Protein Titration Control and Monitoring System: A Collaborative, Real-world Course Project

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Abstract

Currently, The Texas A&M University System Health Science Center College of Medicine uses a manually-operated system for the pH titration of histidine penta-peptides, model compounds and proteins. The data collected from this system will be used to determine the pKa of the histidine side-chain in these different compounds. This information will be added to the world-wide data base of pKa's collected and will contribute to our understanding of how pKa's are affected by their local environment and will contribute to the development of new drugs and/or drug delivery systems. To increase data collection efficiency, the Health Science Center approached the Electronics and Telecommunications Engineering Technology Programs about automating the titration system. The Protein Titration Control and Monitoring system was assigned as the course project in an undergraduate data acquisition and process control class that is taught at the junior-level. The students were required to work in four to five-person teams to develop a complete system-level design, implement the design in hardware and software, and test and deliver an operational system to the sponsor. The software was designed and implemented using National Instruments LabVIEW graphical development environment and a standard data acquisition card installed in a desktop computer. The system accepts inputs from the researcher, automates the entire titration and data collection process, databases the pertinent information, and correctly terminates the experiment. The system can be operated in a local mode or the experimenter can initiate a data collection session, monitor the progress of the experiment in real time, and access the recorded results via the Internet using LabView Data Socket technology.

Introduction

Electronics and Telecommunications Engineering Technology (EET/TET) students at Texas A&M University take a series of technical courses each of which includes an integrated laboratory experience. After receiving feedback and recommendations from industries that hire EET/TET graduates, more emphasis is being placed on laboratories where teams of students are required to design, implement, test, and analyze a project. The experience is concluded with the documentation of the results of each project in both written and oral format. This approach begins in selected sophomore-level courses and continues through the capstone senior design project with less and less faculty intervention and control as the students progress in their curriculum. One of the courses that utilize this approach is a junior-level Computer-based Data Acquisition and Control course. The focus of this course is to learn and apply fundamental data acquisition and signal conditioning concepts. In addition, the students learn to integrate hardware design with graphical software development to provide a highly intuitive user interface for the system. National Instruments has been very supportive of this course and has provided data acquisition hardware and LabVIEW software as the development platform for these student projects.

To provide a higher level of motivation and performance, and thus, a greater degree of learning, the EET/TET faculty seeks to identify projects that provide a relevant and real-world experience for the student design teams. Many of the project requirements will come from an industry representative while in other cases the problem definition is provided by some other group at the university. A sponsor that truly needs the capability that will come from the students' efforts is a major motivational factor and results in much more time and energy being invested in the project. During the Fall 2000 semester, Mr. Richard Thurlkill, a Ph.D. candidate in the Texas A&M College of Medicine, identified an excellent project which met the basic requirements of the course and the College of Medicine provided the necessary support for the implementation of a solution by the students. Mr. Thurlkill needed to replace the existing, manually operated titration system that produced his empirical data with one that could be totally automated. In addition, the system needed to ensure repeatability in the experimental and data collection processes. Most of the experiments require as much as two hours of processing time, and the manually operated system was prone to operator errors. In addition, inconsistencies in the measurement setup between experiments, especially over extended periods, resulted in variations in data repeatability.

The primary requirement of the project from the user's perspective was to provide a fully automated titration system that was capable of

1. accepting user configuration information,
2. controlling the entire experimental environment,
3. collecting and recording all relevant data,
4. monitoring and terminating the experiment via the internet
5. notifying the user of termination and reason for termination via the internet, and
6. transferring the raw data file to a specified email address.

Students generally spend the first five weeks of the course learning the fundamentals of data acquisition and signal conditioning and applying their knowledge of the LabVIEW software development tools to this environment. During this time, the project definition and general requirements are presented. Students are also tasked to form their own teams of four or five individuals. As juniors, the EET/TET students understand the need to select team members that will provide a balance of expertise in hardware design, software development, testing, data reduction and analysis, and technical documentation. During weeks six and seven, the student design teams interact with the customer/sponsor to make sure they understand all of the project's constraints and requirements. This understanding is documented by the groups in two written reports -- one that outlines system requirements and another, referred to as an interface control document, that documents the format and content of the final recorded file. Each team is totally responsible for implementing the entire system; however, teams will volunteer to become specialist in particular areas and share their knowledge with the other teams. In other situations, a member from each team may interact as a subcommittee for the project to set certain standards or conditions for the project so that teams may efficiently share the system resources.

As the project progresses, teams may suggest additional features via engineering change proposals and, if accepted, may earn additional credit for their project. These additional features are only considered if the design team meets all of the minimum system requirements. Not providing all system attributes will generally reduce the team's project grade. Information on these added features is considered proprietary and is not shared with the other teams.

