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A New Model for Advanced Undergraduate Chemistry Laboratory Courses: A Two-semester Integrated Laboratory Experience

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Abstract Chemistry faculty in the School of Science and Technology (SST) at Georgia Gwinnett College (GGC) have instituted a new two-semester laboratory capstone course sequence as part of the Chemistry major program. These integrated laboratory courses emphasize a multidisciplinary approach that provides a unique undergraduate experience for students. Integrated Laboratory I (Chemistry 4701 (IL-I)), which incorporates biochemistry-based laboratory techniques, practices and instrumentation, places students in a research-like setting similar to that found in graduate school. Integrated Laboratory II (Chemistry 4702 (IL-II)), which leverages organic qualitative analysis, synthesis and spectroscopic methods, affords students an understanding of chemical industrial remediation operations. This work describes the design, execution and success of these laboratory experiences. Student attitudinal surveys and course outcome assessments suggest this course sequence strengthens student critical thinking and problem-solving abilities toward answering questions of biochemical and chemical interest.

Keywords Biochemical research, Undergraduate research creative experience (URCE), Organic synthesis, Spectral methods, Science, Technology, Engineering & math (STEM)

1. Introduction

As the Office of Professional Training for the American Chemical Society [1] has noted, undergraduate students majoring in the chemical sciences planning to enter graduate programs of study or those seeking employment in industrial research require a diverse skill set to be competitive for those opportunities. Skills-based chemistry program outcomes that require students to have a firm conceptual foundation in the theory, operation and maintenance of different analytical, spectroscopic and preparative laboratory instrumentation as well as their application in an undergraduate research experience are essential for success. In addition to these "hard" skills, are the expectations that students can apply critical thinking and problem solving to design and implement research plans, analyze data obtained from their work, and present their findings both orally and in writing with technical proficiency.

While upper level undergraduate students can develop many of these skills by participating in typical capstone research courses, incorporating research throughout a student's undergraduate program is a better model for ensuring that they have the requisite skills needed to succeed in industry or graduate school. For the past several years, the School of Science and Technology (SST) at GGC has participated in the University System of Georgia's STEM II initiative [2] by implementing a very successful STEM four-year undergraduate research creative experience (4-Yr URCE) offered in forty-one courses [3]. Beginning at the introductory course level and continuing throughout their undergraduate studies at GGC, students in STEM disciplines engage in a variety of URCE projects [4]. Studies conducted by the National Research Council [5] and Lapatto [6, 7] indicate that students engaged in research in such integrated experiential learning settings throughout their entire STEM undergraduate program of study ensures that their senior-level research experience and internship opportunities are far more productive. Indeed, the positive effect of these URCEs may extend beyond graduation to graduate studies or employment [8].

The new Integrated Laboratory sequence (IL-I and IL-II courses) dovetails nicely with SST's 4-Yr URCE program with one small distinction – the entire course sequence is taught in the laboratory. We engage undergraduate students in experiential learning that successfully melds critical thinking and problem-solving with research project design, advanced experimental technique application and instrumentation use in small-scale interdisciplinary research-like opportunities one might expect to find in

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graduate programs as well as those which emulate research in an industrial setting.

The Integrated Laboratory-I course is a biochemistry-focused research experience similar to what students might expect to find in a graduate school setting, while the Integrated Laboratory-II course is a venue in which students apply advanced organic qualitative and spectral analysis protocols to identify unknown organic waste materials as members of a chemical remediation company.

This paper examines the development of the Integrated Laboratory course sequence, methods used, instrumentation employed, and provides an initial evaluation of the course sequence success.

