Introduction to Chromatography

Learning Objectives

Students should be able to:

Content

- Relate the separation process to features of the chromatogram, such as retention time and resolution.

- Explain how the chemical interactions between a solute and both the stationary phase and the mobile phase impact retention.

- Predict the elution order for a set of compounds given the mobile and stationary phase composition.

Process

- Relate molecular interactions and macroscale processes (Critical Thinking).

Prior knowledge

- An understanding of intermolecular forces at the general chemistry level.

Further Reading

- Harris, D.C. 2010. *Quantitative Chemical Analysis,* 8th Edition, W.H. Freeman: USA, Section 22-2, p.542-3.

- Skoog, D.A., F.J. Holler, S.R. Crouch, 2007. *Principles of Instrumental Analysis,* 6th Edition, Thomson/ Brooks-Cole, 26A-B, pp.763-768.

Authors

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Consider this…

In the experiment shown below in Figure 1, a glass column is packed with a solid material suspended in solvent. The solid material is called the stationary phase and the solvent is the mobile phase. Initially the same amounts of solutes A and B are added to the top of the column as shown in (a) below. As time progresses as shown in (b)-(e) solvent is allowed to flow through the column.

Figure 1 *Diagram showing a chromatographic separation of a mixture of solutes A and B.*
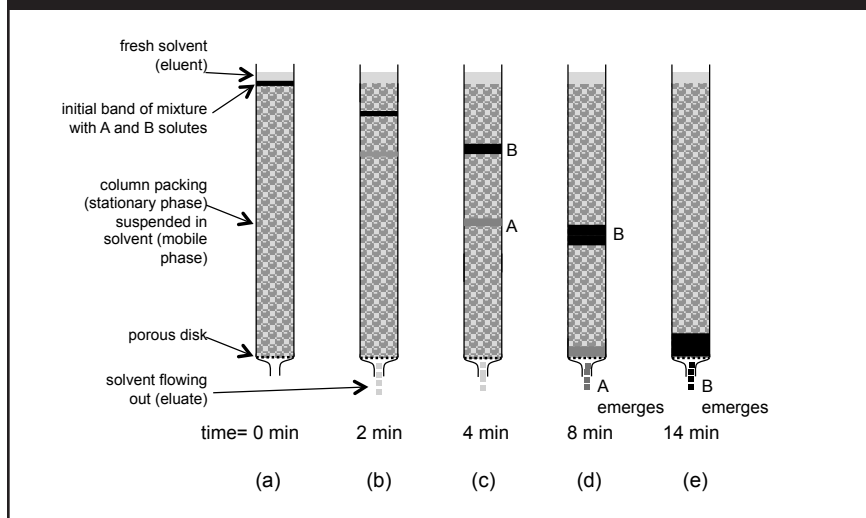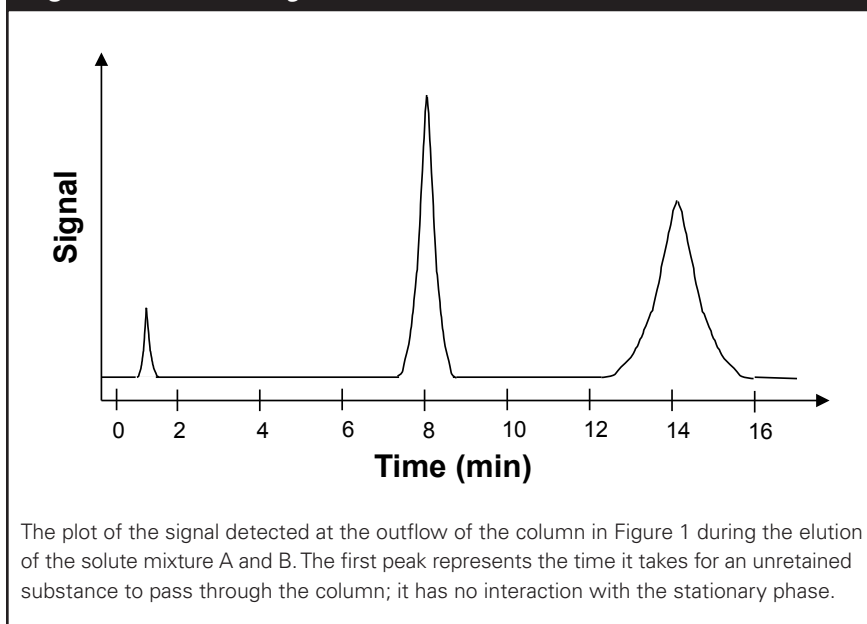

Key Questions

1. In Figure 1a, solutes A and B are mixed together in the initial band. As individuals, list differences observed as time progresses from (a)-(d). Compare your lists within the group. Develop a comprehensive list for the group.

