

LABORATORY WORK NO. 7

THIN LAYER CHROMATOGRAPHY (TLC)

- **PRINCIPLE:** Thin Layer Chromatography (TLC) is a quick analytic method. It deals with division of single substances between movable phase and stationary phase on thin layer.

Chromatographic methods are divided according to the type of stationary and movable phase.

Division according to stationary phase :

- column chromatography – stationary phase is placed into column
- paper chromatography – paper or modified cellulose form stationary phase
- **Thin Layer Chromatography (TLC)** – stationary phase is in the form of thin layer (e.g. on Al plate)

Division according to movable phase:

- gas chromatography
- liquid chromatography
- fluidized chromatography
- plasma chromatography – flow of ions forms movable phase

An aluminium plate covered with silica gel or carbon dioxide is the most often **stationary phase**.

A mixture of organic solvents in different ratio can form **mobile phase** – so-called developer system. Different suitable systems can be used according to the nature of tested substances. Here are some examples:

pure ethanol
chloroform:ethanol 9:1
chloroform:acetone 85:15

Sample is laid on (in the form of a drop) to the beginning of the plate and it is let develop itself in movable phase that drifts the sample as coloured stains towards so-called forefront (that means to the point of capillary rise of movable phase).

A non-dimensional value called **retention factor R_f** is determined for individual substances.

This can be calculated in this way: $R_f = \frac{a}{b}$

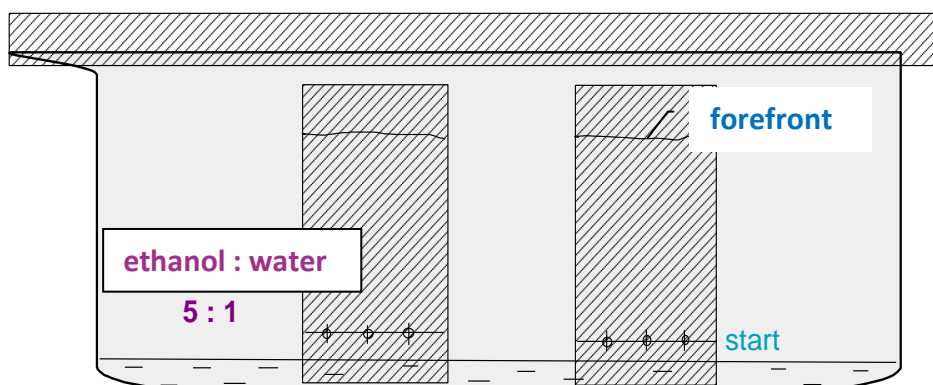
where a is the distance from the centre of the stain to start position
 b – distance of the forefront from the start position

TASK NO.1. CARRY OUT DIVISION OF ORGANIC COLORANT AND CHLOROPHYLL BY MEANS OF TLC METHOD

- **AIDS:** chromatographic paper, chromatographic developer apparatus for TLC, glass rod, mortar with pestle, filtration paper
- **CHEMICALS:** source of chlorophyll (green leaves), ethanol, distilled water, sand
- **PROCEDURE:**
 1. Prepare filtration paper 5 cm x 10-12 cm and draw starting line 1 cm bellow the bottom edge .
 2. Spread a sample of green leaves together with sand in the mortar untill juice creates.
 3. Spread repeatedly the sample by the rod on the surface of the plate – 3 samples side by side.
 4. Immerse the plate into the beaker with movable phase – mixture of ethanol and water in 5:1 ratio and let them develop. The sample must not be immersed below water level.
 5. As soon as water level reaches 1 cm bellow the top bottom, stop developing. Let the plate dry up on the air.
 6. Draw in the centres of the single stains. Determinate the retential factor R_f for each stain.

■ Apparatus:

Developer chromatographic bath





TASK NO. 2. DIVISION OF INORGANIC COLORANTS

- **AIDS:** chromatographic developer apparatus, beaker, petri dish, paper, chalk, wick, round filtration paper

- **CHEMICALS:** coloured markers, food colorants , ethanol, distilled water

- **PROCEDURE:**

Repeat the procedure from task No. 1 for the samples of 3 inorganic colorants (colorant solutions, coloured marker). Water forms the movable phase, filtration paper forms the stationary phase.

Carry out division of marker colours on stationary phase with chalk, water is the developer bath.

- **CONCLUSION:** (common for both tasks or respective to each one)

We carried out division of both inorganic and organic colorants by the means of TLC method TLC. As a sample we usedAs a developer bath we used.....

Retention factor for given sample is

STUDENT'S SHEET NO.7

TLC

1. Vocabulary:

Match the Czech words with their English equivalents:

1. Thin Layer Chromatography	A potravinařské barvivo	1....
2. gas chromatography	B sloupcová chromatografie	2....
3. stationary phase	C chromatografická vyvíjecí aparatura	3....
4. retention factor	D petriho miska	4....
5. chromatographic developer apparatus	E kádinka	5....
6. mortar with pestle	F plynová chromatografie	6....
7. petri dish	G barevné fixy	7....
8. beaker	H třecí miska s tloučkem	8....
9. coloured markers	I retenční faktor	9....
10. food colorant	J chromatografie na tenké vrstvě	10...
11. column chromatography	K stacionární fáze	11...

2. Choose correct answer from two possibilities:

- TLC is a quick **synthetic** / **analytic** method
- Mobile** / **stationary** phase is a mixture of organic solvents in different ratio.
- Column chromatography is a chromatographic method of dividing according to **stationary** / **mobile** phase.
- Gas chromatography is a chromatographic method according to **stationary** / **mobile** phase.
- Retention factor can be calculated: $R_f = \frac{a}{b}$ where **a** is distance **from the centre of the stain** to the start position / distance **of the forefront** from the start position.

3. Complete correct expression

Name the type of chromatography where stationary phase is placed into column.

.....

Name the type of chromatography where stationary phase is in the form of thin layer.

.....

Name the type of chromatography where flow of ions forms movable phase.

.....

Name the non-dimensional value which is important to determine individual substance.

.....



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

What is the name of the apparatus which is necessary to be used for dividing colorants by TLC Metod?

4. Choose the correct spelling of the word:

- a) distilled water
- b) distillet water
- c) distilled water
- d) distiled water

- a) column chromatography
- b) column chromatografy
- c) colony chromatography
- d) column chromatography

- a) retential factor
- b) retencial factor
- d) retencial faktor
- e) retention factor

- a) feed colorants
- b) food colourands
- c) food colours
- d) food colorants