I. NOVEL INSTRUCTIONAL TECHNOLOGY TOOLS IN TEACHING PHARMACEUTICAL ANALYSIS LABORATORIES. II. NEW APPROACHES TOWARDS THE SYNTHESIS OF SUGAR AMINO-ACIDS.

by

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To my mother, brother and the sacred memory of my father.

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Abstract

I. Maintaining high standards of science training is important for pharmacy graduates to practice knowledgeably, responsibly, and confidently. Instrumentation and resource constraints are maximal in the pharmaceutical analysis laboratory due to the nature of the experiments that need to be conducted and the need to provide as much individualized learning experience as possible. Therefore, the 1st year PharmD students perform the laboratory exercises in subgroups, rotating each week in three tri-weekly cycles. Although this arrangement optimizes space, instrument and resources utilization, it creates inevitable educational gaps related to each subgroup's experiment. More specifically, there are gaps with the lectures' progress and the necessary pre-laboratorial instruction for each experiment. Therefore, online instructional tutorials and technique-demos as well as accompanying online quizzes were prepared and delivered through a secure course-website. Each student had to view the tutorials and pass the quizzes before coming to the laboratory. In addition, virtual laboratories were designed as an additional aid for some experiments. As shown by student surveys, these changes were well-received and improved many aspects of this class.

II. Most cancer mortality results after it has metastasized from its primary growth site and spread to remote sites. Metastasis is a complex succession of events that the cancer cell manages to accomplish as the disease progresses. One necessary step is the

breakdown of the physical barriers, such as endothelial basal membrane and extra-cellular matrix. In order to achieve this, cancer cells express heparanase, a β-endoglucuronidase that breaks down the glycan part of heparan sulfate, which is a basic constituent of these physical barriers. Amino-sugars have been shown to inhibit this type of carbohydrate-processing enzymes by virtue of their transition state mimicry. Since the substrate of heparanase carries acidic moieties, its inhibitors also carry acidic groups. Few efficient synthetic routes are available for the synthesis of sugar amino-acids. The feasibility of the use of an unprecedented intramolecular S_N2'Mitsunobu reaction and α-aminomethyl radical cyclizations is explored for the synthesis of α- and β- sugar-amino-acids respectively.

PART I

CHAPTER I

TRENDS IN PHARMACEUTICAL ANALYSIS TEACHING AND NOVEL INSTRUCTIONAL TECHNOLOGY TOOLS IN DRUG ASSAY LABORATORY

1.1 Introduction: Current Trends in Pharmacy Student Professional Education

Pharmacists' training has undergone profound changes in scope, focus, and context towards the end of the 20th and into the 21st century. [1] It once focused on a drug-centered approach whereby the students received training about many aspects of the drug itself. Typical curricula included: medicinal chemistry, pharmaceutics and pharmacology. Because drugs are bioactive chemicals, pharmacists had to know everything about their chemistry and biology; they were even able to get involved in drug research. This drug-centered education was the aftermath of the Durham-Humphrey amendments of 1950s, which limited the exclusive privileges of pharmacists to determine the patients' drug therapy possibilities. It was also at this moment when drugs started to require a doctor's prescription, thereby taking away some of pharmacists' responsibilities. As a result, the education became less patient-centered and more drug-centered. During that historical period, therefore, a degree in pharmacy was essentially a science degree based on these three foundational disciplines, medicinal chemistry, pharmaceutics and pharmacology.

Gradually, a movement emerged within pharmaceutical education to reassume responsibilities over patient-care decision-making.[1] First, the first PharmD programs appeared in the 1960s and, at the same time, education began to shift from a drug-centered curriculum to a more patient-centered one. This new curriculum included more clinical aspects, such as experiential training dealing with patients, which often detracted

from basic scientific training. Finally, in the late 1980s, the *American Association of Colleges of Pharmacy* (AACP) appointed a *Commission to Implement Change in the Pharmaceutical Education* (CICPE) in an effort to rationalize and standardize these evolutions.[2] The Commission created a new mission for the profession, a new objective of pharmacy practice, and, as a result, a new mission for pharmacy education.[3]

Thus, the mission of the profession became "to serve society, the individual needs of the patient, and to produce and distribute drug entities and knowledge related to them." The mission of pharmacy practice, therefore, was "to deliver products and knowledge revolving around the rational use of medications and to provide pharmaceutical care".[3] These changes formalized the shift of pharmaceutical education from drug-centered to patient-centered. After this moment, pharmaceutical education required a strong liberal arts education along with a substantive foundation in the different areas of the field so that graduates could provide pharmaceutical care as qualified health practitioners. In addition, pharmacists had to be able to ensure optimal medication therapy outcomes and specialize in a specific area of practice. They had to educate patients, pharmacist trainees, and other health-care professionals. Finally, they had to, conduct research and to provide service to the community. There was also a greater emphasis placed on drug therapeutic management, which transformed the pharmacist into health information officer within an integrated health-delivery system.[2]

In the early 1990's, the CICPE concluded that a four or five year straight out-of-school curriculum would not be sufficient to educate pharmacists in these competencies.[3] Instead, it proposed to establish the Doctor of Pharmacy (PharmD) degree as the single professional qualification for all new pharmacists. The 4-year PharmD training, as the CICPE outlined, should follow at least 2 years of pre-pharmacy training in basic courses that provide the foundation for the later professional training. The Commission Papers stressed that the pre-professional or pre-pharmacy education should aim to prepare pharmacy practitioners with an understanding and appreciation of society and with a new role as their role within it as health care providers.

At the same time, the Institute of Medicine (IOM) identified five principal competencies that each PharmD graduate should have, which include the ability to: i) provide patient-centered care, ii) work in interdisciplinary teams with other health-care

professionals, iii) employ evidence-based practice, iv) apply quality improvement approaches, and v) utilize informatics.[2] The AACP's Center for the Advancement of Pharmaceutical Education (CAPE) and the Accreditation Council for Pharmaceutical Education (ACPE) Standards 2007[4] summarized these competencies, indicating that graduates should be competent to:

> "i) provide patient-centered care, ii) provide population-based care, iii) manage human, physical, medical, informational, and technological resources, iv) manage medication use systems, and v) promote the availability of effective health and disease prevention services"

These new competencies heralded a substantial change in pharmaceutical education. Most importantly, the incorporation of social and behavioral science - as well as pharmacy practice training- led to the curtailing of the basic science curriculum. This trend was furthered by the vagueness of ACPE's Standards 2000[5] Guideline 11.2 for pharmaceutical education science training, which stated that:

> "The biomedical and pharmaceutical sciences should be of such depth, scope, timeliness, quality, sequence, and emphasis to provide the foundation for and support of the intellectual and clinical objectives of the professional program in pharmacy".

This requirement was open to interpretations, which ultimately lead to the further erosion of the basic sciences in the PharmD curriculum.[6] On the other hand, when it came to the social/behavioral and practice training the Standards 2000 included a far more expansive, extensive, and detailed list of educational guidelines. These conflicting guidelines led to an ongoing debate that often placed basic science training in opposition to (or at the expense of) pharmacy practice training and vice-versa.[6,7] This debate is not restricted in US, but also occurs at an international level too.[8,9,10] It's generally noted that basic science training is in constant retreat within the field.

The chemical composition/levels and properties of drugs determine their interaction with the biological targets and therefore the success or failure of the pharmaceutical therapy. As a result, "[p]harmacists are scientists as well as clinicians, and basic science knowledge is what separates them from technicians".[6] No one is better prepared than a pharmacist to provide a wide-range of information about drugs; they know everything from a very basic scientific level to a clinical one. They are also able to

combine all elements of their education into a comprehensive understanding of drug therapy. All PharmD programs ask for training in General Chemistry/Organic Chemistry with laboratories and other science classes as pre-enrollment requirements. In fact, 2/3 of the PharmD students in the United States have a science degree, which makes sense. Nevertheless, there is a general observation that "pharmacists are not that science inclined and proficient".[6] This common perception is exacerbated by the fact that most professors who teach basic science courses do not have a pharmaceutical background and those in the practice have become so specialized that basic science is marginal to their teaching. Pharmacy students also often feel that basic science is a strenuous, redundant requirement and are negatively predisposed, which compounds the problematic relationship between pharmacy practice and science. Along this debate, leading PharmD educators have rushed to point out that "maintaining high standard science training is important if pharmacy graduates are to practice knowledgeably, responsibly and confidently in any setting".[6] One study even showed that basic science GPA predicts overall PharmD GPA, insinuating that a training in basic sciences is important for academic success in this field.[11]

As a response to the debate following the Standards 2000 and the implementation of PharmD education, ACPE included an appendix in its revised Standards 2007[4] which included specific course requirements and outlines of course content that need to be included in an accredited PharmD program. This revision:

> "has placed greater emphasis on the desired scientific foundation and practice competencies, the manner in which programs need to assess students' achievement of the competencies, provision of pharmacist-delivered patient care, including medication therapy management services, the advancement of the practice of pharmacy and its contributions to society, the pursuit of research and other scholarly activities, and the assessment and evaluation of desired outcomes".

Guideline 1.3 in Standards 2007 (concerning the mission of schools of pharmacy) establishes the biomedical, pharmaceutical, social/behavioral/administrative, and clinical sciences as foundations of the PharmD educational training. These requisites reaffirm of the role of science training in comparison to the Standards 2000, which stress the introduction of pharmaceutical care in the curriculum and mission statement.[4]

Standard No 9 is expanded into the Standards 2007 to emphasize the need for a curriculum that among other things "must develop in graduates' knowledge that meets the criteria of good science".[4] In a footnote, "good science" is described as one that is "evidence-based, convincing, explanatory, honest, testable, and systematic". No such mention was made in Standards 2000.[5] In a newly established Standard (Standard No 10), the need of adequate coursework for the "desired scientific foundation" is included along with a reference to the Appendices that describe this in more detail. Furthermore, Guideline 10.2 asks that each faculty be aware of other professors' course contents. Also, for the first time, it asks that basic science and pharmacy practice faculty collaborate in order to integrate their courses better; more specifically, it wants basic science faculty to provide applications and examples relevant to practice, and wants practice faculty to stress the scientific basis for pharmacotherapy. At the same time, it cautions against course overloading and requests that students achieve a balanced curriculum. Guidance 10.2 concludes by encouraging the adoption of evidence-based teaching methodologies as well as advocating for the introduction of "innovations to promote optimal learning."

This latter point is further expanded in Standard No 11.[4] As in Standards 2000[5], there is a statement about the need to develop critical thinking and problem-solving skills. Consistent with IOM's provisions (*vide supra*), Guideline 11.2 advises the incorporation of "computer and other instructional technologies" as well as "active learning techniques." Expertise in informatics is reiterated in Standard No 12, which entails the competencies and expected outcomes of a PharmD graduate. Also, for the first time, there is an emphasis on evidence-based practice as well as taking into account biomedical sciences that may influence the outcome of pharmacotherapy.

The rather vague Guideline 11.2 regarding course content in Standards 2000 (*vide supra*)[5] is reiterated in Guideline 13.1 of Standards 2007.[4] This time, however, it adds social/behavioral/administrative and clinical sciences on top of the biomedical and pharmaceutical ones as basis not only for understanding but also for developing therapeutic schemes. Again, there is reference made to Appendix B for additional guidance on what is meant by science foundation. Such detail is missing in Standards 2000.

Furthermore, there is a greater focus on the assessment and evaluation of student learning with a much more elaborate and relevant standard (Standard No. 15).[4] According to Standard No. 15, there should be a variety of assessments available that also help develop students' problem solving and critical thinking. It also specifies the goal of having students gain of certain desired competencies in sciences so that they can incorporate them into pharmacy practice. It goes as far as asking for student portfolios as a proof of both the students' progression through the program as well as documentation of the competencies that they achieved.

Finally, Standards 2000 Standard No 16[5] included a provision that allowed pre-professional requirements to include "some elements of the biomedical sciences area" of the professional degree curriculum. This Standard, coupled with the vagueness of Guideline 11.2, led to a greatly diminished basic sciences in the professional program. As a result, the pharmacy practice training remained intact while basic sciences were fulfilled in an earlier, pre-professional level. Standards 2007[4] just omit this clause in the analogous Guideline 17.1 only to reframe it later in Appendix B.

In an attempt to refract the diminution of basic science foundation training of Standards 2000, Standards 2007 include "Appendix B: Additional Guidance on the Science Foundation for the Curriculum."[4] This was the result and the subject of extensive deliberation among faculty, practitioners, and regulators to identify elements of the science foundation that were deemed necessary for pharmacists. It provides particular subject listings along with some brief, indicative content intended to be covered. Although still "some of these areas may be addressed in pre-pharmacy courses" it stressed that the majority of these courses should be part of the professional curriculum. The actual content is left open-ended to accommodate the quickly evolving sciences as well as to account for their significance for pharmacy practice.

In addition to its evolving standards, pharmaceutical education is also influenced by the changes in higher education as a whole. One is the assessment movement[12], which has been defined as "a continuous, systematic process of developing and reviewing student outcomes and collecting, reviewing, and using these data to inform program improvement." These changes have caused instruction and delivery to evolve from being "teaching-based" to "learning-based." This shift was further reinforced by the stated aim

of having life-long education. Assessment assumes a new role of being embedded within instructional delivery. Already included in Standard 15 of Standards 2007[4], the emphasis is placed on the continuous, systematic, and variable way of assessing whether students have achieved the desired outcomes. At the same time, the school "must use the analysis of assessment measures to improve student learning."

Another factor of change is the perceived pharmacist shortage. The United States has too few pharmacists at a time when the population is increasing, life expectancy is rising, baby-boomers are aging, and polypharmacy is ubiquitous[11]; this has been pointed out by several employment surveys.[13] An increase in dispensing errors and patient deaths has also been attributed to this shortage as fewer pharmacists are available for proper dispensing and patient consultation in an ever increasing number of prescriptions. For this reason, pharmacist salaries have experienced a substantial increase, making it a quite attractive professional option. In response to this demand for pharmacists, new pharmacy schools have opened. In 1993, for example, there were 75 colleges and schools of pharmacy and 38,902 total students enrolled, while in fall 2005, there were 92 colleges and schools of pharmacy with a 46,527 student population.[2]

However, both the high cost of tuition and the scarcity of faculty render the effort to increase the number of schools of pharmacy insufficient. On the other hand, existing pharmacy schools have sought to expand their class sizes thus imposing space and faculty resource strains.[11] Thus, quite a few schools have resorted to establishing satellite campuses and/or establishing distance education programs.[14] In fact, for the first time, Standards 2007 provide guidelines and affirm the applicability of such efforts (Guidelines 1.5, 2.5, 3.5, 7.9, 11.5, 17.8, 27.5, and 30.6).[4] At the same time, with the advent of broadband Internet connectivity and the ability to transmit and receive high-quality voice, video and picture files more quickly, higher education is in the midst of a "virtual revolution." Education is no longer restricted by time or distance and pharmaceutical education has attempted to adopt some of these ways of distributing course content.

1.2 Pharmaceutical Analysis (Drug Assay) Training in a Changing Educational Environment

Pharmaceutical Analysis is one of the earliest pharmacy-related science classes taught. A drug's action, therapeutic or toxic, depends on its dosage and levels in

biological systems. The subject of Pharmaceutical Analysis is to establish the identity and provide an estimate of the quantity of chemical substances that are used as drugs; this can be done during and after the production of the drugs or following their administration to the patient recipient of drug treatment. In today's movement towards evidence-based medicine, this discipline generates evidence about drugs that can be directly applied for safe production and use. Thus, one would expect that the Pharmaceutical Analysis class and Drug Assay laboratory would be an integral part of the pharmacist's training.

Yet, it is generally observed that pharmaceutical analysis content instruction has been deemphasized. After the shift of drug-centered to patient-centered pharmacy practice, it is not obviously useful to pharmacists in community or hospital pharmacies. This course treats drugs as drugs (i.e. chemical substances) and, in fact, as early as in the 1970's, few students believed that this course material was relevant to pharmacy practice.[15] The accreditation guidelines of 2007 ask for the PharmD graduates to master the "ability to quantitative methods of measurement in order to provide drug information, evaluation of clinical laboratory data".[4] And in a newly included Appendix B—"Additional Guidance on the Science Foundation for the Curriculum"—it is stated that there should be training in Bioanalysis/Clinical Chemistry. It specifies the instruction of "fundamentals of laboratory medicine and its importance to screening, diagnosis, and evaluation of patients" as well as "clinical data relevant to disease state management." The same appendix allows for PharmD programs to include the required content in individual or integrated courses and allows for an undefined number of sections to be covered in the pre-pharmacy curricular requirements.

The result of all the above changes is that in the 2007-2008 academic year only 8 of the 101 AACP accredited PharmD programs in USA explicitly included training in Pharmaceutical Analysis (Figure 1.1). Some other 12 programs covered some Pharmaceutical Analysis content integrated within other classes such as Pharmacy Practice or Pharmaceutics/Pharmacokinetics. All of them had two semesters of general chemistry with lab as a pre-pharmacy requirement, which is a far-cry from a class focusing exclusively on drug analysis.

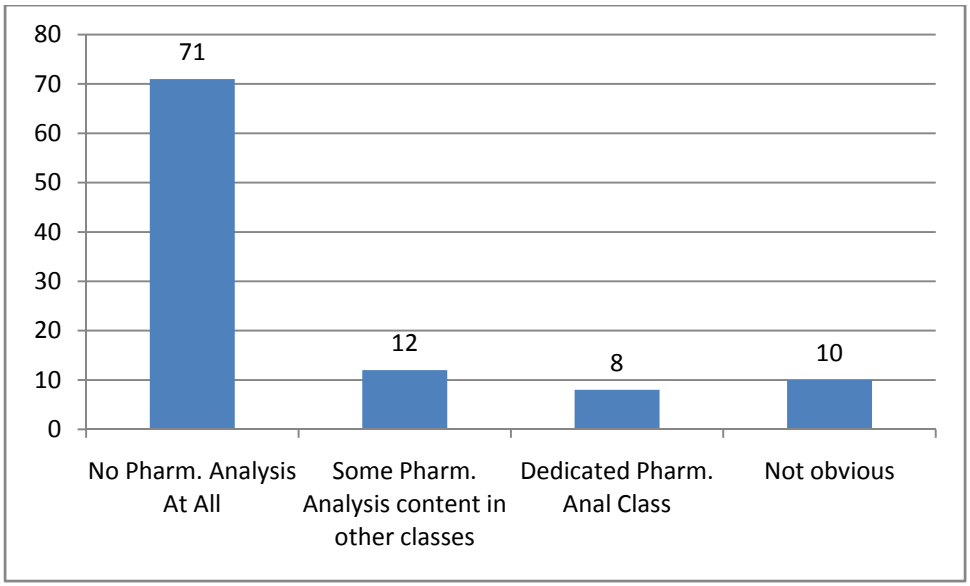

Figure 1.1: Pharmaceutical Analysis content instruction in US Schools of Pharmacy

It was within this framework of transition in pharmaceutical education and education as a whole, that there was an attempt to improve the way the instructor(s) delivered the Pharmaceutical Analysis Laboratory—one of the basic science courses of the PharmD curriculum at the University of Michigan.

1.3 Evaluation of Previous Drug Assay Laboratory Instruction at the University of Michigan

The University of Michigan is one of only 8 PharmD programs in the USA to still offer a full semester course that focuses exclusively on Drug Assay training as well laboratory hands-on training which takes place during the first semester (Fall Term) of the PharmD curriculum. It is a 3-credit hour class with two hours of lecture and four hours of laboratory instruction per week. This means that all ~80 enrolled students have to be trained in one semester using expensive instrumentation and consumables. These factors, along with the need to have an as individualized instruction as possible, make it so that students must split up into different groups in order to complete the different experiment each week. So the class of approximately 80 students is divided in sections in the beginning of the semester and students remain in these sections throughout term. The first half of the students comes to the laboratories on Monday, while the other half comes on Wednesday. These groups are further split into 3 subgroups (A, B, C) and each week one subgroup works on one exercise while the other two subgroups work on a different

set of experiments of the cycle. The following week, they rotate to do another exercise (Appendix A). Thus, in every 3-week cycle, 3 experiments are taught for a total of 3x3 = 9 experiments for the whole semester. This plan (along with the first cycle of experiments) is illustrated diagrammatically below (Tables 1.1 & 1.2):

Day	Monday	Wednesday
Number of Students	38	38
Groups	A, B, C	A, B, C
Students/Group	13+13+12	13+13+12

Table 1.1: Student splitting in groups for Drug Assay Laboratories.

1st Cycle, 1st week, Monday & Wednesday: Group A (Experiment #2), B (Experiment #3), C (Experiment #4)
1st Cycle, 2nd week, Monday & Wednesday: Group A (Experiment #4), B (Experiment #2), C (Experiment #3)
1st Cycle, 3rd week, Monday & Wednesday: Group A (Experiment #3), B (Experiment #4), C (Experiment #2)

Table 1.2: Example of student rotations along 1st cycle of experiments.

This arrangement, indeed, streamlines the students' training from the spatial and logistical point of view. Nevertheless, it has some serious drawbacks. Every week's lab does not necessarily follow the progress of the lectures. Quite a few times students come to the laboratory without knowing anything about the particular experiment. And, even if they had heard the lecture portion of the class, they need time to absorb the material and to relate to the experiment. Moreover, without an exam or a class assignment looming, students are less likely to study the lecture material in advance. As observed from the full schedule of lectures and laboratories (see tables A1 and A2 in Appendix A), it is obvious that quite a few students—at various points in the term—experience this mismatch of lecture and laboratory progress. In the first cycle of experiments, for example, two thirds of the students performed the Fluorescence experiment without having heard about it during lectures while another two thirds did the HPLC experiment of cycle #2 under similarly undesired conditions.

So a pre-laboratorial tutorial session is necessary to ensure that the students gain an adequate understanding of the theory behind each experiment and so that they know what the experimental procedure entails. This could help the experiments run efficiently, promptly, and timely, which is important because they cannot exceed the four hours allocated to them. Furthermore, the students would feel confident and comfortable performing the experiments and they would be able to see more clearly that there is a connection between the content taught in lectures and labs. Finally, it could help the students see a utility in the experiment in relation to the pharmacy practice.

One way of achieving more cohesion between labs and lectures would be to have teaching assistants (TAs) spend 20-30 minutes at every laboratory meeting teaching theory and experimental procedures before each experiment. However, this would be burdensome, time consuming, and inefficient since it would have to be repeated 2x3 = 6 times. Also, most students would come totally unprepared so, among other problems, those who come prepared ones would be impeded and annoyed. In addition, there would be limitations on time, space, and visual aids in which to deliver these presentations in an efficient and effective way during laboratory time. Furthermore, there would be no evaluation of students' understanding or a timely way to identify their possible weaknesses/misunderstandings. Traditional lecturing before lab is a passive way of teaching while requiring immediate results. Active learning is more desirable but it would be impossible with this model.

As a possible alternative 10-12 years ago, in the beginning of each of the 3 cycles, all 3 subgroups (of both sections) would get together and have each TA teach for 20 minutes his/her part experiment of that particular cycle. So, at the expense of ~1 hour of laboratory time in the first experiment day of each cycle, the students would hear a description of the basic analytical principles behind each of the three experiments of the cycle along with a detailed description of the experimental procedures. The danger of this way of organizing the class was that it left the students confused, disconnected, and unable to follow the class coherently since they had to hear about two other experiments that were irrelevant to their own work on the very day of their own experiment. Also, the reality is that not all TAs are as efficient in delivering a complete tutorial in 20 minutes and, by the time of the actual experiment, most of the material covered is forgotten. This

failing was reflected in the student evaluations after class (see Chapter II) where no less than 3/4 of the Fall 2004 students felt that the laboratory exercises were not following what was presented in lecture.

At the same time there, is always a desire to incorporate electronic resources in teaching in order to prepare the students for working in an electronically-managed, professional environment. Also, according to AACP, the aim of the PharmD education is to create committed lifelong self-learners. In summer 2005, there was a general consensus that something needed to be done about the laboratory portion of the class and the course instructors and coordinator conceived and planned out a way to redesign the content and methodology of the course, as discussed below. This revisioning of the way the course was taught took the above points into account.

1.4 Rationale for New Instructional Methodology

As previously discussed, the former way of incorporating a pre-laboratorial tutorial session in the beginning of each experimental cycle created numerous problems. To make up for these problems, an integrated system of pre-recorded tutorials was designed and implemented in order to provide the necessary theoretical background for each laboratory exercise. These tutorials were accompanied by prerecorded experimental demonstrations wherever applicable. Both lessons were made available outside of class through a secure website platform such as a distance education component.[16, 17]

Distance education is defined as a delivery of education where there is a "separation in space between instructor and learner." Distance education's scope and application as an alternate form of curriculum delivery has been increased with the improvement of web-based tools. Depending on the timing of the delivery and the reception of instruction, distance education is defined by two modes: synchronous and asynchronous.[18] In the synchronous category, there is no time separation between the distant instruction and the student learning. An example would be an audiovisual transmission of a lecture from one site to another, where students can interact with the instructor and their classmates in real time. In the asynchronous delivery, there is also a separation in time between content delivery and student reception. The pre-recorded web-based lectures that students access at a later time described in this study constitute an

asynchronous distance education effort. Online lectures serve as a unique way of uniformly delivering information to all students, reducing inconsistencies with learning experiences, delivering material to larger groups of students, offering learning flexibility to students who have part-time jobs or other needs, actively engaging learners, decreasing the need for printed handouts, monitoring learning more effectively, and, finally, increasing students' accountability by having an electronic record of accessed lectures.[19]

It is also important to ensure that students actually watch these tutorials and to evaluate their preparedness before coming to the class. So, once implemented, online instructional videos were followed by online quizzes that students had to view and pass before coming to the actual laboratory. In fact, it has been reported that the longer a web-based module is, the lower the students' persistence.[19] So these quizzes might be able to enhance student persistence by forcing them to view the pre-laboratorial videos in their entirety. Actually, the quiz questions were used as a self-assessment tool in order to encourage students think about the assay and the basis behind it rather than examining and grading them on what they just heard. For this reason, a rather low threshold of 60% was set in order for students to pass the quiz (or 4/6 correct answers). Also, these quizzes were not included in the final course grade. Because of this and because of the way the questions were formatted, quizzes served (and can still serve) as another vehicle of transmitting information, elucidating notions and encouraging students to learn the material as self-learners through active learning.[20, 21] With this model, a lot of subtleties can be better taught if the student actively seeks to choose among various statements revolving around a topic. Active learning is a style of teaching that requires learners to formulate answers to questions based on acquired knowledge while continuing to search for new knowledge that may provide better, more complete answers. Importantly, active learning enhances students' ability to think in an independent and critical manner.

Active learning models of education may also initiate in-class discussions, which are important in helping to evaluate students' trajectories These evaluations are also included in the ACPE guidelines for assessment-based teaching according to which students' learning should be assessed in every step of the didactic process in order for them to move from teacher-based to learner-based teaching.[2,11] These techniques allow faculty members an opportunity to monitor students' learning prior to grading-based

assessments (i.e., quizzes or examinations) and to adjust their teaching strategies appropriately to maximize learning. The techniques are simple, non-graded, anonymous, in-class tools that provide feedback on the teaching-learning process.[22] The goal of these techniques is to offer useful information for faculty members, which will help them to improve their teaching methods.

Finally, quizzes create a sense of live interaction, which appears to be a critical factor for the successful reception of asynchronous teaching methodologies for the students.[23] In this way, professors would be ensured that students have familiarized themselves with the experiment before coming to the laboratory. Also, the students would know their weaknesses and come prepared to ask questions, thereby adding a synchronous component (in-class debriefing with TA) in this asynchronous distant education setting.

One advantage of including these tools is that the laboratory class might feel more logically coherent and could stand alone regardless of the progress of the main lectures. In addition, no class time would be consumed with the pre-laboratorial tutorials and the students could see the instructional materials at their own pace. From the instructors' point of view, they would know where to focus their efforts during the labs and would be able to explain possible points of confusion. There could also be a lot of active learning opportunities. Since everything is done electronically, each student/instructor could keep a portfolio of their activities. This material could be used later as reference or supplemental material (for example, in laboratory report writing or other classes). Electronic portfolios are another recommendation by ACPE in order to help train pharmacists to become lifelong self-learners.[4]

Also, it was envisioned that the students should be provided with problem solving skills beyond the essentially technical ones of executing experimental protocols safely and precisely. Furthermore, a variety of exposure can only be helpful as different students have different learning styles. In addition, it is not always feasible to include a purposefully erroneous experiment and ask from students to resolve it because it could harm the expensive and sensitive instrumentation used. So, in addition to the reasons listed above, complimentary virtual laboratories were introduced as part of a particular experiment in addition to the wet one. Apart from the variety of exposure added, students

could try sparingly erroneous manipulations to better visualize and appreciate the events that take place during assay. In addition, they can substitute for more elegant/expensive experiments.

Taken together, these instructional interventions enable the alignment of the laboratory with Bloom's Taxonomy of Educational Objectives, which pertain to the cognitive domain.[24,25] Briefly, these were a hierarchical order of intended learning outcome categories and it was proposed that in order to achieve the next level of learning, one would have had to achieve the earlier one.[26,27] The categories are as follows: I. Knowledge, II. Comprehension, III. Application, IV. Analysis, V. Synthesis and VI. Evaluation. In the previous laboratory setting, the students were asked to be competent in the exercises and interpretation of the results and generate a lab-report (i.e. objectives III, IV and V) without first ensuring that they mastered I and II. The new methodology may ensure that objectives I and II are achieved and/or provide opportunities for the instructors to rectify students' deficiencies in these domains before starting the lab exercises. After that, the students can advance to categories III, IV and V in a more streamlined and promising way. With the virtual laboratories, there are opportunities for the students to advance to even VI, by encouraging them to actively explore the experiments' applicability and by having them generate their own proper conditions.

In a formative assessment attempt, it was speculated that the students might not appreciate the extra work outside class time.[28] Another possible drawback alluded to previously is the lack of live interaction with the instructor. Interpersonal communication skills are an integral part of pharmacy education culture and this asynchronous teaching methodology operates against the development of these skills.[29, 30] However, within the whole context of this class, there are numerous opportunities for live interaction with the instructors. Also, the current arrangement of delivery may cause the students miss the beneficial effects of note-taking.[31] Furthermore, the students may have an aversion to change and resist accepting a different method of instruction than what they had experienced in their pre-Pharm curricula. Finally, the perceived overlap between the wet and virtual laboratory was another concern lest the students would sense a redundancy in the educational materials provided.

1.5 New Student Schedule of Activities per Experiment/Integration of Laboratory Instruction

The University of Michigan provides a webspace for instructors and students for each class. The sum of those WebPages constitutes the Course Tools (CTools) website, which is individualized and personalized for each instructor and student depending on the classes he/she teaches or takes. In order to access his/her personalized CTools webpage, each participant logs-in through a secure server directing him/her to his/her own CTools site after visiting **http://ctools.umich.edu.** The instructor can control the content and access to all of the material, which is posted on the CTools site; he or she can also communicate with the students s/he selects. In addition, once logged-in, the CTools secure server provides access to other secure sites containing additional instructional materials. Only registered students and instructors have access to this site and the instructors could control the students' access to the various modules.

For each of the 10 experiments, the students followed the same general schedule of activities. In order to ensure equal treatment of both Monday and Wednesday students and to avoid possible confusion, access to the online materials was controlled and granted only to the specific students who would perform the particular experiment. The material was also posted only on certain calendar days before its actual execution in the laboratory. So each student group (A, B, C, Monday or Wednesday) was granted access and notified by e-mail 4 days before the upcoming week's experiment about the availability of the instructional materials; each group was also provided with web-links directing them to the particular sites that hosted them. They were then given 3 -days to complete the assignment, which was due before the eve of the laboratory day. The modules were arranged by experiment using clickable icons. Upon clicking these icons, students would be presented with the Real Player© or Adobe Flash Movie Player© embedded players and they would watch the appropriate material of the particular experiment. In 2006, there was an effort to include all ten experiments in an "adventure or journey" template. Such a method might help to put each experiment in context. The journey was entitled: "The Caribbean Missions," which drew on the popular theme from the movie, *Pirates of the Caribbean*, which was popular at that time. The semester-long activity included 4 missions: Mission 1 was Experiment #1 which was common for all students. Then followed Missions 2 (Drug Analysis of Selected Medications), 3

(Detection of Drug Abuse) and 4 (Approaches to Control Arrhythmias), which reflected each of the experimental cycles (1, 2 and 3 plus Experiment #1).

At his/her discretion and pace, students could access their respective experiment's material. As soon as they logged-in, the students were directed to their particular experiment or "Mission" tutorial. This could only be viewed online using the embedded Real Player© screen and could be started and stopped at will during playback. In addition, the PowerPoint© presentations used were also available in the same website if the students had trouble reading the recorded ones because of problems with the videotaping quality. After viewing the video, the students were asked to click "Submit" and they were then directed to the online quiz where they had to respond to 6 multiple-choice or fill-in the blanks questions with no time limit. Upon finishing, students were asked to click "Submit" and they were shown the results. If they failed to pass a threshold, they were asked to repeat the lecture and quiz. Finally, the students were given access to the online experimental demonstrations utilizing a similar distribution webpage as in the case of the online lectures.

In the eve of each wet laboratory, the course coordinator could easily generate spreadsheets, which would include detailed information about each student's online activity for the particular experiment, such as whether a given student watched the pre-laboratorial clip or not as well as the actual times s/he spent watching them verify that the student had not spent less time than required. Also, a detailed report of all students' online quiz performances was included. Results could be broken down per question so the coordinator knew which students were struggling and in what areas. If, for example, a lot of students failed in a question the coordinator, would advise the TA so that before starting the experiment s/he could spend some time explaining the correct answer to the students accordingly.

On the day of the experiment, the instructor could also verify that the students had seen the online videos and passed the quiz before allowing them to perform the experiment. Those who had failed were required to view the tutorials and retake the tests in the laboratory using one of the computers. At any time, a student might watch any part of the online tutorials if s/he wished to refresh his/her memory or to clarify points of confusion. In addition, as explained above, the laboratory instructor could use the online

quiz students' results to discuss points that students seemed to be struggling with. For this reason, the students maintained access to the online materials for the rest of the course. Appendix B contains examples of all the above mentioned materials.

1.6 Methods:

1.6.1 Online Prelaboratorial Lectures

For Fall 2005, ten lectures of approximately thirty minutes were video-recorded (i.e. one per experiment); and each video used a synchronized 3-18 slide PowerPoint© presentation as visual aid. The instructor was recorded while lecturing, which also showed him pointing to the particular parts he was analyzing using a laser pointer. The viewer could view a video-recording of the instructor's tutorial and the synchronized PowerPoint© presentation as if s/he were attending a real lecture. The video-files could be viewed using clickable Real Player thumbnails embedded in a secure webpage. Since it was the goal to address each student individually, regardless of the progress of the class lectures, each pre-laboratorial lecture was designed to serve as a stand-alone class for a student with the prerequisite general chemistry knowledge. As the class laboratory advanced to the later cycles, knowledge acquired in the earlier parts of the course was also considered to be an important prerequisite.

Thus, each lecture started with a brief, but thorough review of the necessary theory behind each technique, which was introduced with its application and usefulness in drug assay. This step was then followed by a description of the physicochemical principles behind the separation and/or generation of the analytical signal. Wherever possible, in later-cycle experiments, the new technique was introduced in comparison and in relation to earlier applications. After the theoretical part, the specific experiment using the technique was described extensively, which was done in three parts. The first explained why the particular technique was appropriate for the attempted assay. This was done by showing and discussing the chemistry of the involved molecules, which pointed to the structural features that allow for that type of analysis. Then there followed a detailed and illustrative description of the experimental procedure itself. It concluded with an explanation of how the results are interpreted in order to generate the analytical answer/conclusion. The PowerPoint© presentations were enhanced by diagrammatic

depictions of experimental manipulations, illustrations of key instruments, and animation schemes that progressively added to the complexity of each slide. The items on the slides would appear following the instructor's narration, which unfolded in a progressive and interactive manner.

In Fall 2006, the format of some of the tutorials was altered because certain aspects of the virtual laboratories of Fall 2005 *(see description below)* were incorporated into some of the Fall 2006 pre-laboratorial tutorials. More specifically, by employing Adobe Flash©, each PowerPoint© presentation was converted into an animated movie with only the instructor's voice (and not his person) narrating the tutorial. The first theoretical part followed as before, and the movie followed the previously used PowerPoint© presentation with added verbatim and short questions, which one had to answer correctly in order for the animated movie to advance; and as the narration progressed, the text and objects of the presentation were introduced and/or highlighted in an animated way. This part was then followed by a virtual lab demonstration, which covered the technical description of the actual experiment.

1.6.2 Online Pre-laboratorial Quizzes

Each online lecture or movie was followed by a short online quiz. The student was directed directly to the quiz webpage as soon as s/he clicked the "Submit" button after s/he viewed the pre-laboratorial lecture material. The student was asked 6 multiple choice or fill-in-the-blanks questions. For the fill-in-the blank questions, there was a statement or question followed by a multiple-choice option, one of which was the correct one. The fill-in–the-blanks questions included statements about an assay where two words or phrases were purposefully omitted. As the students overlaid the mouse cursor over each blank, a blurb appeared providing the different choices. So a student had to think about which pair of words/phrases made sense, which served to reinforce his/her knowledge of the material and of important concepts.

The content of the questions was variable. Some questions dealt with the theoretical principles behind each assay in order to ensure that students had a firm grasp of them. Many times, the students would be asked to predict the outcome of an experimental manipulation or error. Other questions focused on more technical issues.

