

Virtual Chromatography Laboratory

Part 1: Gas Chromatography

In this exercise, you will be using ACD/Labs GC Simulator 9.0 to explore gas chromatographic behaviour. The software makes use of various known relationships between gas chromatographic retention times and molecular properties, including boiling point/vapour 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 behaviour of molecules based on their chemical structure; these can be imported from ChemSketch directly into the GC Simulator module. Alternatively, experimental data can be imported and used to generate prediction equations; these can in turn be used to optimise 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.

Short glossary:

Retention time, t_R :

Elapsed time from injection to detection of the peak centre for a retained compound

Dead time or void volume, t_0 or t_M :

Time from injection to detection of the peak centre for a completely unretained compound; also the time for one mobile phase volume (V_M) to pass completely through the column, i.e. $t_0 = V_M/F$

Capacity factor, k' :

Retention time expressed relative to dead time, i.e. $(t_R - t_0)/t_0$

Resolution, R_s :

Defined as the separation divided by the average baseline width for two adjacent peaks, i.e. $R_s = 2(t_{R2} - t_{R1})/(w_2 + w_1)$

Exercise 1: Exploring a Stored Chromatogram

1.1 Login to the computer as

User: _____

Password: _____

1.2. Go to **Start** → **Programs** → **ACDLabs 9.0 (NETWORK)** → **GC Simulator**

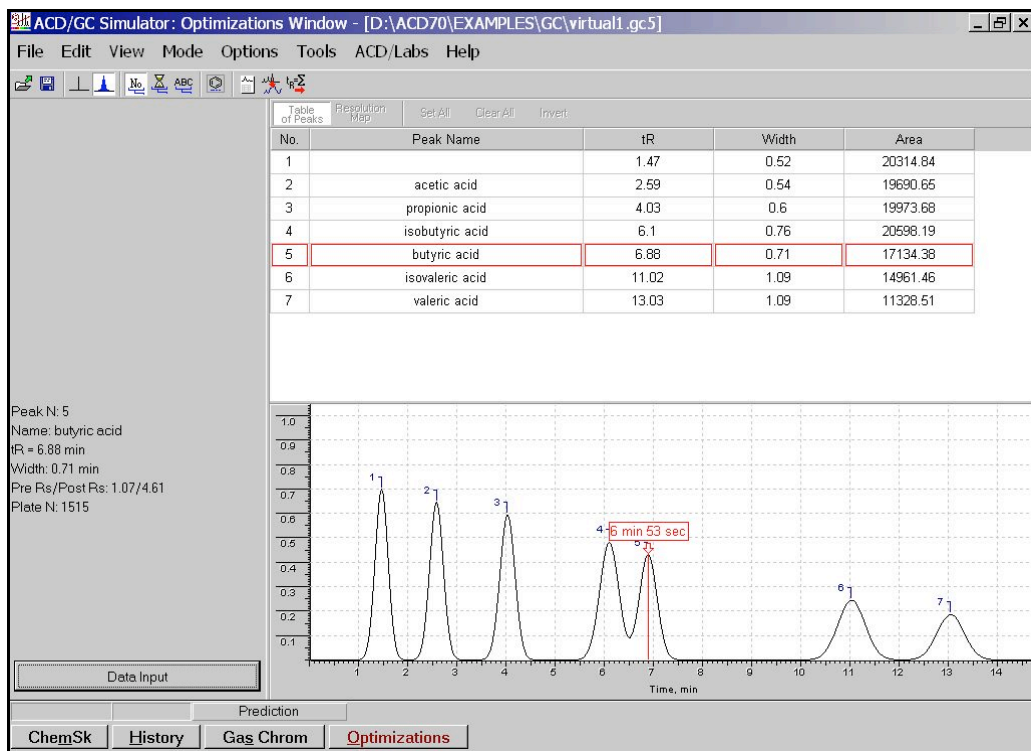
1.3. Open the file





C:\Documents and Settings\chm416\My Documents\virtual1.gc5

Important:

