Focusing Review

Twenty Years of Collaborative Research in Invertebrate Biology and Analytical Chemistry at Lafayette College

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Abstract

This review updates the earlier one in this journal on our collaborative research program in invertebrate biology and analytical chemistry at Lafayette College. This review covers the 2001–2007 period in which 19 different undergraduates published 44 peer reviewed papers with us. Most of the studies used quantitative thin layer chromatography to examine various analytes in medically important snails, leeches. parasitic flatworms, and mouse feces and urine. The analytes studied were lipids, lipophilic pigments, carbohydrates, amino acids, bile pigments and metallic ions. High–performance liquid chromatography, atomic absorption spectrometry, and inductively coupled plasma–atomic absorption emission spectrometry were also used in some of the studies. Involving undergraduate students in research can provide extraordinary benefits for their education and professional development and is the ultimate mechanism in the problem based learning approach that is becoming increasingly used to teach chemistry and other science courses. The interdisciplinary research model described has allowed undergraduate students to independently complete research projects that are publishable in high quality peer reviewed journals. The research experience is usually the defining undergraduate endeavor of these students, most of whom pursue careers in the chemical or biochemical sciences or medicine, and the resultant publications allow them to compete successfully for entrance into highly prestigious graduate programs.

Keywords : invertebrate biology, analytical chemistry, Lafayette College, undergraduate research, HPLC, AAS, TLC, ICP–AES

1. Undergraduate Research as a Teaching and Learning Tool

A unique undergraduate collaborative research program involving the use of analytical techniques to study biological systems, begun in the late 1980 s at Lafayette College, was described in an earlier paper in this journal [1]. This is the 20 th anniversary of the first student coauthored paper resulting from that program [2], and we would like to take this opportunity to provide additional information on its operation and success.

Many reasons for the importance of undergraduate research for faculty and students were outlined in the previous paper [1]. This importance is even greater now with the ever wider adoption of the collaborative or cooperative problem based learning (PBL) strategy in science laboratory courses, especially analytical chemistry courses. PBL [3–5] involves teaching important concepts in the context of solving real analytical chemistry problems to students organized into groups. As the advantages of this teaching/learning approach in analytical laboratory courses become increasingly realized by faculty, it is more appreciated that undergraduate research is the ultimate exercise in PBL and that it can provide dramatic benefits for student education and professional development. It is the best method for exciting students about science and equipping them with the knowledge and skills needed in their career. It teaches critical thinking and how to apply the knowledge gained in their courses. Most of our students who have pursued careers in the sciences and medicine consider research to have been the defining endeavor in their undergraduate experience.

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2. The Biology–Analytical Chemistry Collaborative Program

In 2002, the American Chemical Society's Committee on Professional Training defined the characteristics of an undergraduate research program that meets the requirements for a certified BS–Chemistry degree [6]. We already had incorporated all of these attributes into our individual research programs that were started when we arrived Lafayette College some 40 years earlier and then into the collaborative program we initiated in the early 1980s. Our students carry out important scientific projects with the goal of developing new knowledge and publishing it in peer–reviewed journals. The projects are based on our research interests so students can draw on our expertise and resources. After initial training by us, and by our advanced students in the case of new students in our group, we meet regularly with the student to review results and make plans for the future, but the student has the primary responsibility for the project and provides substantial input into its direction, especially as his or her experience increases. The student–mentor relationship builds student confidence, moves the student towards the completion of the project, and guides the student's future education and career development. We emphasize publication of results because the peer review process insures the quality of the research and exposes the student to the entire research process, including design of a project with well defined objectives, collecting pertinent literature references, completion of the experimental work, preparing a paper for submission to a journal, revision based on referees' comments, and publication. We consider the research to be incomplete without acknowledgement from, and dissemination to, other scientists in the form of a peer–reviewed publication, and a record of published research is vital for students wanting to apply to highly competitive graduate school programs or to be successful in other career goals. We design projects that can be completed by each student in the available time, but, if necessary, a project started by one student can be completed by another, leading to a jointly–authored paper.

A survey conducted in 2000 by Research Corporation and four other foundations [7] found that faculty at primarily undergraduate institutions publish, on average, about one paper every other year, and only about 25% of these papers include an undergraduate student as a coauthor. Our program is dramatic proof that much higher productivity in an undergraduate setting is possible if a program that excites and engages students is developed. Too many faculty believe that research performed by undergraduates cannot have the necessary quality to be published, and they have students perform experiments and work on problems whose results are already known or are easily predictable ; this type of work is not publishable and is not defined as research by us.

