

CHAPTER 28

- 28-1. (a) Species that are somewhat volatile and thermally stable.
- (b) Nonpolar low to moderate molecular mass organics and particularly isomeric organic species.
- (c) Molecular species that are nonvolatile or thermally unstable.
- (d) Most low to moderate molecular mass organic compounds that are nonvolatile or thermally unstable.
- (e) Substances that are ionic or that can be derivatized to form ions.
- (f) High molecular mass compounds that are soluble in nonpolar solvents.
- (g) Low molecular mass nonpolar gases.
- (h) High molecular mass hydrophilic compounds.
- (i) Small organic and inorganic ions.
- 28-2. Three methods for improving resolution include:
- (1) Adjustment of k_A and k_B by employing a multicomponent mobile phase and varying the ratio of the solvents to find an optimal mixture.
- (2) Variation in the chemical composition of the solvent system in such a way as to make α larger.
- (3) Employing a different packing in which α is greater.
- 28-3. In partition chromatography, k is conveniently varied by using a two (or more) component solvent system and varying the ratio of the solvents.
- 28-4. (a) In gas chromatography, α is generally varied by varying the column packing.

- (b) in LC, both column packing and chemical composition of the mobile phase can be varied to yield better α values.
- 28-5. In adsorption chromatography on an alumina packing, it is generally best to increase the polarity of the mobile phase as the elution proceeds. Thus the ratio of acetone to hexane should be increased as the elution proceeds.
- 28-6. The linear response range of a detector is the range of analyte concentration or mass over which the detector responds linearly. It is the same as the dynamic range defined in Section 1E-2.
- 28-7. (a) In an *isocratic elution*, the solvent composition is held constant throughout the elution.
- (b) In a *gradient elution*, two or more solvents are used and the composition of the mobile phase is changed continuously or in steps as the separation proceeds.
- (c) In a *stop-flow* injection, the flow of solvent is stopped, a fitting at the head of the column is removed, and the sample is injected directly onto the head of the column. The fitting is then replaced and pumping is resumed.
- (d) A *reversed-phase packing* is a nonpolar packing that is used in partition chromatography with a relatively polar mobile phase.
- (e) In a *normal-phase packing*, the stationary phase is polar and the mobile phase is relatively nonpolar.
- (f) In *ion-pair chromatography* a large organic counter-ion is added to the mobile phase as an ion-pairing reagent. Separation is achieved either through partitioning of the neutral ion-pair or as a result of electrostatic interactions between the ions in solution and charges on the stationary phase resulting from adsorption of the organic counter-ion.

- (g) In *ion chromatography*, the stationary phase is an ion-exchange resin, and detection is ordinarily accomplished by a conductivity detector.
- (h) A *bulk property detector* responds to some property of the mobile phase (such as thermal or electrical conductivity) that is altered by the presence of analytes.
- (i) A *solute property detector* responds to some property of analytes, such as absorption or fluorescence.
- (j) *Sparging* is a process for removing dissolved gases from a solution by sweeping the liquid with a stream of fine bubbles of an inert gas of low solubility.
- 28-8. A *guard column* is a short column through which the mobile phase flows before it reaches the injection region and the analytical column in HPLC instruments. The composition of the guard column is similar to that of the analytical column except that the particles are generally larger to minimize pressure drop. The purpose of the guard column is to remove particulate matter and contaminants from the mobile phase and to saturate the mobile phase with the stationary phase so that losses of that phase from the analytical column are minimized.
- 28-9. Normal-phase partition chromatography and adsorption chromatography are similar in the respect that the stationary phases in both are polar, whereas the mobile phases are relatively nonpolar.
- 28-10. (a) diethyl ether, benzene, *n*-hexane
(b) acetamide, acetone, dichloroethane
- 28-11. (a) ethyl acetate, dimethylamine, acetic acid
(b) hexane, propylene, benzene, dichlorobenzene

