Column chromatography – Separation of leaf pigments (Item No.: P3120300)

Curricular Relevance

Area of Expertise: Chemistry
Education Level: University
Topic: Organic Chemistry
Subtopic: Distillation, Purification
Experiment: Column chromatography – Separation of leaf pigments

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Intermediate	1 Hour	1 Hour	2 Students

Additional Requirements:

Experiment Variations:

Keywords:

Individual chemical compounds from mixtures

Task and equipment

Introduction

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds.

Notes:
The separation of a mixture as carried out here is simply an introduction to column chromatography. The samples that are collected can be characterised by their adsorption spectra or by their fluorescence under UV-light. Such examinations require:

VIS Spectrophotometer	35656-99	1
Cells for spectrophotometer, 2 pcs	35664-02	1
UV analysis lamp, 254/366 nm	33972-93	1

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Safety instructions

Petroleum ether is a colourless, water insoluble, easily inflammable, volatile liquid that is lighter than water. The vapours are heavier than air and form mixtures capable of explosion with air. There is a danger of ignition of it by electrostatic charges.
n-Propyl alcohol is a combustible, colourless liquid that has an odour typical of alcohols and is miscible with water and most organic solvents. n-Propyl alcohol causes slight irritation to eyes and mucous membranes.
Do not inhale vapours. Avoid contact with eyes and skin. Wear appropriate protective clothing, protective gloves and protective goggles when working with them.

1-Propanol
H225: Highly flammable liquid and vapour.
H318: Causes serious eye damage.
H336: May cause drowsiness or dizziness.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233: Keep container tightly closed.

Quartz glass wool
H332: Harmful if inhaled.
H335: May cause respiratory irritation.
P261: Avoid breathing dust/fumes/gas/mist/vapours/spray.

Petroleum ether, 40-60°C
H225: Highly flammable liquid and vapour.
H304: May be fatal if swallowed and enters airways.
H336: May cause drowsiness or dizziness.
EUH066: Repeated exposure may cause skin dryness or cracking.
P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233: Keep container tightly closed.
P240: Ground/bond container and receiving equipment.

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Equipment

Position No.	Material	Order No.	Quantity
1	Frame for complete experiments	45500-00	1
2	Rear-cover for compl.-exp. panel	45501-00	1
3	Panel for complete experimental setups	45510-00	1
4	Clamping holder,18-25mm	45520-00	2
5	Clamping holder,turnable,8-10mm	45522-00	1
6	Clamp on holder	02164-00	1
7	Universal clamp	37715-00	1
8	Spring plugs, 50 off	45530-00	1
9	G-clamp	02014-00	2
10	Column for ion-exchange chromatography	35025-01	1
11	Vacuum adaptor, straight, GL25/12	35806-15	1
12	Erlenmeyer flask, GL 25/12, 100ml	35844-15	4
13	Closure caps,10, GL25	41221-03	1
14	Secure bottle, 500 ml, 2 x Gl 18/8, 1 x 25/12	34170-01	1
15	Spring manometer, 0...-1000 mbar	34170-02	1
16	Glass tube, right-angled	36701-07	1
17	Stopcock,3-way,t-shaped, glass	36731-00	1
18	Air control valve	37003-00	1
19	Mortar w. pestle,250ml, porcelain	32605-00	1
20	Funnel, glass, top dia. 80 mm	34459-00	1
21	Circular filter,d 110 mm,100 pcs	32977-04	1
22	Erlenm.flask wide neck 250ml PN45	36434-00	1
23	Pipette, w. rubber bulb, long tip	64838-00	1
24	Graduated cylinder 50 ml	36628-00	1
25	Volumetric pipette, 50 ml	36581-00	1
26	Graduated pipette, 0.1 ml	36594-00	1
27	Pipettor	36592-00	1
28	Glass rod,boro 3.3,l=300mm, d=9mm	40485-07	1
29	Water jet pump, plastic	02728-00	1
30	Scissors, straight,180 mm	64798-00	1
31	Rubber tubing,vacuum,i.d.6mm	39286-00	3
32	Quartz glass wool 10 g	31773-03	1
33	Sea sand, purified 1000 g	30220-67	1
34	Starch,soluble 100 g	30227-10	1
35	Petroleum ether, 40-60 °C, 500 ml	30184-50	1
36	Propyl alcohol,normal 250 ml	31754-25	1

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Task

Use column chromatography to purify individual chemical compounds from mixtures of compounds.

