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**  
Easy

**Preparation Time**  
10 Minutes

**Execution Time**  
10 Minutes

**Recommended Group Size**  
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.](image)

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**

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

**Tasks**

Separate 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 fuchsin 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 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 fuchsin 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 fuchsinics 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 simple 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 optimal chromatographic separation method can be found for nearly every