

## Column Chromatography (exp. 07.)

### Important concepts

Heterogeneous phase equilibrium, adsorption chromatography, stationary phase and mobile phase, eluents, Beer Lambert's law. Absorption photometry in visible range, absorbance vs. concentration calibrating curve.

### Objective:

In this experiment you will use column chromatography for the separation of two dyes (Sudan III and Erioglauricine) from their common solution. After separation you will determine the dye concentrations by measuring the absorbance of the solutions at appropriate wavelengths.

### Background:

All the various types of chromatography are based on the fact that different components of a multicomponent gas or liquid system are differently distributed between a phase attached to a porous solid carrier and a fluid phase (gas or liquid) flowing through that solid.

According to the underlying physico-chemical principle we can distinguish between *adsorption*, *partition*, and *ion-exchange* chromatography.

According to the physical state of the fluid phase (mobile phase) we have liquid chromatography (LC) and gas chromatography (GC).

The method that is used here is a simpler version of liquid chromatography and the underlying physico-chemical principle is adsorption.

In the present experiment you will make use of the fact that adsorption of the following substances is different on  $\text{Al}_2\text{O}_3$ :

water(solvent component) > Erioglauricine (dye component) > ethanol (solvent component) > Sudan III (dye component).

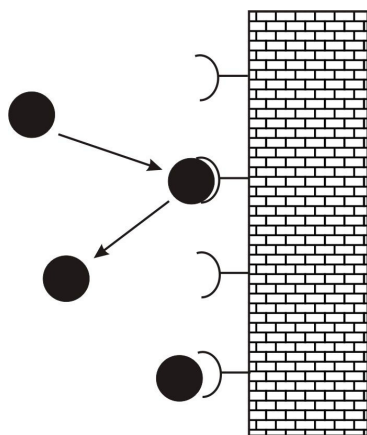


Figure 1. Dye molecules are bounded to active sites of solid (alumina) at the interface.

## Apparatus

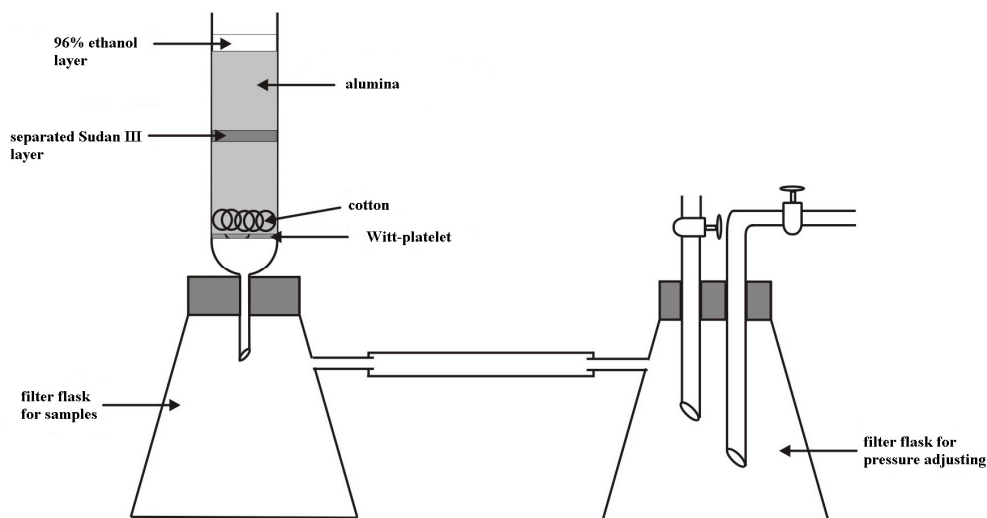


Figure 2. Chromatographic apparatus

## Procedure

1. Assemble the apparatus shown in Fig. 2. Turn on the water aspirator. Vacuum is controlled by the stopcock on the top of the second filter flask (buffer flask). Replace the Witt platelet and put an even layer of 2-3 mm of cotton over it.

2. Measure in a 25 ml beaker 15 ml of alumina and 11 ml 96% ethanol, and mix them well with a glass rod. Wet the cotton layer with 96% ethanol and press against the bottom of the tube with a glass rod. Fill the whole amount of suspension into the tube. Fill a  $\approx 1$  cm thick layer of 96% ethanol over the column. The column is ready for use.

