# Chapter 12

1. (a) To calculate the number of theoretical plates we use equation 12.15; thus

$$N_A = 16 \times \left(\frac{t_{r,A}}{w_A}\right)^2 = 16 \times \left(\frac{8.04 \text{ min}}{0.15 \text{ min}}\right)^2 = 46000 \text{ plates}$$

$$N_B = 16 \times \left(\frac{t_{r,B}}{w_B}\right)^2 = 16 \times \left(\frac{8.26 \text{ min}}{0.15 \text{ min}}\right)^2 = 48500 \text{ plates}$$

$$N_C = 16 \times \left(\frac{t_{r,C}}{w_C}\right)^2 = 16 \times \left(\frac{8.43 \text{ min}}{0.16 \text{ min}}\right)^2 = 44400 \text{ plates}$$

The average number of theoretical plates is 46 300.

(b) The height of a theoretical plate, $H$, is equal to $L/N$ where $L$ is the length of the column and $N$ is the number of theoretical plates. Using the average number of theoretical plates from part (a) gives the average height as

$$H = \frac{20 \text{ m} \times \frac{1000 \text{ mm}}{\text{m}}}{46300 \text{ plates}} = 0.43 \text{ mm/plate}$$

(c) Theoretical plates do not really exist; they are, instead, an artificial construct that is useful for modeling the variables that affect the width of a solute's peak and its resolution relative to other solutes. As we see from equation 12.15, the number of theoretical plates for a solute is defined in terms of its retention time and its peak width. Two solutes may have identical retention times but different peak widths because retention time is a function of the equilibrium between the concentration of solute in the mobile phase and the concentration of solute in the stationary phase, but peak width is a function, in part, of the kinetic effects that control how quickly the solute moves within the stationary phase and within the mobile phase.

2. Using equation 12.1, the resolution between solutes A and B is

$$R_{AB} = \frac{2(t_{r,B} - t_{r,A})}{w_A + w_B} = \frac{2(8.26 \text{ min} - 8.04 \text{ min})}{0.15 \text{ min} + 0.15 \text{ min}} = 1.47 \approx 1.5$$

and the resolution between solutes B and C is

$$R_{BC} = \frac{2(t_{r,C} - t_{r,B})}{w_B + w_C} = \frac{2(8.43 \text{ min} - 8.26 \text{ min})}{0.15 \text{ min} + 0.16 \text{ min}} = 1.10 \approx 1.1$$

To calculate selectivity factors or to calculate resolution using equation 12.19, we first must calculate each solute's retention factor using equation 12.8; thus

$$k_A = \frac{t_{r,A} - t_m}{t_m} = \frac{8.04 \text{ min} - 1.19 \text{ min}}{1.19 \text{ min}} = 5.756 \approx 5.76$$

$$k_B = \frac{t_{r,B} - t_m}{t_m} = \frac{8.26 \text{ min} - 1.19 \text{ min}}{1.19 \text{ min}} = 5.941 \approx 5.94$$

$$k_C = \frac{t_{r,C} - t_m}{t_m} = \frac{8.43 \text{ min} - 1.19 \text{ min}}{1.19 \text{ min}} = 6.084 \approx 6.08$$

With retention factors in hand, we calculate the selectivity factors using equation 12.9; thus

$$\alpha_{AB} = \frac{k_B}{k_A} = \frac{5.941}{5.765} = 1.032 \approx 1.03$$

$$\alpha_{BC} = \frac{k_C}{k_B} = \frac{6.084}{5.941} = 1.024 \approx 1.02$$

Finally, we use equation 12.19 to calculate resolution; thus

$$R_{AB} = \frac{\sqrt{N_B}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_B}{1 + k_B} =$$

$$\frac{\sqrt{48500}}{4} \times \frac{1.032 - 1}{1.032} \times \frac{5.941}{1 + 5.941} = 1.46 \approx 1.5$$

$$R_{BC} = \frac{\sqrt{N_C}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k_C}{1 + k_C} =$$

$$\frac{\sqrt{44400}}{4} \times \frac{1.024 - 1}{1.024} \times \frac{6.084}{1 + 6.084} = 1.06 \approx 1.1$$

To improve the resolution between solute B and solute C, we might pursue the following: increase the number of theoretical plates; increase the resolution factor for solute C; and/or increase the column's relative selectivity for the two solutes. For the latter, we can seek to decrease the retention time for solute B, increase the retention time for solute C, or both.

3.  Depending on your measurements, your answers may vary slightly from those given here: the solute's retention time, $t_r$, is 350 s, the retention time for the non-retained solutes, $t_m$, is 25 s, and the solute's peak width, $_w$, is 22 s. Using these values gives the following additional results

$$t_r' = t_r - t_m = 350 \text{ s} - 25 \text{ s} = 325 \text{ s}$$

$$k = \frac{t_r - t_m}{t_m} = \frac{350 \text{ s} - 25 \text{ s}}{25 \text{ s}} = 13$$

$$N = 16 \times \left(\frac{t_r}{w}\right)^2 = 16 \times \left(\frac{350 \text{ s}}{22 \text{ s}}\right)^2 = 4050 \text{ plates}$$

$$H = \frac{L}{N} = \frac{2 \text{ m} \times \frac{1000 \text{ mm}}{\text{m}}}{4050 \text{ plates}} = 0.49 \text{ mm/plate}$$

4.  Depending on your measurements, your answers may vary slightly from those given here: solute A's retention time, $t_{r,A}$, is 350 s and its peak width, $w_A$, is 19.8 s; solute B's retention time, $t_{r,B}$, is 370 s and its peak width, $w_B$, is 20.3 s. Using these values gives a resolution of

$$R_{AB} = \frac{2(t_{r,B} - t_{r,A})}{w_A + w_B} = \frac{2(370 \text{ s} - 350 \text{ s})}{19.8 \text{ s} + 20.3 \text{ s}} = 0.998 \approx 1.0$$

5.  Increasing the length of the column increases the number of theoretical plates. Using equation 12.19, we see that

$$\frac{(R_{AB})_{new}}{(R_{AB})_{old}} = \frac{1.5}{1.0} = \frac{(\sqrt{N_B})_{new}}{(\sqrt{N_B})_{old}}$$

Rearranging and solving for the number of theoretical plates in the new, longer column gives

$$(\sqrt{N_B})_{new} = 1.5 \times (\sqrt{N_B})_{old}$$

$$(N_B)_{new} = 2.25 \times (N_B)_{old}$$

To increase the number of theoretical plates by a factor of 2.25× by adjusting the column's length only, requires a column that is 2.25× longer than the original column, or 4.5 m in length.

