

Adapting the Chemical Analysis of Paintings in the Context of Art Conservation to
a Project-Based Learning Instrumental Analysis Laboratory

Progress Report for Year 1 for ADSL.

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Abstract.

This manuscript is envisioned as a “living” progress report for my attempt at Rensselaer Polytechnic Institute to use art conservation methods as a means to help better teach analytical chemistry. The intent is to update this manuscript and web site as a means to provide additional information about what works and what does not work with respect to project based learning with this approach in the Instrumental Methods of Analysis laboratory. This report gives a brief introduction to the chemistry of paintings. Additionally, the different instrumental approaches commonly used to identify various chemical components of paintings are described. Next, this report describes a recent effort at Rensselaer Polytechnic Institute to bring project-based learning to the upper-level instrumental analysis laboratory. The molecular analysis of a painting (The Buffalo Room, by Robert Chanler) that needed to be conserved was used as the basis for this project. The unique chemical qualities of paintings that make this type of chemical analysis highly challenging are described. Finally, lessons learned from our first attempt to bring forth this type of learning to our students are described.

Project -Based Learning.

Historically, Instrumental Methods of Chemical Analysis has been taught from the perspective of having a set of defined experiments that allow students hands-on access to various instruments. The pedagogical rationale for this approach is that students learn the instrumentation by using it. While students sometimes get to operate the instrumentation, they are often not truly challenged and maybe not fully aware of the chemical basis behind the different experimental steps that are involved in actually measuring the components of a sample. All practicing analysts know the instrumental step is among the last steps performed for sample chemical analysis. Preceding the analysis step are the sampling steps, dissolution steps, perhaps treatment with specific reagents, extractions, and sometimes sample preconcentration. These steps all require a much greater chemistry understanding than simply using the instrument to analyze a pre-made sample. Therefore, this approach to teaching the instrumental laboratory gives students a mechanical introduction to instrumentation for chemical analysis rather than forcing them to consider the many options available to solve a particular chemical analysis problem. Additionally, the complexities of the chemistry involved with sample preparation processes prior to instrumental analysis are not fully appreciated. For this reason and for other pedagogical reasons, e.g., using real world samples, many instructors have turned to the project-based learning approach for content delivery in the instrumental laboratory course.

The NSF has held a symposium aimed to integrate analytical sciences across college chemistry curricula. In addition to this symposium, there have been many researchers at four-year colleges as well as research universities, who have developed innovative analytical chemistry curricula focused on the chemical analysis of real world samples since the 1980s [1]. Among these different examples, increased student enthusiasm levels as well as a greater appreciation for the problem-solving process have been communicated [2]. However, It has been noted by others at the University of Kansas, which has used this PBL approach in their courses for many years, that students often do not realize is how much work it really takes to perform a real chemical analysis [3].

Art as a Teaching Tool in Science Curricula.

Visual art provides real-world science examples that pique student interest in chemistry and physics (optics) courses. This approach has been used at many levels including K-12 [4], undergraduate [5,6] as well as graduate education [7]. From the perspective of using art pieces as samples for analytical chemistry instruction, there are relatively few examples in the literature. Professor Margaret Merritt at Wellesley College used a project focused on pigment analyses of art objects in an Analytical Chemistry course as a means to illustrate the needs of modern analytical chemistry, which requires understanding the multiple component steps (sampling, sample dissolution, sample preparation) involved in an analysis [8]. In this approach, the students wrote proposals and had to seek the input of other experts including an art curator who was a member of the Wellesley arts faculty as well as experts at Harvard University and the Museum of Fine Arts in Boston. It appears from the information available that art objects were only used for part of the course to illustrate one particular type of instrument.

Paintings provide a unique complex sample that has the potential to require knowledge in nearly all the major sub-disciplines of chemistry (analytical, biochemistry, inorganic, organic, and physical). Any pigment analysis often requires inorganic components. The pigments can also aggregate and form nanocomposites. Binders are made of plant or animal materials (milk or egg proteins) and thus highlight analysis of biochemical components since they have different amino acid contents [9,10]. Thus, the proteomic analysis of binders is often a critical aspect for solving a conservation analysis problem. Sometimes additional varnishes are used which require knowledge of organic chemistry and organic analyses. All of these issues coupled with required chemical analysis provide a highly interdisciplinary project for chemical analysis all contained in one sample.

