

APLICAÇÕES DE CROMATOGRAFIA EM CAMADA DELGADA E CROMATOGRAFIA EM PAPEL

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Separation of Plant Pigments by TLC

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Developing solvent (mobile phase):

100 mL of petrol ether, 11 mL of isopropanol and 5 drops of distilled water .

Petroleum ether is volatile and very flammable. Petroleum ether presents a high fire risk. The toxicity of petroleum ether varies according to its composition. Many of the components are of quite low toxicity, but some formulations may contain chemicals that are suspected carcinogens. Avoid ingestion and inhalation. **Acetone** and **isopropanol** are highly flammable

Preparation of the TLC chamber:

The developing solvent is placed into a TLC chamber. The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm. The chamber is closed and shaken. It is kept covered so that evaporation doesn't change the composition of the developing solvent mixture. After 15 minutes the chamber will be saturated with the solvent vapor.

Extraction of the leaf pigments:

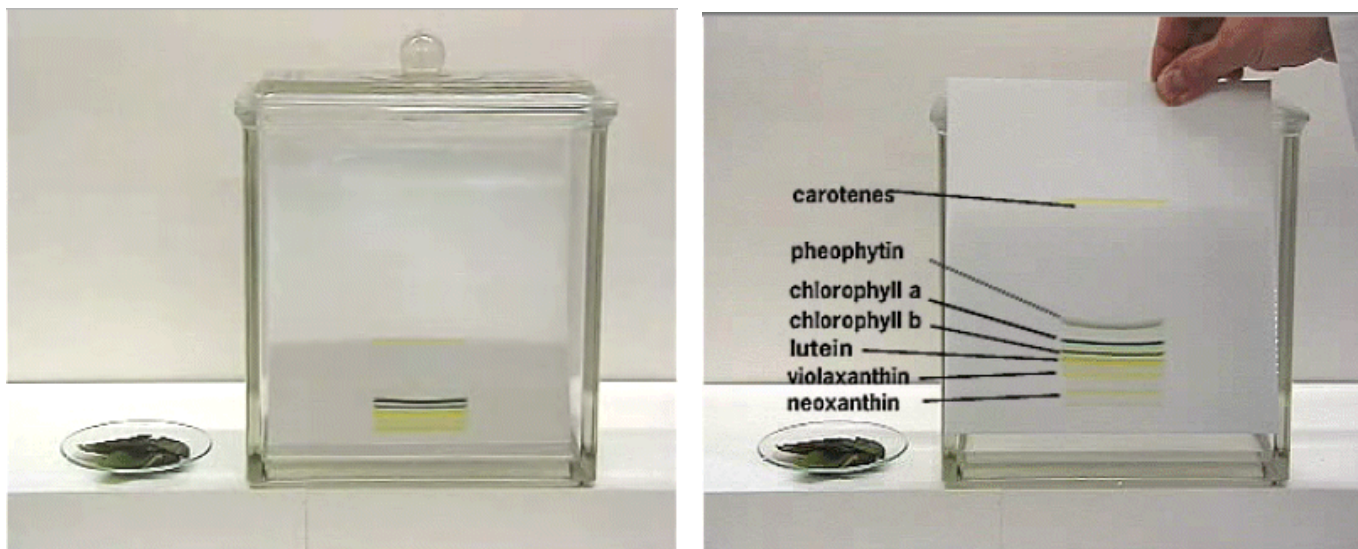
Using a pestle fresh leaves are grinded in a mortar containing 22 mL of acetone, 3 mL of petroleum ether and a spatula tip-ful of CaCO_3 . The pigment extract is filtered. The filtrate is put into a separating funnel and is mixed with 20 mL of petroleum ether und 20 mL of 10% aqueous NaCl solution. The separating funnel is shaken carefully. When the layers have separated the lower layer is allowed to drain into a beaker. This phase is thrown away. The upper layer is washed 3-4 times with 5 mL of dest water. Afterwards the extract is placed in an Erlenmeyer flask and is dried with about 4 spatula tips of Na_2SO_4 . The liquid is carefully decanted into a round bottom flask. Using a rotary evaporator the leaf extract is concentrated to a final volume of about 3 mL.