To increase the number of theoretical plates without increasing the column's length, we must decrease the height of a theoretical plate. First, let's calculate the number of theoretical plates for the second solute in Figure 12.68, as this is the number of theoretical plates that appears in equation 12.19; thus

$$N_B = 16 \times \left(\frac{t_{r,B}}{w_B}\right)^2 = 16 \times \left(\frac{370 \text{ s}}{20.3 \text{ s}}\right)^2 = 5315 \text{ plates}$$

To increase the number of theoretical plates by a factor of 2.25× requires a column that has 11 960 plates, or a height of

$$H = \frac{L}{N} = \frac{2 \text{ m} \times \dfrac{1000 \text{ mm}}{\text{m}}}{11960 \text{ plates}} = 0.167 \text{ mm/plate}$$

6.  Using equation 12.19, we find that for the first row the resolution is

$$R_{AB} = \frac{\sqrt{100000}}{4} \times \frac{1.05 - 1}{1.05} \times \frac{0.5}{1 + 0.5} = 1.25$$

and for the second row, the retention factor for solute B is

$$1.50 = \frac{\sqrt{10000}}{4} \times \frac{1.10 - 1}{1.10} \times \frac{k_B}{1 + k_B}$$

$$1.50 = 2.273 \times \frac{k_B}{1 + k_B}$$

$$0.6599 + 0.6599 k_B = k_B$$

$$k_B = 1.94$$

and for the third row, the selectivity ratio is

$$1.00 = \frac{\sqrt{10000}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{4}{1 + 4}$$

$$1.00 = 20 \times \frac{\alpha - 1}{\alpha}$$
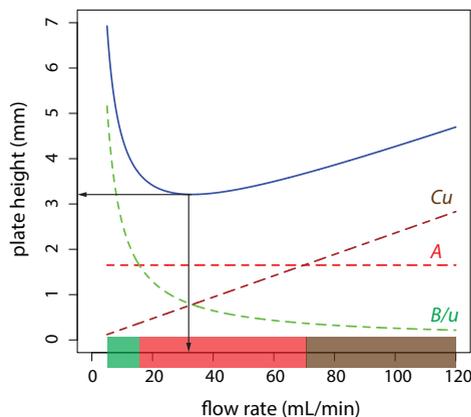
**Figure SM12.1** The van Deemter plot for Problem 7a. The solid **blue** line shows the plate height as a function of flow rate using equation 12.26; the **red**, **green**, and **brown** dashed lines show, respectively, the contribution to the plate height of multiple paths (A), of longitudinal diffusion (B), and of mass transfer (C). The range of flow rates where each term is the limiting factor are shown along the x-axis; from left-to-right, they are B, A, and C. The arrows identify the optimum flow rate of 33 mL/min with a plate height of 3.20 mm.
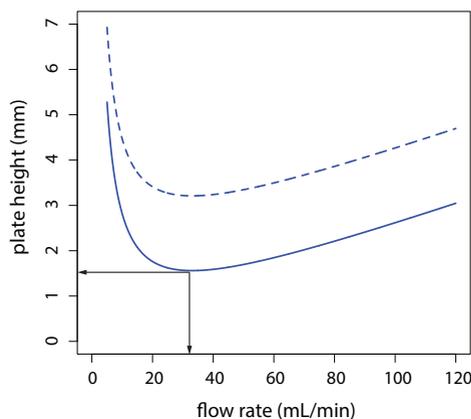


**Figure SM12.2** The van Deemter plot for Problem 7d. The solid **blue** line shows the plate height as a function of flow rate for an open-tubular column and the dashed **blue** line is for the packed column in Problem 7a. The arrows identify the optimum flow rate of 33 mL/min with a plate height of 1.56 mm.

$$0.0500\alpha = \alpha - 1$$

$$\alpha = 1.05$$

and for the fourth row, the number of theoretical plates is

$$1.75 = \frac{\sqrt{N_B}}{4} \times \frac{1.05 - 1}{1.05} \times \frac{3.0}{1 + 3.0}$$

$$1.75 = 8.929 \times 10^{-3} \sqrt{N_B}$$

$$\sqrt{N_B} = 196.0$$

$$N_B = 38400 \text{ plates}$$

7.  (a) Figure SM12.1 shows the van Deemter plot of plate height, $H$, as a function of the mobile phase's flow rate, $u$, with the individual contributions to plate height shown by the dashed lines and their combined contribution shown by the solid line.

(b) The $B$ term (longitudinal diffusion) limits the plate height for flow rates less than 16 mL/min. The $A$ term (multiple pathlengths) limits the plate height for flow rates between 16 mL/min and 71 mL/min. The $C$ term (mass transfer) limits the plate height for flow rates greater than 71 mL/min.

(c) The optimum flow rate is 33 mL/min with a corresponding plate height of 3.20 mm.

(d) Figure SM12.2 shows the van Deemter plot for an open-tubular column along with the original packed column from part (a). The optimum flow rate remains unchanged at 33 mL/min, but the corresponding plate height is 1.56 mm.

(e) Using equation 12.10

$$\frac{N_{open}}{N_{packed}} = \frac{L\big/H_{open}}{L\big/H_{packed}} = \frac{H_{packed}}{H_{open}} = \frac{3.20 \text{ mm}}{1.56 \text{ mm}} = 2.05$$

we find that the open-tubular column has approximately $2\times$ as many theoretical plates as in the packed column.

8.  (a) Figure SM12.3 shows the van Deemter plots for both the first row of data and for the last row of data. For the first row of data, the optimum reduced flow rate is 3.63, which corresponds to an actual flow rate of

$$u = \frac{\nu D_m}{d_p} = \frac{3.63 \times (6.23 \times 10^{-6} \text{ cm}^2\text{s}^{-1})}{(5.44 \times 10^{-6} \text{ m}) \times \frac{100 \text{ cm}}{\text{m}}} = 0.0416 \text{ cm/s}$$

and the optimum reduced plate height is 1.36, which corresponds to an actual plate height of

$$H = hd_p = 1.36 \times (5.44\,\mu m) = 7.40\,\mu m$$

For the last row of data, the optimum reduced flow rate is 3.25, which corresponds to an actual flow rate of

$$u = \frac{\nu D_m}{d_p} = \frac{3.25 \times (6.23 \times 10^{-6}\,cm^2\,s^{-1})}{(5.44 \times 10^{-6}\,m) \times \dfrac{100\,cm}{m}} = 0.0372\,cm/s$$

and the optimum reduced plate height is 0.97, which corresponds to an actual plate height of

$$H = hd_p = 0.97 \times (5.44\,\mu m) = 5.28\,\mu m$$

(b) One of the most important contributions to the multiple paths term ($A$) in the van Deemter equation, is the difference in the stationary phase's packing efficiency near the column's walls relative to that near the column's center. The less compact packing found near the column's walls allows for a shorter pathlength through the column. Solute molecules that spend more time near the column's walls elute more quickly than solute molecules that spend more time near the column's center. The result of this difference, of course, is greater band broadening, fewer theoretical plates, and larger value for $H$. A column with an internal diameter of 12 μm packed with 5.44 μm diameter particles can fit only two particles side-by-side, which means it no longer makes sense to distinguish between the column's center and its walls; the result is a reduction in $A$.