Finally, the website permitted the instructor to post comments, which the student could read after viewing their results and the correct answers on the quiz. These could be comments below a correct or an erroneous entry or a general statement referring to the quiz questions as a whole.

The questions were presented altogether on a single page maintained at **https://lessons.ummu.umich.edu/** , which is directly accessible at the end of the video-taped tutorial and/or virtual laboratories. Once accessed, students could scroll to answer any question they want. To avoid students cross-communicating their answers to each other, all of the questions were shuffled electronically for each student's own online quiz. In addition, the multiple choice answers were also presented in various sequences in order to avoid students' passing the quiz simply by copying off of others. At minimum, the students would have to dig, see, and reflect upon on what the correct answer was instead of just striking the letter answer; this helped to ensure students' minimum mental engagement beyond just clicking the correct letter answer. After finishing answering the 6 questions, students were asked to click "Submit." Students could go back and revise their answers as many times as they wish with no time restriction till the point at which they clicked "Submit. After that, they could view their score and read feedback for all their answers, correct or incorrect. If s/he passed with a certain score, s/he would have then completed the assignment; if s/he did not pass, that student was asked to perform the tutorial or virtual lab again. The website could generate cumulative reports in a spreadsheet form, from which the instructor could see both the start and end time of each student's online attendance for each lesson as well as each score broken down by both question and by student, as well.

1.6.3 Methods: Online Experimental Demonstrations

In addition to the pre-laboratorial lectures, the instructor was videotaped demonstrating the actual use of key instruments that were featured in any given experiment. These videos were shot in the laboratory (and the instructor wore all the necessary safety equipment); they were made available accordingly as each student group progressed through the respective experiments. These videos were actual Real Player© movies with full capacity to go back and forth and to navigate through the topics

discussed. Typically, they began with an overview of the instruments, explaining their application in pharmaceutical analysis and any special precautions that had to be taken when handling them. The presentations continued with an identification of key components, their function, and the way that they were actually used. Whenever necessary, the camera would zoom-in to focus students' attention on a subtle but critical detail. Then, the instructor would perform a mock sample experiment to illustrate at least the initiation of an actual assay. The demonstration usually concluded with a demonstration of the "safe-keeping of the instrument" instructions so that the instruments would be readily available for the next student to use. Also these demonstrations were a good way to stress and reinforce points discussed in class or in the pre-laboratorial instruction.

1.6.4 Methods: Virtual Laboratories

The online virtual laboratories were first used as a compliment to the wet laboratory (Fall 2005). In Fall 2006, they were incorporated in a number of pre-laboratorial tutorials instead of the experimental manipulations' narrations *(see above for the theoretical part preceding the virtual lab in Fall 2006)*. They would start with a pictorial overview of key instrumentation that was used (*set-up tour*), in which overlaying the mouse over a feature would cause a highlighting color to change and to provide a short description of the targeted component. In other cases, the pictures were made clickable, thereby providing a short description for each clicked part of the instrument, which offered an interactive way for the students to explore. Then, a safety measures demonstration slide followed showing the precautions required for the experiment (gloves and goggles). Images or cartoons of the glassware/instrument used were shown moving accordingly in each manipulation. Verbal cues explained what was shown and each distinct part was colored differently to facilitate an easy viewing and understanding of what was being shown on the computer screen. A similarly colored index of key terms was also included in the slides. Diagram-based exhibits would demonstrate the overall accomplishment of an experimental manipulation, which included pictures or drawings of what the analyte and instrument setup should look like at a particular stage. Additionally, phenomena and changes observed during the experiment

were highlighted by color changes and other color-coded arrows, text, boxes etc. which provided visual effects that added clarity. A separate clip/slide was designed for each experimental step and the viewer could replay a slide by clicking on a "replay" button.

Also both parts of the lecture movie were intercepted by instant multiple-choice questions or simple calculations, which the student had to answer correctly in order to advance to the next section. Every answer—correct or incorrect— was provided with a full explanation. If a student answered incorrectly, s/he was given a clue and directed back to the question slide to try to answer again. In other cases, perspective questions where multiple or all statements were correct were used to provoke the students into thinking about what was coming up next and to induce the content of the next slide. So, instead of simply stating exactly what would happen next in the experiment, the student was asked to reflect and speculate about it. In addition, in order to provide a temporal component to the experiment, a virtual timer was displayed and students were asked to adjust it to the appropriate duration for the specific experimental step. These movies were fully navigable and the student could return to the previous clip/slide at will by clicking the "back" button. However, a student had to see a particular slide in its entirety before being able to repeat or access previous slides. An index with all clips along with their sequence was also included. The website could generate a report where the time that a student logged-in and out could be viewed.

1.7 Sample Laboratory Experiment Tutorial descriptions

A detailed description of Experiments 5, "Analysis of OTC Analgesics By High Performance Liquid Chromatography (HPLC), and 10 "Reverse Phase HPLC Determination of Quinidine by Internal Standards Method" follows:

Experiment #5 is the first experiment of the second cycle of experiments for the Pharmaceutical Analysis laboratory. The students are asked to analyze an unknown analgesic tablet, to identify and to quantify its contents. It is performed during weeks 6, 8 and 9 (there are no laboratory exercises on the 7^{th} week). So, the majority of students will not have heard anything about HPLC before the actual experiment and, therefore, the pre-laboratorial preparation is critical for their successful performance. For this reason, an 11-

slide presentation was designed to deliver adequate theoretical background and practical instructions about this experiment.

First, chromatography is introduced as a different analytical method than what students had been working with before because it entails separating a mixture into its components. The key mechanism of this separation is described introducing the two phases, stationary and mobile and the differential affinity for either is stated as the principle of separation. The spatial nature of the separation is then connected with the time each compound spends moving along the mobile or remaining in the stationary phase. These events are visualized in the next slide. At first, a diagram shows the concentration of components A+B of a mixture at various times during the chromatography. In the beginning, they are shown together. In the next time-point, the separation of the two peaks starts to be evident and, in the third picture, compound B has been eluted while A is still in the column which indicates the eventual separation. Each time point is disclosed successively to compare with the previous one. This abstract depiction is contextualized next by introducing the notion of a column filled with the stationary phase along which the mobile phase proceeds. An illustration of a column— with a mixture of colorful compounds being eluted at different time points— shows the events discussed in the previous diagram. The graphic introduction of a detecting device, which is found at the end of the column and which monitors the existence and the amount of analytes in the eluent in real-time, is what completes setting the stage for the chromatographical analyses of complex mixtures.

The third slide introduces the variety of methods of achieving the differential affinity for either phase by focusing on the partition phenomenon employed in the particular experiment. The characterizations of a chromatography as liquid, normal or reverse phase chromatography are then defined. Having addressed the liquid chromatography part of the HPLC acronym, the high performance part is elaborated in the next slide. The longer exposure to the stationary phase is correlated with an eventual better separation. In practical terms, this would mean a larger surface area of the stationary phase particles, which is only achieved by a smaller size. This way of achieving better separation, however, operates at the expense of the time it would take the mobile phase to go through a tightly packed column of the stationary phase. So, it

logically follows that the application of pressure to the mobile phase would force it through in a satisfactory amount of time. The whole high performance concept of HPLC is then summarized, thereby bringing all these elements together. The fifth slide provides a cartoon depiction of the basic components of an HPLC system. Each component's name and function is mentioned successively, starting from the solvent depot and finishing at the recorder of the chromatogram. The narrative follows the travel of the mobile phase along the HPLC machine.

The presentation next focuses on the generation of analytically useful information out of an HPLC experiment. Chromatograms are introduced as plots of the HPLC detector signal as a function of time, including a picture on which the retention time is defined. Below this, the second piece of information is provided and one can see a chromatogram, as well as peak integration. Subsequently applications of how each can be used for a qualitative and quantitative analysis are also discussed. More specifically, the retention time can be compared to that of a known standard substance in order to identify the mixture component while the peak integration areas of standard solutions can be used to construct a regression line from which the unknown sample's peak integration can be related to a concentration. The slide concludes with the introduction of external standard methods of pharmaceutical analysis in order to define such analyses, which will be juxtaposed later with Experiment #10 where an internal standards method is used.

The final four slides include practical instructions on how to conduct a particular HPLC experiment. First, each analgesic active ingredient is analyzed by the HPLC separately in order to record the respective retention time of each one. The UV/Vis detector setting at 280 nm is connected to what was discussed before in UV spectroscopy and is related to the structure of the common analgesics, which contains aromatic rings. This process is followed by directions on how to construct a standard curve for each drug, which involves using mixtures of standard solutions of each analgesic. The power of HPLC in determining which peak corresponds to which substance is also highlighted at this point. Next, the experimental manipulations of the unknown drug sample follow, as do the final instructions for the HPLC experiment and the requisite calculations.

For the online quiz, most of the questions are centered on understanding the principles of HPLC while others ask the students to make predictions. The first one is a

“fill in the blanks” one and asks directly: “The High Performance attribute of HPLC is due to: Using a(n) [____A____] solid support in column allows for extensive interaction of mixture components with stationary phase and [____B____] allows for expedient passage of mobile phase through column.” For blank A, the options include: “extra long and highly hydrophobic” or “finely powdered and tightly packed” and, for blank B: “mobile phase composition” or “application of high pressure.” A related question discusses the practical aspect of HPLC: “If we didn't apply high pressure on the mobile phase during our HPLC experiment: Our experiment would take too long time to complete” as opposed to the possibility of losing optimum separation capability or reproducibility. Another wrong option is “the stationary phase would not be tightly packed,” which is meant to help students understand that the column is already tightly packed and the pressure is applied to just accelerate the passage of mobile phase. Another term introduced was that of the “spatial resolution of a mixture to its components along the stationary phase during a chromatographic separation” and the next question asks what it refers to. Besides the correct “differential migration of the mixture components along the stationary phase when eluted with the mobile phase”, the “space of stationary phase the mixture occupies as it gets resolved moving along it with the mobile phase elution,” “the difference in resolution of a mixture depending on the space available in the chromatography system,” and “the differential migration of the mobile phase over the stationary phase during a chromatography experiment” are given as wrong alternative choices. The final question in this series concerns “the retention time of a peak.” The options include “cannot help us identify a mixture component,” “can give us information about the structure of a compound,” “can help us determine the amount of substance in the mixture,” “is independent of the mobile phase and stationary phase used,” and the correct choice “is influenced by the rate of the column elution,” which helps students to think about and/or clarify critical aspects of this notion.

The remaining two questions ask the students to interpret potential experimental findings. For example: “If a substance A has a retention time of $R_tA = 4.34$ min and a substance B $R_tB = 6.45$ min, which of the following statements is true?” It is stressed that for some answers there is no basis for judgment and the false answers show the range of misunderstandings that students might have had about out this notion. These include:

“substance B is more polar than substance A,” “substance A spends more time bound on the stationary phase,” “substance B has higher concentration than A in the particular sample,” and, finally, “substance A will be eluted out of the column approximately 2 minutes after substance B. The correct answer is: “substance A spends more time migrating along with the mobile phase.” A similar question follows regarding the other piece of information that was collected during an HPLC experiment—the peak integration area. In keeping with this information, a “fill in the blank” asks: “If the peak of substance A has larger integration area (area under the curve) than substance B then it is true that we cannot tell which of the two substances has larger [____A____]. The integration area has to be correlated with the peak integration areas of standards of known [____B____] first.” The options are: “concentration”, “absorbance,” or “polarity” for blank A, while the choices include “retention time”, “concentration” or “absorbance” for blank B.

Finally, there is also a laboratory tutorial video for this experiment which starts with a reiteration of what the HPLC can do and what this acronym means. Then a general overview of the HPLC components is given; it starts by showing the basic units, injection site, pump, column, detector and recorder. A more detailed description is next, which follows the sequence of parts that the mobile phase will encounter during an experiment. First, a beaker which serves as the solvent reservoir is shown. This is where the mobile phase is stored and from there a plastic tube withdraws the solvent system feeding it directly on the HPLC pump. It is pointed out that no pressure has been applied yet, so no specially reinforced tubing is required. Once in the pump, the mobile phase is pressurized, which is highlighted by the fact that the tubing after the pump is made of steel to withstand the pressure applied. The key settings on the pump are also shown, including the pressure and flow-rate as well as how to adjust it. Then the injection site is discussed at the top of the pump compartment. The two settings of the lever (“load” and “inject”) are switched without loading anything and their difference and importance are also discussed. At that point, the camera zooms-in on the injection loop to show where the sample is stored temporarily after it’s loaded and before the experiment starts.

Following this step, the steel tube from the pump that the student is using is directed to the very column. As expected, the column has walls made of steel and its

composition is written on its label, which is shown in another close-up shot of the video. After the column, the UV/Vis detector follows. It is emphasized that UV/Vis is a quick method for detection, as seen in Experiments #3 and #4. Therefore, it can be used to detect and measure the presence of a substance in the column elutes in real-time. After the detector, another tube carries the mobile phase to a discharge container to be discarded. At the same time, the analytical signal generated at the detector is communicated to the recorder/integrator; this also occurs in real-time. So, as a result, the mixture of substances is introduced on the column along the mobile phase, it is then separated to its components and as each component is eluted out of the column, it (what does the "it" refer to? Say that word instead of "it) will be detected and recorded at the very moment of its exit. In this way, the two key experimental parameters—retention time and peak integration—can be monitored, recorded, and presented in a chromatogram output format.

At this point, a sample experiment was run. First, it was stressed that since the system is under pressure, the presence of air-bubbles in the sample or the mobile phase can perturb the assay. For this reason, the solvent system and the sample are degassed before the experiment. Also, the column needs to be soaked and saturated with the mobile phase so the HPLC is turned on and so that the flow rate of the mobile phase is adjusted. After inspecting the HPLC waste outlet, it must be confirmed that the column is not blocked. The instructor shows how to manipulate the sample before loading in order to remove all air-bubbles by attaching a filter in the tip to protect the column for solid impurities. Then, with the injection lever at "load," the injection loop is flushed with the sample. It must be noted that the capacity of the loop is only 20 μL so if they inject more the excess will be discarded, which is shown in a close-up of the injection loop where the excess sample volume flows away into a waste beaker. Finally, the monochromatic of the UV/Vis detector is set at an appropriate value and the experiment is ready to start. This, once again, relates to what the students have done in the other spectrophotometric experiments.

The experiment starts by switching the injection site lever from "load" to "inject, which introduces the analyte to the stream of the mobile phase. The recording should start at the same time: a point that is stressed before and during the initiation of the HPLC

run. During the experiment, the instructor points to several things that the students should look for and be careful of. These things included: First, the flow of the mobile phase out of the end of the tube after the detector and into the waste beaker. Second, the changes in the absorbance in the display of the detector as various substances pass the column. Finally, the real-time recording of the analytical signal in the integrator/recorder. A straight line means that nothing comes out, whereas a peak indicates the presence and amount of a substance in the eluent. When a substance comes out, it is indicated at the detector's display and by the movement of the recorder's ink-head. After some time, the assay is stopped and the presentation focuses on the interpretation of the chromatogram generated. The various parameter records are pointed out and the use of retention times for the qualitative and the peak integral values for quantitative analysis are also repeated.

Experiment #10 is the third experiment of the third cycle of experiments. It refers to the development of a suitable elution method to use HPLC for the analysis of Quinidine in biological samples using an internal standards method. It is conducted during weeks 10, 11, and 12 and all students have heard about HPLC in the lectures and also have already done the related experiment #5 using an external standards method. Therefore, the lecture does not need to repeat the chromatographic theory in depth but, instead, needs to focus more on why a different method is used along with biological sample manipulations. A13-slide presentation was videotaped to provide the necessary theoretical background and technical aspects of this assay.

The first two slides briefly reiterate the basic concepts of chromatography from experiment#5 such as: what is HPLC, how is the separation achieved, the definition of reverse phase, and the two types of information collected as well as their applications (retention time for qualitative and peak integration for quantitative analyses). In the third slide, the actual use of retention time and peak integration in experiment 5 is further analyzed. It is reiterated that in order to do a qualitative analysis, the students compared the retention times of the peaks of their unknown samples with the retention time of a sample of a pure standard of each drug. Also for the quantitation, they ran assays of standard solutions of known concentrations from which they derived a standard curve. Students were then able to calculate the concentration of the unknown sample correlating the peak integrals of their unknown samples with those of the standard curve. At the

conclusion of this slide, it is stressed that the basic assumption behind the external standards method is that the analyte will behave the same in both the standard solutions as well as in the unknown sample.

The next slide introduces the notion that this assumption might not always be the case and that there can be a variety of non-reproducible sample manipulation variations. Compound decomposition, complexation with proteins, and variable yields of derivatization reactions are mentioned as examples of instances in which this basic assumption is not true. The idea of adding to samples a known amount of a substance of similar structure with our analyte is explained next. If this similar substance can suffer to a proportional extent the errors suffered by the analyte, the ratio of their analytical signals (peak integration areas) should be solely related to the concentration of the analyte. The term "internal standards" is added and the whole concept is reiterated once more.

The presentation focuses next on the quinidine analyte, which the students have to assay in a simulated biological sample. Its uses, narrow therapeutic index to justify the assay and its structure are pointed out. It is highlighted that the presence of various proteins in blood serum could potentially lead to complexation problems because of the presence of groups in quinidine that allow such interactions. In a search for a suitable internal standard, the structure of quinine is shown. The similarities are obvious since they only differ by one asymmetric carbon center's absolute configuration and are in fact epimers. So it is concluded that quinine might serve as a valid internal standard in a quinidine assay.

In addition, in this experiment the students are asked to develop their own method of reverse phase HPLC elution to separate and assay quinidine. They are given a starting clue and are asked to consider the capacity factor *k'* in order to adjust the mobile phase content so as to achieve an optimum separation of a 1:1 mixture of quinidine/quinine. Once they identify the optimum mobile phase, they use this solvent system to equilibrate the column. While equilibrating the column, they are asked to prepare standard samples of quinidine of various concentrations, adding bovine serum albumine and an amount of quinidine to them. The substances are shown being added successively to the test-tube cartoons while highlighting the different suffixes (-ine and –idine) for quinidine and quinine as well as the difference in their amounts (concentration gradient for the former

and equal amounts for the latter). The back extraction sample purification is examined next. It is first underscored that both quinidine and quinine contain nitrogens that can function as weak bases (their pK_as are also provided). Then the pH of each tube is adjusted to 8.9, which keeps the molecules uncharged, water-insoluble but organic solvent-soluble. A sample test tube with the aqueous solution in blue is shown. Then an immiscible organic solvent is added, shown in a different color, which forms a top layer because it is lighter than water. A colored arrow points to the organic layer where the quinine/quinidine free bases should reside. The aqueous layer and the water soluble impurities are then discarded and an HCl solution is added to the organic layer which again forms the top layer. Quinidine/quinine are shown protonated in the side and are, at this point, water-soluble and organic solvent-insoluble. So after the back extraction, the charged species will migrate to the aqueous layer; for this reason, if they discard the organic layer, the organic impurities will be eliminated. Successive animation schemes show the back extraction and arrows point to where the analytes exist at all time to help students follow the changes and migrations

The final two slides describe the HPLC assay. The students are advised to calculate both ratios (peak 1 over peak 2 and vice versa) since they don't know which peak corresponds to quinidine or quinine. The one that produces a gradient following the concentration gradient will tell which is which. Then a regression line of ratios of peak integrations vs. concentrations of quinidine can be calculated. In the end, the ratio of the unknown sample is used to calculate the amount of quinidine in this sample.

In Fall 2006, however, the format changed and the pre-laboratorial lecture was limited to discussing the theory behind the assay. The experimental component was presented in the related virtual laboratory (*vide infra*). The first clip includes the general discussion of HPLC and includes a question about the relation of polarity and order of elution on a reverse phase HPLC experiment. The next slide focuses on the type and use of information gained from a chromatogram. Another question asks student to pick the correct statement about retention time among many possible experimental options. Through this question, it is stressed that the retention time is not related to the peak integration area nor does it provide information about the nature or concentration of a compound. The third slide reiterates the use of external standards as stated previously in

experiment #5. Instead of providing reasons why this might go wrong, the students are asked to think about the correct answer. Some reasons are mentioned and the correct answer is “all of the above.” Once the potential deficiencies of the external standards method have been indentified, the lecture focuses on the internal standards method, its applicability, and its advantages. As before, the use of quinine as an internal standard for the assay of quinidine is justified, showing their structural similarity. In addition to highlighting the one stereocenter difference, the two molecular structures are overlaid for better visualization of their similarity.

Having already done an HPLC experiment using an external standards method and GC/MS using internal standards, the students are asked to combine their previous knowledge with a back extraction to develop a method for experiment #10. So the questions of the online quiz ask about this new method of purification and the internal standards used. The first question is a practical exploratory one, inquiring about which of the following cannot be removed by the specific back extraction, which encourages students to think about the various steps where each could be removed: “inorganic salt impurities,” “hydrogen carbon impurities,” “weakly acidic organic impurities,” or “weakly basic organic impurities.” The feedback explains how inorganic salts will not be dissolved in MTBE and the hydrogen carbons cannot return to the aqueous phase when HCl is added to the organic methyl *t*-butyl ether (MTBE) phase. Also, weakly acidic impurities will be deprotonated and, thus, water soluble/organic solvent insoluble in the first step of the back extraction and, finally, weakly basic impurities have similar behavior as quinidine/quinine and will follow it during the back extractions.

Exploring the internal standard method and its capabilities, the next question asks “which of the following potential sources of error cannot be solved using the internal standards method.” The wrong options include statements like: “an error when calculating and mixing the components of mobile phase,” “a random amount of protein complexing various amounts of the analytes across different samples,” and “a failure to extract all amount of the analytes during the back extraction,” which are situations which this method can indeed resolve. “An instability error in the HPLC UV/Vis detector” though will respond differently to the quinidine and quinine and this error cannot be rectified. Continuing on the same pattern, the next question involves s a yes/no response

that asks the student: “By accident a student adds double amount of quinine internal standard in one of the tubes. Can he/she divide the peak integration area of the quinine peak by half and claim that he/she corrected the error?” The answer is “no” and feedback is provided explaining that this mathematical operation assumes that there is a linear relationship between the peak integration area of quinine and its sample concentration, which is not the case on this experiment.

Three other questions ask from students to predict what will happen in various situations. The first question, for example, asks: “if the concentration of quinidine in the standard samples used increases, which of the following should be decreasing? Possible answers include: “retention time,” “ratio of quinine over quinidine peak integration areas,” “ratio of quinidine over quinine peak integration areas,” and “peak integration area.” A general feedback remark explains the correct answer: Absolute values of peak integration areas will vary from sample to sample inconsistently to the concentration gradient. The ratio of quinidine over quinine should be increasing so its reverse, the ratio of quinine over quinidine should be decreasing. Retention times will vary irrespectively of the concentration gradient. Another question describes “a careless student who doesn't transfer the entire amount of MTBE layer into a clean test tube to work on the second phase of the back extraction” and asks about “what type of error will he/she get”? All 4 options are given with “no error at all” as the correct answer since “by neglecting to transfer all of this extract we will be decreasing the concentration of quinidine and quinine proportionally so the ratio of their analytical signals should not be affected.” The final question of the series is another predictive one: “When asked to prepare a suitable mobile phase for this assay a student thought he would be done earlier if he increased the acetonitrile component of the mobile phase to decrease its polarity and accelerate the quinidine/quinine elution. What problem might he/she have when he runs his/her assay?” Among various answers about an increase or a decrease of peak resolution or integration, “the peak resolution would decrease” is the correct choice since “as the retention times decrease there will be considerable overlap between the two peaks resulting in poor peak resolution”.

In addition to the pre-laboratorial lecture and quiz, the students were provided with a virtual laboratory tutorial. The opening slide shows a set with HPLC

instrumentation. An index is shown and the whole virtual laboratory is broken into 3 sections. The general safety slide with the multiple choice question about protective measures taken for this lab follows. An animation shows the preparation of the mobile phase, mixing aqueous buffer with acetonitrile followed by a multiple choice question which asks the student to calculate how much of each solvent would be needed to make a certain amount of mobile phase of the desired percentile content. An incorrect answer returns the screen, where space to work on the problem is provided for students to figure out the response. When the correct answer is clicked, a screen showing the correct way of working on the problem is illustrated. Important factors for the solution are highlighted by using different colors. The active learning technique of asking a question regarding these factors is employed here because all of the answers are correct; this way, students must consider the entire function of the mobile phase in the HPLC; carrying the sample through the column, chemically interacting with sample and cleansing the column, are given as correct answers instead of just mentioning them. Following a feedback slide, the students are asked to turn on the HPLC at a certain flow rate and to set the detector at a specific wavelength; a photograph of the HPLC is shown indicating the critical components. Successive close-up photographs show where to read the actual flow rate and detector wavelength. The next screen shows the loading site prompting students to inject their 1:1 quinine/quinidine sample. A multiple choice question about what could be an optimum capacity factor *k'* for the particular experiments concludes the HPLC equilibration step.

The second step (creating a standard curve) begins by showing how to mix the necessary variable amounts of quinidine and water followed by adding bovine serum albumin (BSA) and internal standard quinine to create standard samples of increasing quinidine concentration. Each component is added separately and indicated by a different color. The next slide summarizes what each test tube should contain. The question next asks why we add a constant amount of quinine in each test tube. Among other options, the correct is "to compare with quinidine." Following these steps, the organic solvent is shown being added along with an aqueous buffer and briefly vortexed. The students are then asked where the organic phase lies, following the mixing of the two phases. In addition, students are also asked what the function is of this solvent in the experiment.

The top layers (organic) are recovered and transferred in new tubes as the next screen shows. To these tubes, a certain amount of HCl solution is shown being added and the whole mixture is again vortexed. Then the top layer is discarded and a question asks about the function of HCl in this back extraction procedure. Finally, the students are asked to flush the injection loop multiple times and run the HPLC experiment for each standard sample. A sample chromatogram is displayed highlighting all the key information (retention time, peak integration area) which can be found there. Also the order of elution of quinidine and quinine is shown.

In the third part, the determination of the unknown sample is described. In the first slide, the students are asked to work with their unknown sample as they did with the standard solutions. Then a plot of the standard curve is shown along with an experimental point lying outside the standard sample bracketed range of concentrations. A question asks if this is an appropriate situation to accurately calculate the unknown concentration. After explaining why this is not possible, the students are given an unknown chromatogram and are asked to calculate the ratio of quinidine over quinine. In case of a wrong number, they are given instructions of reaching the correct answer and are asked to repeat the calculation. Then they are asked to go to the standard curve and find the unknown amount of quinidine in the sample. Again, detailed instructions are given to those who failed to calculate the correct amount. There were a number of different unknowns used. The virtual laboratory concludes with this final calculation.

In Fall 2006, the virtual lab was slightly modified to include instructor narration for most parts. Also, it started with a virtual tour of HPLC where a list of key features of the equipment was listed at the side of an HPLC picture. The student could highlight each component by rolling the mouse over each description. For some hidden parts (such as the column or the injection loop), a new photo and highlight could be displayed on a mouse roll over, and then revert back to the original once rolling the mouse away. During the mobile phase preparation, the necessity of degassing is mentioned. In an attempt to connect this experiment to what the students had learned before, they are asked to provide an experiment in which they could identify a suitable wavelength to set the detector at and monitor the HPLC eluents. A close-up shows how to load the sample and start the experiment. The capacity and resolution factors are discussed, showing a

chromatogram which provides an acceptable separation of the two epimers, whose peaks are highlighted, at a reasonable time. The description of the manipulations necessary to assemble and back-extract the standard quinidine samples follows. The successive changes in the layers are also highlighted by the use of different colors. When vortexing, the two colors of the phases merge to produce a uniform color which gets resolved to the original ones as the two layers reseparate upon standing. The experiments are run and the resultant chromatograms are shown in color coded areas for the quinine, quinidine, solvent peaks and the peak integration information. The virtual laboratory concludes with an animation of a mixture of 4 substances being loaded on an HPLC machine. Their elution is followed as if the students could see how they are separated on the column. The mobile phase is shown continuously eluding the column and collected in a waste jar. Even the substances' analytical signal is recorded in a mock detector as they exit from the column. The virtual lab concludes as before with an unknown sample.

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CHAPTER II

EVALUATION OF NOVEL INSTRUCTIONAL METHODS

2.1 Experimental Design

The novel instructional methodology was first introduced in Fall 2005 and continued to the Fall 2006 term. At the same time, a summative evaluation plan was undertaken before, during and after the actual classes. It was not possible to randomly assign and deliver either the old or the new ways of teaching to students of the same term because it would create a sense of discomfort, disparity, and inequality among them. Lacking the possibility of having a control group throughout the semester-long instruction, the overall experimental design for the summative evaluation of the novel instructional techniques was limited to a simple one-group pre-test/post-test one[1]:

O1 X O2

For pretest data, the students of Fall 2005 and Fall 2006 classes were surveyed in the beginning of the semester about various aspects of the perceptions and expectations that they had for their upcoming class. In the end of the respective semesters, the students were surveyed again about the same questions as in the beginning of the term survey and additionally on other questions regarding their impressions of their educational experience. A similar survey to that posed to the Fall 2004 students was conducted in students in Winter 2006—3 semesters after they had received their Pharmaceutical Analysis training. To adjust for the effects of the time separation of the surveys, the Fall 2005 and Fall 2006 students were surveyed once again 3 semesters later in their studies, that is in Winter 2007 and Winter 2008 respectively. The only opportunity to include a

control group in the evaluation was in the in the first year of application (Fall 2005) where it was possible to have one section of the students perform the HPLC virtual laboratory only (and in addition to their regular preparation) while the other half did the same for the gel electrophoresis experiment. This way, each section of the class that didn't follow the one virtual laboratory would serve as a control group for the other section of the class that did. In the end of the semester, all of the students were given an online quiz on HPLC and Gel Electrophoresis. Thus, the overall experimental design for the effectiveness of the virtual laboratories was an adaptation of the quasi-experimental non-equivalent control group[1] that fits the realities of this laboratory:

X1 O1
- O2

- O1'
X2 O2'

An additional source of data was the formal instructor evaluations, which were performed in-class in the end of their respective sections (end of 5th and 13th weeks). The questionnaires were the same for every year and data were available as far back as 2001. It was assumed that each year's students were otherwise equivalent to each other, which enabled the use of a quasi-experimental time-series setting[1] recording and comparing students' responses to particular questions before and after the introduction of the instructional technology for the Fall 2001 – 2006 terms:

O1 O2 O3 O4 X O5 O6

2.2 Evaluation Methods

The beginning of the semester surveys included the following questions, aiming at capturing the students' expectations about (as well as the relevance/importance of what they will learn) and their familiarity with the topics discussed in this class. For the 1-5 scales, it was assumed that they are equidistant in the students' minds. These were given in the students' first laboratory exercise day (Table 2.1):

Survey Question:	Type of Answer:
"What do you expect to learn in this class?"	Open-ended short answer
"How much familiarity do you feel you have with the concepts and techniques that will be used in this class/laboratory?"	1-5 Scale: Not at all… A lot
"How relevant do you think this class is to your practice as a pharmacist professional? How much do you think you will apply and use the knowledge and skills you will gain in this class?"	1-5 Scale: Not at all relevant… Very relevant

Table 2.1: Beginning-of-term survey questionnaire and type of answer options.

The end of the term surveys as well as the 3-semester follow-up, surveys contained similar questions regarding familiarity and relevance as well as additional questions that reflected the laboratory educational experience itself: (Table 2.2)

Survey Question:	Type of Answer:
"Were your expectations for this laboratory met? Why and why not?"	Open-ended short answer
"How much do you think the laboratory followed the progress of the lecture portion of the class?"	1-5 Scale: Not at all… All of the time.
"How much variety of laboratory techniques do you think you got exposure to?"	1-5 Scale: No variety at all… A lot of variety.
"How was the pace of laboratories concerning the timeline of experiments, deadlines and preparation?"	1-5 Scale: Very hectic and demanding… No hard time at all.

Table 2.2: Additional end-of-term survey and 3-semester follow-up questions and type of answer options.

Only the end of term surveys contained two additional questions directed at each of the new instructional tools used, namely "instructional videos on experiment theoretical background and experimental protocols," "instructional videos on laboratory techniques," "follow-up quizzes on each experiment's video," and "virtual laboratories enhancing the learning of various techniques." The students were asked to judge the merits of each technique and rank them according to their perceived educational value ("Waste of Time," "Little Value Added," "Not Bad," "Somewhat Valuable," "Great Educational Value") as well as to recommend whether they should be "Kept As Is," "Revised," or "Eliminated" in a separate column.

Four particular questions from the formal University of Michigan instructor evaluations were relevant to this evaluation. Students were asked to mark whether they "Strongly Agree," "Agree," are "Neutral," "Disagree," or "Strongly Disagree," with each of the following statements: "overall this was an excellent course,""I learned a great deal from this course," "the laboratory was a valuable part of this course," and "laboratory assignments were relevant to what was presented in class." Student responses were converted to numerical data (1-5 Likert scale "Strongly Disagree"…. "Strongly Agree").

Statistical analyses and graphs were performed using SPSS© 16.0 and/or Excel 2007© statistical programs. Numerical survey data means for two different student groups (years) were compared using two-tailed independent sample *t*-tests, whereas matched-pair *t*-tests were used for comparing the results of two surveys of the same group of students at two different time-points. For the final quiz, mean score comparisons of one-tailed *t*-tests were used to test for improvement among the scores in the intervention group since the two groups underwent the same training except for the addition of the virtual laboratories. Multiple mean comparisons were performed using ANOVA for independent samples and ANOVA with pair-wise comparisons for three or more surveys of the same sample using the Bonferroni adjustment to prevent false rejections of the null hypotheses of equality of means. X^2 tests were performed to inspect whether the year of the surveys for the assessment/ recommendations of the novel educational tools (2005 or 2006) is associated with the results or not. Unless otherwise stated, probabilities of rejecting the null hypotheses p of less than 0.05 were deemed statistically significant.[2]

2.3 Results

2.3.1 Student Surveys' Educational Outcomes

The following tables summarize the mean values for each of the numerical survey answers and the statistical significance of their difference (*p* values):

Survey Question: Familiarity	***Mean/SD***	**Statistical significance of difference of means (*p*)**					
Before* Class '05 *(N=62)	**3.42(1.05)**	***Before* Class '05**	***After* Class '05**	***Before* Class '06**	***After* Class '06**	**One year On '04**	**One year On '05**
After* Class '05 *(N=74)	**4.11(0.59)**	0.023*					
Before* Class '06 *(N=63)	**3.41(0.83)**	0.969	0.000*				
After* Class '06 *(N=73)	**3.97(0.74)**	0.000*	0.222	0.001*			
One year On '04 *(N=35)*	**3.48(0.85)**	0.750	0.000*	0.682	0.032*		
One year On '05 *(N=36)*	**3.39(0.84)**	1	0.001*	0.892	0.000*	1	
One year On '06 *(N=47)*	**3.64(0.76)**	0.230	0.000*	0.163	0.023*	1	0.507

Table 2.3: Means/SD of responses on "Familiarity" per survey and cross-tabulated *p* values of the difference of means.

The students felt equally (and quite) familiar before class in both Fall 2005 and Fall 2006 terms (means of 3.42 and 3.41 respectively, p=0.969) Table 2.3). Their sense of familiarity increased significantly in both end of the term surveys in comparison with their baseline familiarity (3.42 to 4.11 for 2005 (p=0.023) and 3.41 to 3.97 for 2006 (p=0.001)), which reached similar levels for both years (p=0.222). Nevertheless, familiarity levels decreased to similar levels (p=0.507) to the before-class-instruction values in the 3-semester-after-class surveys (3.39 and 3.64, p=1 and p=0.163

respectively), not significantly different than the Fall 2004 3-semester-after-class familiarity survey levels (3.48, p=1 for both years).

Survey Question: Relevance	***Mean/SD***	**Statistical significance of difference of means (*p*)**					
Before* Class '05 *(N=60)	**3.82(0.77)**	***Before* Class '05**	***After* Class '05**	***Before* Class '06**	***After* Class '06**	**One year On '04**	**One year On '05**
After* Class '05 *(N=71)	**3.24(1.02)**	0.033*					
Before* Class '06 *(N=63)	**3.81(0.89)**	0.962	0.001*				
After* Class '06 *(N=71)	**3.10(0.96)**	0.000*	0.398	0.004*			
One year On '04 *(N=36)*	**2.17(0.91)**	0.000*	0.000*	0.000*	0.000*		
One year On '05 *(N=36)*	**2.75(1.02)**	0.000*	0.220	0.000*	0.085	0.031*	
One year On '06	**3.11(0.92)**	0.000*	0.484	0.004*	1	0.000*	0.277

Table 2.4: Means/SD of responses on "Relevance" per survey and cross-tabulated p values of the difference of means.

The students attributed sizable and equal relevance to what they were going to learn before class in both Fall 2005 and Fall 2006 terms (means of 3.81 and 3.82 respectively, p=0.962) (Table 2.4). Their sense of the subject's relevance, however, decreased significantly in both end of the term surveys compared to their before class values (3.81 to 3.24 for 2005 (p=0.033) and 3.82 to 3.10 for 2006 (p=0.004)) to reach similar levels for both years (p=0.398). A decrease in mean values was also observed in the 3-semester-after-class surveys for Fall 2005 and Fall 2006 classes, which were significantly different than the before-class values but not the after-class ones (2.75, p=0.000 and p=0.220 for 2005 and 3.11, p=0.004 and p=1 for 2006). Both 3-semester-

after-class survey were significantly higher than the Fall 2004 3-semester-after-class levels (2.17, p=0.031 and p=0.000 for year 2005 & 2006 after-class-surveys respectively) but not significantly different amongst themselves (p=0.277).

