delta = t_1/t_2$, the ratio of the retention times of the two components). This factor reflects the ratios of all derived variables, e.g. quantity ratios, distribution coefficients, etc..

The retention time of a component is measured by means of a detector positioned at the exit of the column. The gas flowing past changes the temperature of the detector, which can be detected by the change in resistance. Due to the statistical nature of molecular transport (diffusion processes lead to different path lengths of the molecules) the measurement signal of a transported component shows an approximate Gaussian shape. The retention time t_R, the time between injection of the component until the Gauss peak (maximum) appears on the detector, remains constant under the same chromatographic conditions and is therefore characteristic of the component under investigation. The identification of an unknown component can therefore be done by comparing the retention times with a standard.



The retention volume V_R (the amount of mobile phase that has flowed through the separation system in time t_R) can be calculated from the following relation:

1) $$\mathbf{V_R} = \mathbf{t_R}\,\mathbf{F}$$, where F - constant flowrate (mL/min).

A comparison of the absolute retention times is difficult because tR depends on the flow velocity of the mobile phase as well as on the length L of the separation distance. The so-called capacity factor (k* value) is a quantity independent of F and L. This quantity relates the retention time $\mathbf{t_R^*}$ to the dead time $\mathbf{t_0}$:

2) $$\mathbf{k^*} = \mathbf{t_R^*}/\mathbf{t_0} = (t_R - t_0)/t_0 = (t_R/t_0) - 1$$

Thus, The k* value represents the molar ratio of a component in stationary and mobile phase. The k* value is linked to the distribution coefficient K:

3) $$\mathbf{k^*} = (\mathbf{V_s}/\mathbf{V_g})\mathbf{K}$$

where V_s is the volume oft he stationary phase and V_g is the volume of the mobile phase. The capacity factor k* is thus proportional to the volume of the stationary phase (or the specific surface area of the adsorbates, inm^2/g).

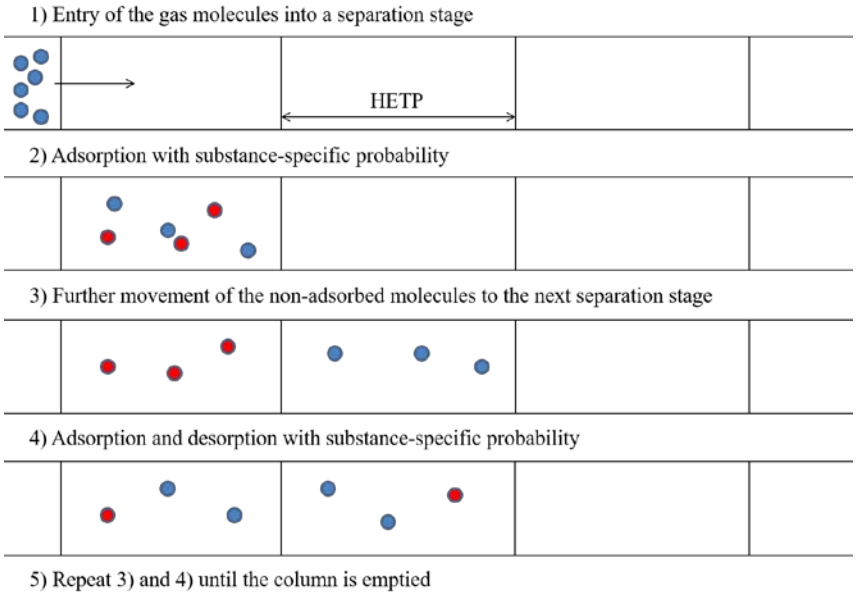
The relative retention α for two components 1 and 2 can thus be represented as follows:

4) $$\alpha = \mathbf{k_2}^*/\,\mathbf{k_1}^* = \mathbf{K_2}\,/\,\mathbf{K_1},$$

i.e. two components with identical k* values remain inseparable.

