respectively. Use a spreadsheet to prepare the calibration curve and calculate the concentration of calcium in the sample, and the standard deviation of the concentration.

Review Equations 16.18 to 16.21 and the spreadsheet that follows those in Chapter 16 for the error analysis. In addition to the statistics described there, we need the standard error in x, S_x:

$$S_x = \frac{S_r}{m}\sqrt{\frac{1}{N} + \frac{(y_c - y_{ave})^2}{m^2 S_{xx}}} \tag{17.2}$$

Note the similarity of this to Equation 16.21. The value of y_c is zero (the volume of the intercept). We use this equation to calculate the standard deviation of the concentration of the unknown:

$$S_c = -\frac{C_x S_x}{b_v} = -\frac{C_x S_x}{-b/m} \tag{17.3}$$

We place a minus sign in front since the intercept is a negative number, and this gives a positive number for S_c.

The total absorbance, A_t, is the sum of the absorbance by the sample and that due to the added standard:

$$A_t = A_x + A_s \tag{17.4}$$

$$A_t = kC_x\frac{V_x}{V_t} + kC_s\frac{V_s}{V_t} \tag{17.5}$$

where V_x is the volume of the unknown (25.0 mL), V_s the volume of added standard, V_t the total volume (50.0 mL), C_x the unknown concentration, C_s the standard concentration (2.50 ppm), and k a porportionality constant. Since V_t is constant,

$$A_t = k'C_xV_x + k'C_sV_s \tag{17.6}$$

where $k' = k/V_t$. A plot of A_t vs. V_s for a series of solutions should give a straight line with slope $k'C_s$ and intercept of $k'C_xV_x$:

$$m = k'C_s \tag{17.7}$$

$$b = k'C_xV_x \tag{17.8}$$

Combining,

$$C_x = \frac{bC_s}{mV_x} \tag{17.9}$$

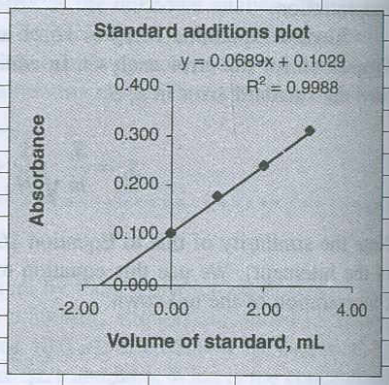
The volume intercept is

$$\frac{-b}{m} = -\frac{C_xV_x}{C_s} \tag{17.10}$$

$$C_x = -\frac{C_s}{V_x}\cdot\frac{-b}{m} \tag{17.11}$$

We use the spreadsheet to calculate b and m, and from these, C_x.

	A	B	C	D	E	F	G	H
1	Multiple standard additions plot and calculation.							
2	Vol. Unk., V_x =	25.0	mL					
3	Conc. Std., C_s =	2.50	ppm					
4	**V_s, mL**	**A_t**						
5	0.00	0.101						
6	1.00	0.176						
7	2.00	0.238						
8	3.00	0.310						
9								
10	Slope, m:		**0.0689**					
11	Intercept, b:		**0.1029**					
12	Vol. Intercept, b_v (-b/m):		**-1.49347**					
13	**C_x, ppm:**		**0.149347**					
14								
15	S_r:		**0.003788**					
16	N:		**4**					
17	S_{xx}:		**5**					
18	y_{ave}:		**0.21**					
19	Std. devn.in vol. = S_v:		**0.078569**					
20	**Std. Devn in $C_x = S_c$:**		**0.007857**					
21	**Cell C10** = SLOPE(B5:B8,A5:A8)							
22	**Cell C11** =INTERCEPT(B5:B8,A5:A8)							
23	**Cell C12** volume intercept (b_v) = -b/m =-C10/C11							
24	**Cell C13** = Unk. concn. = C_x = -(C_s/V_x) x (b_v) = -(B3/B2)*(C12)							
25	**Cell C15** = S_r = STEYX(B5:B8,A5:A8)							
26	**Cell C16** = N = COUNT(B5:B8)							
27	**Cell C17** = S_{xx} = N*VARP(A5:A8) = C16*VARP(A5:A8)							
28	**Cell C18** = y_{ave} = AVERAGE(B5:B8)							
29	**Cell C19** = Std. devn. in vol. = S_r/m(1/N + (($0-y_{ave}$)^2)/((m^2) x S_{xx}))$^{1/2}$							
30	=C15/C10*SQRT(1/C16+((0-C18)^2)/((C10^2)*C17))							
31	**C20** = Std. devn.unk. = S_c = -(C_x x S_v)/b_v							
32	=[-(C13*C19/C12)]							



The concentration is 0.149 ± 0.008 ppm. Note that the volume intercept corresponding to the unknown is −1.49 mL. This corresponds to the addition of 1.49 mL of the 2.50 ppm standard to 50 mL, a 1:33.6 dilution that gives a concentration of 0.0745 ppm in the sample flask. The 25.0-mL sample was diluted twofold, so the sample concentration is 2 × 0.0745, or 0.149 ppm, as calculated in cell C13:

$$-(C_s/V_x)(-b/m) = -(2.5/25)(-1.49)$$

Or, we calculate that 1.49 mL × 2.50 μg/mL = 3.72 μg/50 mL = 0.0745 μg/mL in the sample flask (0.0745 ppm), corresponding to 0.149 ppm in the original sample.

Suppose we didn't dilute to 50 mL after adding the standard aliquots. Then V_t would not be constant but must be treated as a variable. For n spike additions, the total spike volume, V_{S_n}, is

$$V_{S_n} = \sum_{i=1}^{n} V_{S_i} \tag{17.12}$$

So after two spike additions, for example, $V_{S_n} = V_{S_1} + V_{S_2}$, which for the above example is 1.00 + 2.00 = 3.00 mL. The total volume, V_{T_n}, after n spike additions, is

$$V_{T_n} = V_x + V_{S_n} = V_x + \sum_{i=1}^{n} V_{S_i} \tag{17.13}$$

In the above example, after two spike additions, V_{T_n} is 25.0 + 3.00 = 28.0 mL.

We can write an equation similar to Equation 17.5 for the total absorbance, A_{T_n}, for n spike additions:

$$A_{T_n} = kC_x\frac{V_x}{V_{T_n}} + kC_s\frac{V_{S_n}}{V_{T_n}} \quad (17.14)$$

where C_x is the unknown concentration in the original sample volume and C_s is the spike concentration in its stock volume. V_{T_n} is a variable, known for each spike. Multiplying by V_{T_n},

$$A_{T_n}V_{T_n} = kC_xV_x + kC_sV_{S_n} \quad (17.15)$$

Compare with Equation 17.6. A plot of $A_{T_n}V_{T_n}$ vs. V_{S_n}, with n being the independent variable, gives a straight line with slope kC_s and intercept kC_xV_s:

$$m = kC_s \quad (17.16)$$

$$b = kC_xV_x \quad (17.17)$$

Combining, we arrive at Equation 17.9, and subsequently Equations 17.10 and 17.11, for calculating the unknown concentration. We set up a spreadsheet similar to the one above, but plotting $A_{T_n}V_{T_n}$ vs. V_{S_n}, instead of A_t vs. V_s. The plotting of variable total volume is a bit more complicated than just plotting V_s, but it avoids an experimental step of diluting to volume. The spreadsheet can be set up to automatically calculate V_{T_n} from V_x and V_{S_n}, and from this, $A_{T_n}V_{T_n}$.

Learning Objectives

WHAT ARE SOME OF THE KEY THINGS WE LEARNED FROM THIS CHAPTER?

- Flame emission spectrometry, p. 522
- Distribution of atoms in a flame (key equation: 17.1), p. 524
- Atomic absorption spectrometry—flame and electrothermal, p. 525
- Internal standard and standards addition calibration—using spreadsheets (key equations 17.5, 17.11), pp. 533, 534

Questions

PRINCIPLES

1. What fraction of atoms in a flame are typically in the excited state?
2. Describe the principles of flame emission spectrometry and of atomic absorption spectrophotometry.
3. Compare flame emission and atomic absorption spectrophotometry with respect to instrumentation, sensitivity, and interferences.
4. Why is a sharp-line source desirable for atomic absorption spectroscopy?
5. Explain why flame emission spectrometry is often as sensitive as atomic absorption spectrophotometry, even though only a small fraction of the atoms may be thermally excited in the flame.

6. The Maxwell–Boltzmann expression predicts that the fraction of excited-state atoms in a flame is both highly temperature dependent and wavelength dependent, while the fraction of ground-state atoms remains large in all cases. Yet flame emission and atomic absorption spectrometry in practice do not exhibit large differences in dependence for many elements if the wavelength is greater than about 300 nm. Why is this?
7. Explain why absorption spectra for atomic species consist of discrete lines at specific wavelengths rather than broad bands for molecular species.
8. What causes the red feather in a reducing nitrous oxide–acetylene flame?
9. Explain why electrothermal atomizers result in greatly enhanced sensitivity in atomic absorption spectrophotometry.
10. Explain why an internal-standard element can improve the precision of atomic spectrometry measurements.

INSTRUMENTATION

11. Explain the mechanism of operation of a hollow-cathode lamp.
12. Describe the premix chamber burner. What flames can be used with it?
13. Explain why the radiation source in atomic absorption instruments is usually modulated.

INTERFERENCES

14. Lead in seawater was determined by atomic absorption spectrophotometry. The APCD (ammonium pyrrolidinecarbodithioate) chelate was extracted into methylisobutyl ketone and the organic solvent was aspirated. A standard and reagent blank were treated in a similar manner. The blank reading was essentially zero. Measurements were made at the 283.3-nm line. An independent determination using anodic-stripping voltammetry revealed the atomic absorption results to be high by nearly 100%. Assuming the anodic-stripping voltammetry results are correct, suggest a reason for the erroneous results and how they might be avoided in future analyses.
15. Why is a high-temperature nitrous oxide–acetylene flame sometimes required in atomic absorption spectrophotometry?
16. Why is a high concentration of a potassium salt sometimes added to standards and samples in flame absorption or emission methods?
17. Chemical interferences are more prevalent in "cool" flames such as air–propane, but this flame is preferred for the determination of the alkali metals. Suggest why.
18. An analyst notes that a 1-ppm solution of sodium gives a flame emission signal of 110, while the same solution containing also 20 ppm potassium gives a reading of 125. It was determined that a 20-ppm solution of potassium exhibited no blank reading. Explain the results.

Problems

SENSITIVITY

19. A 12-ppm solution of lead gives an atomic absorption signal of 8.0% absorption. What is the atomic absorption sensitivity?
20. Silver exhibits an atomic absorption sensitivity of 0.050 ppm under a given set of conditions. What would be the expected absorption for a 0.70-ppm solution?

BOLTZMANN DISTRIBUTION

21. The transition for the cadmium 228.8-nm line is a $^1S_0$–$^1S_1$ transition. Calculate the ratio of N_e/N_0 in an air–acetylene flame. What percent of the atoms is in the excited state? The velocity of light is 3.00×10^{10} cm/s, Planck's constant is 6.62×10^{-27} erg-s, and the Boltzmann constant is 1.380×10^{-16} erg K^{-1}.

QUANTITATIVE CALCULATIONS

22. Calcium in a sample solution is determined by atomic absorption spectrophotometry. A stock solution of calcium is prepared by dissolving 1.834 g $CaCl_2 \cdot 2H_2O$ in water and diluting to 1 L. This is diluted 1:10. Working standards are prepared by diluting the second solution, respectively, 1:20, 1:10, and 1:5. The sample is diluted 1:25. Strontium chloride is added to all solutions before dilution, sufficient to give 1% (wt/vol) to avoid phosphate interference. A blank is prepared, to give 1% $SrCl_2$. Absorbance signals on the strip-chart recorder, when the solutions are aspirated into an air–acetylene flame, are as follows: blank, 1.5 cm; standards, 10.6, 20.1, and 38.5 cm; sample, 29.6 cm. What is the concentration of calcium in the sample in parts per million?

23. Lithium in the blood serum of a manic-depressive patient treated with lithium carbonate is determined by flame emission spectrophotometry, using a standard additions calibration. One hundred microliters of serum diluted to 1 mL gives an emission signal of 6.7 cm on the recorder chart. A similar solution to which 10 μL of a 0.010 M solution of $LiNO_3$ has been added gives a signal of 14.6 cm. Assuming linearity between the emission signal and the lithium concentration, what is the concentration of lithium in the serum, in parts per million?

24. Chloride in a water sample is determined indirectly by atomic absorption spectrophotometry by precipitating it as AgCl with a measured amount of $AgNO_3$ in excess, filtering, and measuring the concentration of silver remaining in the filtrate. Ten-milliliter aliquots each of the sample and a 100-ppm chloride standard are added to separate dry 100-mL Erlenmeyer flasks. Twenty-five milliliters of a silver nitrate solution is added to each with a pipet. After allowing time for the precipitate to form, the mixtures are transferred partially to dry centrifuge tubes and are centrifuged. Each filtrate is aspirated for atomic absorption measurement of silver concentration. A blank is similarly treated in which 10 mL deionized distilled water is substituted for the sample. If the following absorbance signals are recorded for each solution, what is the concentration of chloride in the water sample?

Blank:	12.8 cm
Standard:	5.7 cm
Sample:	6.8 cm

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GENERAL

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Chapter Eighteen

SAMPLE PREPARATION: SOLVENT AND SOLID-PHASE EXTRACTION

The next chapter introduces chromatographic techniques for analyzing complex samples, whereby multiple analytes are separated on a column and detected as they emerge from the column. But very often, samples need to be "cleaned up" prior to introduction into the chromatographic column. The techniques of solvent extraction and solid-phase extraction and related techniques are very useful for isolating analytes from complex sample matrices prior to chromatographic analysis. Solvent extraction is also useful for spectrophotometric determination.

Solvent extraction involves the distribution of a solute between two immiscible liquid phases. This technique is extremely useful for very rapid and "clean" separations of both organic and inorganic substances. In this chapter, we discuss the distribution of substances between two phases and how this can be used to form analytical separations. The solvent extraction of metal ions into organic solvents is described.

Solid-phase extraction is a technique in which hydrophobic functional groups are bonded to solid particle surfaces and act as the extracting phase. They reduce the need for large volumes of organic solvents.

18.1 Distribution Coefficient

A solute S will distribute itself between two phases (after shaking and allowing the phases to separate) and, within limits, the ratio of the concentrations of the solute in the two phases will be a constant:

$$K_D = \frac{[S]_1}{[S]_2} \tag{18.1}$$

where K_D is the **distribution coefficient** and the subscripts represent solvent 1 (e.g., an organic solvent) and solvent 2 (e.g., water). If the distribution coefficient is large, the solute will tend toward quantitative distribution in solvent 1.

Neutral organics distribute from water into organic solvents. "Like dissolves like."

Fig. 18.1. Separatory funnel.

The apparatus used for solvent extraction is the **separatory funnel**, illustrated in Figure 18.1. Most often, a solute is extracted from an aqueous solution into an immiscible organic solvent. After the mixture is shaken for about a minute, the phases are allowed to separate and the bottom layer (the denser solvent) is drawn off in a completion of the separation.

Many substances are partially ionized in the aqueous layer as weak acids. This introduces a pH effect on the extraction. Consider, for example, the extraction of benzoic acid from an aqueous solution. Benzoic acid (HBz) is a weak acid in water with a particular ionization constant K_a (given by Equation 18.4). The distribution coefficient is given by

$$K_D = \frac{[\text{HBz}]_e}{[\text{HBz}]_a} \tag{18.2}$$

where e represents the ether solvent and a represents the aqueous solvent. However, part of the benzoic acid in the aqueous layer will exist as Bz^-, depending on the magnitude of K_a and on the pH of the aqueous layer; hence, quantitative separation may not be achieved.