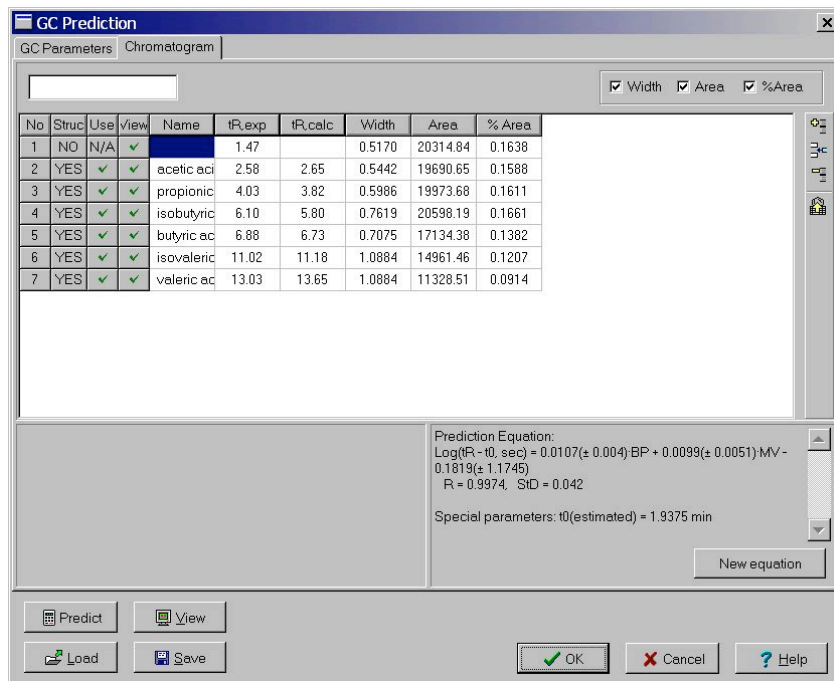
Please do **not** save any changes you make back to the original file. Should you need to interrupt your work, save the modified file using a unique name.

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.



1.4 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 (, , and ) allow you to switch the labelling used in the chromatographic display. The button with the benzene ring () turns the structure display on or off; you can also use the **Structure** → **Show** item in the **View** menu. 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 on either the **OK** or **Cancel** buttons to dismiss the dialog.



The screenshot shows the 'GC Prediction' dialog box with the 'GC Parameters' tab selected. The dialog contains a table with the following data:

No	Struc	Use	View	Name	tR_exp	tR_calc	Width	Area	% Area
1	NO	N/A	✓		1.47		0.5170	20314.64	0.1638
2	YES	✓	✓	acetic aci	2.58	2.65	0.5442	19690.65	0.1588
3	YES	✓	✓	propionic	4.03	3.82	0.5986	19973.68	0.1611
4	YES	✓	✓	isobutyric	6.10	5.80	0.7619	20598.19	0.1661
5	YES	✓	✓	butyric ac	6.88	6.73	0.7075	17134.38	0.1382
6	YES	✓	✓	isovaleric	11.02	11.18	1.0884	14961.46	0.1207
7	YES	✓	✓	valeric ac	13.03	13.65	1.0884	11328.51	0.0914


Below the table, the 'Prediction Equation' is displayed:

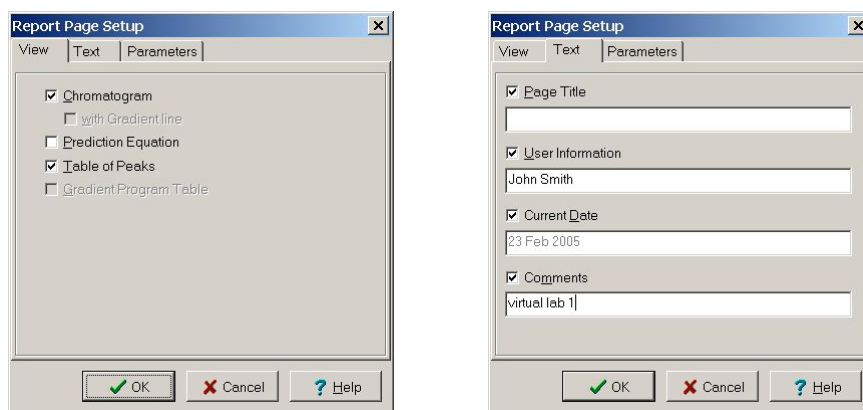
$$\text{Log}(R - t_0, \text{sec}) = 0.0107(\pm 0.004)BP + 0.0099(\pm 0.0051)MV - 0.1819(\pm 1.1745)$$

Additional statistics shown are: $R = 0.9974$, $SD = 0.042$. Special parameters: $t_0(\text{estimated}) = 1.9375 \text{ min}$.

The dialog also includes buttons for 'Predict', 'View', 'Load', 'Save', 'OK', 'Cancel', and 'Help'.

Q1 Was the chromatogram obtained under gradient or isothermal conditions? What was (were) the temperature(s) and flow rate used?

- 1.5 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 the ChemSketch program. You can also choose the **Copy to Report Editor...** item in the **Edit** menu. Make sure that **Chromatogram** is checked in the **View** tab of the **Report Page Setup** dialog box. You can also add your name etc. in the **Text** tab. Click **OK** when you are satisfied with the selected options. You can print out the chromatogram and peak table once the report has opened in ChemSketch.



