Chromatography

ver. 20191104

Chromatography

Chromathography is technique for separating components of a mixture moving in a fluid stream (called the mobile phase) driven through a contiguous stationary phase.

Practical applications:

- separation of different compounds of mixtures
- removal of impurities
- isolation of active constituents
- isolation of metabolite from biological fluids
- estimation of drugs in formulations

Classification according to

- the physical state of the mobile phase
- the polarity of the phases
- the spatial arrangement of the mobile and stationary phases
- the type of interaction

Classification

Classification according to the physical state of the mobile phase

- liquid: **liquid chromatography** (LC)
- gas: **gas chromatography** (GC)

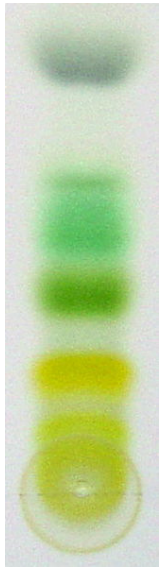
Classification according to the polarity of the mobile and stationary phase

- the stationary phase is more polar than the mobile phase: **normal-phase chromatography**
- the mobile phase is the more polar phase: **reverse-phase chromatography**

Classification

Classification according to the spatial arrangement of the mobile and stationary phases

- solid filling packed in a column: **column chromatography**
- solid coating on a flat surface : **thin layer chromatography** (TLC)
- liquid coating on the inner surface of a thin tube: **gas chromatography with capillary column**



Column chromatography and TLC

- Separation in a column
- The mobile phase is driven by gravity of pressure difference
- Analytical or preparative aims

- Separation in a closed vessel saturated with solvent vapor
- The solvent moves up and carries the components with different speed
- Analitical aim

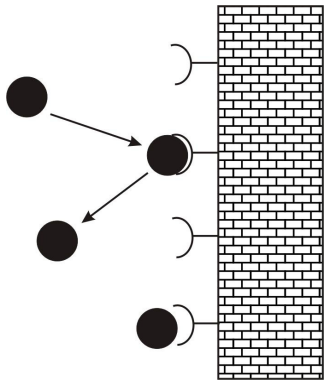
Classification

Classification according to the type of interaction:

- adsorption
- partition
- ion exchange
- affinity
- size exclusion

Adsorption chromatography

The components adsorb on the surface if the stationary phase.
The separation is based on the combination of sorption and desorption processes.



The less adsorptive component runs along the column.
The more adsorptive component binds on the upper part of the column.

The surface of the solid phase filled to the column is energetically inhomogeneous, there are more favorable, called active sites.

The adsorbed component can be washed from the active sites by elution with solvent having more adsorptivity. It can be affected by the eluent mixture composition, the temperature (for GC).

If the stationary phase is more polar than the mobile phase: **normal-phase chromatography**.
In the opposite case: **reverse phase chromatography**.

Adsorption chromatography

Typical stationary phase materials:

- active carbon
- silica based adsorbents
- cellulose based adsorbents
- polystyrene-divinylbenzene based adsorbents
- molecule-sieves (zeolites)
- activated alumina-oxide
- silica gel

Adsorption chromatography applies to only solid-liquid or solid-gas chromatography, because the adsorption phenomenon is an inherent property of solids.

E.g. column chromatography, HPLC chromatography, thin layer chromatography.

Partition chromatography

The partition process is based on the distribution of the compound between two immiscible solvent phases.

The stationary phase:

- a liquid supported on a solid
- a network of molecules, which functions virtually as a liquid, bonded on the solid support

The mobile phase: a liquid which is immiscible with the liquid film.

Partition chromatography applies to liquid-liquid, liquid-gas chromatography and not to solid-gas chromatography, because partition phenomena correspond to liquid phase.

E.g. paper chromatography, gas chromatography, high-performance thin layer chromatography (HPTLC)
(In paper chromatography, the paper is in the solid state, but the pores in between the paper contain moisture which acts as a stationary liquid phase.)