Survey Question: Connection	***Mean/SD***	**Statistical significance of difference of means (*p*)**			
After* Class '05 *(N=74)	**3.86(0.89)**	***After* Class '05**	***After* Class '06**	**One year On '04**	**One year On '05**
After* Class '06 *(N=73)	**3.83(0.94)**	0.847			
One year On '04 *(N=34)*	**2.94(1.04)**	0.000*	0.000*		
One year On '05 *(N=36)*	**3.03(0.88)**	0.000*	0.000*	1	
One year On '06 *(N=46)*	**3.63(0.85)**	0.159	0.119	0.004*	0.012*

Table 2.5: Means/SD of responses on "Connection" per survey and cross-tabulated p values of the difference of means.

The students felt that there was a strong connection between the class lectures and laboratory exercises for both 2005 and 2006 after-class surveys (means of 3.86 and 3.83, p=0.847) (Table 2.5). In the 3-semester-after-class surveys, the picture differs for the 2005 and 2006 surveys. Whereas the sense of connection falls to 2004 levels (2.94) for the 2005 class (3.03, p=1 and p=0.000 for comparison to the mean of after-class 2005 survey), the 2006 class reported a statistically equal sense of connection to that of right after-class (3.63, p=0.119) and much improved over both 2004 and 2005 3-semester-after-class surveys (p=0.004 and p=0.012).

Survey Question: Variety	***Mean/SD***	**Statistical significance of difference of means (*p*)**			
After* Class '05 *(N=74)	**4.01(0.78)**	***After* Class '05**	***After* Class '06**	**One year On '04**	**One year On '05**
After* Class '06 *(N=72)	**4.04(0.78)**	0.828			
One year On '04 *(N=35)*	**3.54(0.91)**	0.008*	0.005*		
One year On '05 *(N=36)*	**3.75(0.77)**	0.059	0.068	0.858	
One year On '06 *(N=46)*	**4.33(0.76)**	0.321	0.323	0.000*	0.006*

Table 2.6: Means/SD of responses on "Variety" per survey and cross-tabulated *p* values of the difference of means.

The students reported that there was a significant amount of variety of laboratory technique exposure in both 2005 and 2006 after-class surveys (means of 4.01 and 4.04, p=0.828). In the 3-semester-after-class surveys the picture differs for the 2005 and 2006 surveys (Table 2.6). Whereas the sense of variety falls to 2004 levels (3.54) for the 2005 class (3.75, p=0.858 and p=0.059 for the mean of after-class 2005 survey), the 2006 class maintained a statistically equal sense of variety to that right after-class (4.33, p=0.323) and much larger than both 2004 and 2005 3-semester-after-class surveys (p=0.000 and p=0.006).

Survey Question: Pace	***Mean/SD***	**Statistical significance of difference of means (*p*)**			
After* Class '05 *(N=74)	**2.90(0.86)**	***After* Class '05**	***After* Class '06**	**One year On '04**	**One year On '05**
After* Class '06 *(N=76)	**3.15(1.01)**	0.115			
One year On '04 *(N=36)*	**2.97(0.94)**	0.712	0.377		
One year On '05 *(N=36)*	**3.00(1.01)**	0.525	0.466	1	
One year On '06 *(N=46)*	**2.93(0.74)**	0.849	0.117	1	1

Table 2.7: Means/SD of responses on "Pace" per survey and cross-tabulated *p* values of the difference of means.

The students reported a statistically equal sense of pace and work-load for all surveys, end-of-term and 3-semester-after class surveys. *P* values vary between 0.115 and 1 (Table 2.7).

For the expectations measurements, the textual data in response to the survey question: "were your expectations met" were codified and discretized into 4 categories, namely: "Yes," "Partially," "No," and "Unclear" (Figure 2.1)

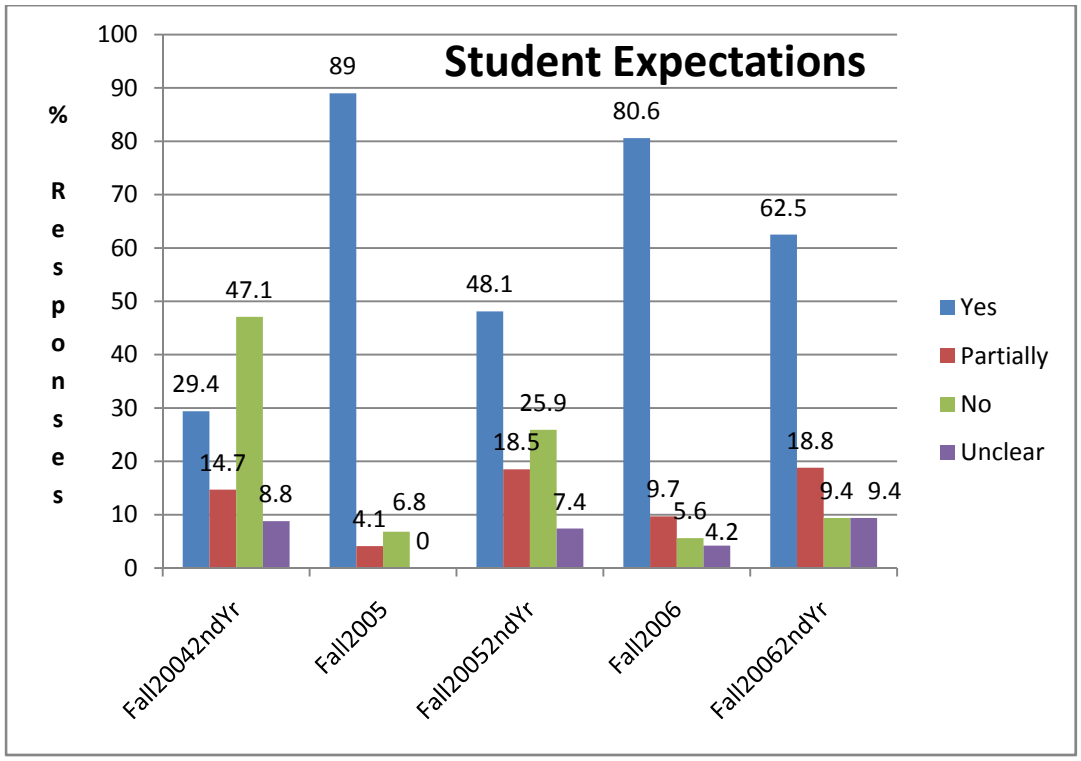

Figure 2.1: Bar-graph of the % frequencies of "Expectation" categories of responses per survey

Survey Question: Expectations	**Statistical significance of difference (*p*)**			
	One year On '04	***After* Class '05**	**One year On '05**	***After* Class '06**
***After* Class '05**	0.000*			
One year On '05	0.403	0.000*		
***After* Class '06**	0.000*	0.164	0.008*	
One year On '06	0.010*	0.002*	0.396	0.269

Table 2.8: *p* values of associations of "Expectations" with the different times of each survey (X^2 tests).

The students reported that their expectations were met to a high degree in surveys right after the end of their 2005 and 2006 classes (89% and 80.6% agreed with the statement, p=0.164) (Table 2.8). The positive responses dropped significantly in the 3-semester-after class surveys for the Year 2005 class but insignificantly for the 2006 one (89% to 48.1%, p=0.000 for 2005 and 80.6% to 62.5%, p=0.269 for 2006 respectively). Compared to the results of the 2004 class 3-semester-after class expectations survey, the 2005 one had higher positive responses (29.4+14.7 = 44.1% for 2004 versus 48.1+18.5= 66.6% for 2005) although not statistically significant ones (p=0.403). In their 3-semester-after-class survey the Fall 2006, students reported that their expectations were met to a significantly higher degree than the Fall 2004 one (44.1% vs. 62.5+18.8 = 81.3% for 2006, p=0.010).

Finally, the results of the final quiz of the Fall 2005 class are presented per section (Monday or Wednesday) below as total scores (%) and furthermore partial scores (%) for either electrophoresis or HPLC related questions (Table 2.9). Finally the p values for the difference of the means per section are displayed:

Final Quiz 2005	**Monday Section (Mean/SD)**	**Wednesday Section (Mean/SD)**	***P* of mean difference**
Total Score	82.63% (15.54)	86.05% (8.86)	0.242
Electrophoresis Questions	85.79% (16.05)	85.26% (16.56)	0.888
HPLC Questions	79.47% (22.05)	86.84% (14.91)	0.092*

Table 2.9: Total and partial scores for electrophoresis and HPLC questions per section and p values of the difference of their means. One-sided t-test p-values are reported for the latter mean score comparisons.

The Monday section performed the electrophoresis virtual lab in addition to the wet lab whereas the Wednesday section had done the HPLC virtual lab. Although the Wednesday section scored overall better than the Monday one (86.05% of Wednesday compared to 82.63% of Monday), this difference was not statistically significant

(p=0.242). The partial scores on the electrophoresis questions were almost identical (85.79% vs. 85.26%, p=0.444). However, the Wednesday section scored significantly better than the Monday section on the HPLC questions (86.84 vs. 79.27%, p=0.046).

2.3.2 Students' Appreciation and Recommendations Regarding the Novel Instructional Methods:

Regarding the new instructional tools, student appreciation and recommendations, all 4 (online lectures, experimental demonstration videos, online quizzes, virtual laboratories) were well received right after the first year of introduction (2005) or later in the 3-semester-after class surveys. In particular, the online lectures were highly appreciated in both 2005 and 2006 by a majority of students; 38.2+40.8 = 79% for 2005 and 47.2+36.1=83.3% for 2006, somewhat but not significantly higher than 2005 (p=0.742) (Figure 2.2).

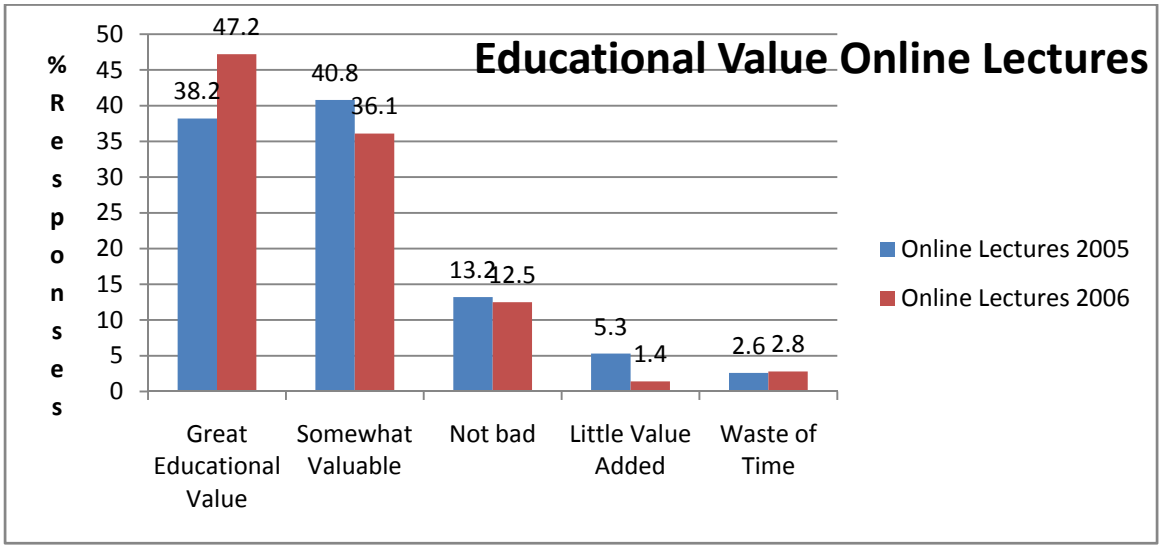

Figure 2.2: Bar-graph of the % frequencies of categories of educational value the students attribute to "Online Lectures" per year of survey.

In addition, a majority of students advised to keep the new tools as they were (55.4% and 61.6% for 2005 and 2006 respectively), a significant minority asked that they be revised (37.8% vs. 32.8%) and only 6.8% and 5.5% chose their elimination (Figure 2.3). There was no significant difference in the recommendations of the two years (p=0.630):

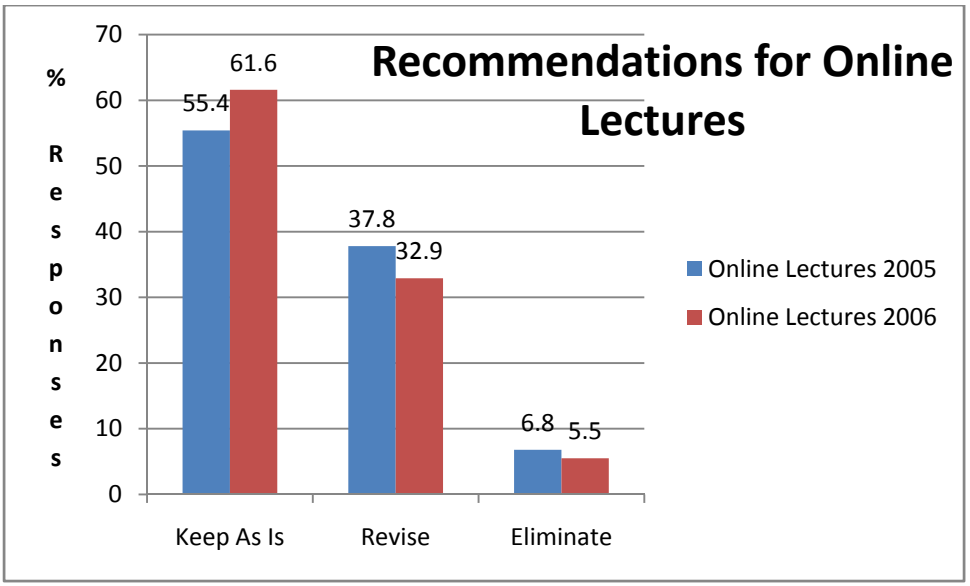

Figure 2.3: Bar-graph of the % frequencies of categories of recommendations the students suggested for "Online Lectures" per year of survey.

The experimental demonstration videos were also highly appreciated in both 2005 and 2006 by a majority of students, 52.7+29.7 = 82.4% for 2005 and 32.4+42.3=74.7% for 2006. A significant decrease in positive opinions was noted for 2006 than 2005 (p=0.023) (Figure 2.4):

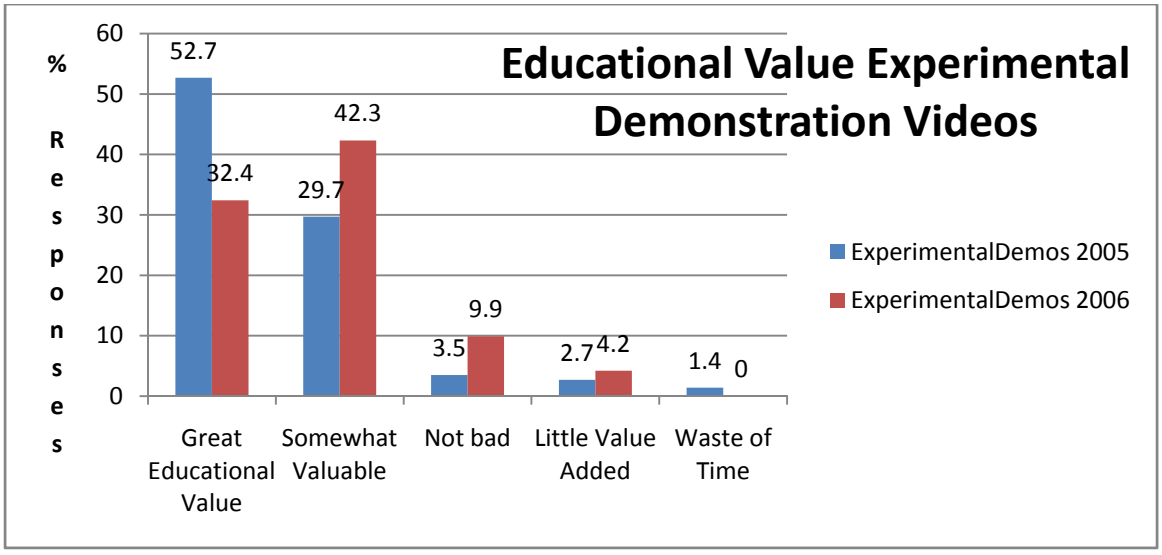

Figure 2.4: Bar-graph of the % frequencies of categories of educational value the students attribute to "Experimental Demonstration Videos" per year of survey.

Also, a vast majority of students advised to keep the experiment demonstration videos as they were (78.1% and 92.3% for 2005 and 2006 respectively), a small minority

recommended that they be revised (16.4% further dropping to 4.2% in 2006) and only 5.5% and 5.6% asked for their complete elimination (Figure 2.5). There was an almost significant increase in the positive opinions in 2006 (p=0.052):

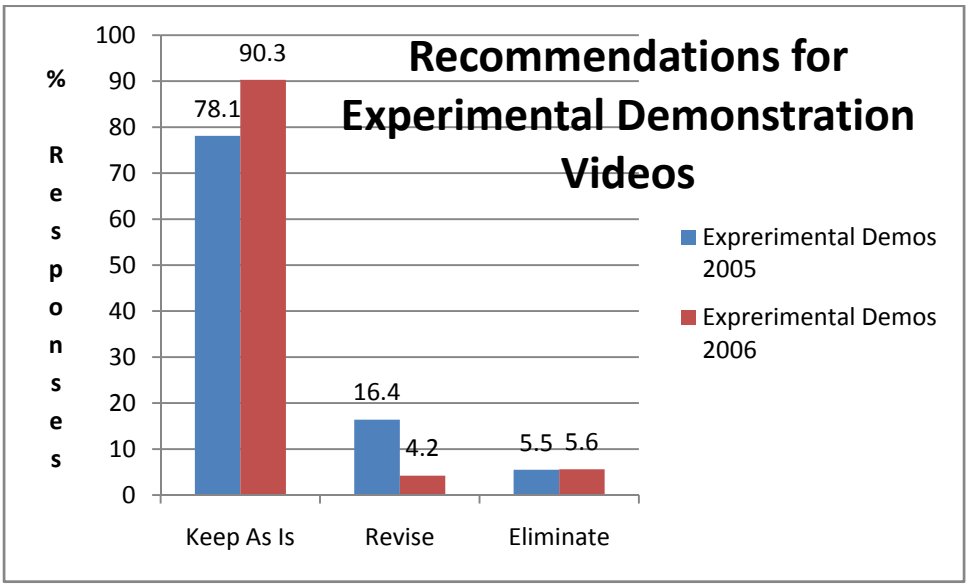

Figure 2.5: Bar-graph of the % frequencies of categories of recommendations the students suggested for "Experimental Demonstration Videos" per year of survey.

Online quizzes were the most poorly received new technique with only 9.5+45.9 = 55.4% positive comments for Fall 2005 students. Though in the Fall 2006 surveys this percentage greatly increased to 30.6+41.7 = 72.3%, (p=0.023) (Figure 2.6):

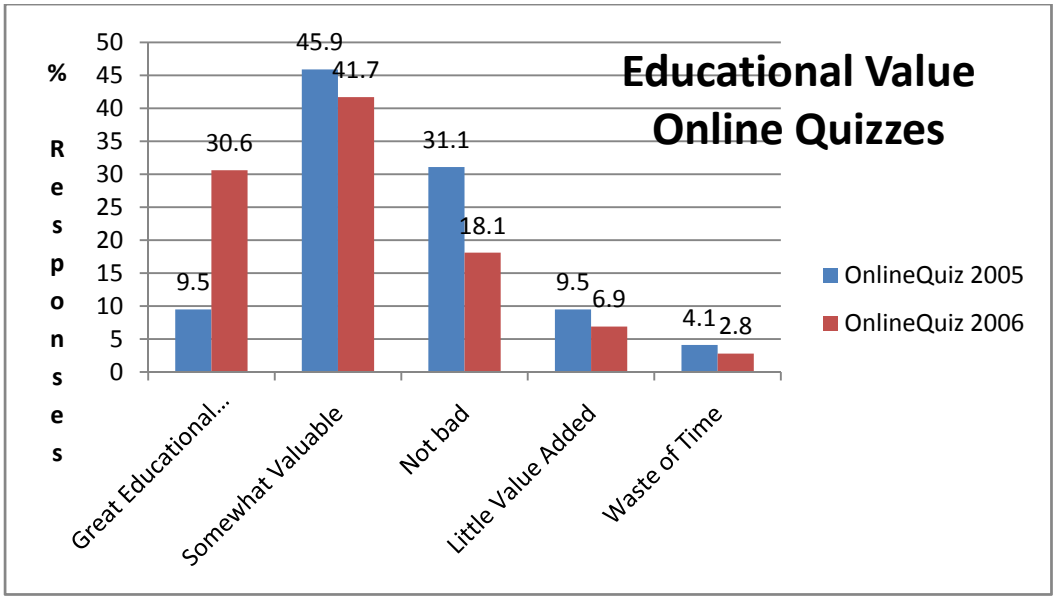

Figure 2.6: Bar-graph of the % frequencies of categories of educational value the students attribute to "Online Quizzes" per year of survey.

Consistently with their appreciation of the new instructional techniques, 57.5% of the students in 2005 asked for revisions in the online quizzes while a significant minority 35.1% asked that they be kept as they were and an 8.1% for their elimination (Figure 2.7) This picture, however, was completely reversed in the Fall 2006 students, 56.8% of whom recommend that they be kept as they were, 38.4% pointed to revisions, and only 4.1% asked for their complete elimination (p=0.023):

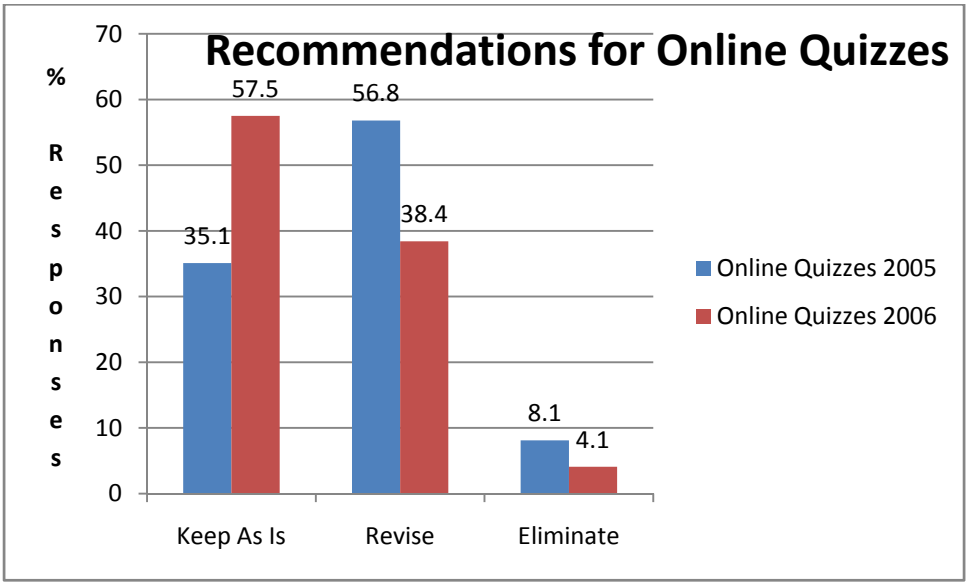

Figure 2.7: Bar-graph of the % frequencies of categories of recommendations the students suggested for "Online Quizzes" per year of survey.

Virtual laboratories were the best-received new instructional module (Figure 2.8). In their first year of application, it was thought to be of great or somewhat educational value by 66.2+20.3 = 86.5% of the students. Even though they were so highly received in 2005, they were received even better in 2006 albeit not statistically significantly, 61.1+30.6 = 91.7% (p=0.454):

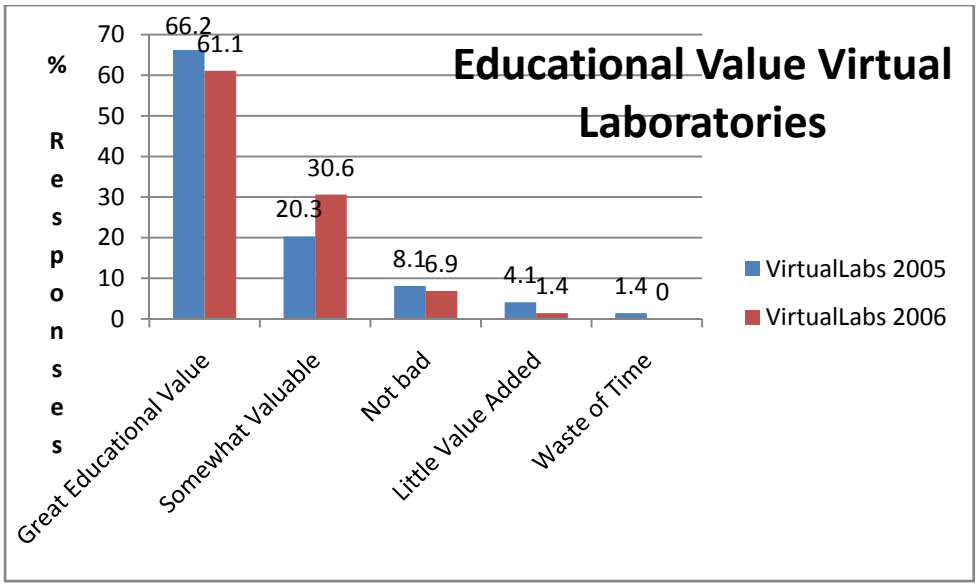

Figure 2.8: Bar-graph of the % frequencies of categories of educational value the students attribute to "Virtual Laboratories" per year of survey.

Similar observations can be made for the students' recommendations about the virtual laboratories. While a vast majority asked that they be kept as they were, this percentage increased from 74.3% in 2005 to 91.8% in 2006 while students asking for revisions dropped from 23% to 6.8% (Figure 2.9). Only negligible 2.7% and 1.4% asked for their elimination (p=0.018):

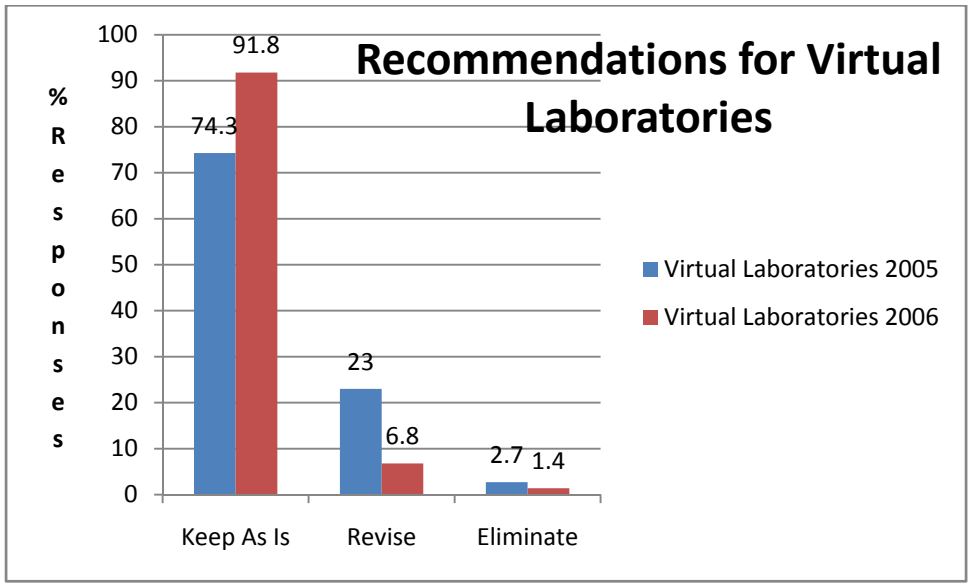

Figure 2.9: Bar-graph of the % frequencies of categories of recommendations the students suggested for "Virtual Laboratories" per year of survey.

The *p* values of the associations of the appreciation/recommendations with the year of the survey (X^2 tests) are summarized in the table below (Table 2.10):

Survey Question:	**Online Lectures**	**Experimental demos**	**Online Quiz**	**Virtual Lab**
A. Educational Value End Term 2005/6	0.630	0.023*	0.023*	0.454
B. Recommendations End Term 2005/6	0.742	0.052	0.023*	0.018*

Table 2.10: *p* values of associations of "Educational Value" and "Recommendations" with the different times of each survey for each of novel instructional module.

2.3.3 Formal Course Evaluations:

The formal course evaluations contained a total of fifteen evaluation statements, four of which were deemed relevant to this research and further analyzed. These included two questions about the class overall (Question 1: "overall this was an excellent course," and Question 3: "I learned a great deal from this course"), and another two questions about the laboratory component of the class (Question 331: "the laboratory was a valuable part of this course," and Question 337: "laboratory assignments were relevant to what was presented in class"). The nominal reflections to these statements "Strongly Agree," "Agree," "Neutral," "Disagree," and "Strongly Disagree" were assigned numerical values according to a 1-5 Likert-scale (5 for Strongly Agree… 1 for Strongly Disagree).

The student evaluations before (2001-2004) and after (2005-2006) Fall 2005 were pooled together and the values and significance of the differences of their means per question and per instructor are presented in the table below (2.11). It should be noted that for Fall 2006, the Instructor 1 was substituted with an instructor who was more involved and, thus, could relate more with the laboratory portion of the class:

Questions Instructor	2001-2004 (Mean/N/SD)	2005-2006 (Mean/N/SD)	*Difference* (2005/6 - 2001/4)	*P* of mean difference
Question 1 Instructor 1	2.59 238 (0.922)	4.44 68 (0.699)	+1.85	0.000*
Question 1 Instructor 2	3.52 238 (0.855)	4.12 68 (0.838)	+0.6	0.000*
Question 3 Instructor 1	2.59 227 (0.914)	3.43 123 (1.460)	+0.84	0.000*
Question 3 Instructor 2	3.81 227 (0.822)	4.20 112 (0.826)	+0.39	0.000*
Question 331 Instructor 1	3.32 226 (1.013)	3.92 121 (1.046)	+0.6	0.000*
Question 331 Instructor 2	3.51 227 (1.138)	3.88 112 (0.978)	+0.37	0.004*
Question 337 Instructor 1	3.49 225 (0.897)	4.16 122 (0.903)	+0.67	0.000*
Question 337 Instructor 2	3.76 227 (1.013)	4.04 112 (1.046)	+0.28	0.012*

Table 2.11: Values and significance of the differences of the means of the pooled formal evaluation scores per question and per instructor before and after the introduction of the new instructional methodology.

Statistically significant increases were recorded for each evaluation question for both instructors. P values of 0.000 were calculated for all comparisons except for Instructor 2's laboratory related Questions 331 and 337 ($p=0.004$ and $p=0.012$ respectively) In addition, the increases were larger for Instructor 1 (Weeks 1-5) than those of Instructor 2 (Weeks 6-13) (+1.85 vs. +0.6 for Question 1: "overall this was an excellent course," +0.84 vs. +0.39 for Question 3: "I learned a great deal from this course," +0.6 vs. +0.37 for Question 331: "the laboratory was a valuable part of this course," and +0.67 vs. +0.28 for Question 337: "laboratory assignments were relevant to what was presented in class."

In addition, the results are presented per question and per year for each of the two instructors below. Each cell contains the difference of mean responses for either academic year as well as its statistical significance expressed by the respective p value.

- ***Question 1: "Overall this was an excellent course"***

Instructor 1 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2006***	**J / I**
	-0.42 **0.090**	**-0.43** **0.090**	**-0.08** **1.000**	**+1.63** **0.000***	***2001***
		-0.01 **1.000**	**+0.35** **0.423**	**+2.06** **0.000***	***2002***
			+0.36 **0.41**	**+2.06** **0.000***	***2003***
				+1.71 **0.000***	***2004***

Table 2.12: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 1, Instructor 1 among each survey year.

For instructor 1 (1st – 5th week of the class), the 2006 class was evaluated significantly higher than all years before the introduction of the instructional technology (p=0.000 for all years and differences of +1.63, +2.06, +2.06 and +1.71 for the years 2001-2004 respectively) (Table 2.12). Data for 2005 evaluations are missing. The differences between the other years show a mixed picture of insignificant differences but 2001 and 2004, in general, scored higher than all other pre-2005 years and in parity among themselves.

Instructor 2 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2006***	**J / I**
	-0.51 **0.08**	**-0.42** **0.078**	**-0.57** **0.002***	**+0.25** **1.000**	***2001***
		+0.10 **1.000**	**-0.06** **1.000**	**+0.76** **0.000***	***2002***
			-0.16 **1.000**	**+0.66** **0.000***	***2003***
				+0.82 **0.000***	***2004***

Table 2.13: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 1, Instructor 2 among each survey year.

For instructor 2, with 2005 data missing again, 2006 year scored significantly higher compared to 2002-2004 (p = 0.000 for all years) years but not compared to 2001 (p=1.000) although still better (+0.25 difference) (Table 2.13). The differences were less than those observed for Instructor 1 evaluations (+0.76, +0.66, +0.82 compared to 2x+2.06 and +1.73 for the years 2002-2004 respectively). Year 2001 also reported significantly higher evaluations compared to year 2004 (+0.57, p=0.002) and almost achieving significance compared to 2002 and 2003 (+0.51, p=0.08 and +0.42, p=0.078 respectively). 2004 fared worse than all the other years but not to a significant level. The rest of comparisons give a mixed picture of small, insignificant differences.

- **Question 3: "I learned a great deal from this course"**

Instructor 1 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2005***	***2006***	**J / I**
	-0.39 **0.021***	**-0.33** **0.052**	**-0.37** **0.027***	**-0.70** **0.000***	**+1.59** **0.000***	***2001***
		+0.05 **0.751**	**+0.02** **0.915**	**-0.31** **0.069**	**+1.98** **0.000***	***2002***
			-0.04 **0.829**	**-0.36** **0.036***	**+1.93** **0.000***	***2003***
				-0.33 **0.053**	**+1.96** **0.000***	***2004***
					+2.29 **0.000***	***2005***

Table 2.14: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 3, Instructor 1 among each survey year.

For instructor 1 Year 2006 gave again a significantly higher evaluation score compared to all years 2001-2005 (p=0.000 for all years and differences ranging from +1.59 (vs 2001) to +2.29 (vs 2005) (Table 2.14). Year 2005 performed significantly worse than 2001 and 2003 (-0.70, p=0.000 and -0.36, p=0.036) and almost significantly worse for years 2002, 2004 (-0.31, p=0.069 and -0.33, p=0.053). Year 2001 also reported significantly higher evaluations compared to years 2002 & 2004 (+0.39, p=0.021 and +0.37, p=0.027) and almost achieving significance compared to 2003 (+0.33, p=0.052).The rest comparisons didn't have significant differences or trends.

Instructor 2 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2005***	***2006***	**J / I**
	-0.32 **0.745**	**-0.50** **0.020***	**-0.43** **0.077**	**+0.36** **0.525**	**-0.10** **1.000**	***2001***
		-0.19 **1.000**	**-0.12** **1.000**	**+0.67** **0.001***	**+0.21** **1.000**	***2002***
			+0.07 **1.000**	**+0.86** **0.000***	**+0.40** **0.138**	***2003***
				+0.79 **0.000***	**+0.33** **0.462**	***2004***
					-0.46 **0.001***	***2005***

Table 2.15: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 3, Instructor 2 among each survey year.

For instructor 2, the results seem to be reversed (Table 2.15); it is Year 2005 which is ranked significantly higher than the rest of the years but 2001 before the introduction of the instructional techniques (+0.67/p=0.001 for 2002, +0.86/*p*=0.000 for 2003 and +0.79/*p*=0.000 for 2004) and even than the second year of application 2006 (+0.46, p=0.001). Year 2006 had positive but not statistically significant differences with the years 2002-2004 (+0.21/*p*=1.000 for 2002, +0.40/*p*=0.138 for 2003 and +0.33/*p*=0.462 for 2004). Year 2001 also reported higher evaluations compared to the pre-2005 ones, significant compared to year 2003 (+0.50, *p*=0.020) and almost achieving significance compared to 2004 (+0.43, *p*=0.077). The rest of the comparisons didn't show significant differences.

- **Question 331 "The laboratory was a valuable part of this course"**

Instructor 1 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2005***	***2006***	**J / I**
	-0.23 **1.000**	**-0.74** **0.002***	**-0.41** **0.495**	**-0.26** **1.000**	**+0.74** **0.001***	***2001***
		-0.52 **0.133**	**-0.19** **1.000**	**-0.03** **1.000**	**+0.96** **0.000***	***2002***
			+0.33 **1.000**	**+0.48** **0.245**	**+1.48** **0.000***	***2003***
				+0.15 **1.000**	**+1.15** **0.000***	***2004***
					+1.00 **0.000***	***2005***

Table 2.16: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 331, Instructor 1 among each survey year.

For instructor 1, once again, Year 2006 fared significantly higher than the rest of the years before the introduction of the instructional techniques and also Year 2005 (+0.74/p=0.001 for 2001, +0.96/p=0.000 for 2002, +1.48/p=0.000 for 2003, +1.15/p=0.000 for 2004 and +1.00/p=0.000 for 2005) (Table 2.16). Year 2005 had a mixed picture in comparison with other years with no difference reaching statistical significance. Year 2001 also reported higher values than all other pre-2005 years, achieving significance compared to year 2003 (+0.74, p=0.002).Years 2003 and 2004 fared worse than all the rest of the years. The rest of the comparisons didn't have significant differences or apparent trends.