Table 1 summarizes the published results of our collaborative studies from 2001 to 2007. The table provides information on the analytical techniques used, a brief summary of each study, and the citation (without the title) of the work. Most of the names mentioned in the table, other than our own, are those of student collaborators.

The number of our collaborative publications in refereed journals averaged 6.3 per year. All of the research students who worked with us had at least one coauthored paper with us. Some of the students published two or more papers, and three students (Ponder, Schneck, and White) each published four papers with us. We continued to publish mainly in the same journals (No. 1, 3, 4, 6, 12, 21) mentioned in our earlier review, although two papers (No. 2, 39) appeared in journals new to us in regard to our collaborative studies. During our 20–year collaborative program, we have published a total of 108 papers in 17 different peer–reviewed journals, approximately one–half concerned mainly with chemistry and the other half with biology. We continued to use TLC as our main analytical technique, but as seen in papers numbered 12, 23, 26, 32, 41, 42, various other analytical procedures were used. In addition to the usual analytes we have studied in the past, i.e., lipids, lipophilic pigments, carbohydrates, amino acids, and metallic ions, we analyzed bile pigments during the 2001–2007 period.

Our work, as in the past, continued to examine various analytes in the larval stages of the medically important trematodes *Schistosoma mansoni* and *Echinostoma caproni* (for example No. 5, 6, 11). We also made similar analyses on adults of *E. caproni* experimentally raised in laboratory mice (No. 8). We continued our work on the pathochemical and pathobiochemical effects of larval trematodes on their snail hosts with emphasis on the *E. caproni–Biomphalaria glabrata* system and the *S. mansoni–B. glabrata* system (No. 5, 6, 23). We expanded our studies to look at multiple effects on analytes in larval trematode–snail systems under conditions of parasitism and estivation or conditions that included diet alteration and parasitism (No. 1, 17, 35, 37, 38).

We tested the the hypercalcification hypothesis, which states that larval trematode parasitism induces an increase in the concentration of Ca in snails' shells ; it was found that this was not so, at least in the snail–parasite systems we used, and in fact we had evidence in certain cases for significant hypocalcification (No. 26). We also looked at the supposed concentration of Ca in snail shells by testing Ca in numerous species of snails and confirmed the fact that the Ca concentration was fixed within a relatively narrow range in snail shells (No. 39).

We explored new model systems including the edible apple snail *Pomacea bridgesii* and the medically important leech *Hirudo medicianlis* and contributed new information on various analytes in these invertebrates (No. 24, 28, 29). Likewise, we explored the lipid concentration of *B. glabrata* subjected to various different salinities and were surprised by the tolerance of this medically impor-

tant snail to relatively high salinities (No.44). Lastly, we began metabolic profiling studies to determine differences in various analytes in the feces and urine of mice experimentally infected with *E. caproni* versus the uninfected controls. Our first studies on lipids in the feces of infected mice showed significant differences in certain classes of neutral lipids in the feces of the infected mice versus the controls but insignificant differences in polar lipids (No. 40, 43). The ultimate goal of this research is to use the mice as models to develop a simple medical test based on TLC to diagnose parasitism in humans

The analytical techniques that have been used by our students in their research are essentially the same as described in the earlier paper, including TLC and HPTLC on silica gel adsorption, cellulose normal phase partition, silica gel–silver nitrate argentation, C–18 chemically bonded reversed phase partition, and anion exchange resin layers with scanning densitometry ; cation exchange HPLC with a suppressed conductivity detector for determination of metals ; ion exclusion HPLC with an ultraviolet absorption detector for determination of carboxylic acids ; and inductively coupled plasma–atomic emission spectrometry and flame and graphite furnace atomic absorption spectrometry for determination of metals. The Chemistry Department has obtained new instruments for documentation and quantification of TLC and HPTLC and for HPLC, as well as a new gas chromatography/quadrupole mass spectrometry instrument that we plan to use in future projects that can benefit from its ability to absolutely identify and quantify very low levels of analytes in biological samples. One of our metal analysis studies (No. 23) involved collaboration with a chemistry alumnus (K. M. Koehnlein) and his coworker (G. L. Bosavage) working at a nearby pharmaceutical company, who had access to instrumentation not available at Lafayette (ICP–AES), and our HPLC and AAS analyses (No. 12, 23, 26, 32, 39, 42) have been aided by collaboration with the Chemistry Department's Instrumentation Specialist (M. Chejlava), who has needed expertise in these analytical methods. One of our TLC studies (No. 2) involved collaboration with a biology alumnus (L. R. Brunet) who maintained the life cycle of *S. mansoni* in her laboratory in the United Kingdom.