- 28-12. In *adsorption chromatography*, separations are based on adsorption equilibria between the components of the sample and a solid surface. In *partition chromatography*, separations are based on distribution equilibria between two immiscible liquids.
- 28-13. In *size-exclusion chromatography* separations are based upon the size, and to some extent the shape, of molecules with little interactions between the stationary phase and the sample components occurring. In *ion-exchange chromatography*, in contrast, separations are based upon ion-exchange reactions between the stationary phase and the components of the sample in the mobile phase.
- 28-14. Nonvolatile and thermally unstable compounds can be separated by HPLC by not GC.
- 28-15. *Pneumatic pumps* are simple, inexpensive and pulse free. They consist of a collapsible solvent container housed in a vessel that can be pressurized by a compressed gas. This pump has limited capacity and pressure output and is not adaptable to gradient elution. The pumping rate depends on solvent viscosity. *Screw-driven syringe pumps* consist of a large syringe in which the piston is moved by a motor-driven screw. They are pulse free and the rate of delivery is easily varied. They suffer from lack of capacity and are inconvenient when solvents must be changed. *Reciprocating pumps* are versatile and widely used. They consist of a small cylindrical chamber that is filled and then emptied by the back-and-forth motion of a piston. Advantages include small internal volume, high output pressures, adaptability to gradient elution, and constant flow rates that are independent of viscosity and back pressure. The pulsed output must be damped.
- 28-16. In *suppressor-column ion chromatography* the chromatographic column is followed by a suppressor column whose purpose is to convert the ions used for elution to molecular species that are largely nonionic and thus do not interfere with conductometric detection

of the analyte species. In *single-column ion chromatography*, low capacity ion exchangers are used so that the concentrations of ions in the eluting solution can be kept low. Detection then is based on the small differences in conductivity caused by the presence of eluted sample components.

28-17. A gas-phase sample is needed for mass spectrometry. The output of the LC column is a solute dissolved in a solvent, whereas the output of the GC column is a gas and thus directly compatible. As a first step in LC/MS, the solvent must be vaporized. When vaporized, however, the LC solvent produces a gas volume that is 10-1000 times greater than the carrier gas in GC. Hence, most of the solvent must also be removed.

28-18. Comparison of Table 28-1 with Table 27-1 suggests that the GC detectors that are suitable for HPLC are the mass spectrometer, FTIR and possibly photoionization. Many of the GC detectors are unsuitable for HPLC because they require the eluting analyte components to be in the gas-phase.

28-19. A number of factors that influence separation are clearly temperature dependent including distribution constants and diffusion rates. In addition, temperature changes can influence selectivity if components A and B are influenced differently by changes in temperature. Because resolution depends on all these factors, resolution will also be temperature dependent

(a) For a reversed phase chromatographic separation of a steroid mixture, selectivity and, as a consequence, separation could be influenced by temperature dependent changes in distribution coefficients.

(b) For an adsorption chromatographic separation of a mixture of isomers, selectivity and,