Application of the extract to the TLC plate:

With a pencil a line is drawn approximately 1,5 cm from the bottom of the plate. The coating of the plate should not be scraped! Using a paint brush or a Pasteur pipet the leaf extract is applied as a line to the TLC plate. The procedure is repeated until the line is very dark green. The transferred extract is allowed to dry thoroughly after each addition. The line is kept as thin and straight as possible.

Experimental procedure:

The loaded TLC plate is carefully placed in the TLC chamber with the sample line toward the bottom. The plate whose top is leaned against the jar wall should sit on the bottom of the chamber and be in contact with the developing solvent (solvent surface must be below the extract line). The TLC chamber is covered. When the solvent front has reached three quarters of the length of the plate, the plate is removed from the developing chamber and the position of the solvent front is immediately marked.

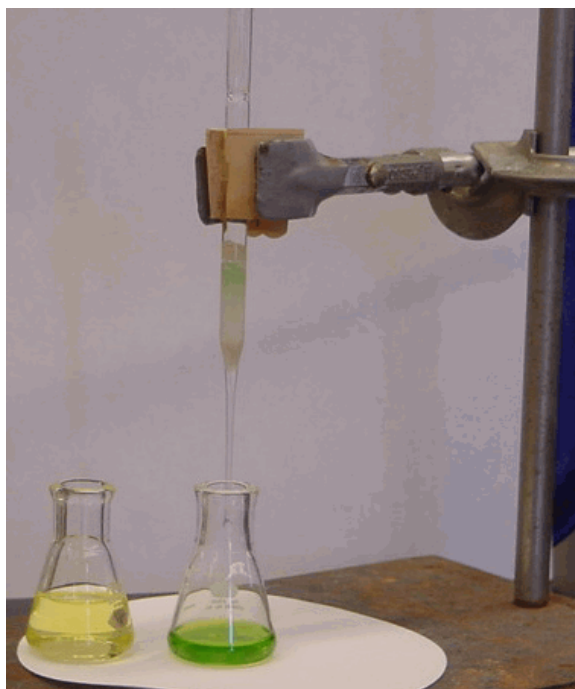


Results and discussion:

As the solvent rises by capillary action up through the TLC plate, the components of the pigment mixture are partitioned between the mobile phase (solvent) and the stationary phase (silica gel) due to their different adsorption and solubility strength. The more strongly a given component is adsorbed to the stationary phase, the less easily it is removed by mobile phase. The more weakly a component is adsorbed the faster it will migrate up the TLC plate. On the other hand, the running distance depends on the solubility of the pigment in the solvent. Since the experiment employs a high non-polar solvent (petroleum ether), the pigments that are least polar (carotenes) will be best solved in the non-polar solvent and will thus have the largest running distance.

leaf pigments	color
carotenes	golden
pheophytin	olive green
chlorophyll a	blue green
chlorophyll b	yellow green
lutein	yellow
violaxanthin	yellow
neoxanthin	yellow

Chromatographic separation of spinach pigments.



Chromatography of Plant Pigments

Experiment adapted from **Experience the Extraordinary Chemistry of Ordinary Things**, B.C. Richardson and T.G. Casteen, Third edition, John Wiley & Sons, Inc., 1998.

Goals

1. To determine the number of pigments in an extract of spinach leaves by thin-layer chromatography, an analytical technique
2. To isolate two fractions of pigments by column chromatography, a preparative technique

A common analytical method is **silica-gel thin-layer chromatography** (TLC), in which the stationary phase is a thin coating of the very polar silica gel (oxides of silicon) on a glass or plastic plate, and the mobile phase is a solvent (or mixture of solvents) that is less polar than silica gel. Typical solvents are hydrocarbons like hexane (C_6H_{14} , very nonpolar), acetone (CH_3COCH_3 , moderately polar), and ethanol (C_2H_5OH , very polar).

Procedure:

Part 1. Extraction of Spinach Pigments

Weigh out approximately 2 grams of spinach leaf. Record the mass. Tear the leaf into small (3-5 mm) pieces, and place them into a mortar with about 5 mL of an 80-20 mixture of petroleum ether and acetone.

