

# CHM416/1104 Virtual Chromatography Laboratory Instructions

## Part 1—Gas Chromatography

In this exercise, you will be using ACD/Labs Method Development Suite 7.0 to explore gas chromatographic behavior. The software makes use of various known relationships between gas chromatographic retention times and molecular properties, including boiling point/vapor pressure and octanol-water partition coefficients ( $\log P_{ow}$ , a measure of the hydrophobicity of the molecule). The software can predict boiling points and, therefore, the retention behavior of molecules based on their chemical structure; these can be imported from ACD/ChemSketch directly into the ACD/GC Simulator module. Alternatively, experimental data can be imported and used to generate prediction equations; these can in turn be used to optimize the separation and determine if additional sample components could be resolved under such conditions.

There are various questions throughout this exercise that you should answer and submit at the end of the exercise. The exercise is broken down into a number of separate exercises; these should be completed in order. Please keep track of the total time spent on this exercise, and report that together with your answers to the questions. Any additional comments or suggestions on this exercise are also welcome.

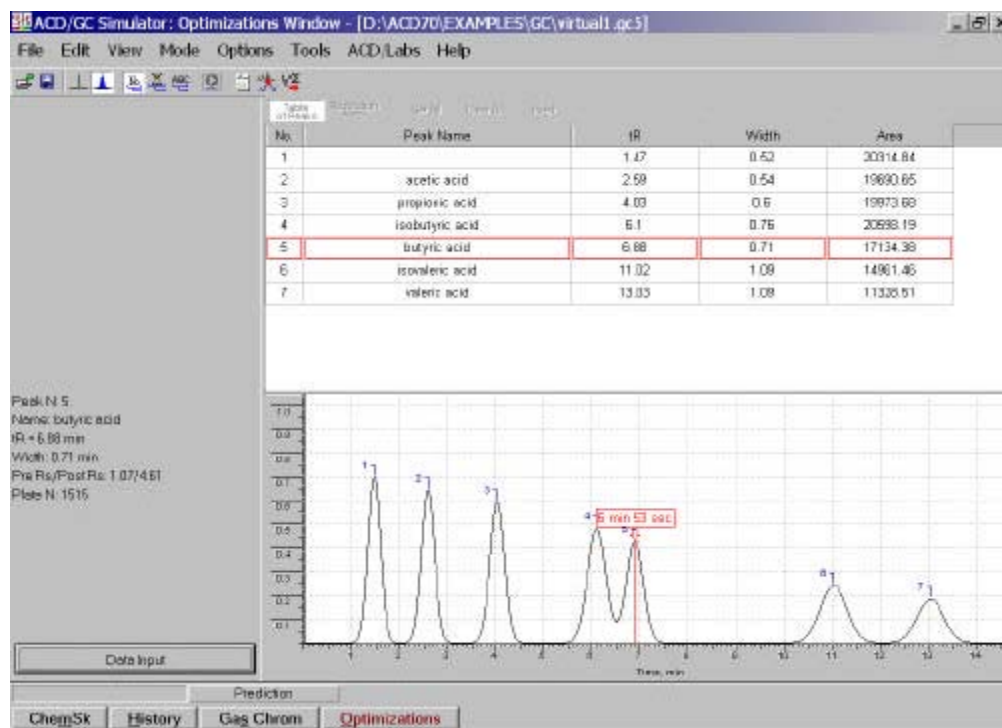
### Exercise 1: Exploring a Stored Chromatogram

- 1.1 Go to **Start >Programs>ACDLabs 7.0>GC Simulator**
- 1.2 Open the file C:\ACD70\EXAMPLES\GC\VIRTUAL1.GC5