9.  The order of elution in both cases is determined by the relative polarities of the solutes, which, from least polar-to-most polar are $n$-heptane, tetrahydrofuran, 2-butanone, and $n$-proponal. When using a more polar stationary phase, such as Carbowax, the more polar solutes are retained longer—and, thus, elute later—than the less polar solutes. The order of elution is reversed when using a less polar stationary phase, such as polydimethyl siloxane.

10. For a single standard we assume that $S = k_A C_A$, where $S$ is the signal, $k_A$ is the analyte's sensitivity, and $C_A$ is the analyte's concentration. Given the data for the standard that contains all four trihalomethanes, we obtain the following values of $k_A$

$$k_{CHCl_3} = \frac{S}{C_{CHCl_3}} = \frac{1.35 \times 10^4}{1.30\,ppb} = 1.038 \times 10^4\,ppb^{-1}$$

$$k_{CHCl_2Br} = \frac{S}{C_{CHCl_2Br}} = \frac{6.12 \times 10^4}{0.90\,ppb} = 6.800 \times 10^4\,ppb^{-1}$$

$$k_{CHClBr_2} = \frac{S}{C_{CHClBr_2}} = \frac{1.71 \times 10^4}{4.00\,ppb} = 4.275 \times 10^3\,ppb^{-1}$$

$$k_{CHBr_3} = \frac{S}{C_{CHBr_3}} = \frac{1.52 \times 10^4}{1.20\,ppb} = 1.267 \times 10^4\,ppb^{-1}$$



**Figure SM12.3** The van Deemter plot for Problem 8a. The solid **blue** line shows results for the first row of data and the solid **green** line shows the results for the last row of data. The arrows identify the optimum reduced flow rate and the optimum reduced plate height for each set of data.
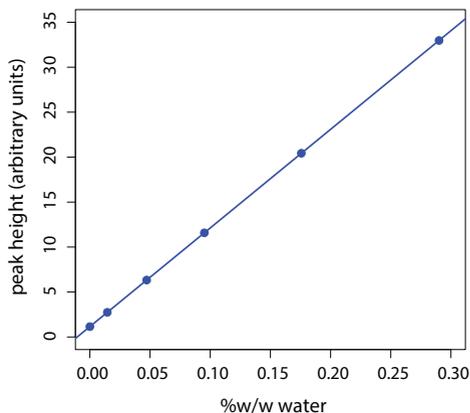
**Figure SM12.4** Calibration data (**blue** dots) and calibration curve (**blue** line) for the data in Problem 11.



**Figure SM12.5** Calibration data (**blue** dots) and calibration curve (**blue** line) for the data in Problem 13.

Now that we know each analyte's sensitivity, we can calculate each analyte's concentration in the sample; thus

$$C_{CHCl_3} = \frac{S}{k_{CHCl_3}} = \frac{1.56 \times 10^4}{1.038 \times 10^4 \text{ ppb}^{-1}} = 1.50 \text{ ppb}$$

$$C_{CHCl_2Br} = \frac{S}{k_{CHCl_2Br}} = \frac{5.13 \times 10^4}{6.800 \times 10^4 \text{ ppb}^{-1}} = 0.754 \text{ ppb}$$

$$C_{CHClBr_2} = \frac{S}{k_{CHClBr_2}} = \frac{1.49 \times 10^4}{4.275 \times 10^3 \text{ ppb}^{-1}} = 3.49 \text{ ppb}$$

$$C_{CHClBr_3} = \frac{S}{k_{CHClBr_3}} = \frac{1.76 \times 10^4}{1.267 \times 10^4 \text{ ppb}^{-1}} = 1.39 \text{ ppb}$$

11. (a) Figure SM12.4 shows the calibration data and the calibration curve, for which the equation is

$$\text{peak height} = 1.151 + 109.7\%\text{w/w}^{-1} \times C_{water}$$

Substituting in the sample's peak height of 8.63 gives the concentration of water as 0.0682% w/w.

(b) Substituting in the sample's peak height of 13.66 gives the concentration of water as 0.114%w/w as analyzed. The concentration of water in the original sample is

$$\frac{\frac{0.114 \text{ g H}_2\text{O}}{100 \text{ g CH}_3\text{OH}} \times 4.489 \text{ g CH}_3\text{OH}}{0.175 \text{ g sample}} \times 100 = 2.92\%\text{w/w H}_2\text{O}$$

12. The two equations for this standard additions are

$$2.70 \times 10^5 = kC_{water}$$

$$1.06 \times 10^6 = k(C_{water} + 5.0 \text{ mg H}_2\text{O/g soil})$$

Solving the first equation for $k$ and substituting into the second equation gives

$$1.06 \times 10^6 = \frac{2.70 \times 10^5}{C_{water}}(C_{water} + 5.0 \text{ mg H}_2\text{O/g soil})$$

which we solve for $C_{water}$

$$1.06 \times 10^6 = 2.70 \times 10^5 + \frac{1.35 \times 10^6 \text{ mg H}_2\text{O/g soil}}{C_{water}}$$

$$7.90 \times 10^5 = \frac{1.35 \times 10^6 \text{ mg H}_2\text{O/g soil}}{C_{water}}$$

$$C_{water} = \frac{1.35 \times 10^6 \text{ mg H}_2\text{O/g soil}}{7.90 \times 10^5} = 1.7 \text{ mg H}_2\text{O/g soil}$$

13. The three standard additions in this case are of pure methyl salicylate. Figure SM12.5 shows the calibration data and the calibration curve, plotting peak height on the *y*-axis versus the volume of methyl salic-

ylate added on the *x*-axis. A regression analysis gives the calibration equation as

$$\text{peak height} = 57.51 \, \text{mm} + (150.66 \, \text{mm/mL}) \times V_{\text{added}}$$

When we plot a standard addition in this way, the *y*-intercept is $k_A C_A V_o / V_f$, where $k_A$ is the method's sensitivity for methyl salicylate, $C_A$ is the concentration of methyl salicylate, $V_o$ is the volume of sample taken (20.00 mL), and $V_f$ is the sample's final volume after dilution (25.00 mL). The slope is $k_A C_{\text{std}} / V_f$, where $C_{\text{std}}$ is the concentration of the standard solution of methyl salicylate (100%). Solving both the equation for the slope, $b_1$, and the equation for the *y*-intercept, $b_0$, for $k$, and setting the equations equal to each other gives

$$\frac{b_0 V_f}{C_A V_o} = k_A = \frac{b_1 V_f}{C_{\text{std}}}$$

Solving for $C_A$ gives its value as

$$C_A = \frac{b_0 C_{\text{std}}}{b_1 V_o} = \frac{57.51 \, \text{mm} \times 100\%}{150.66 \, \text{mm/mL} \times 20.00 \, \text{mL}} = 1.91\%$$

