1. On the course website near the link for this problem set there is a link to a web-based HPLC simulator that is quite sophisticated in terms of functionality (www.multidlc.org/hplcsim). Choose analytes phenol, 3-phenyl propanol, acetophenone, p-chlorophenol, and p-nitrotoluene initially, and set the temperature to 30 °C. Now choose acetonitrile as the organic solvent, and adjust ϕ, the fraction of organic solvent in the mobile phase to 0.48. Clicking on a compound name in the property table will highlight the chromatogram for the pure component in the chromatogram window. Adjust the mobile phase composition until you get baseline resolution between acetophenone and p-chlorophenol, and record the value of ϕ at that point. Now change ϕ significantly (higher or lower) until you see the peaks for acetophenone and p-chlorophenol overlap; keep adjusting ϕ until one peak effectively moves through the other and you get baseline resolution of the pair again, and record this value. What causes this dramatic change in the relative shifting of these peaks? What implications does this have for the optimization of separations of mixtures of compounds?

2. Use the HPLC simulator to come up with 'rules of thumb' for the following scenarios. In each case, systematically vary the solvent type or composition and build a table of retention factors for three or four solutes as a function of the variable you change. Then, use those data to build your statement.

    a. When changing the fraction of acetonitrile in the mobile phase over a relatively small range (e.g., 30%), decreasing the acetonitrile by _____% increases retention by a factor of _____.

    b. When changing from methanol to acetonitrile as the organic component of the mobile phase, approximately _____% more/less acetonitrile must be used to obtain a retention factor similar to that obtained when using methanol.

3. To answer the following questions, access the HPLC simulator at www.multidlc.org/hplcsim. In each case, present your results along with an explanation of your observations that is supported by theory.

    a. Vary the flow rate from low (say 0.3 mL/min) to high (say 3 mL/min). What happens to the retention times, resolution and peak height? For a peak which is not badly overlapping any other peak, plot the peak height (from baseline to the apex) vs. flow rate. Explain the fundamental basis of the trend you observe. What are the practical consequences of this relationship?
    b. Set the flow rate to 1 mL/min and vary the particle size from 1.8 to 3.5 then 5 microns. What happens to the retention times, resolution, peak height, and backpressure? Explain your observations in terms of peak broadening theory.
    c. Set the particle size to 5 microns. Vary the column length from 5 to 10 to 15 cm. What happens to the retention times, resolution, peak height, and backpressure? Explain your observations in terms of peak broadening theory.