Instructor 2 **(Mean difference)** **(J-I)/*p***	***2002***	***2003***	***2004***	***2005***	***2006***	**J** **I**
	-0.56 **0.086**	**-0.79** **0.001***	**-0.18** **1.000**	**-0.22** **1.000**	**+0.13** **1.000**	***2001***
		-0.23 **1.000**	**0.38** **1.000**	**+0.34** **1.000**	**+0.69** **0.005***	***2002***
			0.61 **0.042***	**-0.57** **0.151**	**+0.92** **0.000***	***2003***
				-0.04 **1.000**	**+0.31** **1.000**	***2004***
					-0.36 **1.000**	***2005***

Table 2.17: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 331, Instructor 2 among each survey year.

For instructor 2, the picture looks very varied. Year 2006 reported significantly higher means compared to years 2002 & 2003 (+0.69/p=0.005 for 2002, +0.92/p=0.000 for 2003) and higher, yet insignificantly, scores for the other years before the introduction of the new techniques (Table 2.17). Year 2005 had mixed and insignificant differences from the other years. Other significant differences observed include 2001 and 2004 over 2003 (+0.79/ p=0.001 and +0.61/p=0.042 respectively). 2003 fared worse than the other years of the survey except 2005.

- **Question 337: "Laboratory assignments were relevant to what was presented in class"**

Instructor 1 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2005***	***2006***	**J / I**
	-0.25 **1.000**	**-0.43** **0.272**	**-0.67** **0.001***	**+0.10** **1.000**	**+0.57** **0.012***	***2001***
		-0.18 **1.000**	**-0.43** **0.223**	**+0.35** **0.882**	**+0.81** **0.000***	***2002***
			-0.24 **1.000**	**+0.53** **0.049***	**+1.00** **0.000***	***2003***
				+0.78 **0.000***	**+1.24** **0.000***	***2004***
					+0.46 **0.103**	***2005***

Table 2.18: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 337, Instructor 1 among each survey year.

For instructor 1, once again, Year 2006 fared significantly higher than the rest of the years before the introduction of the instructional techniques and not significantly but still higher than Year 2005 (+0.57/p=0.012 for 2001, +0.81/p=0.000 for 2002, +1.00/p=0.000 for 2003, +1.24/p=0.000 for 2004 and +0.46/p=0.103 for 2005) (Table 2.18). Year 2005 also presented a higher score than the previous years and this was significant for the years 2003 & 2004 (+0.10/p=1.00 for 2001, +0.35/p=0.882 for 2002, +0.53/p=0.049 for 2003, +0.78/p=0.000 for 2004). Year 2001 also reported significantly higher evaluations compared to year 2004 (+0.67, p=0.001) and in general higher than all other pre-2005 years. In general, Year 2004 fared worse than all other years. The rest comparisons didn't achieve statistically significant differences or display trends.

Instructor 2 (Mean difference) (J-I)/*p*	***2002***	***2003***	***2004***	***2005***	***2006***	**J / I**
	-0.39	**-0.45**	**-0.28**	**+0.08**	**-0.05**	***2001***
	0.503	**0.198**	**1.000**	**1.000**	**1.000**	
		-0.06	**+0.11**	**+0.47**	**+0.34**	***2002***
		1.000	**1.000**	**0.250**	**0.857**	
			+0.17	**+0.53**	**+0.40**	***2003***
			1.000	**0.099**	**0.168**	
				+0.36	**+0.23**	***2004***
				1.000	**1.000**	
					-0.13	***2005***
					1.000	

Table 2.19: Values and significance of the differences of the means of evaluation scores -reported as Column J – Row I survey year results- for question 337, Instructor 2 among each survey year.

For instructor 2, the picture looks greatly mixed with no difference achieving statistical significance (Table 2.19). Still Year 2006 reported consistently higher means compared to years 2002 – 2004 but not than 2001. Year 2005 had insignificant differences from the other years yet scored higher than all previous ones. 2001 also fared better than the years 2002 – 2004 and 2003 worse than all other years.

2.4 Discussion

The results stated in the previous section provide multiple insights into addressing different facets of the efforts to improve Pharmaceutical Analysis instruction at the University of Michigan. Starting from the educational outcomes, the high scores of perceived familiarity of the students with this course before class were expected since all of the incoming PharmD students have to have taken 2 semesters of General Chemistry and 2 semesters of Organic Chemistry with laboratories in their pre-Pharmacy curriculum. The significant increase of the sense of familiarity in the end-of-term surveys can be explained on the basis of the temporal proximity of the class instruction. The drop

observed in the 3-semester-after-class surveys to the before-class levels is of serious concern. The fact that ratings fell to the corresponding 2004 levels, before the instructional techniques were introduced, points to a more generalized problem with the lack of retention of the knowledge gained and its integration in the PharmD curriculum on top of the instructional problems noted before (*cf. Chapter 1)*. As a result, Drug Assay class content needs to be revisited and reestablished within the context of other PharmD classes for the students to maintain a high level of familiarity throughout their studies.

A similar picture emerges after considering the survey results about the relevance and applicability which the students attribute to this class regarding their future practice as pharmacists. Both 2005&2006 years reported a rather high degree of relevance before classes began. It was untoward that the same students gave a lower score of relevance when they were surveyed at the end of the class. So, before they attended the class, students had a rather high regard for what they were about to learn and this actually diminished after having seen all that this class entailed. These low levels persist in the 3-semester-after-class surveys; that is after the PharmD students have completed the first two years of their PharmD curriculum, including classes in Pharmaceutics, Pharmacokinetics and others where the relevance of Drug Assay might become evident and reinforced.

It is encouraging that both year 3-semester-after-class surveys showed higher relevance than the 2004 and, at least, were in parity with the right after-class surveys and not worse. These favorable outcomes after the initiation of the new instructional methodology imply that the changes introduced were to the right direction in contextualizing what the students learnt. In fact this could be an asset on which other classes' instructors can build to relate more to Drug Assay within their own classes' syllabi and help improving on the familiarity levels at the same time too.

Another major issue identified when designing the interventions to improve the instruction of this class was the disconnection of the lectures' progress from the laboratory exercises *(cf. Chapter 1).* The students reported a satisfactory sense of connection after-class in both 2005 and 2006. However, this fell sense to the before-the-intervention levels of 2004 for 2005 yet remained significantly higher in the 2006 3-semester-after-class surveys, which was statistically equal to the levels in the after-class

2006 survey. This apparent paradox can be attributed to the fact that 2005 was the first year that the new techniques were introduced and both teaching staff and students needed to adjust to the new methodologies. Also it should be noted that when interpreting these results, the levels of reported connection in the 3-semester-after-class survey come after the students had attended other classes with laboratory exercises which had been taught for longer time and, therefore, present, perhaps, fewer challenges than the expensive instrumental analysis one. This means that the students could compare their sense of connection between class time and laboratory to those of other classes with laboratories and assume a more critical attitude towards this class, which was not the case for the after-class surveys in their first semester of studies, which might result in a reduced sense of reported connection in the 3-semester-after-class surveys than the ones right after-class.

Similar observations can be made about the results of the survey question regarding the variety of exposure the students felt they had experienced at points after the class. Again, the students reported a high level of variety in their educational experience right after class. Also, in the 3-semester-after-class surveys, the 2006 results exhibited the same sense of variety as after-class while the 2005 one fell to the 2004 levels. A similar argument as before can be made for the cause of this: the lack of previous experience of everyone involved in the educational process, both students and instructors alike.

There could be a lot of reasons for why the introduction of the new techniques could be adding to the sense of workload of the students. Gratifyingly, the students reported a statistically equal feeling of pace and workload when they were polled right after-class in 2005&2006 and also 3-semester-after-class for 2004, 2005&2006. Thus, the students would be, at most, equally as likely to complain about the class burden as before, which is important because this is a 3-credit hour only class and it wouldn't be fair to demand a disproportionally high commitment of time and effort for studying and preparation on behalf of the students

The final question about educational outcomes was an overarching one about students' expectations. A large majority of students for both 2005 and 2006 indicated that their expectations were met in surveys right after-class. These positive answer profiles did not last, though as is evident in the decrease of the positive opinions in the 3-

semester-after class follow-up surveys with the one for the Year 2005 class reaching statistical significance. These later-term surveys are of particular value since the students can compare and reflect on their expectations after having participated in four full semesters of classes and laboratories. On the other hand, this time separation could impair students' recalling and reflecting on a class offered 3 semesters ago. Consistently with the picture of the questions about "connection" and "variety" above, the 2005 class had higher percentage of positive responses in comparison to the 2004 class 3-semester-after survey albeit not statistically significant, while the Fall 2006 students surveys achieved statistically significantly higher results. The 2005 Year was complicated again by the fact that it was the first exposure to the new educational methods for everyone involved. Year 2006, on the other hand, maintained its high after-class survey score perhaps because it was the second year of implementation when a lot of technical issues might have been tackled more efficiently.

The 2005 Final Quiz scores also provide some additional interesting insights about the educational value of the virtual laboratories. The mean overall scores were not statistically significantly different for the Monday (virtual lab on electrophoresis only) or Wednesday sections (virtual lab on HPLC only). After inspection of the partial scores for the electrophoresis questions, it can be seen that there was no statistically different mean scores for either sections. This can be explained by the fact that the TA of this particular experiment was highly knowledgeable, motivated, dedicated to his students, and helped them extensively during the wet-lab sessions diluting the additional beneficent educational effect of the virtual laboratories. In addition, this was his second year in the row that he was assigned to assist in the electrophoresis experiment, which may also explain his teaching efficiency.

In the case HPLC questions' partial scores, the section that received the virtual laboratory in addition to the wet laboratory instruction on HPLC fared significantly better ($p = 0.046$). When interpreting these quiz scores, it has to be taken into consideration that there is an upper limit of 100% that these scores can have and that with means >80%, their distribution is skewed towards higher values. This makes it more difficult to effect and show a statistically significant improvement of the already high-scoring students. In view of these the p value of 0.046, which was calculated for the difference of the Monday

vs. Wednesday sections' HPLC question scores consists a far more significant improvement. The TA for this experiment was less efficient in his instruction (per students' evaluations and also because this was his first time teaching such laboratory). This evidence may imply that virtual laboratories can substitute for less-motivated and committed in-class effort on the TA's behalf, ensuring an equally high level of content delivery regardless of the TA's involvement, or lack thereof.

All four novel instructional techniques were well received by large majorities of students in both Fall 2005 and 2006 classes. The virtual laboratories received the highest positive opinions while the online quizzes received the lowest student appreciation. In addition, for the online lectures, online quizzes and virtual laboratories there was an increase in the overall positive responses among the 2005 and 2006 surveys, even achieving statistical significance in the case of the online quizzes. This improvement from 2005 to 2006 scores can be explained by the fact that 2006 was the second year of implementation. Also, better explanations about the need, context, and usefulness of the online quizzes as instructional, not for-credit and not examining, for-credit tools might have helped increase the students' appreciation for them. As expected, since this was the second year of application, all four techniques fared better in the recommendation questions than in the 2005 surveys, with all but online lectures achieving statistically significant higher number of "keep as is" responses.

Online experimental demos were the only technique whose educational value was received less positively in 2006 than 2005; this could be attributed to the better integration and more extensive use of virtual laboratories in 2006, which may have eroded the perception of the educational value of the online experimental demonstration videos. Indeed, although the students' appreciation for the online experimental demonstrations dropped, the percentage of students who advised to "keep as is" increased almost statistically significantly in 2006. At the same time, the corresponding results improved, significantly too, in the case of the recommendations, for the virtual labs.

The formal end-of-class surveys also agree with the general improvement trends mentioned above. When pooled together, 2005-2006 students reported a significantly higher sense of agreement than the 2001-2004 students with the following statements for both instructors: 1: "overall this was an excellent course," 2: "I learned a great deal from

this course," 3: "the laboratory was a valuable part of this course," and 4: "laboratory assignments were relevant to what was presented in class." The mean reported improvement in students' perceptions was higher for instructor 1 (weeks 1-5) than instructor 2 (weeks 6-13). With incoming P1 students with varied previous academic backgrounds in the material (ranging from BS degrees in Sciences to BS in Humanities with only pre-Pharm curriculum completed) and with Drug Assay being the first and most science centered class in their PharmD training, instructor 1 had to teach in a sensitive period of the students academic course. If nothing else, students during their 6th-13th weeks of classes would come to feel more familiar with the class, program, and University as a whole. It's very encouraging to observe that after the implementation of the novel instructional methodology the formal survey, scores tend not only to improve but also to be more unified between the two parts of the class.

2001 was a year of transition for the class instruction since this was the year that the professor who introduced the earlier curricular reforms departed. As a result, 2001 fared better than the years 2002, 2003, and 2004 consistently in all evaluation questions (with or without statistical significance). In addition, 2001 fared as well or better than the 2005 in quite a few questions and even than some of the 2006 evaluations that is after the introduction of the instructional methodology. This was expected since any class attains a certain level of optimization after years of repetitive instruction and coordination, which is not easy to reproduce when changing the personnel who are in charge. At the same time, the new educational tools helped to achieve a convergence between 2005, 2006, and 2001. Year 2005 was a cumbersome experience for all involved since this was the first effort to apply the improvements and the sense of disarray made this year fare worse than the previous years 2001-2004 for Question 3, which stated: "I learned a great deal from this course," and 337 "Laboratory assignments were relevant to what was presented in class" for instructor 1's section. The reverse is true for Question 3 - Instructor 2 year 2005 survey, which fared better than 2001-2004, indicating again that it takes time for all involved to get accustomed to the new realities of the class. In summary, the new instructional methods fulfilled the expectations or were found to be in the right direction.

2.5 Conclusion/Future Directions

The University of Michigan has chosen to keep the Pharmaceutical Analysis and Drug Assay laboratory contrary to the general trend of limiting its share in pharmaceutical education *(cf. Chapter 1)*. This class offers unique opportunities for PharmD students to get training in measuring and handling drugs with precision and in performing calculations with confidence, which is all essential for a safe pharmacy practice. Beyond these necessary practical skills, students have the chance to explore drug-as-chemicals properties such as acidity, solubility, partition, radiation absorbance, reaction catalysis, chemical reactivity etc, all of which are dose/concentration dependent and, therefore, used to generate the analytical signal and are related ultimately to their biological effects and use as drugs in general. Thus Drug Assay offers more competencies to the pharmacist trainees than the "ability to quantitative methods of measurement in order to provide drug information, evaluation of clinical laboratory data" demanded by Standards 2007, Appendix B.[3] It is the first chance for the students to apply knowledge in order to analyze a situation and to improve their higher thinking and decision-making skills early in their PharmD program. Therefore, it is an important step to educate pharmacists in the practice of "good science" which is "evidence-based, convincing, explanatory, honest, testable, and systematic."[3]

Also, as health care moves to evidence-based-medicine, knowing how to determine and interpret the amount of a drug present in a biological system is a critical piece of evidence, for "[e]vidence-based medicine uses the scientific method of using observations and literature searches to form a hypothesis as a basis for appropriate medical therapy".[4] Training in scientific method and "good science" are both important prerequisites for anyone to practice in this capacity. In keeping with this move, the increased use of self-diagnose and monitor kits (i.e. evidence-finding devices), is also a growing trend towards personalized medicine, which may lead patients to the pharmacists for expert free knowledge, interpretation, and troubleshooting of those tests. In addition, pharmacists may be asked to run simple toxicological analyses, for example, those of "street-drugs" or chemical weapon poisoning, as part of public health infrastructure.[5] Pharmaceutical analysis offers a complete experience in how to generate evidence using

solid scientific principles, collecting and interpreting data, and translating the results into something meaningful to the pharmacy practice.

At the same time, it's true that large amount of resources need to be dedicated to this class in order to provide a high quality of education. Understandably, the cost of the laboratory equipment and the practicality of having as high of individualized instruction as possible have driven down the possibility of the adoption of a rotation system in the University of Michigan and elsewhere. Although this has perhaps optimized the material resource use, there are numerous issues regarding the cohesiveness and plausibility of the delivery of the class content. The changes introduced in Fall 2005 term attempted to streamline the laboratory component delivery while leaving the lecture part intact. The availability of online services and electronic resources from the University enabled the incorporation of asynchronous distance education elements while keeping the traditional hands-on wet lab student exercises. These resources included tutorials, experimental demonstrations, quizzes and virtual laboratories all in an electronic form, which were distributed from a secure dedicated course website.

All of the new techniques proved their value over a two-year program-evaluation. The students affirmed the educational value of all of them in the first year of application, even to increase for all but one in the second year (Fall 2006). Also, the large majority of students advised to keep them as they were and while some were asking for improvements, only few asked for their complete elimination. The highest scoring module was the virtual laboratories, followed by the pre-laboratorial lectures, experimental demos, and the quizzes. Even though they weren't part of the final grade, it was expected that the quizzes would be the lower scoring due to the students' aversion to any form of exam. However, the results were greatly improved next year when its educational scope was perhaps better explained and the wording of some of the questions revisited. The drop in the educational value of the experimental demos, while advising to keep them at same time, coincides with the more wide use further integration of the virtual laboratories in 2006. It could be that the students had become saturated with the amount and variety of electronic tools available and/or grew "screen-fatigued."[6] The merging of the virtual labs into the online tutorials could have created a sense of redundancy about these demos, as well.

The first year of the changes 2005 was a transition year for both instructors and students, which was reflected in numerous evaluation outcomes such as the sense of connection between class and lab, the variety of educational experience provided, and students' expectations in places where the scores of the three-year after-class surveys fell to the levels of 2004 (before the changes). The same trend is evident in the formal course evaluations, where 2005 scores only marginally higher than the previous years in most questions. Year 2006, the second year of application, had a consistently higher scoring profile by most measures. On a general level, having the same instruction personnel every year definitely helps the class run effectively. The students in Fall 2001, the last term before the departure of the previous professor, performed almost as equally as 2005. The same stands true for the students who also needed time to get acquainted to the way the course is organized and delivered. Instructor 2, who taught later in the semester, achieved higher scores than Instructor 1. Here, the new instruction methodology has overall been proven valuable in making up for inconsistent efficiency of high-turnover TAs and it also lessens the gap between Instructor 1 and Instructor 2 in the formal teaching evaluations. At the same time, the students didn't report any higher sense of burden than before the introduction with the techniques.

Two major issues of concern identified are the familiarity and relevance that the students find in this class. For evaluations of familiarity, while elevated right after class, drops to the much lower levels of 2004 3-semester-after surveys. For relevance to pharmacy practice, the levels of appreciation dropped after the class as compared to the before-class rather high number. At least the later 3-semester-after surveys show a stabilization of the sense of relevance and score much higher than the same question in the 2004 year survey. In sum, the new way of instruction had a beneficial effect on the relevance but not enough to revert it to the pre-class levels. These two issues share a lot in common, as for a student to keep his/her familiarity levels up he/she needs to see more applications of Drug Assay in the context of other classes later in the PharmD education, at the same time showing examples of the relevance of this class to the practice of pharmacy.

It has been shown that faculty of basic science classes should constantly strive to show the relevance of their class to therapeutic decision-making.[7] The provision of a

portfolio of easily retrievable electronic instructional materials can help both the students and instructors of other classes to quick refer back to and reconnect with what was covered in Pharmaceutical Analysis and to also contextualize this knowledge in the other class. The ready exchange of education materials between faculty is included in the guidelines of Standards 2007 (10.2).[3]

The new format of the Pharmaceutical Analysis lab provides ample opportunity for further development and explorations of this class's scope and context. In particular, it includes more scenarios of possible errors in the actual wet lab and illustration of the applicability of the various techniques to the practice of Pharmacy[7]. For example, the students may use their Pharmaceutical Analysis skills in a compounding laboratory to verify the amount of the drug in their preparations, referring to the corresponding tutorial from this class. In fact, drug assay may have a closer relationship with pharmaceutics where analysis is used in context (stability, therapeutic dose monitoring etc).[8]

The incorporation of electronically delivered content is also compatible with a move towards a more computerized world and in line with Guideline 11.2 and with the related core competency requested by IOM[9]. The pre-laboratory preparation of the students doesn't solely rely in the course teaching's progress, operating as stand-alone pieces of information. At the same time, these tutorials may help the in-class lecture instruction as an alternative, readily available source of information. Asynchronous video streaming can enhance the student's abilities as self-directed and life-long learners and provide students who failed a means to review specific content on the recorded material in order to achieve the desired level of competence.

The better integration of pre-laboratorial material bridges the gap in Bloom's Taxonomy objectives that existed before.[10,11] Objectives I (Knowledge) and II (Comprehension) are now adequately addressed and students' attainment of them is assured or, if there are any problems, they are promptly detected and tackled before asking the students to come to the laboratory (III Application) to analyze the results (IV Analysis) and before asking the author for a complete lab report (V Synthesis).[8,9] Virtual laboratories, the best received new tool, have also provided opportunities to challenge students to the next level (VI Evaluation) without jeopardizing sensitive and expensive laboratory equipment.

Finally, this study offers new ideas for development of evidence-based-education in pharmacy education and in general. Most educational studies focus on studies before-and-after the introduction of new instructional methodology. The educational outcomes in the present one are surveyed in a later time (3-semester-after) in addition to before-and-after class, which provides useful insights since the students have seen other classes with similar content by then and can relate to and express more informed perspectives and opinions. The whole concept of integrating web-based tools into the laboratorial exercise in order to develop practical skills may also be applied to other disciplines where expensive and/or scarce resources are available such as sciences, medicine, nursing, dentistry, education, etc.

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PART II

CHAPTER III

HEPARAN SULFATE AND HEPARANASE AS ANTICANCER DRUG TARGETS AND THEIR INHIBITION BY SUGAR-AMINO ACIDS

3.1 Metastatic Process and Heparan Sulfate/Heparanase Role

Most cancer mortality results from the metastasis of tumors to regional and distant sites.[1] The high mortality rates associated with cancer are caused by the metastatic spread of tumor cells from their site of origin. In fact, metastases are the cause of 90% of cancer deaths. Tumor cell invasion and secondary spread through the blood and lymphatic ducts are the hallmark of a malignant disease.

The acquisition of metastatic potential requires the accumulation of various genetic and cellular changes—the combination of which will allow for one or several of tumor cells to achieve the whole metastatic process.[2] The critical steps are: escape from the primary tumor, dissemination through the circulation, lodgment in small vessels at distant sites, penetration through the vessel wall, and growth in the new sites as a secondary tumor (Figure 3.1).[3] In addition, the expansion of tumor mass beyond a size of a few cubic millimeters depends entirely on de novo formation of a vascular network that provides the growing tumor with oxygen and nutrients (neoangiogenesis).[4] Accordingly, key processes include changes in cell adhesion, the production of enzymes capable of degrading the physical barriers and secretion of cytokines and other factors, which attract and activate stroma and endothelial cells during invasion and angiogenesis.

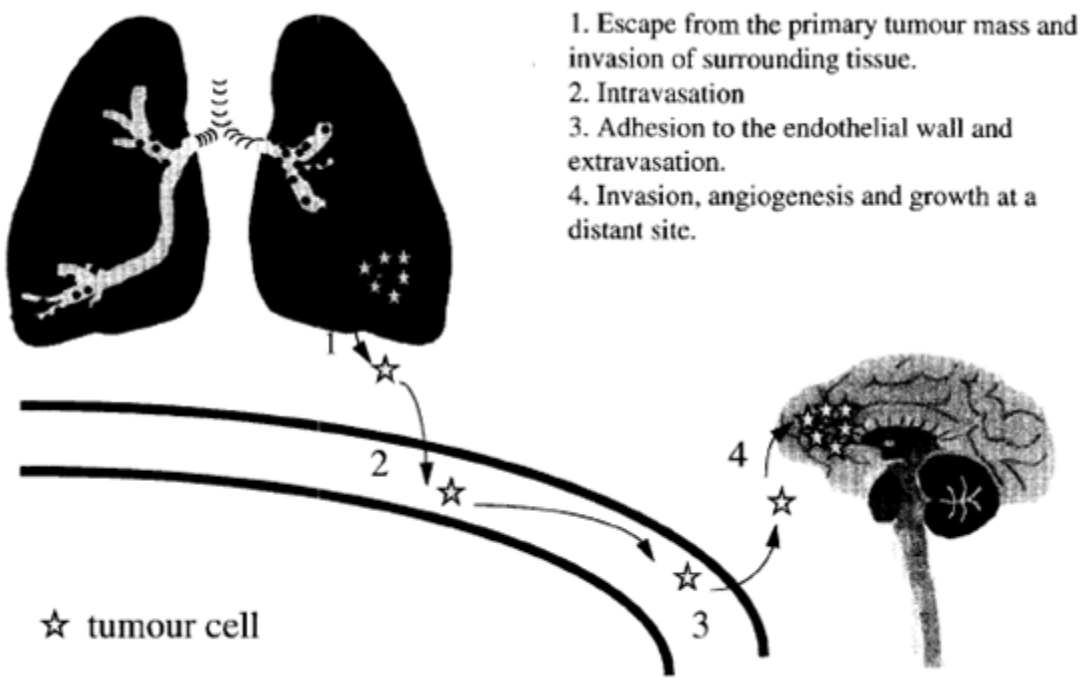


Figure 3.1[2] Stages of Metastasis.

Once in the blood circulation (hematogenous metastasis), the cancer cells survive the mechanical stress by forming emboli and aggregates with themselves and/or other host cells.[5] The arrest of circulating cancer cells to the point of blood vessel invasion is a requisite for their emigration from the blood stream and subsequent growth into metastatic foci in the tissues. Selectins, integrins, cadherins and immunoglobulins and other yet-to-be classified molecules have been shown to be involved in the adhesive interactions between cancer cells and the endothelium. Some of these factors are expressed constitutively and seem to have organ specificity, while others are inducible by the influence of microenvironmental signals such as cytokines and/or free radicals. Initial contacts between the cancer cell and endothelium are weak and transient; they cause the cancer cell to slow down and "roll" along the vessel wall ("docking phase"). This motion initiates activation of both the endothelium and the cancer cells through cytokines, free radicals, bioactive lipids, and growth factors. These mediators cause an expression of an inducible adhesion molecules by both parts reinforcing and finally "locking" the cells onto the vessel wall.

Once the cancer cells are anchored onto the walls of the blood vessels, they degrade the physical barriers in order to invade the tissue.[2,4,5] The major physical barriers to the migration of tumor cells are the extracellular matrix (ECM) and the endothelial basement membranes (BMs). These barriers consist of protein (collagens, laminin, fibronectin etc) and glycosaminoglycan components (dermatan sulfate, chondroitin sulfate and heparan sulfate). The cancer cells secrete proteases such as matrix metalloproteinases, cystein/serine proteases to cleave the protein, and uronidases to degrade the saccharidic part.

For example, heparanase[6], an endo-β-glucuronidase, cleaves the β-glycosidic bond between acetylglucosamine and glucuronic acid units of heparan sulfate (HS) to yield shorter chains (Figure 3.2).

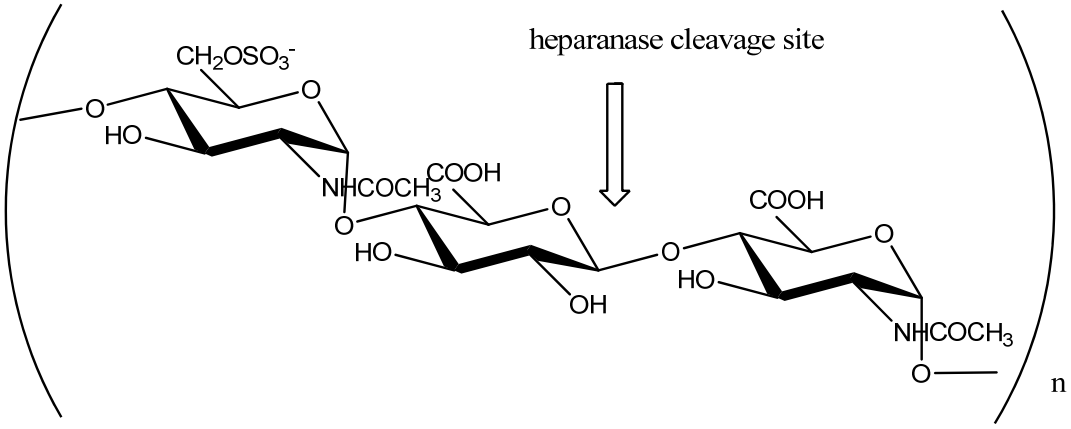


Figure 3.2 The heparan sulfate basic repeated structure. The scissile bond is also indicated.

Heparanase is normally expressed only by platelets, placental trophoblasts, and leukocytes during wound healing, embryonic development, and inflammatory response, respectively.[7-9] The cancer cells once again recruit a physiological process to assist the metastatic process and express heparanase ectopically.[10-14] Contrary to what was previously believed ECM and BMs are not just passive barriers. Among other important functions, they are involved in the regulation of growth factor and cytokine activity, which is critical for the tumor growth after the invasion. These are sequestered by macromolecules such as HS and get released upon breakdown of HS promoting new tissue growth, neoangiongenesis etc (Figure 3.3).

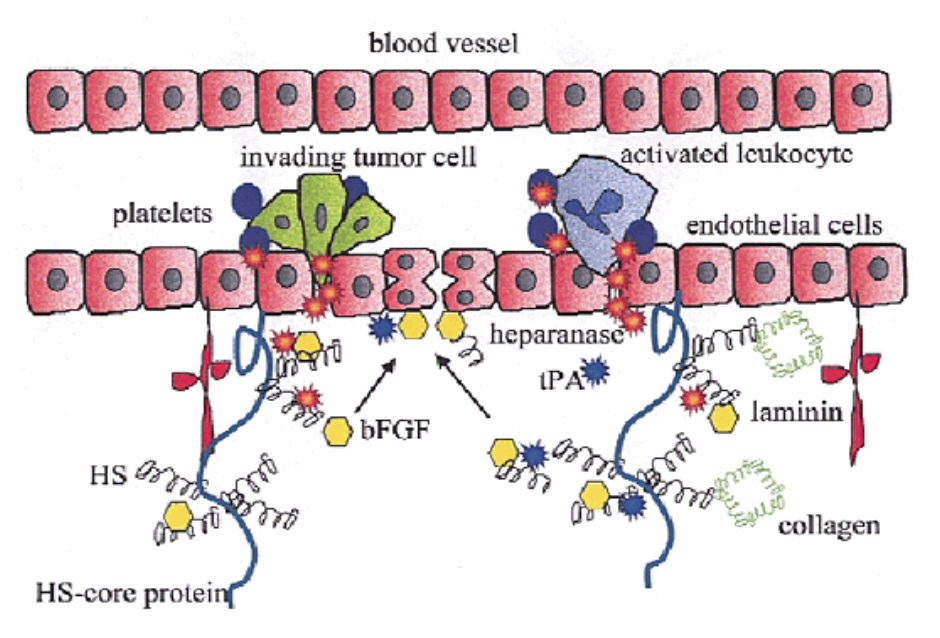


Figure 3.3[10] Heparanase breaking down barriers in tumors.

All these events promote neoangiogenesis to the benefit of the invading tumor cells. In addition, release of urokinase and tissue plasminogen activators (uPA and tPA) results in generation of plasmin, which further degrades ECM to reinforce the proteolytic and mitogenic cascades in a positive feedback fashion. It is also possible that similar tissue specific growth factors are involved in the organ specificity of metastasis.[5] In another demonstration of its importance, heparanase cDNA, when transfected to non-metastatic tumors, caused their transformation to highly metastatic ones.[9]

3.2 Aza-sugars as Sugar-Processing Enzyme Inhibitors

The breakdown and the addition of sugars are catalyzed by many different carbohydrate-processing enzymes—respectively named glycosidases and glycosyl-transferases[15]—that regulate the adhesion/recognition properties of various cellular components. Generally, these processes involve a cleavage of the glycoside bond linking a sugar's anomeric carbon with an oligo- or polysaccharide or nucleotide diphosphate group. The liberated glycosyl group may be then transferred to water (glycosidases) or to some other nucleophile (transferases) (Figure 3.4). Glycosidases are categorized as exoglycosidases, which remove sugars one at a time from the non-reducing end of the

oligo- or polysaccharide and endoglycosidases, which are capable of cleaving internal glycosidic bonds within polysaccharides. They are categorized as α or β, depending on whether they catalyze the cleavage of α or β glycosidic bonds.

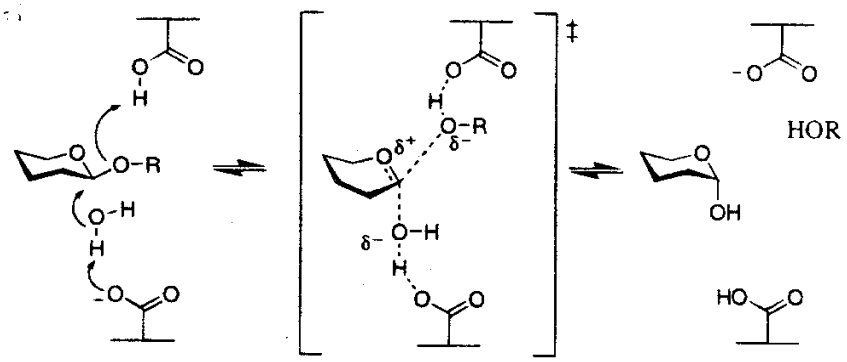
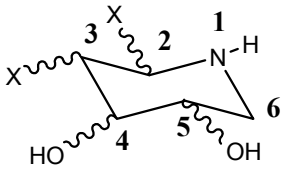


Figure 3.4[15] General mechanism of glycosidases, such as heparanase.

A common transition state exists for all these glycosidases. As the incoming general-base primed nucleophile approaches, the glycosidic bond becomes partially cleaved under the influence of general acid catalyst (Figure 3.4). As is evident from isotope effect studies, there is a significant oxocarbonium character that places a partial positive charge on the endocyclic oxygen and anomeric carbon of the acceptor sugar.[15] Accordingly, a number of natural poluhydroxylated cyclic amines that resemble the sugar structure and have a positively ionizable nitrogen atom instead of the endocyclic sugar oxygen exhibited competitive inhibitory activity towards various such enzymes, by virtue of their substrate and transition state mimicking ability (Figures 3.5, 3.6). [16-18]



X=CH_2OH, COOH or other polar group

Figure 3.5 General aza-sugar structure.

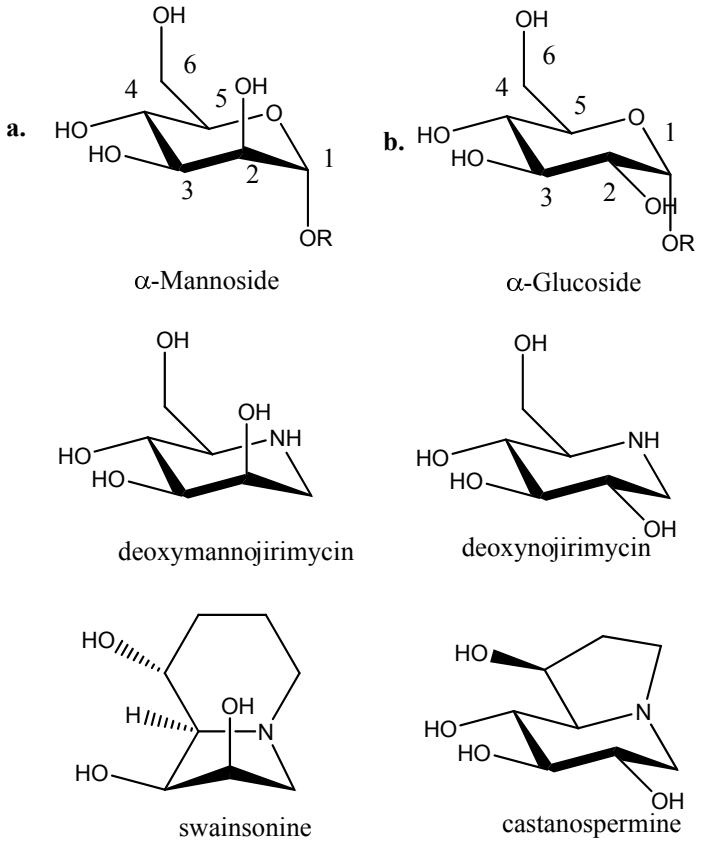
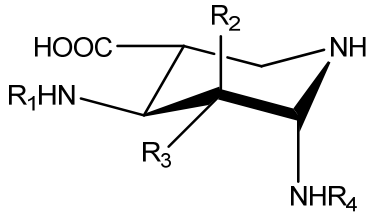


Figure 3.6 Some azasugar glycosidase inhibitors
(a: α-mannosidase and b: α-glucosidase inhibitors).

There are numerous examples in the literature of such molecules and their synthetic analogs that show profound inhibitory glycosidase effect in various disease states.

In the case of heparanase and other uronidases, the substrate contains a number of highly acidic moieties, sulfates and carboxylates (Figure 3.2). Accordingly aza-sugar type uronidase inhibitors feature acidic moieties promoting their transition state mimicking ability. There are increasing numbers of examples in the literature of such molecules and their synthetic analogs that inhibit various uronidases blocking invasion in in vitro and in vivo metastasis models.[19-21] Since they combine a sugar scaffold with amine and acidic groups they are considered a case of sugar amino-acids (Figure 3.7).[22]



R_1=R_3=H R_2=OH R_4=Ac

R_1=R_2=H R_3=OH R_4=Ac

R_1=R_3=H R_2=OH R_4=$COCF_3$

R_1=-C(=NH)NH_2 R_2=OH R_3=H R_4=Ac

R_1=-C(=NH)NH_2 R_2=OH R_3=H R_4=$COCF_3$

Figure 3.7 Some sugar amino-acid type of uronidase inhibitors.

