Complete LabVIEW-Controlled HPLC Lab: An Advanced Undergraduate Experience

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ABSTRACT: Virtually all modern chemical instrumentation is controlled by computers. While software packages are continually becoming easier to use, allowing for more researchers to utilize more complex instruments, conveying some level of understanding as to how computers and instruments communicate is still an important part of the undergraduate curriculum. Understanding how digital and analog signals are used to allow the smooth operation of an instrument allows the user to better understand the possible sources or problems when instruments stop working optimally. The experiment presented here allows students to investigate the communication between the computer and a high-performance liquid chromatography (HPLC) system by allowing them to construct a LabVIEW-based control program. Student understanding of instrument control is reinforced by having them control pump speed, detector zeroing, and a solvent recycling valve while also monitoring the injection port status and the detector output. Students gain an understanding of the HPLC process by incorporating all of the instrument tasks into their control software and then use their system to analyze a mixture of five small organic molecules, thereby gaining insight into the chemical concepts underlying HPLC separations.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, Chromatography, HPLC, Instrumental Methods, Laboratory Computing/Interfacing

INTRODUCTION

Computer-controlled instrumentation is a crucial aspect of all areas of chemistry. While instruments are becoming easier to use, a fundamental understanding of the principles behind their operation is still important. Knowing how an instrument functions allows the user to recognize when it is not performing correctly and can guide the troubleshooting process. Additionally, it allows the user to understand what parameters should be changed, and in what fashion, in order to affect a desired change or create a new method. Laboratory experiments that allow students to have hands-on access to chemical instrumentation are critical in order to build this understanding. Allowing students to build their own methods or construct their own instruments or create an instrument control program provides examples of more advanced laboratory activities that allow for a deeper understanding of the instrumentation than is often obtained by simply using an instrument with a preset method for the analysis of a given sample.

Even though most users do not need to design their own software to control chemical instrumentation, doing so in a teaching setting is an excellent way for students to learn about not only the instrument hardware, but also how the various components work together to form the entire instrument. One way that instrument control has been taught in undergraduate laboratories is through the use of the LabVIEW software platform by creating a virtual instrument (VI). LabVIEW has been used to simulate laboratory experiments via computer. It has also been incorporated into the undergraduate laboratory where it has been used for data acquisition for differential thermal analysis as well as gas chromatography, calorimetry, and emission spectroscopy. Experiments have been designed that use LabVIEW to control a cyclic voltammetry instrument, a sequential injection analyzer, a fluorimeter, and a photometer.

Separations are an important part of many chemical processes. High-performance liquid chromatography (HPLC) is a major analytical tool used for a wide range of sample types. A variety of previous teaching lab experiments have been developed that utilize HPLC for both qualitative and quantitative analyses. These include the analysis of various foods, pharmaceuticals, and beverages. Two previous examples of coupling HPLC and LabVIEW have been reported. In one paper, LabVIEW was used to create a pulsed amperometric detection waveform and collect the resulting data. Aside from the detector, there was no LabVIEW control of other aspects of the HPLC system. The platform by creating a virtual instrument (VI). LabVIEW has been used to simulate laboratory experiments via computer.

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Laboratory Experiment

other experiment describes a LabVIEW-controlled HPLC system where LabVIEW is used to monitor the signal from the detector, control a fraction collector, and control the pump.21 LabVIEW was used to control both the pump speed and the formation of a linear gradient.

The experiment presented here expands on the previous descriptions of LabVIEW-based HPLC systems by incorporating a detector autotzero, setting how long data will be collected (essentially defining the length of the chromatogram), allowing the injection port valve to trigger the start of data collection, and adding a solvent recycling system. In addition, this paper uses the newer DAQ Assistant LabVIEW sub-VIs rather than the legacy Ni-DAQ sub-VIs, allowing current National Instruments hardware to be used.

Finally, the experiment described here includes an HPLC separation of up to five components that can be incorporated into the lab to allow students to use the LabVIEW control VIs that they have created. Mixtures containing various combinations of bisphenol A, n-butylbenzene, ethylbenzene, toluene, and p-xylene are presented to the students once they have a functioning HPLC system for them to analyze and qualitatively determine the mixture components. Quantitative measurements could be made as well if standards of known concentration were provided, but that is not the focus of the experiment presented here.

■ EXPERIMENTAL PROCEDURES

The HPLC used in this experiment was purchased from ChromTech (Apple Valley, MN) and consists of an ISO-2000 isocratic pump, a Rheodyne 7725I manual injector with an internal microswitch contact, a 20 μL sample loop, and a DVW-10 variable wavelength UV–vis detector. In addition, a Cole-Parmer 12 V three-way valve was added to the standard HPLC system. The valve is controlled by a 12 V signal that is itself controlled with a solid-state relay.