Ion exchange chromatography

The principle of this technique are the electrostatic interactions of ioninc or polar compounds with opposite charges on the stationary phase matrix.

The stationary phase:

- an immobile matrix (resin or gel) that contains charged ionizable functional groups or ligands linked with covalent bonds
- can be cation-exchange or anion-exchange

The mobile phase: a buffered water solution

The separation can be controlled by the pH and the ionic strength.

Affinity chromatography

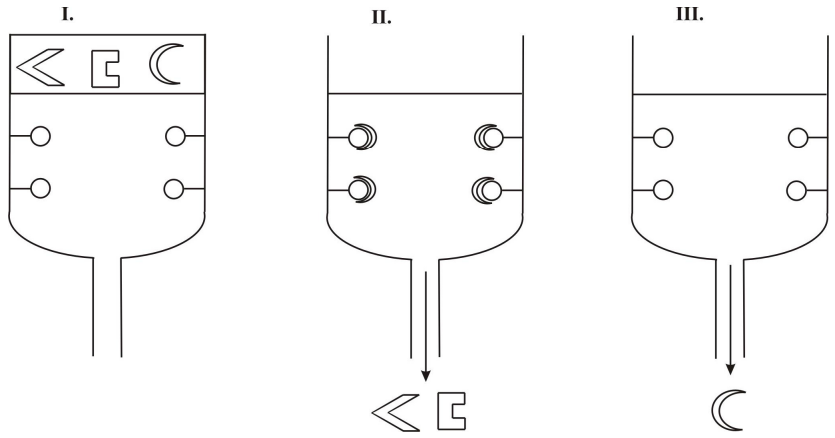
Relies on the highly specific binding between an analyte and its counterpart.

The stationary phase: the counterpart of the analyte is immobilized on the stationry phase, called affinity column.

Only the desired analyte is retained on the column, everything else passes through. The analyte is then eluted as a sharp band by some eluent that can remove the analyte from its counterpart.

Needs: appropriate counterpart and releasing agent (the analyte should remain intact during this process).

Some example pairs of analyte and counterpart:
enzyme – substrate analogue; antibody – antigen;
nucleic acid – complementary base sequence;
hormon – receptor.



Size exclusion chromatography

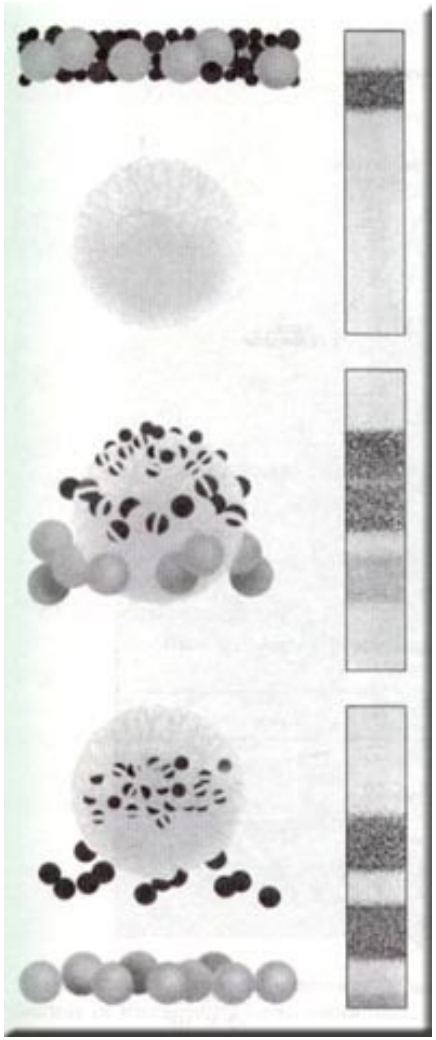
Molecules are separated according to their size by their ability to penetrate into pores.

The stationary phase: contains large number of pores.

