Chromatography:

Separation of Dyes with Sep-Pak Cartridges

Introduction and Theory

Chromatography is a technique that allows for the separation of a liquid/dissolved mixture/solution of molecule[s] based upon molecular weight, polarity (hydrophobic versus hydrophilic) or charge, to give three examples.

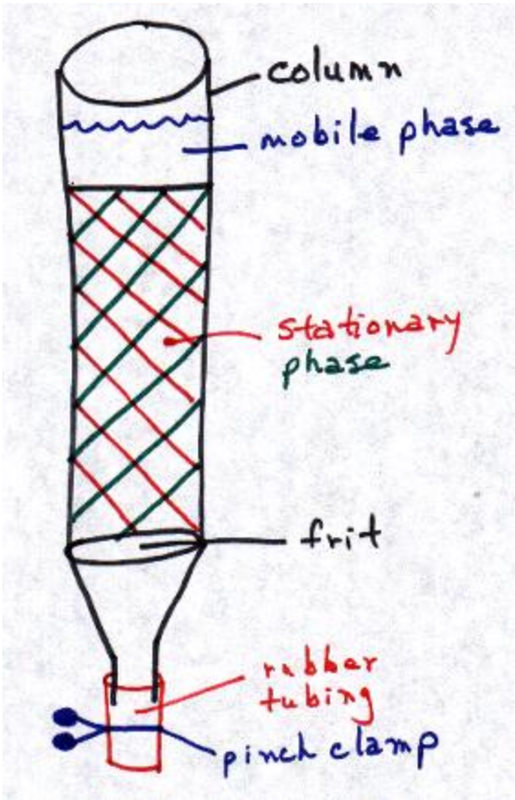
In order to cause the separation of molecules, some must separate from the solution and interact with some sort of solid support. This process is called partitioning. Partitioning may be viewed arithmetically to give us an idea of how the molecules are distributed between the solid support (stationary phase) and a liquid (mobile phase). This is done by looking at the partition coefficient (a constant similar to K_a, K_b, K_{eq}, etc), the K_p:

$$K_p = \frac{[solute]\,in\ stationary\ phase}{[solute]\,in\ mobile\ phase} = \frac{[S]_s}{[S]_M}$$

A partition coefficient of 1 says that the solutes (molecules) are equally distributed (partitioned) between both phases. A partition coefficient less than one says that the solutes are primarily in the mobile phase. A partition coefficient greater than one says that the solutes are primarily in the stationary phase.

What are these phases? The stationary phase may be complex carbohydrates, a silicate bonded to octadecane, octane or ethane or it may be a resin that may be modified to change its ionic characteristics (remember cation exchangers for water softening? -- same thing). The mobile phase is some liquid, e.g., may be water, a buffer, an organic solvent or a mixture of organic/aqueous solvents.

Where are these phases? They are kept in a column, a tube made out of glass, metal or non-reactive plastic. A simple column consists of a glass tube that has a ceramic frit or a plug of angel hair in the bottom of the tube, just above the exiting tip: The graphic at right illustrates and labels a simple chromatography column.



How does this simple column work? In general, a sample in liquid form (may be a vapor if gas chromatography is used -- here we are restricting our discussion to liquid samples) is added to the top of the wet column. The pinch clamp is opened just enough to allow the mobile phase to migrate into the stationary phase (known as packing). The pinch clamp is then closed and solvent is carefully added to the top of the column. The pinch clamp is then opened and the mobile phase is allowed to leave the column, i.e., the mobile phase is eluted. The eluant is collected in fixed, pre-determined volumes (aliquots) for later analysis. Solvent is continuously added so that the column never runs dry -- this may cause cracking of the column, which disrupts the whole process.