A successful project generally provides the customer/sponsor with a set of solutions from which to select the "best". In addition, the students will have a feeling of accomplishment and camaraderie as well as an enhanced understanding of the fundamental course objectives. Finally, the faculty will feel that the students have learned from the experience, both increasing their technical knowledge and their ability to work in a team environment. The project undertaken by the students during the Fall 2000 semester for the College of Medicine was extremely successful. This paper provides an overview of the project and the solution that was selected by the sponsor. An additional benefit of the interaction was to build a stronger bridge between the College of Engineering and the College of Medicine at Texas A&M University.

Background

The Automated Titration System was developed as a project in the Engineering Technology Department's Junior level ENTC 359 class for the College of Medicine at Texas A&M University. The system was developed by a group of 5 students over a time period of eight weeks. The system integrates all the hardware essential to perform automatic chemical titration of acids and bases. Before the Automated Titration System was developed the College of Medicine performed time consuming chemical titrations by hand. The system is currently being used by the College of Medicine to perform titrations of histidine.

Histidine is one of the 20 naturally occurring amino acids, the building blocks of proteins. Its uniqueness is based on the fact that its side chain is acid titratable with an acid ionization constant, pKa, of free histidine of 7.02. This property makes histidine a key amino acid in the functional properties of many proteins. The pKa of a compound is that pH where 50% of the molecules present are acidic and 50% are basic. Since the pKa of histidine is very near physiologic pH, histidine can function as either an acid or a base. It's pKa can shift when inserted into a protein depending on the chemical makeup immediately surrounding the histidine, and this is proven by the fact that histidine pKa's in proteins vary from 6.2-9.3. How and to what extent nearest neighbor amino acids perturb the pKa of histidine is of interest to the College of Medicine, and the system will allow them to explore and characterize those effects in depth. It may even lead to large advances in drug delivery to different areas of the body. Another important application of the system will be the determination of the pH dependence on protein stability for many different proteins. This analysis is extremely sensitive to outside influences such as carbon dioxide present in the atmosphere. The automated titration system allows the College of Medicine to perform analyses with a high degree of confidence.