14. For the internal standard we have

$$\frac{S_A}{S_{\text{IS}}} = \frac{67.3}{19.8} = K \times \frac{C_A}{C_{\text{IS}}} = \frac{45.2 \, \text{mg camphor}}{(2.00 \, \text{mL}) \times (6.00 \, \text{mg terpene/mL})}$$

which we solve for $K$, obtaining 0.902 mg camphor/mg terpene. Using this value for $K$ and the data for the sample, we have

$$\frac{24.9}{13.5} = \frac{0.902 \, \text{mg camphor}}{\text{mg terpene}} \times \frac{C_A}{2.00 \, \text{mL} \times \dfrac{6.00 \, \text{mg terpene}}{\text{mL}}}$$

which we solve for $C_A$, obtaining 24.54 mg camphor in the sample as analyzed. The concentration of camphor in the original sample is

$$\frac{24.45 \, \text{mg camphor}}{53.6 \, \text{mg sample}} \times 100 = 45.8\% \text{w/w camphor}$$

15. Figure SM12.6 shows the calibration data and the calibration curve, for which the equation is

$$\frac{A_{\text{analyte}}}{A_{\text{int std}}} = -0.01983 + (3.206 \times 10^{-3} \, \text{ppb}^{-1}) \, C_{\text{analyte}}$$

Substituting in the sample's peak area ratio of 0.108 gives the concentration of heptachlor epoxide as 39.87 ppb in the sample as analyzed. The concentration of heptachlor epoxide in the original sample of orange rind is

$$\frac{\dfrac{39.86 \, \text{ng}}{\text{mL}} \times 10.00 \, \text{mL}}{50.0 \, \text{g sample}} = \frac{7.97 \, \text{ng}}{\text{g}} = 7.97 \, \text{ppb}$$
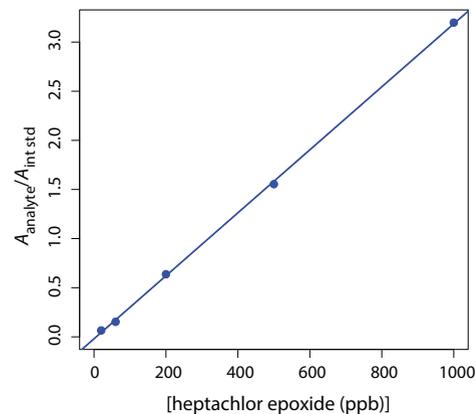


**Figure SM12.6** Calibration data (**blue** dots) and calibration curve (**blue** line) for the data in Problem 15.

Recall that 1.00 ppb is equivalent to 1.00 ng/mL or to 1.00 ng/g.
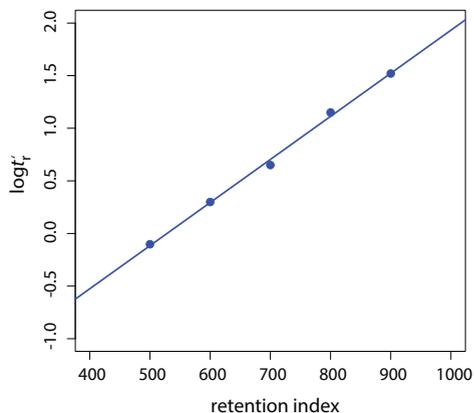
**Figure SM12.7** Calibration data (**blue** dots) and calibration curve (**blue** line) for the data in Problem 17.
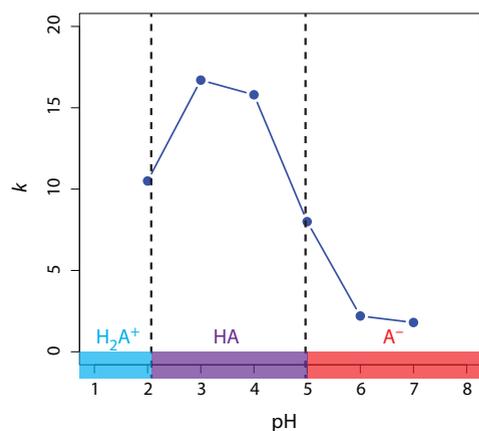


**Figure SM12.8** Plot showing the effect of pH on the retention factor for 2-amino-benzoic acid. The x-axis also displays the ladder diagram for 2-aminobenzoic acid, which shows, in **blue**, that its full protonated form, $H_2A^+$, is the predominate species below a pH of 2.08, that shows, in **purple**, that its neutral form, HA, is the predominate species between a pH of 2.08 and a pH of 4.96, and that shows, in **red**, that its fully deprotonated form, $A^-$, is the predominate species above a pH of 4.96.

16. The retention indices for octane and for nonane are, by definition, 800 and 900, respectively. The retention index for toluene is calculated using equation 12.27; thus

$$I_{\text{toluene}} = 100 \times \frac{\log(17.73) - \log(15.98)}{\log(20.42) - \log(15.98)} + 800 = 842$$

17. Figure SM12.7 shows a plot of the data where the y-axis is the log of adjusted retention time and where the x-axis is the retention index (100×number of C atoms). A regression analysis of the data gives the calibration curve's equation as

$$\log t_r' = -2.163 + (4.096 \times 10^{-3})I$$

Substituting in the analyte's retention time of 9.36 min gives its retention index, I, as 765.

18. In a split injection, only a small portion of the sample enters the column, which results in peaks with smaller areas and smaller widths when compared to a splitless injection, where essentially all the sample enters the column. Because it takes longer for the sample to enter the column when using a splitless injection, retention times are longer and peak widths are broader.

19. Figure SM12.8 shows a plot of the retention factor for 2-amino-benzoic acid as a function of pH. Superimposed on the x-axis is a ladder diagram for 2-aminobenzoice acid, a diprotic weak acid with $pK_a$ values of 2.08 and of 4.96. The neutral form of 2-aminobenzoic acid, HA, partitions into the stationary phase to a greater extent and, therefore, has a longer retention time and a larger retention factor than either its fully protonated form, $H_2A^+$, or its fully deprotonated form, $A^-$.

20. (a) For a reverse-phase separation, increasing the %v/v methanol in the mobile phase leads to a less polar mobile phase and to smaller retention times; the result is a decrease in each solute's retention factor.

(b) The advantage to using a smaller concentration of methanol in the mobile phase is that the resolution between caffeine and salicylamide is better ($\alpha = 1.8$ when using 30%v/v methanol and $\alpha = 1.3$ when using 55% methanol); the disadvantage of using a smaller concentration of methanol is that the separation requires more time.

21. (a) The retention time for benzoic acid ($pK_a$ of 4.2) shows a sharp decrease between a pH of 4.0 and 4.5 as its predominate form changes from a neutral weak acid, HA, to an anionic weak base, $A^-$, that is less strong retained by the stationary phase. The retention time for aspartame (reported $pK_a$ values are in the range of 3.0–3.5 and 7.3–8.5) increases above a pH of 3.5 as its predominate form changes from $H_2A^+$ to HA, with the neutral form being more strong retained by the stationary phase. Caffeine is a neutral base throughout this

pH range; thus, the modest change in its retention times cannot be explained by its acid-base chemistry.

