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**Purpose:** To learn about chromatography.

**Learning Objectives:**
1. Chromatography is how scientists separate substances that are mixed together.
2. Chromatography uses the differing properties of each substance in order to separate them (such as using size to separate various coins).
3. Chlorophyll is what causes tree leaves to be green, but tree leaves also contain other compounds that cause them to be yellow, red, and orange in the fall.

**Next Generation Science Standards (est. 2013):**
- LS1.A: Structure and Function (partial)
- LS2.B Cycles of Matter and Energy Transfer in Ecosystems (partial)
- ETS1.C: Optimizing the Design Solution

**National Science Education Standards (valid 1996-2013):**
- Standard B: Physical Science  
  - Properties and changes in properties of matter  
  - Transfer of energy
- Standard C: Life Science  
  - Structure and function in living systems
- Standard D: Science and Technology  
  - Abilities of technological design  
  - Understandings about science and technology
- Standard F: Science in Personal and Social Perspectives  
  - Science and technology in local, national, and global challenges

**Grade Level:** 2-8

**Time:** approx. 1 hour

**Materials:**
- Paper bags
- Paper cups
- Scissors
- Assorted coins
- Tape
- Pencils or pens
- Green leaves from deciduous trees
- Thin layer chromatography sheets (you will need a flexible sheet with a layer of silica gel which can purchased from a scientific supplier such Flinn Scientific or Sigma-Aldrich)
- Isopropyl alcohol
- Beaker or cup, 1 per student

**Safety:**
Students should be careful with the scissors when poking holes in the cups or cardboard, keeping them closed and using both points.

**Preparation ahead of time:**
Cut thin layer chromatography sheet into 2cm x 5cm strips. Build sample coin sorter.

**Introduction Chlorophyll Chromatography:**
Deciduous trees lose their leaves every fall. Many of types of these trees also undergo color changes in their leaves, going from green to yellow, orange, or red, and finally, brown. As days become shorter and nights become cooler, the tree makes less chlorophyll. Chlorophyll is continuously replenished throughout the summer, giving leaves their green appearance. Once the chlorophyll disappears from the leaves, the remaining colored substances (carotenoids) are visible, causing the leaf to be yellow or orange.

Thin layer chromatography, or TLC, is a technique that is used to separate a mixture of compounds. The mixture is placed, or spotted, on a TLC plate (today, this activity will use a thin sheet of plastic coated in silica gel) and then placed in the developing chamber. At the bottom of the chamber (today, this activity will use beakers) is a tiny amount of solvent. This is known as the stationary phase. Because each compound adheres to the plate differently, they travel different distances as the solvent moves up the plate. In a plant leaf, the carotenoids and chlorophyll are mixed together.

**Introduction Coin Sorters:**
Tell the students that they will be making coin sorters, and that coin sorters use the physical properties of the coins in order to separate out coins of different monetary value. The coin sorters they will be making use the coins’ circumference to separate the coins. The students can also be told that scientists use a similar technique to separate molecules. Often chemical reactions can produce multiple types of molecules. In order to analyze them, scientists need to separate the molecules by type. This is often done by a procedure called chromatography. Explain that chromatography uses the properties of molecules to make them separate. Illustrate this with a model of a column, if available (see procedures pt. 3).
Tell the students that today they will be using chromatography to learn why leaves change colors in the fall. Just as coin sorters separate coins using the physical property of size, scientists use a method called TLC to separate molecules based on their chemical properties. Show the students the TLC plates and explain that they are very fragile and delicate and that the silica coating flakes off easily. Point out that the silica is the substance that the plant molecules will be using to travel through, so if it is removed, the molecules will be stuck. Remind them throughout the session to not bend or rub the plates too hard.

**Procedures:**

1. **Chlorophyll Chromatography**
   a. Take the students outside to collect leaves. A variety of leaves should be collected.
   b. Help the students identify what type of leaf they have collected.
   c. The students should mash up the leaf with their fingers. Tell the students this is necessary to break open the plants’ cells.
   d. The students should gently rub the mashed parts of the leaves against the plate. They should not rub the plate very hard, or the silica gel will rub off.
   e. If no green is visible on the plate, repeat steps 4 and 5.
   f. Pour some isopropyl alcohol into a beaker or cup (developing chamber). It should just cover the bottom and be no more than ½ inch deep. Do not allow students to pour the isopropyl alcohol.
   g. The students should place their TLC plate into the cup, making sure that the green mark is above the level of isopropyl alcohol. The isopropyl alcohol will begin to travel up the plate.
   h. Wait 10-15 minutes, or until the isopropyl alcohol reaches the top of the plate. During this time, discuss the chromatography related vocabulary words, or move on to the photosynthesis activities.
   i. Once the solvent has reached the top of the plate, allow the students to remove from the developing chamber.

2. **Coin Sorters**
   a. Begin by showing the students the model coin sorter, and explain how each junction between the cups selects a different size to remain in the cup and allows the others fall through: The top junction has holes the size of a nickel. This will allow nickels, dimes, and pennies to fall through to the next cup, leaving quarters isolated in the top. The next junction has holes the size of a penny. This will allow dimes and pennies to fall through, leaving nickels isolated in the second cup. The third junction has holes the size of a dime. This will allow dimes to fall through, separating the pennies and dimes.
b. Help the students trace three circles on the paper bags: two the size of a cup top, one the size of a cup bottom. You can refer to these as “sorting plates”. Have the students cut out the circles.
c. Students should cut the bottom off of three cups.
d. The students will trace the outlines (1-3) of quarters and pennies on the larger sorting plates and nickels on the smaller sorting plate. Have them trace as closely as possible to the coins, and to space the tracings evenly on the circles. They should carefully cut out these circles, being careful not to cut any bigger than the tracing marks.
e. The students should securely tape each sorting plate onto a cup top or bottom. At this point, they should do a preliminary test on the effectiveness of the sorting. Do the coins fall through the appropriate holes in the sorting plates? (i.e. Pennies should fall through all but the smallest of the holes, dimes should fall through all, etc.)
f. The stack of cups should be assembled by taping halfway around the pair of cups at each junction. (This allows the cups to remain stacked while shaking coins through the coin sorter, but also allows the coins to be removed after sorting.)

3. (Optional) Size Exclusion Chromatography Column Model
   (Instructions to build this model can be purchased online at http://ice.chem.wisc.edu/Catalog/Manuals.html#Anchor-ICE-47217)
   a. Allow students to observe sorting of various size beads within the model.
      i. Note: Students should not be allowed to use model unsupervised. Model is easily broken and/or disassembled, causing beads to scatter.

Discussion:
Chlorophyll is just one of the many of the colored compounds in a tree leaf. Chlorophyll is what traps the light energy. Its green color covers up the other colors until fall. When a tree stops producing chlorophyll, the other colors become apparent. They can be separated using a technique called thin layer chromatography. In TLC, a mixture of compounds travels in the mobile phase (isopropyl alcohol in today’s activity) across a stationary phase (TLC plate). Some of the compounds adhere strongly to the stationary phase and do not move very much. Other compounds do not adhere strongly and move farther across the stationary phase. This allows us to separate and see the different colors of the compounds in plant leaves.

Throughout the activity, reinforce the analogy between chromatography and coin sorting. Highlight the key point that sorting occurs based on the substances’ properties.
Evaluation:
What is the similarity between a coin sorter and chromatography?
Why do the different substances separate in the TLC?
Do you think substances would separate if you used water instead of alcohol?
What is the difference between a stationary phase and a mobile phase?
Why do you think it is important to separate compounds?