The added valve allows the mobile phase (solvent) to be recycled back to the reservoir when no sample is flowing through the system. Most HPLC systems have mobile phase flowing constantly with all of the liquid going directly to a waste container. If an autosampler is being used such that there is little time between analyses, this is not overly wasteful. However, if some time is going to be allowed to pass between the end of one analysis and the start of the next, due to sample preparation time for example, then the mobile phase flowing between runs is completely wasted and needlessly adds to the chemical waste of the experiment. The three-way valve added in the HPLC system described here allows for the mobile phase to be sent back to the reservoir during this delay between runs, thus reducing the amount of mobile phase sent to the waste container and making the experiment more “green”. While it is true that mobile phase passing through the column, even when no sample has been introduced, can elute off compounds still adsorbed to the stationary phase, the amount of material eluted off is normally quite minimal and thus does not significantly contribute to mobile phase contamination. This can be confirmed by observing no noticeable increase in the background signal over time even when mobile phase is being recycled.

Other HPLC systems could be used as long as they support output monitoring and external control for at least some pump and detector functions. If not all functions described in this experiment are available, the instructor can modify the scope of the experiment to remove student control of those functions.

Detailed pictures of the instrument and the connections described here are presented in the online Supporting Information.

Two National Instrument data acquisition boards are used to monitor and control various HPLC functions. A PCI-6503 digital I/O (DIO) card is used to monitor the status of the injection port microswitch and to control the three-way recycling valve. A PCIe-6341 Multiple I/O (MIO) card is used to turn the pump on and off, set the pump flow rate, zero the detector, and monitor the detector output. To save time and allow the entire experiment to be completed in one 4 h lab period, the connections between the HPLC and the I/O cards are made by the instructor before the students start lab. These connections could be made by the students if so desired, but this would either require the removal of some other aspect of the experiment, or a two-session lab experiment. An example diagram of the HPLC system and computer connections is shown in Figure 1.

Students are told which color wires correspond to which HPLC function, but they have to decide if these functions are digital or analog signals and if they are input or output signals. On the basis of the wire colors, they can trace each wire pair to the connections to determine which I/O card channel or line is connected to which HPLC function. This knowledge is crucial when they begin to build the LabVIEW VI that will control the HPLC. Currently, LabVIEW 2015 is used for this experiment, but several previous versions of LabVIEW have been used in the past. Any version of LabVIEW capable of controlling the two I/O cards should be adequate. One student example of a working LabVIEW VI, along with a description of how it operates, is shown in the online Supporting Information.

Once the LabVIEW control and acquisition program has been completed, the functional HPLC system can be tested. The mobile phase consists of 75% methanol/25% water that has been filtered and degassed with a flow rate of 1.0 mL/min. The detector is set to 254 nm since all analytes used in this experiment are aromatic. Instructors can choose other analytes which might require a different wavelength to be monitored. Students are instructed to inject 30–40 μL of sample, to ensure...
that the entire 20 μL sample loop is full. Excess volume is collected in a waste container. The column used in this experiment is a PerkinElmer Pecosphere 33 mm long C18 column with an internal diameter of 4.6 mm containing 3 μm particles. This short column provides relatively good separation of the five analytes, with only two compounds not fully resolved, while allowing for the entire separation to be completed in approximately 5 min. Longer columns could be used to provide better separation, but this will also increase the separation time or require faster flow rates.

By having the connections already made for the students, and by using a short HPLC column, the students complete the entire experiment in a single 4 h lab period. Students typically spend about 3 h working and testing their LabVIEW VIs, with the final hour spent analyzing the individual analytes and the instructor-prepared mixtures. Each lab section is capped at four students, and with four HPLC systems, each student is allowed to work independently on their LabVIEW control VI. While this is ideal from a pedagogical standpoint, it may be impractical for courses with larger enrollments or for institutions that do not have four HPLC systems. Students could work in pairs to allow more students in each lab section, but this would severely limit the pedagogical outcomes as it is difficult for two students to simultaneously build a LabVIEW VI. One option would be to have one student focus on the LabVIEW VI, while another student makes the wire connections between the HPLC and the National Instrument cards. If quantitative analysis were added, one student could be tasked with making a series of standards of known concentration as well.

■ HAZARDS

There are no hazards associated with the LabVIEW VI creation, but once the HPLC is to be turned on and mobile phase pumped through the system, gloves and goggles should be worn. Methanol, butylbenzene, ethylbenzene, toluene, and p-xylene are flammable, so care should be taken to keep heat and flames away. Bisphenol A, ethylbenzene, toluene, and p-xylene are toxic so care should be taken to avoid exposure, and all waste should be collected and disposed of appropriately. A blunt-end syringe is used, but care should still be taken to avoid puncturing gloves or skin with the syringe.

■ RESULTS AND DISCUSSION

Students have used LabVIEW in several lab experiments prior to the one described here. They have spent several weeks learning how to create VIs using LabVIEW both in other lab experiments as well as in out of lab exercises. While they still require some help in their VI development, by this point in the semester they are fairly adept at using LabVIEW for digital and analog I/O.

After a brief introduction to the HPLC hardware, students start determining which wires are connected to which terminals on the National Instruments cards. The students are told which colored wires correspond to which HPLC functions, so once they determine how the wires are connected to the boards, they can establish which channels or lines are related to which HPLC function. For the example connections shown in Table S1, the three-way recycling valve is connected to the DIO card line 1 (used as an output), and the injection port microswitch is connected to the DIO card line 2 (used as an input). The pump on/off control is connected to the MIO card’s digital I/O line 1 (used as an output) while the pump flow rate is controlled through the MIO card’s analog output line 1. The detector’s autozero function is connected to the MIO card’s digital I/O line 2 (used as an output) while the detector signal is connected to the MIO card’s analog differential input lines 1–2. Diagrams of the connections between the computer and the HPLC system are given in the online Supporting Information.