Most of our recent students have been BS–Chemistry or BS–Biochemistry majors, but AB–Chemistry majors have also been successful researchers. One of our students was a BS–Biochemistry–Neuroscience double major. Students work for academic credit during the school year in our Independent Research or senior Honors Research courses, while others have been supported by the college EXCEL Scholar Program for part–time research during the school year or full–time research during the three–week interim (January) session between semesters or for 10 weeks during the summer. In addition to funding from Lafayette College through the EXCEL Scholar Program and special accounts available to us as emeritus professors with endowed chairs, summer salaries for research students have been provided by three awards made to J. Sherma by the Camille and Henry Dreyfus Foundation Senior Scientist Mentor Program in 2001, 2004, and 2007.

Students who published with us during the 2001–2007 period are in graduate school pursuing doctoral programs in chemistry, biochemistry, molecular biology, immunology, genetics, and the marine sciences ; other graduates are working in various capacities in industrial or pharmaceutical chemistry ; two others are in medical school ; and two students will graduate in 2008 and are applying to Ph.D. programs in the biomedical sciences.

Despite the doubts of many research scientists, our experience has shown undergraduate students can succeed greatly and produce much very high quality, publishable research if a program is well designed with projects of interest to the students. The publications allow the faculty member to build a national, or even international, reputation and can be critical for students who want to enter highly competitive graduate or medical school programs or compete for a position as a chemist in industry upon graduation. This is particularly important for students at undergraduate institutions that are not generally known for the quality of their research. A research experience alone is helpful, but a record of publication is the best way for students to prove their accomplishments and establish their own reputations.

Our analytical chemistry–biology interdisciplinary model has worked especially well, but there is no doubt that collaborations in other areas are possible and can benefit students and faculty. In the period under review, one of us has collaborated with a developmental biologist and two undergraduate chemistry majors on the determination of phospholipids in fruit flies (*Drosophila melanogaster*) by quantitative HPTLC [8], and we are beginning a project involving the determination of neutral lipids in the side–blotched lizard *Uta stansburiana* in collaboration with an herpetologist. Successful collaborations have also occurred between a physical chemist and chemical engineer and an environmental chemist and civil engineer working together with chemistry and engineering majors at Lafayette.

This program involving thin layer and column chromatography and atomic spectrometry will be continued with students majoring in chemistry, biochemistry, biology, or other science or engineering programs who show an interest in interdisciplinary research. New analytical techniques will be used as the necessary instrumentation becomes available, and studies of different analytes and sample biological systems will be carried out as important new research areas are identified. For example, we will determine bile acids in mouse feces and urine as possible markers for parasitism. We will continue to stress the publication of research results and the benefits of the program on the education of the students while

at Lafayette and their success after graduation.

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References

[1] Sherma, J. ; Fried, B. Chromatography– Journal of Separation and Detection Sciences. **2001**. *22,* 17–24.

[2] Duncan, M ; Fried, B ; Sherma, J. *Comp. Biochem. Physiol*. **1987**, *86 A*, 663–665.

[3] Larive, C. K. *Anal. Bioanal. Chem*. 2004, *380*, 357–359.

[4] Yuzhi, W. *The China Papers*. July, **2003**, 28–33.

[5] Wenzel, T. J. Microchim. Acta. **2003**, *142*, 161–166.

[6] "Characteristics of Undergraduate Research". American Chemical Society Committee on Professional Training Newsletter., **2002**, Vol. III, No. 4 (Spring issue), 3.

[7] Rawls, R. L. *Chem Eng. News*. **2002** September 30 issue, 30 –31.

[8] Nyako, M. ; Marks, C. ; Sherma, J. ; Reynolds, E. E. *Biochem. Genet*. **2001**, *39*, 339–349.