2. Integrated Laboratory Course Development

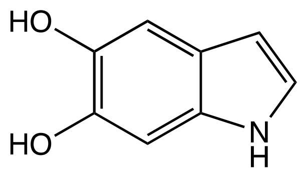
2.1. Backwards Design from Course Outcomes

Because these courses are capstone experiences, only students who met specific course pre-requisites and co-requisites were enrolled. For IL-I, these include both CHEM 3000K (Instrumental Analysis) and BCHM 3100K (Biochemistry I). For IL-II, students must have successfully completed IL-I and either have satisfactorily completed or be currently enrolled in CHEM 4201K (Physical Chemistry I). Taking these requirements into account and using a backwards design approach [9], faculty members selected to teach the Integrated Laboratory courses developed each course based on the course outcomes (COs) [10] shown in Table 1.

2.2. IL-I Course Layout and Requirements

Owing to the success of the 4-yr URCE model being implemented at GGC, students enrolled in IL-I were treated from the beginning of the course as if they were graduate students entering the laboratory with an expectation of independent thought and action. Rather than being handed specific protocols, students were informed, through reading and discussion early in the course, of the nature of the problem and of the overarching aims of the laboratory, with the instructor serving as Principal Investigator of a large grant. Their first assigned task was to choose from among several possible research pathways or tracks under the overarching hypothesis and develop a set of specific aims toward this goal using guidance from the National Institutes of Health (NIH) [11]. They were challenged with finding protocols using their own literature and internet search skills (CO 2). As a starting point for their literature search, a set of twenty core manuscripts was placed into the GGC *My*Courses D2L-Brightspace learning management system online course content folder [12].

The research problem chosen for IL-I and presented to the students allowed for maximum flexibility and use of techniques in both chemistry and biochemistry. The overarching hypothesis involves the pigment neuromelanin (NM) which forms spontaneously in the brain from the cyclization of dopamine to compounds such as 5,6-dihydroxyindole and their subsequent polymerization. See Figure 1.



5,6-dihydroxyindole

Figure 1. Monomeric Component of Neuromelanin

Table 1. Integrated Laboratory Course Outcomes

CO	Chemistry 4701 (IL-1)	Chemistry 4702 (IL-2)
1	Use instrumentation at an appropriate level of sophistication to analyze and answer substance life-cycle questions of green chemical and biochemical interest	Use spectroscopic instrumentation, inert atmosphere reaction apparatus and computational methods at an appropriate level of sophistication to analyze and answer questions of chemical interest
2	Effectively retrieve and critically evaluate articles from peer-reviewed technical journals	
3	Write well-organized, concise laboratory reports in a scientifically appropriate style; communicate written details of reports to peers in a clear and concise manner	Write well-organized, concise laboratory reports in a variety of scientific styles; communicate oral and written details of reports to peers in a clear and concise manner
4	Understand and apply the aspects of modern chemical safety in each of the following: general safety awareness, correct disposal techniques, minimizing hazards in the laboratory, use of safety data sheets (SDS)	
5	Apply critical thinking skills and their understanding of all chemistry sub disciplines to design and execute experiments that incorporate the principles of green chemistry; analyze data and draw appropriate conclusions	Apply critical thinking skills and knowledge of fundamental chemistry to design and execute experiments which may require oxygen and/or moisture-free environments; analyze data and draw appropriate conclusions
6	Demonstrate sound ethical principles when conducting laboratory work	
7	Interact productively with peers and work effectively as part of a team pursuing chemical inquiries and/or common goals	

