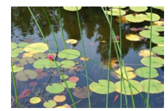
Chromatography

Separation of mixtures of substances in solutions based on different migration speeds

Example applications

- **Research:** "Are there impurities in the medication?"
- **Food analysis:** "Is there alcohol in non-alcoholic beer?"
- **Environmental analysis:** "Is this body of water contaminated with chemicals?"
- **Forensics:** "What kind of felt-tip pen was used to write this note?"
- **Sports:** "Did this athlete take a performance-enhancing drug?"



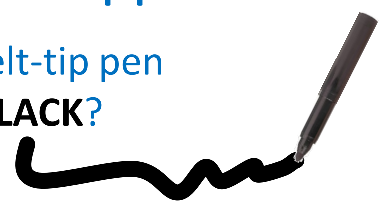



? How can substances that are present in a common solution be separated?



? What colors are in a felt-tip pen?

Is a black felt-tip pen really **BLACK**?



Experiment:
Paper chromatography of felt-tip pens

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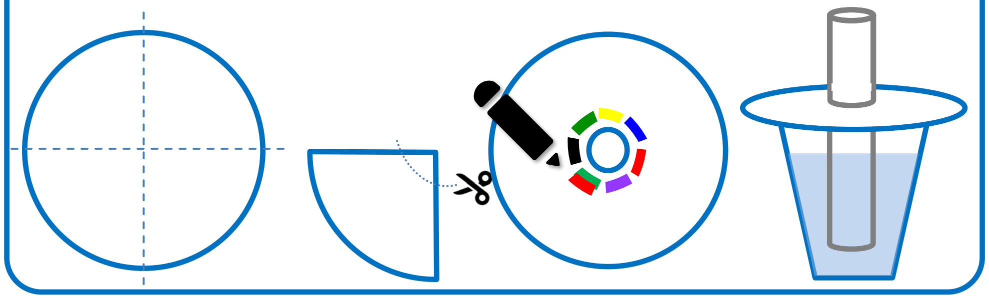
Materials & setup

4 filter papers, 2 plastic cups, felt-tip pens, scissors, water, baby oil, funnel



Observations

What are felt-tip pen colors composed of?

Do the individual felt-tip pen colors consist of only one color each? ____________________________

Which colors can you see on the filter paper? ____________________________

Which color moves the slowest? ____________________________
Which moves the fastest? ______________________

What colors make up the black felt-tip pen color? ____________________________

Which solvent (liquid) works best for this experiment? ____________________________

Instructions

- Fold the filter paper twice in the middle to form a quarter circle. Then cut off the tip (3-4 mm) and unfold the circle. You will now have a small circle in the middle.
- Choose different felt-tip pens and draw lines around the hole. If you like, you can also mix colors.
- Roll up the other piece of filter paper and insert it through the hole like a straw.
- Fill the cup ¾ full with water and place the filter paper construction over it so that the roll is immersed about 2 cm into the liquid.
- Wait until the liquid has been sucked up (almost) to the edge of the painted filter paper.
- Remove the filter papers from the liquid and take them apart again. Allow the filter paper to dry for best results.
- Repeat the experiment using oil as the liquid. The hole in the filter paper should be as small as possible, otherwise it will take a very long time.

Conclusions

The dyes in the pens are highly soluble in _____________ but poorly soluble in _________. This means they can be separated chromatographically using _____________ as the mobile phase. The colors consist of different basic dyes, which are found in printer cartridges, for example. They are called ___________, ___________, and __________. After chromatography, the individual dyes can be recognized separately again. Think about why this is.

Experiment:
Chalk chromatography with M&Ms

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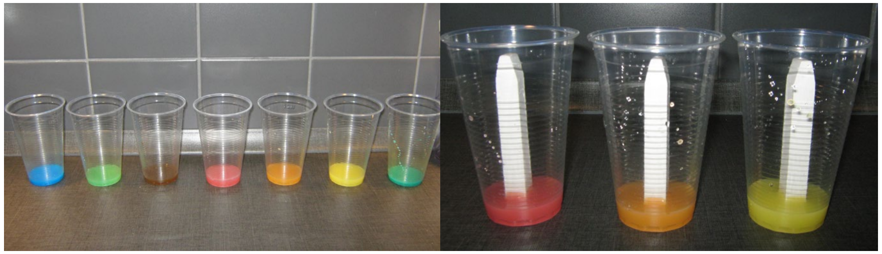
Materials & setup

4 plastic cups, 3 M&Ms of each of 4 colors, 4 pieces of chalk, 1 teaspoon, water



Instructions

- Put a few M&Ms in the cups, always keeping the same colors together.
- Add a little water (1 cm high) and stir until the dye has dissolved from the M&Ms
- If possible, remove the M&Ms from the water
- You have now produced extracts that contain all the water-soluble components from the colorful surface
- Place a piece of white chalk in each cup so that the liquid rises up inside it
- When the liquid has risen almost to the top of the chalk, you can remove it
- Compare the different pieces of chalk with each other.
- Mix two different M&Ms (e.g., yellow and blue) and repeat the experiment

Observations

Which colors were contained in the mixture?