Once students have determined all of the connections, they begin constructing their HPLC control VIs in LabVIEW. They need to set the recycling valve to recycle the mobile phase so that solvent is recycled when the instrument is turned on and remains so until a sample is injected onto the column. This is accomplished by sending a 0 V signal from the DIO board to a
Figure 3. Portion of LabVIEW VI demonstrating collection and saving of data. LabVIEW images used with permission. Copyright 2014 National Instruments.

Figure 4. Example front panel for HPLC control VI. LabVIEW images used with permission. Copyright 2014 National Instruments.
solid-state relay, which opens the relay, sending 0 V to the three-way valve causing it to be in its default state. They also need to turn on the pump, set a flow rate, and monitor the status of the injection port microswitch, as shown in Figure 2, an example of a student-created LabVIEW VI (more details are provided in the online Supporting Information). This monitoring is accomplished by using a While Loop in the LabVIEW VI that continuously monitors the microswitch state until the injection port is moved from the load to the inject position. Once the sample has been injected onto the column, the state of the microswitch will change, allowing the LabVIEW VI to move past the While Loop. Students use this state change to autozero the detector, and begin collecting data points, as shown in Figure 3.

The recycling valve is also switched to allow solvent to flow to the waste container so that the sample does not contaminate the mobile phase reservoir. They build a real-time chart to display the active chromatogram on the front panel display and save the data into a spreadsheet file. By setting the number of points collected, and the time between data point collection, the total time of the chromatogram is established. Once the analysis is complete, the recycling valve is changed back to send the mobile phase back to the solvent reservoir until the next analysis is started. This eliminates mobile phase being sent to waste between injections.

An example of a student front panel is shown in Figure 4. This panel allowed the student to turn the pump on or off, set a flow rate, set the number of data points and the time between data points, control if the detector was autozeroed at the beginning of the analysis or not, and control if the data was saved or not and if so what the file was called. It also contained a running chromatogram as well as a final smoothed chromatogram that was displayed once all data had been collected.

Once the students have a working HPLC control VI, they are asked to analyze an unknown mixture and determine which compounds are in their unknown sample. They must recognize that, once this happens, the recycling valve should send mobile phase to waste, and data should be collected from the detector. Without a fundamental understanding of the hardware, no control program can be constructed. This experiment has been offered annually for over 20 years, although the HPLC hardware and LabVIEW versions have changed over that time. Each year 4–15 students have completed this experiment. Each student works independently on their own VI with their own HPLC system in order to maximize their exposure to the instrument and allow them to create their own control VI. The ability of each student to create a functioning HPLC control VI is itself an achievement, with virtually every student completing a working VI by the end of the lab period. Over 90% of the students analyze their unknown sample and correctly identify the components in their mixture.

### STUDENT OUTCOMES

The act of creating a LabVIEW VI to control the HPLC requires more than just knowledge of LabVIEW programming. Students must understand what each component in the HPLC does in order to decide what parameters they need to control and an appropriate range for the values. They need to understand how each component works with each other and the order of events in a separation in order to compile a chronological order for their control programs. For example, they need to understand that when the injection port valve is switched from the “load” to the “inject” position, the sample is sent to the column and the state of the microswitch changes. They must recognize that, once this happens, the recycling valve should send mobile phase to waste, and data should be collected from the detector. Without a fundamental understanding of the hardware, no control program can be constructed.

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over the past five years, although HPLC is also discussed in the lecture portion of the class making it impossible to determine the true impact of the lecture versus the lab experience on student learning.

■ CONCLUSIONS
The experiment presented here allows students to use the LabVIEW instrument interface language to build an inclusive control program for an HPLC instrument. This allows them to investigate both digital and analog signals used as both inputs and outputs from the HPLC. In addition to a fairly advanced LabVIEW instrument control application, students also are required to develop an in-depth understanding of how the HPLC system works. They need to understand that various aspects of the HPLC system (i.e., pump speed, detector signal, start trigger, detector zeroing, etc.) in order to know how to control or monitor each aspect of the experiment. They are also able to reinforce the advantages of employing green chemistry methods by incorporating the solvent recycling valve, thereby reducing the amount of mobile phase needed instead of discarding 100% of the mobile phase during the lab period. While a specific HPLC system was used for the LabVIEW VI described here, many other HPLC systems could be used allowing this experiment to be replicated, although certain parameters might not be available to be controlled or monitored in all systems.

■ ASSOCIATED CONTENT

1 Supporting Information
The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.7b00041.

Copy of the student handout, instructor notes that may be helpful for others interested in adopting a similar experiment in their curriculum, and copies of student-built LabVIEW VI front and back panels (PDF, DOCX)

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Notes
The authors declare no competing financial interest.

■ REFERENCES