If you select the **Table of Peaks** option, the chromatogram and peak table can end up on different pages. There is a *very* small page control in the main window. Make sure you print all pages if you also selected the peak table option. To return to the GC Simulator, select **Optimizations** from the taskbar at the bottom of the ChemSketch window and dismiss any error message. You may now exit the program(s) and logout from the computer workstation if you need to.

- 1.6 Answer the following questions regarding the specimen chromatogram:

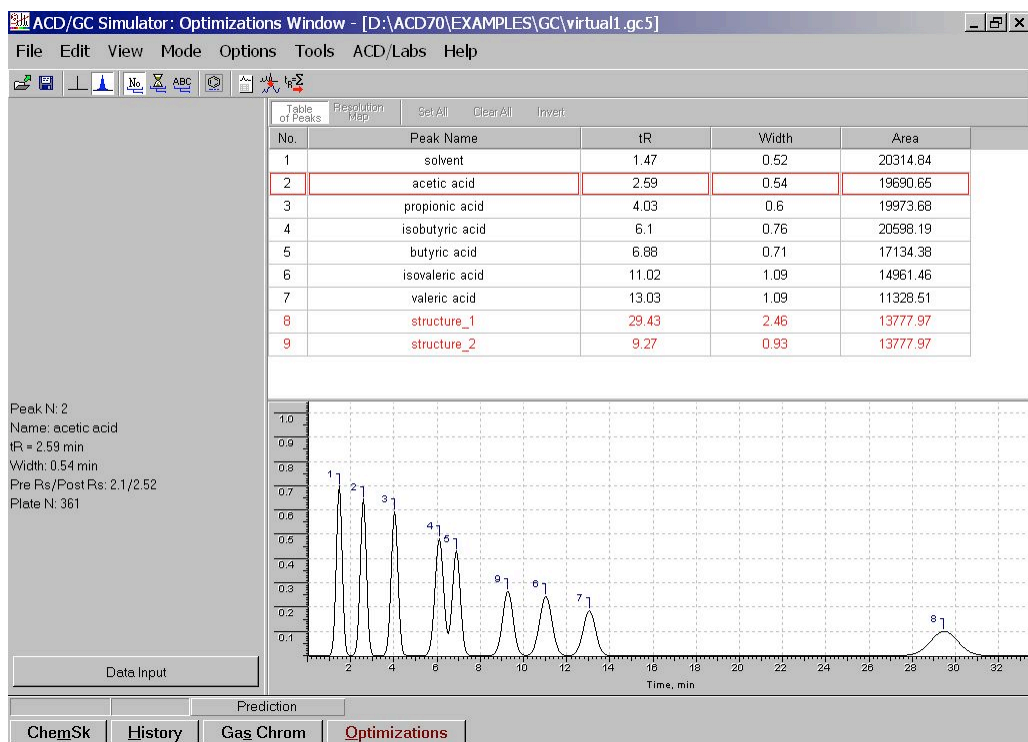
Q2 Look at the structures of the organic acids separated in this chromatogram.

- (i) What determines the elution order of linear (straight-chain) acids? Explain your answer.
- (ii) What determines the elution order of structural isomers (*i.e.* linear versus branched with the same number of carbon atoms)? Explain your answer.

Q3 Based on your observations in question 2, estimate the retention time that you would expect to observe under identical chromatographic conditions for (a) hexanoic acid, $\text{CH}_3(\text{CH}_2)_4\text{CO}_2\text{H}$ and (b) pivalic acid, $(\text{CH}_3)_3\text{CCO}_2\text{H}$ (2,2-dimethylpentanoic acid). Show your calculations.

Exercise 2: Checking Predictions

- 2.1 Open the same stored chromatogram used in exercise 1 (virtual1.gc5), and click on the ChemSk button in the bottom-left corner of the main window to transfer to ChemSketch. Once in ChemSketch, open the file 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 ChemSketch, click on the **Optimizations** button at the bottom of the window. This will transfer both structures to the GC Simulator module, 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 matches which entry. You can enter the correct names by clicking on the **Data Input** button. 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 on the **OK** button 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 $t_R(\text{exp})$ with $t_R(\text{calc})$. You will notice that the errors in predicted retention time are typically about 5% or less.

Now open the file `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? If there was a significant difference (*i.e.* more than 5%) what might explain this?

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

- 2.4 Answer the following questions relating to the chromatograms used in parts 1 and 2:

- 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 the 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?
- Q7 When is it potentially a disadvantage to use a capillary rather than a packed column?

Exercise 3: Optimising a Temperature Gradient

In this final exercise with the 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.