In contrast to distillation and extraction, chromatography does not have any physical separation stages; in simple terms, the process is modelled as follows: a certain amount of the gas mixture enters a section of the column at a certain point in time, which is regarded as the theoretical separation stage. In this section each gas molecule is adsorbed with a substance-specific

probability (red). All non-adsorbed molecules (blue) move to the next separation stage in the next time step, all adsorbed molecules remain. Now all gas molecules can be adsorbed again with a substance-specific probability, while all adsorbate molecules desorb with a substance-specific probability. Again all non-adsorbed molecules move one separation stage further, while all adsorbed molecules remain in their respective stage. This is repeated until all molecules have passed all separation stages. As long as the column is not overloaded by too large sample volumes, incorrectly selected temperature or flow rate, the separation is statistical and the individual components leave the column Gaussian-distributed. Otherwise the measured peak will be deformed.



According to the theory of Martin and Synge from 1941, the resolving power of a column of length L can be described with the parameter HETP (height equivalent to a theoretical plate):

5) $$\mathbf{HETP} = \mathbf{L} / \mathbf{N} \quad \text{(in mm)}$$

where N, the number of separation stages in the column (plate number), can be calculated for Gaussian peaks as follows:

6) $$\mathbf{N} = 16\,(\mathbf{t_R} / \mathbf{W})^2$$

Where t_R is the residence time at the peak maximum and W is the peak base width (distance of the intersection of the turning tangents with the base line). For peaks that deviate from the Gaussian shape, the base width W is replaced by the half-width $W_{1/2}$:

7) $$\mathbf{N} = 8 \ln 2\,(\mathbf{t_R} / \mathbf{W_{1/2}})^2 \quad \text{therefore} \quad \mathbf{HETP} = \mathbf{L} / [8 \ln 2\,(\mathbf{t_R} / \mathbf{W_{1/2}})^2]$$

The resolution **R** of a GC separation path is defined by the ratio of the retention time difference of the adjacent peaks Δt_R, and the arithmetic mean of the corresponding base widths W:

8) $$\mathbf{R} = (\mathbf{t_{R2}} - \mathbf{t_{R1}}) / (\mathbf{W}(1) + \mathbf{W}(2))/2 = 1{,}198\,(\mathbf{t_{R2}} - \mathbf{t_{R1}}) / [\mathbf{W}_{1/2}(1) + \mathbf{W}_{1/2}(2)]$$

The linear flow rate **u** of the mobile phase is calculated from the chromatogram as follows

9) $$\mathbf{u} = \mathbf{L_g} / \mathbf{t_0}$$

L_g Length of the separation column incl. inlet and outlet in cm

t_0 Dead time of the column in s at this flow rate

If the dead time signal is not visible in the chromatogram t_0 can be calculated approximately in this way:

10) $\mathbf{t_0 = A * L_g / F}$ **(→ u=F/A)**

A= Cross sectional area of the column tube
F= Flow rate according to flow meter

According to Van Deemter, the HETP parameter depends on the flow of the mobile phase u This equation describes chromatographic separation as a dynamic process and takes into account the role of diffusion and mass transfer:

11) $\mathbf{HETP} = A + B/\mathbf{u} + C\,\mathbf{u}$.

A - Contribution of the Eddy diffusion (diffusion caused by turbulences)
B - Contribution of longitudinal diffusion
$C=[\ K_1 d_f^2/D_S + K_2 d_P^2/D_M]$ Mass transfer from and to stationary phase.
u – Linear flow rate
λ - Constant, that describes particle shape and homogeneity oft he package.
d_P - Particle diameter
D_M - Diffusion coefficient of the mobile phase
Ψ – Correction factor for the free spaces between the particles
d_f - Thickness of the liquid film
K_1, K_2: Correction factors for the specific geometry of the column and the column capacity.