18.2 Distribution Ratio

It is more meaningful to describe a different term, the **distribution ratio**, which is the ratio of the concentrations of *all* the species of the solute in each phase. In this example, it is given by

$$D = \frac{[\text{HBz}]_e}{[\text{HBz}]_a + [\text{Bz}^-]_a} \tag{18.3}$$

We can readily derive the relationship between D and K_D from the equilibria involved. The acidity constant K_a for the ionization of the acid in the aqueous phase is given by

$$K_a = \frac{[\text{H}^+]_a[\text{Bz}^-]_a}{[\text{HBz}]_a} \tag{18.4}$$

Hence,

$$[\text{Bz}^-]_a = \frac{K_a[\text{HBz}]_a}{[\text{H}^+]_a} \tag{18.5}$$

From Equation 18.2,

$$[\text{HBz}]_e = K_D[\text{HBz}]_a \tag{18.6}$$

Substitution of Equations 18.5 and 18.6 into Equation 18.3 gives

$$D = \frac{K_D[\text{HBz}]_a}{[\text{HBz}]_a + K_a[\text{HBz}]_a/[\text{H}^+]_a} \tag{18.7}$$

$$D = \frac{K_D}{1 + K_a/[\text{H}^+]_a} \tag{18.8}$$

This equation predicts that when $[H^+]_a \gg K_a$, D is nearly equal to K_D, and if K_D is large, the benzoic acid will be extracted into the ether layer; D is maximum under these conditions. If, on the other hand, $[H^+] \ll K_a$, then D reduces to $K_D[H^+]_a/K_a$, which will be small, and the benzoic acid will remain in the aqueous layer. That is, in alkaline solution, the benzoic acid is ionized and cannot be extracted, while in acid solution, it is largely undissociated. These conclusions are what we would intuitively expect from inspection of the chemical equilibria.

Equation 18.8, like Equation 18.1, predicts that the *extraction efficiency will be independent of the original concentration of the solute*. This is one of the attractive features of solvent extraction; it is applicable to tracer (e.g., radioactive) levels and to macrolevels alike, a condition that applies only so long as the solubility of the solute in one of the phrases is not exceeded and there are no side reactions such as dimerization of the extracted solute.

In solvent extraction, the separation efficiency is usually independent of the concentration.

Of course, if the hydrogen ion concentration changes, the extraction efficiency (D) will change. In this example, the hydrogen ion concentration will increase with increasing benzoic acid concentration, unless an acid–base buffer is added to maintain the hydrogen ion concentration constant (see Chapter 7 for a discussion of buffers).

In deriving Equation 18.8, we actually neglected to include in the numerator of Equation 18.3 a term for a portion of the benzoic acid that exists as the dimer in the organic phase. The extent of dimerization tends to increase with increased concentration, and by Le Châtelier's principle, this will cause the equilibrium to shift in favor of the organic phase with increased concentration. So, in cases such as this, the efficiency of extraction will actually increase at higher concentrations. As an exercise, derivation of the more complete equation is presented in Problem 12.

18.3 Percent Extracted

The distribution ratio D is a constant independent of the volume ratio. However, the fraction of the solute extracted will depend on the volume ratio of the two solvents. If a larger volume of organic solvent is used, more solute must dissolve in this layer to keep the concentration ratio constant and to satisfy the distribution ratio.

The fraction of solute extracted is equal to the millimoles of solute in the organic layer divided by the total number of millimoles of solute. The millimoles are given by the molarity times the milliliters. Thus, the percent extracted it given by

$$\%\,E = \frac{[S]_o V_o}{[S]_o V_o + [S]_a V_a} \times 100\% \qquad (18.9)$$

where V_o and V_a are the volumes of the organic and aqueous phases, respectively. It can be shown from this equation (see Problem 11) that the percent extracted is related to the distribution ratio by

$$\boxed{\%\,E = \frac{100D}{D + (V_a/V_o)}} \qquad (18.10)$$

If $V_a = V_o$, then

Extraction will be quantitative (99.9%) for D values of 1000.

$$\boxed{\%\,E = \frac{100D}{D + 1}} \qquad (18.11)$$

In the case of equal volumes, the solute can be considered quantitatively retained if D is less than 0.001. It is essentially quantitatively extracted if D is greater than 1000. The percent extracted changes only from 99.5 to 99.9% when D is increased from 200 to 1000.

Example 18.1

Twenty milliliters of an aqueous solution of 0.10 M butyric acid is shaken with 10 mL ether. After the layers are separated, it is determined by titration that 0.5 mol butyric acid remains in the aqueous layer. What is the distribution ratio, and what is the percent extracted?

Solution

We started with 2.0 mmol butyric acid, and so 1.5 mmol was extracted. The concentration in the ether layer is 1.5 mmol/10 mL = 0.15 M. The concentration in the aqueous layer is 0.5 mmol/20 mL = 0.025 M. Therefore,

$$D = \frac{0.15}{0.025} = 6.0$$

Since 1.5 mmol was extracted, the percent extracted is (1.5/2.0) × 100% = 75%. Or

$$\%\ E = \frac{100 \times 6.0}{6.0 + (20/10)} = 75\%$$

Equation 18.10 shows that the fraction extracted can be increased by decreasing the ratio of V_a/V_o, for example, by increasing the organic phase volume. However, a more efficient way of increasing the amount extracted using the same volume of organic solvent is to perform successive extractions with smaller individual volumes of organic solvent. For example, with a D of 10 and $V_a/V_o = 1$, the percent extracted is about 91%. Decreasing V_a/V_o to 0.5 (doubling V_o) would result in an increase of % E to 95%. But performing two successive extractions with $V_a/V_o = 1$ would give an overall extraction of 99%.

18.4 Solvent Extraction of Metals

To extract a metal ion into an organic solvent, its charge must be neutralized, and it must be associated with an organic agent.

Solvent extraction has one of its most important applications in the separation of metal cations. In this technique, the metal ion, through appropriate chemistry, distributes from an aqueous phase into a water-immiscible organic phase. Solvent extraction of metal ions is useful for removing them from an interfering matrix, or for selectively (with the right chemistry) separating one or a group of metals from others. The technique is widely used for the spectrophotometric determination of metal ions since the reagents used to accomplish the extraction often form colored complexes with the metal ion. It is also used in flame atomic absorption spectrophotometry for introducing the sample in a nonaqueous solvent into the flame for enhanced sensitivity, and removal of matrix effects.

The separation can be accomplished in several ways. You have noted above that the uncharged organic molecules tend to dissolve in the organic layer while

the charged anion from the ionized molecules remains in the polar aqueous layer. This is an example of "like dissolves like." Metal ions do not tend to dissolve appreciably in the organic layer. For them to become soluble, their charge must be neutralized and something must be added to make them organiclike. There are two principal ways of doing this.

EXTRACTION OF ION-ASSOCIATION COMPLEXES

In one method, the metal ion is incorporated into a bulky molecule and then associates with another ion of the opposite charge to form an **ion pair**, or the metal ion associates with another ion of great size (organiclike). For example, it is well known that iron(III) can be quantitatively extracted from hydrochloric acid medium into diethyl ether. The mechanism is not completely understood, but evidence exists that the chloro complex of the iron is coordinated with the oxygen atom of the solvent (the solvent displaces the coordinated water), and this ion associates with a solvent molecule that is coordinated with a proton:

$$\{(C_2H_5)_2O{:}H^+,\ FeCl_4[(C_2H_5)_2O]_2^-\}$$

Similarly, the uranyl ion UO_2^{2+} is extracted from aqueous nitrate solution into isobutanol by associating with two nitrate ions (UO_2^{2+}, $2NO_3^-$), with the uranium probably being solvated by the solvent to make it solventlike. Permanganate forms an ion pair with tetraphenylarsonium ion [$(C_6H_5)_4As^+$, MnO_4^-], which makes it organiclike, and it is extracted into methylene chloride. There are numerous other examples of ion-association extractions.

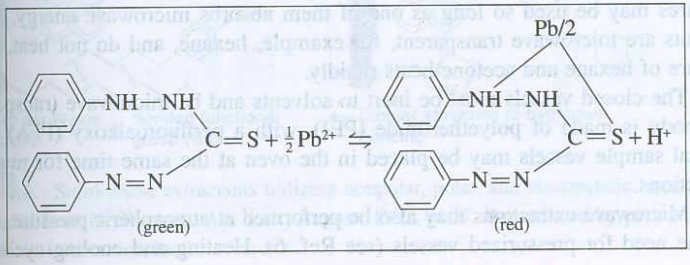
EXTRACTION OF METAL CHELATES

The most widely used method of extracting metal ions is formation of a chelate molecule with an organic chelating agent.

As mentioned in Chapter 9, a chelating agent contains two or more complexing groups. Many of these reagents form colored chelates with metal ions and form the basis of spectrophotometric methods for determining the metals. The chelates are often insoluble in water and will precipitate. They are, however, usually soluble in organic solvents such as methylene chloride. Many of the organic precipitating agents listed in Chapter 10 are used as extracting agents.

EXTRACTION PROCESS FOR METAL CHELATES

Most chelating agents are weak acids that ionize in water; the ionizable proton is displaced by the metal ion when the chelate is formed, and the charge on the organic compound neutralizes the charge on the metal ion. An example is *diphenylthiocarbazone (dithizone)*, which forms a chelate with lead ion:



The usual practice is to add the chelating agent, HR, to the organic phase. It distributes between the two phases, and in the aqueous phase it dissociates as a weak acid. The metal ion, M^{n+}, reacts with nR^- to form the chelate MR_n, which then distributes into the organic phase. The distribution ratio is given by the ratio of the metal chelate concentration in the organic phase to the metal ion concentration in the aqueous phase. The following equation can be derived:

$$D = \frac{[MR_n]_o}{[M^{n+}]_a} = K\frac{[HR]_o^n}{[H^+]_a^n} \tag{18.12}$$

where K is a constant that includes K_a of HR, K_f of MR_n, and K_D of HR and MR_n. Note that the distribution ratio is independent of the concentration of the metal ion, provided the solubility of the metal chelate in the organic phase is not exceeded. HR is often in large excess and is considered constant. The extraction efficiency can be affected only by changing the pH or the reagent concentration. A 10-fold increase in the reagent concentration will increase the extraction efficiency the same as an increase in the pH of one unit (10-fold decrease in $[H^+]$). Each effect is greater as n becomes greater. By using a high concentration of reagent, extraction can be performed in more acid solution.

Chelates of different metals extract at different pH values, some in acid to basic solution, some only in alkaline solution. By appropriate adjustment of pH, selectivity can be achieved in the extraction. Also, judicious use of masking agents, complexing agents that prevent one metal ion from reacting with the chelating agent, can enhance the selectivity.

18.5 Accelerated and Microwave-Assisted Extraction

Accelerated solvent extraction is a technique for the efficient extraction of analytes from a solid sample matrix into a solvent. The sample and solvent are placed in a closed vessel and heated to 50 to 200°C. The high pressure allows heating above the boiling point, and the high temperature accelerates the dissolution of analytes in the solvent. Both time of extraction and the volume of solvent needed are greatly reduced over atmospheric extraction.

In **microwave-assisted extraction** (MAE), the solvent is heated by microwave energy. The analyte compounds are again partitioned from the sample matrix into the solvent. This approach is an extension of closed-vessel acid digestion described in Chapter 2. A closed vessel containing the sample and solvent is placed in a microwave oven similar to the one described in Figure 2.27. The kinetics of extraction is affected by the temperature and the choice of solvent or solvent mixture. Atmospheric heating for extraction is limited to the boiling point of the solvent. Closed-vessel temperatures at 175 psig typically reach on the order of 150°C, compared with boiling points of 50 to 80°C for commonly used solvents. Solvent mixtures may be used so long as one of them absorbs microwave energy. Some solvents are microwave transparent, for example, hexane, and do not heat, but a mixture of hexane and acetone heats rapidly.

The closed vessels must be inert to solvents and be microwave transparent. The body is made of polyetherimide (PEI), with a perfluoroalkoxy (PFA) liner. Several sample vessels may be placed in the oven at the same time for multiple extractions.

Microwave extractions may also be performed at atmospheric pressure, without the need for pressurized vessels (see Ref. 6). Heating and cooling cycles are

employed to prevent boiling of the solvent. This technique also reduces extraction times substantially. For information on commercial MAE systems, see www.cem.com.

18.6 Solid-Phase Extraction

Liquid–liquid extractions are very useful but have certain limitations. The extracting solvents are limited to those that are water immiscible (for aqueous samples). Emulsions tend to form when the solvents are shaken, and relatively large volumes of solvents are used that generate a substantial waste disposal problem. The operations are often manually performed and may require a back extraction.

Many of these difficulties are avoided by the use of **solid-phase extraction** (SPE), which has become a widely used technique for sample cleanup and concentration prior to chromatographic analysis (next chapter) in particular. In this technique, hydrophobic organic functional groups are chemically bonded to a solid surface, for example, powdered silica. A common example is the bonding of C_{18} chains on silica, with particle sizes on the order of 40 μm. These groups will interact with hydrophobic organic compounds by van der Waals forces and extract them from an aqueous sample in contact with the solid surface. The same solid phases used in high-performance liquid chromatography (Chapter 21) are used for solid-phase extraction.

The powdered phase is generally placed in a small cartridge, similar to a plastic syringe. Sample is placed in the cartridge and forced through by means of a plunger (positive pressure) or a vacuum (negative pressure), or by centrifugation (see Figure 18.2). Trace organic molecules are extracted, preconcentrated on the column, and separated away from the sample matrix. Then they can be eluted with a solvent such as methanol and then analyzed, for example, by chromatography (Chapters 19–21). They may be further concentrated prior to analysis by evaporating the solvent.

The nature of the extracting phase can be varied to allow extraction of different classes of compounds. Figure 18.3 illustrates bonded phases based on van der Waals forces, hydrogen bonding (dipolar attraction), and electrostatic attraction.

When silica particles are bonded with a hydrophobic phase, they become "waterproof" and must be conditioned in order to interact with aqueous samples. This is accomplished by passing methanol or a similar solvent through the sorbent bed. This penetrates into the bonded layer and permits water molecules and analyte to diffuse into the bonded phase. After conditioning, water is passed to remove the excess solvent prior to adding the sample.

In solid-phase extraction, the bonded C_{18} chains take the place of the organic solvent.

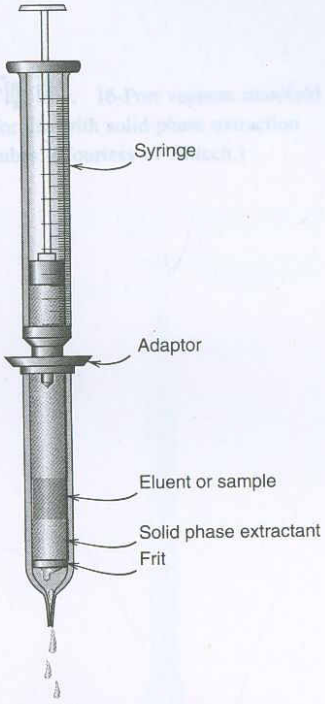


Fig. 18.2. Solid-phase cartridge and syringe for positive pressure elution.

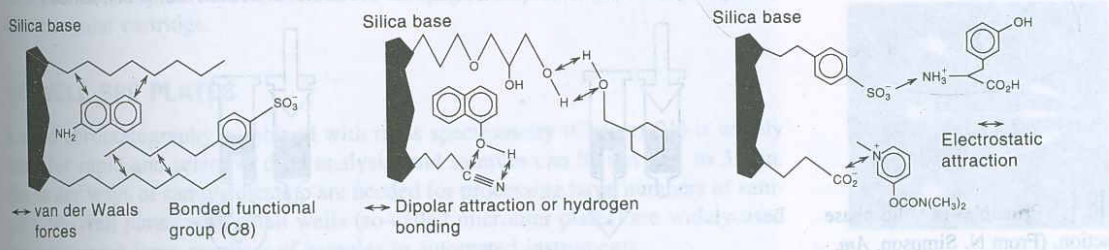


Fig. 18.3. Solid-phase extractants utilizing nonpolar, polar, and electrostatic interactions. (Adapted from N. Simpson, *Am. Lab.*, August, 1992, p. 37. Reproduced by permission of American Laboratory, Inc.)