Warning:

1. **Many organic solvents emit harmful vapors, irritate the skin, and are flammable.**
2. **Avoid breathing the vapors.**

3. **Immediately wipe spills from your skin or the bench with a dry towel.**
4. **Wash affected skin with soap and water.**
5. **Keep organic solvents away from flames and electrical equipment.**
6. **Finally, do not put organic solvents like hexane and acetone into the sink or down the drain. Your instructor will tell you how to dispose of them.**

Grind the mixture slowly and firmly with the pestle until the solvent is dark green. Most of the solvent will evaporate or become mixed with the organic matter. There will need to be about 2 mLs of the dark green solvent in order to complete the following two procedures, another small amount of the solvent may need to be added to the mortar to obtain the sample. Transfer as much (1-2 mLs) of the **liquid extract** as possible into a test tube, avoiding ground leaves and stems. Centrifuge the extract. Be sure to balance the centrifuge with another tube and liquid of roughly the same mass. Use a dropper to transfer the supernate (liquid on top layer) to a clean tube. Avoid transferring solids or any water that forms a layer at the bottom. Keep this extract tightly stoppered when not in use. The extract should be deeply colored, but should not be cloudy or contain suspended solids. If it is not transparent, centrifuge again for a longer time.

Part 2. Analytical Chromatography of Pigment Mixture

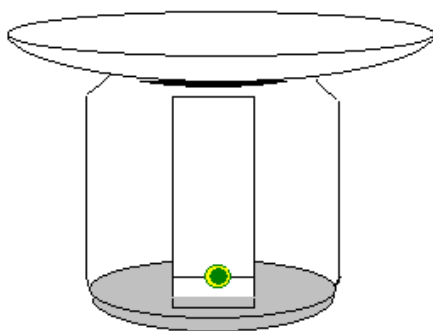
Reminder: Keep your extract and all organic solvents away from flames.

Obtain one of the 2-cm by 7-cm TLC plates provided. Handle the plate gently. Do not touch the coated surface of the plate with your fingers. With clean gloves or forceps, hold the TLC plate by the edges so as not to contaminate it or to loosen the white silica gel coating.

Using a pencil (NOT a pen), gently draw a line across one end of a TLC plate, about 1 cm from one end of the plate, on the side that is coated with powdery silica gel. Do not mark with enough pressure to loosen the coating on the plate. Using a clean capillary tube, make a **small** spot (no more than 2 mm in diameter!). Allow the spot to dry thoroughly. Spot again in the same place after the first sample is dry, again keeping the spot very small. Continue until you have a small, **dark** green spot with a **clearly visible yellow ring around it**. Allow the plate to air dry thoroughly.

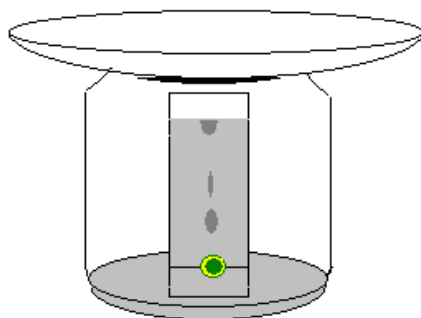
Prepare the developing tank with the filter paper, leaving a path for viewing the contents of the tank. Add about 5 mL of 80/20 petroleum ether/acetone mixture to the tank. Cap the tank, and swirl it gently to wet the paper liner. After the paper is wet, the depth of the solvent should be no more than 5 mm, or half the height of the spot on your silica-gel plate. If the solvent is too deep, pour some out into a waste container.

Using the forceps provided carefully set the plate into the tank, with the spotted end down, place a watch glass on the tank, and leave the tank undisturbed.



Thin layer Chromatography Development Tank
with a 80/20 Petroleum ether/ Acetone solution
about half way up to the sample .

The solvent should not immerse the spot, but it will quickly rise across it. This process is called developing the plate.



When the solvent rises up the TLC plate to about 1cm
from the top unscrew the lid, remove the plate with
clean gloves or forceps and quickly mark the top
boundary of the solvent front.

Watch as solvent rises and the components separate on the plate. Record in your observations and shape size or color changes. Allow the solvent line, called the front, to rise until it is within 1 cm of the top of the plate. Do not allow it to reach the top. Remove the developed plate. Immediately, gently mark the final solvent level on the plate with a pencil.

Observe the plate in room light and under a black light. How many different pigment spots can you distinguish? How does fluorescence change their color?