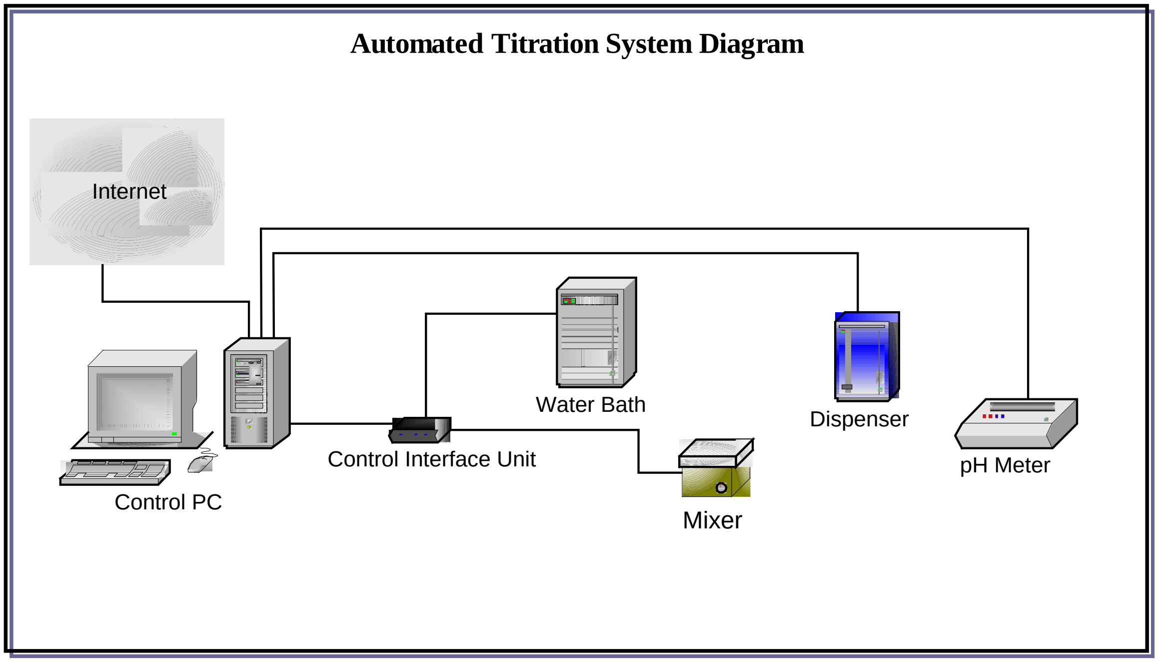

Figure 1 - System Diagram

The titration system is comprised of six main hardware devices and include: a water bath, a pH / temperature meter, a chemical dispenser, a magnetic mixer, a control interface unit, and a standard PC with a data acquisition card. A block diagram of the system can be seen in Figure 1. The PC uses the LabVIEW 6i software package and a data acquisition card by National Instruments to control all aspects of the titration. The system is capable of performing acid to base and base to acid titration in a temperature-controlled environment. The temperature of the titration can be held constant with in a +/- .5 ^{o}C range. If for whatever reasons the experiment ever exceeds user input maximum or minimum abort temperatures the system will automatically shut down and send an alert to either an E-mail address or to an E-mail enabled cell phone (or pager) specified by the user. The chemical dispenser is capable of automatically adding user-defined amounts of titrant at several rates and time intervals. The pH meter works in conjunction with the dispenser in taking pH readings during the titration. All pH readings and temperature values are stored in an output text file on the PC for easy analysis after the titration is completed. A desired pH stop value is entered by the user at the beginning of each titration experiment in order to indicate when the experiment is to be terminated. The system will send an alert to the users E-mail address or to his/her cell phone or pager when the experiment has been completed. The user can monitor the progress of the experiment from either the LabVIEW main control panel on the PC controlling the titration or from any other PC connected to the Internet. When the titration is completed, the system is capable of sending the output log as an attachment to an E-mail address that the user can specify.   The user also has the option of aborting the experiment at any time during the titration by pressing an “abort” button on the main control panel or by running a special LabVIEW abort program on any other PC connected to the Internet. The system also incorporates an easy to use “configuration wizard” that guides the user through the setup and configuration of the experiment before the titration is started. The Automated Titration System provides the elements necessary to automatically perform, control and monitor all aspects of time consuming and sometimes tedious titration.

Hardware System Components

The Automated Titration System as shown in Figure 2 is comprised of six main hardware devices and includes: a water bath, a pH / temperature meter, a chemical dispenser, a magnetic mixer, a control interface unit, and a standard PC with a PCI-6025 data acquisition card. All software control is implemented on the PC using National Instruments' LabVIEW 6i software package. The system also takes advantage of the Internet control, observation, and notification features of LabVIEW.

Figure 2 - System Picture

The water bath is used to control the temperature of the experiment by circulating temperature-controlled water around the vessel where the titration is being carried out. The particular unit used in the system is a Brinkmann - RM 6 Lauda model water bath and can be seen in Figure 3. Slight modifications were made to the unit so that it could be automatically controlled by the PC.

Figure 3 - Water Bath

The water bath has three physical parameters (heater, cooler and pump) that are controlled and one parameter (water temperature) that is monitored. DC controlled AC relays were added to the water bath so that the heater, cooler and pump could be controlled by the PC. A temperature probe (National Semiconductor LM35 model) immersed in the water bath is used to monitor the temperature of the water. The temperature that is acquired is used to control the heater and cooler. (Another temperature probe in the vessel where the actual titration is taking place is also used in the water bath temperature control.) During the initial configuration setup the user is able to set a desired temperature (in ^{o}C) the acceptable range of temperature variation. All input temperatures are limited to a range between 10 ^{o}C and 80 ^{o}C in .1 ^{o}C increments. The variation temperature that the experiment can be held between will default to +/-1 ^{o} C and can be changed in .5 ^{o}C increments. The user can also set both a maximum abort and minimum abort temperature value. If the temperature ever reaches the maximum or minimum abort temperatures the experiment will be terminated and the entire system will be shut down. The pump control consists simply of an on/off switch controlled automatically when the experiment is started and completed.

The particular unit that is used to monitor the titration pH and temperature is a Corning Model 450 pH meter. A picture of the meter can be seen in Figure 4. The pH meter consists of four probes that can each be placed into the titration vessel. Two of the probes monitor the pH of the solution and the other two probes are used to measure the temperature of the solution. (All probes do not need to be placed in the experiment vessel at the same time, just one pH probe and one temperature probe.) During the setup the user can select the wait time for pH data collection. This number will be the wait time after titrant is added to the vessel before pH readings are taken. It will be used to calculate the rate at which the system will take pH readings. This wait time defaults to 12 sec and can be varied between 1 sec and 60 sec in steps of 1 second. The user can also choose to set the number of pH values to be averaged during the experiment setup. This number of readings are averaged and the resulting average pH reading is logged to an output file and displayed on the main control panel. The number of averages ranges between one and ten. During experiment setup the user can enter a pH stop value. When this desired pH is reached the experiment will be considered complete and will shutdown the system. It will range between values of 0-14 in increments of .1 pH. The system creates a text database file that includes all the measured pH values collected during the experiment referenced to the amount (moles) of titrant added. The file also includes user input data describing the experiment including the concentration of titrant (in moles), moles of sample and volume of sample. During the experiment the user will be able to view the current pH of solution in the vessel, the elapsed experiment time, and the current temperature of the solution and the water bath on the PC screen. The user will also be able to terminate the experiment at any time by hitting an “abort” button placed on the front panel displayed on the PC.