Table 1. Collaborative Research in Invertebrate Biology – Analytical Chemistry, 2001–2007

No.	Techniques	Studies	Citations
1	TLC	Diet affects the neutral lipid content of *Biomphalaria glabrat*a infected with *Schistosoma mansoni*	Fried, B. ; Muller, E. E. ; Broadway, A. ; Sherma, J. *J. Parasitol.* **2001**, 87, 223–225.
2	TLC	*S. mansoni* infection alters the neutral lipid content of mice	Muller, E. ; Brunet, L. R. ; Fried, B. ; Sherma, J. *Int. J. Parasitol.* **2001**, 31, 285–287.
3	TLC	Carbohydrates in *B. glabrata* maintained on a high fat diet	Kim, Y. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2001**, 14, 61–63.
4	TLC	Effects of leaf lettuce versus mid–rib diet on lipids, pigments, and carbohydrates in *B. glabrata*	Eidam, P. M. ; Schariter, J. A. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol.* **2001**, 24, 1467–1478.
5	TLC	Glucose, maltose, and raffinose in *B. glabrata* infected with Echinostoma caproni	Wagner, S. D. ; Kim, Y. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2001**, 14, 459–461.
6	TLC	Glucose and maltose in *B. glabrata* infected with *S. mansoni*	Schariter, J. A. ; Fried, B. ; Sherma, J. *Acta Chromatogr*. **2001**, 11, 102–107.
7	TLC	Lutein and β–carotene in *B. glabrata* on a high fat diet	Kim, Y. Y. ; Fried, B. ; Sherma, J. *Veliger* **2002**, 45, 256–258.
8	TLC	Amino acids and carbohydrates in *E. caproni* adults	Pachuski, J. ; Wagner, S. D. ; Fried, B. ; Sherma, J. *Comp. Parasitol*. **2002**, 69, 202–205.
9	TLC	Lipids in *S. mansoni* cercariae	Schariter, J. A. ; Pachuski, J. ; Fried, B. ; Sherma J. *J. Liq. Chromatogr. Relat. Technol*. **2002**, 25, 1615–1622.
10	TLC	Carbohydrates and amino acids in *S. mansoni* cercariae	Wagner, S. D. ; Pachuski, J. ; Fried, B. ; Sherma J. *Acta Chromatogr*. **2002**, 12, 159–169.
11	TLC	Amino acids in B. glabrata infected with *S. mansoni*	Pachuski, J. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol.* **2002**, 25, 2345–2349.
12	AAS, HPLC	Metallic ions in *Cerithidea californica* infected with *Euhaplorchis californiensis*	Kaufer, S. W. ; Chejlava, M. ; Fried, B. ; Sherma, J. *Parasitol. Res*. **2002**, 88, 1080–1082.
13	TLC	Amino acid release of *E. caproni* adults is affected by the tonicity of the media	Ponder, E. L. ; Fried, B. ; Sherma, J. *Parasitol. Res.* **2003**, 89, 242–244.
14	TLC	Amino acid content of various larval stages of *E. caproni*	Ponder, E. L. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2003**, 26, 2697–2702.
15	TLC	Lipids in adult *Helisoma trivolvis* (CO strain) on a high fat diet	Schneck, J. L. ; Fried, B. ; Sherma, J. *Veliger* **2003**, 46, 325–328.
16	TLC	Lipids in juvenile *H. trivolvis* on a high fat diet.	Schneck, J. L. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2003**, 16, 405–407.
17	TLC	Diet and larval trematode parasitism affect lutein and β–carotene concentrations in planorbid snails	Evans, R. T. ; Fried, B. ; Sherma, J. *Comp. Biochem. Physiol.* **2004**, 137 B, 179–186.
18	TLC	Neutral lipid release of *E. caproni* adults is affected by the tonicity of the medium	Schneck, J. L. ; Fried, B. ; Sherma, J. *Parasitol. Res.* **2004**, 92, 285–288.
19	TLC	Hydrophilic vitamins in standards and *H. trivolvis*	Ponder, E. L. ; Fried, B. ; Sherma, J. *Acta Chromatogr.* **2004**, 14, 70–81.