Studies of the molecular aspects of paintings has such great historic as well as scientific importance that the Dutch government via the Dutch Organization for Scientific Research (NWO) developed a cooperative project entitled “MOLART” – Molecular Aspects of Aging of Painted Art. The goals of the researchers involved with MOLART aimed to develop a scientific framework at the molecular level for art conservation [7]. Approximately seven Ph.D. theses,

many of them with a chemical analysis focus have emerged as output from the MOLART project.

Analytical Chemistry applied to Art Studies.

Chemical analysis applied to problems in art have focused in three major areas – 1) determination of forgeries (e.g., is the painting completely forged or merely touched up?), 2) degradation studies since knowing the process of degradation for a painting allows for suitable methods to be developed to prevent accelerated damage of the object and 3) conservation studies. This project developed at RPI focused on the latter – conservation studies. In particular, this project is focused on conservation of historical paintings in NY State.

Conservation studies are highly local. To truly conserve an object that has cultural as well as historical value requires an accurate, detailed knowledge of the material used in the creation of the object. This is a difficult challenge that requires both an understanding of the historical context of the object as well as general scientific knowledge to begin the conservation process. Furthermore, many of the objects that have to be conserved are of significant age (100 years old or greater). The significant object age can cause problems since oftentimes the historical records are either incomplete or missing. The effects of chemical aging (deterioration of paint and binder, etc.) combined with incomplete knowledge of storage conditions for the painting throughout its lifetime are common problems that painting conservators face on a daily basis. In addition to these environmental factors, pigment and binder creation were often individualistic preferences of the artist and are tremendously variable from one artist to the next. Painters often created their own pigments and rarely kept records. Moreover, the aging processes that occur during the painting lifetime are actually initiated from the time the first layer is applied. Paint drying can occur within a few hours and depends on numerous environmental factors – humidity, temperature, light intensity and the specific pigments or other additives used. Even after drying, chemical reactions continue throughout the lifetime of the painting. These chemical reactions cause discoloration, yellowing, fading, and darkening of the pigments.

Appreciating the heterogeneity of a painting's physical structure is critical for approaching the chemical analysis of these samples [11]. A painting consists of many

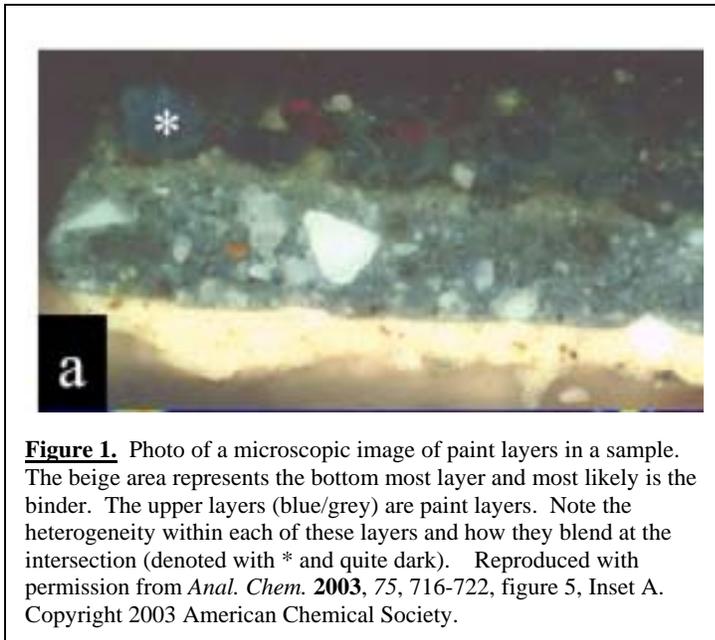


Figure 1. Photo of a microscopic image of paint layers in a sample. The beige area represents the bottom most layer and most likely is the binder. The upper layers (blue/grey) are paint layers. Note the heterogeneity within each of these layers and how they blend at the intersection (denoted with * and quite dark). Reproduced with permission from *Anal. Chem.* **2003**, 75, 716-722, figure 5, Inset A. Copyright 2003 American Chemical Society.