3.3 Known Heparanase Inhibitors

After its discovery, isolation and cloning[7-9] heparanase has attracted considerable interest as a target for cancer treatment.[23,24]

These include small molecules discovered after high throughput screening and lead optimization efforts (Figure 3.8). [25,26] Two chemical categories were identified: dibenzimidazole ureas and benzoxazol-5-yl acetic acid derivatives.

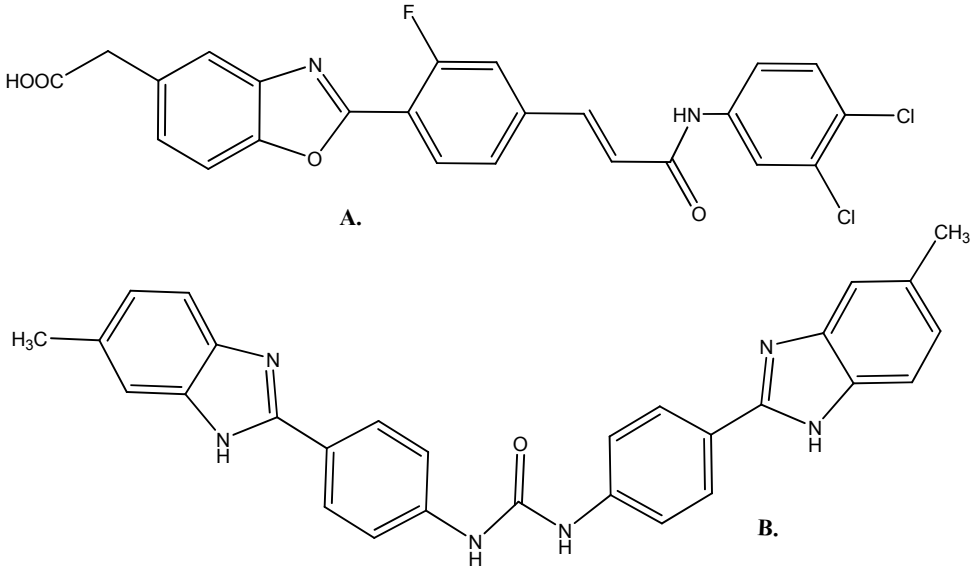


Figure 3.8: Small molecule non aza-sugar heparanase inhibitors. A. Benzoxazol-5-yl acetic acid derivative B. Dibenzimidazole ureas

In addition, oligosaccharides were discovered as part of a natural extracts screening. In particular PI-88 is the only drug candidate to have reached clinical phase II

studies. PI-88 is a mixture of highly sulfonated mannan oligosaccharides, consisting of predominantly penta- and tetra-sized species. They were isolated from the yeast *pichia pastoris*.[27] It is postulated that it has dual mode of action not only inhibiting the enzymatic activity but also perturbing the binding or action of HS-bound growth factors. Also combinatorial chemistry efforts have yielded synthetic analogs of PI-88 with promising activity.[28] It has been suggested that Heparins, and in particular the low-molecular-weight species (LMWH), have a beneficial effect on patients with advanced cancer.[29]

Another natural product inhibitor is the trachypsic acid: (Figure 3.9)[30]

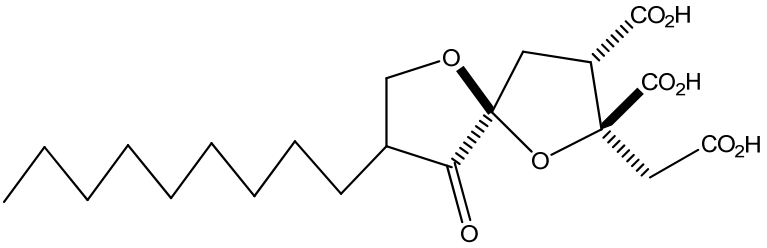
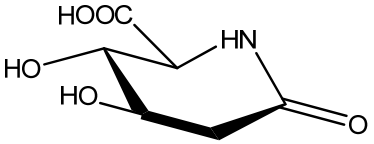
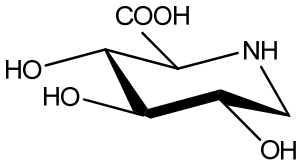


Figure 3.9 Trachypsic acid

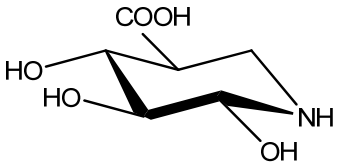
Finally, some aza-sugar inhibitors are known to inhibit β-glucoronidases such as heparanase.[31] (Figure 3.10):



D-Glucaro-δ-lactam (Ki ~39nM



Uronic acid deoxynojirimycin derivative Ki~80μM



Glucuronic acid-type iminosugar Ki~79nM)

Figure 3.10 Some sugar amino-acid inhibitors of heparanase

From all the previous data, the need to develop inhibitors of these carbohydrate processing enzymes becomes apparent. Thus far, these polyhydroxylated cyclic amines (termed aza- or 1-imino-sugars) have proved tedious to synthesize in satisfactory quantities. The presence of 3-4 asymmetric centers and the lack of a well-established methodology for introducing the amino (methylene) functionality in a stereoselective fashion make their synthesis problematic. In the case of heparanase, the use of macromolecules such as heparins as inhibitors is not practical, for they can have non-

specific effects and may not susceptible to chemical modifications that will improve their specificity/activity.

The synthetic efforts stated in Chapters IV and V focus on the achievement of the crucial ring forming cyclization step with concomitant stereoselective introduction of precursors to carboxyl in positions 2 or 3 (relative to the endocyclic nitrogen), which seem to be necessary structural elements for the activity of these heparanase inhibitors. For the other stereocenters, there are available or readily accessible precursors that contain them in the proper stereochemistry.

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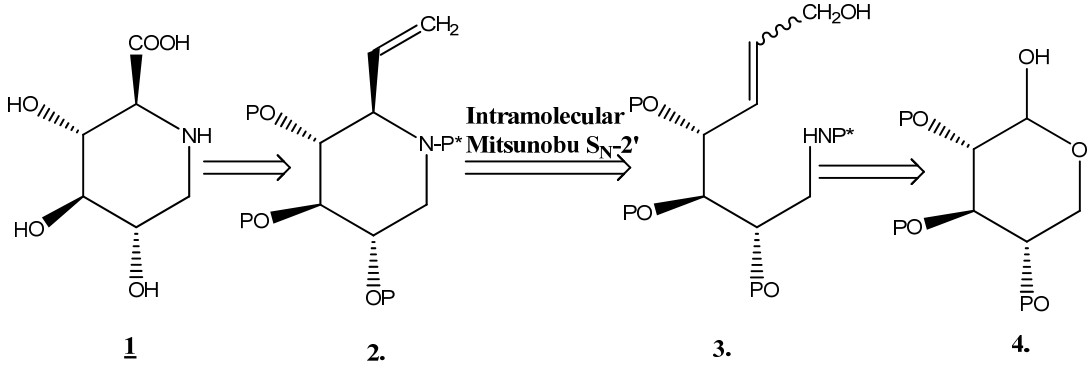
CHAPTER IV

α-SUGAR AMINO-ACID STEREOGENIC CENTER INTRODUCTION

4.1 Initially Proposed Synthetic Approach

In the case of 2-carboxy substituted or α-sugar amino-acids and their analogs an application of an unprecedented intramolecular S_N-2' Mitsunobu 6-exo-trig reaction for the critical cyclization step was proposed. As exemplified in the retrosynthetic analysis below (Scheme 4.1), (2S,3R,4R,5S)-3,4,5-trihydroxy-piperidine-2-carboxylic acid **(1)** can be derived from intermediate **(2)** using standard oxidation/deprotection reactions. **(2)** is the product of the intramolecular Mitsunobu reaction product. The substrate for this reaction **(3)** should be readily accessible from the suitably protected L-xylose **(4)**.

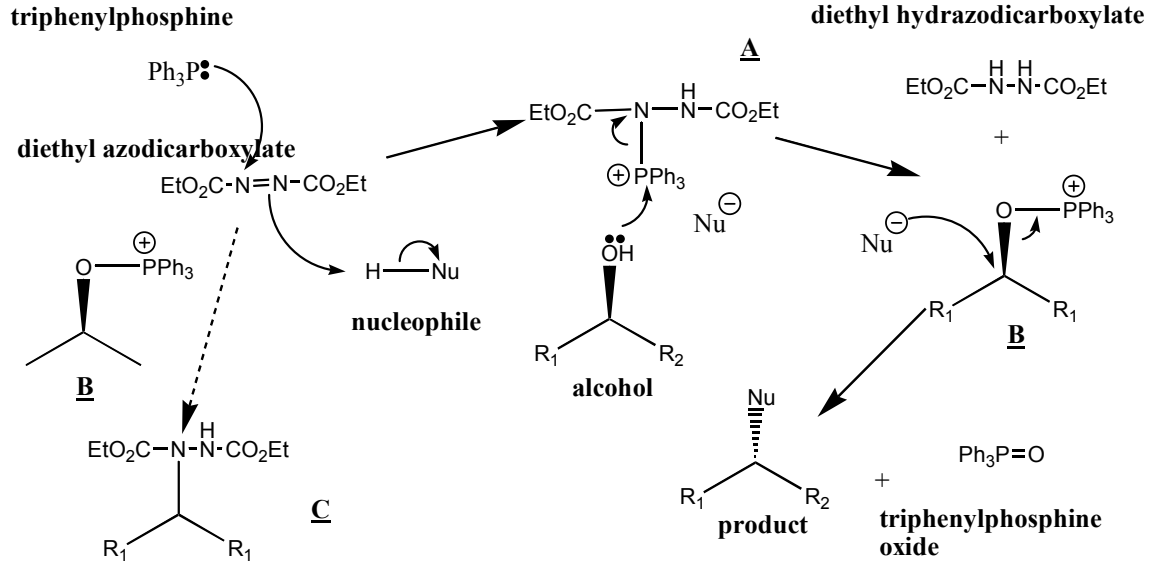


Scheme 4.1 Retrosynthetic analysis for (2S,3R,4R,5S)-3,4,5-trihydroxypiperidine-2-carboxylic acid.

4.2 Mitsunobu Reaction: S_N2 vs S_N2' and syn vs anti attack dichotomies

The Mitsunobu reaction involves the one pot nucleophilic substitution of alcohols with clean inversion of stereochemistry, without any prior activation.[1,2,3] With this reaction, a variety of Bronsted-Lewis acid nucleophiles can be transformed into a single

step and high yields to esters, ethers, amides, imides, sulfonamides, azides, nitriles, halides, which can be further converted to amines and other products. This transformation is achieved using a "redox" system of trialkylphosphine and an azodicarboxylate (typically triphenyl-phosphine and diethyl azodicarboxylate). The reaction is believed to proceed in three steps (Scheme 4.2):[2,3]

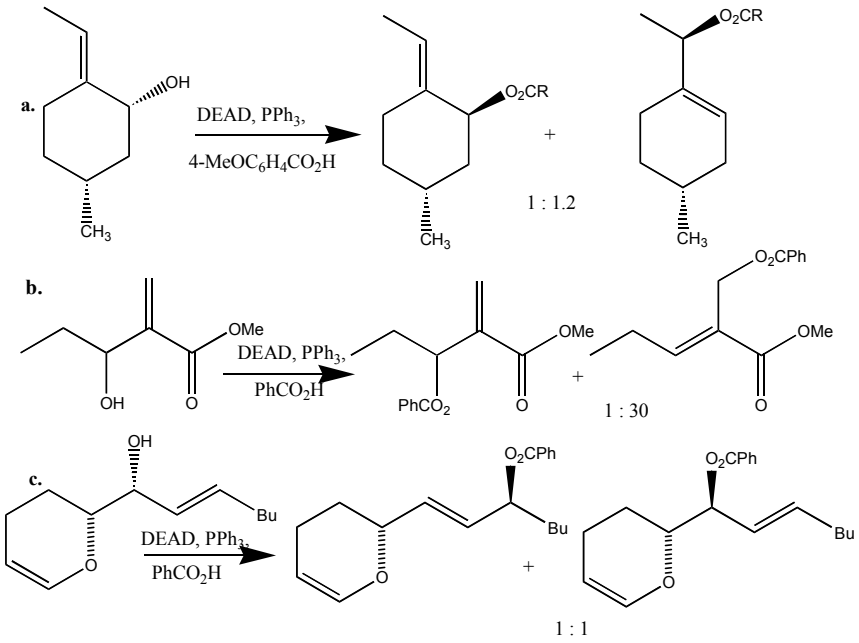


Scheme 4.2 Current mechanism for Mitsunobu reaction.

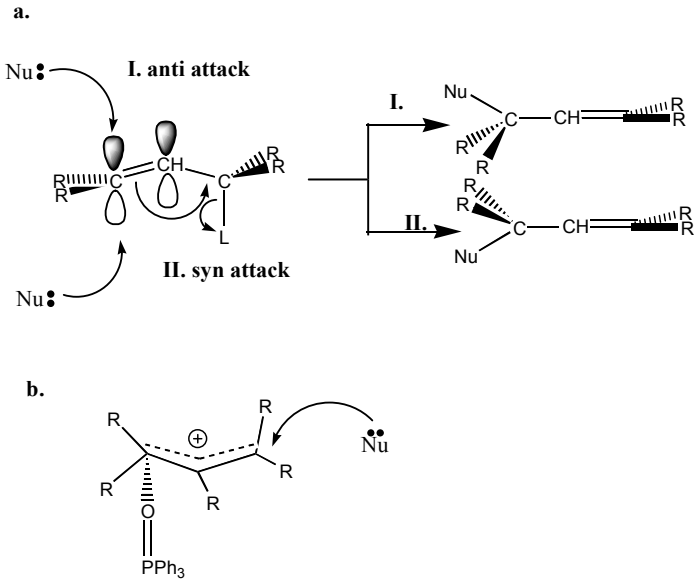
In the first step, triphenylphosphine rapidly reacts with DEAD to form the phosphonium salt adduct **A** after protonation by the acid nucleophile Nu-H. Then, the alcohol gets activated as an oxyphosphonium salt **B** that is primed for nucleophilic attack to give the product with inverted configuration along with triphenylphosphine oxide as a byproduct. Although versatile, the Mitsunobu reaction has a serious limitation; if the acidic proton on Nu-H has a pK_a larger than 11, the yield drops significantly and if larger than 13, the reaction does not occur at all.[3] In these cases the Zwitterionic adduct from the first step does not get protonated to form **A** but instead attacks any **B** formed to furnish alkylated hydrazine derivative **C**. Later new "redox" systems have been developed which allow for efficient reactions with nucleophiles of pK_a up to 15.[4,5,6,7]

The intramolecular versions of Mitsunobu reaction are, in many cases, the exception to the acidity requirement and several cyclizations with even simple amines displacing hydroxyls have been reported.[7] In addition, some intermolecular S_N2' displacements of allylic alcohols have also been observed in cases where the double bond

is *exo* to a ring[8] (Scheme 4.3a), or is prone to Michael attack[9] (Scheme 4.3b) or due to steric hindrance of the alcohol[10] (Scheme 4.3c). There are reports that decreased acidity of Nu-H,[9] addition of a Pd catalyst[11] or addition of stoichiometric amounts of triethylamine[9] improves the S_N2':S_N2 ratio. Two cases of an intramolecular S_N2' Mitsunobu reaction have been reported in the literature.[12,13]



Scheme 4.3 Some cases of S_N2' Mitsunobu reactions.

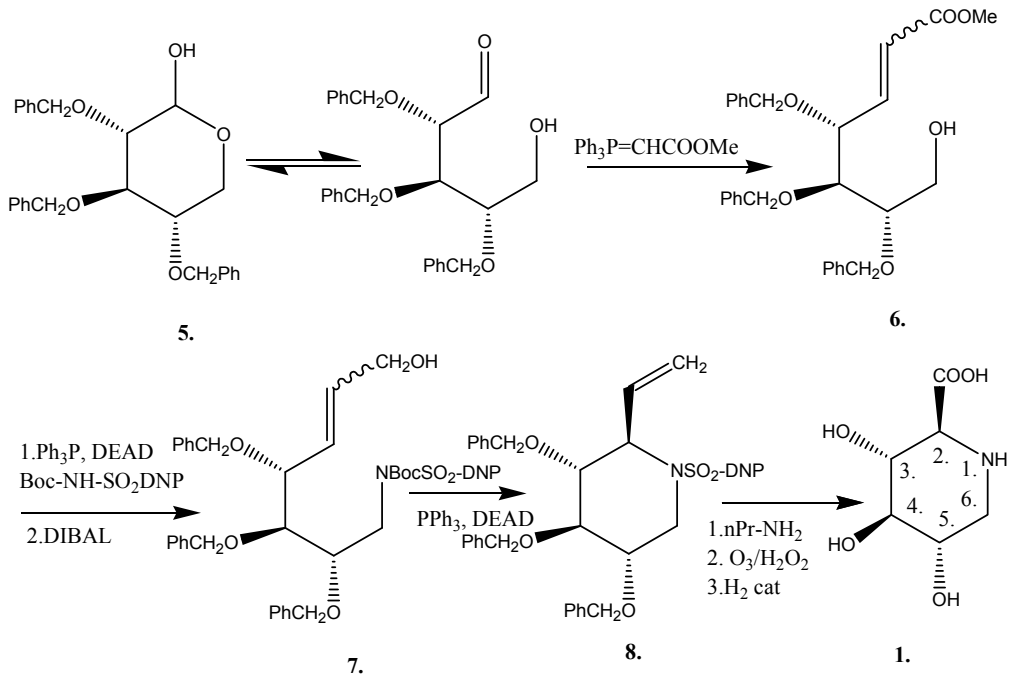


Scheme 4.4 The two possible ways of S_N2' attack.[16,18]

Generally, S_N2' reactions occur through a *syn* process rather than an *anti* one (Scheme 4.4a), although this is still controversial.[14-17] Three factors are believed to influence the trajectory of attack: i) the aromaticity of the *syn* transition state[15], ii) an energy consuming "inversion" in the central carbon atom occurring only in the *anti* reaction coordinate[14], and iii) an electrostatic repulsion between the incoming nucleophile and the departing leaving group in the *syn* mode of attack. Experimentally, though, it has been consistently observed that intermolecular S_N2' Mitsunobu reactions give the product resulting from *anti* mode of attack.[18] To rationalize it, it has been proposed that the reaction rather proceeds by a partial S_N1 pathway in which the leaving group $Ph_3P{=}O$ shields the *syn* phase of the allylic cation allowing for attack only in an *anti* fashion (Scheme 4.4b).[18] A similar stereochemical outcome is expected for this case and considering the steric factors of the ring to be formed (that can be manipulated accordingly), both epimers at that center can be accessed, as it is shown later in the discussion (see Scheme 4.6).

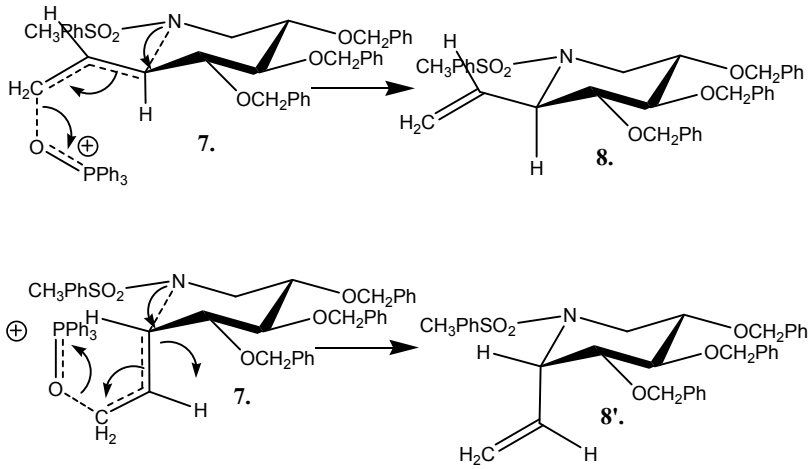
4.3 Progress in Synthesis of (2S,3R,4R,5S)-3,4,5-trihydroxypiperidine-2-carboxylic Acid

We propose that the uronic acid derivative of deoxynojirimycin (2S,3R,4R,5S)-3,4,5-trihydroxypiperidine-2-carboxylic acid **(1)**, a moderate inhibitor of heparanase[19], can be synthesized starting from $_L$-xylose, (Scheme 4.5). Thus, Wittig's condensation of benzylated $_L$-xylose **(5)** with (carbomethoxymethylidene)-triphenyl-phosphorane yields the conjugated ester **(6)**, which upon Mitsunobu coupling with 2,4-dinitro-sulfonamide and reduction with DIBAL yields **(7)**, the precursor for the intramolecular Mitsunobu reaction. After the intramolecular Mitsunobu reaction of **(7)**, piperidine **(8)** is oxidized and deprotected to give the title compound **(1)**.



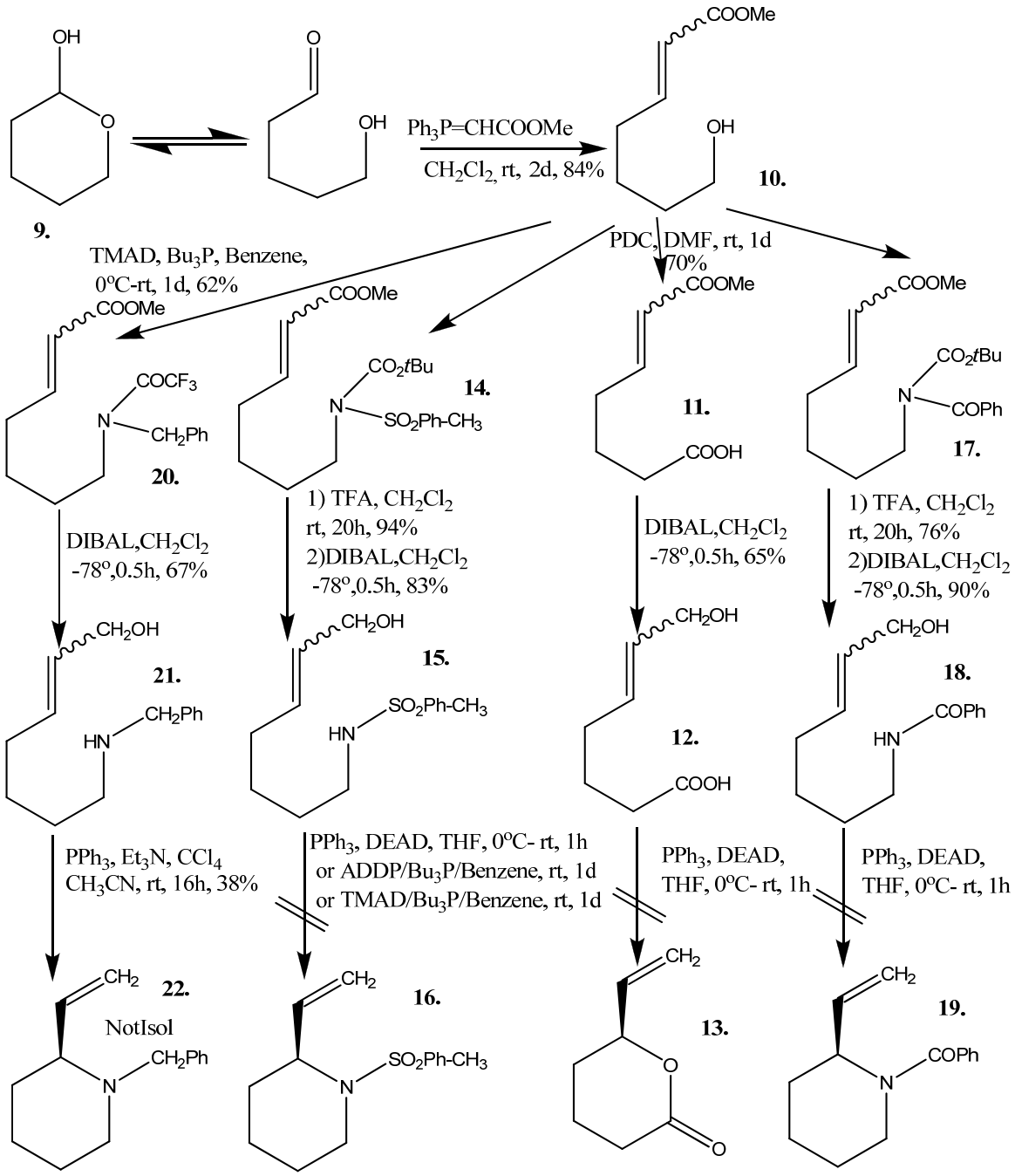
Scheme 4.5 Proposed synthesis of (2S,3R,4R,5S)-3,4,5-trihydroxypiperidine-2-carboxylic acid.

The stereochemical outcome can be rationalized and it assumes a chair-like transition state in the reaction of the E-isomer of **(7)**, (Scheme 4.6). The substituents on the ring are preferentially accommodated in the equatorial plane and this preorganization of the ground state allows the nucleophile attack proceed in an *anti*-trajectory shown, that gives rise to the desired product **(8)**. The alternative conformer of the alkene would place the allylic oxyphosphonium group in an axial position, which is strongly disfavored due to diaxial steric interactions.

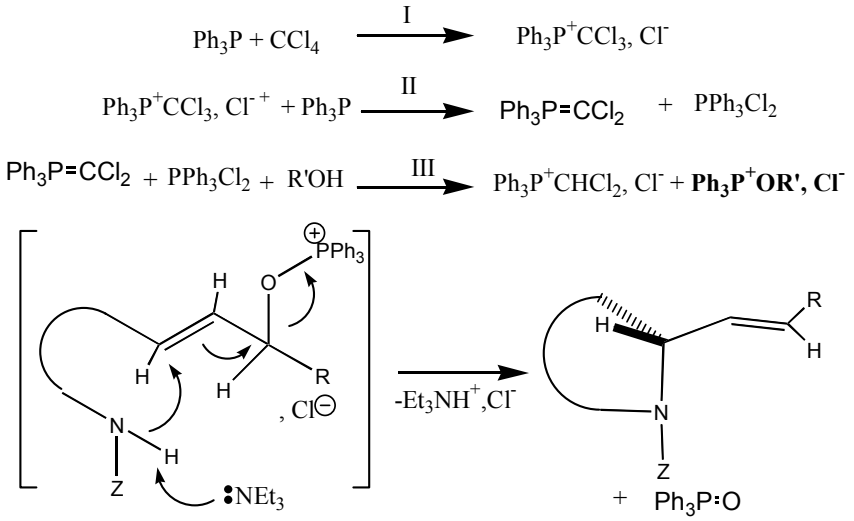


Scheme 4.6 Rationalization of the expected stereoselectivity

Preliminary model reactions have been performed to examine the feasibility of the intramolecular Mitsunobu reaction (Scheme 4.7). Initially, intramolecular ring closure was attempted using carboxylic acid **(12)** a typical Mitsunobu nucleophile. Thus, 2-hydroxy-2H-tetrahydropyran **(9)** was reacted with methyl (triphenylphosporanylidene)-acetate to give methyl 7-hydroxy-hept-2-enoate **(10)**. This was oxidized with PDC to carboxylic acid **(11)**, which was then reduced with DIBAL to give allylic alcohol **(12)**, a suitable intramolecular Mitsunobu precursor. Repeated attempts to cyclize it in high dilution led to unidentifiable products of intermolecular condensations. Next, a different less acidic nucleophile was made, a sulfonamide **(10)** underwent Mitsunobu condensation with the quite acidic *t*-Boc derivative of *p*-toluenesulfonamide to give product **(14)**. Removal of the *t*-Boc group with TFA followed by DIBAL reduction as afforded the Mitsunobu precursor **(15)**. Repeated attempts using some novel Mitsunobu reagents—suitable for compounds with larger pK_a and tributyl-phosphine—failed to produce any of the desired product **(16)**. Another approach involved the construction of a secondary amine precursor **(18)**. Alcohol **(10)** was condensed with *N*-benzyl- trifluoroacetamide using the modified "redox" system TMAD/Bu_3P, which is effective for nucleophiles of larger pK_a (up to 15). Removal of the trifluoroacetamido group and reduction of the ester was accomplished in a single step with DIBAL to afford **(21)** in a fair yield. Then a different combination of reagents to activate the allylic hydroxyl via its oxyphosphonium derivative was used. Triphenylphosphine is known to react with alcohols in the presence of CCl_4 to generate in three steps the same type of reactive intermediates (Scheme 4.8).[20,21] When **(18)** was subjected to this reaction, a mixture of products that contained NMR evidence of piperidine **(19)** as the major product formed. The small scale of the reaction made further characterization difficult but at least we have a clue that our strategy may work.

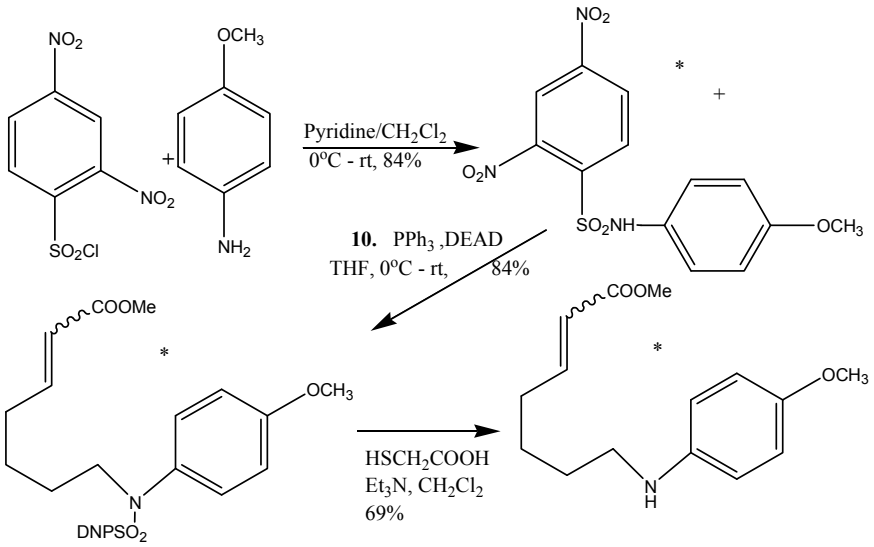


Scheme 4.7 Model reactions to probe intramolecular S_N2' Mitsunobu.



Scheme 4.8 Mechanism for generation of oxyphoshonium alcohol derivative and subsequent triethylamine promoted cyclization.[21]

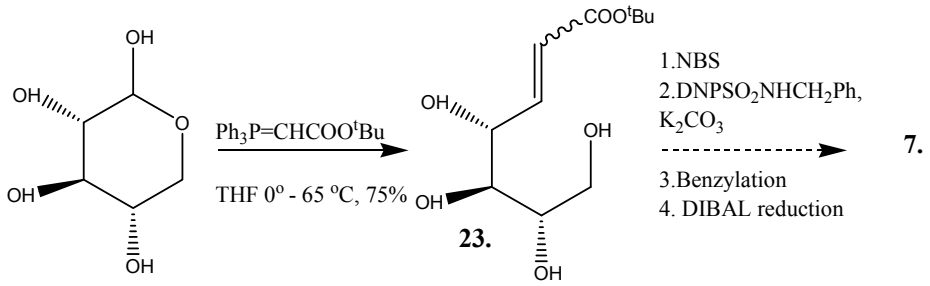
Subsequently, higher yielding access to S_N2' precursors was achieved using dinitrophenylsulfonyl chemistry. [22] (Scheme 4.9)



Scheme 4.9 Improved access to intramolecular S_N2' Mitsunobu precursors.

Our initial success prompted us to continue pursuing the intramolecular S_N2' Mitsunobu reaction and to continue with the actual synthesis. The preorganization of the substituted precursor is expected to favor the intramolecular closure. However, the HWE condensation of 2,3,4-tribenyl-L-xylose with methyltriphenylpsosphoranylidene acetate didn't produce the desired α,β-unsaturated ester **(6)** under various attempted conditions.

Intramolecular Michael reaction of the product and/or eliminations complicated the reaction and resulted in unresolvable mixtures. So according to literature[23], we sought an alternative route using the unprotected L-xylose and *t*-butyl ylide (as opposed to the methyl one).



Scheme 4.10 Proposed completion of the synthesis.

Subsequent bromination of the primary hydroxyl, benzylation under neutral conditions and introduction of the nitrogen moiety should afford an intermediate which can be used for the successful synthesis of the cyclization substrate **(7)**. (Scheme 4.10) Alternatively selective protection with TBDMS group on the primary hydroxyl followed by benzylation, TBDMS deprotection and introduction of the nitrogen containing group is also a viable route.

4.4 Conclusion

The feasibility of the critical intramolecular S_N2' Mitsunobu reaction was demonstrated. The model substrates were unsubstituted aliphatic chains and, thus, less likely to assume the proper 3D conformation that will bring the reactive centers proximal to each other. Instead intermolecular reactions seem to have dominated all the attempts. It is expected that because of the stereochemical biases of the actual substrate it will provide better results regarding the intramolecular reaction.

It was untoward that xylose did not furnish the methyl-ester synthetic intermediate following literature procedures. It seemed that a lot of side-reactions occurred, thereby diminishing the amount of the conjugated methyl ester produced. The t-butyl ester provided a plausible alternative and the completion of the synthesis can be attained.

4.5 References

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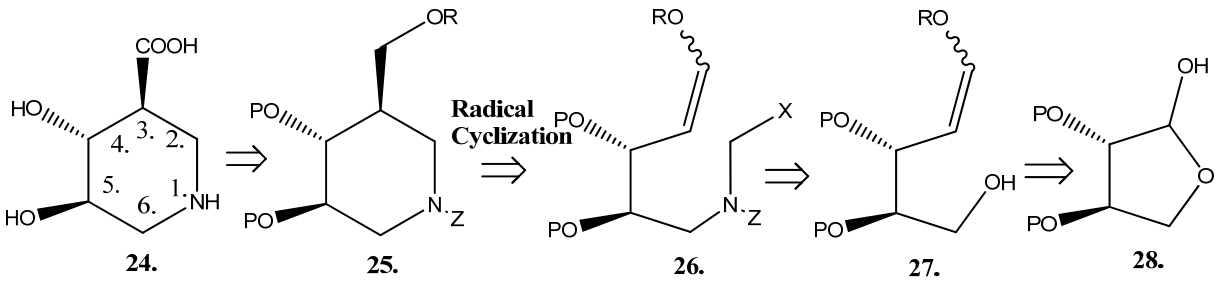
CHAPTER V

β–SUGAR AMINO-ACID STEREOGENIC CENTER INTRODUCTION

5.1 Initially Proposed Synthetic Approach

For the case of 3-substituted azasugars (or β-sugar amino-acids), we propose a radical cyclization approach[1]. As exemplified in the retrosynthetic analysis below (Scheme 5.1), potent β-glucuronidase inhibitor (3S,4R,5R)-4,5-dihydroxy-piperidine-3-carboxylic acid[2,3] **(24)** can be derived from **(25)** using oxidation/deprotection reactions. **(25)** is the product of an α-aminomethyl radical cyclization reaction of a suitable precursor **(26),** which should be accessible from alcohol **(27)** using an intermolecular Mitsunobu reaction. The substrate for this reaction can be obtained from the protected D-threose **(28)**.



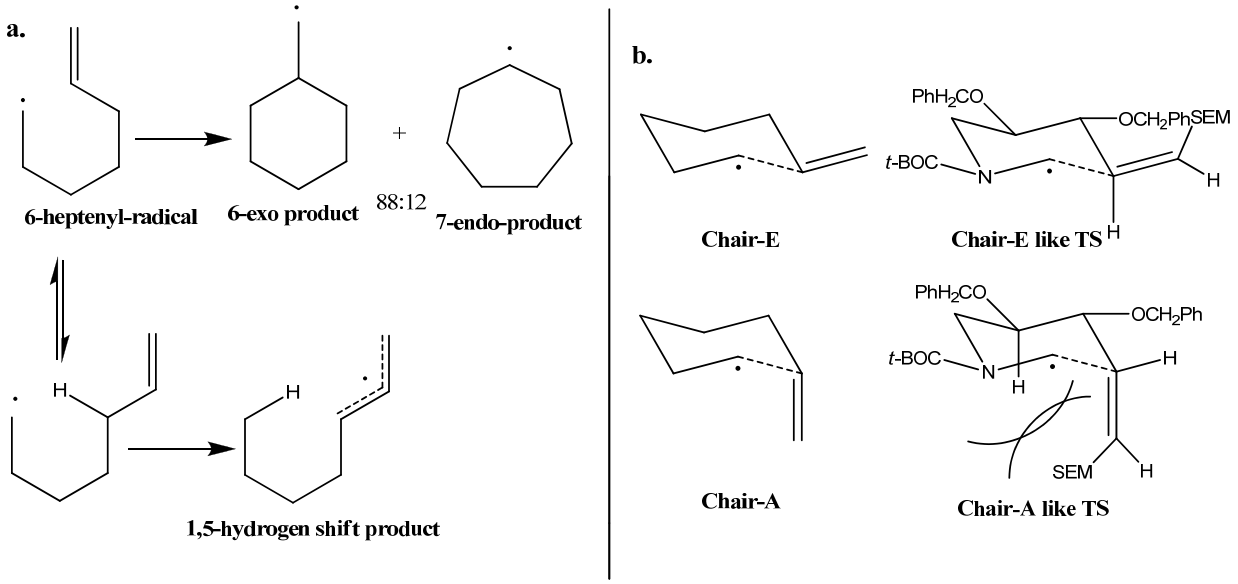
24. **25.** **26.** **27.** **28.**

Scheme 5.1 Retrosynthetic analysis for 3S,4R,5R-4,5-dihydroxy-piperidine-3-carboxylic acid.

