Chromatographic separation processes: thin-layer chromatography (Item No.: P3120400)

Curricular Relevance

Area of Expertise: Chemistry
Education Level: University
Topic: Analytical Chemistry
Subtopic: Chromatography
Experiment: Chromatographic separation processes: thin-layer chromatography

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Easy	10 Minutes	10 Minutes	2 Students

Additional Requirements:

- pencil
- ruler

Experiment Variations:

Keywords:

thin-layer chromatography, separation procedure, adsorbent material, stationary phase, mobile phase, capillary action

Overview

Short description

Principle

Chromatographic separation processes are very important for analytical chemistry. Their relatively simply technique and the possibility to separate even the smallest portions of mixtures explain the rapid development of these processes. There are numerous variations of this method. As a result, the optimum chromatographic separation method can be found for nearly every separation task. The method that is described here can be used to demonstrate the fundamental principles and possibilities of this method with relatively simple means.

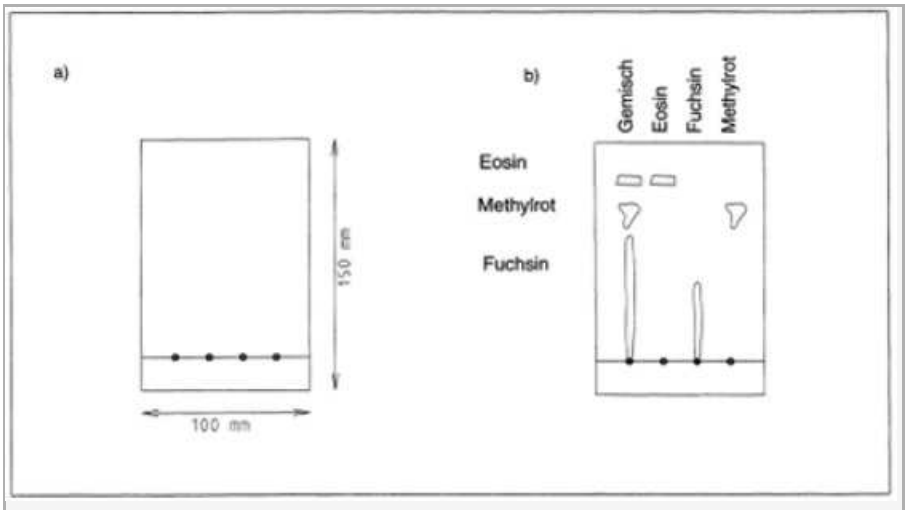

Fig. 1.

Safety instructions

Ethyl alcohol is a highly flammable liquid that can be mixed with water. In combination with air, its vapours may form explosive mixtures.
First aid: Wash the affected skin areas with water and soap. Let splashes to the eyes evaporate with the lid gap wide open (blow carefully into the eyes). Then, rinse the eyes with water.
If inhaled: Fresh air.
Disposal: Collect flammable, halogen-free, organic solvents and solutions in a collecting vessel that is marked accordingly.

Ethyl alcohol
H225: Highly flammable liquid and vapour,.
H319: Causes serious eye irritation.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

Eosin
H319: Causes serious eye irritation.
P260: Do not breathe dust/fumes/gas/mist/vapours/spray.

Fuchsine
H350: May cause cancer.
P201: Obtain special instructions before use.
P260: Do not breathe dust/fumes/gas/mist/vapours/spray.

Equipment

Position No.	Material	Order No.	Quantity
1	Separation chamber, 180x120x50 mm	35010-06	1
2	Capillary holder	35010-07	1
3	Micro-capillaries, 2 / 1000 ml, 100	35010-08	1
4	Watch glass, dia.80mm	34572-00	4
5	Graduated cylinder 100 ml	36629-00	1
6	Pasteur pipettes, 250 pcs	36590-00	1
7	Rubber caps, 10 pcs	39275-03	1
8	Test tube, 160 x 16 mm, 100 pcs	37656-10	1
9	Test tube rack for 12 tubes, holes d= 22 mm, wood	37686-10	1
10	Spoon, special steel	33398-00	1
11	Scissors, straight,180 mm	64798-00	1
12	TLC-foil, silica gel F254, 25 off	31503-04	1
13	Ethyl alcohol, absolute 500 ml	30008-50	1
14	Eosin for microscopy 25 g	31296-04	1
15	Fuchsine powder 25 g	31320-04	1
16	Methyl red 25 g	31574-04	1
17	Water, distilled 5 l	31246-81	1

Tasks

Seperate a dye mixture by thin-layer chromatography.