Table 2. Research Tracks and Techniques Presented to IL-I Students

Track	Techniques	Possible aims
Reverse Transcriptase -Polymerase Chain Reaction (RT-PCR)	Neuronal cell culture, Ribonucleic acid extraction, RT-PCR	Regulation of tyrosine hydroxylase (TH) mRNA upon pigmentation.
Immuno-Histochemistry (IHC)	Neuronal cell culture, IHC, fluorescence spectroscopy	Regulation of TH protein levels upon pigmentation, dopamine localization.
Polymers	Polymer chemistry, macro-scale Ultra Violet/Visible (UV/Vis) spectroscopy, fluidics	Influence of alternate catecholamines on polymerization rate, pH effects, hydrogel formation.
Binding assays	Microscale UV/Vis spectroscopy, kinetics and binding assays, High Performance Liquid Chromatography (HPLC) separation	Predict binding and measure dissociation constant of 6-hydroxy dopamine, Methyl-Phenyl Tetrahydropyridine (MPTP) and related compounds.
Competition assays	Microscale fluorescence spectroscopy, competition binding assays, kinetics	Predict competition with nicotine binding to neuromelanin; provide proof of principle for competition assay.
Lipids and vesicles	Critical micellular concentration, vesicle formation and pigment inclusion	Determine threshold concentration for dopamine polymerization, test incorporation of melanins into lipid vesicles.
Behavior	Fish or Caenorhabditis elegans	Test induction of neuromelanin in model organisms treated with dopamine precursor, test nicotine for protection against Parkinsonian-inducing agents in pigmented organisms.

In brief, this macromolecule, as with all melanins, clearly affects the function of cells harboring it, in this case, dopaminergic neurons. Oddly however, melanins have been largely overlooked and are not typically listed among the functional macromolecules of the cell in typical biology or biochemistry textbooks. Pigment of the *substantia nigra* is not present at birth but forms throughout a person's lifetime and is never lost except in the diseased state, suggesting that there is a very important reason for retaining it. However, the presence of pigmented granules also appears to increase susceptibility to toxin-induced necrosis and the loss of grey matter. Many amines resembling dopamine fall among these toxins, suggesting an endogenous role for NM in binding dopamine [13]. All projects under the umbrella then involved development of different model systems in an attempt to understand the functional role of this polymer. As such, students were presented with a set of tracks and supporting experimental techniques (Table 2) from which they could choose according to their interests and ability. Upon choosing a track, students then developed research aims (COs 5 and 6) that would enable them to contribute to the overall research program.

With their research aims in hand, students were tasked to design, execute and if necessary, troubleshoot their assigned problem to completion (COs 1, 3-6). Throughout the semester, students held journal club, reported progress on a regular basis, and maintained a proper laboratory notebook to contribute data toward the 'grant' aims. Although students worked independently, sharing and communication were key (CO 7). For a final report, students prepared a paper in the form of a journal publication and presented their work in poster form. A draft outline of this paper was required at mid-term. Instructions given to the students for these tasks follow:

1. Journal Club: Students give a 20-minute presentation on a paper related to their topic of research (COs 2 & 7). The presentation includes a polished summary of the major findings, relevant figures and tables from the paper and is to be delivered in a coherent, logical format using PowerPoint or other suitable presentation graphics.
2. Mid-Term Research Paper: This document should be a detailed outline of what will become your final research paper. Many of sections of the paper, including the introduction, methods used throughout the semester, and references can all be completed before your research project is finished. The more you write at mid-term, the less work you'll have to do near finals!
3. Final Research Paper: A detailed summary of your work in this course will be presented in the style of a journal publication such as the Journal of Biological Chemistry.

2.2. IL-II Course Layout and Requirements

Students are welcomed to the course by the Chief Executive Officer (CEO) (instructor) of the Forensichem Chemical Remediation Corporation. In advance of chemical operations in the laboratory, students are provided with documents on the GGC *My*Courses D2L-Brightspace online course content page that outline the company's mission statement, analytical techniques, company capacities, reporting responsibilities, and disposition recommendations. Students are "hired" as bench chemists who are responsible for identifying unlabeled organic waste materials found in an abandoned chemical storage site which the company has been contracted to remediate. Students sign a non-disclosure agreement and are issued company laboratory notebooks that remain the property of the company.

For the course, the unlabeled organic waste materials bench chemists would identify using both classical chemical testing methods and spectroscopic techniques, were, in fact, a large library of organic compounds assembled and placed in numbered vials [14]. In keeping with "green" chemistry principles, chemists were only provided with small quantities of the "unknowns" – usually less than ten grams. The compounds for the library were selected so as to have a broad array of solids and liquids containing aliphatic, unsaturated and/or aromatic components. Additionally, analytes were chosen that had a wide range of functional groups. See Figure 2.