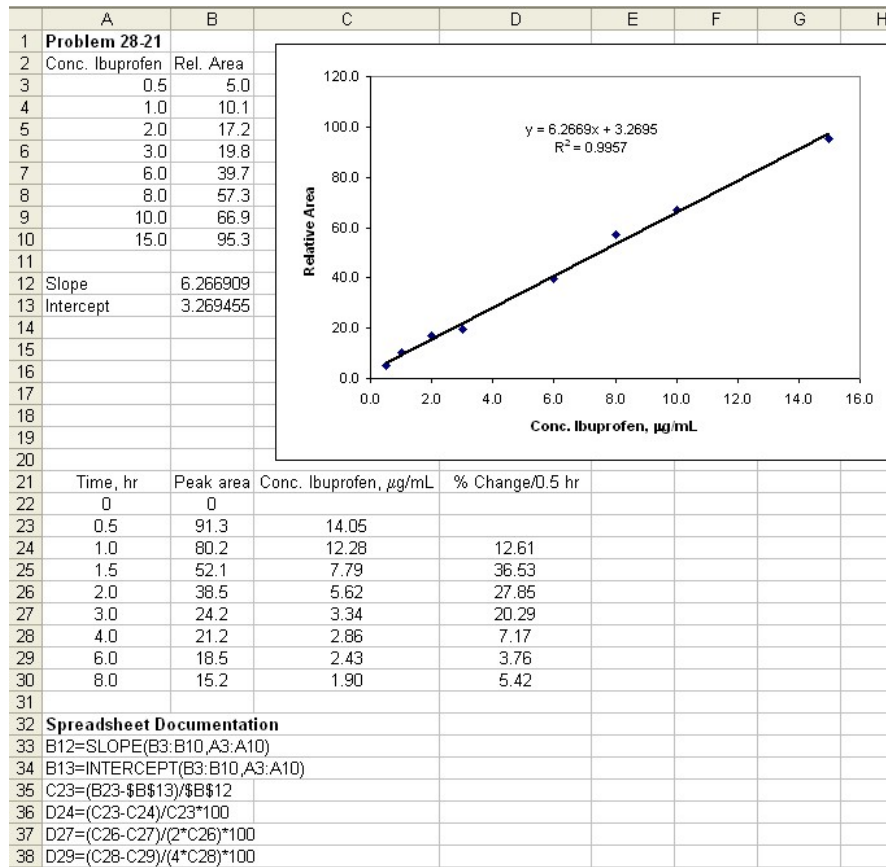
as a consequence, separation could be influenced by temperature dependent changes in distribution coefficients.

28-20.

	A	B	C	D	E
1	Problem 28-20				
2	t_{R1}	9.00			
3	t_{R2}	9.25			
4	t_M	1.083333			
5	α	1.031579			
6	k_2	7.538462			
7			R_s	N	
8			0.50	5476	
9			0.75	12321	
10			0.90	17742	
11			1.0	21904	
12			1.10	26504	
13			1.25	34225	
14			1.50	49284	
15			1.75	67081	
16			2.0	87616	
17			2.5	136900	
18	Spreadsheet Documentation				
19	B3=9+15/60				
20	B4=65/60				
21	B5=(B3-B4)/(B2-B4)				
22	B6=(B3-B4)/B4				
23	D8=16*C8^2*(B5/(B5-1))^2*((1+B6)/B6)^2				

If the second peak were twice as broad as the first, R_s and N would be smaller.

28-21.



From the spreadsheet the largest percentage loss occurs between 1.0 and 1.5 hrs.

28-22. For a normal-phase packing, Equation 28-3 applies. That is

$$\frac{k_2}{k_1} = 10^{(P'_1 - P'_2)/2}$$

where P'_1 and P'_2 are the polarity indexes of chloroform and *n*-hexane respectively.

(a) $k_1 = (29.1 - 1.05)/1.05 = 26.7$ (Equation 26-12)

(b) $P'_{AB} = 0.50 \times 4.1 + 0.50 \times 0.1 = 2.10$ (Equation 28-2)

Substituting into the equation for k_2/k_1 gives

$$\frac{10}{26.7} = 10^{(2.1 - P_2')/2}$$

Taking the logarithm of both sides of this equation gives

$$\log \frac{10}{26.7} = -0.427 = (2.1 - P_2')/2$$

$$P_2' = 2 \times 0.427 + 2.1 = 2.95$$

Substituting P_2' for P_{AB}' in Equation 28-2 gives

$$2.95 = \phi_A \times 4.1 + \phi_B \times 0.1$$

where $\phi_A + \phi_B = 1.00$

$$2.95 = 4.1\phi_A + 0.1(1.00 - \phi_A)$$

$$\phi_A = (2.95 - 0.1)/4.0 = 0.712$$

Thus the mixture should be 71% CHCl_3 and 29% *n*-hexane.