Do the individual M&M colors consist of only one color each? ______________________________

Which colors can you find in the chalk with the mixture? ______________________________

Which dye rises to the top the most? ______________________________

Are the dyes the same as those in felt-tip pens? ______________________________

What happens when you mix two colors? ______________________________

Conclusions

The colorful coatings of M&Ms are highly soluble in ____________, which makes them easy to extract. They can then be separated chromatographically, e.g., in a piece of ______ or with a strip of cardboard. If you separate a mixed color, you can see the individual dyes on top of each other as ___________. Then you know which dyes are contained in the mixture. Felt-tip pens and M&Ms have __________________ basic dyes.

Experiment:
Chromatography of chlorophyll

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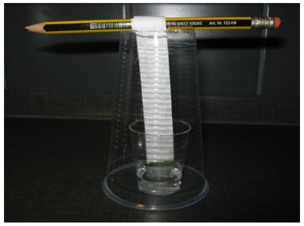
Chlorophyll – pigments in plants

Plants need their green pigments not only to look pretty; chlorophyll plays a crucial role in photosynthesis. In this process, plants convert water and carbon dioxide into oxygen and sugar. The two chlorophylls a and b help to bind the carbon dioxide in the cell. There are also other pigments in the leaf. Not all leaves contain the same amount of the different pigments. When the leaves turn yellow and red in autumn, they contain less and less chlorophyll (green) and eventually only carotene (orange/yellow) and xanthophylls (red/orange/yellow).

Materials & setup

Leaves, coin, pen, scissors, coffee filter paper, beaker, mini beaker



Instructions

- Collect a few leaves (preferably green and "juicy").
- Cut the filter paper into long strips.
- Place the filter strip on a leaf and roll about 1.5 cm from the end of the strip with a coin so that a green stripe is created. Repeat this in several places on the leaf until the strip is thickly coated.
- Cut the bottom off the plastic cup and turn it upside down.
- Fill the small cup with isopropanol (about 1 cm high), label it, and place it in the large cup.
- Attach the filter strip to the top of a pen. When the pen is placed across the cut cup, the filter strip should be immersed in the isopropanol, but the green strip should remain above it (see illustration).
- Observe how the liquid rises and only remove the strip when only clear liquid and no more dye is rising up the filter paper.

Observations

	With water	With isopropanol
How many different stripes do you see?	____________________	____________________
What colors are there?	____________________	____________________ ____________________
Which color is most prevalent?	____________________	____________________

Conclusions

The green plant components are soluble in ___________, but not in _________.
Therefore, the pigments can only be separated using __________ as a solvent.
The extract does not only contain chlorophyll, but
__ types of chlorophyll and several other colored substances, all of which are drawn through the filter paper at different speeds. These substances are
____________ a, ____________ b, _________ and ____________.

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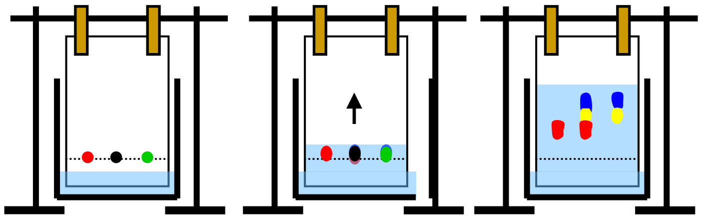
Chromatography
Basic explanation