Figure 18.4 illustrates a typical sequence in a solid-phase extraction. Following conditioning, the analyte and other sample constituents are adsorbed on the sorbent extraction bed. A rinsing step removes some of the undesired constituents, while elution removes the desired analyte, perhaps leaving other constituents behind, depending on the relative strengths of interaction with the solid phase or solubility in the eluting solvent. Such a procedure is used for the determination of organic compounds in drinking water in the official Environmental Protection Agency (EPA) method (Ref. 9).

SPE CARTRIDGES

The SPE sorbent is prepackaged in polypropylene syringe barrels, with typically 500 mg of packing in 3- or 5-mL syringe barrels. Smaller 1-mL syringes packed with 100 mg are becoming more popular because of reduced sample and solvent requirement and faster cleanup times, and even smaller packed beds down to 10 mg are available. These smaller packings, of course, have smaller capacity. Larger ones may be required for preparing large volumes of environmental samples such as polluted water that has large amounts of contaminants to be removed.

The SPE cartridges are used for the isolation and concentration of drugs from biological samples and are typically processed in batches of 12 to 24 using vacuum manifolds (Figure 18.5). There are automated liquid bundling systems to improve the efficiency.

SPE PIPET TIPS

Solid-phase extraction has been automated. The first application systems utilized robotic systems, and then automated *xyz* liquid-handling systems. The automated liquid-handling systems are designed to handle pipet tips to dispense liquids. So

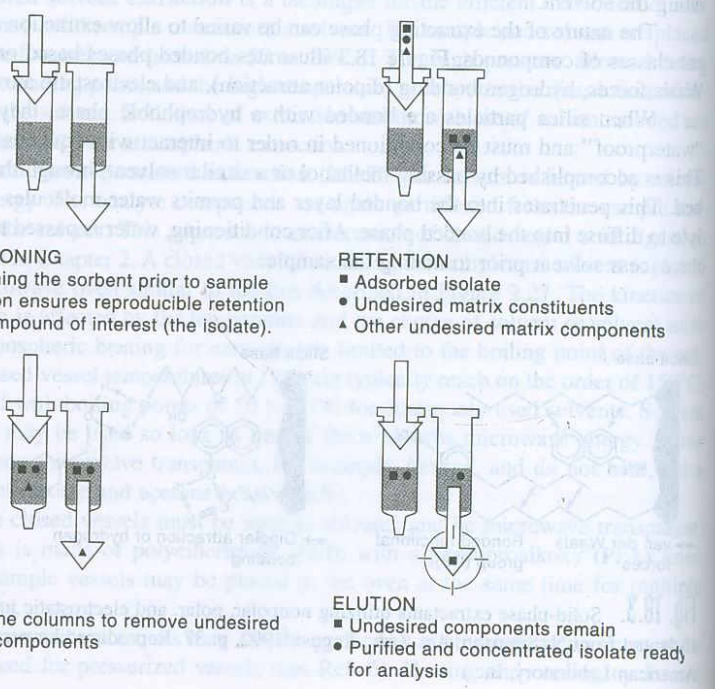


Fig. 18.4. Principles of solid-phase extraction. (From N. Simpson, *Am. Lab.*, August, 1992, p. 37. Reproduced by permission of American Laboratory, Inc.)

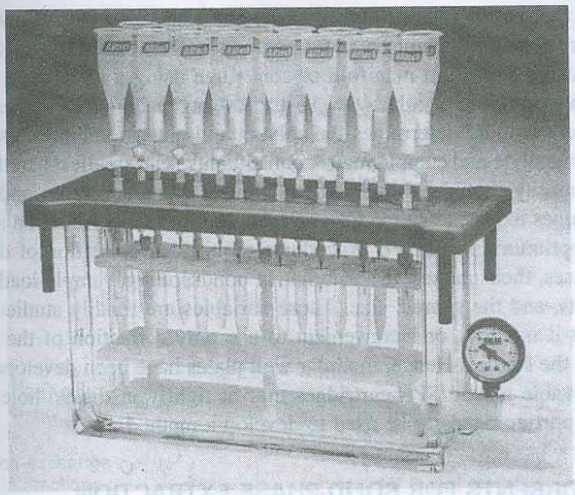


Fig. 18.5. 16-Port vacuum manifold for use with solid-phase extraction tubes. (Courtesy of Alltech.)

sorbent-filled pipet tips for SPE were introduced (Figure 18.6) for use with the automated systems. These pipet tips can be used with multichannel pipettors (Chapter 2). The flow can be bi-directional, with liquid samples pulled from the bottom and eluent dispensed from the top. Commercially prepared tips are available for specific applications. For example, the Millipore ZipTipC_4 can be used to desalt 1 μL of 100 femtomole (10^{-15} mol) amounts of peptides prior to being analyzed by liquid chromatography–mass spectrometry. Pipet tips are used only once and discarded, eliminating any cross-contamination problem. See the EST Analytical Web page for example separations using SPE pipet tips; gas chromatograms of cleaned-up complex samples are illustrated (www.estanalytical.com).

SPE DISKS

The small cross-sectional area SPE pipet tips are prone to plugging by protein samples. So solid-phase extractants are also available in filter form (extraction disks) in which 8-μm silica particles are enmeshed into a web of PTFE [poly(tetrafluoroethylene)] fibrils. Fiberglass-based disks, which are more rigid, are also available. The greater cross-sectional area disks with shorter bed depths allow higher flow rates for large-volume samples with low concentrations of analyte, typically encountered in environmental analysis. The disks are less prone to channeling found with packed cartridges. They tend to plug if samples contain particulate matter, and so a prefilter may have to be used. Disk cartridges are also available that operate like a regular cartridge.

96-WELL SPE PLATES

Liquid chromatography combined with mass spectrometry (Chapter 21) is widely used for rapid and selective drug analysis, and samples can be run in 1 to 3 min. So, faster ways of sample cleanup are needed for processing large numbers of samples. 96-Well plates with small wells (so-called microtiter plates) are widely used for processing large numbers of samples in automated instruments.

Solid-phase extraction systems have been designed in a 96-well microtiter plate format, so they can be processed automatically. Single-block plates with 96 wells contain either packed beds or disks of sorbent particles, in an 8-row ×

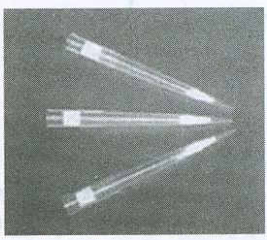


Fig. 18.6. Disposable solid-phase extraction pipet tips. [Courtesy of EST Analytical (www.estanalytical).]

12-column rectangular format (Figure 18.7). The plates sit on top of a 96-well plate collection system. The chemistry is the same as in the above formats. Samples are processed using a vacuum manifold or centrifuge using a microplate carrier. The SPE columns are 1 to 2 mL, with 10 to 100 mg packing of sorbent particles. The bed mass loading determines the solvent and elution volumes, as well as the capacity for analyte and sample matrix constituents. The smallest bed that provides adequate capacity should be used. This minimizes extraction times and the smaller elution volumes require less time for evaporation prior to reconstitution and analysis.

The optimum use of SPE procedures requires investigation of different stationary phases, their masses, the volumes of conditioning, sample load, wash, elution solvents, and the sample size. These variables are readily studied in column format. But it is costly or inconvenient to use only a fraction of the 96 wells to perform all the studies. Hence, modular well plates have been developed that have small removable plastic SPE cartridges that fit tightly in the 96-hole base plate, and only a portion needs to be used to develop a method.

OTHER SORBENTS FOR SOLID-PHASE EXTRACTION

Sorbents are available in long chain lengths (C_{20} and C_{30}) for isolation of hydrophobic molecules. "Universal sorbents" have been developed that will sorb a group of structurally similar compounds. An example in Figure 18.8*a* is a synthetic polymer of N_2–vinylpyrrolidone (top half of molecule) and divinylbenzene (bottom half). It provides hydrophilicity for wetting and hydrophobicity for analyte retention. A sulfonated version (Figure 18.8*b*) is a mixed-mode sorbent that has both ion exchange and solvent extraction properties and will retain a range of acidic, neutral, and basic drugs. These wettable sorbents do not require conditioning.

POLYMERIC PHASES

Besides the common silica-based SPE particles, polymer-based supports are also available. These have advantages of being stable over a wide pH range, and they do not possess residual silica groups that can interact with, for example, metal ions

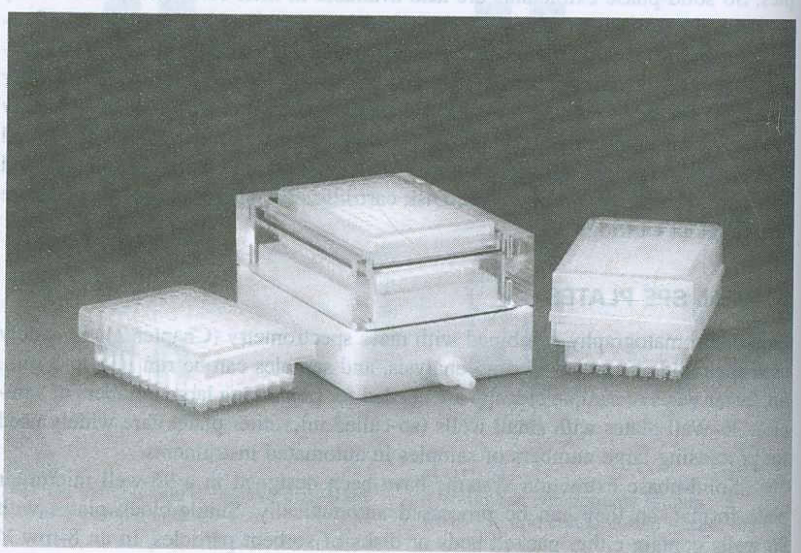


Fig. 18.7. 96-Well extraction plates and vacuum manifold with collection plate.

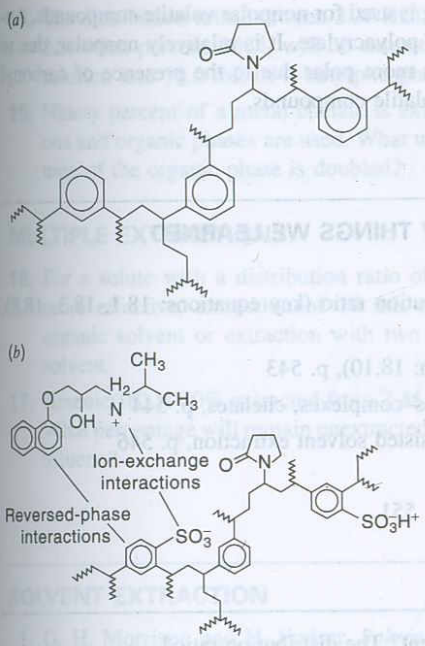


Fig. 18.8. "Universal sorbents": Chemical structures of Waters' Oasis (*a*) HLB and (*b*) MXC polymer sorbents. The top structure in (*b*) is the basic drug propanolol demonstrating drug–sorbent interaction. [From D. A. Wells, *LC.GC*, **17**(7) (1999) 600. Reproduced by permission of LC.GC.]

or other cationic species. The particles are spherical, while silica-based SPE particles are irregular in shape, and the polymeric particles have been designed to be wettable. They typically have higher capacity than silica-based particles.

DUAL PHASES

The use of two different phases can extend the range of compounds extracted. Three types are used, mixed mode, layered, and stacked phases. In the mixed mode, two different types of chemically bonded phases are mixed together in the cartridge. An example is a mixture of C8 and cation exchange particles. In the layered mode, the two different phases are packed one on top of the other. Stacked phases use two cartridges in series to provide enhanced separations. The first two modes are more readily adapted to automation since only a single cartridge is used.

SOLID-PHASE MICROEXTRACTION (SPME)

Solid-phase microextraction is a solvent-less extraction technique, usually used for analyte collection for determination by gas chromatography (Chapter 20) and is based on adsorption. A fused silica fiber is coated with a solid adsorbent or an immobilized polymer, or a combination of the two. Figure 18.9 illustrates an SPME fiber. Typical fiber dimensions are 1 cm × 110 μm. The fiber is inserted in a syringe needle device. Solid, liquid, or gaseous matrices can be sampled by SPME. The fiber is exposed to a gaseous or liquid sample, or the headspace above a solid or liquid sample for a fixed time and temperature; samples are often agitated to increase efficiency of analyte adsorption. Following adsorption, the analyte is thermally desorbed, usually directly in the injection port of a gas chromatograph for introduction into the GC column.

There are limited adsorbents. A widely used one is poly(dimethylsiloxane), which is useful for screening for volatile flavor components of beverages, foods,

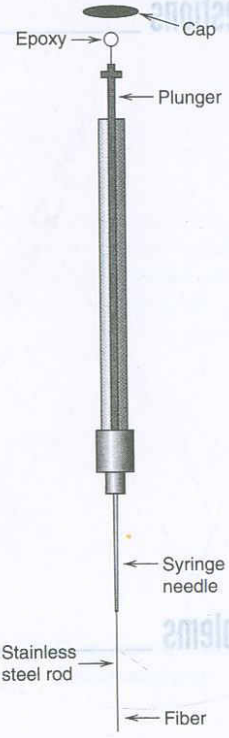


Fig. 18.9. Schematic of a solid-phase microextraction device. [From C. L. Arthur, D. W. Potter, K. D. Buchholz, S. Motlagh, and J. Pawliszn, *LC.GC*, **10**(9) (1992) 656. Reproduced by permission of LC.GC.]

and the like. A 100-μm layer coating is used for nonpolar volatile compounds. Another example is an 85-μm layer of polyacrylate. It is relatively nonpolar, due to the presence of methyl groups. It is more polar due to the presence of carbonyl groups, and so extracts polar semivolatile compounds.

Learning Objectives

WHAT ARE SOME OF THE KEY THINGS WE LEARNED FROM THIS CHAPTER?

- Distribution coefficient, distribution ratio (key equations: 18.1, 18.3, 18.8), pp. 541, 542
- Percent extracted (key equation: 18.10), p. 543
- Solvent extraction of metal ions–complexes, chelates, p. 544
- Accelerated and microwave-assisted solvent extraction, p. 546
- Solid-phase extraction, p. 547
- Solid-phase microextraction, p. 551

Questions

1. What is the distribution coefficient? The distribution ratio?
2. Suggest a method for the separation of aniline, $C_6H_5NH_2$, an organic base, from nitrobenzene, $C_6H_5NO_2$ (extremely toxic!).
3. Describe two principal solvent extraction systems for metal ions. Give examples of each.
4. Describe the equilibrium processes involved in the solvent extraction of metal chelates.
5. What is the largest concentration of a metal chelate that can be extracted into an organic solvent? The smallest concentration?
6. Discuss the effect of the pH and of the reagent concentration on the solvent extraction of metal chelates.
7. What is the basis of accelerated solvent extraction?
8. What is the basis of microwave-assisted extraction?
9. How does solid-phase extraction differ from solvent extraction?
10. What is solid-phase microextraction?

Problems

EXTRACTION EFFICIENCIES

11. Derive Equation 18.10 from Equation 18.9.
12. In deriving Equation 18.8, we neglected the fact that benzoic acid partially forms a dimer in the organic phase ($2HBz \rightleftharpoons (HBz)_2$; $K_p = [(HBz)_2]/[HBz]^2$, where K_p is the dimerization constant). Derive an expression for the distribution ratio taking this into account.
13. Ninety-six percent of a solute is removed from 100 mL of an aqueous solution by extraction with two 50-mL portions of an organic solvent. What is the distribution ratio of the solute?

14. The distribution ratio between 3 *M* HCl and tri-*n*-butylphosphate for $PdCl_2$ is 2.3. What percent $PdCl_2$ will be extracted from 25.0 mL of a 7.0×10^{-4} *M* solution into 10.0 mL tri-*n*-butylphosphate?
15. Ninety percent of a metal chelate is extracted when equal volumes of aqueous and organic phases are used. What will be the percent extracted if the volume of the organic phase is doubled?