(b) Figure SM12.9 shows a plot of the retention times for each species as a function of pH. The two shaded areas show ranges of pH values where an adequate separation is likely (defined here as a difference in retention time of at least 1.0 min). For pH values between 3.5 and 4.1, the retention times for benzoic acid and aspartame are similar in value, with the two coeluting at a pH of approximately 3.9. Above a pH of 4.3, the retention times for benzoic acid and caffeine are similar in value with the two coeluting a pH of 4.4.
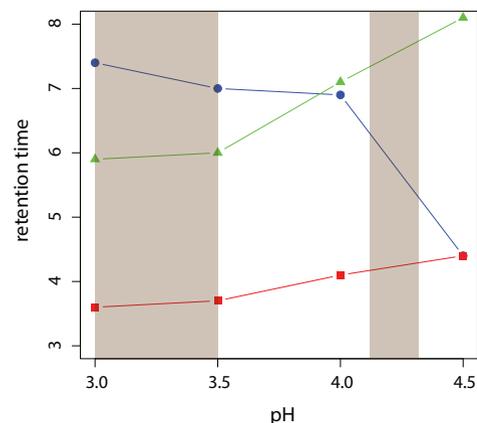


**Figure SM12.9** Plot showing the effect of pH on the retention times for benzoic acid (in **blue**), for aspartame (in **green**), and for caffeine (in **red**). The areas highlighted in **brown** show mobile phases where an adequate separation of all three compounds is possible.

22. For a single standard we assume that $S = k_A C_A$, where $S$ is the signal, $k_A$ is the analyte's sensitivity, and $C_A$ is the analyte's concentration. Given the data for the standard that contains all seven analytes, we obtain the following values of $k_A$

$$k_{\text{vit C}} = \frac{S}{C_{\text{vit C}}} = \frac{0.22}{170\ \text{ppm}} = 1.29 \times 10^{-3}\ \text{ppm}^{-1}$$

$$k_{\text{niacin}} = \frac{S}{C_{\text{niacin}}} = \frac{1.35}{130\ \text{ppm}} = 1.04 \times 10^{-2}\ \text{ppm}^{-1}$$

$$k_{\text{niacinamide}} = \frac{S}{C_{\text{niacinamide}}} = \frac{0.90}{120\ \text{ppm}} = 7.50 \times 10^{-3}\ \text{ppm}^{-1}$$

$$k_{\text{pyridoxine}} = \frac{S}{C_{\text{pyridoxine}}} = \frac{1.37}{150\ \text{ppm}} = 9.13 \times 10^{-3}\ \text{ppm}^{-1}$$

$$k_{\text{thiamine}} = \frac{S}{C_{\text{thiamine}}} = \frac{0.82}{60\ \text{ppm}} = 1.37 \times 10^{-2}\ \text{ppm}^{-1}$$

$$k_{\text{folic acid}} = \frac{S}{C_{\text{folic acid}}} = \frac{0.36}{15\ \text{ppm}} = 2.40 \times 10^{-2}\ \text{ppm}^{-1}$$

$$k_{\text{riboflavin}} = \frac{S}{C_{\text{riboflavin}}} = \frac{0.29}{10\ \text{ppm}} = 2.90 \times 10^{-2}\ \text{ppm}^{-1}$$

Now that we know each analyte's sensitivity, we can calculate each analyte's concentration in the sample; thus

$$C_{\text{Vit C}} = \frac{S}{k_{\text{Vit C}}} = \frac{0.87}{1.29 \times 10^{-3}\ \text{ppm}^{-1}} = 674\ \text{ppm}$$

$$C_{\text{niacin}} = \frac{S}{k_{\text{niacin}}} = \frac{0.00}{1.04 \times 10^{-2}\ \text{ppm}^{-1}} = 0\ \text{ppm}$$

$$C_{\text{niacinamide}} = \frac{S}{k_{\text{niacinamide}}} = \frac{1.40}{7.50 \times 10^{-3}\ \text{ppm}^{-1}} = 187\ \text{ppm}$$

$$C_{\text{pryidoxine}} = \frac{S}{k_{\text{pryidoxine}}} = \frac{0.22}{9.13 \times 10^{-3}\ \text{ppm}^{-1}} = 24.1\ \text{ppm}$$

$$C_{\text{thiamine}} = \frac{S}{k_{\text{thiamine}}} = \frac{0.19}{1.37 \times 10^{-2}\ \text{ppm}^{-1}} = 13.9\ \text{ppm}$$
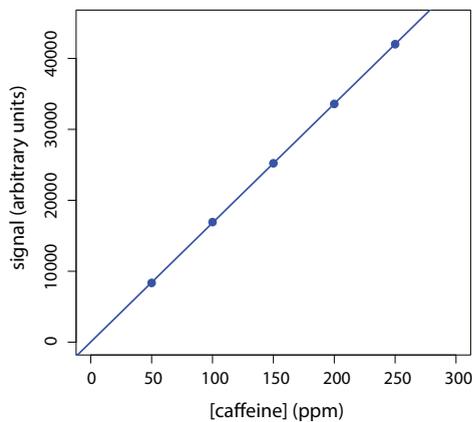
**Figure SM12.10** Calibration data (**blue** dots) and calibration curve (**blue** line) for the data in Problem 23.
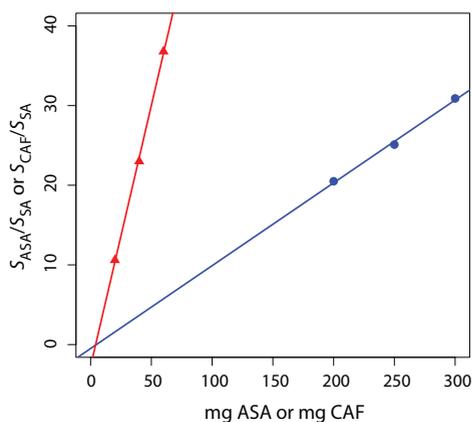


**Figure SM12.11** Calibration data and calibration curve for the analytes in Problem 24: data and results for acetylsalicylic acid (ASA) shown in **blue**, and data and results for caffeine (CAF) shown in **red**.

$$C_{\text{folic acid}} = \frac{S}{k_{\text{folic acid}}} = \frac{0.11}{2.40 \times 10^{-2} \text{ ppm}^{-1}} = 4.58 \text{ ppm}$$

$$C_{\text{riboflavin}} = \frac{S}{k_{\text{riboflavin}}} = \frac{0.44}{2.90 \times 10^{-2} \text{ ppm}^{-1}} = 15.2 \text{ ppm}$$

These are the concentrations as analyzed. To prepare the tablet for analysis, we dissolved it in 100 mL of solvent (10 mL of 1% v/v $NH_3$ in dimethyl sulfoxide and 90 mL of 2% acetic acid); thus, we multiply each concentration by 0.100 L to arrive at the mass of each analyte in the original tablet: 67 mg of vitamin C; 0 mg of niacin; 19 mg of niacinamide; 2.4 mg of pyridoxine; 1.4 mg of thiamine; 0.46 mg of folic acid; and 1.5 mg of riboflavin.