Figure 4 - pH Meter

The dispenser adds the titrant to the solution. The particular dispenser used in the system is a Hamilton – Microlab 500 model. A picture of the dispenser can be seen in Figure 5. During experiment setup the user can select from a list of predefined syringe sizes to be used in the dispenser. The user will be required to enter the concentration of the titrant being used in the experiment. The user will also be required to input the volume of titrant to be added to the solution. This volume will be dependent on the syringe size that the

Figure 5 - Dispenser

user has chosen to use in the experiment, but it has a resolution equal to .1% of the syringe size.

The mixer is the device that is used to simply stir/agitate the solution in the vessel where the titration is taking place. A picture of the mixer can be seen in Figure 6. A few modifications were made to the mixer unit so that the PC could control it. The PC provides a pulse width modulated output to a DC controlled motor that spins a magnet underneath the mixer table. The magnet spins at different speeds as the duty cycle of the pulse width modulated output is changed. This magnet makes a special pill, which is inside the solution, spin due to the magnetic field created by the magnet. In order to control the mixer from the output of the data acquisition card, a special counter/timer algorithm was used to vary the duty cycle of a 0 to 5V square wave. At the time of experiment setup the software allows the user to select one of eleven different predefined agitation speeds for the mixer. The mixer commences agitation when the experiment is started and ceases agitation when the experiment is completed

Figure 6 - Mixer

The Control Interface Unit (CIU) provides a central location to interface the PC's data acquisition card to the, temperature probe, mixer and water bath. A picture of the CIU can be seen in Figure 7. A 50 pin male connector is located on one side of the CIU. The data acquisition card's ribbon cable plugs into that connector. Three color-coded RCA cables used for connection to the water bath are permanently attached at the back of the CIU. The outer rings of these plugs are grounded to the CIU input power supply ground and the inner plugs supply a TTL "on" or "off" signal to each of the water bath control ports. Next to the RCA plugs is a four-pin male "microphone" plug used to attach the water bath temperature probe. The female mini connection on the right side of the CIU is used as the power connection. A 9 Volt DC power supply is used to supply power to the entire unit. The black RCA cable, also on the right side serves as the connection to the mixer. The outer ring on the RCA plug supplies 9 Volts to the mixer and the inner plug supplies a TTL pulse width modulated signal to the mixer. A single blue LED is on the front of the CIU and indicates when the power is on. The circuitry in the CIU serves only to protect the PC's data acquisition card. A 4-gate tri-state buffer acts to isolate the Water Bath from the PC. The temperature probe is powered with only five volts. The analog lines connected to this probe are capable of handling 24 volts without damage to the card. Thus, no isolation is necessary. A single LM7805 voltage regulator takes the input power and converts it to 5V and supplies power to both the buffer and the LM35 temperature sensor. Figure 7 shows the interior and all external connections of the CIU.