#### **Important:**

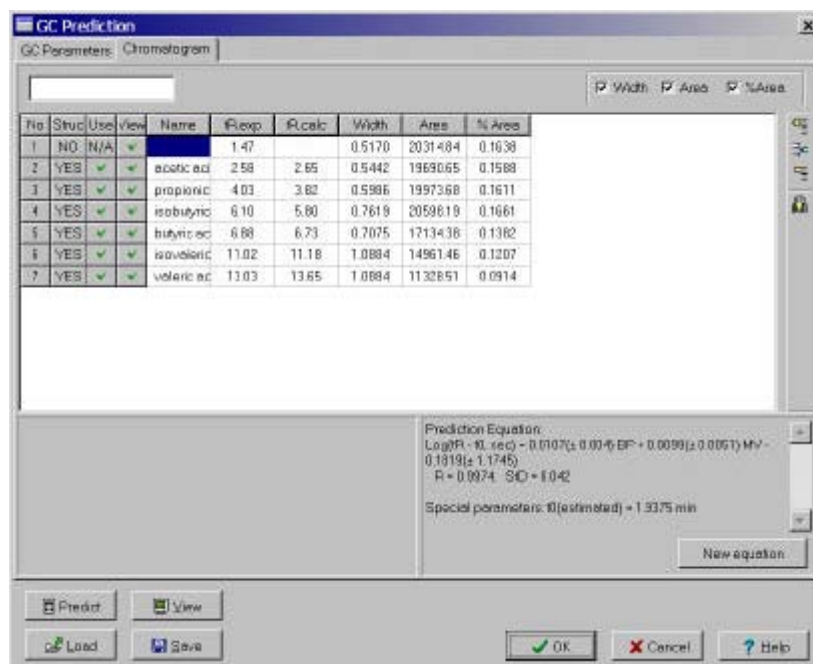
Please do **not** save any changes you make back to the original file. Should you need to interrupt your work, save the modified file using a unique name.

You will notice that the file opens with various graphical panels across the main window, as well as a menu bar, tool bar, and various status displays and buttons along the bottom.



- 1.3 Move the cursor across either the chromatogram (lower panel) or peak table (upper panel). As you do so, the information on the left-hand side of the window will also change. The term Pre Rs/Post Rs refers to the resolution between the current peak (in this case #5, butyric acid) and the peaks immediately before (#4) and after (#6). The three buttons in the tool bar ('No', hourglass, and 'ABC') allow you to switch the labeling used in the chromatographic display. The button with the benzene ring turns the structure display on or off; as well, from the **View** menu, you can point to **Structure**, and then choose **Show**. The **Structure** submenu also lets you choose whether the structure is displayed in a floating or popup window. The floating window should default to within the chromatogram panel; the popup window can be dragged and resized to any convenient area of the screen.

You can obtain further chromatographic details by clicking on the **Data Input** button on the lower left-hand side of the main **GC Simulator** window. Doing this opens a dialog box (below) featuring two tabbed displays, **GC Parameters** and **Chromatogram**. Click on the **GC Parameters** tab to view the experimental conditions used to obtain the actual chromatogram displayed. When you are done, simply click either **OK** or **Cancel** to dismiss the dialog.