Set-up and procedure

Set-up

Set-up:

Fig. 1

Position the clamping holders on the panel for complete experiments as shown in Fig. 2. Attach the clamp on holder for the demonstration board to the bottom of a stand leg.
Fix a universal clamp to this and use to secure the security bottle. Assemble the apparatus as shown in Fig. 1 and fix it to the clamping holders.

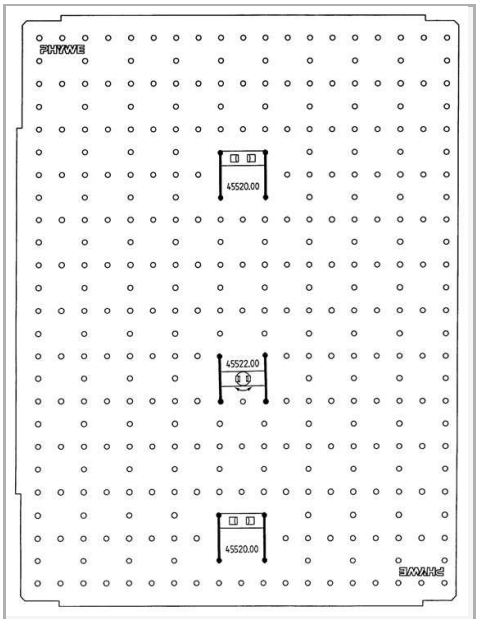

Fig. 2

Procedure

- Insert a small wad of quartz glass wool into the column and push it down to the bottom.
- Now fill the column (press the starch together a little at intervals).
- Position a second wad of quartz glass wool on top to close the column.
- Wet the starch with petroleum ether by filling approx. 20 ml into the top of the column and letting it flow through. **From now on, never let the column run dry!**

Chlorophyll is now to be removed from the greens. To do this, cut the leaves up somewhat and grind them with sufficient sea sand in the mortar. Use the least possible amount of petroleum ether to take up the grindings and filter them into a 250 ml Erlenmeyer flask through a funnel containing a filter. The green solution so obtained can be concentrated a little with a water jet pump if necessary.The more concentrated the chlorophyll solution is the better. It is not very stable, however, and should only be used when fresh.

- Adjust a pressure of about 800 hPa (a vacuum of -200 hPa against atmospheric pressure) with the fine regulating valve on the security flask, add the chlorophyll sample to the column and open the tap at the lower end of the column.
- As soon as the sample has completely entered the column packing, pass in the mobile phase consisting of petroleum ether and n-propyl alcohol (in the ratio 500:1). About 30 ml of this is sufficient for this experiment.
- When it can be seen that a fraction is coming into the vacuum adapter, change the Erlenmeyer flask. Up to four fractions can be collected in this manner, when the mobile phase in the first Erlenmeyer flask is returned back into the large flask.
- As soon as a distinct separation has occured, briefly close the tap to show the separating effect. A swan-neck camera is very suitable for projecting this.

Results and evaluation

Observations:
The sample completely passes into the column within about five minutes, and the front of the mobile phase has reached the lower third of the column within a further five minutes. A separation into a wide yellow region and a narrow green band is clearly recognisable. A closer look shows that the wide yellow fraction is also separated into two. Each fraction that is collected is presented in an Erlenmeyer flask.

Explanation:
It can be clearly seen that there must be several leaf pigments. These are, alongside the chlorophylls, the xanthophylls. In this experiment, separation is made principally between the xanthophylls (yellow) and the chlorophylls (green). The separation is distinctly better with less vacuum, but it takes considerably longer. Under the conditions used, chlorophyll a and chlorophyll b are not separated. Should a quantitative examination to be carried out on the fractions that are collected, then we recommend separation without suction (vacuum). Please refer to technical literature on Biology for details on leaf pigments.
The separation takes place because of the differing adsorption of the pigments to the carrier material (in this case, starch). The more strongly adsorbed pigments are held longer in the column. The separation is better, the longer the column and the greater the difference in the adsorptions to the carrier material.

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