MULTIPLE EXTRACTIONS

16. For a solute with a distribution ratio of 25.0, show by calculation which is more effective, extraction of 10 mL of an aqueous solution with 10 mL organic solvent or extraction with two separate 5.0-mL portions of organic solvent.
17. Arsenic(III) is 70% extracted from 7 *M* HCl into an equal volume of toluene. What percentage will remain unextracted after three individual extractions with toluene?

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Chapter Nineteen

CHROMATOGRAPHY: PRINCIPLES AND THEORY

GC and HPLC are the most widely used forms of chromatography.

In 1906, the Russian scientist Tswett reported separating different colored constituents of leaves by passing an extract of the leaves through a column of calcium carbonate, alumina, and sucrose. He coined the term **chromatography**, from the Greek words meaning "color" and "to write." Tswett's original experiments went virtually unnoticed in the literature for decades, but eventually other methods were developed and today there are several different types of chromatography. Chromatography is taken now to refer generally to the separation of components in a sample by distribution of the components between two phases—one that is stationary and one that moves, usually but not necessarily in a column.

The International Union of Pure and Applied Chemistry (IUPAC) has drafted a recommended definition of chromatography: "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase), while the other (the mobile phase) moves in a definite direction" [L. S. Ettre, "Nomenclature for Chromatography," *Pure & Appl. Chem.*, **65**(4) (1993) 819–872]. The stationary phase is usually in a column, but may take other forms, such as a planar phase (flat sheet). Chromatographic techniques have been more valuable in the separation and analysis of highly complex mixtures than any other and revolutionized the capabilities of analytical chemistry. In this chapter, we introduce the concepts and principles of chromatography, including the different types, and describe the theory of the chromatographic process in columns.

The two principal types of chromatography are gas chromatography (GC) and liquid chromatography (LC). Gas chromatography separates gaseous substances based on adsorption on or partitioning in a stationary phase from a gas phase and is described in Chapter 20. Liquid chromatography includes techniques such as size exclusion (separation based on molecular size), ion exchange (separation based on charge), and high-performance liquid chromatography (HPLC—separation based on adsorption or partitioning from a liquid phase). These are presented in Chapter 21, along with thin-layer chromatography (TLC), a planar form of LC, and electrophoresis where separation in an electrical gradient is based on the sign and magnitude of solute charge.

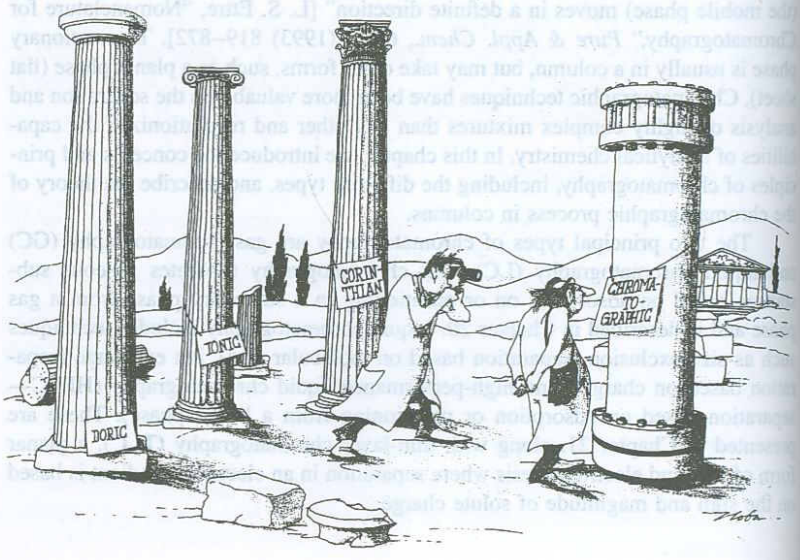
Birth of Modern Liquid and Gas Chromatography

In June 1941, the British chemists A. J. P. Martin and R. L. M. Synge presented a paper at the Biochemical Society meeting in London on the separation of monoamino monocarboxylic acids in wool using a new liquid–liquid chromatography technique called partition chromatography. The details are published in *Biochem. J.*, **35** (1941) 91. For this work, they received the 1952 Nobel Prize in Chemistry (www.almz.com/nobel). In a second paper, they stated "The mobile phase need not be a liquid but may be a vapour . . ." and "Very refined separations of volatile substances should therefore be possible in columns in which permanent gas is made to flow over gel impregnated with a nonvolatile solvent." But this was largely missed during World War II, when many libraries did not receive journals, and it was not until 1950 that Martin, along with a young colleague A. T. James, successfully demonstrated "liquid–gas partition chromatography" at the October meeting of the Biochemical Society [A. T. James and A. J. P. Martin, *Biochem. J. Proc.*, **48**(1) (1950) vii.]. Thus were born two of the most powerful analytical techniques in use today. For a fascinating historical account of these developments, see L. S. Ettre, "The Birth of Partition Chromatography," *LC-GC*, **19**(5) (2001) 506.

19.1 Principles of Chromatographic Separations

A solute equilibrates between a mobile and a stationary phase. The more it interacts with the stationary phase, the slower it is moved along a column.

While the mechanisms of retention for various types of chromatography differ, they are all based on establishment of an equilibrium between a stationary phase and a mobile phase. Figure 19.1 illustrates the separation of these components on a chromatographic column. A small volume of sample is placed at the top of the column, which is filled with the chromatographic particles (stationary phase) and solvent.



Courtesy of Merck KGaA. Reproduced by permission.

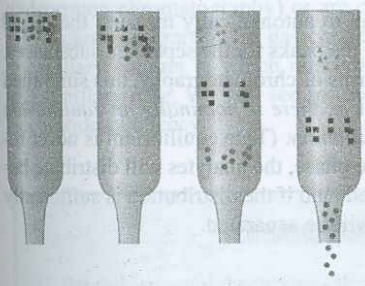


Fig. 19.1. Principle of chromatographic separations.

Mobile-phase solvent is added to the column and is allowed to slowly emerge from the bottom of the column. The individual components interact with the stationary phase to different degrees,

$$X_m \rightleftharpoons X_s \tag{19.1}$$

The distribution equilibrium is described by the distribution constant

$$K_c = \frac{[X]_s}{[X]_m} \tag{19.2}$$

where $[X]_s$ is the concentration of component X on or in the stationary phase at equilibrium and $[X]_m$ its concentration in the mobile phase. This equilibrium constant is governed by the temperature, the type of compound, and the stationary and mobile phases. It is also called the distribution coefficient or the partition coefficient in partition chromatography. Solutes with a large K_c value will be retained more strongly by the stationary phase than those with a small value. The result is that the latter will move along the column (be eluted) more rapidly. Because true equilibrium between the two phases is not achieved, there will be some lag of the analyte molecules between the two phases, which depends on the flow rate of the mobile phase and on the degree of interaction with the stationary phase, and results in band broadening. Figure 19.2 illustrates the distribution of two species A and B along a column as they move down the column. If we measure the concentration of eluted molecules as they emerge from the column and plot this as a function of time or of the volume of mobile phase passed through the column, a chromatogram results. Note that as the substances move down the column, each band becomes more spread out. The areas under the peaks remain the same. Band-broadening effects are treated below. In modern chromatography, a flow cell and

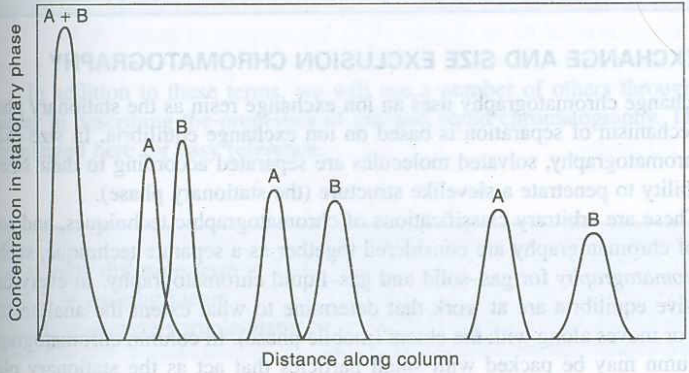


Fig. 19.2. Distribution of two substances, A and B, along a chromatographic column in a typical chromatographic separation.

detector are placed at the end of the column to automatically measure the eluted compounds and print out a chromatogram of the peaks for the separated substances.

Although there are several different forms of chromatography, this simplified model typifies the mechanism of each. That is, *there is nominally an equilibrium between two phases, one mobile and one stationary.* (True equilibrium is never really achieved.) By continually adding mobile phase, the analytes will distribute between the two phases and eventually be eluted, and if the distribution is sufficiently different for the different substances, they will be separated.

19.2 Classification of Chromatographic Techniques

Chromatographic processes can be classified according to the type of equilibration process involved, which is governed by the type of stationary phase. Various bases of equilibration are: (1) adsorption, (2) partition, (3) ion exchange, and (4) pore penetration.

ADSORPTION CHROMATOGRAPHY

The stationary phase is a solid on which the sample components are adsorbed. The mobile phase may be a liquid (*liquid–solid chromatography*) or a gas (*gas–solid chromatography*); the components distribute between the two phases through a combination of sorption and desorption processes. Thin-layer chromatography (TLC) is a special example of adsorption chromatography in which the stationary phase is a *plane*, in the form of a solid supported on an inert plate, and the mobile phase is a liquid.

PARTITION CHROMATOGRAPHY

The stationary phase of partition chromatography is a liquid supported on an inert solid. Again, the mobile phase may be a liquid (*liquid–liquid partition chromatography*) or a gas (*gas–liquid chromatography*, GLC).

In normal-phase chromatography, polar compounds are separated on a polar stationary phase. In reversed-phase chromatography, nonpolar compounds are separated on a nonpolar stationary phase. The latter is more common!

In the normal mode of operations of liquid–liquid partition, a polar stationary phase (e.g., methanol on silica) is used with a nonpolar mobile phase (e.g., hexane). This favors retention of polar compounds and elution of nonpolar compounds and is called **normal-phase chromatography**. If a nonpolar stationary phase is used, with a polar mobile phase, then nonpolar solutes are retained more and polar solutes more readily eluted. This is called **reversed-phase chromatography** and is actually the most widely used.

ION EXCHANGE AND SIZE EXCLUSION CHROMATOGRAPHY

Ion exchange chromatography uses an ion exchange resin as the stationary phase. The mechanism of separation is based on ion exchange equilibria. In size exclusion chromatography, solvated molecules are separated according to their size by their ability to penetrate a sievelike structure (the stationary phase).

These are arbitrary classifications of chromatographic techniques, and some types of chromatography are considered together as a separate technique, such as *gas chromatography* for gas–solid and gas–liquid chromatography. In every case, successive equilibria are at work that determine to what extent the analyte stays behind or moves along with the eluent (mobile phase). In column chromatography, the column may be packed with small particles that act as the stationary phase

(adsorption chromatography) or are coated with a thin layer of liquid phase (partition chromatography). In gas chromatography, the more common form today is a capillary column in which microparticles or a liquid are coated on the wall of the capillary tube. We will see in Chapter 20 that this results in greatly increased separation efficiency.

Chromatography Nomenclature and Terms

In the fundamental discussions that follow, we use the IUPAC recommended symbols and terms, published in 1993 (Ref. 5). The listing is very extensive, filling 54 pages. L. S. Ettre, who chaired the IUPAC committee, has published an abbreviated list of symbols and the most significant changes from traditional use [L. S. Ettre, "The New IUPAC Nomenclature for Chromatography," *LC.GC*, **11**(7) (July) (1993) 502]. Majors and Carr published a very useful updated "Glossary of Liquid-Phase Separation Terms," R. E. Majors and P. W. Carr, *LC.GC*, **19**(2) (February) (2001) 124, www.chromatographyonline.com. Full text is also available at www.zirchrom.com/pdf/glossary.pdf. They incorporate the recommended IUPAC terms.

Some of the older terms and the corresponding recommended terms are given in the following table:

Old		New	
Symbol	Term	Symbol	Term
α	Selectivity factor	α	Separation factor
HETP	Height equivalent to a theoretical plate	H	Plate height
k'	Capacity factor	k	Retention factor
n	Number of theoretical plates	N	Efficiency, number of plates
n_{eff}	Effective number of theoretical plates	N_{eff}	Effective theoretical plates; effective plate number
t_m	Mobile-phase holdup time	t_M	Mobile-phase holdup time
t_r	Retention time	t_R	Retention time
t'_r	Adjusted retention time	t'_R	Adjusted retention time
w	Base peak width	w_b	Bandwidth of peak

In addition to these terms, we will use a number of others throughout the chapter in describing the properties of gas and liquid chromatography. These are summarized here for easy reference.

A = eddy diffusion term = $2\lambda d_p$
λ = packing factor
d_p = average particle diameter

B = longitudinal diffusion term = $2\gamma D_M$
γ = obstruction factor
D_M = diffusion coefficient

C = interphase mass transfer term = $\dfrac{1}{6}\dfrac{d_p^2}{D_M}$

C_m = mobile-phase mass transfer term
C_s = stationary-phase mass transfer term
L = column length
u = mobile-phase linear velocity, cm/s
$\overline{u}$ = average mobile-phase linear velocity, cm/s
v = reduced velocity
h = reduced plate height$u$
R_s = resolution

19.3 Theory of Column Efficiency in Chromatography

The band broadening that occurs in column chromatography is the result of several factors, which influence the efficiency of separations. We can quantitatively describe the efficiency of a column and evaluate the factors that contributed to it.

THEORETICAL PLATES

A theoretical plate represents a single equilibrium step. The more theoretical plates, the greater the resolving power (the greater the number of equilibrium steps).

The separation efficiency of a column can be expressed in terms of the number of theoretical plates in the column. A theoretical plate is a defined concept derived from distillation theory, whereby each theoretical plate in chromatography can be thought of as representing a single equilibrium step. In reality, they are a measure of the efficiency of a column. For high efficiency, a large number of plates is necessary. The **plate height**, H, is the length of a column divided by the number of theoretical plates. To avoid a long column, then, H should be as short (thin or small) as possible. These concepts apply to all forms of column chromatography, but the parameters are easier to determine in gas chromatography.

Experimentally, the plate height is a function of the variance, σ^2, of the chromatographic band and the distance, x, it has traveled through the column, and is given by σ^2/x; σ is the standard deviation of the Gaussian chromatographic peak, and is equal to the width of the peak at the steepest potion of the curve (the inflection point). The width at half-height, w_h, corresponds to 2.35σ, and the peak base width, w_b is equal to 4σ (Figure 19.3). The number of plates, N, for a solute eluting from a column of length, L, is $L/H = Lx/\sigma^2 = L^2/\sigma^2$ (for the full column length: $x = L$) $= 16\ L^2/w_b^2$.

The narrower the peak, the greater the number of plates.

The **number of plates** or **efficiency** can be obtained from a chromatogram from the expression

$$N = 16\left(\frac{t_R}{w_b}\right)^2 \tag{19.3}$$

where N is the number of plates of a column *toward a particular compound*, t_R is the *retention time*, and w_b is the peak width measured at the base in the same units

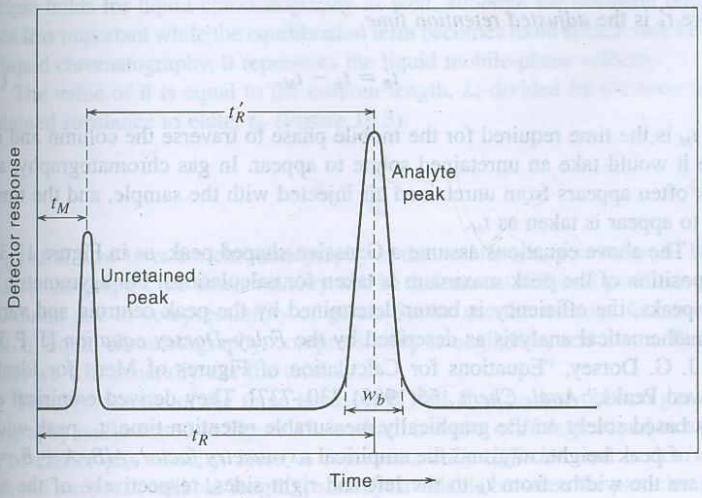


Fig. 19.3. Determination of number of plates. $w_b = 4\sigma$.

as t_R. These are illustrated in Figure 19.3. *Retention volume* V_R may be used in place of t_R. It should be noted that w is not the base width of the peak but the width obtained from the intersection of the baseline with tangents drawn through the inflection points at each side of the peak.