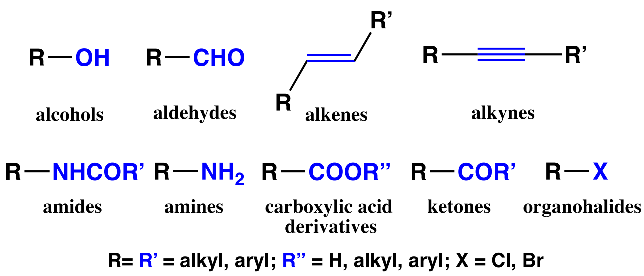


Figure 2. Functional Groups Present on IL-II Unknowns

A series of solubility and chemical test reagents were prepared and made available. These include distilled deionized water, acids and bases for solubility tests as well as functional group testing reagents such the Lucas, Hinsberg, Brady, Tollens, ceric nitrate, bromine, iodoform, potassium permanganate, chromic acid and ferric chloride reagents. Other tests may be provided if requested by the chemists. A wide variety of solvents are available and may be freshly distilled if required for inert atmosphere/dry solvent processes.

Table 3. IL-II Materials, apparatus and instrumentation available

Technique	Materials/Apparatus
Boiling Point	Hickman 12/20 microscale column
Thin Layer chromatography	Analtech silica gel plates
Column chromatography	60 mesh silica gel column
Synthesis/distillation	19/22 miniscale laboratory kit
Inert atmosphere reactions	Konte nitrogen line
Reduced pressure reactions	PLAS Labs glove box
	Konte Schlenk line
Instrumentation	
pKa	Vernier pH meter
Melting Point	SRS Digimelt MPA-160
Refractive Index	Abbe-3 refractometer
Optical Activity	Azzota Disc polarimeter
Spectrophotometry	Perkin Elmer UV-Vis
IR Spectroscopy	Nicolet 15S FTIR
NMR Spectroscopy	Anasazi-90 ($^{1}H/^{13}C$), Picospin-45 (^{1}H) NMR
GC-Mass Spectrometry	Shimadzu QP 20105 GC-MS
Separation	Agilent 1260 HPLC Chromatotron radial chromatograph
Solvent removal	Buchi rotary evaporator
Constant temperature reactions	J-Kem 210 temperature controller

Materials, apparatus and instrumentation made available for physical constant and chemical property determinations, synthesis, separations as well as spectral analysis are shown in Table 3.

The overarching goals for the course are for the bench chemists to employ critical thinking and problem-solving in bringing to bear their knowledge, skills, and abilities to identify four liquid or solid organic compounds. In addition, they must apply their knowledge of organic synthesis to prepare, purify and characterize a derivative of one of their identified organic compounds.

Specific bench chemist tasks outlined in the course syllabus include:

1. The preparation and delivery (both orally and in written form) of an Unknown Identification and Derivatization Briefing to the CEO, ForensiChem (CO 2). The briefing (in Powerpoint format) will describe in detail the following subtasks:

   a. A comprehensive analysis regime required to

   identify all unknown compounds in accord with the timeline (COs 1-6). The plan satisfactorily addresses: the student's specific approach to identify their unknowns (physical constant determination, solubility and chemical testing, spectroscopic analysis, etc); the derivatization plan (based on a carbonyl functionality) which includes at least two references from the scientific peer-reviewed literature; and any laboratory safety precautions required.

   b. A timeline that describes the major activities to

   be accomplished in sufficient detail and includes identification of four unknowns, the derivatization experiment and all report submissions.
2. Unknown identification reports are prepared using the standard company report form (CO 3). Each bench chemist must complete and submit four reports to the CEO, ForensiChem for approval [14].
3. Derivative synthesis of an identified unknown compound (CO 5). Bench chemists select one of the organic unknowns they have identified and upon CEO approval, synthesize a derivative of the molecule. Chemists are also responsible for the complete purification and characterization of the compound by all means at their disposal.
4. Preparation of a journal article describing the synthesis, isolation and characterization of their derivative (COs 2, 3, 6). Bench chemists will use the Journal of Organic Chemistry (JOC) guidelines for authors [15] to construct the manuscript in the proper format. At a minimum, the article will contain an abstract, introduction, results and discussion, conclusion, and reference sections. The reference citation format is prescribed by the American Chemical Society [16].