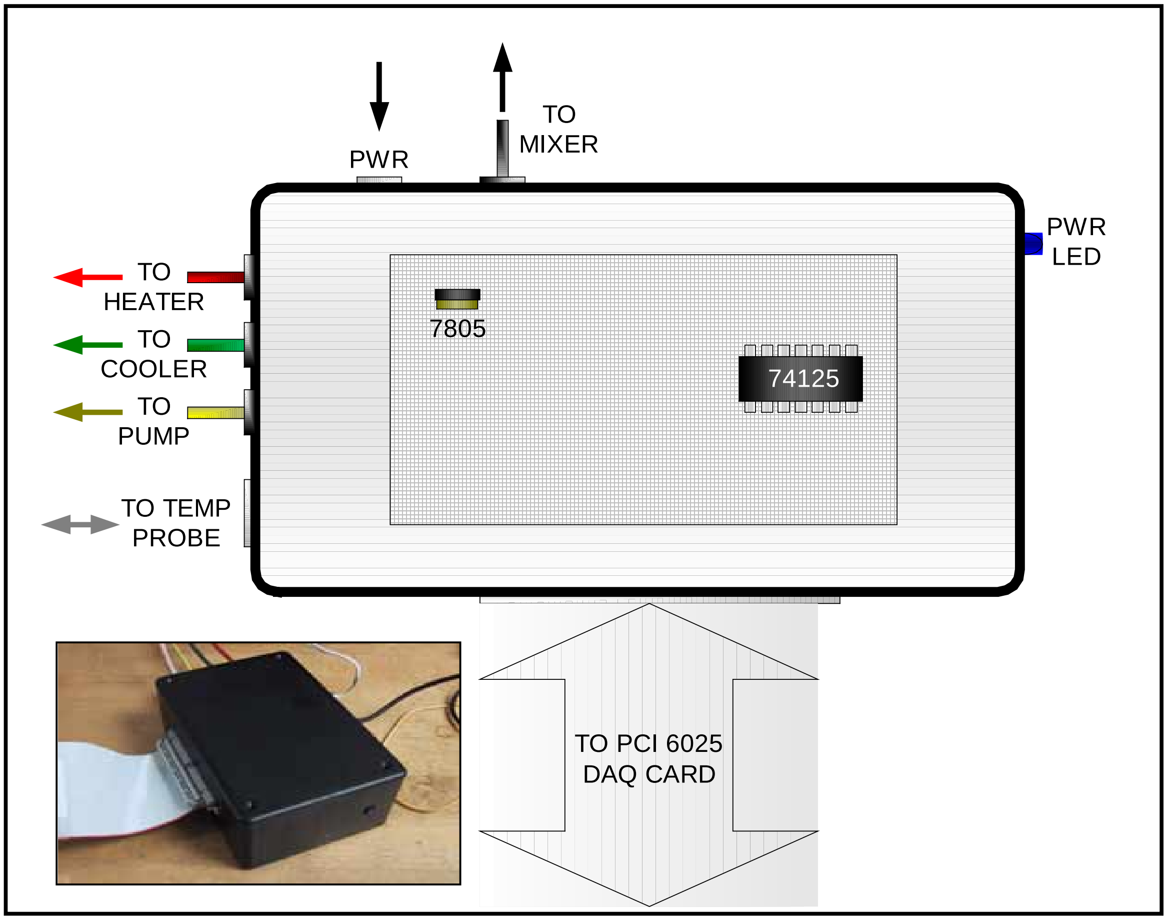

Figure 7 - Control Interface Unit

Operational Software

All of the software in the system was created with LabVIEW 6i. LabVIEW is a graphical programming language that is specially designed to work with National Instrument's data acquisition cards. It provides a quick way to create easy to use "windows" based environments that can be used to collect data and control a wide range of devices. The software for the system was written as a series of sequences. Each of the sequences contains code that asks the user to enter data, controls the system hardware, collects data from the hardware, or displays data to the user. A software flow diagram that shows the sequencing of events can be seen in Figure 8. After the user starts the LabVIEW program, the software displays a series of pop-up windows that provide instructions to the user or request that the user enter experiment parameters. After all parameters are initialized, the system performs a temperature probe calibration and then brings the water bath to the desired temperature before the titration is started.

While the titration is in progress the user can view all aspects of the system status from the main control panel. The system also displays a graph depicting the pH vs amount of solution dispensed. A picture of the main control panel is shown in Figure 9. Two examples of the pop-up parameter windows are shown in Figure 10. After the titration is completed the system shuts down all components and displays a pop-up window that allows the user to rinse the dispenser. The user can then choose to perform another titration.