Reverse Phase Chromatography (RPC)

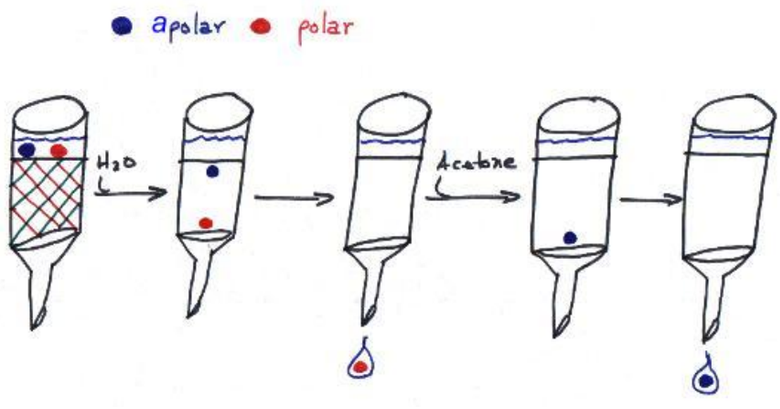
In the case of RPC, a hydrophobic bonded packing phase (stationary phase) is combined with a hydrophilic mobile phase. The bonded phase is stable over a pH range of 2-7.5. The stationary phase is typically a silicate with a C-18, C-8 or C-2 functional group attached to it. The mobile

phase is either a graduated or 100% aqueous solvent. As a rule, hydrophilic particles partition into the solvent and will elute first. Apolar compounds partition into the stationary phase and have longer retention times (time of being "bound" to the packing material). The retention times of both kinds of compounds may be altered by using appropriate solvent mixtures. The table, below, provides some introductory RPC applications:

Polarity of Particles	Particles	Stationary Phase	Solvent	Polarity of Solvent
Low	Aliphatic hydrocarbon soluble	C-18	Aqueous methanol	High
Moderate	Methyl ethyl ketone soluble	C-8	Aqueous acetonitrile	Moderate
High	Lower alcohol soluble	C-2	Aqueous dioxane	Low

As one might expect, water is the weakest eluent in RPC. Typically, RPC is accomplished with a pump that pushes the mobile phase through the column. The pump may be mechanical and adjustable -- this is called RP-HPLC (Reverse Phase-High Pressure [or Performance] Liquid Chromatography). The pump may be as simple as a syringe placed onto a Sep-Pak™ from Waters, **figure at right**:

This is also RPC, but lacks the finesse to be called RP-HPLC. The figure, below, illustrates simple RPC on a column:



Note the application of the organic solvent following the aqueous solvent.

This experiment takes advantage of the fact that a simple water flavoring (Grape Kool-Aid) contains compounds (dyes, flavorings) of differing polarities. These compounds may be easily separated on C-18 (this is an 18-carbon hydrocarbon chain that is bonded to the silicate) columns in the form of Sep-Paks™ attached to syringes.

Materials and Methods

Supplies		
Grape Kool Aid powder	1-Sep-Pak	Ethanol
Methanol	1-10 mL Syringe	Acetone
Isopropyl alcohol	9 beakers (100-150 mL beakers work very well)	2-5 cuvets
Water	Scanning UV-Vis Spectrophotometer	

Methods

Groups will be assigned by the faculty member. Groups will be assigned to use differing solutions of different solvents. The solvents and concentrations needed are as follow:

Methanol	Ethanol	Isopropyl alcohol	Acetone
5%	5%	5%	5%
20%	20%	20%	20%
60%	60%	60%	60%
100%	100%	100%	100%

Each group needs to make 100 mL of the each of the assigned alcohol solutions, i.e., one group makes dilutions of methanol, another of ethanol, another of 2-propanol, another of acetone. Concurrently, a student volunteer will pour approximately half an envelope of the powdered Kool Aid into roughly 900 mL water and stir thoroughly.

Once all alcohol solutions are made and the Kool Aid is mixed, follow these steps:

1. Remove the plunger from the 10 mL syringe.
2. Attach the syringe barrel to the Sep-Pak.
3. Pour about 10 mL of your 100% solvent into the syringe while holding it over the sink.
4. Put the plunger back in the syringe and slowly push the solvent through the Sep-Pak.
5. When you have pushed all of the solvent through the Sep-Pak, remove the syringe from the Sep-Pak.
6. Pull the plunger back to the catch.
7. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink.