Grading rubrics for the briefing and final journal article are supplied to the bench chemists so that the performance standards for the tasks are clearly understood [14].

3. Course Execution – Results and Discussion

3.1. IL-I

In the first semester that IL-I was offered, three students registered for the course and chose the following research projects: HPLC analysis of dopamine and caffeine binding to NM, RT-PCR of pigmented vs non-pigmented cells, and fluorescence spectroscopy of amines in the presence of NM. In the second term, eight students registered for IL-I. To aid in scale up due to class size, students were assigned to work in pairs rather than individually. Students were still expected to produce independent specific aims and conclusions at the end of the term, but in this case, those with the most similar projects had a dedicated partner to work with and were asked to consult amongst themselves whenever possible to alleviate instructor time requirements.

Projects chosen by IL-I students upon the second course offering built upon the data and conclusions of prior IL-I students and included: Influence of buffers and pH on melanin polymerization and the formation of hydrogels, investigation of competition binding against nicotine using nano-drop fluorescence spectroscopy, cell culture, RT-PCR, IHC, and effects of alternate catecholamines on melanin polymerization kinetics. Students were highly encouraged to follow interesting leads they encountered during their project to capitalize on serendipitous discovery. Indeed, one such discovery during the first IL-I term led to the development [17] of a nicotine binding assay with possible utility in measuring the binding constants of compounds which do not induce a unique spectroscopic signal of their own. This competition assay was then utilized by a pair of students in the following IL-I term, each choosing an individual compound for testing. In this manner, students were truly able to observe and benefit from the slow, steady progression of science in real time.

3.2. IL-II

In the first semester that IL-II was offered, four students registered for the course and each selected four unknown organic compounds from among sixteen choices. In the second term, five students registered for IL-II. To aid in scale up due to class size, twelve more unknown organic compounds were added to the compound library to allow for rotation of compounds.

All bench chemists successfully conducted their unknown analysis and derivatization briefing, identified their unknown compounds, synthesized their derivatives, and completed the reports and journal article as prescribed. Throughout the semester, students were encouraged to discuss their efforts with each other and post literature resources and protocols located via literature searches on the course D2L-Brightspace discussion boards. Additionally, students were assessed with three quizzes that measured their mastery of qualitative analysis methods, chemical testing mechanisms and spectroscopic analysis. During the first two terms, two students prepared 2,4-dinitrophenylhydrazones of aldehydes, two students prepared 2,4-dinitrophenylhydrazones of ketones, one student prepared a 2,4-dinitrophenylpyrazole, one prepared aspirin from salicylic acid, one prepared a carboxylic acid from an alcohol, one student prepared a tetrabromoalkane from an alkyne and one prepared an acetamide from an aromatic amine.

3.3. Success of the Integrated Laboratory Course Sequence

Assessing the initial success of integrated laboratory course sequence was a function of several metrics. For IL-I, publication of scholarly products with student co-authorship related to the NM research project was a key indicator of the research value of the course. For both IL-I and IL-II, instructor-generated course assessment reports provided a measure of course outcome mastery. Finally, student end of course attitudinal surveys for the integrated laboratory provided a qualitative assessment of success with the courses in terms of instructional design and delivery as well as student-teacher engagement.