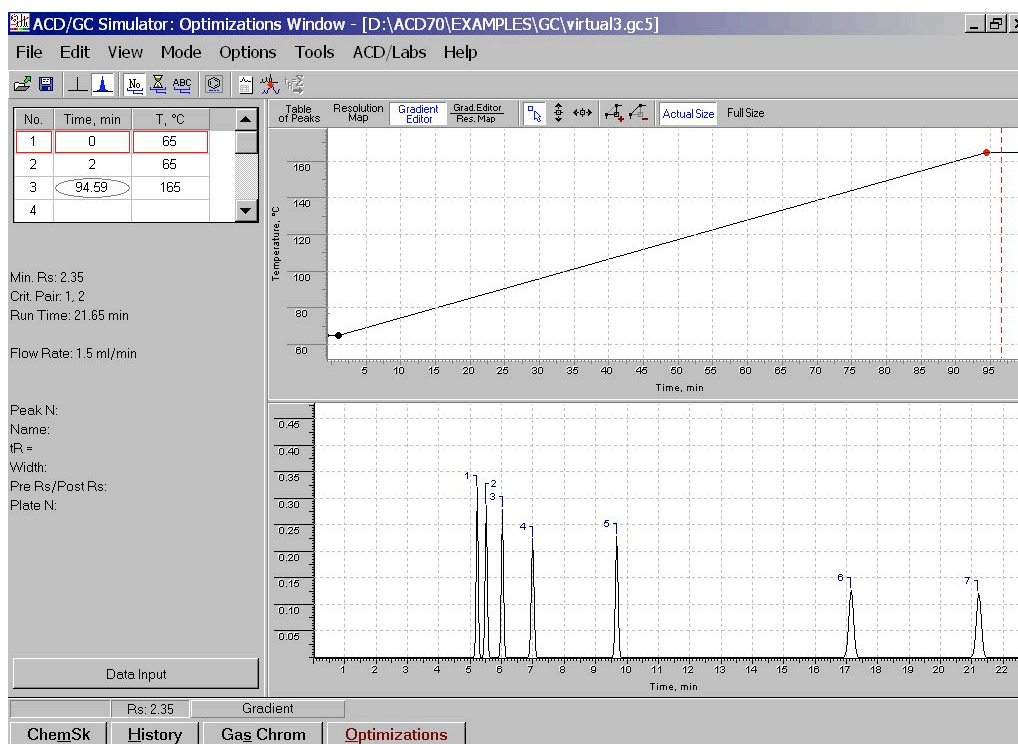
3.1 Launch the ACD/Labs GC Simulator (or switch to the GC Simulator module if you still have the ACD/Labs suite open) and load the file:

C:\Documents and Settings\chm416\My Documents\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 use 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 using the **Copy to Report Editor...** toolbar button or menu item as in previous exercises. Once you have printed out the report, switch back to the GC Simulator module by clicking on the **Optimizations** button at the bottom of the 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 for the last peak in the chromatogram). Finally, note 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 to 85 °C at 30 min; this corresponds 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: the hold time is the duration of the initial isothermal segment of the temperature program. It allows the solvent to be separated from the sample components at the head of the column, so that the full column length is available for separating individual sample components.

Q7 What would be the effect of increasing the hold duration on the retention time and resolution of the first four peaks?

Q8 What would be the effect of decreasing the hold duration on the retention time and resolution of the first four peaks?

- 3.4 After recording your prediction to questions 7 and 8, click on the button to constrain dragging to the horizontal 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 slope.

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, and what is the shortest attainable retention time?

Q9 What affect would increasing the temperature of the initial isothermal hold have on the chromatogram?

3.5 Find out whether or not you were correct in your answer to the last part of question 9 by changing the temperature of the initial hold. Note: you *must* change the temperatures of both the first *and* second points in the temperature gradient to the same value. You can do this by setting the drag mode to vertical (temperature axis) and raising or lowering both points, or by editing the gradient table directly. Determine the initial hold conditions (temperature and duration) that give you the shortest possible retention time for the first peak whilst keeping it resolved from the second peak (i.e. $R_s \geq 1.5$). When you are satisfied with your changes, print a copy of the results and return to the GC simulator module as you did in step 3.1.

Q10 What ultimately limits the shortest retention time that can be attained for the first peak, assuming the same column and flow rate are used?

3.6 Adjusting the temperature gradient: the temperature gradient is defined by the heating rate (i.e. slope of the temperature-time plot) and final temperature (i.e. duration of change). It is usual to have a final hold, in which the final temperature attained by the program is maintained isothermally until the chromatographic run is completed.

Try adjusting the time and temperature for the third point in the temperature program so that the overall run time is reduced while retaining full baseline resolution between all peaks. For practical purposes, 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 whilst maintaining resolution, print a final report.