20	TLC	Amino acids in *B. glabrata* infected with *E. caproni*	Ponder, E. L. ; Fried, B. ; Sherma, J. *J. Parasitol.* **2004**, 90, 665–666.
21	TLC	Effects of aging on lipids in *B. glabrata*	Schneck, J. L. ; Fried, B. ; Sherma, J. *Veliger* **2004**, 47, 100–102.
22	TLC	Neutral lipids in snail conditioned water and feces of *B. glabrata* infected with *E. caproni*	Schneck, J. L. ; Bandstra, S. R. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2004**, 27, 2039–2045.
23	ICP–AES	Inorganic elements in *B. glabrata* infected with *S. mansoni*	Ong, J. H. L. ; Chejlava, M. ; Fried, B. ; Koehnlein, K.M. ; Bosavage, G. L. ; Sherma, J. *J. Helminthol.* **2004**, 78, 343–346.
24	TLC	Lipids in the apple snail *Pomacea bridgesii*	Jarusiewicz, J. A. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2004**, 17, 454–458.
25	TLC	Neutral lipids in snail conditioned water and feces of *B. glabrata* infected with *S. mansoni*	Bandstra, S. R. ; Schneck, J. L., Fried, B. ; Sherma, J. *Chemia* **2004**, 7(2), 3–9.
26	HPLC, AAS	Effects of larval trematodes on $CaCO_3$ in snails' shells	White, M. M. ; Chejlava, M. ; Fried, B. ; Sherma, J. *Parasitol. Res*. **2005**, 95, 252–255.
27	TLC	Sterols in snails	Jarusiewicz, J. A. ; Sherma, J. ; Fried, B. *J. Liq. Chromatogr. Relat. Technol*. **2005**, 28, 2607–2617.
28	TLC	Lipids in *Hirudo medicinalis*	Martin, D. L. ; Sherma, J. ; Fried, B. *J. Liq. Chromatogr. Relat. Technol*. **2005**, 28, 2597–2606.
29	TLC	Absence of (β–carotene and presence of biliverdin in *H. medicinalis*	Martin, D. L. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2005**, 18, 400–402.
30	TLC	Diet affects carotenoids and lipids in *P. bridgesii*	Jarusiewicz, J. A. ; Fried, B. ; Sherma, J. *Comp. Biochem. Physiol*. **2006**, 143B, 244–248.
31	TLC	Diet affects lipids in *H. medicinalis*	Martin, D. L. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2006**, 19,167–170.
32	AAS, HPLC	Effects of a high fat diet on inorganic elements in *H. trivolvis* (CO strain)	Ong, J. H. L. ; Chejlava, M. ; Fried, B. ; Sherma, J. *Veliger* **2006**, 48, 1–7.
33	TLC	Estivation and starvation affect lipid content of planorbid snails	White, M. M. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2006**, 29, 2167–2180.
34	TLC	Estivation affects lipophilic pigments in planorbid snails	Arthur, B. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2006**, 29, 2159–2165.
35	TLC	Effects of diet and trematode parasitism on lipids in snails	Bandstra, S. R. ; Fried, B. ; Sherma, J. *J. Planar Chromatogr.* **2006**, 19, 180–186.
36	TLC	Effects of *E. caproni* parasitism on lipids in *B. glabrata*	Bandstra, S. R. ; Fried, B. ; Sherma, J. *Parasitol. Res*. **2006**, 99, 414–418.
37	TLC	Effects of parasitism and estivation on sugars in *B. glabrata*	Jarusiewicz, J. A. ; Sherma, J. ; Fried, B. *Comp. Biochem. Physiol*. **2006**, 145B, 346–349.
38	TLC	Effects of parasitism and estivation on lipids in *B. glabrata*	White, M. M. ; Fried, B. ; Sherma, J. *J. Parasitol*. **2007**, 93, 1–3.
39	HPLC	Concentration of $CaCO_3$ in shells of freshwater snails	White, M. M. ; Chejlava, M. J. ; Fried, B. ; Sherma, J. *Amer. Malacol. Bull*. **2007**, 22, 139–142.
40	TLC	Neutral lipids in the feces of mice infected with *E. caproni*	Bandstra, S. R. ; Murray, K. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2007**, 30, 1437–1445.
41	HPLC	Carboxylic acids in *B. glabrata* infected with *S. mansoni*	Massa, D. R. ; Chejlava, M. J. ; Fried, B. ; Sherma, J. *Parasitol. Res*. **2007**, 101, 925–928.
42	TLC, HPLC	Carboxylic acids in standards and *H. trivolvis* snails	Massa, D. R. ; Chejlava, M. J. ; Fried, B. ; Sherma, J. *J. Liq. Chromatogr. Relat. Technol*. **2007**, 30, 2221–2229.
43	TLC	Polar lipids in the feces of mice infected with *E. caproni*	Murray, K. E. ; Fried, B. ; Sherma, J. *Acta Chromatogr*. **2007**, 18, 190–198.
44	TLC	Effects of salinity on the lipid content of *B. glabrata*	Martin, D. L. ; Fried, B. ; Sherma, J. *Veliger* **2007**, 49, 101–104.

Abbreviations : AAS = flame and/or graphite furnace atomic absorption spectrometry ;
HPLC = ion exchange or ion exclusion column high performance liquid chromatography ;
ICP–AES = inductively coupled plasma–atomic emission spectrometry ;
TLC = thin layer chromatography and/or high performance thin layer chromatography.

Note : In No. 25, the full title of *Chemia is Acta Universitatis Cibiensis, Seria F, Chemia*.