Make sure that there always remains some liquid (this time 96% ethanol) over the settled  $\text{Al}_2\text{O}_3$  column otherwise it will dry out or crack (even if you can not see that happen) and you will have to prepare a new one.

## Separating the dyes.

3. Pipette **1 ml** from your assigned unknown solution over the column. Start suction.

Handling of *vacuum apparatus*.

3a. The vacuum control stopcock and the vacuum inlet on the filter flask are opened.

3b. Open the tap of water aspirator completely, and close gently the vacuum control stopcock.

3c. Having finished the suction open the vacuum control stopcock first, and **after that** close the tap of water aspirator.

4. Stop suction when the red ring originating from Sudan III left the column. Disassemble the apparatus. Wash the content of filter flask into a 25 ml volumetric flask and fill it up to ring mark with 96% ethanol.

5. Fit the column again onto the cleaned and dried filter flask and continue experiment by feeding the column with 30% ethanol. Start suction. Stop suction when the blue ring originating from Erioglaucline left the column. Disassemble the apparatus. Wash the content of filter flask into a 25 ml volumetric flask and fill it up to ring mark with 30% ethanol.

Be careful in filling a proper amount of solvent. Use as maximum volume only 15 ml for the separation process and 7 – 10 ml for washing the content of filter flask.

It is probable, that some alumina will get into the filter flask together with the blue dye. The opalescent solution should be filter before photometry.

### Photometry

The spectrum of SUDAN III. and Erioglaurine, i.e. the absorbance vs. wavelength function are taken on Metertech 880 single beam spectrophotometer.

For the sake of the *highest signal to noise ratio* in detecting absorbances we determine the wavelength belonging to the highest absorbance,  $\lambda_{\max}$  for the separated dyes.

Component dye Sudan III

**Sample solution:** Sudan III solution (in a 25.0 ml volumetric flask, solvent: 96% ethanol).

**Reference solution:** 96% ethanol.

Component dye Erioglaurine

**Sample solution:** Erioglaurine solution (in a 25.0 ml volumetric flask, solvent: 30% ethanol)

**Reference solution:** 30% ethanol.

In a single beam instrument like Metertech 880 the lowest measuring error is reached when we fill both the reference and the sample solution in the same cuvette.

### Scanning spectrum

1. Choose 'Spectrum' from 'Function' of menu bar and a screen for function Spectrum will show up. A dialog box of 'Setup Spectrum' also shows up. Press 'OK' after setting to execute the program according to new parameters.

Set the scan parameters as in the Table below:

Table 1a. for scanning parameters for Sudan III

Parameters	values
Start wavelength	500 nm
Stop wavelength	560 nm
Measure mode	ABS
Low value (ABS)	0
High value (ABS)	3.000
scan speed	medium ( 600 nm/s)

Table 1b. for scanning parameters for Erioglaurine

Parameters	values
Start wavelength	600 nm
Stop wavelength	650 nm
Measure mode	ABS
Low value (ABS)	0
High value (ABS)	3.000
scan speed	medium ( 600 nm/s)

1. Fill some 96% ethanol in a 1 cm **glass** cuvette, put a Teflon lid on and place the cuvette in the instrument's cuvette holder. On activating knob **0A** instrument will scan the  $I_0$  values for reference solution, i.e. zeroing the instrument in the wavelength range in the table.
2. Fill some Sudan III solution into the cuvette formerly used. Put a Teflon lid on the cuvette and place the cuvette in the instrument's cuvette holder. On activating knob **Run** instrument run the spectrum of the solution. The maximum wavelength of the absorbance band (centre of absorption band) is determined first. Read *seven absorbance, wavelength data* by nanometers symmetrically distributed at the centre of absorption band.
3. Fill some 30% ethanol in a 1 cm glass cuvette, put a Teflon lid on and place the cuvette in the instrument's cuvette holder. On activating knob **0A** instrument will zero the instrument.
4. Fill some Erioglaurine solution into the cuvette formerly used. Put a Teflon lid on the cuvette and place the cuvette in the instrument's cuvette holder. On activating knob **Run** instrument run the spectrum of the solution. The maximum wavelength of the absorbance band (centre of absorption band) is determined first. Read the *seven closest absorbance, wavelength data pair* symmetrically distributed at the centre of absorption band, by nm precision.

### Evaluation

In Table 2a. and 2b. calibrating functions of seven wavelengths are given in the form

$$A(\lambda) = a + b \cdot c_x \quad 1.$$

where  $c_x$  is the concentration of your diluted unknown,  $A(\lambda)$  is the measured absorbance of your unknown.