The **retention time** (t_r) for a solute is the time needed after the mixture is placed on the column until that component emerges from the column. The retention factor (k') is the ratio of the time a solute spends in the stationary phase to the time spent in the mobile phase. Retention time for an unretained solute is the void time (t_m).

2. Using the elution times from Figure 1, what are the retention times for solutes A and B?

Consider this…

Figure 2 *A chromatogram.*

The plot of the signal detected at the outflow of the column in Figure 1 during the elution of the solute mixture A and B. The first peak represents the time it takes for an unretained substance to pass through the column; it has no interaction with the stationary phase.

Key Questions

3. Which peak on the chromatogram corresponds to solute A and which peak corresponds to solute B? Label each peak as either A or B. Does your group agree with these labels?

4. The retention time determination should be consistent from person to person. From the chromatogram what part of the peak should be used to give the most consistent retention times? Explain your reasoning to your group.

5. Looking at the chromatogram, determine the void time for this column.

6. The void time is the same for all the solutes in this chromatogram. Discuss with your group why the void time is equal to the time the solutes spend in the mobile phase. Record your agreed upon explanation.

7. If the retention time of a solute is the time the solute spends in both the mobile phase and the stationary phase, how could you determine the time the solute spends in only the stationary phase?

8. Write an equation to show how the retention factors for each solute are determined from the chromatogram in Figure 2 and calculate the retention factors for solutes A and B. Note that the retention factor is a unitless number.

9. What is the relationship between the broadness of the peaks A and B in Figure 2 and their bandwidths shown in Figure 1?

10. Describe the relationship between the retention factor and characteristics of the peaks on the chromatogram such as retention time and peak broadness.

In chromatography the term resolution (R_s) is used to express the quality of a separation between two peaks.

11. Look at Figure 2 and discuss with your group how changes in retention time and the broadness of peaks A and B would influence R_s. Write a summary of your group consensus below.

12. Resolution can be computed by dividing the difference in retention times between the two peaks by their average width along the baseline. If a value of R_s=1.50 represents baseline resolution between two peaks in time units, will the R_s value for the separation shown in Figure 2 be greater than or less than 1.50? Briefly explain your answer, and then verify it with a quick calculation by estimating these values.

13. Based on your understanding of resolution, speculate as to how a chemist might improve the resolution of two chromatographic peaks.

Consider this…

Figure 3
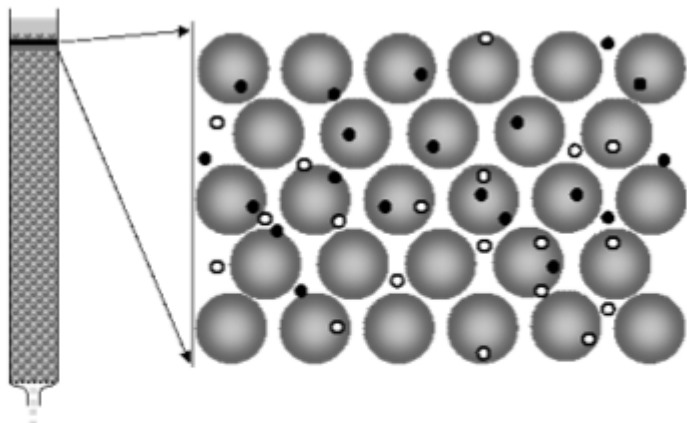
This is a snapshot microscopic view of individual solute molecules A (open circles) and B (black circles) in the vicinity of a solid stationary phase porous spherical particle (silica, SiO2 with a large number of surface Si-OH groups) in a column. Those solute molecules touching a stationary phase particle should be considered in the stationary phase. The mobile phase (hexane, C_6H_{14}) in which the solute is dissolved is not shown.