superimposed layers placed onto a support that is either a wooden panel or a canvas as shown in Figure 1 [12]. The first layer is what is known as the "ground" layer and is sometimes called the "binder" or the support layer. The binding layer can be made up of either carbohydrates or proteins. Then paint layers are applied and may consist of several layers for large areas coupled with detailed regions and highlights. The paint layer can contain inorganic as well as organic components. The

heterogeneity provides locally distributed micro- and nanostructures throughout the paint layer. It is common to apply a varnish layer after pigment drying to provide gloss as well as saturate the paint colors. Thus, the end result is a multi-layered sample that becomes blended. Furthermore, painted objects are not homogenous and adjacent areas on the painting can be quite different from each other with respect to their paint layers.

Outline of Analytical Methods used for Paint Analysis.

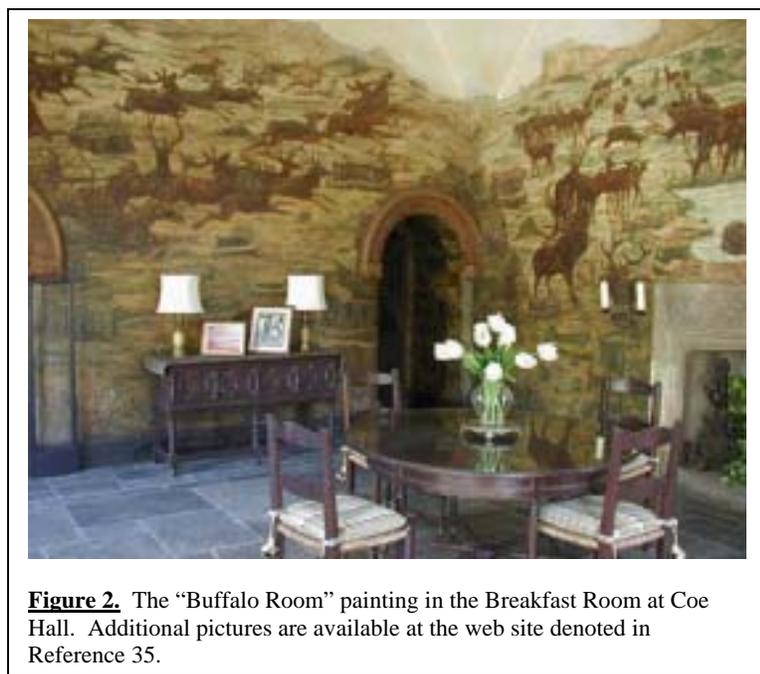
Paintings can be analyzed for their molecular content by using a variety of different instrumental methods of analysis [13]. The principal methods applied to paintings are chromatographic and spectrometric. Chromatographic analysis of paintings can involve the use of thin-layer chromatography (TLC), gas chromatography (GC) and liquid chromatography (LC). TLC is typically only used to obtain rapid information about a particular class of analyte being present in the painting. The lack of resolving power forces the use of GC and LC methods for separation and quantitative analysis.

Gas chromatographic methods have been widely used in the chemical analysis of paint samples [14]. GC has been applied to amino acid analysis [15,16] and fatty acid analysis [17,18]. Pyrolysis-GC allows for the online decomposition of materials to yield volatile products

that can be chromatographically separated. Pyrolysis-GC is commonly applied to analyze synthetic resins or the varnishes that are applied to different paintings [19,20].

High performance liquid chromatographic separation of derivatized amino acids has been applied to determining amino acid content of paint binders [21]. LC analysis of individual amino acids in different proteinaceous binders (gums, egg yolk, and egg white) has been reported [22]. More recently, various LC-MS approaches to molecular analysis of paintings for determination of amino acids, fatty acids, and organic dyes using either atmospheric pressure ionization (APCI) or electrospray ionization (ESI) have been described [23,24,25].