- 3.7 Click on the ChemSK (Chem Sketch) button 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 _b (°C)	M _m (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

- Q11 Considering the structure, size and boiling points of these compounds, predict roughly where you would expect benzene, toluene, and *n*-propylbenzene to elute relative to the rest of the peaks in the chromatogram under the conditions derived in the preceding step.
- 3.8 Select the structures of benzene, toluene, and *n*-propylbenzene in the ChemSketch file by holding down the **shift** key down whilst clicking below each one. Transfer these to the GC Simulator by clicking on the **Predict for Gradient** button. Print any final chromatogram, and exit the ACD/Labs Method Development Suite.
- Q12 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?
- Q13 Given the results obtained in this exercise, what aspects of molecular structure contribute to elution order in GC? What role, if any, do features such as aromatic rings and ring substitution play? (Compare your findings in part 3 with those from parts 1 and 2.)

Virtual Chromatography Laboratory

Part 2: Liquid Chromatography

In this exercise, you will use the ACD/Labs LC Simulator 9.0 module to examine the effects of solvent composition on elution in liquid chromatography. In this particular case, you will make use of chromatograms that have already been combined and prepared for use in the LC Simulator module. As was the case for the GC Simulator, this module makes use of a variety of experimental databases and physical parameters (both measured and calculated) in order to predict retention behaviour in HPLC. Since we are now dealing with a liquid mobile phase, one important difference between the LC and GC modules is the use of $\log D$ as a parameter; this is the same as $\log P_{ow}$, but is pH-dependent. This reflects the importance of pH on elution in HPLC, especially given the number of acidic and basic compounds analysed by this technique.

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 the Effect of pH

1.1 Login to the computer as

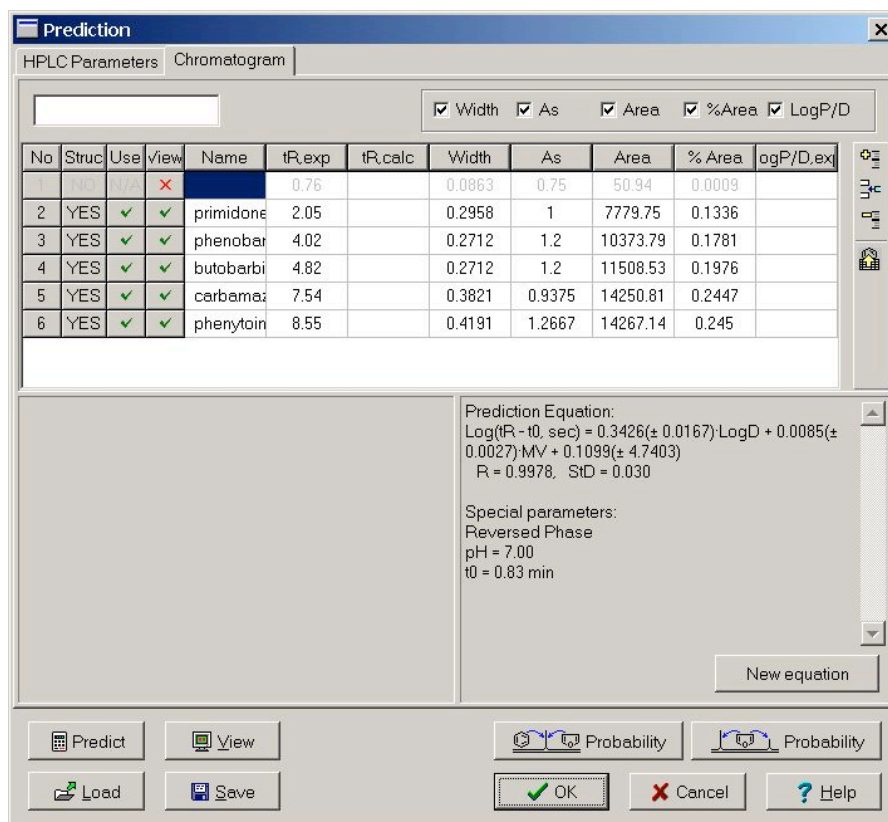
User: _____
Password: _____

1.2. Go to **Start** → **Programs** → **ACDLabs 9.0 (NETWORK)** → **LC Simulator**

1.3 Open the file

C:\Documents and Settings\chm416\My Documents\drugs.lc7

You will see an alert box warning you that some data need to be recalculated – click **OK**, and you will find the following dialog box displayed on the screen:



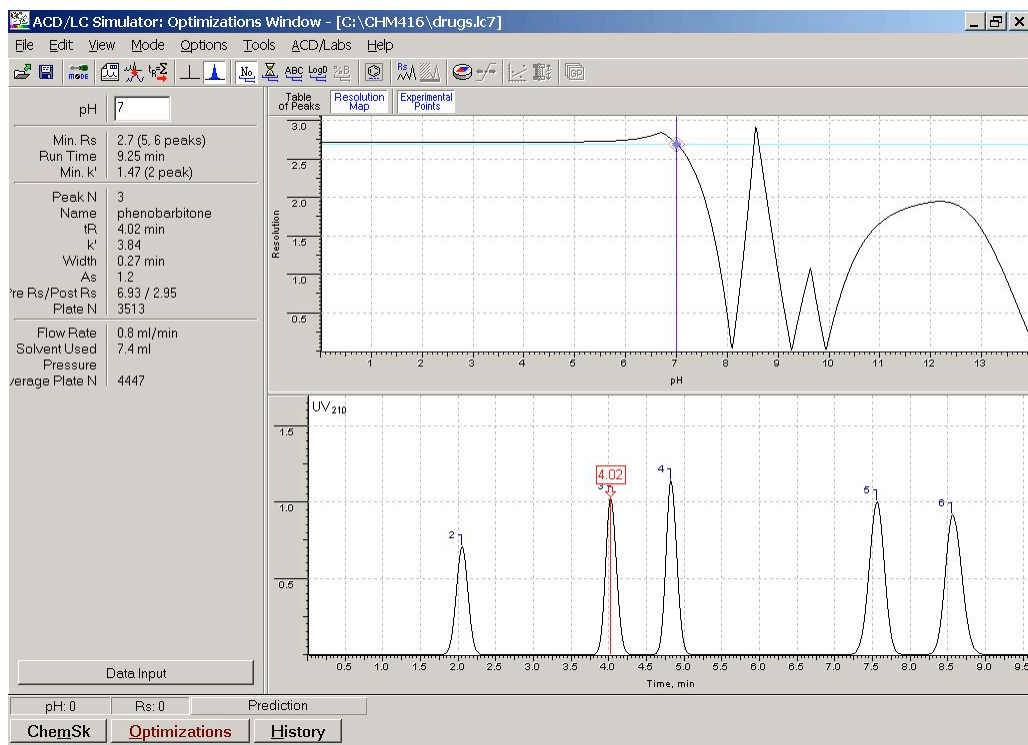
This is the same dialog you see whenever you click on the **Data Input** button in the main simulator window. As you can see, it lists the names of a series of compounds, together with relevant parameters such as retention time, peak width, and area. Click on the **Predict** button – the software will then calculate values of log P or log D, and predict retention times for each peak based on these parameters. Finally, click on the **OK** button to close the dialog box and see the actual chromatogram.

Important:

Please do **not** save any changes you make back to the original file. Save the modified file using a unique name.

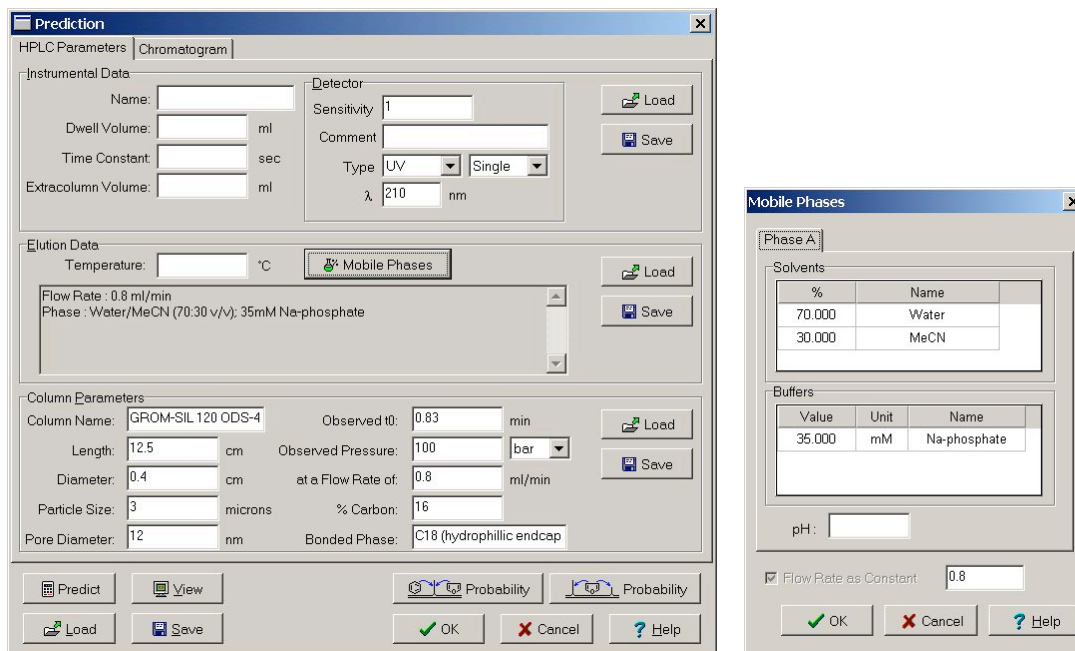
- 1.4 Navigating through the LC Simulator Module: you will notice that the layout of this module is similar to the GC one, and many of the tools work in the same way. You will also notice some significant differences between the LC and GC modules. You will notice in the screen-capture below, for example, that the window shows something called a “resolution map” panel with pH as a parameter. The resolution map uses the prediction equation generated from experimental data, and the structures of the compounds in the chromatogram, to predict the effect of pH on the resolution between the

“critical pair” – the two peaks with the lowest resolution of any pair in the chromatogram for the specified parameter value.