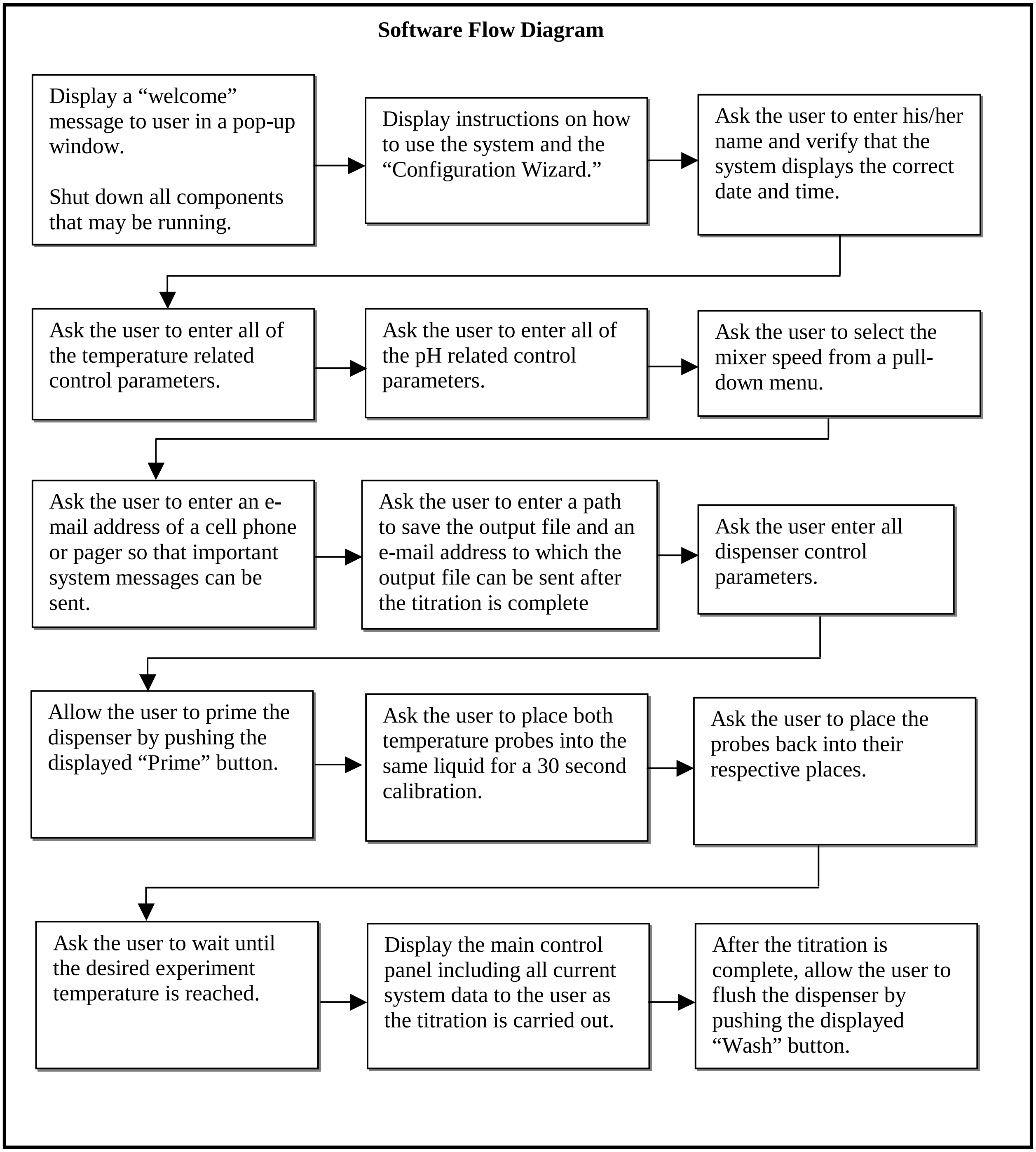

Figure 8 - Software Flow

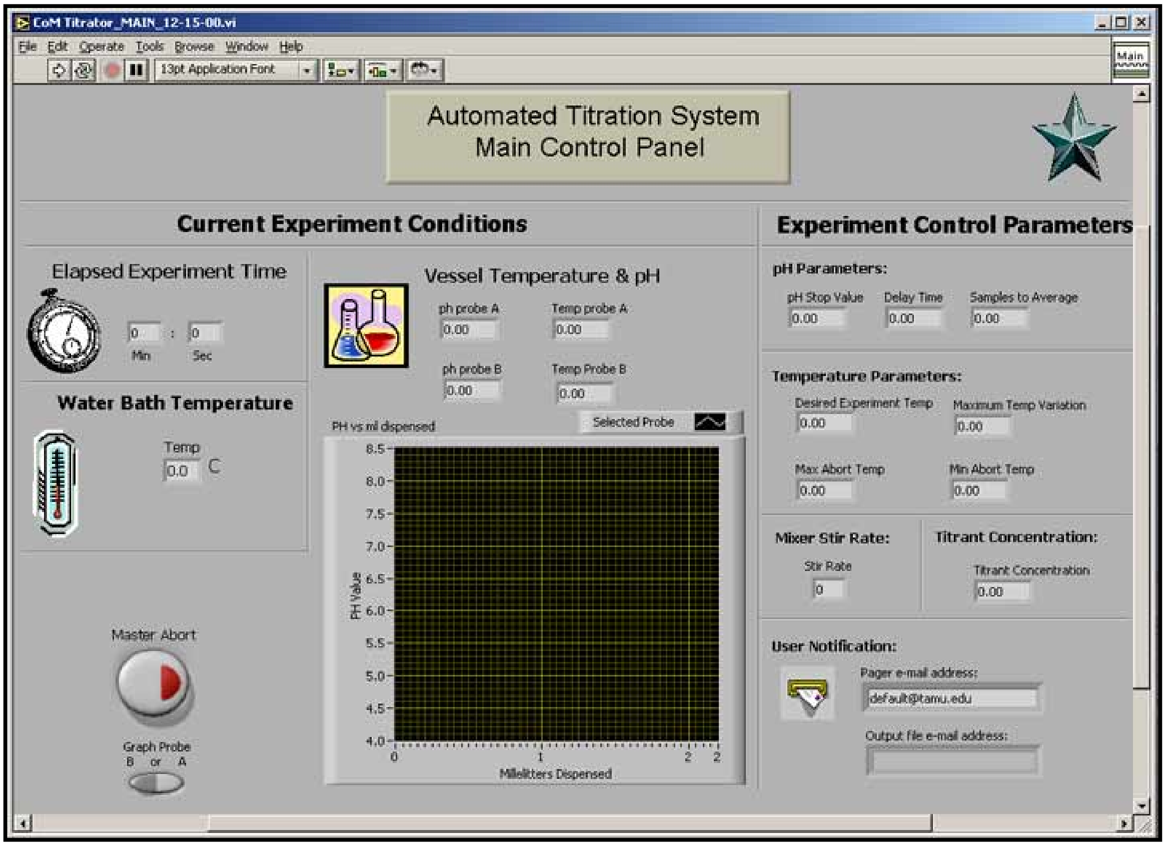

Figure 9 - Main Control Panel

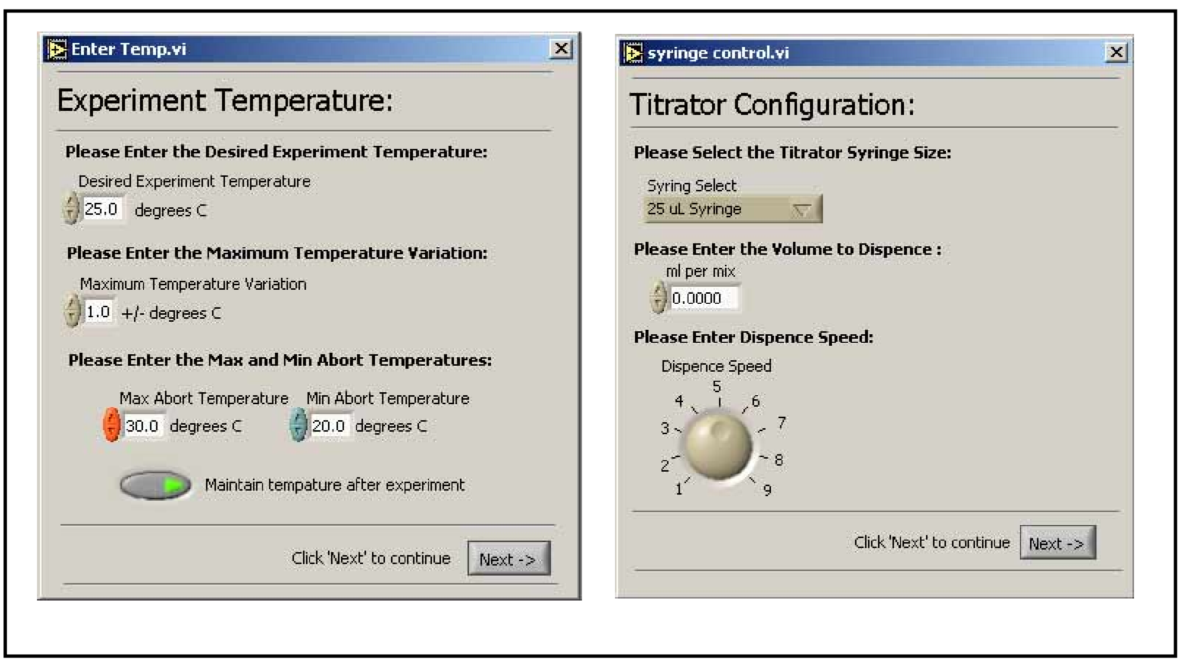

Figure 10 - Pop-Up Windows

System Products

The system produces certain items that the user can use during and after performing a titration. The main product the system creates is an output text file. The output file can be stored locally on the PC controlling the system, and it can also be e-mailed to any e-mail address after each titration is complete. This file contains a header containing all experimental parameters that were used while performing the titration and a data section that with the pH and temperature related data acquired during the titration. An example of this output file can be seen in Figure 11. The user can import this output file directly into a data analysis software package and use it to perform calculations and draw conclusions about the titration. This file can also be used to generate graphs in any spreadsheet program. The system also has a notification feature that will alert the user to system errors or experiment completion via a pager or message-enabled cell phone. This allows the user to perform other tasks while the titration is in progress. The user is not tied to the lab watching over the experiment the entire time. The system can also be accessed through the Internet. The user can view the main control panel from any computer with Internet access while a titration is in progress. This allows the user to periodically check the progress of the experiment from multiple locations.

```
------------------------------------------------------------------------------------------
Histidine Titration          12/22/00 09:09:05 AM          Richard T.
IP Address of Experiment Computer:          128.194.57.240
Initialization Setup:
------------------------------------------------------------------------------------------
Desired Experiment Temperature:(Degree Celsius)                 25.0
Maximum Control Limit Temperature:(Degree Celsius)              25.5
Minimum Control Limit Temperature:(Degree Celsius)              24.5
Maximum Abort Temperature:(Degree Celsius)                      30.0
Minimum Abort Temperature:(Degree Celsius)                      20.0
Measurement Delay Time:(seconds)                                5
pH Sample to Average:                                           3
Mixer Stir Rate:(0~100)                                         6
Email Address:
Syringe Size:                                                   0.2500
Volume to Dispense:(mL)                                         0.0100
Dispense Speed:(0~9)                                            5
Titrant Concentration:(Moles)                                   0.010000

Results:
------------------------------------------------------------------------
 pH A   TempA    pH B   TempB   Titrant  Min    Sec
------------------------------------------------------------------------
9.073   25.167   7.002  25.167  0.010   0.000  22.790
8.888   25.067   7.001  25.067  0.020   0.000  44.050
8.709   24.967   7.000  24.967  0.030   1.000  2.060
8.562   24.833   7.002  24.833  0.040   1.000  22.000
8.446   24.800   7.001  24.800  0.050   1.000  40.890
8.350   24.967   7.001  24.967  0.060   1.000  59.950
8.271   25.167   7.000  25.167  0.070   2.000  18.460
```

Figure 11 - Output File

Project Benefits

From a faculty viewpoint, real-world projects add a significant educational dimension to undergraduate engineering technology courses. These include:

- an increased motivation to learn
- a greater depth of understanding of technical concepts
- the opportunity to develop initiative and to bring fresh ideas and perspectives to the solution process.

Because students are generally eager to contribute, working on real-world projects provides them the inspiration to go beyond the confines of the classroom and laboratory to develop unique approaches in meeting design requirements. When a group of students realize that the system they are developing is actually needed by someone and that the results of their efforts will be put into service, they become highly enthusiastic. This enthusiasm coupled with an appreciation that the project is more than they can handle on their own, causes the student to become an active and supportive member of the team. All students realize early in the project that they must clearly define the tasks for which they will assume responsibility. Because a particular student's grade can be reduced based on the confidential assessment of his or her contributions made by other team members, all students understand that they must contribute to the team's overall success.