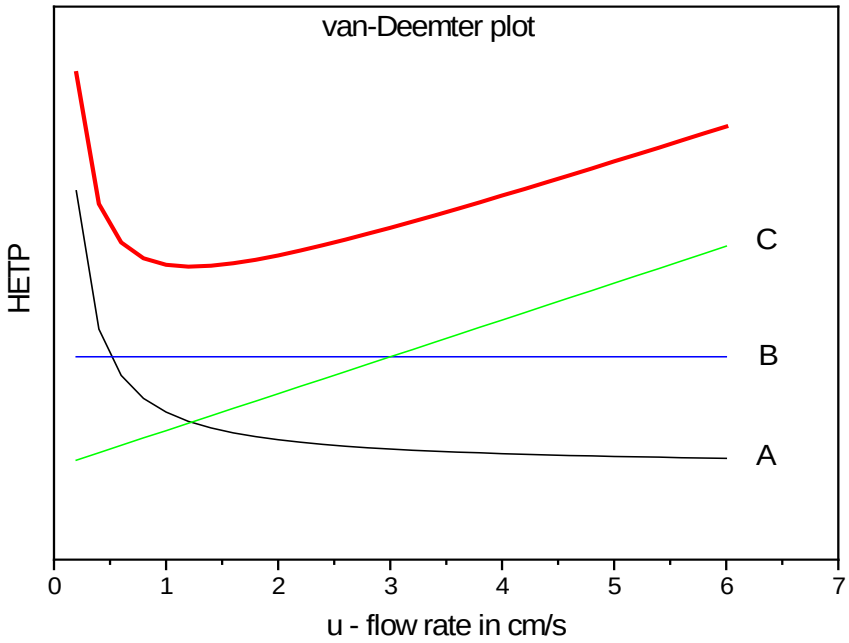
The eddy diffusion A is only dependent on the packing of the stationary phase and the associated path that the mobile phase must travel in it. The smaller and more homogeneously packed the column, the smaller the eddy diffusion. It is independent of the flow velocity u. $A=2*\lambda*d_P$
The longitudinal diffusion B is only dependent on the mobile phase. It occurs in both packed and unpacked columns and describes the widening of the phase due to diffusion away from the center. The greater u the smaller the contribution of B. $B=2*\lambda* D_M$.
C is the mass transfer between stationary and mobile phase and thus describes the actual chromatographic process. C differs from particle to particle and thus enables the separation of different components of the mobile phase. The slower the flow velocity, the more effective is the separation of the individual components.$C=[\ K_1 d_f^2/D_S + K_2 d_P^2/D_M]$

This equation shows how to find the optimum separation performance of the column (optimum: shortest possible analysis time and good separation performance). By analyzing the specific parameters (λ, d_P, d_M …) it is possible to make statements about the optimal column composition: columns with a small diameter, which are tightly and homogeneously packed with fine-grained material, show the best separation performance.
At low flow rates u the role of the A and C terms can be neglected and eq. 9 allows to determine the effective coefficient of longitudinal diffusion (eq.11):