23. Figure SM12.10 shows the calibration data and the calibration curve, for which the equation is

$$\text{signal} = 30.20 + (167.91 \text{ppm}^{-1})\, C_{\text{caffeine}}$$

Substituting in the sample's signal of 21 469 gives the concentration of caffeine as 127.7 ppm in the sample as analyzed. The amount of caffeine in the original sample, therefore, is

$$\frac{127.7 \text{ mg caffeine}}{L} \times \frac{10.00 \text{ mL}}{1.00 \text{ mL}} \times 0.02500 \text{ L} = 31.9 \text{ mg caffeine}$$

24. (a) Figure SM12.11 shows the calibration data and the calibration curves for both acetylsalicylic acid (ASA) and for caffeine (CAF), using salicylic acid (SA) as an internal standard. The calibration equation for acetylsalicylic acid is

$$\frac{S_{\text{ASA}}}{S_{\text{SA}}} = -0.5000 + (0.1040 \text{ mg}^{-1})\, m_{\text{ASA}}$$

and the calibration curve for caffeine is

$$\frac{S_{\text{CAF}}}{S_{\text{SA}}} = -2.733 + (0.6550 \text{ mg}^{-1})\, m_{\text{CAF}}$$

Substituting in the peak area ratio of 23.2 for ACA gives the amount of acetylsalicylic acid as 228 mg, and substituting in the peak area ratio of 17.9 for CAF gives the amount of caffeine as 31.5 mg. Because the standards and the sample were prepared identically, these are the amounts of acetylsalicylic acid and of caffeine in the original tablet.

(b) Analgesic tablets contain some insoluble materials. If we do not remove these insoluble materials before we inject the sample, we will clog the column and degrade its performance.

(c) When we use an internal standard, the relative amount of solvent is not important as it does not affect the ratio of analyte-to-internal standard in any standard or sample. What does matter is that we know the mass of acetylsalicylic acid and the mass of caffeine in each standard, and that we know that each standard contains the same

mass of the internal standard, salicylic acid; we ensure this by adding exactly 10.00 mL of the same standard solution of salicylic acid to each standard and to each sample.

(d) If there is some decomposition of acetylsalicylic acid to salicylic acid, then the analysis is no longer possible as an unknown portion of salicylic acid's peak area will come from acetylsalicylic acid. One way to determine if this is a problem is to inject a sample without adding any salicylic acid and then look to see whether a peak appears at the retention time for salicylic acid; if a peak is present, then we cannot use this method to determine the concentration of acetylsalicylic acid or caffeine.

25. We begin by letting $m_A$ represent the milligrams of vitamin A in a 10.067 g portion of cereal. Because we use a different amount of cereal in the standard addition, 10.093 g, the cereal's contribution of vitamin A to the standard addition is

$$m_A \times \frac{10.093 \text{ g}}{10.067 \text{ g}}$$

The following two equations relate the signal to the mass of vitamin A in the sample and in the standard addition

$$S_{sample} = km_A$$

$$S_{std\ add} = k\left\{ m_A \times \frac{10.093 \text{ g}}{10.067 \text{ g}} + 0.0200 \text{ mg} \right\}$$

Solving both equations for $k$ and setting them equal to each other leaves us with

$$\frac{S_{sample}}{m_A} = \frac{S_{int\ std}}{m_A \times \dfrac{10.093 \text{ g}}{10.067 \text{ g}} + 0.0200 \text{ mg}}$$

Making appropriate substitutions and solving gives

$$\frac{6.77 \times 10^3}{m_A} = \frac{1.32 \times 10^4}{m_A \times \dfrac{10.093 \text{ g}}{10.067 \text{ g}} + 0.0200 \text{ mg}}$$

$$(6.7875 \times 10^3)\, m_A + 135.4 \text{ mg} = (1.32 \times 10^4)\, m_A$$

$$6412.5 m_A = 135.4 \text{ mg}$$

$$m_A = 0.0211 \text{ mg}$$

The vitamin A content of the cereal, therefore, is

$$\frac{0.0211 \text{ mg vitamin A}}{10.067 \text{ g sample}} \times 100 = 0.211 \text{ mg vitamin A/100 g cereal}$$

26. (a) The separation is based on an anion-exchange column, which will not bind with $Ca^{2+}$ or $Mg^{2+}$. Adding EDTA, a ligand that forms stable complexes with $Ca^{2+}$ and $Mg^{2+}$, converts them to the anions $CaY^{2-}$ and $MgY^{2-}$.

(b) For a single standard we assume that $S = k_A C_A$, where $S$ is the signal, $k_A$ is the analyte's sensitivity, and $C_A$ is the analyte's concentration. Given the data for the standard that contains all seven analytes, we obtain the following values of $k_A$

$$k_{HCO_3^-} = \frac{S}{C_{HCO_3^-}} = \frac{373.5}{1.0 \text{ mM}} = 373.5 \text{ mM}^{-1}$$

$$k_{Cl^-} = \frac{S}{C_{Cl^-}} = \frac{322.5}{0.20 \text{ mM}} = 1612 \text{ mM}^{-1}$$

$$k_{NO_2^-} = \frac{S}{C_{NO_2^-}} = \frac{264.8}{0.20 \text{ mM}} = 1324 \text{ mM}^{-1}$$

$$k_{NO_3^-} = \frac{S}{C_{NO_3^-}} = \frac{262.7}{0.20 \text{ mM}} = 1314 \text{ mM}^{-1}$$

$$k_{SO_4^-} = \frac{S}{C_{SO_4^-}} = \frac{341.3}{0.20 \text{ mM}} = 1706 \text{ mM}^{-1}$$

$$k_{Ca^{2+}} = \frac{S}{C_{Ca^{2+}}} = \frac{458.9}{0.20 \text{ mM}} = 2294 \text{ mM}^{-1}$$

$$k_{Mg^{2+}} = \frac{S}{C_{Mg^{2+}}} = \frac{352.0}{0.20 \text{ mM}} = 1760 \text{ mM}^{-1}$$

Now that we know each analyte's sensitivity, we can calculate each analyte's concentration in the sample; thus

$$C_{HCO_3^-} = \frac{S}{k_{HCO_3^-}} = \frac{310.0}{373.5 \text{ mM}^{-1}} = 0.83 \text{ mM}$$

$$C_{Cl^-} = \frac{S}{k_{Cl^-}} = \frac{403.1}{1612 \text{ mM}^{-1}} = 0.25 \text{ mM}$$

$$C_{NO_2^-} = \frac{S}{k_{NO_2^-}} = \frac{3.97}{1324 \text{ mM}^{-1}} = 0.0030 \text{ mM}$$

$$C_{NO_3^-} = \frac{S}{k_{NO_3^-}} = \frac{262.7}{1314 \text{ mM}^{-1}} = 0.12 \text{ mM}$$

$$C_{SO_4^-} = \frac{S}{k_{SO_4^-}} = \frac{324.3}{1706 \text{ mM}^{-1}} = 0.19 \text{ mM}$$

$$C_{Ca^{2+}} = \frac{S}{k_{Ca^{2+}}} = \frac{734.3}{2294 \text{ mM}^{-1}} = 0.32 \text{ mM}$$

$$C_{Mg^{2+}} = \frac{S}{k_{Mg^{2+}}} = \frac{193.6}{1760 \text{ mM}^{-1}} = 0.11 \text{ mM}$$