Table 2a. calibrating functions of Sudan III

Absorbance at given wavelength	parameter $a$ in Eq. 1.	parameter $b$ in Eq. 1.
A(514 nm)	-0.00755	801.475
A(515 nm)	-0.00740	802,700
A(516 nm)	-0.00834	804.700
A(517 nm)	-0,00767	805.025
A(518 nm)	-0.00729	804,657
A(519 nm)	-0.00740	804.600
A(520 nm)	-0.00817	804.650

Table 2a. calibrating functions of Erioglaurine

Absorbance at given wavelength	parameter $a$ in Eq. 1.	parameter $b$ in Eq. 1.
A(627 nm)	0.02268	1030.025
A(628 nm)	0.02252	1039.375
A(629 nm)	0.02412	1043.175
A(630 nm)	0,02416	1044.200
A(631 nm)	0.04095	1027.875
A(632 nm)	0.02321	1033.250
A(633 nm)	0.02275	1024.725

By using the equations you can calculate the dye concentration of separated solution. Finally, take the average of seven concentration data for both of the dyes.

Take care for the concentration calculation, you are asked to give the concentration of your original unknown (not separated) solution from which you filled 1 cm<sup>3</sup> onto the top of the column.

**Graphs, results, data to be reported:**

14 absorbance wavelength data pair.

The average of concentration for Sudan III and Erioglauicine.

**Minimum level tests (MLT).**

**Column chromatography (Exp. 7.)**

Heterogeneous equilibrium, adsorption chromatography, stationary phase and mobile phase, eluents. Beer Lambert's law. Absorption photometry in visible range, absorbance vs concentration calibrating curve.

**MLT Q.and A**

Q1. What is the difference between **adsorption** and **absorption**?

A1. Adsorption process takes place on the surface, and absorption in the bulk.

Q2. What is the objective (purpose) of the column chromatography experiment?

A2. To carry out the separation of four component dye mixture with a well-known separation technique, and determine the separated components quantitatively by photometry.

Q3. What are the stationary phase and mobile phase used in this experiment?

A3. The stationary phase: Al<sub>2</sub>O<sub>3</sub>, and mobile phase: water – ethanol mixture.

Q4. What eluent mixtures are applied?

A4. Eluent mixtures are: 96% ethanol comes first, 30% ethanol is the next.

Q5. What are the components of slurry (the column material), and how do you prepare that?

A5. A slurry is prepared by mixing the first eluent, 96% ethanol with the powder of stationary phase and then carefully poured into the column. Care must be taken to avoid air bubbles. The solution of the dye mixture is pipetted on top of the stationary phase.

Q6. What is the strongest *adsorbate* of the four components?

A6. The surface of Al<sub>2</sub>O<sub>3</sub> is hydrophilic and water adsorbs on it as a strongest adsorbate.

Q7. What adsorption order develops on alumina in the four component liquid system?

A7. H<sub>2</sub>O > Erioglauicine > Ethanol > Sudan III. The most polar molecule water adsorbs to polar alumina the strongest way.

Q8. Describe the separation process of dye mixture.

A8. At first eluent 96% ethanol extracts Sudan III. Erioglauicine and water are strongly adsorbed to alumina and they remain on the top of the column. Sudan III, the red component on active sites is replaced by ethanol along the length of the column.

In the second stage eluent 30% ethanol washes Erioglauicine from column, because of its increased water content. Water is capable to desorb blue component Erioglauicin.

Q9. Describe the concentration determination process of separated dye components.

A9. The separated dye components are diluted to known volume and their absorbances are measured by photometer. From absorbance vs dye concentration straight line the measured absorbance of separated dyes are read.

Q10. Light intensity – concentration function: the Beer Lambert's law.

A10. At a single wavelength mostly at  $\lambda_{\max}$  of a spectral band the Beer Lambert's law can be written as

$$A(\lambda) = \log \frac{I_0}{I} = \epsilon(\lambda) \cdot c \cdot l$$

where  $A(\lambda)$  is the absorbance,  $\epsilon(\lambda)$  is the molar absorbance,  $c$  is the concentration of absorbing species and  $l$  is the optical pathlength of the solution.  $\epsilon(\lambda)$  is a material coefficient which depends on the wavelength.  $I_0$  and  $I$  are the incoming and outgoing intensity of radiation.