12) $\mathbf{HETP} = 2\,\Psi\,\mathbf{D_M}/\mathbf{u}$,

whereby in practice the parameter $\Psi \approx 1$ is set,

13) $\mathbf{D_{eff}} = \mathrm{HETP}\ u\ /2.$

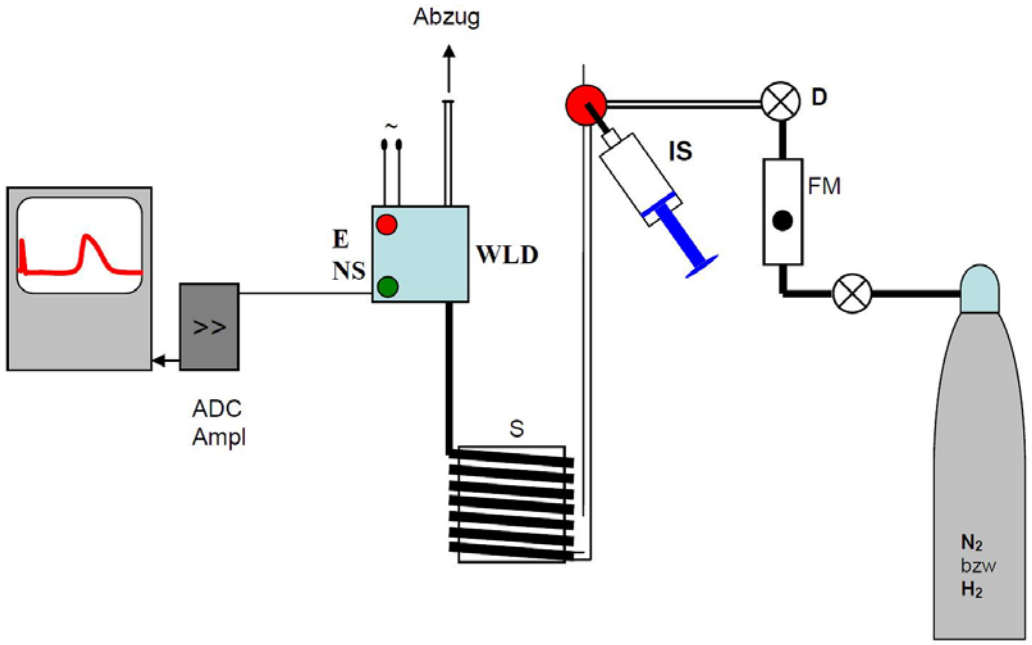


3. Aims of the experiment

The analysis of the chromatograms of pure substances should first clarify the most important basic relations of gas chromatography. The characteristic quantities of gas chromatography, HETP, N and D_{eff}, should be determined as functions of the experimental parameters (amount of substance M, flow velocity u) with nitrogen as carrier gas. In the second part, the most important components of an unknown commercial gas mixture will be identified by comparing the retention times of pure reference substances.

4. Experimental setup

The main component of the apparatus is a long spiral column (**S**) filled with porous oxidic material (L=2 m, inner diameter of the glass tube ϕ=5 mm, ength including inlet and outlet L_g=2,7 m). The analysed gas flows through the material of the column (pipe from the N2 gas bottle through the column to the hood). The flow velocity of the carrier gas or mixture is measured with a flow meter (**FM**). The scale of the flow meter is in Nl/min.
The detection of the transported components of the mixture is realized by means of a thermal conductivity detector (**WLD**) positioned at the outlet of the column. The carrier gas flowing past heating wire of the WLD detector. The mixture in the carrier gas has a lower thermal conductivity than the pure carrier gas, so the heating wire is cooled less. The resulting temperature increase is measured as the resistance change of the heating wire compared to a reference element in a Wheatstone bridge. Such gases as H_2, N_2 and He guarantee the achievement of the highest temperature differences because they also have the highest thermal conductivity. The signal, the compensation voltage from the Wheatstone bridge, is proportional to the temperature difference generated and this is proportional to the number of gas molecules reaching the area of the heating wire (to the partial pressure of the mixture). A sensitivity of the WLD detector of 235 has proven to be optimal. Before each measurement, the zero point should be reset by varying the resistance Z.



D – Throttle valve
FM – Flow meter
S – column of length 2m
WB – Water bath
WLD – thermal conductivity detector)
ADC+**Ampl** – amplifier with ad converter
DA – Data acquisition and analysis
IS – Injection syringe
E – Sensitivity (optimised; do not change)
NS – Zero position (compensation oft the reference signal)

The signal from the Wheatstone bridge as a function of time, U(t), represents the molecules of the mixture flowing past. Ideally, the signal has a Gaussian shape. The analog signal (a few mV) is amplified, digitized and transmitted to the computer via an interface. The signals are recorded using a Labview program (GC), for which you will receive instruction on site. The time between injection of the mixture (IS) and the appearance of the maximum of the Gauss peak corresponds to the retention time, t_R. If the peak has a different shape than a Gaussian curve, t_R corresponds to the center of gravity.
In all experiments the pressure of the carrier gas is set to **0.5** bar by means of the reducing valve on the gas cylinder. The flow rate u is determined by means of a throttle valve (**D**) at the outlet of the flow meter.
A GC curve is not recorded until a stable flow velocity has been reached. In the next step, the signal is to be set to 0 mV as a reference with the control knob **NS**. Then the recording is started and the injection of the sample gas can be performed.

5. Experimental procedure

A. HETP (V)

Using the example of iso-butane (N_2-carrier gas, F = 0,037 Nl/min → Nl/min=net liters/min) measurements are to be carried out with different amounts of the gas (V= 1, 2, 3 und 4 cm^3). A syringe is used to take the determined quantity of gas. For each experiment the HETP value should be calculated and the calculated HETP values should be presented as a function of **V**.

The calculation of retention times and half-widths from the chromatograms is performed using the Origin Baseline and Peaks function (See Chap. 7).