5.2 α-Aminomethyl Radical Cyclization

The key step in this synthesis is the 6-exo-trig cyclization of the 6-heptenyl radical. For a successful radical cyclization, the rate of ring closure is of great importance because it must be faster than the reaction with the radical trapping reagent. Also, each of

the reaction steps must be faster than the unwanted side reactions (reaction with solvent, radical recombination, 1,5-hydrogen shift etc). 6-exo cyclizations are much slower that the 5-exo and also slower than the respective competing 7-endo pathway; the 6-heptenyl radical reacts 7 fold faster in a 6-exo fashion than the competing 7-endo way. For a comparison, 5-exo is 50 times faster than 6-endo.[1] (Scheme 5.2 a).



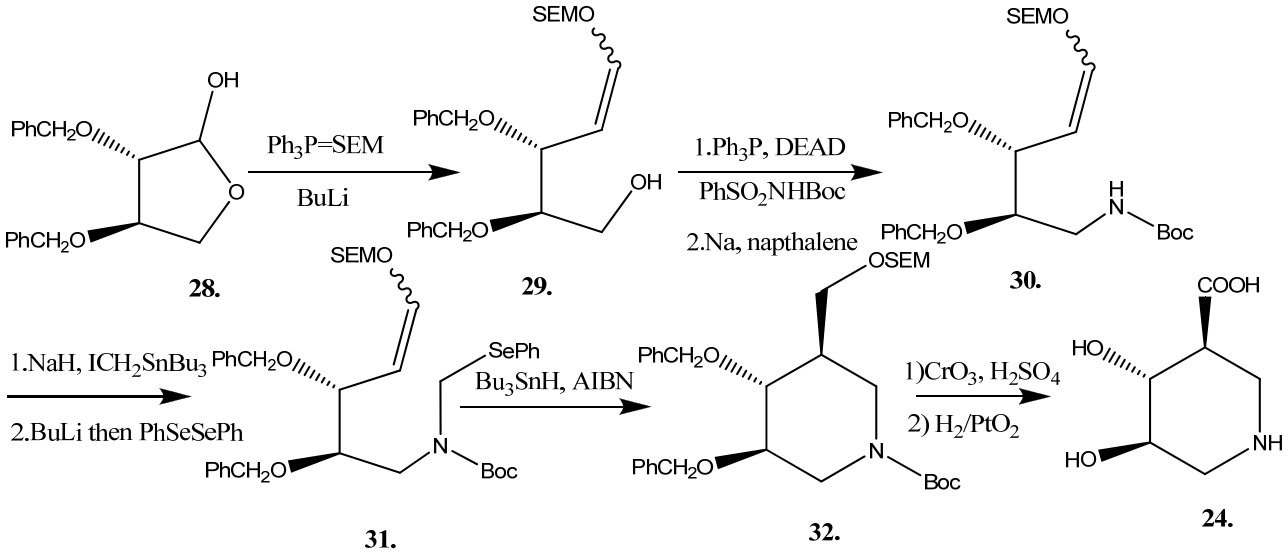
Scheme 5.2 a. Regioselectivity of 6-heptenyl radical cyclization. b. Stereoselectivity of the proposed radical cyclization.

The stereoselectivity is explained (Scheme 5.2 b) by the greater stability of the "chair-E" transition state compared to the "chair-A" in which the double bond suffers repulsive diaxial interactions. In this radical cyclization, assuming that all the substituents on the ring lie in the equatorial orientation, it is anticipated that for both E- and Z-isomers there will be a strong preference for the chair-E like TS that should result in the desired stereoselectivity.

The existence of a nitrogen atom adjacent to the free-radical greatly influences its stability/reactivity/formation.[4] Because of its lone pair of electrons, the nitrogen can stabilize the radical through resonance.[5] Consequently, it is easy to form but less reactive than what one might desire. It is also very nucleophilic, which means that it will not react with electron rich alkenes i.e. there is a polarity mismatch.[6] One way to circumvent these problems is to attach an electron withdrawing group (EWG) on the nitrogen. This way, the lone pair of electrons will be delocalized towards the EWG and less towards the

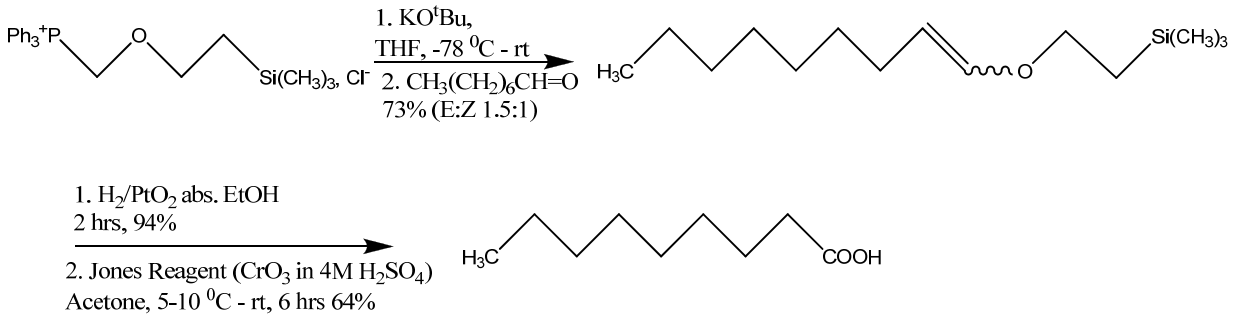
free radical, reducing its SOMO and nucleophilicity rendering it more reactive. Therefore, in the current synthesis there is a Boc group attached on the nitrogen. Also there is a preference to use the trimethylsilylethoxy (SEM) O- protecting group because it can be introduced and provide an additional carbon atom on an aldehyde substrate and then be removed and oxidized to the homologated carboxylic acid in one step with the Jones reagent (*vide infra*).

5.3 Progress in Synthesis of (3S,4R,5R)-4,5-dihydroxypiperidine-3-carboxylic Acid



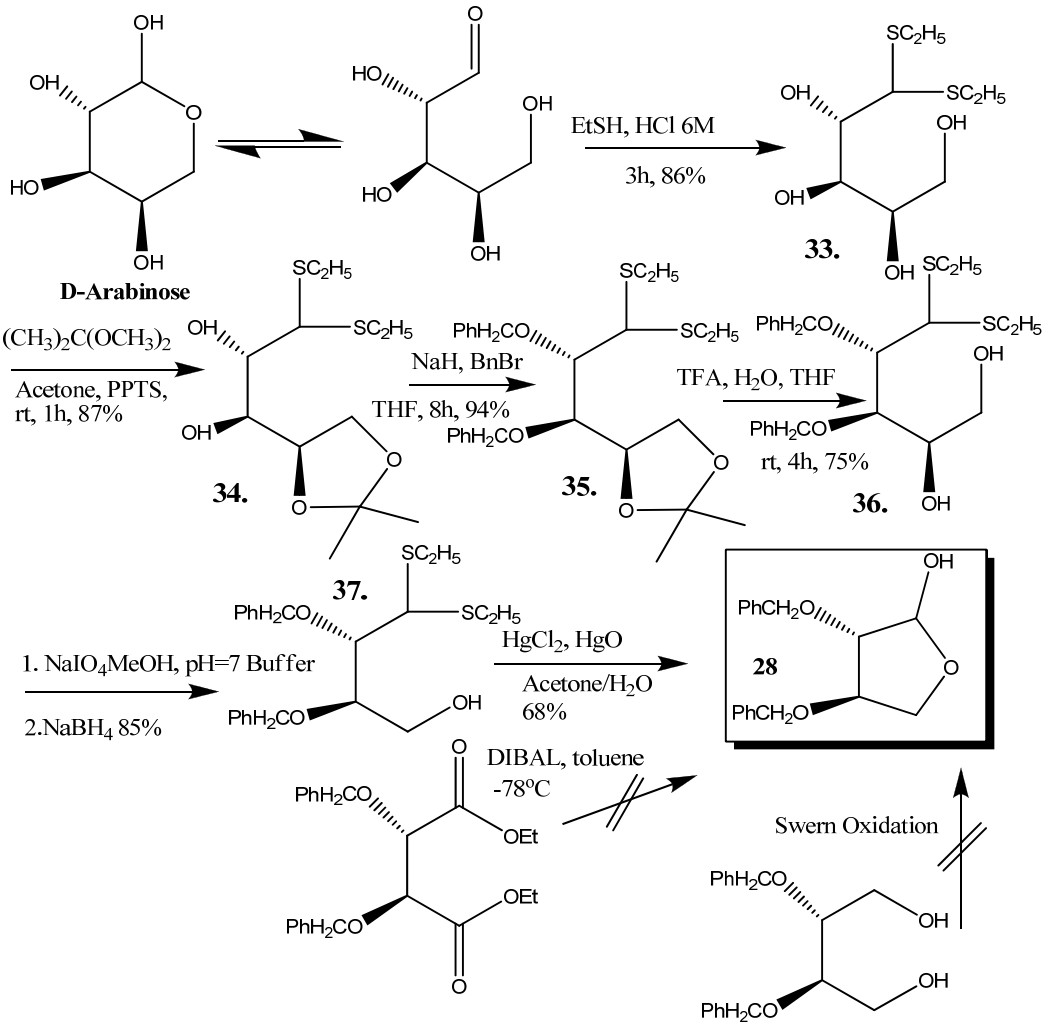
Scheme 5.3 Proposed synthesis of (3S,4R,5R)-4,5-dihydroxypiperidine-3-carboxylic acid, **(24)**.

The proposed synthesis of the potent heparanase inhibitor (3S,4R,5R)-4,5-dihydroxypiperidine-3-carboxylic acid **(24)**, is shown in Scheme 5.3. This compound was previously synthesized in a tedious, multistep way[3] with a stereoselectivity of 4:1 for the newly introduced stereocenter. Starting from 1-hydroxy-2,3-bis(phenylmethyloxy)tetrahydrofuran **(28)**, Wittig condensation with [(2-trimethylsilyl)ethoxymethylidene]-triphenyl phosphorane should afford olefine **(29)**. Mitsunobu[7] substitution of the hydroxyl group with N-Boc-benzenesulfonamide and reductive removal of the sulfonamido group gives **(30)**, which should give the radical precursor **(31)** in two steps. Radical cyclization is expected to afford cyclic product **(32),** which upon Jones oxidation and catalytic hydrogenation should give the desired product **(24)**. The one step deprotection-oxidation of the homologated SEM-enolether was examined and optimized on a model substrate. (Scheme 5.4)

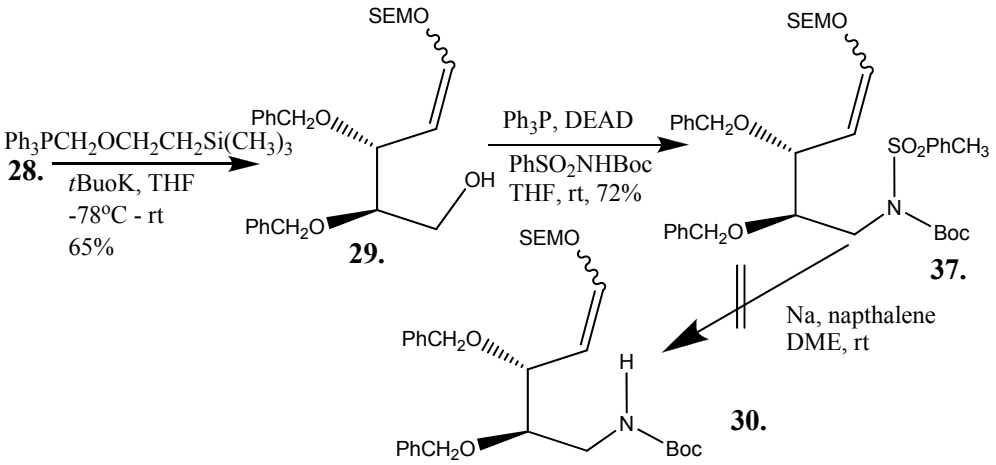


Scheme 5.4 Model reactions for the one-step oxidation/deprotection of the SEM O-protecting group to carboxylic acid.

When attempted, oxidation of 2S, 3S-dibenzyloxy-1,4-butandiol or partial reduction of 2,3-dibenzyl-L-(+)-diethyl tartrate both failed to produce the desired protected D-Threose **(28)**. So a longer and more tedious procedure was sought[8]: (Scheme 5.5) D-Arabinose diethyl thioacetal **(33)** was formed followed by kinetic acetonide **(34)** formation. Subsequent benzylation of the remaining hydroxyls followed by selective removal of the acetonide protecting group afforded diol **(35)**. Hyperiodic acid oxidation followed by reduction with $NaBH_4$ and final deprotection of the thioacetal furnished **(28)** in satisfactory amounts. With **(28)** in-hand, the synthesis continued with the Wittig reaction of **(28)** with (2-trimethylsilyl)-ethoxy-methylidene-triphenyl-phosphorane (Scheme 5.4). Mitsunobu reaction of product **(29)** with 4-methyl-benzensulfonyl-*t*-butoxy-carbonyl-amide led to product **(37)**. Attempts to remove the benzensulfonyl group under dissolving metal conditions led to an intractable mixture of products (Scheme 5.6).

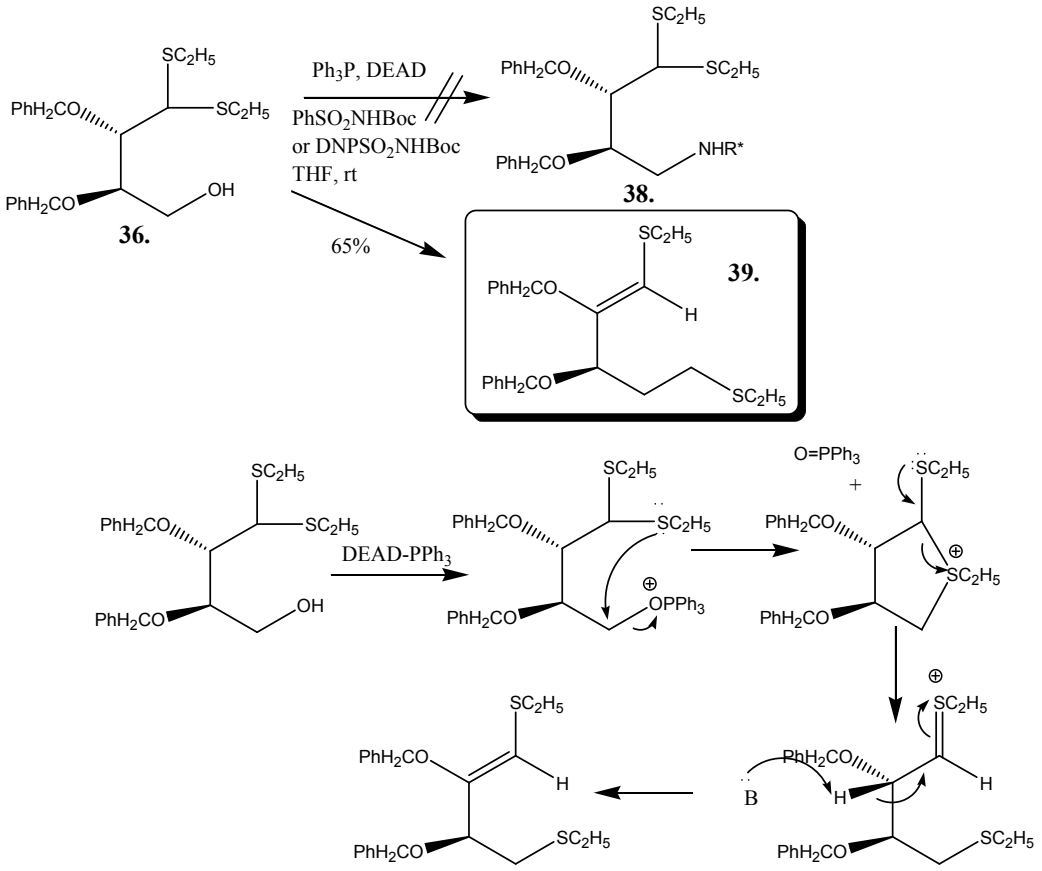


Scheme 5.5 Synthesis of 1-hydroxy-2,3-bis(phenylmethyloxy)-tetrahydrofuran **(28)**



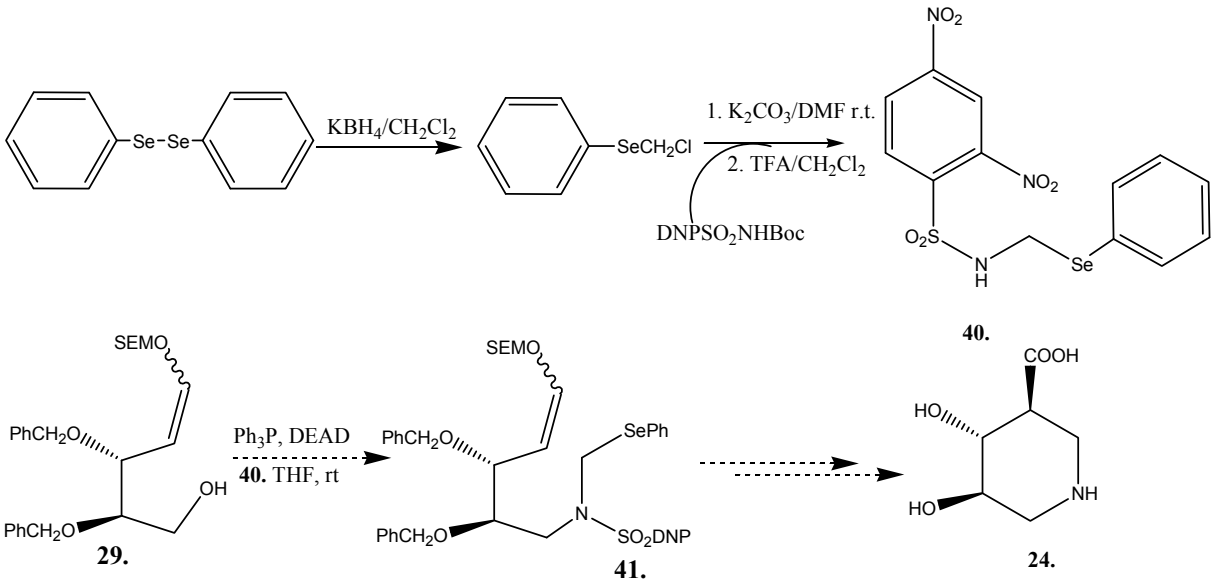
Scheme 5.6 Failed attempts to carry synthesis further.

An alternate route was pursued which would install the trimethylsilylethoxyalkylidene group after the Mitsunobu reaction. So the still protected thioacetal **(36)** was subjected to the same Mitsunobu reaction that worked before. Instead of the expected thioacetal **(38)** the product turned out to be an unprecedented thioenolether **(39)**. A possible mechanism is shown below (Scheme 5.7).



Scheme 5.7 Unprecedented intramolecular Mitsunobu reaction and mechanism.

An alternative route using dinitrophenylsulfonyl based Mitsunobu reagents[9] to introduce the endocyclic nitrogen can resolve the problem of the phenylsulfonyl group deprotection. Diphenyl diselenide is converted to chloromethyl phenyl-selenide[10] and reaction with the quite acidic N-Boc-dinitrophenylsulfonamide affords selenide **(40)** after removal of the Boc protecting group. (Scheme 5.8) Then it can be used to carry out the synthesis as planned. The strong electron withdrawing effect of the dinitrophenylsulfonyl group could also promote the radical cyclization reaction as explained above.



Scheme 5.8 Alternative route incorporating the benzyl-seleno moiety using Mitsunobu chemistry.

5.4 Conclusion

The radical cyclization precursor was not synthesized. Still, considerable knowledge was gained by exploring the different ways to reach the radical substrate. Literature procedures didn't seem to work all the time, so different paths were sought out. The unexpected thioenolether formation upon Mitsunobu conditions remains to be further explored—both mechanistically and also for organic syntheses. It would be very interesting to see the outcome of a reaction of a mixed thioselena- or thio-oxa acetal. The resulting molecule has an endocyclic double-bond and a thioether. If selenium of a mixed thioselena-acetal is more nucleophilic and migrates then a useful carbocyclic radical cyclization precursor may be available in this one step.

5.5 References:

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Appendices

Appendix A: UM Drug Assay Class schedule

Instructor 1

Week	Lecture Topic (T-Th)	Lab Exercise (M-W)
1st	Biopharmaceutical Analysis	
	Statistical Treatment of Analytic Data	
2nd		**Introduction to LabTechniques (All Groups)**
	Titration and Buffers	
		Introduction to LabTechniques (All Groups)
	UV Spectroscopy	
3rd		Titration (Group A)
		Visible Spectroscopy (Group B)
		UV/Fluorescence Spectroscopy (Group C)
	UV Spectroscopy	
		Titration (Group A)
		Visible Spectroscopy (Group B)
		UV/Fluorescence Spectroscopy (Group C)
	Visible Spectroscopy	
4th		Titration (Group B)
		Visible Spectroscopy (Group C)
		UV/Fluorescence Spectroscopy (Group A)
	Fluorescence spectroscopy	
		Titration (Group B)
		Visible Spectroscopy (Group C)
		UV/Fluorescence Spectroscopy (Group A)
	Fluorescence spectroscopy	
5th		Titration (Group C)
		Visible Spectroscopy (Group A)
		UV/Fluorescence Spectroscopy (Group B)
	Mass Spectroscopy	
		Titration (Group C)
		Visible Spectroscopy (Group A)
		UV/Fluorescence Spectroscopy (Group B)
	Mass Spectroscopy	
6th		**HPLC (Group A)**
		GC/MS (Group B)
		Enzyme Stability (Group C)
	Exam 1	
		HPLC (Group A)
		GC/MS (Group B)
		Enzyme Stability (Group C)

Table A1: Instructor 1 schedule of classes and labs. Schedule conflicts are highlighted

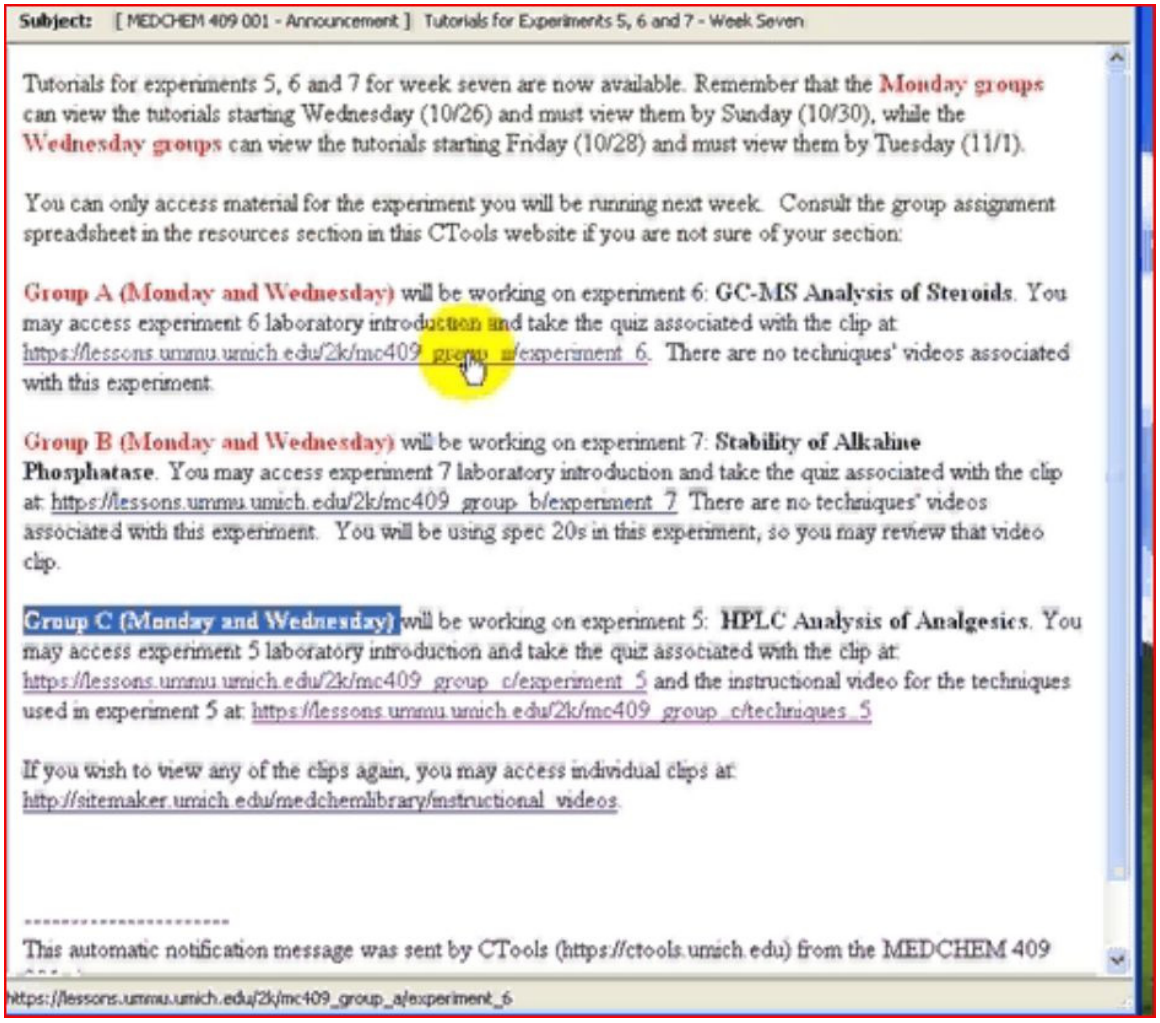
Instructor2

Week	Lecture Topic (T-Th)	Lab Exercise (M-W)
6th	Liquid/liquid extraction	
7th	Chromatography Theory	Fall Break
8th		**HPLC (Group B)**
		GC/MS (Group C)
		Enzyme Stability (Group A)
	Gas Chromatography	
		HPLC (Group B)
		GC/MS (Group C)
		Enzyme Stability (Group A)
	Reverse and Normal Phase HPLC	
9th		HPLC (Group C)
		GC/MS (Group A)
		Enzyme Stability (Group B)
	Ion Exchange and gel permeation HPLC	
		HPLC (Group C)
		GC/MS (Group A)
		Enzyme Stability (Group B)
	Separation of Stereoisomers	
10th		Colorimetric assay (Group A)
		Gel Electrophoresis (Group B)
		HPLC Internal Standards (Group C)
	Electrophoresis	
		Colorimetric assay (Group A)
		Gel Electrophoresis (Group B)
		HPLC Internal Standards (Group C)
	Electrophoresis	
11th		Colorimetric assay (Group B)
		Gel Electrophoresis (Group C)
		HPLC Internal Standards (Group A)
	Analysis of Protein and Enzyme Drugs	
		Colorimetric assay (Group B)
		Gel Electrophoresis (Group C)
		HPLC Internal Standards (Group A)
	Immunoassay	

Week	Lecture Topic (T-Th)	Lab Exercise (M-W)
12th		Colorimetric assay (Group C)
		Gel Electrophoresis (Group A)
		HPLC Internal Standards (Group B)
	Exam 2	
		Colorimetric assay (Group C)
		Gel Electrophoresis (Group A)
		HPLC Internal Standards (Group B)
13th	Immunoassays	
	Guest Lecture	
14th	Guest Lecture	
		Lab Competency Exam
	Radioisotopes	
		Lab Competency Exam
15th	Final Exam	

Table A2: Instructor 2 schedule of classes and labs. Schedule conflicts are highlighted

Appendix B: Representative samples of new Drug Assay laboratory instruction methods



Subject: [MEDCHEM 409 001 - Announcement] Tutorials for Experiments 5, 6 and 7 - Week Seven

Tutorials for experiments 5, 6 and 7 for week seven are now available. Remember that the **Monday groups** can view the tutorials starting Wednesday (10/26) and must view them by Sunday (10/30), while the **Wednesday groups** can view the tutorials starting Friday (10/28) and must view them by Tuesday (11/1).

You can only access material for the experiment you will be running next week. Consult the group assignment spreadsheet in the resources section in this CTools website if you are not sure of your section.

Group A (Monday and Wednesday) will be working on experiment 6: **GC-MS Analysis of Steroids**. You may access experiment 6 laboratory introduction and take the quiz associated with the clip at: https://lessons.ummu.umich.edu/2k/mc409_group_a/experiment_6. There are no techniques' videos associated with this experiment.

Group B (Monday and Wednesday) will be working on experiment 7: **Stability of Alkaline Phosphatase**. You may access experiment 7 laboratory introduction and take the quiz associated with the clip at: https://lessons.ummu.umich.edu/2k/mc409_group_b/experiment_7 There are no techniques' videos associated with this experiment. You will be using spec 20s in this experiment, so you may review that video clip.

Group C (Monday and Wednesday) will be working on experiment 5: **HPLC Analysis of Analgesics**. You may access experiment 5 laboratory introduction and take the quiz associated with the clip at: https://lessons.ummu.umich.edu/2k/mc409_group_c/experiment_5 and the instructional video for the techniques used in experiment 5 at: https://lessons.ummu.umich.edu/2k/mc409_group_c/techniques_5

If you wish to view any of the clips again, you may access individual clips at: http://sitemaker.umich.edu/medchemlibrary/instructional_videos.

This automatic notification message was sent by CTools (https://ctools.umich.edu) from the MEDCHEM 409

https://lessons.ummu.umich.edu/2k/mc409_group_a/experiment_6

Illustration B1: Weekly class e-mail inviting each group to work on the online tutorials of their respective laboratory exercise including their website links

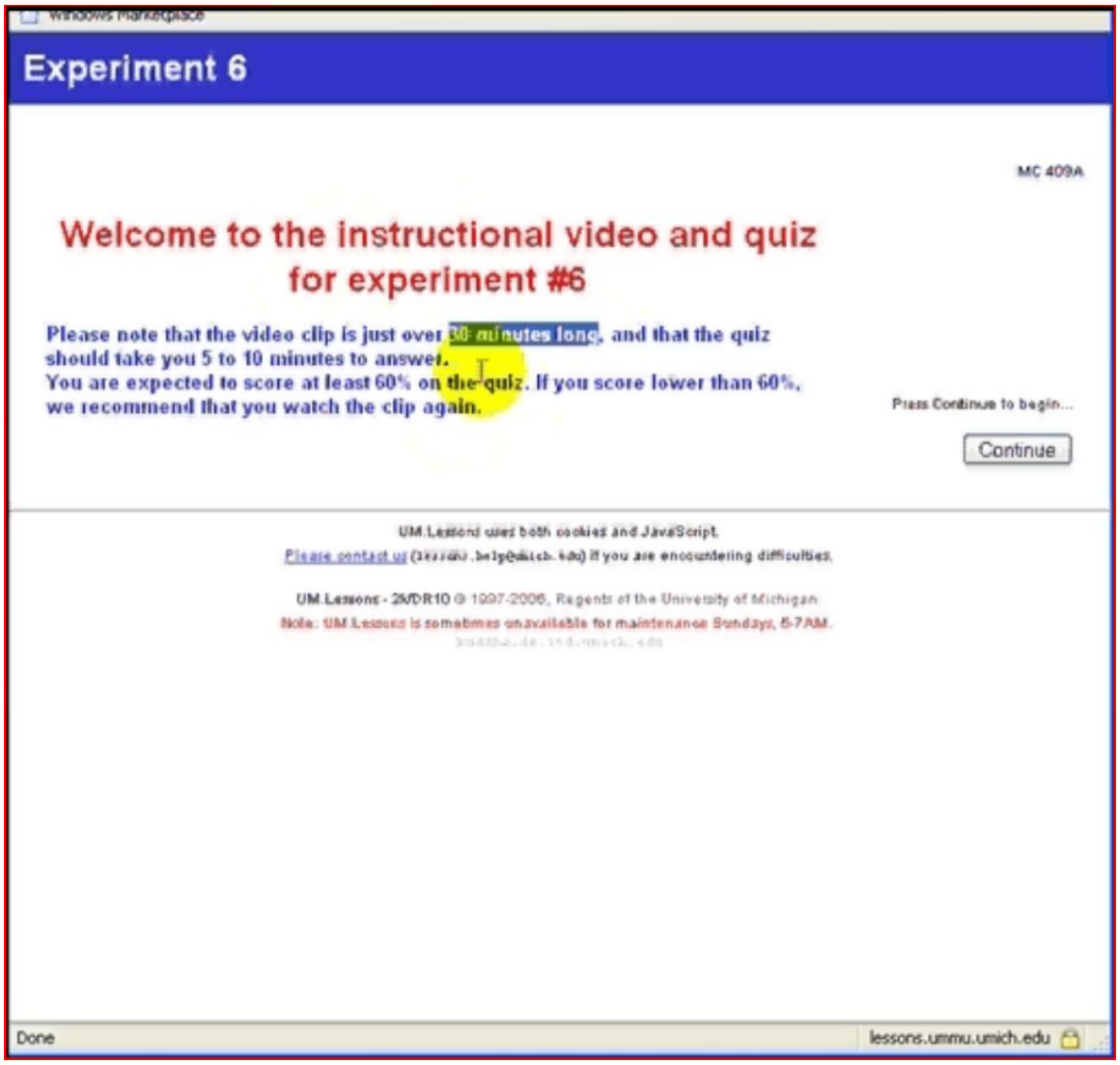


Illustration B2: Entry slide to online lecture indicating approximate time-length and instructions about the quiz in the end.

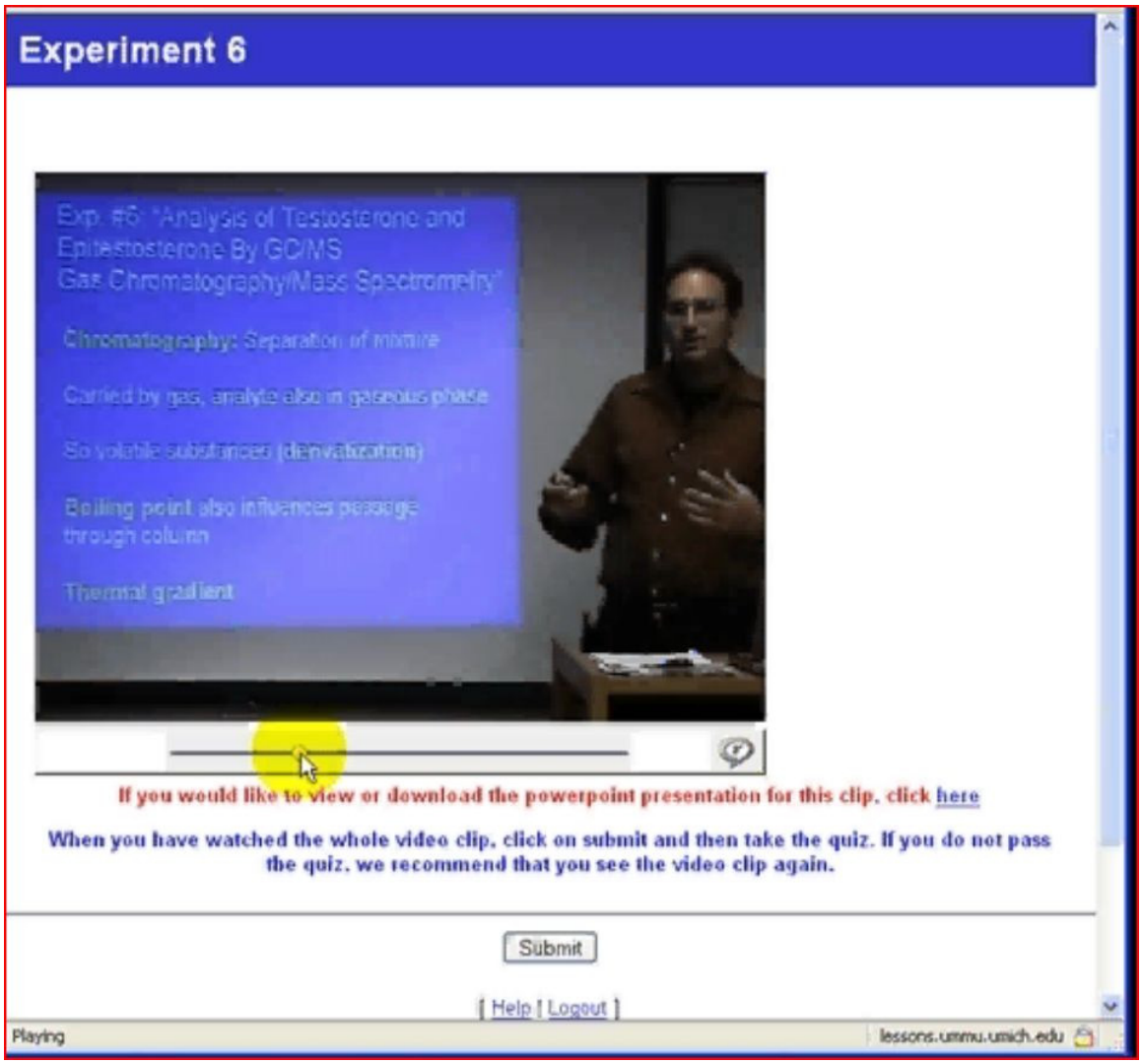


Illustration B3: Online lecture instruction about chromatography theory.