From the Greek:
chromos: color
graphein: to write

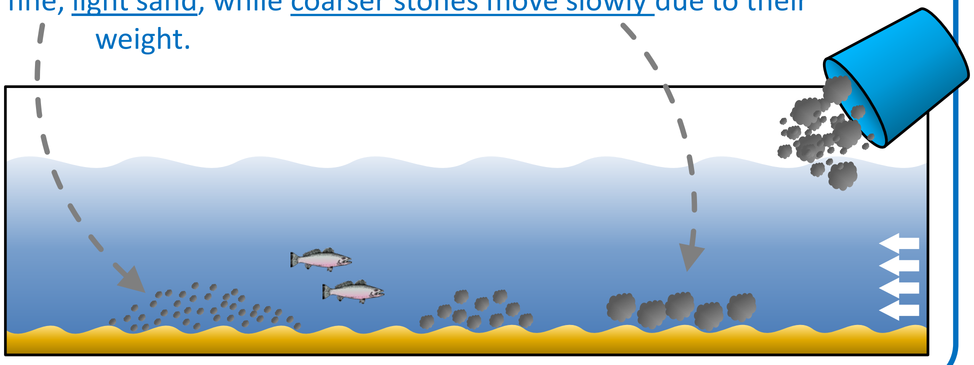
Chromatography

Chromatography is a separation process. The sample to be separated is dissolved in a liquid (e.g., water) that flows through an absorbent material with special separation properties (e.g., filter paper). The components of the solution migrate through the material at different speeds because they "adhere" to it to varying degrees. They can then be measured using a suitable detector (e.g., photometer).



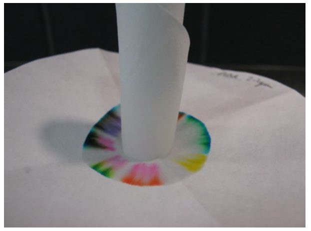
Riverbed as an illustration

Like pebbles in a rushing river: the current quickly washes away the fine, light sand, while coarser stones move slowly due to their weight.



Paper chromatography

If you draw on filter paper with a felt-tip pen and place one end of the paper in a liquid, the liquid travels through the paper and partially carries the dyes from the felt-tip pen with it.



Chalk chromatography

Chalk consists of many small particles that have been compressed together. Water can now climb up the chalk, taking the soluble components with it. This could go on forever if it weren't for gravity counteracting the whole process. That's why the heavy/large particles get "stuck" first, while the water continues to climb up the chalk.

Application examples in chemistry

- For analysis:
  - How much starting material is still in the sample, or how much product has already been produced?
  - Have impurities or by-products been produced?
- For production:
  - To separate different products from each other

Chromatography in the laboratory
In-depth explanation

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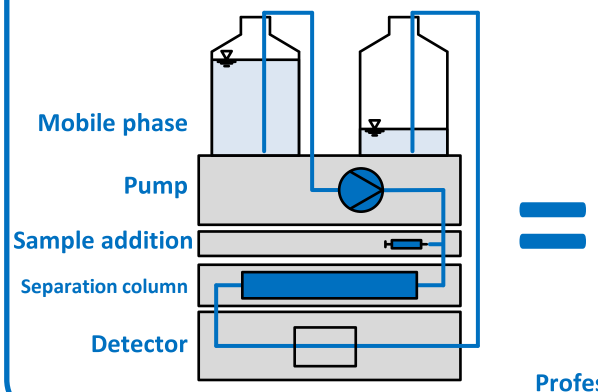
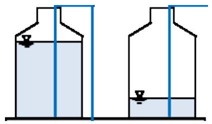
HPLC = High Performance Liquid Chromatography

High Performance Liquid Chromatography



Professional design for the laboratory

The mobile phase



The **mobile phase** flows continuously through a solid, fine-pored carrier material in the separation column.
The sample is injected into the mobile phase at only one point in time. The various molecules in the sample interact with the carrier material, i.e., they adhere to it and then detach again. The stronger the interactions, the more the substances are slowed down on their way, meaning they migrate more slowly. The sample must be soluble in the mobile phase, which is why there are different mobile phases, such as isopropanol for chlorophyll. A suitable mobile phase must be found for each separation task.

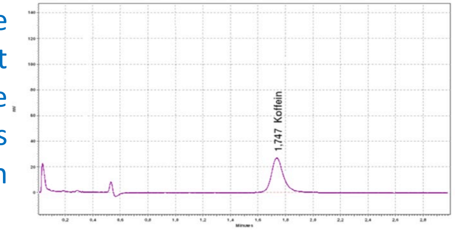
The separation column

The **separation column** consists of a tube filled with fine grains, known as the "**stationary phase**." Depending on the task, the grains in the column can be made of very different materials, such as pure sand or plastics. They are selected so that the various substances from the sample adhere to them to varying degrees, but are also released again by the mobile phase. As a result, the various substances reach the detector at the end of the column at different times (the retention times).

The detector

The **detector** at the end of the separation column makes the separation measurable and visible on a diagram. The detector measures a property of whatever is flowing past it. Depending on the type of detector, this can be, for example, color* or electrical conductivity, but in any case a property in which the sample and the mobile phase differ. If only the mobile phase flows past the detector, nothing or only a small value is measured. The more of the substance that reaches the detector, the greater the measured value. These are visible on the diagram as peaks.

The concentration in the sample can be calculated from the height or area of the peak. To do this, the diagram is compared with diagrams of known substances in known concentrations.



Beispiel eines Chromatogramms

* For more details, see photometer cards