B. **HETP** (**u**): Van Deemter - Plot
The dependence of the **HETP** parameter on the linear flow rate u shall be recorded for n-butane in the N2 carrier gas. Record chromatograms of n-butane (1ml) at different flow velocities in the range 0.07 - 0.015 Nl/min. In the upper range, measurements should be taken at intervals of about 5 points (scale divisions), downwards from 0.025 Nl/min in smaller steps (3 points). Calculate **HETP**, **N** and $\mathbf{D_{eff}}$ at the optimum flow velocity **u***. By fitting the measured curve HETP(u) to the van Deemter function, parameters A and C should be determined. The parameter B cannot be determined with the setup used here, because the low flow velocities required for this cannot be set in a stable way. The optimal flow velocity **u***(cm/s) is therefore in this case the minimum achievable stable flow velocity.

C. Identification of an unknown gas mixture
At the lowest achievable stable flow rate (approx. 0.015Nl/min) the components of the unknown gas mixture are determined. For this you need chromatograms of 1 ml each of propane, iso-butane and n-butane as well as 2 ml of the gas mixture. The components of the mixture are assigned by comparing the retention times with those measured for the pure substances. The volume fractions of the respective components should be determined.
Using the example of the neighbouring peaks of iso- and n-butane of the chromatogram of the gas mixture, the resolution **R** of the separation distance is to be calculated according to formula (8)

6. Auswertung und Datenanalyse

A) Volume dependence: ***HETP (V)***
Determine from the chromatograms iso-butane **HETP** and $\mathbf{D_{eff}}$. Tragen Sie die erhaltenen Werte gegen V auf. Plot the values obtained against V. The measured dependencies HETP(V) and $D_{eff}(V)$ and the change in peak shape should be explained.
Perform a maximum error estimate for the values determined here:

$$\Delta HETP = \left|\frac{\partial HETP}{\partial L}\right| \Delta L + \left|\frac{\partial HETP}{\partial t_r}\right| \Delta t_r \; + \left|\frac{\partial HETP}{\partial W_{1/2}}\right| \Delta W_{1/2}$$

For the column length error you can assume 2cm due to possible packing errors; make your own assumptions for retention time and half-value width, which should be justified.

B) Flow rate dependence: ***HETP(u)***
Determine **HETP**, **N** and $\mathbf{D_{eff}}$. from the chromatograms of n-butane. Apply HETP against u and determine the van Deemter parameters A and C. Interpret the calculated parameters of the van Deemter equation, A and C.

C) Component determination of the gas mixture
Integrate the peaks of the individual measurements and determine the peak integral/volume ratio (Origin). From this you can calculate the composition of the mixture by comparing it with the integrals of the peaks in the mixture:

$$V_{Probe} = \frac{V_{Referenz}}{I_{Referenz}} * I_{Probe}$$

How good is the identification of the components of the unknown gas mixture achieved here (calculation of the resolution **R**)?

7. Evaluation of chromatograms with Origin 2016 and newer version

1. Import the Excel data sheet in origin *File/Import/Excel(xls)*
2. Enter the measured data in a diagram. Scale the diagram so that all measurement events are clearly visible. The injection deflection is not required for evaluation and therefore does not have to be included in the shown *y*-axis
3. Under *Analysis* select the function *Peaks and Baselinie → Peak analyzer → open Dialog*
4. Select as aim *Peaks integrieren* → next
5. Select „*user defined*“ als Basislinien-Modus → next
6. Under „Connect“ select the „*Interpolation*“ option and set the anchor points (*modify*), in a way that the baseline is completely captured but no peak is changed in its shape → next
7. Auto substract baseline → next
8. Disable *Auto Find, Add* Peaks and select all oft hem with a doublke click → next
9. For the integration choose the option „Adjust on preview diagram“ and select as integration range the whole width of the peak
10. Complete the process. The retention time is under „Center“, FWHM under „FWHM“ and the peak integral under „Area“.

What you should know:
1) Chromatography as a method for the separation of substances
2) The ideal chromatographic process
3) Characteristics of gas chromatography
4) Peak shapes in gas chromatography (Gaussian and leading)
5) The theory of separation stages
6) Gas-solid chromatography
7) van Deemter-Analysis of the HETP-values

Literatur

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