Molecules that are larger than the largest pores cannot enter any pores and hence are “excluded” from the pores and come out in the void volume.
Molecules that are smaller than the smallest pores, in contrast, can explore the entire pore space in the stationary phase and come out last.

As a result, the molecules above a certain size (exact value depending on the pore size distribution in the stationary phase) coming out first, and molecules below a certain size coming out together at the end.

Molecules of intermediate size are separated in the retention window.



Detectors

The chromatograph performs the separation, but somehow we have to know what and when comes out from the device: detectors.

Non-specific detectors: we can see if anything comes out.
Specific detectors: we can see if something comes out.

Or

Destuctive detectors: the effluent is destructed while analysed.
Non-destuctive detectors: the effluent is only analysed.

Detectors

Destructive detectors:

- Charged aerosol detector (CAD)
- Evaporative light scattering detector (ELSD)
- Flame ionization detector (FID)
- Flame photometric detector (FPD)
- Nitrogen Phosphorus Detector (NPD)
- Atomic-emission detector (AED)
- Mass spectrometer (MS)

Non-destructive detectors:

- UV detectors (fixed or variable wavelength)
- Fluorescence detector
- Refractive index detector (RI or RID)
- Radio flow detector.
- Chiral detector
- Conductivity monitor
- Thermal conductivity detector (TCD)
- Electron capture detector (ECD)
- Photoionization detector (PID)

One example: the thermal conductivity detector

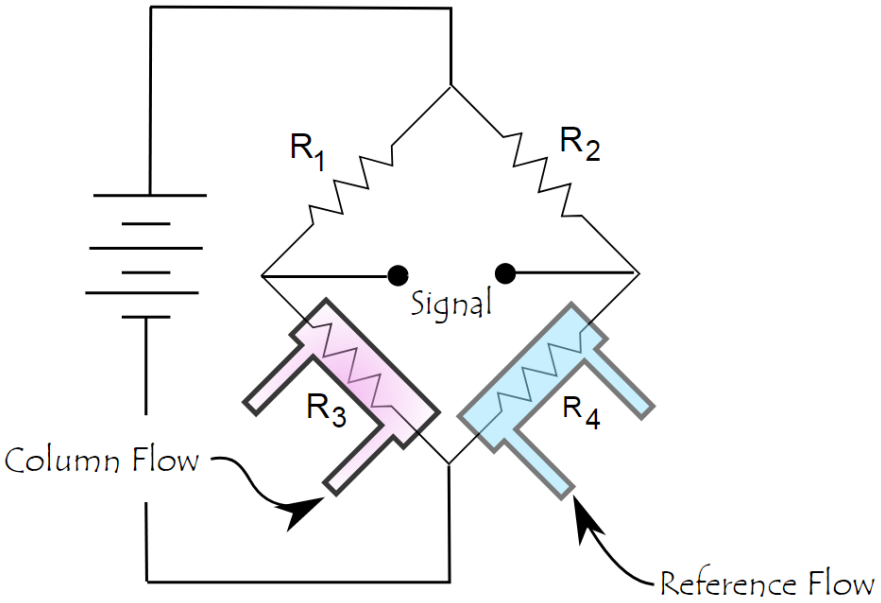
Used in GC, also known as katharometer.

Mesures the changes of the thermal conductivity of the effluent compared to the reference gas flow.

Non-specific and non-destructive detector.

The conductivity measurement is based on the resistance change of a heated wire surrounded by the effluent gas.

Technical solution for the sensitive measurement of resistance change: the Wheatstone bridge.



