THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS

**Required Prelab reading:** Padias, 167-174



**Procedure**

You will be given an unknown sample containing two of the five possible knowns (shown above) dissolved in an appropriate solvent. You will be asked to identify the two compounds in your sample by TLC using three different solvent systems.

Record your unknown code number.

Spotting the Plate

You will be allowed a total of ***five TLC plates*** for this experiment.



 For spotting TLC plates, you will be given very fine glass capillaries. Prepare three plates by very lightly drawing a pencil line 0.7-1.0 cm from the bottom edge of each plate. This line must be above the level of the solvent in the developing chamber. Make small marks evenly across the line where you want to spot your compounds and label each (1-5 for knowns; u for unknown). Use a capillary to spot each known and your unknown. To do this take some of the solution into the capillary and just touch the tube to the plate for as short a time as you can to make the spot as small as possible; never bigger than this letter "o". Unless you have had previous experience do not place more than four spots on one plate. Do not let the spot get too big as this will produce a streak rather than distinct spots. Spotting the plate too heavily will also produce the same result. Allow time for the solvent to evaporate and spot the plate again. You can do this by gently blowing on the plate. Make sure the spot is dry before proceeding further. Check the spot periodically under the UV lamp to see if you have spotted enough material; it will show up as a dark spot. Each sample (knowns and unknowns) is a 1-1.5% solution in an appropriate solvent.

At this point you have only used three TLC plates, you will use the other two to confirm the identity of your unknowns.

Chromatography and Development

 The three solvent systems available for your TLC's are hexane:toluene, 1:1; chloroform; and chloroform:methanol, 9:1. Read the theoretical material in your text carefully and refer Table 1-2, p. 42 so you will know before coming to lab the relative polarity of these solvents.

Use the tall form beakers provided for your TLC chambers. Cut a piece of filter paper to fit inside the beaker; it should encircle about half the inside of the vessel so that a window is available to watch the progress of the chromatography. When the paper is in place, add about 0.5 cm of solvent, cover with the watch glass, and gently shake the beaker to completely wet the paper. The purpose of the paper is to facilitate the equilibrium of the solvent and its vapor. You do not change the paper with each new TLC. It should remain in the beaker for the duration of the experiment. To make things more efficient TLC chambers with different solvents can be shared between several students.

 Place one TLC plate in each of the three solvent tanks by leaning them against the paper. Remember, the spots must be above the solvent. Position the plate so that you can observe the progress of the chromatography through the window of the paper liner; replace the cover glass. This does not require constant attention, but when the solvent reaches approximately 1 cm from the top (about 5 minutes) of the plate, remove the plate, mark the solvent front and allow the solvent to evaporate.

Visualization of Spots

Unless one of the materials you have analyzed is colored, you will not be able to see any thing on the plate surface. The most useful method for visualizing your plate is with the UV light. Your plates are impregnated with a very polar phosphor that does not move up the plate as they are eluted but does fluoresce when exposed to UV light. Consequently, your plates will appear green or pink when held under a UV lamp. If there is another material on the plate one of two things can happen. First, this other material can prevent the fluorescence of the plate phosphor, in which case, the spot on the plate appears a dark spot. To describe this type of behavior, we say that the fluorescence is "quenched." The second result is the component itself will fluoresce even better than the plate phosphor in which case you will observe a bluish-white spot. In this experiment, you should note the type of behavior exhibited by each material since it may help you in identifying your unknown. Each unknown in this experiment is able to exhibit either quenching or fluorescence. What functional group, common to each unknown, must be responsible for this phenomenon?

 Carefully circle the spots you see on the TLC plate with a pencil while under the UV lamp.

 Calculate the Rf value. The Rf is calculated by dividing the distance the spot has traveled by the distance traveled by the solvent front (Figure 3-29, p 170). Report all Rf values to two decimal places.

 Sketch your TLC plates with Rf values and elution solvent in your lab notebook. This should be done each time you perform TLC in this course.

Identification of your Unknown

When you think you have the identity of your unknown, run a TLC plate with three spots: your unknown, a co-spot (both known and unknown spotted in the same place) and the suspected known. Identical compounds should have identical Rf values. Develop the plate with a solvent that gives a reasonable Rf value (0.20 < Rf < 0.80.)

When choosing a solvent there are a few important points to remember. The first is that 99% of compounds move based on their polarity (*i.e.* the least polar travel fastest and the most polar travel slowest); this holds true no matter what the solvent is. However, very polar solvents may move everything very fast and non-polar solvents may move nothing at all. So, when choosing a solvent to try at first, pick one of medium polarity - one in the middle of the chart. Once you look at the results, you should be able to decide whether to go up or down in polarity of your solvent. To be a reasonable solvent, it should move your compound away from the origin but not all the way up with the solvent front.

**TLC**

**DATA SHEET**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name:** |  |  | **Section:** |  |

**Unknown number:**

Use chemical drawing software and electronically paste structures of the unknowns in the boxes.

|  |  |  |  |
| --- | --- | --- | --- |
| Structure of unknown #1 | | Structure of unknown #2 | |
|  |  | |  |

Name of 1st unknown Name of 2nd unknown

|  |  |  |
| --- | --- | --- |
| **Compound name** | a**Solvent system** | **Rf** |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
| **Unknown #1** |  |  |
| **Unknown #2** |  |  |

aThe Rf value for each unknown must be in the same solvent system as the corresponding known. The three remaining knowns must have Rf values from a “reasonable” solvent (0.20 < Rf < 0.80.)

Calculations: (you may include these on a separate attached sheet)

**Turn in you TLC plates, properly labeled, taped to a single sheet of paper.**

**Postlab questions**

1. Rank the five knowns from most polar to least polar. Explain your reasoning based on your experimental results. You may wish to refer to specific TLC plates or data to support your answer. Your instructor should be able to easily confirm your answer by looking at your TLC plates.

2. Explain why running a TLC co-spot (two compound spotted on top of each other) is better at confirming identity than simply running the two compounds next to each other.

3. When 2-propanol was used as the developing solvent, two substances moved with the solvent front (Rf = 1) during TLC analysis on a silica gel plate. Can you conclude that the two compounds are identical? If not, what additional TLC experiments would you perform?