(c) A mass balance for $HCO_3^-$ requires that

$$C_{NaHCO_3} = 0.83 \text{ mM} = [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

Given that the pH of 7.49 is closer to $pK_{a1}$, which is 6.352, than it is to $pK_{a2}$, which is 10.329, we will assume that we can simplify the mass balance equation to

$$C_{NaHCO_3} = 0.83 \text{ mM} = [H_2CO_3] + [HCO_3^-]$$

Using the $K_a$ expression for $H_2CO_3$

$$K_a = 4.45 \times 10^{-7} = \frac{[H_3O^+][HCO_3^-]}{[H_2CO_3]}$$

and substituting in for $[H_3O^+]$ using the pH, and substituting in the mass balance equation for $[H_2CO_3]$, gives

$$4.45 \times 10^{-7} = \frac{(3.24 \times 10^{-8})[HCO_3^-]}{0.83 \text{ mM} - [HCO_3^-]}$$

which we solve to find that

$$3.69 \times 10^{-7} \text{mM} - (4.45 \times 10^{-7})[HCO_3^-] = (3.24 \times 10^{-8})[HCO_3^-]$$

$$(4.77 \times 10^{-7})[HCO_3^-] = 3.69 \times 10^{-7} \text{ mM}$$

$$[HCO_3^-] = 0.77 \text{ mM}$$

(d) The ion balance, $IB$, for this sample is

$$IB = \frac{[Na^+] + [NH_4^+] + [K^+] + 2[Ca^{2+}] + 2[Mg^{2+}]}{[HCO_3^-] + [Cl^-] + [NO_2^-] + [NO_3^{2-}] + 2[SO_4^{2-}]}$$

$$IB = \frac{0.60 + 0.014 + 0.046 + 2(0.32) + 2(0.11)}{0.77 + 0.25 + 0.0030 + 0.12 + 2(0.19)}$$

$$IB = \frac{1.520}{1.523} = 0.998 \approx 1$$

Note that each ion's concentration is multiplied by the absolute value of its charge as we are interested in the concentration of charge, not the concentrations of ions.

This is a reasonable result as the total concentration of positive charge equals the total concentration of negative charge, within experimental error, as expected for an electrically neutral solution.

27. For a single standard we assume that $S = k_A C_A$, where $S$ is the signal, $k_A$ is the analyte's sensitivity, and $C_A$ is the analyte's concentration. Given the data for the standard that contains all three analytes, we obtain the following values of $k_A$

$$k_{Cl^-} = \frac{S}{C_{Cl^-}} = \frac{59.3}{10.0 \text{ ppm}} = 5.93 \text{ ppm}^{-1}$$

$$k_{NO_3^-} = \frac{S}{C_{NO_3^-}} = \frac{16.1}{2.00 \text{ ppm}} = 8.05 \text{ ppm}^{-1}$$

$$k_{SO_4^-} = \frac{S}{C_{SO_4^-}} = \frac{6.08}{5.00 \text{ ppm}} = 1.22 \text{ ppm}^{-1}$$

Now that we know each analyte's sensitivity, we can calculate each analyte's concentration in the sample; thus

**Figure SM12.12** Calibration data (**blue dots**) and calibration curve (**blue** line) for the data in Problem 28. Note that this is an unusual calibration curve in that we place the dependent variable—what we measure, which in this case is retention volume for the standards—on the *x*-axis instead of the *y*-axis, and the independent variable—what we control, which in this case is the formula weight of our standards—on the *y*-axis instead of the *x*-axis. There is nothing wrong with this choice, although we cannot use equation 5.25 to estimate the uncertainty in our determination of a sample's formula weight.

$$C_{Cl^-} = \frac{S}{k_{Cl^-}} = \frac{44.2}{5.93 \text{ ppm}^{-1}} = 7.45 \text{ ppm}$$

$$C_{NO_3^-} = \frac{S}{k_{NO_3^-}} = \frac{2.73}{8.05 \text{ ppm}^{-1}} = 0.339 \text{ ppm}$$

$$C_{SO_4^-} = \frac{S}{k_{SO_4^-}} = \frac{5.04}{1.22 \text{ ppm}^{-1}} = 4.13 \text{ ppm}$$

These are the concentrations as analyzed; because the original sample was diluted by a factor of $10\times$, the actual concentrations in the wastewater are $74.5$ ppm $Cl^-$, $3.39$ ppm $NO_3^-$, and $41.3$ ppm $SO_4^{2-}$.

28. In size-exclusion chromatography, the calibration curve is a plot of log(formula weight) as a function of retention volume. Figure SM12.12 shows the calibration data and the calibration curve for the standards, for which the calibration equation is

$$\log(\text{formula weight}) = 9.062 - (.5107 \text{ mL}^{-1})\, V$$

Substituting in the sample's retention volume of 8.45 mL, gives a result of 4.747 for log(formula weight), or a formula weight of 55,800 g/mol.

29. Given the $pK_a$ values and a pH of 9.4, caffeine is present in its neutral form, and benzoic acid and aspartame are present as singly charged anions. Caffeine, therefore, is the first of the three analytes to elute because the general elution order for CZE is cations, neutrals, and anions. Benzoic acid is smaller than aspartame, which means its electrophoretic mobility, $\mu_{ep}$, is more negative than that for aspartame, and that it total electrophoretic mobility, $\mu_{tot}$, is less positive than that for aspartame; thus, aspartame elutes before benzoic acid.

30. Substituting in the area of $15\,310$ for the first sample into the calibration equation gives the concentration of $Cl^-$ as 2.897 ppm in the sample as analyzed. The %w/w $Cl^-$ in the original sample is

$$\frac{\left\{ \begin{array}{c} \dfrac{2.897 \text{ mg}}{L} \times \dfrac{50.00 \text{ mL}}{0.250 \text{ mL}} \\[2mm] \times\, 0.1000 \text{ L} \times \dfrac{1 \text{ g}}{1000 \text{ mg}} \end{array} \right\}}{0.1011 \text{ g sample}} \times 100 = 57.3\%\text{w/w } Cl^-$$

The remaining two samples give concentrations of 57.4%w/w $Cl^-$ and %57.2%w/w $Cl^-$. The mean and the standard deviation for the three samples are 57.3%w/w $Cl^-$ and 0.1%w/w $Cl^-$, respectively.

To evaluate the method's accuracy, we use a *t*-test of the following null and alternative hypotheses

$$H_0{:}\,\overline{X} = \mu \qquad H_A{:}\,\overline{X} \neq \mu$$

where $\mu$ is 57.22%w/w $Cl^-$. The test statistics is $t_{exp}$, for which

$$t_{exp} = \frac{|\mu - \overline{X}|\sqrt{n}}{s} = \frac{|57.22 - 57.3|\sqrt{3}}{0.10} = 1.39$$

The critical value for $t(0.05,2)$ is 4.303. Because $t_{exp}$ is less than $t(0.05,2)$, we have no evidence at $\alpha = 0.05$ that there is a significant difference between our experimental mean of 57.33%w/w Cl⁻ and the accepted mean of 57.22%w/w Cl⁻.