An alternative way to estimate the number of plates is from the width of the peak measured at a height of one-half of the peak height, w_h:

$$N = \frac{5.545 t_R^2}{w_h^2} \tag{19.4}$$

Example 19.1

Calculate the number of plates in the column resulting in the chromatographic peak in Figure 19.3.

Solution

Measuring with a ruler, $t_R = 52.3$ mm and $w_b = 9.0$ mm

$$N = 16\left(\frac{52.3}{9.0}\right)^2 = 54_0$$

This is not a very efficient column, as we will see below.

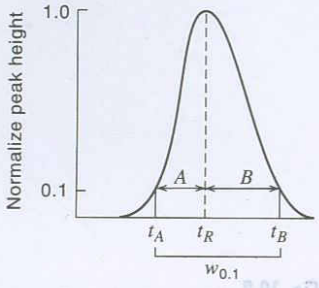
The *effective plate number* corrects theoretical plates for dead (void) volume and hence is a measure of the true number useful of plates in a column:

$$N_{\text{eff}} = 16\left(\frac{t'_R}{w_b}\right)^2 \tag{19.5}$$

where t'_R is the *adjusted retention time*.

$$t'_R = t_R - t_M \tag{19.6}$$

and t_M is the time required for the mobile phase to traverse the column and is the time it would take an unretained solute to appear. In gas chromatography, an air peak often appears from unretained air injected with the sample, and the time for this to appear is taken as t_M.



Asymmetric peak and Foley–Dorsey equation terms.

The above equations assume a Gaussian-shaped peak, as in Figure 19.3, and the position of the peak maximum is taken for calculations. For asymmetric (tailing) peaks, the efficiency is better determined by the peak centroid and variance by mathematical analysis as described by the *Foley–Dorsey equation* [J. P. Foley and J. G. Dorsey, "Equations for Calculation of Figures of Merit for Ideal and Skewed Peaks," *Anal. Chem.*, **55**(1983) 730–737]. They derived empirical equations based solely on the graphically measurable retention time, t_R, peak width at 10% of peak height, $w_{0.1}$, and the empirical *asymmetry factor*, A/B. $A + B = w_{0.1}$, and are the widths from t_R to the left and right sides, respectively, of the asymmetric peak. (When the peak is symmetrical, $A = B = \frac{1}{2}$ the peak width at 10% height).

Foley and Dorsey derived the number of theoretical plates as:

$$N_{sys} = \frac{41.7(t_R/w_{0.1})^2}{A/B + 1.25} \tag{19.7}$$

This equation corrects the retention time and plate count for peak tailing and extra-column sources of broadening.

For a symmetric peak ($A/B = 1$), this becomes $N_{sys} = 18.53(t_R/w_{0.1})^2$, which is close to the theoretical equation of $N_{0.1} = 18.42(t_R/w_{0.1})^2$, that is, the equation holds for ideal as well as asymmetric peaks.

Once the number of plates is known, H can be obtained by dividing the length of the column, L, by N ($H = L/N$). The width of the peak, then, is related to H, being narrower with smaller H. H may be expressed in centimeters/plate, millimeters/plate, and so forth. The *effective* plate height, H_{eff}, is L/N_{eff}.

The term H is usually determined for the last eluting compound. For a well-packed high-performance liquid chromatography (HPLC) column of 5-μm particles H should be about 2 to 3 times the particle diameter. Values of 0.01 to 0.03 mm are typical.

GAS CHROMATOGRAPHY EFFICIENCY—THE VAN DEEMTER EQUATION

We want H to be minimum. But velocities greater than $\bar{u}_{opt}$ are usually used to shorten separation times.

van Deemter showed for a packed gas chromatography column that the broadening of a peak is the summation of somewhat interdependent effects from several sources. The **van Deemter equation** expresses these in terms of the plate height, H:

$$H = A + \frac{B}{\bar{u}} + C\bar{u} \tag{19.8}$$

where A, B, and C are constants for a given system and are related to the three major factors affecting H, and $\bar{u}$ is the average linear velocity of the carrier gas in cm/s. While the van Deemter equation was developed for gas chromatography, it in

principle holds for liquid chromatography as well, although the diffusion term becomes less important while the equilibration term becomes more critical (see below). For liquid chromatography, $\bar{u}$ represents the liquid mobile-phase velocity.

The value of $\bar{u}$ is equal to the column length, L, divided by the time for an unretained substance to elute, t_M (Figure 19.3):

$$\bar{u} = \frac{L}{t_M} \tag{19.9}$$

The general flow term for chromatography is the *mobile-phase velocity*, u. But in gas chromatography, the linear velocity will be different at different positions along the column due to compressibility of gases. So we use the *average linear velocity*, $\bar{u}$. In liquid chromatography, compressibility is negligible, and $\bar{u} = u$. Because of this, we will generally use the term u.

The significance of the three terms A, B, and C in packed-column gas chromatography is illustrated in Figure 19.4, which is a plot of H determined as a function of carrier gas velocity. Here, A represents *eddy diffusion* and is due to the variety of tortuous (variable-length) pathways available between the particles in the column and is independent of the gas- or mobile-phase velocity. The heterogeneity in axial velocities (eddy diffusion) is related to particle size and geometry of packing by:

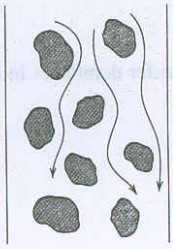
$$A = 2\lambda d_p \tag{19.10}$$

where λ is an empirical column constant (packing factor), with typical values of 0.8 to 1.0 for a well-packed column, and d_p is the average particle diameter. It is minimized by using small and uniform particles, and tighter packing (which creates backpressure, though). But an extremely fine solid support is difficult to pack uniformly, which affects eddy diffusion.

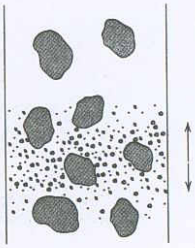
The term B represents **longitudinal** (axial) or **molecular diffusion** of the sample components in the carrier gas or mobile phase, due to concentration gradients within the column. That is, there is a gradient at the interface of the sample zone and the mobile phase, and molecules tend to diffuse to where the concentration is smaller. The diffusion in the mobile phase is represented by:

$$B = 2\gamma D_M \tag{19.11}$$

Peaks are broadened by eddy diffusion, molecular diffusion, and slow mass transfer rates. Small, uniform particles minimize eddy diffusion. Faster flow decreases molecular diffusion but increases mass transfer effects. There will be an optimum flow.



Eddy diffusion.



Molecular diffusion.

Molecular diffusion in usually negligible in LC but important in GC.

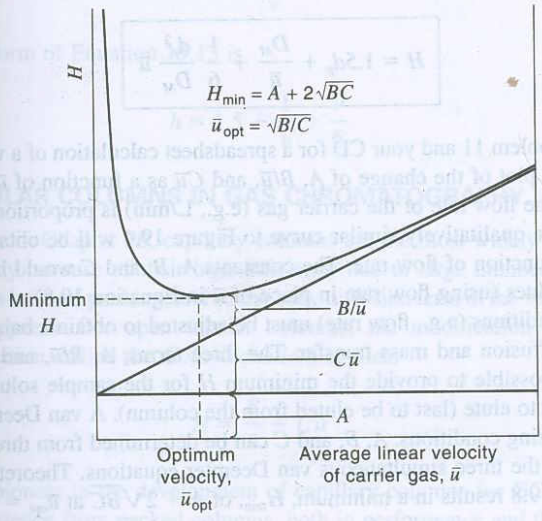


Fig. 19.4. Illustration of the van Deemter equation.

where γ is an obstruction factor, typically equal to 0.6 to 0.8, and D_M is the diffusion coefficient. Molecular diffusion is a function of both the sample and the carrier gas (in GC where it is important). Since the sample components are fixed in a given analysis, the only way to change B or $B/\bar{u}$ is by varying the type, pressure, and flow rate of the carrier gas. High flow rates reduce molecular diffusion, as do denser gases, such as nitrogen or carbon dioxide versus helium or hydrogen. In liquid chromatography, molecular diffusion in the stationary phase is very small compared to that in gases. In GC, it dominates only at flow rates less than $\bar{u}_{opt}$, and for LC is generally negligible under normal operating conditions. We usually operate at flow rates greater than $\bar{u}_{opt}$ since H does not increase very much and separations are faster.

Mass transfer dominates in LC.

The constant C is the **interphase mass transfer** term and is due to the finite time required for equilibrium of the solute to be established between the two phases as it moves between the mobile and stationary phases. It is dependent on the diffusion coefficient and the particle size (since this influences the distances between particles through which the solute must diffuse, approximated by:

$$C = \frac{1}{6}\frac{d_p^2}{D_M} \tag{19.12}$$

It is influenced by the partition coefficient and, therefore, by the relative solubility of the solute in the stationary liquid phase (i.e., by the type and amount of liquid phase as well as the temperature). Or, in the case of adsorption chromatography, it is influenced by the adsorbability of the solute on the solid phase. Increasing the solubility of the vapor components of the sample (for gas chromatography) in the stationary liquid phase by decreasing the temperature may decrease C, provided the viscosity of the liquid phase is not increased so much that the exchange equilibrium becomes slower. The term $C\bar{u}$ is also decreased by decreasing the flow rate, allowing more time for equilibrium. In addition, it is minimized by keeping the stationary liquid-phase film as thin as possible to minimize diffusion within this phase. In liquid chromatography, this term dominates due to the slow diffusion in the liquid mobile phase. It is minimized by using small particles, thin stationary phase films, low-viscosity mobile phases, and high temperatures.

From Equations 19.10, 19.11, and 19.12, the rule of thumb for a normal packed column is

$$\boxed{H = 1.5d_p + \frac{D_M}{\bar{u}} + \frac{1}{6}\frac{d_p^2}{D_M}\bar{u}} \tag{19.13}$$

See Problem 11 and your CD for a spreadsheet calculation of a van Deempter equation and plot of the change of A, $B/\bar{u}$, and $C\bar{u}$ as a function of $\bar{u}$.

Since the flow rate of the carrier gas (e.g., L/min) is proportional to the linear velocity, a qualitatively similar curve to Figure 19.4 will be obtained by plotting H as a function of flow rate. The constants A, B, and C would have different numerical values (using flow rate in place of $\bar{u}$ in Equation 19.8).

The conditions (e.g., flow rate) must be adjusted to obtain a balance between molecular diffusion and mass transfer. The three terms, A, $B/\bar{u}$, and $C\bar{u}$ are kept as small as possible to provide the minimum H for the sample solute that is the most difficult to elute (last to be eluted from the column). A van Deemter plot can aid in optimizing conditions. A, B, and C can be determined from three points and a solution of the three simultaneous van Deemter equations. Theoretically, a plot of Equation 19.8 results in a minimum, H_{min}, of $A + 2\sqrt{BC}$ at $\bar{u}_{opt} = \sqrt{B/C}$. Note

the importance of the slope beyond $\bar{u}_{opt}$. The smaller the slope, the better since the efficiency will then suffer little at velocities in excess of $\bar{u}_{opt}$.

An efficient packed gas chromatography column will have several thousand theoretical plates, and capillary columns will have in excess of 10,000 theoretical plates. The H value for a 1-m column with 10,000 theoretical plates would be 100 cm/10,000 plates = 0.01 cm/plate. In a high-performance liquid chromatography (below), efficiency on the order of 400 theoretical plates per centimeter is typically achieved, and columns are 10 to 50 cm in length.

REDUCED PLATE HEIGHT IN GAS CHROMATOGRAPHY

For comparing the performance of different columns, a dimensionless plate height term is used, called the *reduced plate height*, h, obtained by dividing by the particle diameter:

$$h = \frac{H}{d_p} \tag{19.14}$$

A well-packed column should have an h value at the optimum flow of 2 or less. For open tubular columns

$$h = \frac{H}{d_c} \tag{19.15}$$

where d_c is the inner diameter of the column.

The reduced plate height is used with the *reduced velocity*, ν, for comparing different packed columns over a broad range of conditions; ν relates the diffusion coefficient in the mobile phase and the particle size of the column packing:

$$\nu = \bar{u}\frac{d_p}{D_M} \tag{19.16}$$

(For open-tubular columns, d_p is replaced by d_c.) The *reduced form of the van Deempter equation* is

$$h = A + \frac{B}{\nu} + C\nu \tag{19.17}$$

The reduced form of Equation 19.13 is

$$h = 1.5 + \frac{1}{\nu} + \frac{\nu}{6} \tag{19.18}$$

OPEN TUBULAR COLUMNS IN GAS CHROMATOGRAPHY

There is no eddy diffusion in open tubular columns.

As we will see in Chapter 20, capillary columns are the most widely used in gas chromatography because of their high efficiency due to large numbers of plates. These columns have no packing, and so the eddy diffusion term in the van Deempter equation disappears. For open tubular columns, the modification of the van Deempter equation, called the *Golay equation*, applies:

$$H = \frac{B}{\bar{u}} + C\bar{u} \tag{19.19}$$

Golay was a pioneer in the development of capillary columns for GC and recognized the difference from packed columns, both in performance and theory.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: THE HUBER AND KNOX EQUATIONS

LC must contain a correction for mass transfer in both the mobile and stationary phases.

When a van Deempter plot is applied to HPLC, there is a curvature away from the theoretical curve at high velocities. Huber pointed out that in relating plate height to mass transfer kinetic factors, we need to add an additional term to account for mass transfer in both the stationary phase and the mobile phase:

$$H = A + B/\bar{u} + C_m\bar{u} + C_s\bar{u} \tag{19.20}$$

This is known as the *Huber equation*. Here, $\bar{u}$ is the mobile-phase linear velocity. The constant C_m is the mobile-phase mass transfer term and C_s the stationary-phase term. The B (longitudinal diffusion) term, except at very low mobile-phase velocities, is nearly zero and can be neglected. It is a function of the mobile-phase viscosity and the analyte molecule. The A (eddy diffusion) term turns out to be small compared with diffusion in the liquid phases and almost a constant value and is, therefore, usually neglected. So, H is estimated as:

$$H = C_m\bar{u} + C_s\bar{u} \tag{19.21}$$

The term C_s is relatively constant; C_m, in this case, includes stagnant mobile-phase transfer (in the pores of the particles). Representative H versus u plots for HPLC are shown in Figure 19.5 for different size particles. (See below for particle size and efficiency.)

At very slow velocities for small particles, molecular diffusion does become appreciable and H increases slightly. Note the lesser dependence on flow velocity compared to gas chromatography for the smaller particles. For well-packed columns of typical 5-μm particles, H values are usually in the range of 0.01 to 0.03 mm (10 to 30 μm). Note the scale in Figure 19.5 is in that range.