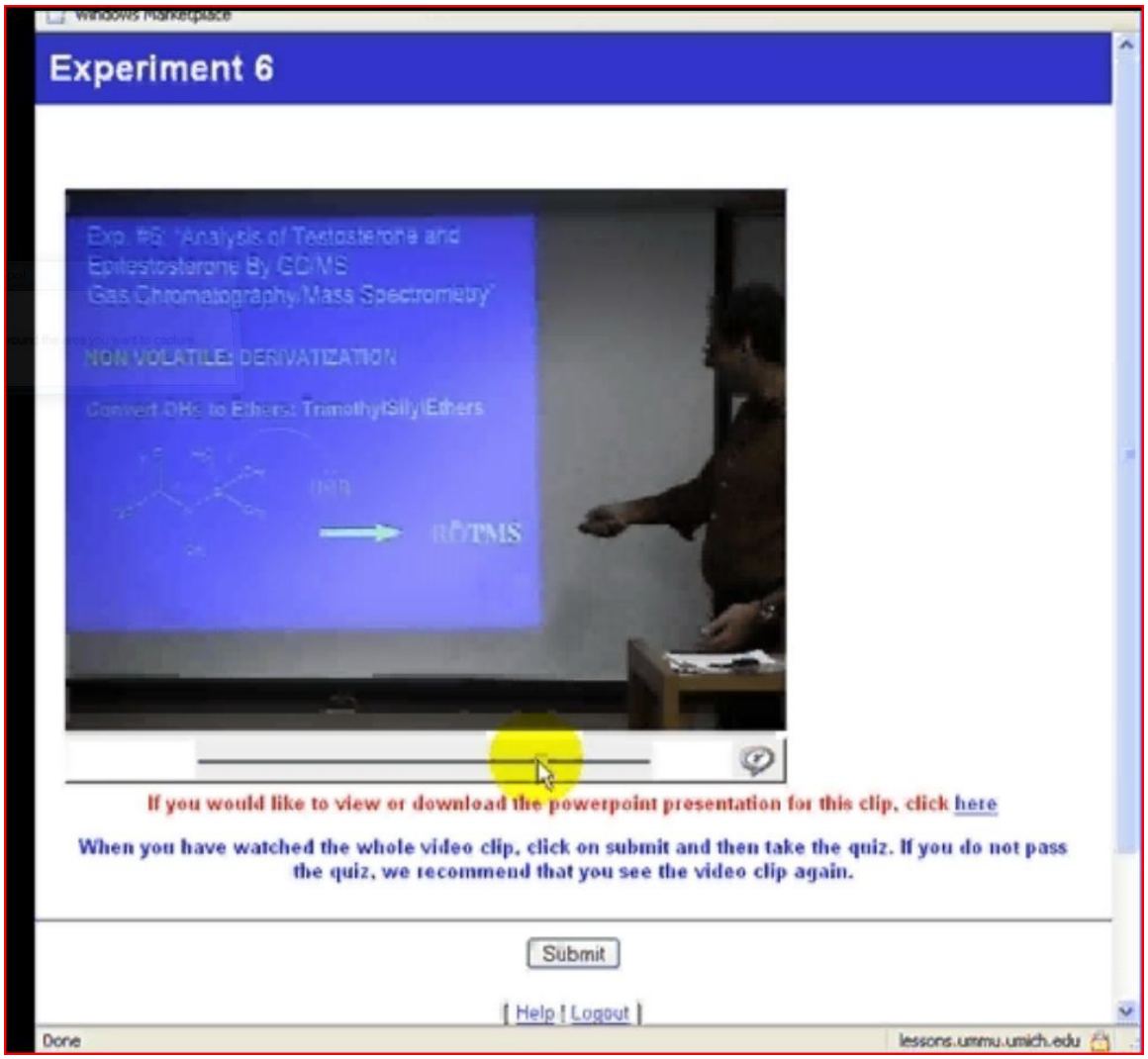


Illustration B4: Explaining why the analytical technique is appropriate for the particular analyte after discussing its chemical structure and properties.

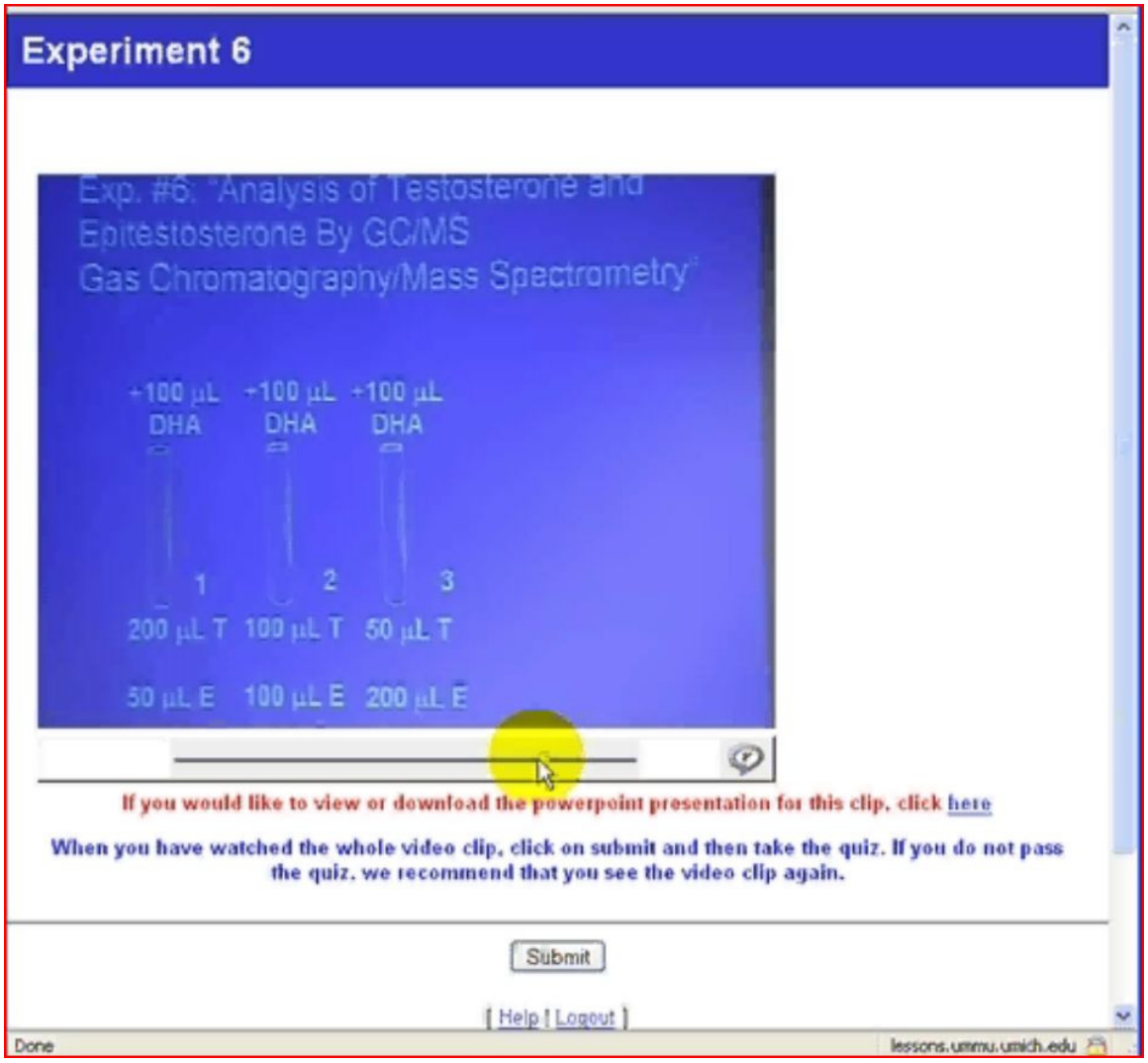


Illustration B5: Illustrating experimental protocol step-by-step.

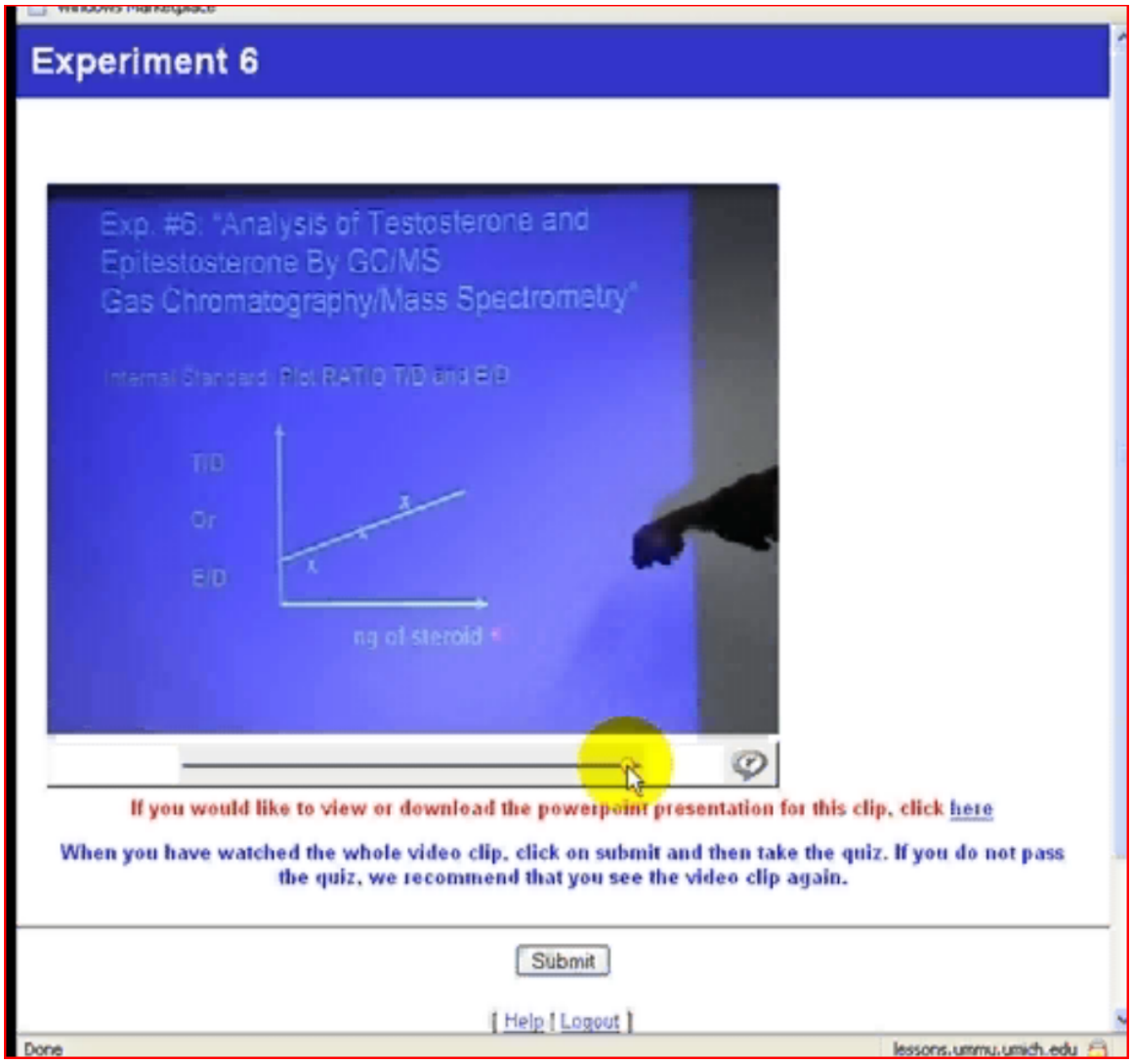


Illustration B6. Explaining how to use the data collected to derive an analytical answer.

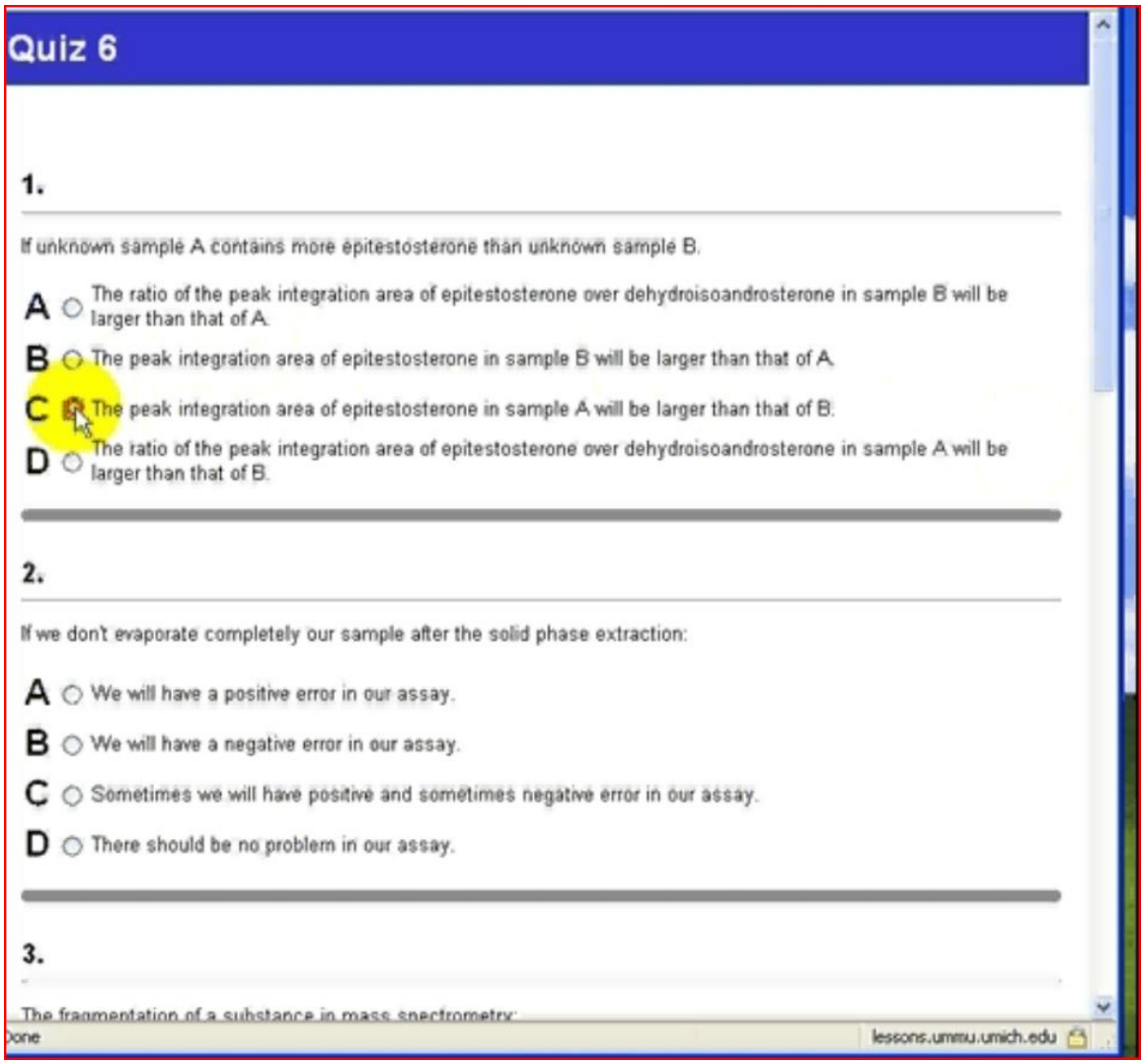


Illustration B7: Answering a multiple choice quiz question.

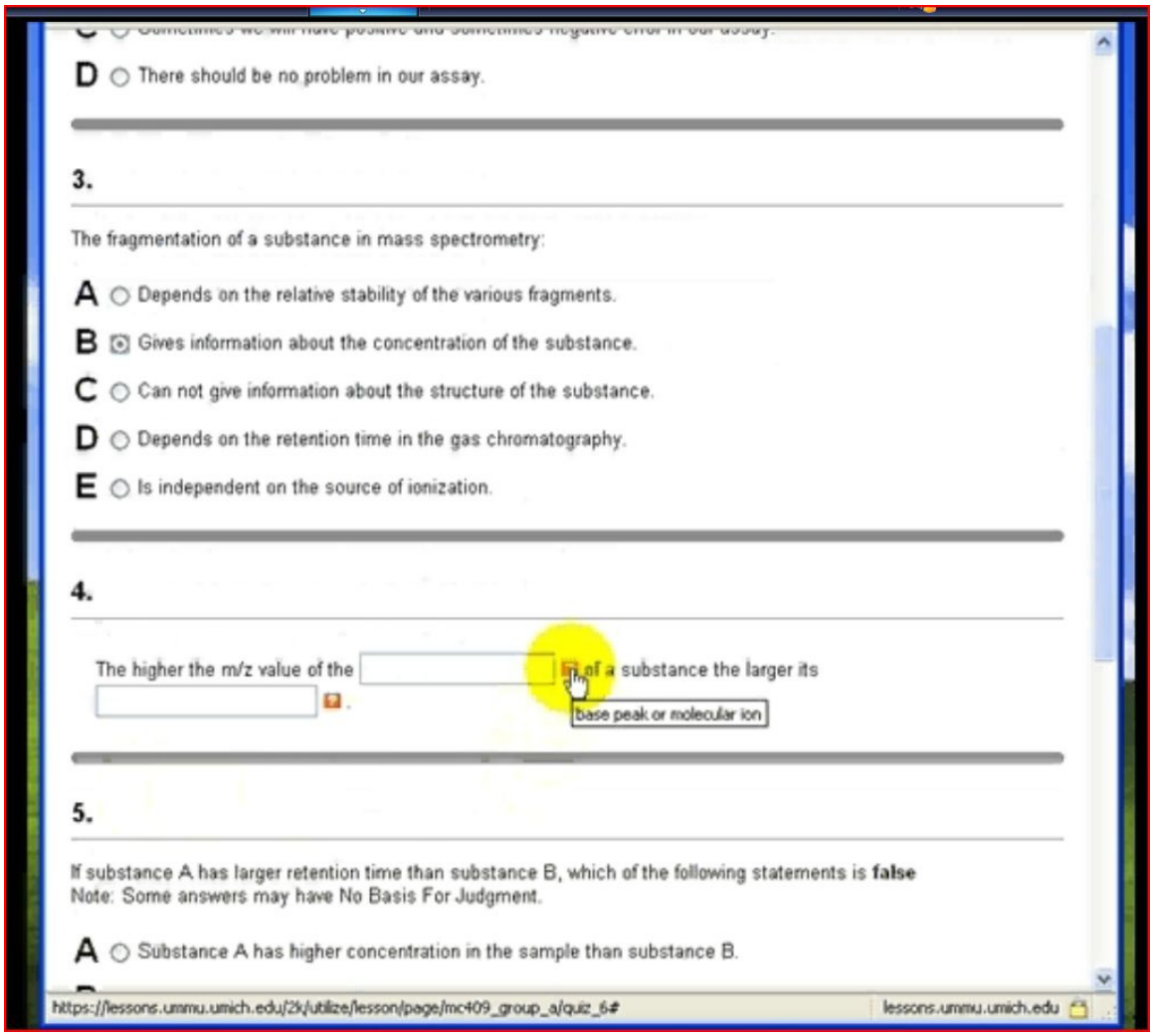


Illustration B8: Answering a "fill-in-the-blank" quiz question overlaying the cursor over a blank and choosing among multiple prompts.

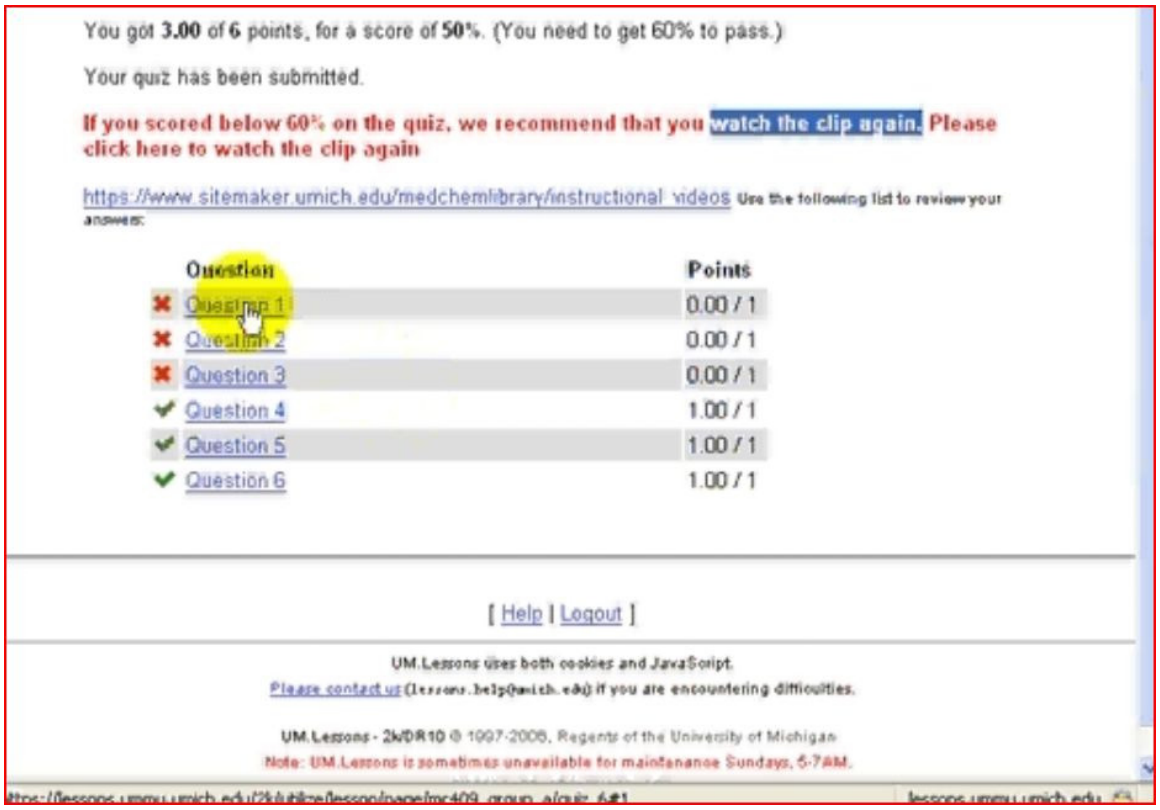


Illustration B9: Quiz results screen.

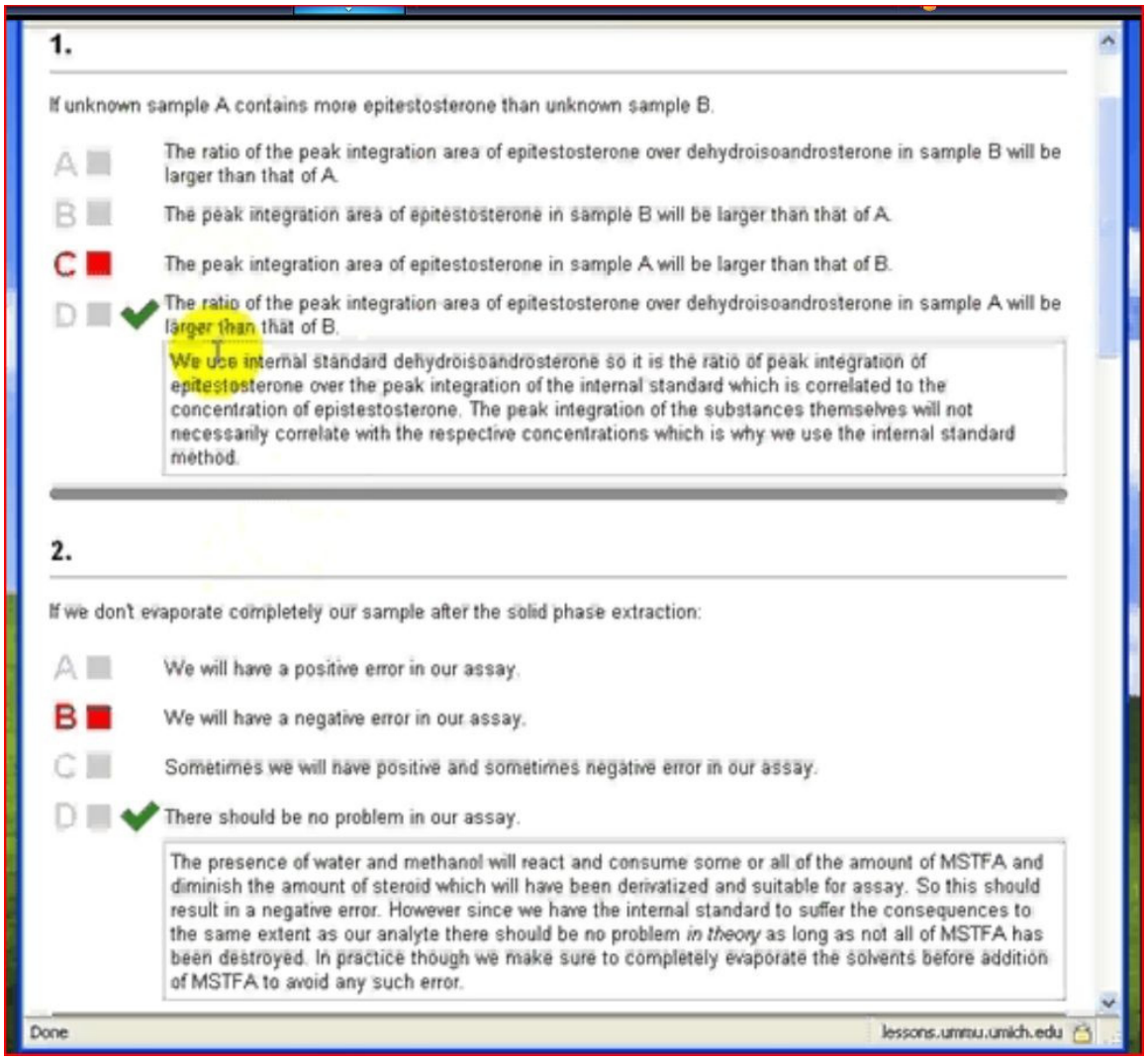


Illustration B10: Providing on-screen feedback on online quiz answers.

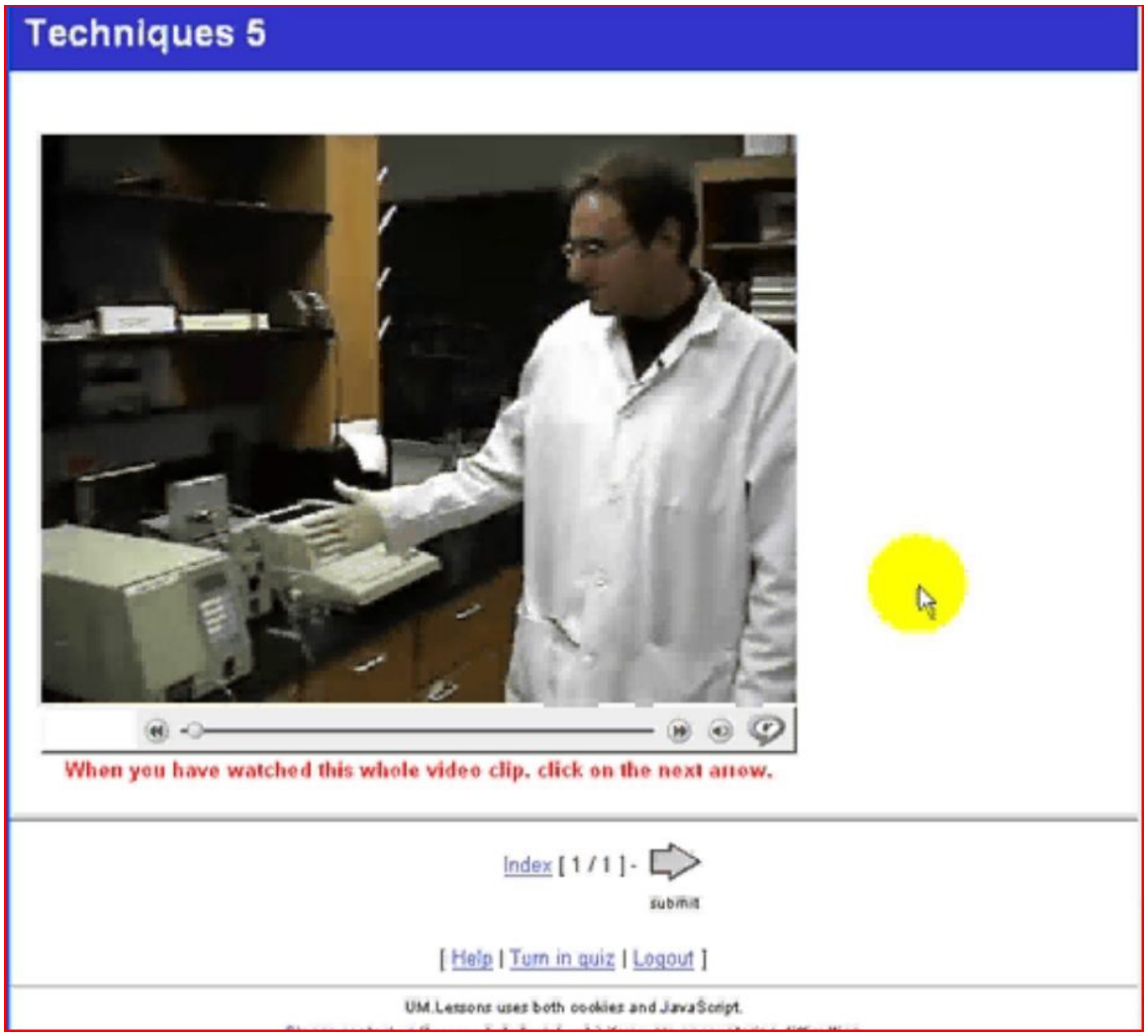


Illustration B11: Experimental demonstration of analytical instruments.

Illustration B12: Close-up shot of an analytical instrument.

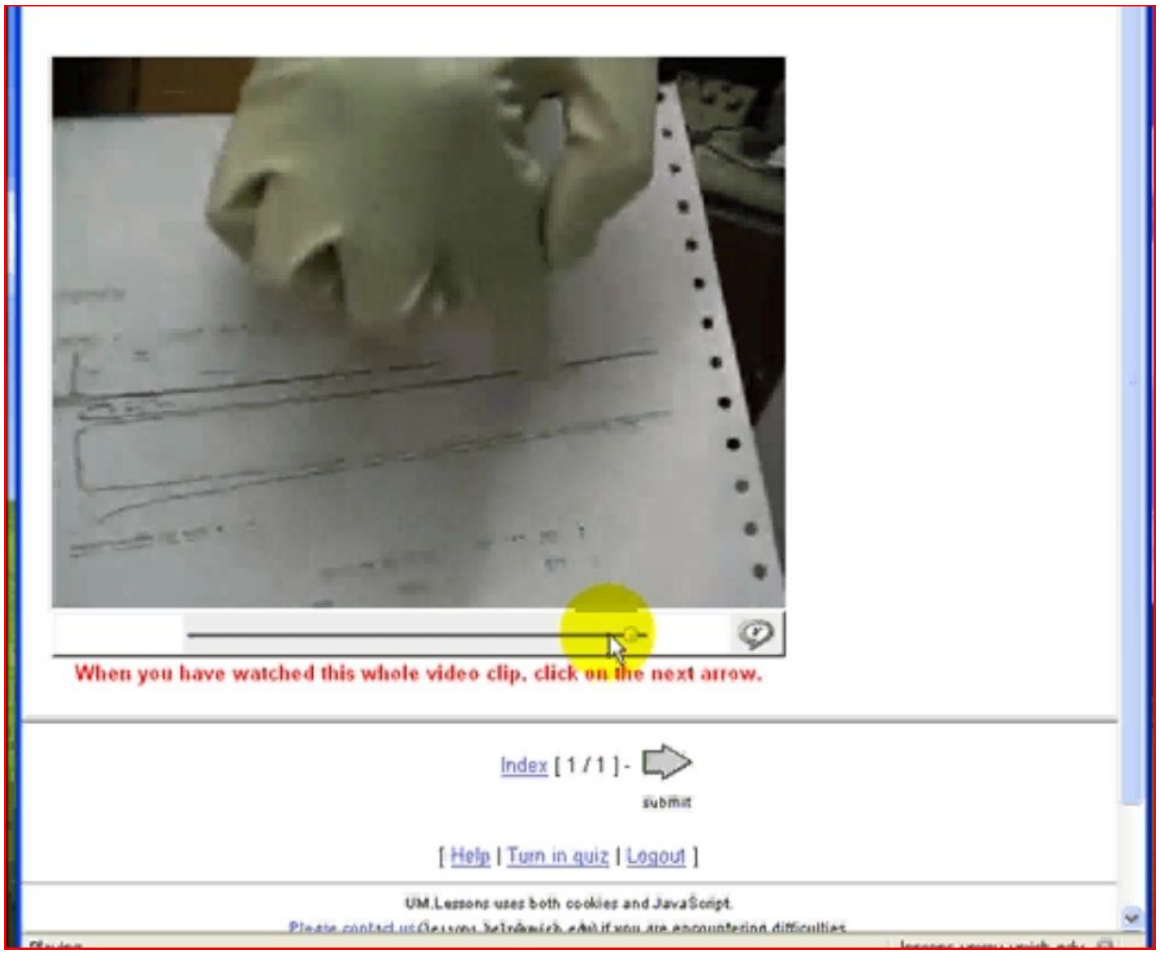


Illustration B13: Explanation on the data generated by the instrument.

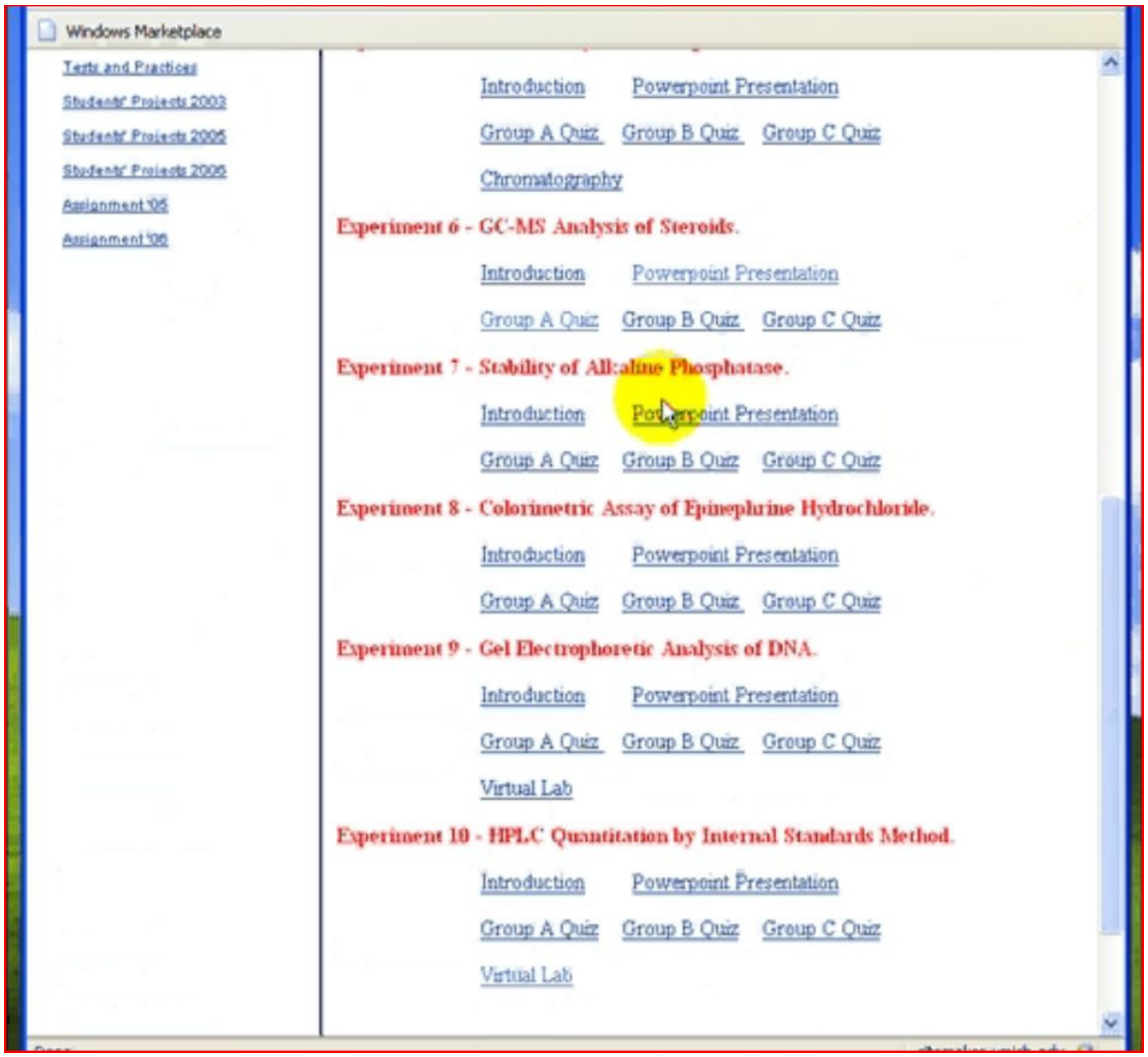
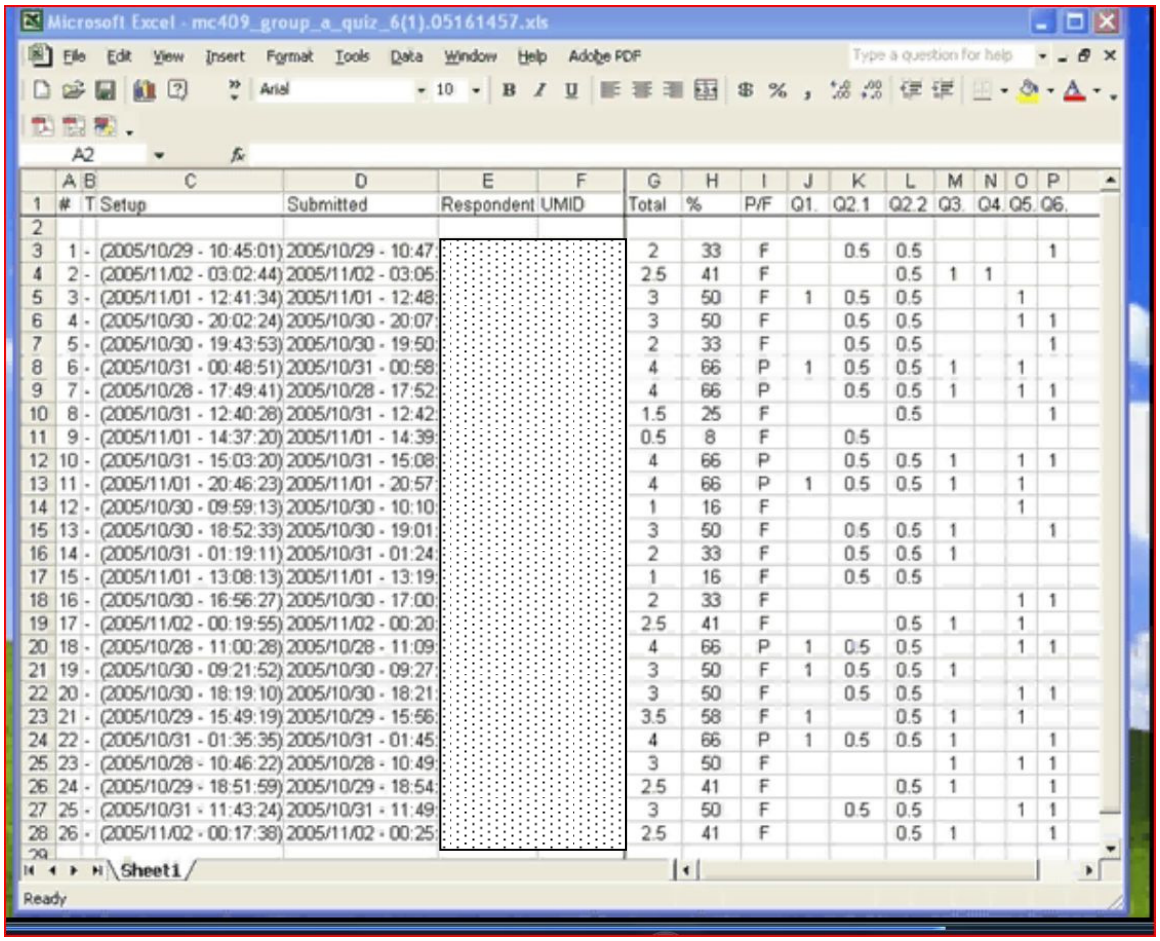


Illustration 14: Central depository website for all tutorial resources.