Using the forceps provided carefully set the plate into the Iodine tank, with the spotted end down, put the cap on the tank, and leave the tank undisturbed. The spots should begin to appear and darken. Remove the plate from the jar, carefully replace the top, and immerse the plate gently into a beaker of tap water. Remove the plate and allow it to air dry.

Compute R_f for each of the major spots on your plate.

Part 3. Preparative Chromatography of Pigment Mixture

Label two small, clean test tubes #1 and #2.

Set up a **Pasteur pipet**, which will serve as your chromatography column, on a ring stand as illustrated at the top of this lab procedure.

Wet a small amount of glass wool with petroleum ether and insert it into the column to make a small (less than 0.5 cm deep), soft plug where the tip begins to taper.

Warning: Glass wool is irritating to the skin. Handle it lightly or with gloves, and wash your hands as soon as you have your plug in place.

Hold a gloved finger up to the small bottom opening of the pipet to keep the liquid from flowing out and add petroleum ether until the pipet is about half full.

Use a cone of weighing paper to pour in sand to form a 0.25-cm layer on top of the glass wool. Next, pour a 3-cm layer of silica gel powder. Complete the chromatography **bed** by topping the silica gel with 0.25 cm of sand. A small wooden stirrer can be used to tap down the solids and to remove any air bubbles from the column.

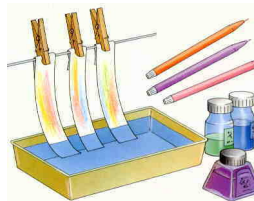
The liquid layer should always be above the solid layers.

Use a dropper to add petroleum ether to the column gently, without disturbing the top of the bed. Fill the column completely, and let the column drip into a small waste container (this is called eluting the column, and collecting the eluate). **From now on, do not let the level of liquid fall below the top of the bed.** When the petroleum ether level is within 0.25 cm of the bed, gently fill the pipet with your pigment extract. Allow the sample to flow through the column, keeping watch for any colored bands moving down the column.

As soon as any colored material approaches the bottom of the column, replace the waste container with sample tube #1. As the level of pigment extract in the column falls, keep adding more until all of the extract has been placed onto the column.

Observe and record the color of the pigment that comes through the column into sample tube #1. What color is the pigment that remains at the top of the silica gel?

When the extract level falls to within 0.25 cm of the bed, begin eluting the column with petroleum ether and replace tube #1 with the waste container and stopper tube #1. Allow about 5 mL of petroleum ether to pass through the column into the beaker. When the petroleum ether level falls to within 0.25 cm of the bed, start eluting the column with acetone. As soon as green pigment approaches the bottom of the column, replace the waste container with tube #2 and continue eluting with acetone until a second colored fraction is collected in the tube. As the last of the pigment comes out of the column, replace tube #2 with the waste container and stopper tube #2. The column can now run dry. You now have two stoppered tubes of pigments, one that eluted as you added the extract to the column, and one that eluted with acetone.



CROMATOGRAFIA EM PAPEL

Separate pigments from green leaves with paper chromatography

1. Collect green leaves and cut them into very small pieces. Use a mortar and pestle to grind the leaves for five minutes with a small volume of methylated spirit and clean sand until a deep green solution forms. Draw a fine pencil line 5 cm from the end of a 1 cm wide strip of absorbent paper. Suspend the absorbent paper in a test-tube without touching the bottom. Use a fine eye dropper to put one small drop of the solution on the centre of the fine pencil line and let it dry. Add more solution to the same place to make a small concentrated spot. Hang the paper strip with the lower end in the methylated spirit solvent and the spot of green solution above the solvent level. Leave the paper strip in the solvent until the methylated spirit has almost reached the top of the absorbent paper. Capillary attraction draws up the solvent. Mark the chromatogram on the paper to show a top orange band of xanthophyll and a lower green band of chlorophyll. A band of carotene is visible if the solvent is toluene.