Key Questions

14. Examine the distribution of solutes A and B between the mobile phase and the stationary phase. Which solute has more molecules in the mobile phase?

15. This distribution of the solute molecules is similar to a partitioning between the stationary phase and the mobile phase. We can define a distribution constant (K) as the ratio of the concentration of the solute in phase 2 to the concentration in phase 1. In chromatography the mobile phase is considered phase 1 and the stationary phase is phase 2. Write the distribution constant expressions for solute A (K_A) and B (K_B).

16. Using the number of solute molecules in each phase in Figure 3, quickly approximate the distribution constants for solutes A and B.

17. If the interaction between the mobile phase and the solute is stronger than the interaction between the stationary phase and the solute, which phase do solute molecules partition preferentially into, the mobile or stationary phase? One group member should explain his/her reasoning to the group.

> **Keep in mind that Figure 3 is a snapshot as if all movement magically stopped in the column. Obviously, this does not occur during the separation process. Remember that this is a dynamic process and solute molecules are constantly moving between the mobile phase and the stationary phase. In addition, the process of separation is not an equilibrium process.**

18. The solute will move through the column only if it is in which phase?

19. If a solute molecule partitions preferentially into the mobile phase, would you expect it to have a relatively long or short retention time? Do the relative values of K_A and K_B in KQ 16 support your conclusion? Explain.

20. Re-read the description of the mobile phase and stationary phase in Figure 3. Describe the mobile and stationary phases as polar or non-polar.

21. Discuss with your group whether solute A or B is the most polar, given that Solute B is retained longer on the column. Once you have reached a consensus, describe your group's reasoning below.

22. Discuss and explain in complete grammatically correct sentences your group's consensus of why Solute A elutes before solute B.

23. How confident are you in predicting the elution order of compounds in a separation? If you are not confident, what additional questions do you have?

24. What is one way this activity improved your ability to visualize the microscopic separation processes embedded in the appearance of a chromatogram?

Applications

25. In the experiment illustrated in Figures 1-3, which of these variables could change the elution order? Explain your reasoning.

Mobile phase composition   Length of column   Solvent flow rate
Solute composition   Stationary phase composition

26. Suppose you wish to decrease the amount of time for all the peaks to elute but continue to completely separate the compounds in Figure 2. Which of the following variables would affect this change? Explain your reasoning.

Mobile phase composition   Length of column   Solvent flow rate
Solute composition   Stationary phase composition

27. Which of the variables you identified in Q26 would be easiest to change and give the desired effect? Explain your reasoning.

28. Suppose you repeat the experiment in Figures 1 and 2 with the same mobile phase and stationary phase, but using methanol and cyclohexane as solutes. Which solute would elute first? Explain your reasoning.

29. In the following chromatogram, a mixture of pyridine (1), phenol (2), and toluene (3) was separated. Determine the retention times of the components. Given this order of elution, would you expect the stationary phase to be polar or non-polar? Explain why the toluene peak is much shorter than the other two peaks even though it is approximately the same concentration. (Chromatogram from Grace Davidson Discovery Sciences (Alltech))
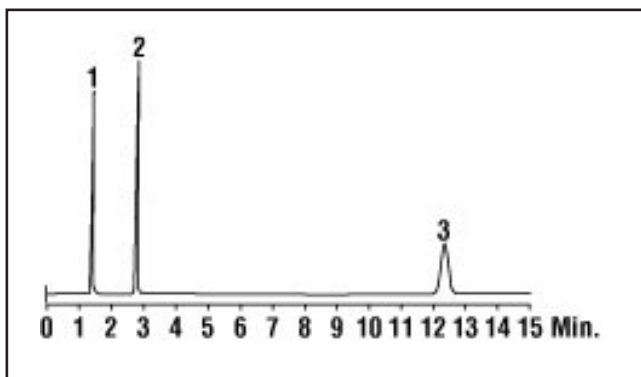
30. Stationary phases come in different varieties. Which of the two analytes listed for each stationary phase has the greater retention time (or is retained more)? Explain your answer.

Stationary Phase	Retention mechanism	Analytes
Polar Solid	adsorption	H_2O and benzene
Nonpolar Liquid	solubility	H_2O and benzene
Ionic	electrostatic interaction	Na^+ and Mg^{2+}
Porous solid	pore penetration	ethylene and polyethylene