Knox developed an empirical equation for liquid chromatography that contains a term useful for correcting the deviation from the van Deempter equation,

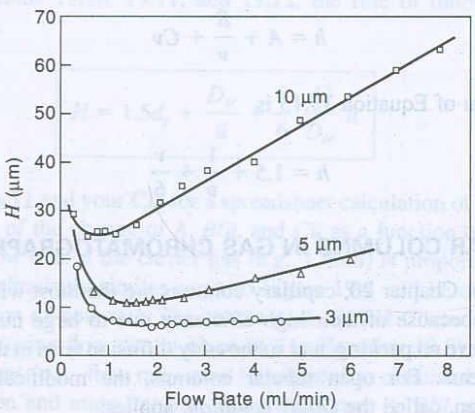


Fig. 19.5. van Deempter plots for different particle sizes in HPLC. The smaller particle sizes are more efficient, especially at higher flow rates. Column i.d.: 4.6 mm; mobile phase: 65% acetonitrile/35% water; sample: *t*-butylbenzene. [From M. W. Dong and M. R. Gant, *LC.GC*, **2** (1984) 294. Reprinted with permission.]

containing the third root of the velocity. The *Knox equation* is usually expressed in the dimensionless reduced form since the physical meaning of the term is not clear:

$$h = Av^{1/3} + \frac{B}{v} + Cv \tag{19.22}$$

The term A is typically 1 to 2, with a large value representing a poorly packed bed; B is about 1.5 and C about 0.1. So a typical good column follows:

$$h = v^{1/3} + \frac{1.5}{v} + 0.1v \tag{19.23}$$

EFFICIENCY AND PARTICLE SIZE IN HPLC

Column efficiency is related to particle size. It turns out that for well-packed HPLC columns, H is about two to three times the mean particle diameter, that is,

$$H = (2 \text{ to } 3) \times d_p \tag{19.24}$$

Figure 19.6 shows the variation of N as a function of linear velocity for different particle sizes. Note N is on a logarithmic scale. Particles of different size distribution packed in columns of equal diameter will exhibit essentially the same longitudinal diffusion. Larger particles exhibit larger stagnant mobile phase transfer (C) since the solute molecule has a longer path to traverse in the pores, which increases band broadening.

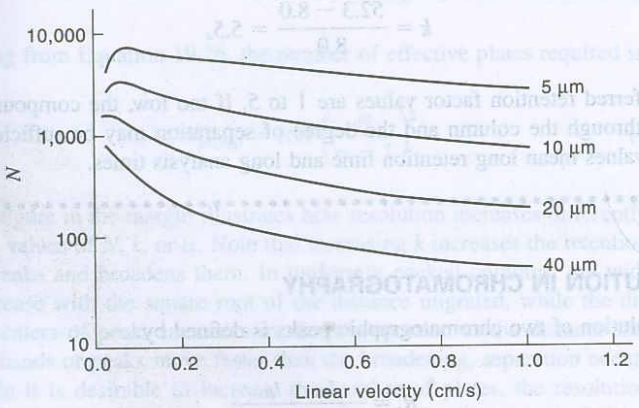


Fig. 19.6. Plate number as a function of linear velocity for different size particles in a 10-cm column. [From J. Maclennan and B. Murphy, *Today's Chemist at Work*, February (1994) 29. Copyright 1994 by the American Chemical Society. Reproduced by permission of Waters Corporation.]

RETENTION FACTOR IN CHROMATOGRAPHY

The **retention factor** k for a sample peak is defined by

Retention factor is a measure of retention time and, therefore, resolution capacity.

$$k = \frac{t_R - t_M}{t_M} = \frac{t'_R}{t_M} \tag{19.25}$$

where t_R is the **retention time** (time required for the analyte peak to appear) and t_M is the time it would take for an unretained solute to appear (see Equation 19.6). A large retention factor favors good separation. However, large capacity factors mean increased elution time, so there is a compromise between separation efficiency and separation time. The retention factor can be increased by increasing the stationary phase volume. A change in the retention factor is an indication of degradation of the stationary phase.

The effective plate number is related to the retention factor and effective plate number via:

$$N_{\text{eff}} = N\left(\frac{k}{k+1}\right)^2 \tag{19.26}$$

The volume of a chromatographic column consists of the stationary-phase volume and the **void volume**, the volume occupied by the mobile phase. The latter can be determined from t_M and the flow rate. One void volume of the mobile phase is required to flush the column once.

Example 19.2

Calculate the retention factor for the chromatographic peak in Figure 19.3.

Solution

Measuring with a ruler, $t_R = 52.3$ mm and $t_M = 8.0$ mm.

$$k = \frac{52.3 - 8.0}{8.0} = 5.5_4$$

The preferred retention factor values are 1 to 5. If too low, the compounds pass rapidly through the column and the degree of separation may be sufficient. And large k values mean long retention time and long analysis times.

RESOLUTION IN CHROMATOGRAPHY

The resolution of two chromatographic peaks is defined by:

You should strive for a resolution of at least 1.0.

$$R_s = \frac{t_{R2} - t_{R1}}{(w_{b1} + w_{b2})/2} \tag{19.27}$$

where t_{R1} and t_{R2} are the retention times of the two peaks (peak 1 elutes first), and w_b is the baseline width of the peaks. This is a measure of the ability of a column to separate two peaks. A resolution of 0.6 is needed to discern a valley between

two peaks of equal heights. A value of 1.0 results in 2.3% overlap of two peaks of equal width and is considered the minimum for a separation to allow good quantitation. A resolution of 1.5 is an overlap of only 0.1% for equal width peaks and is considered sufficient for baseline resolution of equal height peaks.

We can describe resolution in thermodynamic terms, without regard to peak width. The **separation factor**, α, is a thermodynamic quantity that is a measure of the relative retention of analytes, and is given by:

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{k_2}{k_1} \tag{19.28}$$

where t'_{R1} and t'_{R2} are the adjusted retention times (Equation 19.6) and k_1 and k_2 are the corresponding retention factors (Equation 19.25). This describes how well the peaks are separated without taking peak width into consideration. The resolution can, then, be written as:

$$R_s = \frac{1}{4}\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_{ave} + 1}\right) \tag{19.29}$$

where k_{ave} is the mean of the two peak capacity factors. This form relates resolution to efficiency, that is, band broadening and retention time (Equation 19.3), and is known as the *resolution equation* or the *Purnell equation*. Note that since N is proportional to L, the resolution is proportional to the square root of the column length, $\sqrt{L}$; this holds strictly only for packed columns. So doubling the length of the column increases the resolution by $\sqrt{2}$ or 1.4. A fourfold increase would double the resolution. Retention times, of course, would be increased in direct proportion to the length of the column. For asymmetric peaks, the centroids of the peaks should be used in calculating retention times for calculating α values.

The number of plates required for a given degree of resolution is given by:

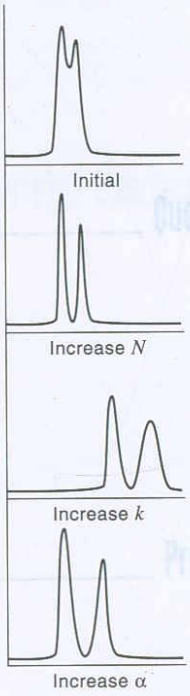
$$N_{req} = 16R^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{k_{ave} + 1}{k_2}\right)^2 \tag{19.30}$$

Substituting from Equation 19.26, the number of effective plates required is

$$N_{eff(req)} = 16R^2\left(\frac{\alpha}{\alpha - 1}\right)^2 \tag{19.31}$$

The figure in the margin illustrates how resolution increases differently with increasing values of N, k, or α. Note that increasing k increases the retention time for both peaks and broadens them. In uniformly packed columns, the widths of bands increase with the square root of the distance migrated, while the distance between centers of peaks increases in direct proportion to the distance traveled. Since the bands or peaks move faster than the broadening, separation occurs.

While it is desirable to increase the number of plates, the resolution in a packed column, as noted above, increases only with the square root of N (e.g., by increasing L), and the pressure drop increases. It is more effective to try to increase the selectivity (α) or retention factor (k) by varying the stationary and mobile phases. Increasing the retention time, of course, lengthens the analysis time, and a compromise is generally chosen between speed and resolution.



Example 19.3

Ethanol and methanol are separated in a capillary GC column with retention times of 370 and 385 s, respectively, and base widths (w_b) of 16.0 and 17.0 s. An unretained air peak occurs at 10.0 s. Calculate the separation factor and the resolution.

Solution

Use the longest eluting peak to calculate N (Equation 19.3):

$$N = 16\left(\frac{385}{17.0}\right)^2 = 8.21 \times 10^3 \text{ plates}$$

From Equation 19.28,

$$\alpha = \frac{385 - 10}{330 - 10} = 1.04_2$$

From Equation 19.25,

$$k_1 = \frac{370 - 10}{10.0} = 36.0$$

$$k_2 = \frac{385 - 10}{10.0} = 37.5$$

$$k_{ave} = (36.0 + 37.5)/2 = 36.8$$

From Equation 19.29,

$$R_s = \frac{1}{4}\sqrt{8.21 \times 10^3}\left(\frac{1.042 - 1}{1.04_2}\right)\left(\frac{37.5}{36.8 + 1}\right) = 0.91$$

Or, from Equation 19.27, we obtain

$$R_s = \frac{385 - 370}{(17.0 + 16.0)/2} = 0.91$$

19.4 Chromatography Simulation Software

You are in charge of developing a new chromatographic separation. This involves selecting the proper column (stationary phase) and dimensions, mobile phase, and optimizing variables such as percent organic solvent, solvent or temperature gradient, and so forth. Optimization normally will require many repetitive chromatographic runs. But help is here! There are commercial software packages that assist the analyst in method development and optimization. Some of these are posted on the text website, with detailed descriptions of their capabilities. They are listed here: *DryLab* (LC Resources): www.lcresources.com; *ACD/GC Simulator, ACD/LC Simulator,* and *ACD/ChromManager* (ACD/Labs): www.acdlabs.com; and *ChromSword® AUTO* (Merck KGaA): www.hii.hitachi.com/LC%20ChromSword.htm.

19.5 Freebies: Company Searchable Chromatogram Databases

Agilent Technologies (www.chem.agilent.com) provides a database that contains an extensive library of GC and LC chromatograms based on applications conducted by Agilent chemists. See www.chem.agilent.com/scripts/chromatograms.asp. See also www.chem.agilent.com/scripts/chromatograms.asp for a tutorial site about HPLC columns. A description of these sites is given on the text website, as well as of Supelco (www.sigmaaldrich.com) and Hamilton (www.hamiltoncompany.com) sites for chromatogram databases.

Learning Objectives

WHAT ARE SOME OF THE KEY THINGS WE LEARNED FROM THIS CHAPTER?

- How chemicals are separated on a column, p. 556
- Types of chromatography: adsorption, partition, ion exchange, size exclusion, p. 558
- Chromatographic nomenclature (see tables of terms), p. 559
- Theory of column efficiency, p. 560
  - Plate number (key equation: 19.3), p. 560
  - van Deempter equation for packed GC columns (key equations: 19.8, 19.13), p. 562
  - Golay equation for open tubular GC columns (key equation 19.19), p. 565
  - Huber and Knox equations for HPLC (key equations: 19.20, 19.22), p. 566
- Retention factor (key equation: 19.25), p. 568
- Chromatographic resolution (key equations: 19.27, 19.29), p. 568
- Separation factor (key equation: 19.28), p. 569
- Chromatography simulation software and databases, pp. 570, 571

Questions

1. What is the description of chromatography?
2. Describe the principles underlying all chromatographic processes.
3. Classify the different chromatographic techniques, and give examples of principal types of applications.
4. What is the van Deemter equation? Define terms.
5. How does the Golay equation differ from the van Deemter equation?
6. How do the Huber and Knox equations differ from the van Deempter equation?

Problems

CHROMATOGRAPHY RESOLUTION

7. A gas-chromatographic peak had a retention time of 65 s. The base width obtained from intersection of the baseline with the extrapolated sides of the peak was 5.5 s. If the column was 3 ft in length, what was H in cm/plate?

8. It is desired to just resolve two gas-chromatographic peaks with retention times of 85 and 100 s, respectively, using a column that has an H value of 1.5 cm/plate under the operating conditions. What length columns is required? Assume the two peaks have the same base width.

9. The following gas-chromatographic data were obtained for individual 2-μL injections of *n*-hexane in a gas chromatograph with a 3-m column. Calculate the number of plates and H at each flow rate, and plot H versus the flow rate to determine the optimum flow rate. Use the *adjusted* retention time t'_R.

Flow rate (mL/min)	t_M (Air Peak) (min)	t'_R (min)	Peak Width (min)
120.2	1.18	5.49	0.35
90.3	1.49	6.37	0.39
71.8	1.74	7.17	0.43
62.7	1.89	7.62	0.47
50.2	2.24	8.62	0.54
39.9	2.58	9.83	0.68
31.7	3.10	11.31	0.81
26.4	3.54	12.69	0.95

10. Three compounds, A, B, and C, exhibit retention factors on a column having only 500 plates of $k_A = 1.40$, $k_B = 1.85$, and $k_C = 2.65$. Can they be separated with a minimum resolution of 1.05?

SPREADSHEET PROBLEM

11. Prepare a spreadsheet for a van Deemter plot for the following hypothetical, A, B, and C terms: $A = 0.5$ mm, $B = 30$ mm · mL/min, and $C = 0.05$ mm · min/mL. Plot H vs. $\bar{u}$ at linear velocities of 4, 8, 12, 20, 28, 40, 80 and 120 mL/min. Also, on the same chart, plot A vs. $\bar{u}$, $B/\bar{u}$ vs. $\bar{u}$, and $C\bar{u}$ vs. $\bar{u}$, and note how they change with the linear velocity, that is, how their contributions to H change. Calculate the hypothetical H_{min} and $\bar{u}_{opt}$ and compare with the H_{min} on the chart. Also calculate $B/\bar{u}_{opt}$ and $C\bar{u}_{opt}$. Look on the chart and see where the $B/\bar{u}$ and $C\bar{u}$ lines cross. Check your results with those in your CD, Chapter 19.

Recommended References

GENERAL

1. J. C. Giddings, *Unified Separation Science*, New York: Wiley-Interscience, 1991.
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HISTORICAL ACCOUNTS

These references give a good review of how separation techniques have evolved, particularly the stationary phases used and why we use the ones we do today.

6. H. J. Isaaq, ed., *A Century of Separation Science*, New York: Marcel Dekker, 2002.

7. L. S. Ettre, "The Birth of Partition Chromatography," *LC.GC*, **19**(5) (2001) 506.
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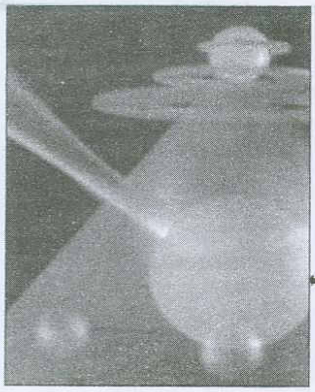
COMPUTER SIMULATION

10. J. W. Nolan and L. R. Snyder, "Gradient Elution Chromatography," in *Encyclopedia of Analytical Chemistry*, Vol. 13, *Theory and Instrumentation*, 11342 Chichester: Wiley, 2000.
11. R. G. Wolcott, J.W. Dolan, and L. R. Snyder, "Computer Simulation for the Convenient Optimization of Isocratic Reversed-Phase Liquid Chromatography Separations by Varying Temperature and Mobile Phase Strength," *J. Chromatogr. A*, **869** (2000) 3.
12. J. W. Dolan, L. R. Snyder, L. C. Sander, R. C. Wolcott, P. Habner, T. Baczek, and R. Kaliszan, *J. Chromatogr. A*, **857** (1999) 41.



Chapter Twenty

GAS CHROMATOGRAPHY

Gas chromatography (GC) is one of the most versatile and ubiquitous analytical techniques in the laboratory. It is widely used for the determination of organic compounds. The separation of benzene and cyclohexane (bp 80.1 and 80.8°C) is extremely simple by gas chromatography, but it is virtually impossible by conventional distillation. Although Martin and Synge invented liquid–liquid chromatography in 1941, the introduction of gas–liquid partition chromatography by James and Martin a decade later had a more immediate and larger impact for two reasons. First, as opposed to manually operated liquid–liquid column chromatography, GC required instrumentation for application, which was developed by collaboration among chemists, engineers, and physicists; and analyses were much more rapid and done on a small scale. Second, at the time of its development, the petroleum industry was required to have improved analytical monitoring and immediately adopted GC. Within a few short years, GC was used for the analysis of almost every type of organic compound.

Very complex mixtures can be separated by this technique. When coupled with mass spectrometry as a detection system, virtually positive identification of the eluted compounds is possible at very high sensitivity, creating a very powerful analytical system.