#	T	Setup	Submitted	Respondent	UMID	Total	%	P/F	Q1.	Q2.1	Q2.2	Q3.	Q4.	Q5.	Q6.
1	-	(2005/10/29 - 10:45:01)	2005/10/29 - 10:47:			2	33	F		0.5	0.5				1
2	-	(2005/11/02 - 03:02:44)	2005/11/02 - 03:05:			2.5	41	F			0.5	1	1		
3	-	(2005/11/01 - 12:41:34)	2005/11/01 - 12:48:			3	50	F	1	0.5	0.5			1	
4	-	(2005/10/30 - 20:02:24)	2005/10/30 - 20:07:			3	50	F		0.5	0.5			1	1
5	-	(2005/10/30 - 19:43:53)	2005/10/30 - 19:50:			2	33	F		0.5	0.5				1
6	-	(2005/10/31 - 00:48:51)	2005/10/31 - 00:58:			4	66	P	1	0.5	0.5	1		1	
7	-	(2005/10/28 - 17:49:41)	2005/10/28 - 17:52:			4	66	P		0.5	0.5	1		1	1
8	-	(2005/10/31 - 12:40:28)	2005/10/31 - 12:42:			1.5	25	F			0.5				1
9	-	(2005/11/01 - 14:37:20)	2005/11/01 - 14:39:			0.5	8	F		0.5					
10	-	(2005/10/31 - 15:03:20)	2005/10/31 - 15:08:			4	66	P		0.5	0.5	1		1	1
11	-	(2005/11/01 - 20:46:23)	2005/11/01 - 20:57:			4	66	P	1	0.5	0.5	1		1	
12	-	(2005/10/30 - 09:59:13)	2005/10/30 - 10:10:			1	16	F						1	
13	-	(2005/10/30 - 18:52:33)	2005/10/30 - 19:01:			3	50	F		0.5	0.5	1			1
14	-	(2005/10/31 - 01:19:11)	2005/10/31 - 01:24:			2	33	F		0.5	0.5	1			
15	-	(2005/11/01 - 13:08:13)	2005/11/01 - 13:19:			1	16	F		0.5	0.5				
16	-	(2005/10/30 - 16:56:27)	2005/10/30 - 17:00:			2	33	F						1	1
17	-	(2005/11/02 - 00:19:55)	2005/11/02 - 00:20:			2.5	41	F			0.5	1		1	
18	-	(2005/10/28 - 11:00:28)	2005/10/28 - 11:09:			4	66	P	1	0.5	0.5			1	1
19	-	(2005/10/30 - 09:21:52)	2005/10/30 - 09:27:			3	50	F	1	0.5	0.5	1			
20	-	(2005/10/30 - 18:19:10)	2005/10/30 - 18:21:			3	50	F		0.5	0.5			1	1
21	-	(2005/10/29 - 15:49:19)	2005/10/29 - 15:56:			3.5	58	F	1		0.5	1		1	
22	-	(2005/10/31 - 01:35:35)	2005/10/31 - 01:45:			4	66	P	1	0.5	0.5	1			1
23	-	(2005/10/28 - 10:46:22)	2005/10/28 - 10:49:			3	50	F				1		1	1
24	-	(2005/10/29 - 18:51:59)	2005/10/29 - 18:54:			2.5	41	F			0.5	1			1
25	-	(2005/10/31 - 11:43:24)	2005/10/31 - 11:49:			3	50	F		0.5	0.5			1	1
26	-	(2005/11/02 - 00:17:38)	2005/11/02 - 00:25:			2.5	41	F			0.5	1			1

Illustration B15: Course coordinator's report based on students' pre-laboratorial preparation before each lab.

Illustration B16: Virtual laboratory animation snap-shot showing preparation of HPLC mobile phase.

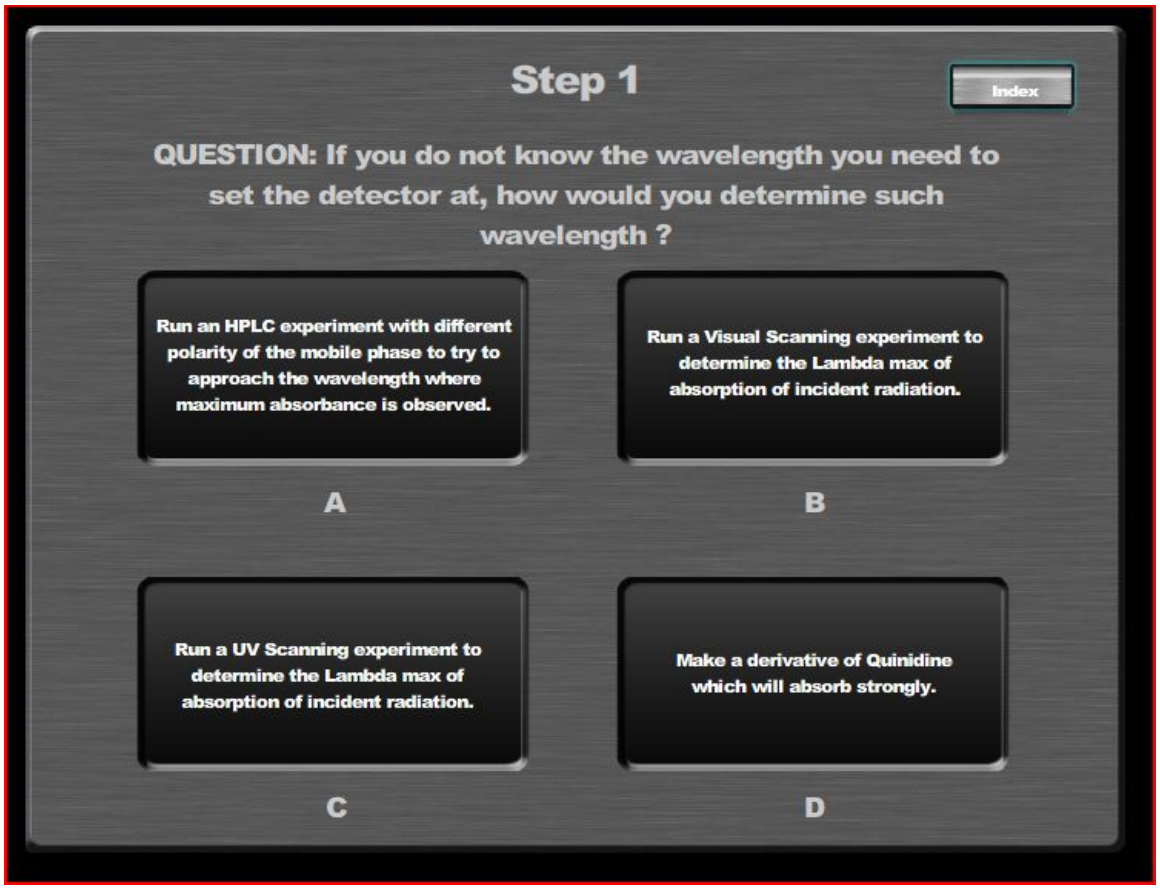


Illustration B17: Example of a multiple choice question intercepting the virtual laboratory.

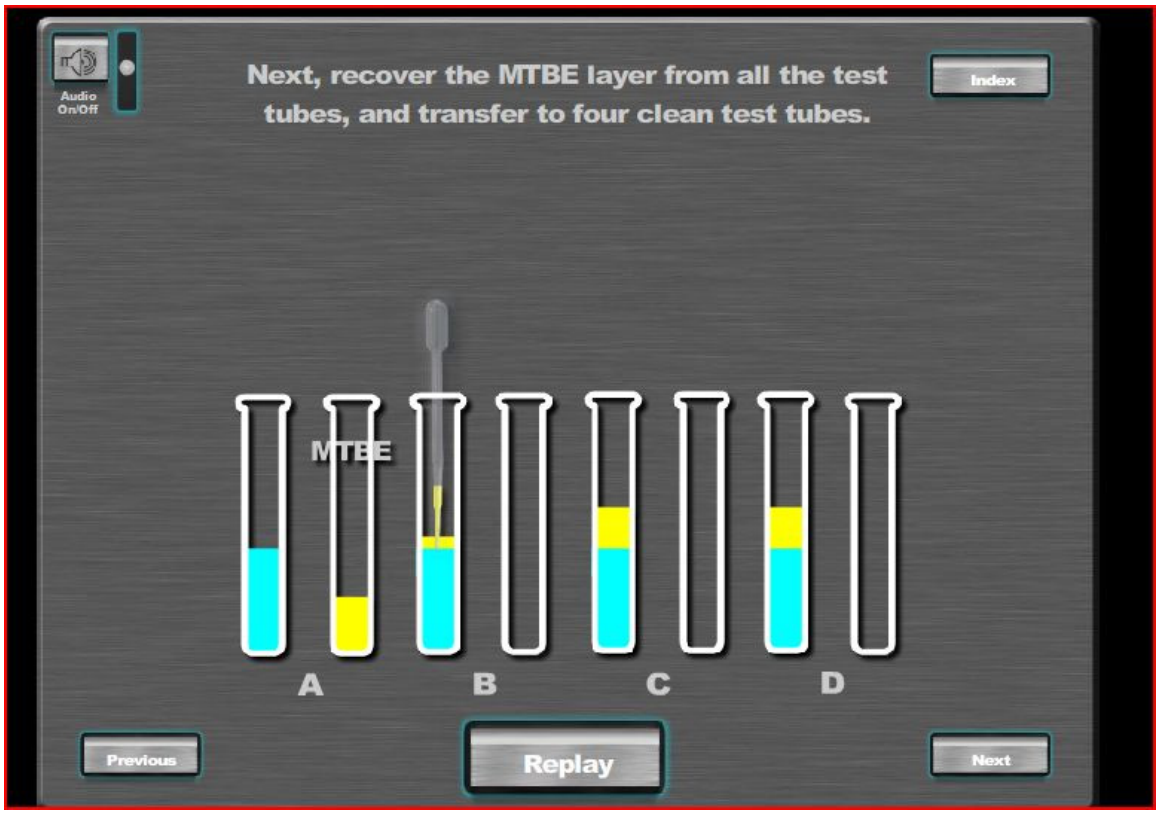


Illustration B18: Snap-shot of animation on how to conduct back-extraction.

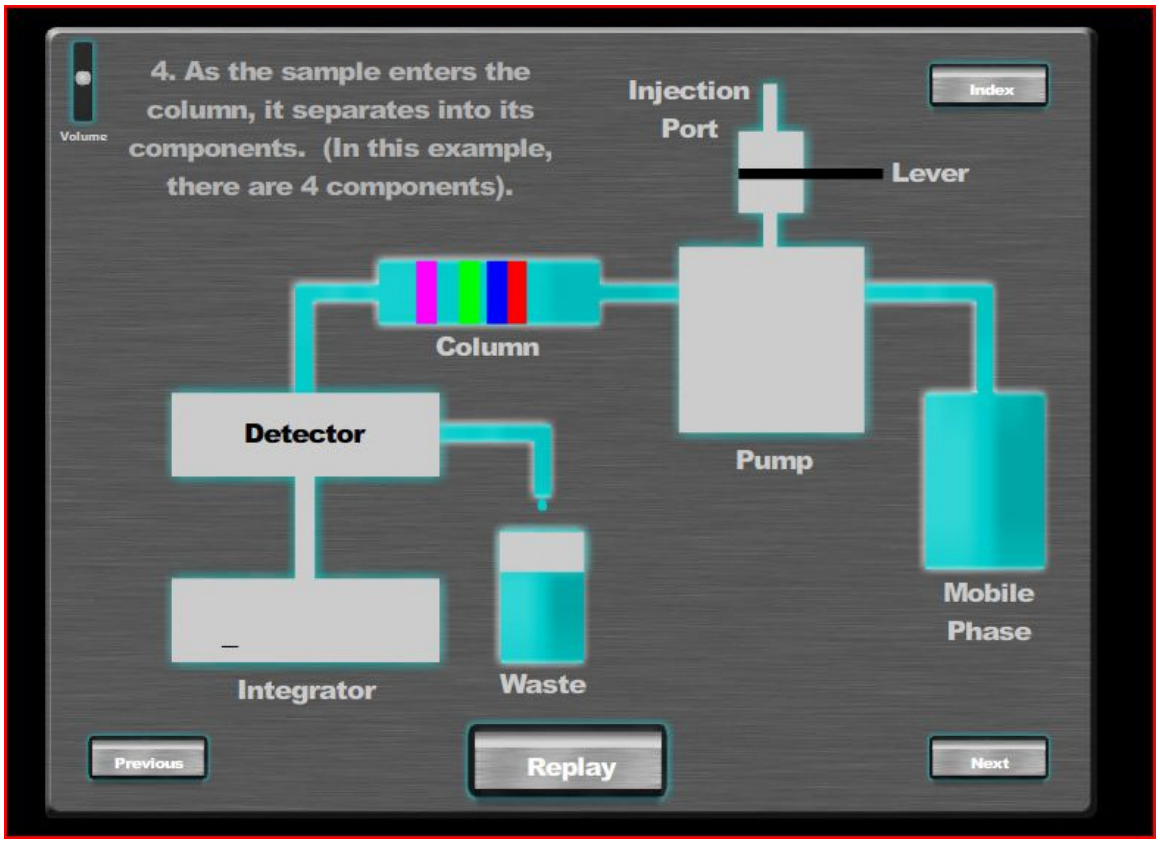


Illustration B19. Snap-shot of animation depicting the separation occurring during the elution of the injected mixture on a chromatographic column.

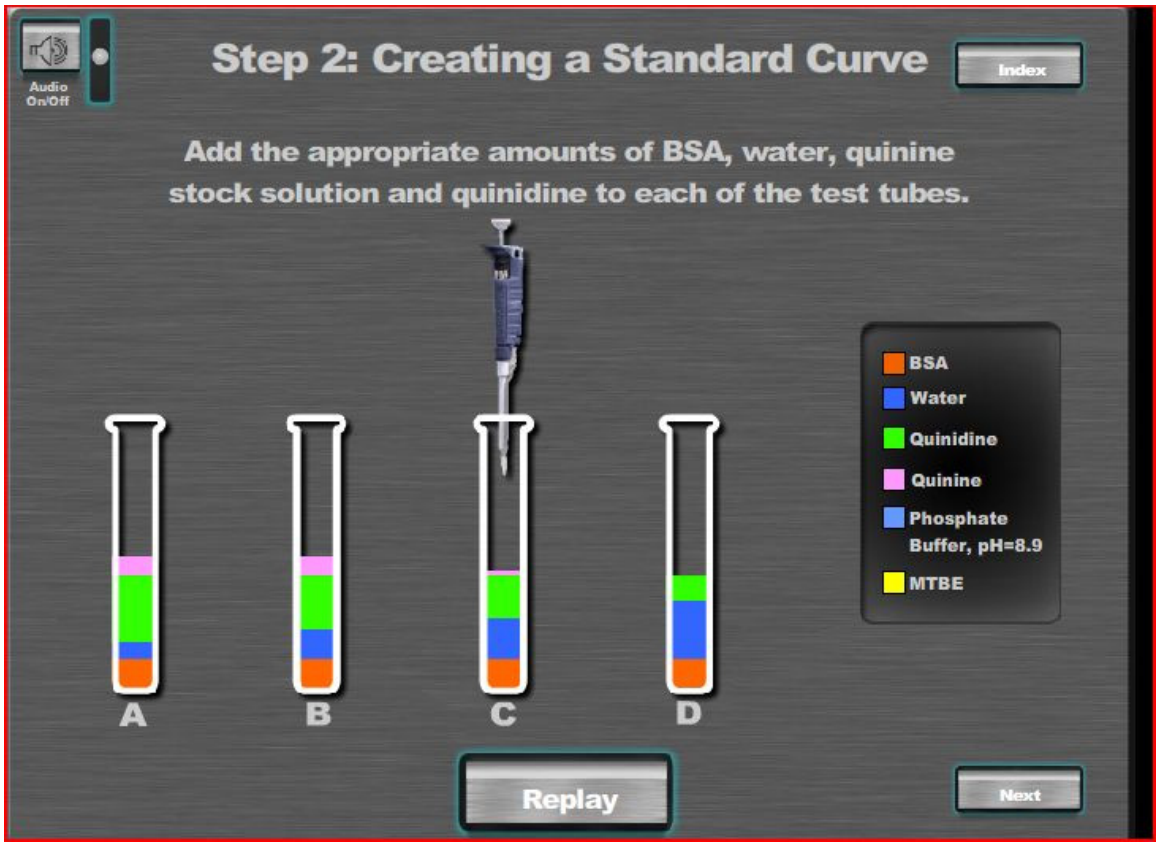


Illustration B20: Snap-shot of animation illustrating how to prepare standard samples.

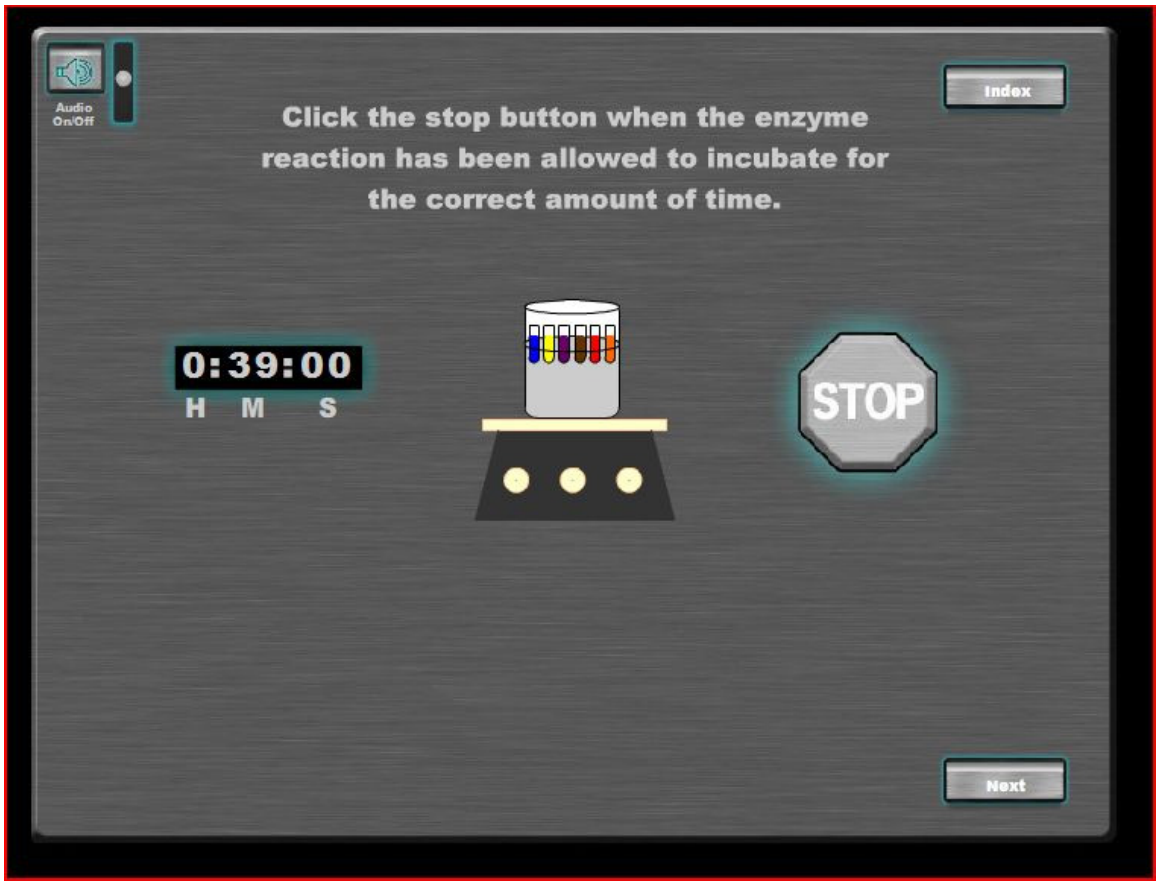


Illustration B21: Timer used in electrophoresis virtual lab for the students to incubate their samples for the proper amount of time.

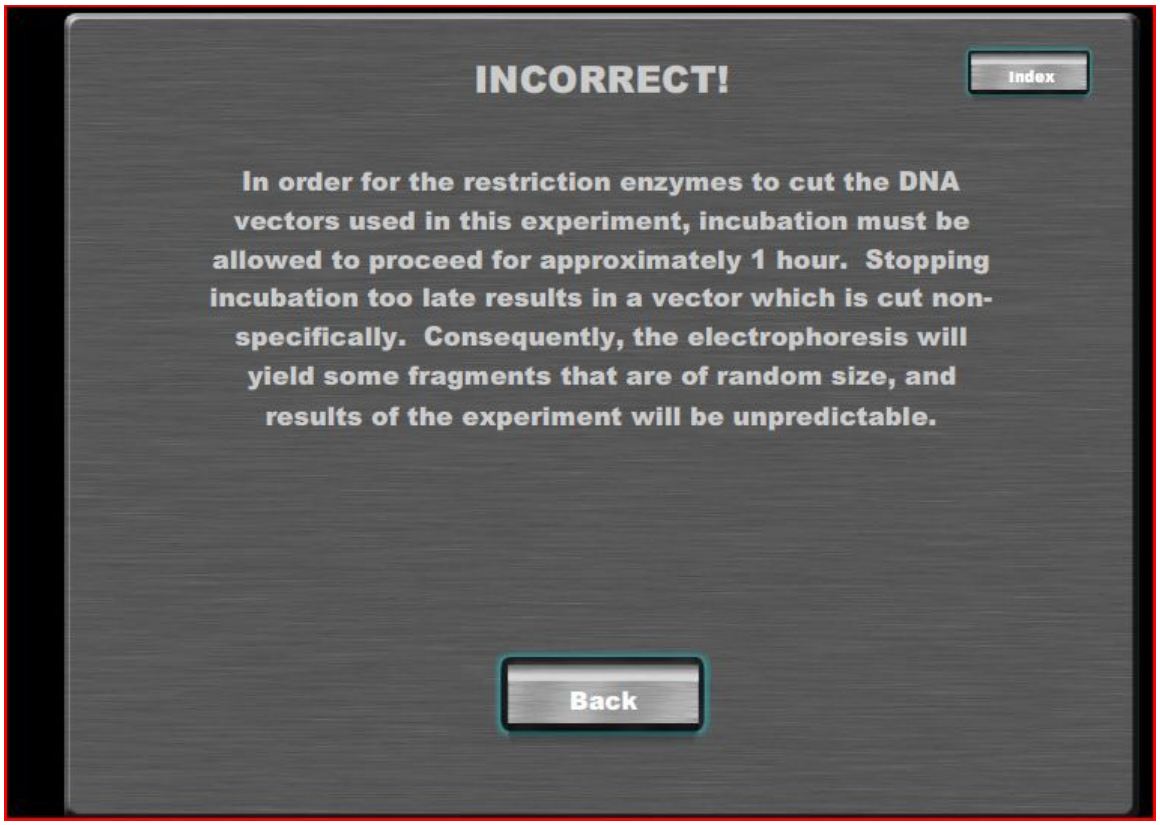


Illustration B22: Response displayed if the students exceed the prescribed time of incubation.

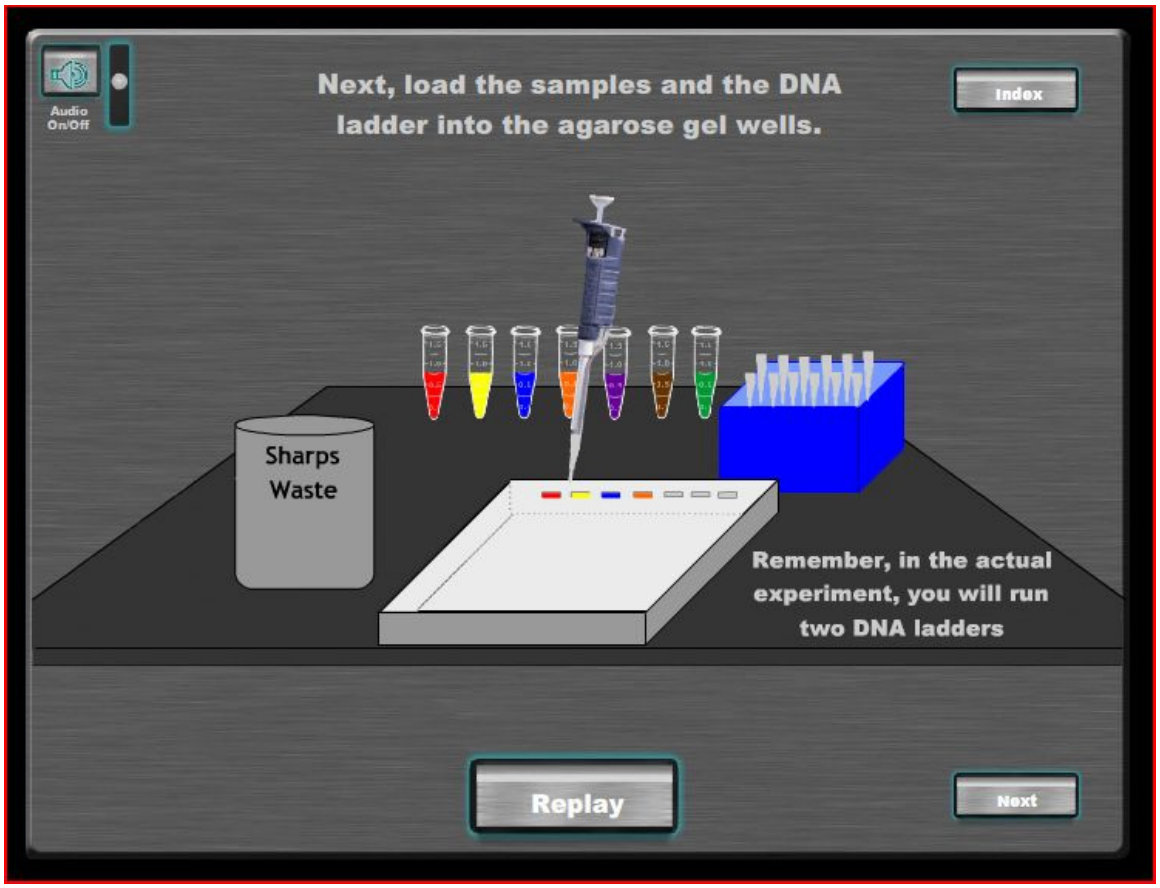


Illustration B23: Loading of DNA samples on the agar gel of an electrophoresis virtual laboratory.

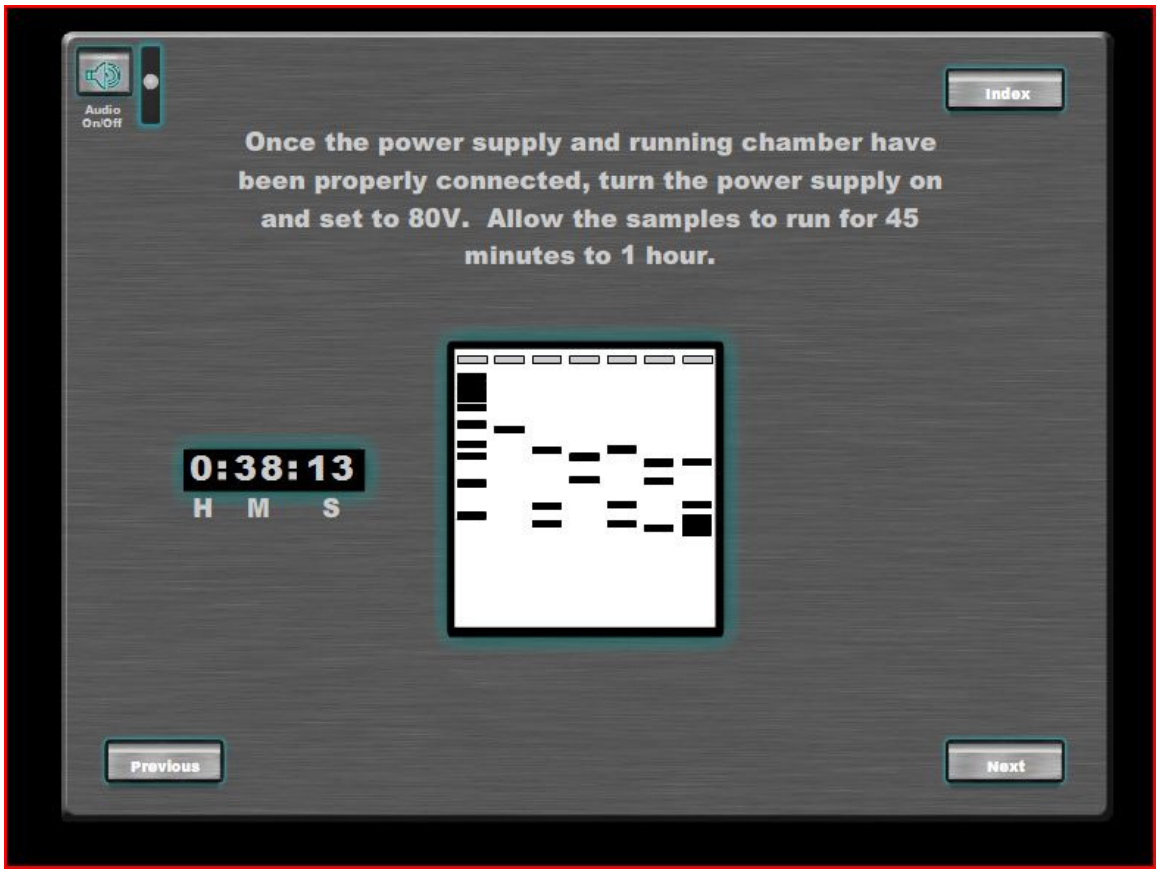


Illustration B24: Snap-shot of the animation showing the advance of DNA fragments on a gel of an electrophoresis virtual laboratory plus a timer.

Appendix C: Experimental methods and characterization of new Compounds

t-Butyl N-(4-methyl-benzenesulfonyl)-N-(6-methoxycarbonyl-hept-5-enyl)-carbamate (14)

To a solution of 406 mg of *N*-Boc-p-toluenesulfonamide (1.5 mmol, 1.5 equiv.) and 785 mg of triphenylphosphine (3 mmol, 3 equiv.) in 9 mL of anhydrous THF, a solution of 158 mg of methyl 7-hydroxy-2-heptenoate (1 mmol, 4:1 E:Z mixture) **(10)** in 6 mL of THF was added. The solution was cooled to 0 ^{o}C and 0.32 mLs of diethyl azodicarboxylate (2 mmol, 2 equiv.) was added dropwise and the reaction mixture was stirred for 1 hour at room temperature. The solvent was evaporated leaving a yellow thick oil that was purified by silica gel column chromatography (hexanes – ethyl acetate 9:1). Finally 341 mg (0.82 mmols) of a 4:1 Z/E mixture of product was obtained (82%), as a colorless oil.

^{1}H-NMR (E-isomer) ($CDCl_3$) δ (ppm) 1.34 (s, 9H, ($C\mathbf{H}_3$)$_3$C-O), 1.53 (quintet, 2H, *J*=7.5 Hz, $C\mathbf{H}_2CH_2CH$=), 1.79 (quintet, 2H, *J*=7.3Hz, $C\mathbf{H}_2CH_2NH$), 2.28 (dt, 2H, *J*=7.2, 1.8Hz, $C\mathbf{H}_2CH$=CH), 2.44 (s, 3H, $C\mathbf{H}_3$-Ar), 3.73 (s, 3H, $OC\mathbf{H}_3$), 3.83 (t, 2H, *J*=7.5Hz, $C\mathbf{H}_2N$), 5.85 (dt, 1H, *J*=15.8, 1.5 Hz, =C**H**CO), 6.96 (dt, 1H, *J*=15.8, 7 Hz, =C**H**CH_2), 7.30 (d, 2H, *J*=8.0Hz, Ar-**H**), 7.77 (d, 2H, *J*=8.0Hz, Ar-**H**)

^{13}C-NMR (E-isomer) 21.70, 25.17, 27.95, 29.74, 31.81, 46.81, 51.46, 84.20, 121.21, 127.66, 128.12, 129.14, 137.32, 143.96, 148.69, 150.81, 166.87.

E-N-7-hydroxyhept-5-enyl-4-methylbenzenesulfonamide (15)

147 mg of *E*-N-(6-methoxycarbonyl)-hept-5-enyl-4-methylbenzenesulfonamide (0.47 mmol) was dissolved in 6 mL of anhydrous CH_2Cl_2 and the solution was cooled to –78 ^{o}C. 1.04 mL of DIBAL (1M solution in CH_2Cl_2, 1.04 mmol, 2.2 equiv.) was added dropwise and the reaction mixture was stirred at that temperature for 25 minutes. The reaction was quenched with methanol and poured in a saturated potassium sodium tartrate solution. EtOAc was added and the whole mixture was stirred till both layers were clear. The organic layer was then washed with brine, dried over $MgSO_4$ and reduced to colorless oil. Silica gel column chromatography (hexanes/EtOac 85:15, 80:20, 70:30) afforded 108 mg (0.38 mmol) of the pure allylic alcohol (81%) as colorless oil.

^{1}H-NMR ($CDCl_3$) δ (ppm) 1.40 (m, 4H, C**H**$_2$C**H**$_2$CH=), 1.99 (dd, 2H, *J*=12.8Hz, C**H**$_2$CH=CH), 2.43 (s, 3H, C**H**$_3$-Ar), 3.93 (t, 2H, *J*=6Hz, C**H**$_2$N), 3.93 (t, 2H, *J*=6Hz, C**H**$_2$N), 4.06 (m, 2H, C**H**$_2$OH), 5.60 (m, 2H, C**H**=C**H**), 7.31 (d, 2H, *J*=8Hz, Ar-**H**), 7.74 (d, 2H, *J*=8Hz, Ar-**H**)

^{13}C-NMR 21.49, 25.84, 28.98, 31.48, 42.98, 63.58, 127.07, 129.60, 129.67, 132.21, 136.93, 143.37

Appendix D: Selected NMR spectra