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Gas chromatography (GC)

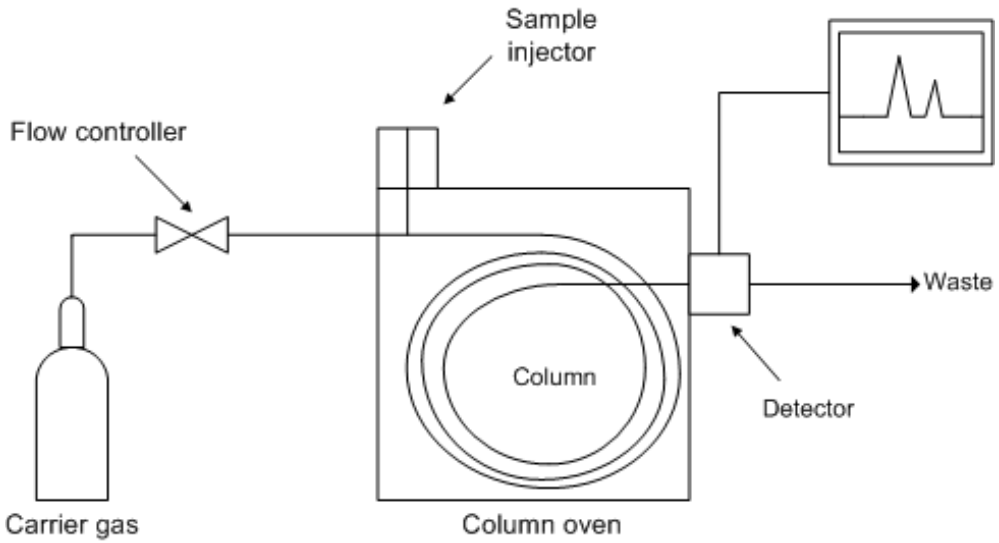
The mobile phase is gas.
The stationary phase can be solid (GSC) or liquid (GLC).

GLC is to a great extent more widely used than GSC, therefore we focus on GLC.

The separation is based on the partition of the component between the mobile and stationary phase:

$$K_c = c_{\text{stationary}} / c_{\text{mobile}}$$

K_c (the distribution constant) depends on
- temperature
- the chemical nature of the stationary phase