There are two types of GC: **gas–solid (adsorption) chromatography** and **gas–liquid (partition) chromatography**. The more important of the two is gas–liquid chromatography (GLC), used in the form of a capillary column. In this chapter, we describe the principles of operation of gas chromatography, the types of GC columns, and GC detectors. The principles of mass spectrometry (MS) are described, along with coupling of the gas chromatograph with a mass spectrometer (GC-MS).

20.1 Performing GC Separations

Analyte in the vapor state distributes between the stationary phase and the carrier gas. Gas-phase equilibria are rapid, so resolution (and the number of plates) can be high.

In gas chromatography, the sample is converted to the vapor state (if it is not already a gas) by injection into a heated port, and the eluent is a gas (the **carrier gas**). The stationary phase is generally a nonvolatile liquid supported on a capillary wall or inert solid particles such as diatomaceous earth (kieselguhr—derived from skeletal remains of microscopic marine single-celled algae, consisting mainly of silica); the kieselguhr is usually calcined to increase particle size, creating what is known as firebrick, sold as Chromosorb P or W, for example. There are a large number of

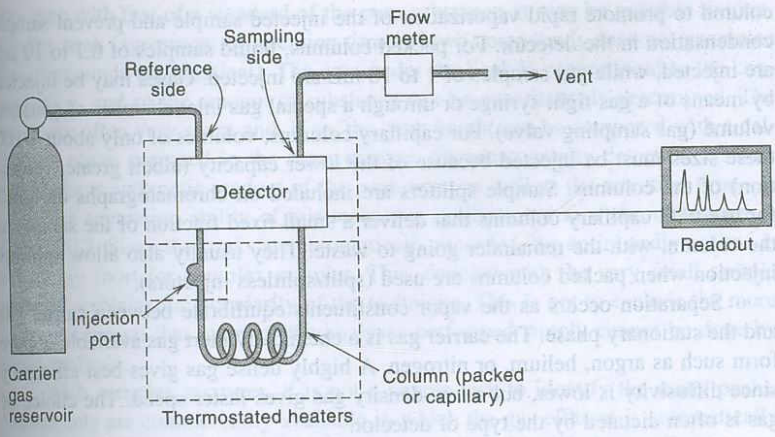


Fig. 20.1. Schematic diagram of gas chromatograph.

liquid phases available, and it is by changing the liquid phase, rather than the mobile phase, that different separations are accomplished. The most important factor in gas chromatography is the selection of the proper column (stationary phase) for the particular separation to be attempted. The nature of the liquid or solid phase will determine the exchange equilibrium with the sample components; and this will depend on the solubility or adsorbability of the sample, the polarity of the stationary phase and sample molecules, the degree of hydrogen bonding, and specific chemical interactions. Most of the useful separations have been determined empirically, although more quantitative information is now available.

A schematic diagram of a gas chromatograph is given in Figure 20.1, and a picture of a modern GC system is shown in Figure 20.2. The sample is rapidly injected by means of a hypodermic microsyringe (see Figure 2.11) through a silicone rubber septum into the column. The sample injection port, column, and detector are heated to temperatures at which the sample has a vapor pressure of at least 10 torr, usually about 50°C above the boiling point of the highest boiling solute. The injection port and detector are usually kept somewhat warmer than the

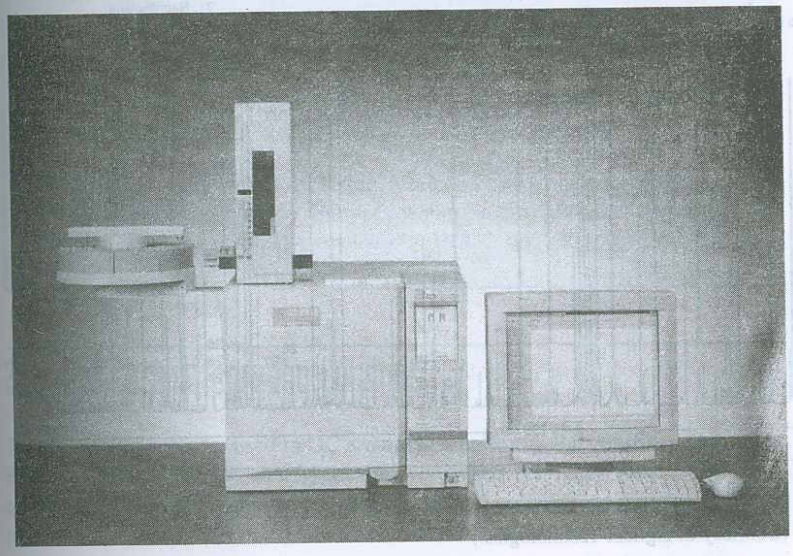


Fig. 20.2. Modern automated gas chromatography system. (Courtesy of Shimadzu North America.)

column to promote rapid vaporization of the injected sample and prevent sample condensation in the detector. For packed columns, liquid samples of 0.1 to 10 μL are injected, while gas samples of 1 to 10 mL are injected. Gases may be injected by means of a gas-tight syringe or through a special gas inlet chamber of constant volume (gas sampling valve). For capillary columns, volumes of only about 1/100 these sizes must be injected because of the lower capacity (albeit greater resolution) of the columns. Sample splitters are included on chromatographs designed for use with capillary columns that deliver a small fixed fraction of the sample to the column, with the remainder going to waste. They usually also allow splitless injection when packed columns are used (split/splitless injectors).

Separation occurs as the vapor constituents equilibrate between carrier gas and the stationary phase. The carrier gas is a chemically inert gas available in pure form such as argon, helium, or nitrogen. A highly dense gas gives best efficiency since diffusivity is lower, but a low-density gas gives faster speed. The choice of gas is often dictated by the type of detector.

Automatic detection of the analytes as they emerge from the column makes measurements rapid and convenient. Retention times are used for qualitative identification. Peak areas are used for quantitative measurements.

The sample is automatically detected as it emerges from the column (at a constant flow rate), using a variety of detectors whose response is dependent upon the composition of the vapor (see below). Usually, the detector contains a **reference side** and a **sampling side**. The carrier gas is passed through the reference side before entering the column and emerges from the column through the sampling side. The response of the sampling side relative to the reference side signal is fed to a recording device where the chromatographic peaks are recorded as a function of time. By measuring the **retention time** (the minutes between the time the sample is injected and the time the chromatographic peak is recorded) and comparing

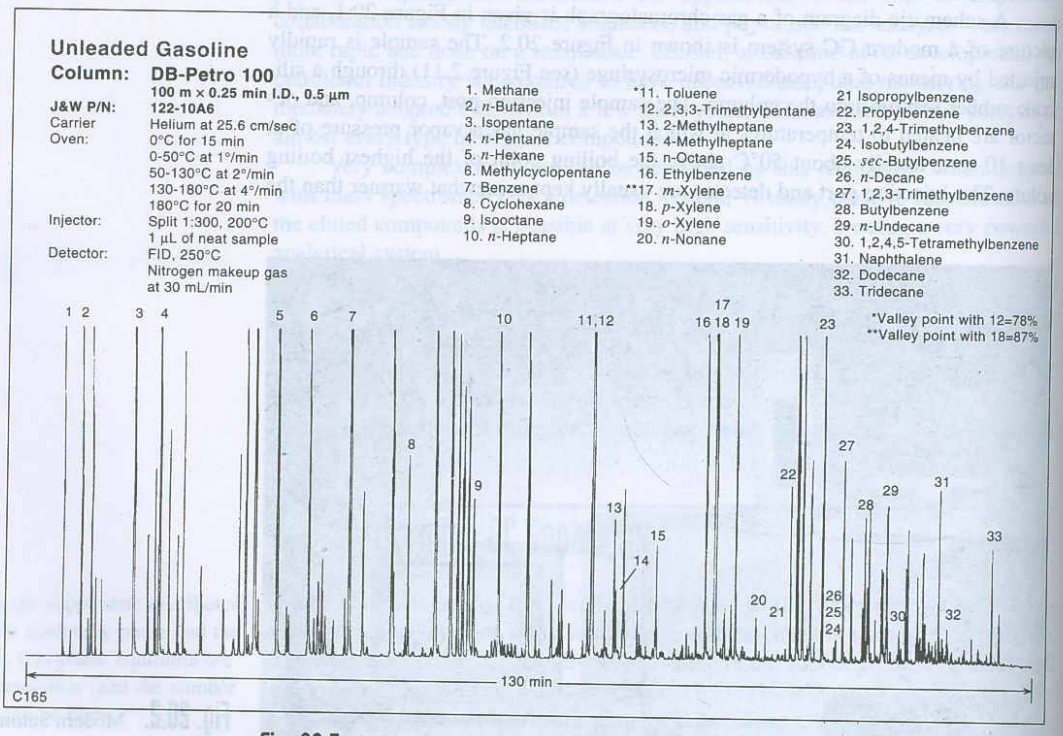


Fig. 20.3. Typical gas chromatogram of complex mixture using a capillary column. (Courtesy of Agilent Technologies.)

this time with that of a standard of the pure substance, it may be possible to identify the peak (agreement of retention times of two compounds does not guarantee the compounds are identical). The area under the peak is proportional to the concentration, and so the amount of substance can be quantitatively determined. The peaks are often very sharp and, if so, the peak height can be compared with a calibration curve prepared in the same manner. Chromatography detection systems usually have automatic readout of the peak area, as well as the retention time.

The separation ability of this technique is illustrated in a chromatogram in Figure 20.3. Since the peaks are automatically recorded, the entire analysis time is amazingly short for complex samples. This, coupled with the very small sample required, explains the popularity of the technique. This is not to exclude the more important reason that many of the analyses performed simply cannot be done by other methods.

With complex mixtures, it is not a simple task to identify the many peaks. Instruments are commercially available in which the gas effluent is automatically fed into a mass spectrometer where they are positively identified according to mass (formula weight and fragmentation pattern). This important analytical technique is called **gas chromatography–mass spectrometry** (GC–MS). The mass spectrometer is a sensitive and selective detector, and when a capillary GC column (very high resolution—see Section 20.2) is used (capillary GC–MS), this technique is capable of identifying and quantifying unbelievably complex mixtures of trace substances. For example, hundreds of compounds may be identified in sewage effluents, and traces of complex drugs in urine or blood or pollutants in water can be determined. For best sensitivity, though, some of the element or compound-type specific detectors listed later offer extraordinary detection limits.

See more on GC–MS below.

What Compounds Can Be Determined by GC?

Many, many compounds may be determined by gas chromatography, but there are limitations. They must be volatile and stable at the temperature employed, typically from 50 to 300°C. GC is useful for:

- All gases
- Most nonionized organic molecules, solid or liquid, containing up to about 25 carbons
- Many organometallic compounds (volatile derivatives of metal ions may be prepared)

If compounds are not volatile or stable, often they can be derivatized to make them amenable to analysis by GC. GC cannot be used for salts nor macromolecules, but these can be determined by HPLC, one of its major uses.

20.2 Gas Chromatography Columns

The two types of columns used in GC are **packed columns** and **capillary columns**. Packed columns were the first type and were used for many years. Capillary columns are more commonly used today, but packed columns are still used for applications that do not require high resolution or when increased capacity is needed.

PACKED COLUMNS

Packed columns can be used with large sample sizes and are convenient to use.

Columns can be in any shape that will fill the heating oven. Column forms include coiled tubes, U-shaped tubes, and W-shaped tubes, but coils are most commonly used. Typical packed columns are 1 to 10 m long and 0.2 to 0.6 cm in diameter. Well-packed columns may have 1000 plates/m, and so a representative 3-m column would have 3000 plates. Short columns can be made of glass, but longer columns may be made of stainless steel so they can be straightened for filling and packing. Columns are also made of Teflon. For inertness, glass is still preferred for longer columns. The resolution for packed columns increases only with the square root of the length of the column. Long columns require high pressure and longer analysis times and are used only when necessary (e.g., for long eluting solutes when high capacity is used). Separations are generally attempted by selecting columns in lengths of multiples of 3, such as 1 or 3 m. If a separation isn't complete in the shorter column, then the next longer one is tried.

The column is packed with small particles that may themselves serve as the stationary phase (adsorption chromatography) or more commonly are coated with a nonvolatile liquid phase of varying polarity (partition chromatography). Gas–solid chromatography (GSC) is useful for the separation of small gaseous species such as H_2, N_2, CO_2, CO, O_2, NH_2, and CH_4 and volatile hydrocarbons, using high surface area inorganic packings such as alumina (Al_2O_3) or porous polymers (e.g., Chromosorb—a polyaromatic crosslinked resin with a rigid structure and a distinct pore size). The gases are separated by their size due to retention by adsorption on the particles. Gas–solid chromatography is preferred for aqueous samples.

The solid support for a liquid phase should have a high specific surface area that is chemically inert but wettable by the liquid phase. It must be thermally stable and available in uniform sizes. The most commonly used supports are prepared from diatomaceous earth, a spongy siliceous material. They are sold under many different trade names. Chromosorb W is diatomaceous earth that has been heated with an alkaline flux to decrease its acidity; it is light in color. Chromosorb P is crushed firebrick that is much more acidic than Chromosorb W, and it tends to react with polar solutes, especially those with basic functional groups.

The polarity of Chromosorb P can be greatly decreased by silanizing the surface with hexamethyldisilazane, $[(CH_3)_3Si]_2NH$. Ottenstein (Ref. 7) has reviewed the selection of solid supports, both diatomaceous earth and porous polymer types.

Column-packing support material is coated by mixing with the correct amount of liquid phase dissolved in a low-boiling solvent such as acetone or pentane. About a 5 to 10% coating (wt/wt) will give a thin layer. After coating, the solvent is evaporated by heating and stirring; the last traces may be removed in a vacuum. A newly prepared column should be conditioned at elevated temperature by passing carrier gas through it for several hours. The selection of liquid phases is discussed below.

Particles should be uniform in size for good packing and have diameters in the range of 60 to 80 mesh (0.25 to 0.18 mm), 80 to 100 mesh (0.18 to 0.15 mm), or 100 to 120 mesh (0.15 to 0.12 mm). Smaller particles are impractical due to high pressure drops generated.

CAPILLARY COLUMNS—THE MOST WIDELY USED

Capillary columns can provide very high resolution, compared with packed columns.

In 1957 Marcel Golay published a paper entitled "Vapor Phase Chromatography and the Telegrapher's Equation" [*Anal. Chem.*, **29** (1957) 928]. His equation predicted increased number of plates in a narrow open-tubular column with the stationary phase supported on the inner wall. Band broadening due to multiple paths (eddy diffusion) would be eliminated. And in narrow columns, the rate of mass transfer is increased since molecules have small distances to diffuse. Higher flow

rates can be used due to decreased pressure drop, which decreases molecular diffusion. Golay's work led to the development of various **open-tubular columns** that today provide extremely high resolution and have become the mainstay for gas-chromatographic analyses. These columns are made of thin fused silica (SiO_2) coated on the outside with a polyimide polymer for support and protection of the fragile silica capillary, allowing them to be coiled. The polyimide layer is what imparts a brownish color to the columns, and it often darkens on use. The inner surface of the capillary is chemically treated to minimize interaction of the sample with the silanol groups (Si–OH) on the tubing surface, by reacting the Si–OH group with a silane-type reagent (e.g., dimethyl dichlorosilane).

Capillaries are also made of stainless steel. Stainless steel interacts with many compounds and so is deactivated by treatment with dimethyl dichlorosilane (DMCS), producing a thin lining of fused silica to which stationary phases can be bonded. Stainless steel columns are more robust than fused silica columns and are used for applications requiring very high temperatures.

The capillaries are 0.10 to 0.53 mm i.d., with lengths of 15 to 100 m and can have several hundred thousand plates, even a million. They are sold as coils of about 0.2 m diameter (Figure 20.4). Capillary columns offer advantages of high resolution with narrow peaks, short analysis time, and high sensitivity (with modern detectors) but are more easily overloaded by too much sample. Split injectors by and large alleviate the overload problem.

Figure 20.5 illustrates the improvements in separation power in going from a packed column (6.4 × 1.8 m) to a very long but fairly wide stainless steel capillary column (0.76 mm × 150 m), to a narrow but shorter glass capillary column (0.25 mm × 50 m). Note that the resolution increases as the column becomes narrower, even when the capillary column is shortened.