One of the most important benefits of real-world projects is the transformation that takes place within each student. In the beginning of the project, individually and as teams, they have no way to visualize the total solution. Although this is somewhat inhibiting, as they work together as a team subdividing the problem into smaller, more manageable pieces, they begin to develop confidence in their abilities until they actually see light at the end of the tunnel. When a successful demonstration is achieved, the students experience a near euphoric feeling that is almost addictive. Once experienced, students look for other opportunities to replicate the feeling. Students who form teams early in their academic program tend to work together on multiple course projects. They also look for more and more challenging projects to stretch their capabilities. During the Fall 2000 semester project, the efforts of all the student teams paid tremendous dividends. Although there were varying degrees of success, each design was significantly different and included diverse features that the team felt would be valuable to the customer/sponsor. The winning team spent additional time and effort adding extra features to the system based on the customer/sponsor's feedback. The system was delivered to the College of Medicine during the second week of December, 2000 and is currently undergoing final testing. Initial results show that the system is performing well and in fact is performing beyond the sponsor's original expectation. And the students that delivered their system will never forget the experience.

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Anthony Vaughan was born in El Paso, Texas in 1978. He is currently a Senior majoring in Electronics Engineering Technology at Texas A&M University. He is a member of the Tau Alpha Pi Engineering Technology Honor

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JAY PORTER
Jay R. Porter joined the Engineering Technology program at Texas A&M University in 1998 as an Assistant Professor and currently works in the areas of mixed-signal circuit testing and virtual instrumentation development. He received the BS degree in electrical engineering (1987), the MS degree in physics (1989), and the Ph.D. in electrical engineering (1993) from Texas A&M University.

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Joseph A. Morgan joined the Engineering Technology program at Texas A&M University in 1989 as the Program Coordinator for Electronics and Telecommunications Engineering Technology. His areas of interest included radar systems, data acquisition, and control systems. He received the BS degree in electrical engineering (1975), the MS degree in industrial engineering (1989), and the D.E. in industrial engineering (1993) from Texas A&M University.