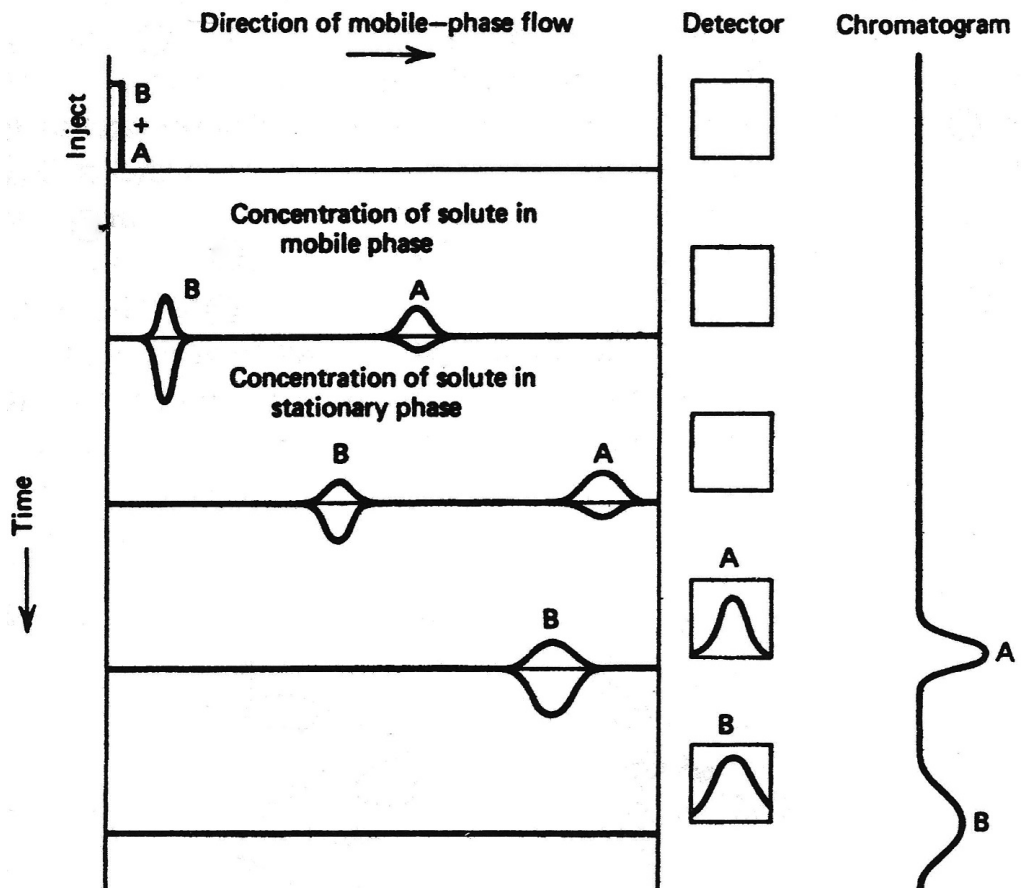


A GC instrument. *wikipedia*

GC separation process

Schematic representation of the chromatographic process.

Adapted from Harold M. McNair, James M. Miller, *Basic Gas Chromatography*, John Wiley & Sons, New York,1998.



Efficiency of the GC separation

The carrier gas is inert and does not interact with the sample, and thus GC separation's selectivity can be attributed to the stationary phase alone.

The separation efficiency is described by the Height Equivalent to a Theorethical Plate (HETP). The lower the HETP value is the narrower the GC peaks are and the separation of the components is better.

HETP can be estimated by the **Van Deemter equation**:

$$\mathrm{HETP} = \mathrm{A} + \frac{\mathrm{B}}{u} + (\mathrm{C_s} + \mathrm{C_m})u$$

where A, B, C_s and C_m empirical constants, u is the flow rate.

Jan Jozef van deemter
Born: 31 March 1918
Died: 10 October 2004
German physicist and engineer

Efficiency of the GC separation

$$\mathrm{HETP} = \mathrm{A} + \frac{\mathrm{B}}{u} + (\mathrm{C_s} + \mathrm{C_m})u$$

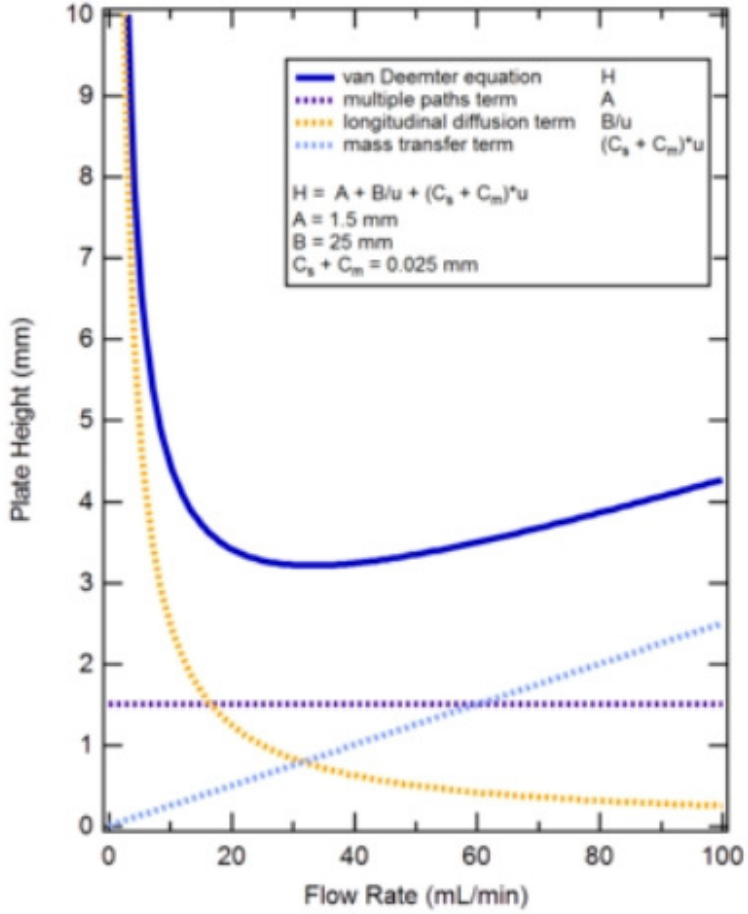
where A, B, C_s and C_m empirical constants, *u* is the flow rate.