Research conducted during the first two cycles of the IL-I course led to the preparation, submission and publication of two peer-reviewed articles. The first article, published in *Neurochemical Research* [18] featured GGC undergraduate students who participated in the integrated laboratory course. The second journal article, published in *Neural Regeneration Research* [13] featured GGC faculty who collaborated on neuromelanin research.

As part of the overall Chemistry program assessment strategy, faculty teaching the integrated laboratory course sequence prepared course assessment reports following each semester. Evaluations of course success, based on the course outcomes (Table 2), formed a principal component of that assessment. Shown in Table 4 are the data for the first two cycles of the integrated laboratory course sequence.

Table 4. Integrated Laboratory Course Outcome Assessment

Course	IL-I[1]		IL-II[2]	
Outcome	%[3]	SD[4]	%[3]	SD[4]
1	89.09	7.01	87.56	0.53
2	79.09	7.01	87.56	0.53
3	86.50	2.42	87.56	0.53
4	100.00	0.00	96.56	0.53
5	82.73	4.67	94.22	2.11
6	96.39	2.34	89.00	0.00
7	92.73	4.67	89.00	0.00

[1]n=11. [2]n=9. [3]% denotes % mastery of course outcomes.
[4]SD=Standard Deviation.

Instructors noted successful accomplishment of course outcomes suggesting that student abilities in instrumentation use and applications, oral presentations, writing abilities, critical thinking skills and problem-solving improved during the courses.

Students participated in an end-of course survey to provide instructors a gauge of how successful the course design and execution had been. The Likert-type attitudinal survey consisted of questions designed to provide feedback to instructors on whether students viewed the instructional design, instructional delivery and student-teacher engagement as satisfactory. The scale ranged from a score of 1 (strongly disagree) to 4 (strongly agree). Table 5 contains a summary of the results.

Table 5. End of Course Survey Results

Survey Area	IL-I[1]		IL-II[2]	
	Rating	SD	Rating	SD[3]
Instructional Design	3.74	0.20	4.00	0.00
Instructional Delivery	3.85	0.09	3.94	0.05
Student-Teacher Engagement	3.75	0.09	4.00	0.00

[1]n=11. [2]n=9. [3]SD=Standard Deviation

Students provided narrative feedback on the end-of-course surveys as well. Sample comments are provided below.

- What I found most valuable about this course was:
  - The experience of designing an experiment with many instrument options.
  - Combining the different techniques I have learned and using them to answer a real-world problem in the field of science.
  - The experience of being independent and simulate a possible work environment in the future.
  - The amount of knowledge I had to apply and the ability to complete a final product by myself.
  - This course took chemistry lab to a whole new level. The most valuable thing was incorporating everything I learned in organic and somewhat from instrumental chemistry to identify an unknown compound. The techniques that are used in this course can benefit my future as a chemist.
- What I found most challenging about this course was:
  - Interpreting the experimental results.
  - Learning to be persistent.
  - Having to recall principles and ideas from previous courses. The instructor challenged us to research answers we arrived at to make sure we had a firm grasp of them.
  - The need to understand IR, NMR, MS, MP, TLC, and other physical characteristics to determine the identity of an unknown.

Thus, survey results for both IL-I and IL-II suggested that students found satisfaction with the open-ended nature of the course design, the manner in which the content was delivered and the high level of student-teacher engagement. Students comments indicate the courses were extremely challenging yet rewarding.

4. Conclusions

The first two cycles of the integrated laboratory course sequence were successful examples of how STEM students at Georgia Gwinnett College have benefitted from 4-Yr undergraduate research creative experiences. These courses provided unique opportunities for students to participate in projects and activities that mimic graduate research programs as well as chemical industry remediation operations. The open-ended laboratory-based nature of the courses creatively challenged students to use problem-solving skills to answer questions of biochemical and chemical interest. While future iterations of the course will doubtless see increased enrollment, new instructors, and different research opportunities for IL-I and chemical industrial applications for IL-II, the adaptability of the course sequence to new research directions will ensure its continued success as an integrated capstone research experience for GGC STEM majors.

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