2. Repeat the experiment with other solvents, e.g. toluene, acetone (propanone)

Separate mixed inks with paper chromatography

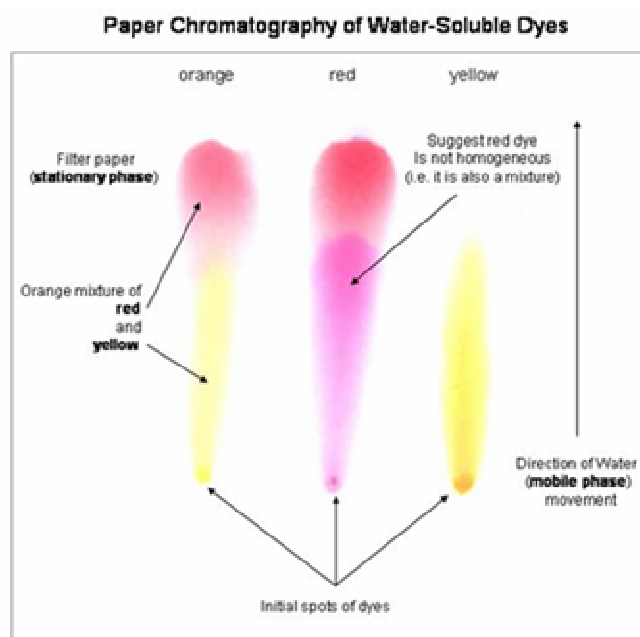
Prepare a mixed solvent from 6 parts of water, 3 parts of methylated spirit, and 1 part of ammonia solution. Put 5 mL of mixed solvent in a test-tube. Prepare mixed ink from equal quantities of red and blue ink. Put a drop of the mixed ink near one end of a 2 cm wide paper strip. Lower the paper strip so that its lower end is in the mixed solvent. Use a stopper to prevent evaporation. As the solvent moves up the paper strip, the component colours of the ink separate to form different coloured bands with red above and blue below. Try other solvents and other inks to obtain good separation of colours.

Repeat the experiment by drawing a line with a ball pen or an ink pen near the end of the paper strip.

Separate food colours in coloured sweets, e.g. jelly beans, "Smarties"

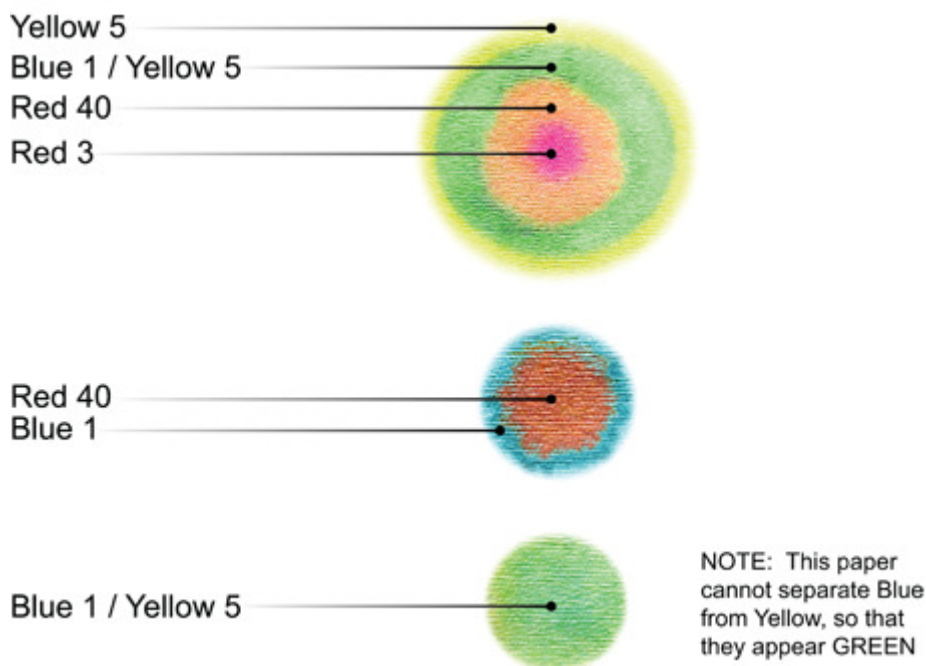
1. Use this method for sweets where the colour is dispersed through the sweet, e.g. jelly beans. This method uses white thread to separate the extracted dye from the extracted sugar. This method extracts only acid dyes. Prepare white woollen thread: Use 1 meter of prepared wool for each colour of the jelly beans. Boil one metre of white woollen thread for 10 minutes in 2% ammonia solution, rinse under the tap to remove fluorescing dyes, then dry. Add 25 mL of warm water to 5 jelly beans of each colour in 5 different containers and leave to stand until they look white. The solutions now contain dye and sugar, which can be separated by dyeing the wool. For each colour, put a 1 metre piece of prepared white woollen thread in a 100 mL beaker and add the dye extract. Add 3 drops of 0.1 M acetic acid. Heat until boiling and simmer for 5 minutes. Remove the dyed wool and wash gently under the tap until no stickiness remains. Re-extract the dye from the wool by placing it in a beaker containing 20 mL of 2% ammonia solution and simmer for 5 minutes. Remove the wool from the beaker. Evaporate the extract in the beaker to dryness on a hot plate in a fume cupboard. Ammonia will be given off. Dissolve the dry residue, with constant stirring, in the minimum number of drops of water to maintain high concentration. Use a capillary tube spotter to make < 2 cm spots of solutions of each colour. Use the following solvent mixture: butanol: ethanol: 2% ammonia, in a 3:1:1 ratio.