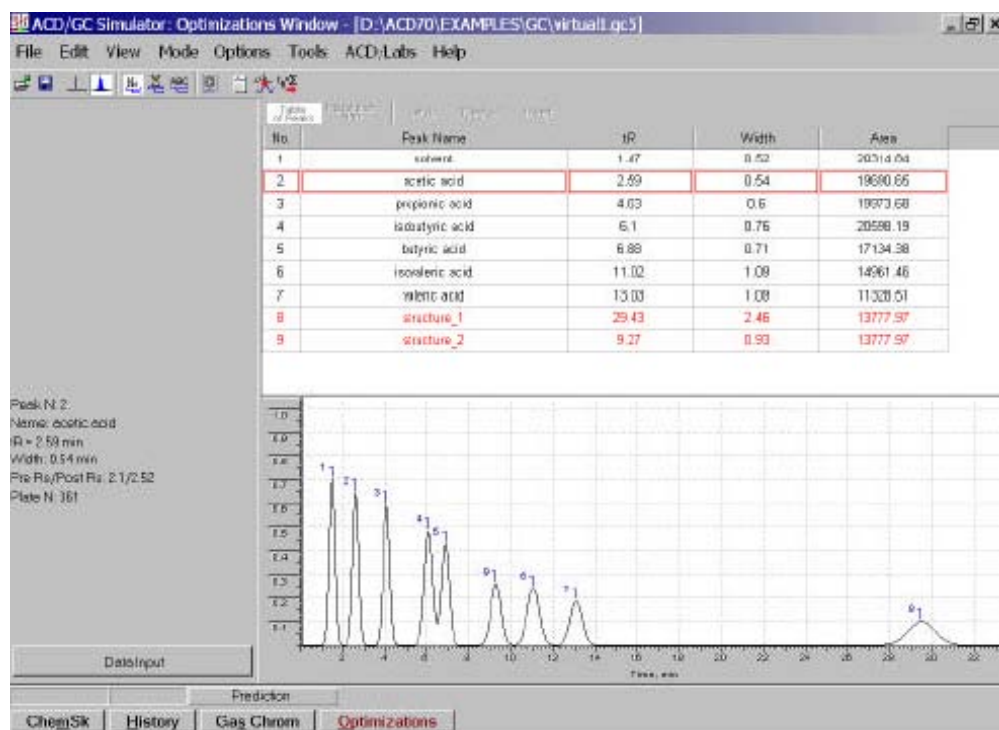


- 1.4 Obtaining a hard-copy of the chromatogram: you will notice that one of the tool bar buttons has an icon that looks like a printed page; clicking on this button generates a report within ACD/ChemSketch. From the **Copy** menu, choose **Copy to Report Editor** as another way of generating this report. Click **OK** in the **Report Page Setup** dialog box. Once the report has opened in ACD/ChemSketch, you can print out the chromatogram and peak table. To return to ACD/GC Simulator, from the **ACD/Labs** menu (in ChemSketch), choose **GC Simulator**.
- 1.5 Answer the following questions regarding the specimen chromatogram:
- Q1 Was the chromatogram obtained under gradient or isothermal conditions? What was the temperature(s) and flow rate used?
- Q2 Look at the structures of the organic acids separated in this chromatogram. What determines the elution order of the linear (straight-chain) fatty acids? What determines the elution order of fatty acids with the same number of atoms but a different carbon skeleton (i.e., linear versus branched)?
- Q3 Based on your observations, estimate the retention time that you would expect to observe under identical chromatographic conditions for (a) hexanoic acid and (b) pivalic acid,  $(\text{CH}_3)_3\text{CCO}_2\text{H}$ .

You may now exit the program(s) and logout from the computer workstation if you need to.

## Exercise 2: Checking Predictions

- 2.1 Open the same stored chromatogram used in exercise 1 (VIRTUAL1.GC5), and click **ChemSk** in the bottom-left corner of the main window to transfer to ACD/ChemSketch. Once in ACD/ChemSketch, open the file C:\ACD70\EXAMPLES\GC\FATTYACIDS.SK2. This file contains structures for the two acids whose retention times you were asked to estimate in the previous exercise.
- 2.2 Still in ACD/ChemSketch, click **Optimizations** at the bottom of the window. This will transfer both structures to ACD/GC Simulator, which will use the existing data to predict the retention times for these new compounds.



- You will notice that there are two new entries in the peak table: 'structure\_1' and 'structure\_2'. Move the cursor over the table entries to determine which peak corresponds to which entry. You can enter the correct names by clicking **Data Input**. Click on the appropriate cell in the spreadsheet-like area of the **Chromatogram** tab, and enter the name in the editable field in the top-left corner of the tab panel. Once you are done, click **OK** to return to the main window, and obtain a hard copy of the report (see step 1.5 above).
- 2.3 The report should list the retention times predicted by the software for all peaks, not just the structures you added. Compare the values of tR(exp) with tR(calc).

You will notice that the errors in predicted retention time are typically about 5% or less.