Thermal analysis can be used to determine chemical composition changes in the binding media [26,27]. Different laboratory methods have been described for studies of paint aging as well as accelerated aging [16,28,29]. Numerous methods have been described for protein analysis of binders including standard chromatographic methods [18,30], immunofluorescence methods [31], as well as more sophisticated LC-MS [32] and MALDI methods [33]. Analysis of the pigment is challenging, as it can be inorganic or organic and often requires an art historian or conservator consultant with knowledge of the painting style of the artist. Inorganic pigments can be identified using x-ray fluorescence [34].



Experience using PBL of Art Objects for Instrumental Analysis Lab.

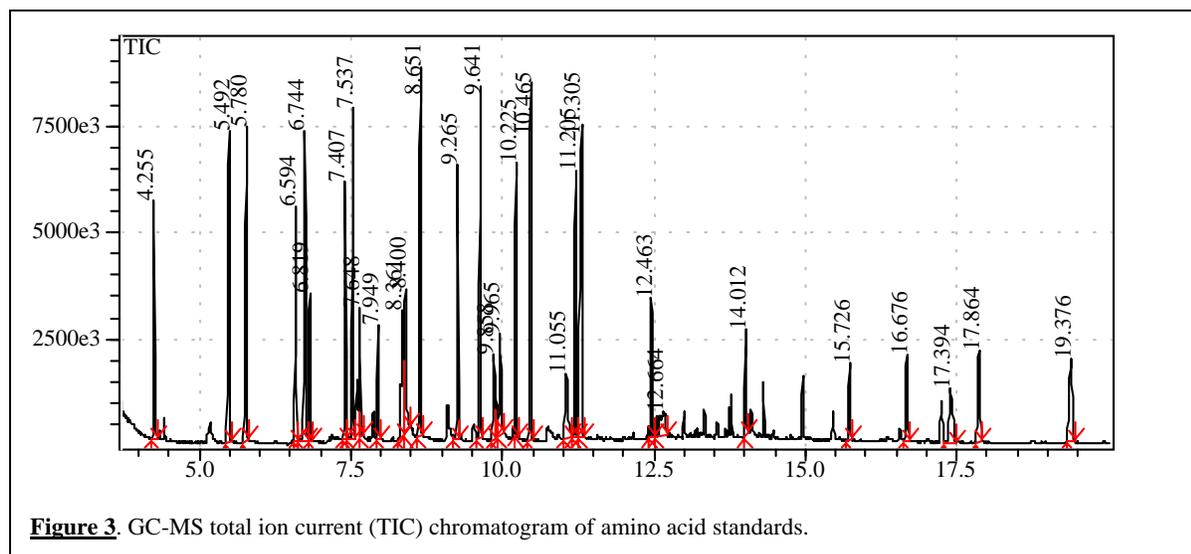
In August 2003, Professor Ron Bailey, acting Chemistry chair at RPI was approached by Joyce Zucker, Painting Conservator, New York State Office of Parks, Recreation and Historic Preservation, Bureau of Historic Sites, regarding a fragile mural painting at Coe Hall at the Planting

Fields Arboretum State Historic Park located on Oyster Bay, Long Island. This painting called the “Buffalo Room” (see Figure 2) was painted by Robert Chanler [35]. According to Joyce Zucker, Chanler was known as a “quirky” artist with respect to the binders and media that he used. This mural painting began to rapidly deteriorate after completion and has undergone numerous coating treatments in an effort to conserve it. The difficulty with conserving this painting is that it is not clear what type of binder was used for this wall painting. Furthermore, since it was directly painted onto a wall, the layers are different than typical canvas paintings.

In September 2003, a meeting with Joyce Zucker, Ron Bailey and the PI, occurred to discuss the chemical analysis needs for the Buffalo Room painting. Many chemical analysis issues came up including the needs to identify the chemical components of the top coating layers as well as the binding layer with the wall. We decided to focus on the binding layer in the Instrumental Analysis course where the PI taught the lecture and Professor Bailey supervised the laboratory in the spring 2004. The primary issue was how to best integrate this project into the existing lecture and laboratory course. It was decided to cut two of the required experiments towards the end of the term so that students would have time to work on the project at the end of term as well as have time to devise a plan of attack after being exposed to many of the instrumental methods needed to solve the complex analysis problem.