There are three types of open-tubular columns. **Wall-coated open-tubular** (WCOT) columns have a thin liquid film coated on and supported by the walls of the capillary. The walls are coated by slowly passing a dilute solution of the liquid

Increasing the film thickness increases capacity but increases plate height and retention time.

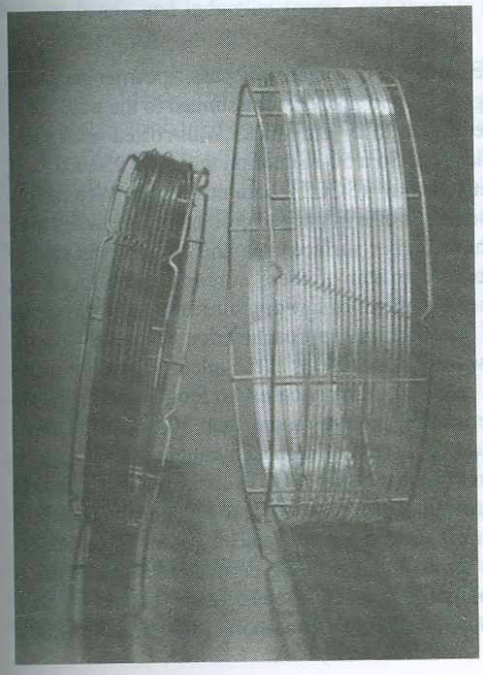


Fig. 20.4. Capillary GC columns. (Courtesy of Quadrex Corp., Woodbridge, CT.)

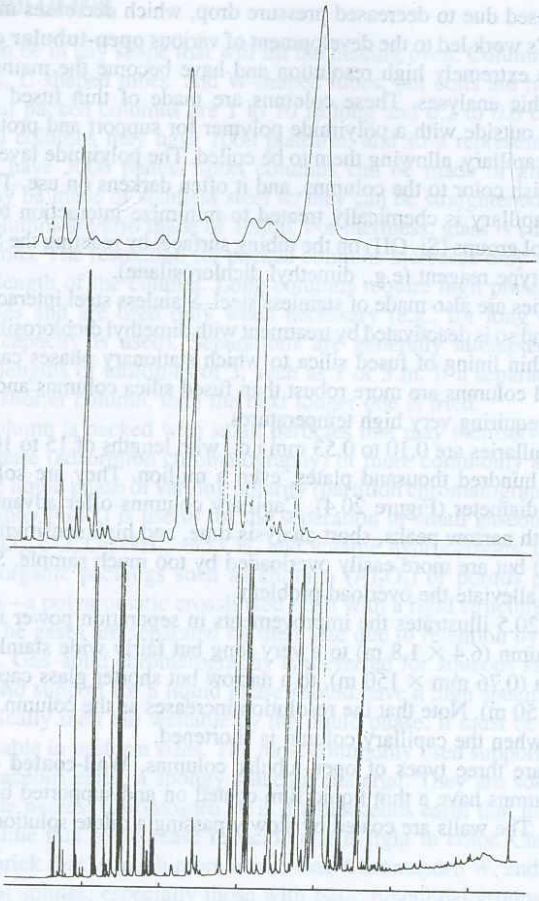


Fig. 20.5. Three generations in gas chromatography. Peppermint oil separation on (top) $\frac{1}{4}$-in. × 6-ft packed column; (center) 0.03-in. × 500-ft stainless steel capillary column; (bottom) 0.25-mm × 50-m glass capillary column. [From W. Jennings, *J. Chromatogr. Sci.*, **17** (1979) 363. Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, A Division of Preston Industries, Inc.]

phase through the columns. The solvent is evaporated by passing carrier gas through the columns. Following coating, the liquid phase is crosslinked to the wall. The resultant stationary liquid phase is 0.1 to 5 μm thick. Wall-coated open-tubular columns typically have 5000 plates/m. So a 50-m column will have 250,000 plates.

In **support coated open-tubular** (SCOT) columns, solid microparticles coated with the stationary phase (much like in packed columns) are attached to the walls of the capillary. These have higher surface area and have greater capacity than WCOT columns. The tubing diameter of these columns is 0.5 to 1.5 mm, larger than WCOT columns. The advantages of low pressure drop and long columns is maintained, but capacity of the columns approaches that of packed columns. Flow rates are faster and dead volume connections at the inlet and detector are less critical. Sample splitting is not required in many cases, so long as the sample volume is 0.5 μL or less. If a separation requires more than 10,000 plates, then a SCOT column should be considered instead of a packed column.

The resolution for open-tubular columns is WCOT > SCOT > PLOT. SCOT columns have capacities approaching those of packed columns.

The third type, **porous layer open-tubular** (PLOT) columns, have solid-phase particles attached to the column wall, for adsorption chromatography. Particles of alumina or porous polymers (molecular sieves) are typically used. These columns, like packed GSC columns, are useful for separating permanent gases, as well as volatile hydrocarbons. The resolution efficiency of open-tubular columns is generally in the order: WCOT > SCOT > PLOT. Wide-bore (0.5-mm) open-tubular

columns have been developed with thicker stationary liquid phases, up to 0.5 μm, that approach the capacity of SCOT and packed columns, but their resolution is decreased.

Columns can tolerate a limited amount of analyte before becoming overloaded, causing peak distortion and broadening, and shifts in retention time. Sample capacity ranges are from approximately 100 ng for a 0.25-mm-i.d. column with 0.25-μm-thick film, up to 5 μg for a 0.53-mm-i.d. column with a 5-μm-thick stationary phase.

Open-tubular columns, being the mainstay for gas-chromatographic separation, are manufactured by numerous companies. Some of the major ones include Alltech Associates, Inc. (www.alltechweb.com), Agilent Technologies (www.chem.agilent.com/Scripts/PHome.asp), Perkin-Elmer Instruments (http://instruments.perkinelmer.com), Quadrex Corp. (www.quadrexcorp.com), Restek Corp. (www.restekcorp.com), SGE, Inc. (www.sge.com), and Sigma-Aldrich (Supelco) (www.sigmaaldrich.com).

STATIONARY PHASES—THE KEY TO DIFFERENT SEPARATIONS

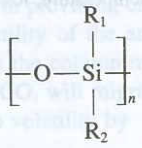
Over a thousand stationary phases have been proposed for gas chromatography, and numerous phases are commercially available. Hundreds of phases have been used for packed columns, necessitated by their low overall efficiency, and stationary-phase selection is critical for achieving selectivity. Several attempts have been made to predict the proper selection of liquid immobile phase without resorting exclusively to trial-and-error techniques (see below).

Liquid stationary phases are selected based on polarity, determined by the relative polarities of the solutes.

Phases are selected based on their polarity, keeping in mind that "like dissolve like." That is, a polar stationary phase will interact more with polar compounds, and vice versa. A phase should be selected in which the solute has some solubility. Nonpolar liquid phases are generally nonselective because there are few forces between the solute and the solvent, and so separations tend to follow the order of the boiling points of the solutes, with the low-boiling ones eluting first. Polar liquid phases exhibit several interactions with solutes such as dipole interactions, hydrogen bonds, and induction forces, and there is not necessarily the same elution correlation with volatility.

Polysiloxanes are the most common stationary phases for capillary GC.

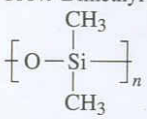
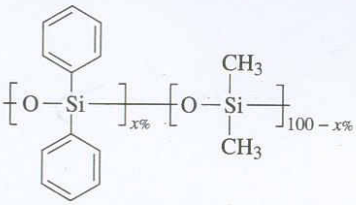
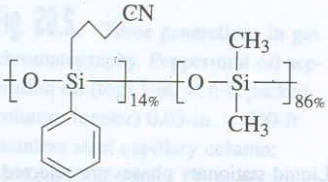
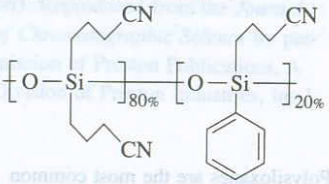
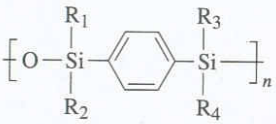
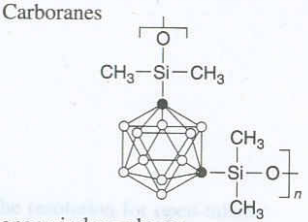
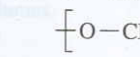
For fused silica columns, the majority of separations can be done with fewer than 10 bonded liquid stationary phases of varying polarity. This is because with their very high resolving power, and selectivity of the stationary phase is less critical. The stationary phases are high-molecular-weight, thermally stable polymers that are liquids or gums. The most common phases are polysiloxanes and polyethylene glycols (Carbowax), with the former the most widely used. The polysiloxanes have the backbone:



The R functional groups determine the polarity, and include methyl (CH_3), phenyl (), cyanopropyl (CH_2CH_2CN), and trifluoropropyl ($CH_2CH_2F_3$). Table 20.1 lists several commonly used stationary phases. Those with cyano functions are susceptible to attack by water and by oxygen. The carbowaxes must be liquid at operating temperatures. Incorporating either phenyl or carborane groups in the

Table 20.1

Capillary Fused Silica Stationary Phases

Phase	Polarity	Use	Max. Temp. (°C)
100% Dimethyl polysiloxane 	Nonpolar	Basic general-purpose phase for routine use. Hydrocarbons, polynuclear aromatics, PCBs.	320
Diphenyl, dimethyl polysiloxane 	5% Low 35%, 65% Intermediate 65%, 35% Intermediate	General-purpose, good high-temperature characteristics. Pesticides.	320 300 370
14% Cyanopropylphenyl–86% dimethylsiloxane 	Intermediate	Separation of organochlorine pesticides listed in EPA 608 and 8081 methods. Susceptible to damage by moisture and oxygen.	280
80% Biscyanopropyl–20% cyanopropylphenyl polysiloxane 	Very polar	Free acids, polysaturated fatty acids, alcohols. Avoid polar solvents such as water and methanol.	275
Arylenes 	Vary R as above to vary polarity	High temperature, low bleed	300–350
Carboranes  open circles = boron filled circles = carbon	Vary R as above to vary polarity	High temperature, low bleed	430
Poly(ethyleneglycol) (Carbowax) 	Very polar	Alcohols, aldehydes, ketones, and separation of aromatic isomers, e.g., xylenes	250

siloxane polymer backbone strengthens and stiffens the polymer backbone, which inhibits stationary-phase degradation at higher temperatures, and results in lower column bleed (loss of stationary phase). These columns are important when coupling to a highly sensitive mass spectrometer for detection (see below), where bleeding must be minimized.

RETENTION INDICES FOR LIQUID STATIONARY PHASES

We mentioned above the challenge of selecting the proper packed-column stationary phase from the myriad of possible phases. Methods have been developed that group phases according to their retention properties, for example, according to polarity. The **Kovats indices** and **Rohrschneider constant** are two approaches used to group different materials. Supina and Rose (Ref. 8) have tabulated the Rohrschneider constants for 80 common liquid phases, which enables one to decide, almost by inspection, if it is worth trying a particular liquid phase. Equally important, it is easy to identify phases that are very similar and differ only in trade name. McReynolds described a similar approach, defining phases by their **McReynolds constants** (Ref. 6). McReynolds used a standard set of test compounds for measuring retention times at 120°C on columns with 20% loading to classify stationary phases.

Another useful literature reference for the selection of stationary phases is a booklet entitled *Guide to Stationary Phases for Gas Chromatography*, compiled by Analabs, Inc., North Haven, CT, 1977.

The **Kovats retention index** is useful also for identifying a compound from its retention time relative to those of similar compounds in a homologous series (those that differ in the number of carbon atoms in a similar structure, as in alkane chains). The index I is defined as

$$I = 100\left[n_s + \frac{\log t'_{R(\text{unk})} - \log t'_{R(n_s)}}{\log t'_{R(n_l)} - \log t'_{R(n_s)}}\right] \tag{20.1}$$

where n_s is the number of carbon atoms in the smaller alkane, and n_l refers to the larger alkane; t'_R is the adjusted retention time (Equation 19.6). The Kovats index for an unknown compound can be compared with cataloged indices on various columns to aid in its identification. The logarithm of the retention time, $\log t'_R$, is generally a linear function of the number of carbon atoms in a homologous series of compounds.

WHAT ABOUT ANALYTE VOLATILITY?

In the above discussions, we have emphasized the role of the polarity of the stationary phase (and of the analyte) in providing effective separations. The other important factor is the relative volatility of the analyte species. The more volatile species will tend to migrate down the column more rapidly. Gaseous species, especially small molecules such as CO, will migrate rapidly. The retention factor, k (see Equation 19.25), is related to volatility by

$$\ln k = \Delta H_v/RT - \ln \gamma + C$$

where ΔH_v is the analyte heat of vaporization, so a higher value (higher boiling point) results in lower volatility and a larger k. Increasing the temperature T decreases this contribution to retention. The $\ln \gamma$ term is a function of the stationary-phase interaction (polarity, etc.), and is an activity term that decreases from unity for the pure state as interaction increases, causing k to increase; C is a constant

(and R is the gas constant). Quite a bit of boiling point selectivity and separation tuning capability is provided by the T-dependent term in the equation. This is why people do temperature programming (see below).

So the selection of chromatographic conditions (column, temperature, carrier flow rate) will be influenced by the compound volatility, molecular weight, and polarity.

20.3 Gas Chromatography Detectors

Since the initial experiments with gas chromatography were begun, over 40 detectors have been developed. Some are designed to respond to most compounds in general, while others are designed to be selective for particular types of substances. We describe some of the more widely used detectors. Table 20.2 lists and compares some commonly used detectors with respect to application, sensitivity, and linearity.

Thermal conductivity detectors are very general detectors, but not very sensitive.

The original GC detector was the **thermal conductivity**, or **hot wire, detector** (TCD). As a gas is passed over a heated filament wire, the temperature and thus the resistance of the wire will vary according to the thermal conductivity of the gas. The pure carrier is passed over one filament, and the effluent gas containing the sample constituents is passed over another. These filaments are in opposite arms of a Wheatstone bridge circuit that measures the *difference* in their resistance. So long as there is no sample gas in the effluent, the resistance of the wires will be the same. But whenever a sample component is eluted with the carrier gas, a small resistance change will occur in the effluent arm. The change in the resistance, which is proportional to the concentration of the sample component in the carrier gas, is registered on the recorder. The TCD is particularly useful for the analysis of gaseous mixtures, and of permanent gases such as CO_2.

Hydrogen and helium carrier gases are preferred with thermal conductivity detectors because they have a very high thermal conductivity compared with most other gases, and so the largest change in the resistance occurs in the presence of sample component gases (helium is preferred for safety reasons). The thermal conductivity of hydrogen is 53.4×10^{-5} and that of helium is 41.6×10^{-5} cal/°C-mol at 100°C, while those of argon, nitrogen, carbon dioxide, and most organic vapors are typically one-tenth of these values. The advantages of thermal conductivity detectors are their simplicity and approximately equal response for most substances. Also, their response is very reproducible. They are not the most sensitive detectors, however.

The flame ionization detection is both general and sensitive. It is the most commonly used detector.

Most organic compounds form ions in a flame, generally cations such as CHO^+. This forms the basis of an extremely sensitive detector, the **flame ionization detector** (FID). The ions are measured (collected) by a pair of oppositely charged electrodes. The response (number of ions collected) depends on the number of carbon atoms in the sample and on the oxidation state of the carbon. Those atoms that are completely oxidized do not ionize, and the compounds with the greatest number of low oxidation state carbons produce the largest signals. This detector gives excellent sensitivity, permitting measurement of components in the ppb concentration range. This is about 1000 times more sensitive than the thermal conductivity detector. However, the dynamic range is more limited, and samples of pure liquids are generally restricted to 0.1 μL or less. The carrier gas is relatively unimportant. Helium, nitrogen, and argon are most frequently employed. The flame ionization detector is insensitive to most inorganic compounds, including