The efficiency is determined by three factors:

A: due to the **possible multiple length pathways in packed columns** (in capillary columns it is zero)

B: due to the **longitudional diffusion**

C: due to the **mass transfer in the stationary and the mobile phases**

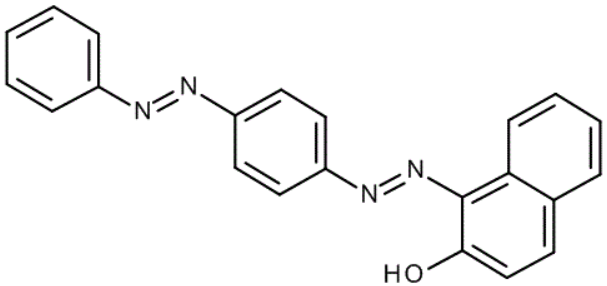


wikipedia

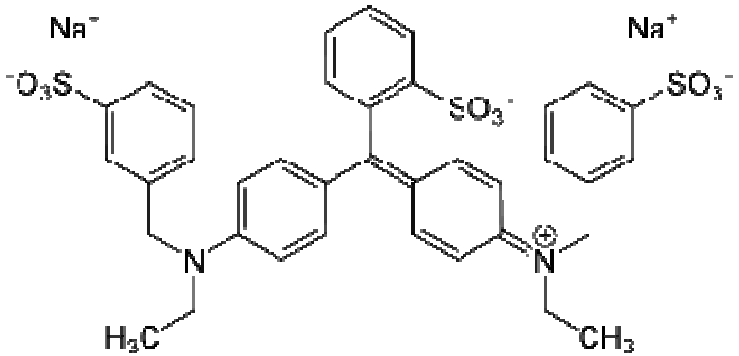
Adsorption column chromatography
case study

Experimental task:

separation of a mixture of a red (Sudan III) and blue dye (Erioglaucine)