31. For the internal standard we have

$$\frac{S_{NO_3^-}}{S_{IO_4^-}} = \frac{95.0}{100.1} = K \times C_{NO_3^-} = K \times (15.0 \text{ ppm NO}_3^-)$$

for which K is $0.06327$ ppm$^{-1}$. Using this value for $K$, for the sample we find that

$$\frac{S_{NO_3^-}}{S_{IO_4^-}} = \frac{29.2}{105.8} = 0.06327 \text{ ppm}^{-1} \times C_{NO_3^-}$$

the concentration of $NO_3^-$ is 4.36 ppm in the sample as analyzed. Because the sample is diluted by a factor of $100\times$, the concentration of nitrate in the original sample is 436 ppm.

32. One approach to separating the compounds is to find a pH where one of the compounds is present as a cation, one of the compounds is present as a neutral species, and one of the compounds is present as an anion. Figure SM12.13, which you will recognize as an alternative form of a ladder diagram, shows the pH ranges where each of a compound's different forms is the predominate species, using blue to represent cations, green to represents neutrals, and red to represent anions. For pH levels between the two dashed lines—a range of pH values from 4.96 to 9.35—the three analytes have different charges and should elute as separate bands. The expected order of elution is benzylamine (as a cation), 4-methylphenol (as a neutral), and 2-aminobenzoic acid (as an anion).

33. (a) Using equation 12.42, we find that the electrophoretic mobility, $\mu_{ep}$, is

$$t_m = \frac{lL}{(\mu_{ep} + \mu_{eof})\,V}$$

$$8.20 \text{ min} \times \frac{60 \text{ s}}{\text{min}} = \frac{(50 \text{ cm})(57 \text{ cm})}{(\mu_{ep} + 6.398 \times 10^{-5}\,\text{cm}^2\text{V}^{-1}\text{s}^{-1})(15 \times 10^3\,\text{V})}$$

$$(7.38 \times 10^6\,\text{Vs})\,\mu_{ep} + 472\,\text{cm}^2 = 2850\,\text{cm}^2$$

$$\mu_{ep} = 3.22 \times 10^{-4}\,\text{cm}^2\text{V}^{-1}\text{s}^{-1}$$

(b) From equation 12.43, the number of theoretical plates, $N$, is

$$N = \frac{(\mu_{ep} + \mu_{eof})\,El}{2DL}$$

Because the internal standard's concentration is the same in the standard and in the sample, we do not need to include it in this equation. If we did include it, then the equation is

$$\frac{S_{NO_3^-}}{S_{IO_4^-}} = K \times \frac{C_{NO_3^-}}{C_{IO_4^-}}$$

and the value for $K$ is 0.6327.
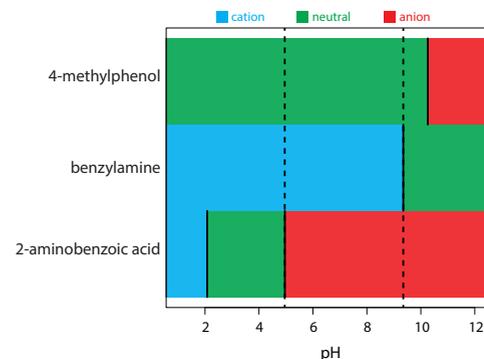


Figure SM12.13 Ladder diagram showing the predominate forms for 2-aminobenzoic acid, benzylamine, and 4-methylphenol as a function of pH. The color indicates the predominate form of each compound with **blue** representing cations, **green** representing neutrals, and **red** representing anions.

$$N = \frac{\left(\begin{array}{l}3.22 \times 10^{-4}\,cm^2V^{-1}s^{-1} + \\ 6.398 \times 10^{-5}\,cm^2V^{-1}s^{-1}\end{array}\right)(15000\,V)(50\,cm)}{2(1.0 \times 10^{-5}\,cm^2s^{-2})(57\,cm)}$$

$$N = 253934 \approx 254000$$

(c) Resolution is calculated using equation 12.43; first, however, we need to calculate the average electrophoretic mobility, $\mu_{avg}$, for the two solutes

$$\mu_{avg} = \frac{3.366 \times 10^{-4}\,cm^2V^{-1}s^{-1} + 3.397 \times 10^{-4}\,cm^2V^{-1}s^{-1}}{2}$$

which gives $\mu_{avg}$ as $3.3815 \times 10^{-4}\,cm^2V^{-1}s^{-1}$. The resolution, therefore, is

$$R = \frac{0.177(\mu_{ep,2} - \mu_{ep,1})\sqrt{V}}{\sqrt{D(\mu_{avg} + \mu_{eof})}}$$

$$R = \frac{0.177\left(\begin{array}{l}3.397 \times 10^{-4}\,cm^2V^{-1}s^{-1} - \\ 3.366 \times 10^{-5}\,cm^2V^{-1}s^{-1}\end{array}\right)\sqrt{15000\,V}}{\sqrt{(1.0 \times 10^{-5}\,cm^2s^{-2})\left(\begin{array}{l}3.3815 \times 10^{-4}\,cm^2V^{-1}s^{-1} + \\ 6.398 \times 10^{-5}\,cm^2V^{-1}s^{-1}\end{array}\right)}}$$

$$R = 1.06 \approx 1.1$$

(d) From equation 12.35, we know that there is an inverse relationship between a solute's electrophoretic mobility, $\mu_{ep}$, and its radius, $r$. For this set of compounds, the longer the alkyl chain attached to pyridine, the larger the compound; thus, electrophoretic mobility decreases from 2-methylpyridine to 2-hexylpyridine.

(e) These three isomeric ethylpyridines have the same effective radius, suggesting that they should have essentially identical electrophoretic mobilities. Equation 12.35, however, treats the solutes as if they are spheres. Of course, they are not spheres, and solutes that are of similar size but have a different shape may show a difference in their relative electrophoretic mobilities due to friction as they move through the buffer. At a pH of 2.5, all three solutes are present in their fully protonated, cationic form and are aligned with the applied field as shown in Figure SM12.14. Of the three solutes, 4-ethylpyridine is the most "stream-lined" and, therefore, has the largest electrophoretic mobility. Of the other two isomers, 2-ethylpyridine is the less "stream-lined" and, therefore, has the smallest electrophoretic mobility.

(f) At a pH of 7.5, the predominate form of pyridine is its neutral, weak base form. As it is neutral, its electrophoretic mobility is zero.



**Figure SM12.14** Structures of the isomeric ethylpyridines in Problem 33e. In an applied field, the compounds are oriented so that their center of charge and their center of mass are aligned with the field's direction. For a more detailed discussion, see the reference in the text.