Now open the file C:\ACD70\EXAMPLES\GC\VIRTUAL2.GC5. This shows a specimen chromatogram of a mixed fatty acid standard obtained using capillary GC with a temperature gradient. Obtain a copy of the report for this chromatogram also, and answer the following questions:

- Q4 How well did the predictions (yours and the computer's) compare to the actual elution order of the fatty acids?
- Q5 How much longer would the packed column from the first chromatogram have to be in order for butyric acid and isobutyric acid (2-methylpropionic acid) to be resolved with  $R_s = 1.50$ ? (The original column was 1.83 m long.)
- Q6 In light of the two different chromatograms obtained for the volatile fatty acids, what would you consider to be the primary advantage(s) of a capillary over a packed column? Are there any potential disadvantages to using a capillary rather than a packed column?

### Exercise 3: Optimizing a Temperature Gradient

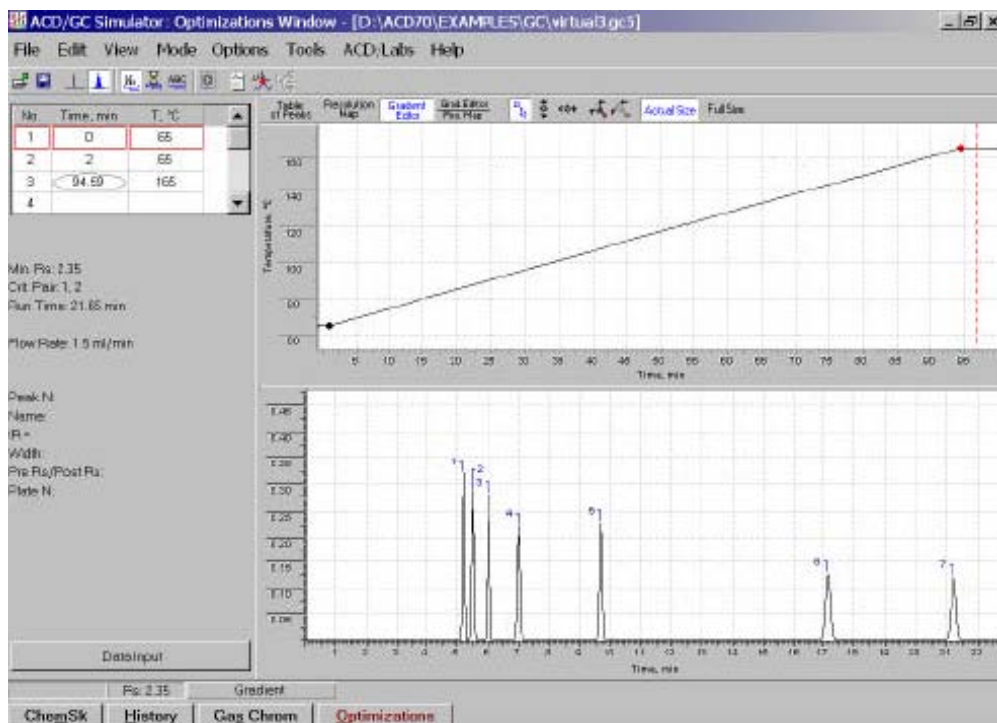
In this final exercise with GC Simulator, you will explore the effects of temperature programming on a specimen chromatogram. In this particular case, two experimental separations of the same compounds on the same column have been obtained at different temperatures and pre-loaded into a prediction file. The software uses this information together with the properties of the sample components to predict the effect of column temperature on resolution and run time.

- 3.1 Launch ACD/GC Simulator (or switch to the GC Simulator module if you still have the ACD/Labs suite open) and load the file:  
C:\ACD70\EXAMPLES\GC\VIRTUAL3.GC5

#### Important:

As with the other sample files in this exercise, please do not make any changes to the actual file. If you need to save your work to resume later, please save the file using a different name.

You will notice that the main window contains an additional panel and some extra buttons.