Sudan III – nonpolar molecule

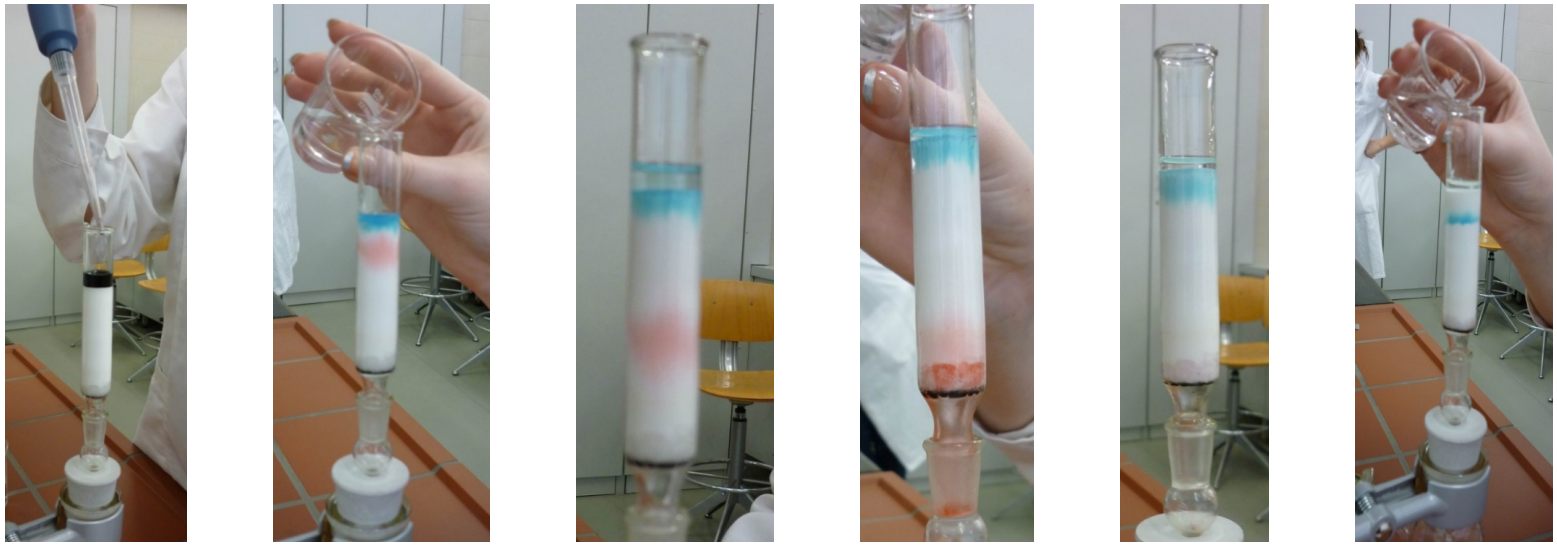


Erioglaucine – polar molecule

Due to the large difference in polarity we can use normal-phase adsorption column chromathography.

Adsorption column chromatography
case study

The stationary phase: Al_2O_3
The mobile phase: 96% and 30% ethanol solutions



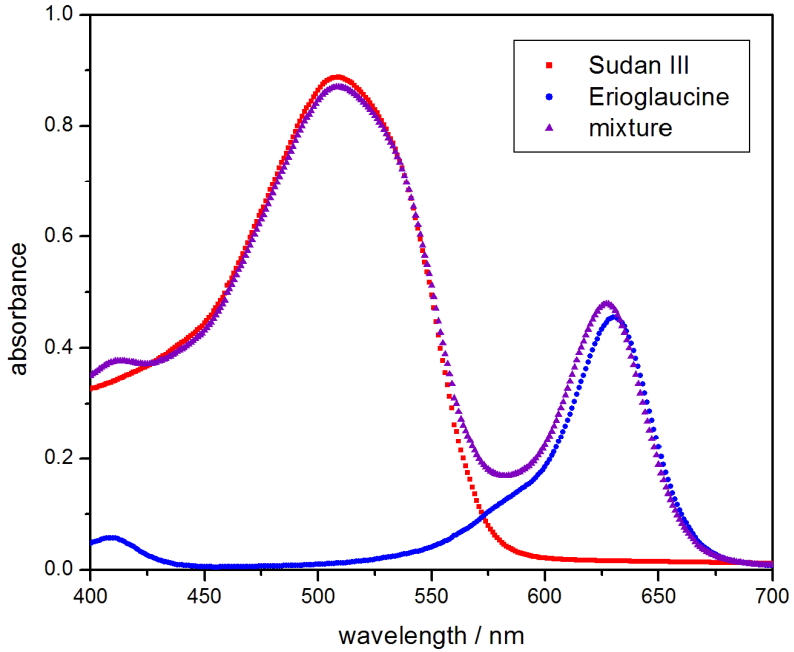
Steps of the separation. a. Sample injection, b-d. Washing of Sudan III with 95% ethanol, e. Only Erioglaucine remained on the column, f. Washing of Erioglaucine with 30% ethanol.

Adsorption column chromatography
case study

Reasoning of the separation order and eluent usage: the polarity order and the adsorptivity of the components on alumina.

The adsorption strengths of the components on Al_2O_3 are the following:
water(solvent component) > Erioglaucine (dye component) > ethanol (solvent component) > > Sudan III (dye component).

Detection of the dyes:
visible absorption spectroscopy.



Visible spectra of the separated dyes and the diluted mixture.