2. Use this method for sweets where the colour is only on the outside layer of the sweet, e.g. "Smarties". Wet the surface of the sweet then rub it directly on chromatography paper. To simplify the separation, dip the paper in 1% sodium chloride solution to reduce the electrostatic attractive forces between the dye and paper molecules. Use a capillary tube spotter to make < 2 cm spots of solutions of each colour. Use the following solvent mixture: butanol: ethanol: 2% ammonia, in a 3:1:1 ratio.



Paper Chromatography

Samples are spotted onto paper [stationary phase]. Solvent [mobile phase] is then added to the center of the spot to create an outward radial flow. This is a form of paper chromatography. [Classic paper chromatography is performed in a manner similar to that of TLC with linear flow.] In the upper image, the same black FD&C dye sample is applied to the paper.



Notice the difference in separation power for this particular paper when compared to the TLC plate. The green ring indicates that the paper cannot separate the yellow and blue dyes from each other, but it could separate those dyes from the red dyes. In the bottom image, a green sample, made up of the same yellow and blue dyes, is applied to the paper. As you would predict, the paper cannot separate the two dyes. In the middle, a purple sample, made up of red and blue dyes, was applied to the paper. They are well separated.

Each bench will have a complete set of spotting solutions. A Spot Plate will be provided to hold several drops of the individual solutions in separate wells. A SINGLE capillary tube will be used for the spotting ALL of the metals. **The capillary tube will be rinsed in a small beaker of DI water between the application of DIFFERENT metal ions or solutions to the chromatography paper.**

Paper chromatography of selected cations

Objectives

Known and unknown solutions of the metal ions Ag^+ , Fe^{3+} , Co^{2+} , Cu^{2+} and Hg^{2+} will be analyzed using paper chromatography. An unknown solution containing some of these cations will be identified by comparison to the R_f values and colors of the stained spots of known solutions.

In this experiment, similar principles are used to separate several metal cations by a paper chromatography procedure. The metal ions— Ag^+ , Fe^{3+} , Co^{2+} , Cu^{2+} , and Hg^{2+} —have differing solubility in the mobile phase—aqueous HCl with ethyl and butyl alcohol—and will move at different rates up the paper. The different metal-ion solubilities are probably due to the formation of various compounds with the chloride ion and their varying ability to dissolve in the organic solvent.

A diagram showing how to prepare the paper is shown below. Standard solutions containing each of these ions will be spotted onto the paper using a capillary tube, along with a standard solution containing all five ions. An unknown will also be spotted onto the paper. Once the paper is prepared, it will be developed by placing the paper into the eluent. After 75-90 minutes, the paper is visualized by wetting it with an aqueous solution containing potassium iodide, KI, and potassium ferrocyanide, $\text{K}_4[\text{Fe}(\text{CN})_6]$. The unique color observed for each ion is produced by a chemical reaction with the visualization solution. This is one useful way to identify which ions are present in an unknown mixture.

Materials and Equipment

Chemicals: 0.1 M aqueous solutions of AgNO_3 , $\text{Hg}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Co}(\text{NO}_3)_2$, and $\text{Cu}(\text{NO}_3)_2$, each with dedicated capillary tubes; eluting solution (aqueous HCl with ethyl and butyl alcohol); visualizing solution (aqueous solution of KI and $\text{K}_4[\text{Fe}(\text{CN})_6]$).