You will also notice that some additional data is provided on the left-hand side of the main window, just below the editable text field indicating the current pH value for the mobile phase. As with the GC simulator, you can have the software display the compound structures in a panel or floating window by either clicking on the structure button in the toolbar or selecting the appropriate options in the **View** → **Structure Window** sub-menu. The same set of peak-labelling options are also there, with the addition of a “log D” button – this displays the calculated log D value for each peak.

- 1.5 Click on the **Data Input** button on the lower left-hand side of the main **LC Simulator** window. This will allow you to check the details of both the sample components and elution conditions through the **HPLC Parameters** and **Chromatogram** tabs. Click on the **Mobile Phases** button in the **HPLC Parameters** tab to see a table of the mobile phase components and buffer composition. Note that the “water” refers to an aqueous buffer, whose composition is listed to the side.



1.6 Answer the following questions regarding the specimen chromatogram:

- Q1 Was the chromatogram obtained under gradient or isocratic conditions?
- Q2 What was the mobile phase used in the experiment?
- Q3 What type of HPLC is being performed – normal or reverse phase? Give the reason for your answer.

Close both dialog boxes to return to the main **LC Simulator** window.

1.7 You can vary the pH conditions in two ways:

- (a) Manually, by entering values into the editable text box in the upper-left corner of the window and pressing the **enter** key.
- (b) Graphically, by clicking and dragging the blue diamond in the resolution map display. (Note: the blue bar shows the original pH setting.) This works in the same way as the temperature gradient in the GC simulator module.

Using either method, examine the effect of changing the pH from 4 to 12 in steps of 1 pH unit, paying particular attention to retention time and elution order within the chromatogram.

- Q4 Peaks 2 and 5 show hardly any variation in t_R over this pH range, whereas the other peaks move considerably. Why is this? (Hint: review the structures of the individual compounds; you can do this either by turning on the structure display feature, or by clicking on the different entries within the **Chromatogram** tab of the **Data Input** dialog box.)
- Q5 What is the consequence of this difference in pH-dependence of the sample components on the overall elution order? What general principles can be inferred from this for optimizing the separation of acidic and/or basic compounds in HPLC?
- Q6 What pH would you have to use in order to reduce the run-time below 7 minutes?
- Q7 In practice, silica-based columns cannot be exposed to such high pH values. Suggest two practical alternatives that could be used to reduce the run-time.

Exercise 2: The Effect of Mobile Phase Composition

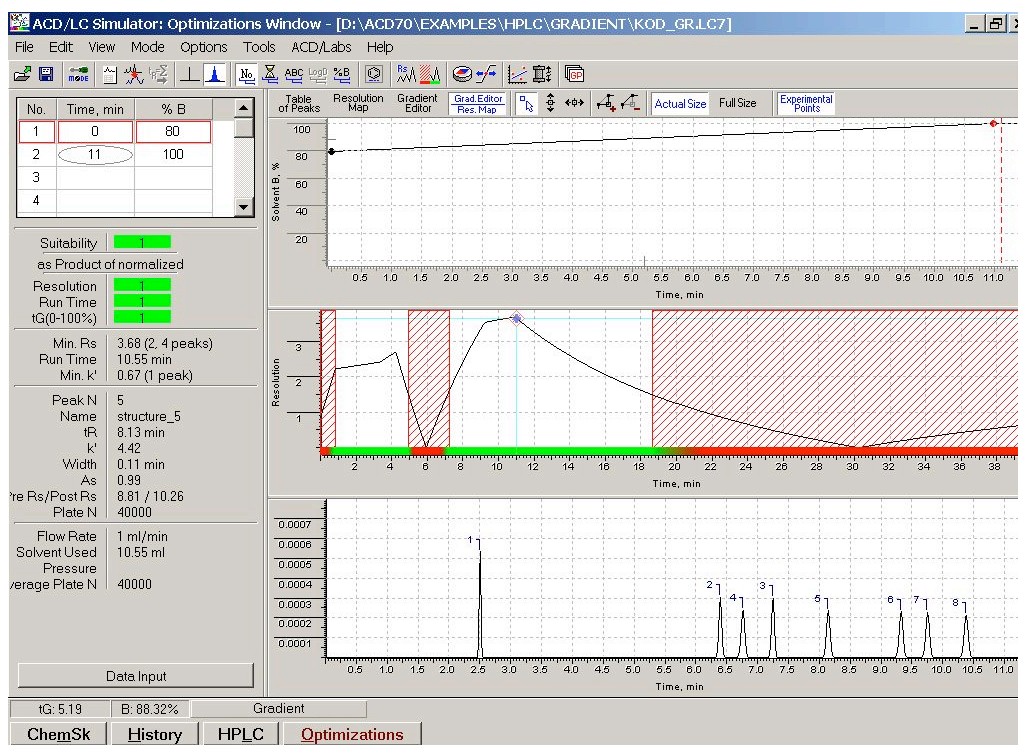
2.1 From within the **LC Simulator** module, open the file