8. Remove the Syringe from the Sep-Pak.
9. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
10. Pour about 10 mL of water into the syringe while holding it over the sink.
11. Put the plunger back in the syringe and slowly push the water through the Sep-Pak.
12. When you have pushed all of the water through the Sep-Pak, remove the syringe from the Sep-Pak.
13. Pull the plunger back to the catch.
14. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink.

15. Remove the Syringe from the Sep-Pak.
16. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
17. Add about 3 mL of the Kool Aid solution to the syringe while holding it over your first beaker.
18. Put the plunger back in the syringe and slowly push the Kool Aid through the Sep-Pak.
19. When you have pushed all of the Kool Aid through the Sep-Pak, remove the syringe from the Sep-Pak.
20. Pull the plunger back to the catch.
21. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink.

22. Remove the Syringe from the Sep-Pak.
23. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
24. Add about 10 mL of water to the syringe while holding it over the sink.
25. Put the plunger back in the syringe and slowly push the water through the Sep-Pak.
26. When you have pushed all of the water through the Sep-Pak, remove the syringe from the Sep-Pak.
27. Pull the plunger back to the catch.
28. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink. .

29. Remove the Syringe from the Sep-Pak.
30. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
31. Add about 10 mL of your 5% solvent to the syringe while holding it over your second beaker.
32. Put the plunger back in the syringe and slowly push the 5% solvent through the Sep-Pak.
33. When you have pushed all of the 5% solvent through the Sep-Pak, remove the syringe from the Sep-Pak.
34. Pull the plunger back to the catch.
35. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink.

36. Remove the Syringe from the Sep-Pak.
37. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
38. Add about 10 mL of your 20% solvent to the syringe while holding it over your third beaker.
39. Put the plunger back in the syringe and slowly push the 20% solvent through the Sep-Pak.
40. When you have pushed all of the 20% solvent through the Sep-Pak, remove the syringe from the Sep-Pak.
41. Pull the plunger back to the catch.
42. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink. You may have to repeat Steps 36-42, 3-6 times to collect all of this fraction.

43. Remove the Syringe from the Sep-Pak.
44. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
45. Add about 10 mL of your 60% solvent to the syringe while holding it over your fourth beaker.
46. Put the plunger back in the syringe and slowly push the 60% solvent through the Sep-Pak.
47. When you have pushed all of the 60% solvent through the Sep-Pak, remove the syringe from the Sep-Pak.
48. Pull the plunger back to the catch.
49. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink. This ought to clear the Sep-Pak, if it doesn't make one more pass with your 60% solvent.
50. To complete this part of the experiment,

51. Remove the Syringe from the Sep-Pak.

52. Pull the plunger all the way out of the syringe and re-attach the syringe barrel to the Sep-Pak.
53. Add about 10 mL of your 100% solvent to the syringe while holding it over the sink.
54. Put the plunger back in the syringe and slowly push the 100% solvent through the Sep-Pak.
55. When you have pushed all of the 100% solvent through the Sep-Pak, remove the syringe from the Sep-Pak.
56. Pull the plunger back to the catch.
57. Put the syringe back on the Sep-Pak and blow air through the Sep-Pak -- you may do this over the sink.
58. Store the Sep-Pak and syringe as instructed.

When everyone has completed this part of the experiment, take your test tube racks with your test tubes back to the scanning UV-Vis spectrophotometer. Your instructor will assist you in obtaining visible spectral scans of the colored solutions you obtained by this separatory method.

Questions

Complete these questions on separate paper and attach to the lab for turn-in.

1. Which dye is more polar? Apolar? How do you know?
2. Describe the colored solutions you obtained during the separation.
3. Using what you learned in CHEM 121 (and in your current pre-lab reading) about spectroscopy, what conclusions can you draw about the color of the solutions and the absorbance scans you obtained of each solution?
4. Speculate as to one way in which Sep-Paks might be used in either the clinical or research laboratory.
5. Based on the class' results, which solvent is the best solvent for the separation of the two dyes?

Sources

1. California Lutheran University's Enriched Science (CLUES) Program: High Performance Liquid Chromatography, Versions A & B.
2. Waters Corporation.

This page reconstructed 11 August 2008, 1549 hours, PDT.