Equipment: Clean piece of chromatography paper; disposable Latex gloves (nitrile gloves are available in the stockroom for people with allergies to Latex); 600 mL beaker; plastic wrap; forceps or beaker tongs; ruler

Preparation of the paper for chromatography

1. Each pair of students should obtain a piece of filter paper with the dimensions shown in Figure 3. Make sure the paper is clean and without tears or folds. Use a *pencil*—not a pen—and a ruler to draw a line across the paper one cm from the long edge of the paper. You will spot the

metal ion solutions on this line. Write your name in pencil in the upper left-hand corner of the paper.

2. Practice spotting water and/or ion solutions onto a strip of filter paper so that you know how to create spots of the correct size. Use glass capillary tubes to spot the ions onto the paper. Solution is applied by lightly and quickly touching a capillary tube containing the solution to the line you drew on the paper. The spots should be between 5–8 mm in diameter. Spots larger than this will excessively spread out during the experiment and make analysis difficult.

3. Known 0.1 M aqueous solutions of AgNO_3 , $\text{Hg}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, $\text{Co}(\text{NO}_3)_2$, and $\text{Cu}(\text{NO}_3)_2$ are provided in test tubes, each containing two or three capillary tubes. Starting on the left, mark the identity of the ion underneath each spot with a pencil; then spot each known ion carefully onto the line. Be careful to avoid contaminating the capillary tube with other ions and replace the capillary tubes back into the correct test tube. A test tube containing a known mixture of all five ions is also provided with a set of capillary tubes. Spot this mixture onto the line as well. Because this solution is more dilute than the single-ion known solutions, apply the known mixture three times, letting the spot dry between each application. A heat lamp will help to dry the spot more quickly.

4. Several unknowns are also provided in test tubes, along with capillary tubes. Your instructor will tell you which unknown should be used. The unknowns will contain between one and four cations, and are more dilute than the single-ion known solutions. The unknown will also need to be applied two and four times for the two trials, letting the spot dry between each application. In case of error, you should spot the unknown in two places along the line so that two trials are available for analysis.

Developing the chromatography paper

1. Place a piece of tape along the upper right edge. Then form a cylinder by connecting the two short edges of the paper with the tape. Make sure the edges do not touch.

2. Obtain 15 mL of the eluting solution. Carefully pour some of this solvent into a 600 mL beaker and carefully swirl for a second or two. **Caution:** Do not breathe the vapors from this solution! Make sure that the level of the liquid will be *below* the spot line on the paper once the paper is placed in the developing chamber

3. Place the paper cylinder into the beaker with the marked edge down. The spots should be *above* the level of the solvent. The paper should not be touching the sides of the beaker. Carefully cover the beaker with plastic wrap and place it in the hood for 75-90 minutes. The solvent should start to move up the paper. Once the beaker is covered, make sure it is level and do not disturb it during the development period. Your instructor may have an assignment for you to work on while you wait.

Visualization and analysis of the paper

1. Once the development period is over, wear disposable gloves and remove the paper from the beaker. Latex gloves are available in the lab and nitrile gloves are available in the stockroom for people with Latex allergies. Let any solvent drip back into the beaker, then remove the tape. Lay the chromatography paper on a paper towel and immediately mark the solvent front with a pencil. Pour the used eluting solvent into the waste container provided. Dry the paper under a heat lamp in the hood. **Caution:** Do not breathe the vapors! Be careful not to burn the paper under the lamp.
2. Once the paper is dry, bring it to the visualization station on the paper towel. *Briefly* dip the paper into the visualizing solution located in a shallow dish in the fume hood. Lift the paper out of the solution immediately and let any excess drip off at the station. Place the wet paper onto a dry paper towel and dry it under a heat lamp immediately, then carry it to your bench for analysis.
3. Find each known single-ion first and record the colors you observe. Some spots may fade over time, so record the colors while the paper is still wet. Measure the distance each spot moved, D , with a ruler. Measure to the center of each spot. Record your data in the data table.
4. Measure the distance to the solvent front, F . The value of F should be approximately the same across the entire paper. Use these values to calculate the R_f for each ion. Each observed spot has its own R_f value. Record your results in the data table.
5. In the lane containing the mixture, find each ion and record the distance moved by each ion. Calculate the R_f for each ion in this lane. The values should closely match those observed in the single-ion knowns.
6. In the lane containing the unknowns, locate the center of each spot observed and record its distance and calculate the R_f values. Use the lane that has the clearest spots. The color and R_f values for the unknown spots should closely match some of the known ions. You should now be able to identify which ion or ions are found in your unknown. Record your data in the corresponding table.
7. Make a sketch of your chromatogram in the space provided on your lab report form, being sure to indicate the position and approximate size and shape of each spot on the paper. Dispose of the paper in the designated waste container.