The spreadsheet-like area at the top-left allows you to change the temperature program, which is also displayed graphically above the chromatogram. You can switch between the gradient editor display and the peak table using the buttons directly above this upper panel. Before you continue, obtain a hardcopy of the chromatogram by clicking **Copy to Report Editor** on the toolbar or, from the **Copy** menu, choose **Copy to Report Editor** (as in previous exercises). Once you have printed out the report, switch back to ACD/GC Simulator by clicking **Optimizations** at the bottom of the ACD/ChemSketch window.

- 3.2 Drag the mouse across the chromatogram, and note which peaks have the lowest resolution between them (this will be displayed for each peak as PreRs/PostRs values in the area on the left of the screen above the **Data Input** button). Also note the run time (retention time of the last-eluting peak in the chromatogram). Finally, compare the times at which the different points of the temperature gradient occur, and compare this to the run time. Change the final (third) point in the temperature gradient so that it corresponds roughly to the column temperature obtained just after the last peak elutes from the column. You can do this either by editing the entries in the spreadsheet area, or by clicking and dragging the point in the gradient editor window. Note that two of the buttons directly above the gradient editor allow you to constrain the direction of dragging to either horizontal or vertical, while the button immediately to the left of them restores simultaneous horizontal and vertical motion.

### 3.3 Adjusting the initial hold time.

Q7 What do you think the effect of increasing the initial hold time (the position of the second point in the temperature program) would be?

After recording your prediction to the above question, click on the button to constrain dragging to the horizontal (time axis) direction, and try increasing and decreasing this value. Note that, as you increase the initial hold time, the temperature ramp will have a correspondingly steeper gradient.

Q8. Is it possible to reduce the retention time for the first peak by changing the duration of the initial hold? If so, by how much can you change it? What effect would increasing the initial temperature have on the chromatogram?

Find out whether or not you were correct in your answer to the last part of Q8 by changing the drag mode to the vertical (temperature axis) direction, and raising and lowering the temperature of the initial hold. (You can also do this by editing the gradient table directly.) Note that you will need to change the temperatures of both the first and second points in the gradient. Find the initial hold conditions (temperature and time) which will give you the shortest possible elution time for the first peak while making sure that it remains resolved from the second peak ( $R_s = 1.5$ ). When you are satisfied with your results, print a copy of the results then return to ACD/GC Simulator as you did in step 3.1.

### 3.4 Adjusting the temperature gradient: Now try adjusting the time and temperature for the third point in the temperature program so that the overall run time is reduced whilst retaining baseline resolution between all peaks.

For practical considerations, assume that the maximum column temperature is limited to 250°C. You may also vary the initial hold parameters if necessary. When you are satisfied that you have obtained the shortest run time while maintaining resolution, print a final report.

### 3.5 Click **ChemSK** (ChemSketch) in the lower-left of the window, and open the file AROMATICS.SK2 (in the same directory as the previous file). This contains the structures of the following compounds, some of which are already in the sample chromatogram:

Compound	T <sub>b</sub> (°C)	M <sub>m</sub> (g/mol)
benzene	80.1	78.12
toluene	110.6	92.15

ethylbenzene	136.2	106.17
<i>o</i> -xylene	144.4	106.17
<i>p</i> -xylene	138.3	106.17
<i>n</i> -propylbenzene	159.2	120.20

- Q9. Considering the structure, size, and boiling points of these compounds, predict roughly where you would expect benzene, toluene, and *n*-propylbenzene to elute under the conditions you derived in the preceding step.
- 3.6 Select the relevant structures in the ChemSketch file by holding down SHIFT while clicking below each one. Transfer these to ACD/GC Simulator by clicking **Predict for Gradient**.
- Q10. Are all the peaks resolved? If not, adjust the temperature gradient so that they are. How well did your prediction match up with the computer prediction?
- Q11. Given the results obtained in this exercise, what aspects of molecular structure contribute to elution order in GC?