Setup and procedure

Procedure

Separation of a dye mixture by thin-layer chromatography

Solutions to be prepared:

Fill 40 ml of a mixture of 4 parts by volume of ethyl alcohol and 1 part by volume of water as the eluent into the separation chamber. This liquid mixture should cover the bottom of the chamber approximately 10 mm high. Seal the chamber immediately with the supplied ground cover so that the mixing ratio does not change. Prepare the following red dye solutions in test tubes : Dissolve a small amount of eosin (covering the tip of a spatula) in approximately 4 ml of water. Dissolve a small amount of fuchsine powder (covering the tip of a spatula) also in approximately 4 ml of water. Dissolve a small amount of methyl red (covering the tip of a spatula) in approximately 4 ml of ethyl alcohol.

Fill approximately 1 ml of these three solutions together into a fourth test tube in order to obtain a mixture. Preparation of the foil: Use a sharp pair of scissors to cut a large TLC foil (the silica gel F254 foil has the dimensions 200 mm x 200 mm) to size so that it fits into the chamber. It should have the dimensions 100 x 150 mm. Ensure that the foil is slimmer than the internal width of the chamber because it must not touch the walls of the chamber during the experiment. When cutting the foil, ensure that the silica gel coating does not come off. This can happen if the foil is folded. It is recommended to scrape the coating off by approximately 1 mm on both lateral edges (knife). This measure prevents the lateral diversion of the liquid stream due to capillary effects when the foil touches the wall of the chamber. Use a soft pencil to draw a "starting line" on the silica gel coating on the cut foil approximately 20 mm away from the lower end. Apply the dye solutions to the starting line as follows: Fill some drops of the solutions into individual watch glasses. Use the capillary holder to grab a micro-capillary by pressing the holding springs lightly onto one of the capillaries. Hold one end of this capillary against the dye solution on the watch glass. The capillary will be filled immediately with the dye solution. Then, hold one end of it against the starting line on the foil. While doing so, a small amount of the solution flows out, thereby forming the starting point. Transfer the other solutions to the starting line in the same manner, but use a fresh capillary tube for each of them. Ensure that the starting points are approximately 20 mm apart. After the starting points of the dyes have dried, place the foil in a slightly tilted position into the separation chamber as shown in Fig. 1A and seal the chamber immediately with the cover.

Theory and evaluation

Observation

The liquid inside the chamber, i.e. the eluent, ascends slowly in the silica gel. The dyes ascend more or less quickly together with the eluent. Of the three dyes used here, eosin ascends the quickest. Methyl red is slightly slower and fuchsine is the slowest. Based on these different speeds of ascension, the three colours can be clearly distinguished in a separate manner on the path of the mixtures. Eosin is on top of the path with methyl red below. This is followed by fuchsine at the lowest position. After a period of 1 to 2 hours during which the eluent has ascended by approximately 10 to 12 cm, take the foil out of the chamber and let it dry in the air. The result is a thin-layer chromatogram as is shown in Fig. 1b.

Result

Dissolved substances migrate at different speeds in certain porous materials, such as silica gel, cellulose, polyamides, and others. Due to this different migration speeds, they can be separated from mixtures, taken up individually, and analysed further if desired.

Notes

Chromatographic separation processes are very important for analytical chemistry. Their relatively simply technique and the possibility to separate even the smallest portions of mixtures explain the rapid development of these processes. There are numerous variations of this method. As a result, the optimum chromatographic separation method can be found for nearly every

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