Extração em Fase Sólida

A extração em fase sólida (SPE, do inglês *solid phase extraction*) tem sido amplamente utilizada para o preparo de amostras, principalmente para a determinação de compostos orgânicos por métodos cromatográficos.

Em geral os composto de interesse estão em concentrações muito baixas nas amostras para serem quantificados de forma adequada. A SPE pode ser empregada para concentrar os analitos de interesse, eliminando a maior parte dos interferentes. Também pode ser empregada para remoção de interferentes de forma seletiva.

A primeira decisão do analista é a seleção do tipo de sorvente capaz de resolver o problema da análise de baixas concentrações. Por muitos anos n-alkilsilica foi o sorvente universal da SPE. Atualmente, vários tipos de materiais são disponibilizados comercialmente. Pode-se destacar os diferentes copolímeros altamente entrecruzados como materiais com maior capacidade de retenção de misturas de compostos com características diversas.

Assim, dependendo da aplicação, empregam-se materiais com diferentes mecanismos de retenção:

- Adsorção (ex. sílica SiOH, florisil Mg₂SiO₃ e alumina Al₂O₃)
- Partição: polares (ex. ciano, amino, diol)
apolares (ex. C-8, C-18, fenila), suportados em sílica ou polímeros.
- Troca iônica: catiônica (sulfônico - forte, carboxílico - fraca)
aniônica (amônio quaternário - forte, amino - fraca)



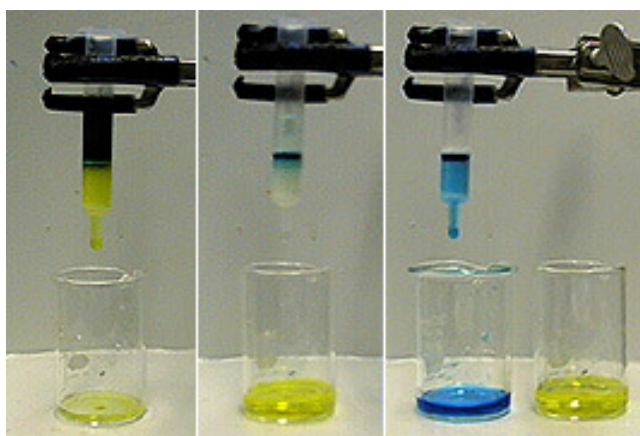
Também são disponíveis cartuchos de SPE com leitos mistos, empregando mecanismos de partição e troca iônica. Os tamanhos dos cartuchos e as quantidades de sorvente são variáveis. A escolha do solvente e da quantidade necessária para a eluição depende do tipo de sorvente utilizado e dos analitos de interesse.

A SPE pode ser realizada no modo off-line, com preparação da amostra separada da análise cromatográfica, ou on-line pela conexão direta com o sistema de análise.

O princípio da SPE baseia-se nas seguintes etapas: condicionamento, retenção, lavagem e eluição.



O experimento visa enfatizar a facilidade do emprego de cartuchos C-18 na separação por fase reversa de componentes de corantes.



Preparo da amostra: (1) Pesar 0,2 g de corante alimentício verde e dissolver em 10 mL de água purificada.

Condicionamento SPE: (1) Colocar um cartucho SPE no suporte; (2) Adicionar 1,0 mL de metanol; (3) Adicionar 1,0 mL de água purificada.

Aplicação da amostra: (1) Adicione 3,0 mL da amostra no cartucho SPE; (2) Aplicar vácuo e coletar o eluato em um tubo.

Lavagem: (1) Adicionar 2,0 mL de água purificada; (2) Aplicar vácuo e descartar o eluato.

Eluição: (1) Colocar o cartucho SPE no suporte; (2) Adicionar 2,0 mL de metanol; (3) Aplicar vácuo e coletar o eluato em um segundo tubo. Observar a coloração.

ATENÇÃO: Use os equipamentos de proteção: óculos, avental e luvas.

Literatura consultada

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