C:\Documents and Settings\chm416\My Documents\KOD_GR.lc7

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.

Note that the software may ask if you want to switch to gradient mode when you do this; answer “yes”. The main **LC Simulator** window should now look like this:



Note that the display can be set to show either or both the resolution map and the solvent gradient. You can change the display mode using the appropriate buttons just above the display area (**Table of Peaks**, **Resolution Map**, **Gradient Editor**, and **Resolution Map/Gradient Editor**). Study the information provided in both the main window and the **HPLC Parameters** and **Combined** tabs of the **Gradient** dialog box opened by clicking on the **Data Input** button, then answer the following questions:

- Q8 What are the two solvents used in the mobile phase? What are the initial and final compositions of the mobile phase, expressed as volume% of each component, in the two experimental chromatograms used to generate the prediction data? (These can be found on the **1st** and **2nd** tabs of the **Gradient** dialog box.)
- Q9 Is this a normal or reverse phase separation? Give the reason for your answer.
- Q10 Pick one peak from the combined chromatogram, and predict on the basis of the compound structure and the solvent mixture whether decreasing the initial amount of solvent B to 50% will increase or decrease its retention time. Explain your answer.
- Q11 Use the k' value of the peak you chose to calculate the effect of making such a change using the polarity index P' and assuming isocratic conditions (see Skoog or the lecture notes for details.) Show your calculation, and clearly state any additional assumptions you made. Note: the k' value is included in the peak information on the left-side of the window.
- 2.2 The gradient can be edited in the same way as the temperature gradient in the GC module; you can edit the values directly in the spreadsheet area in the upper left-hand corner, or you can click and drag the points in the graphical panel on the right. (You can also drag the blue diamond on the resolution map, which will change the time for the second gradient point.) Modify the gradient so that the initial amount of solvent B is 50%.
- Q12 Compare your initial prediction, your calculation, and the software prediction of the new retention time for your chosen peak. Given that polarity index calculations are, by nature, estimates rather exact calculations, under what conditions can they be used to estimate the effect of changes in solvent composition on retention under gradient conditions?
- 2.3 Examine the effect of changing the solvent gradient from an initial value of 0% B to isocratic elution (i.e. 100% B from 0 to 11 min).
- Q13 Summarise the effect of changing the solvent gradient on (a) the overall run-time, and (b) the resolution of the sample components.

2.4 Set the gradient to run from 50% to 100% B, starting at 0 and ending at 11 min. Make sure that the resolution map is displayed (click on the **Resolution Map** button.) You will notice that there are two regions with a white background and a green bar underneath, indicating two possible sets of conditions within which good separation may be obtained. Drag the blue diamond within the resolution map panel to vary the steepness of the gradient (this simply changes the time at which 100% B will be reached).

Q14 What happens to the run-time in comparing these different regions?

Q15 What happens to the elution order as you vary the steepness of the solvent gradient? What is affecting this order?

Q16 Is it possible to obtain an isocratic condition that will provide the same or better run-time whilst maintaining full resolution of all peaks? Which would be better from a practical stand-point: isocratic or gradient elution?

Virtual Lab. Survey:

Please take the time to answer the following questions, and attach them to your report for this virtual laboratory exercise. Thanks!!

S1 Did you find the GC and LC exercises helpful?

(not at all) 1 2 3 4 5 6 7 (extremely)

S2 Were the instructions provided clear and easy to follow?

(not at all) 1 2 3 4 5 6 7 (extremely)

S3 Have you done much practical chromatography prior to taking this course?

Yes

No

If yes, how many chromatograms do you estimate you have run? _____

S4 Do you think these exercises helped you better understand elution

(i) in GC?

(not at all) 1 2 3 4 5 6 7 (extremely)

(ii) in HPLC?

(not at all) 1 2 3 4 5 6 7 (extremely)

S5 Are there any specific aspects of chromatography that you would like to see included in these exercises?

S6 Are there any suggestions you have for improving these exercises?