At the beginning of the course, Ms. Zucker came to lecture on the 2nd day of class (Jan 15, 2004) to introduce the chemical analysis problem from an art conservator’s perspective to the students. There was active student participation during this hour. We overviewed the different challenges associated with the chemical analysis of these samples. In particular, the complexities of sampling with an inhomogeneous painting with layered structure were mentioned. Furthermore, we discussed the extreme limitations with sample size and quantity. Ms. Zucker was generous in the very large samples (many were approximately 5 x 5 mm or smaller), in her opinion, that were given to the class for analysis. Many of these samples were removed from the wall behind the radiator to allow for larger sample size. (The students readily recognized this as an additional complication.)

Several class lectures were used for discussion of analytical methods that could be used to determine the chemical source of the deterioration. The students hypothesized that chemical information about the binder was needed. This decision was based on knowing the sample deterioration history. LC and GC analyses for the amino acid contents were chosen. The binder content is important since binders can be classified by either their composition (proteinaceous or carbohydrate) or by the solubility of the binder (soluble or insoluble in water) [36]. Protein-containing binders include casein (milk), egg yolk, egg white, and collagen (animal glue). Each group of approximately six to seven students had a team captain. The GC analysis was based on a paper found by the students in that group [37]. The GC analysis was performed using the department's Shimadzu QP5050 GC-MS instrument in the shared instrumentation facility and is

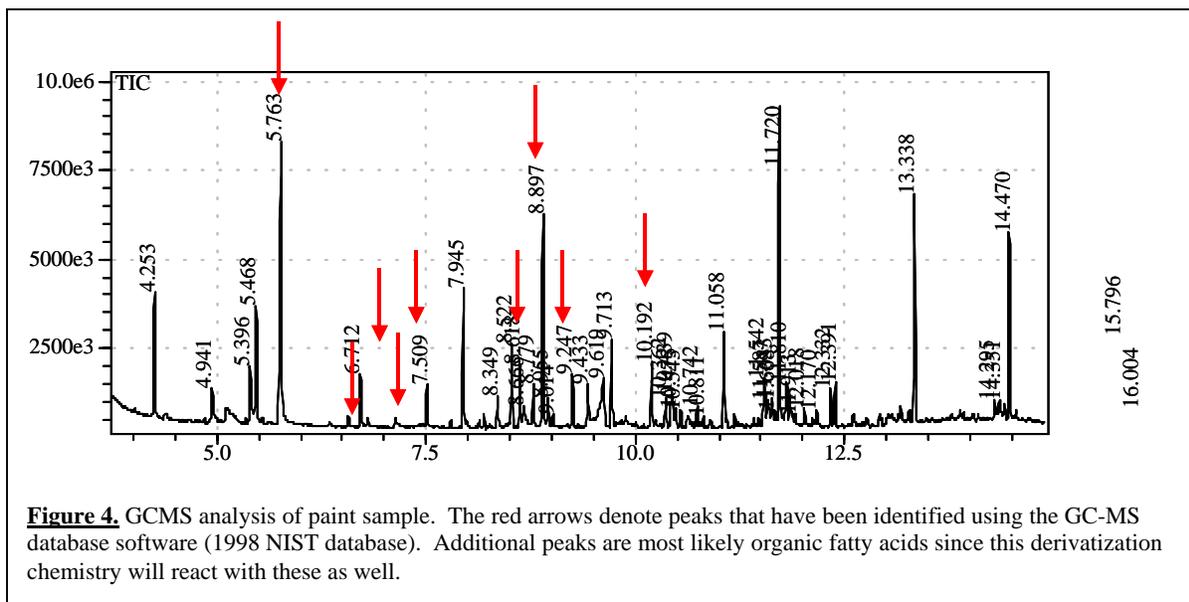


shown in Figure 3. The LC analysis was performed using UV amino-acid derivatization chemistry that has also been previously described [38]. The LC analysis was performed in Professor Stenken's research laboratory with her Shimadzu gradient LC-UV instrument with autosampler and computer control. The current HPLC system in the undergraduate lab is an isocratic system with a diode array detector and manual injection with a strip chart recorder for data collection and is not suitable for the large data set collected.

Each sample set required acid dissolution to solubilize the paint and binder. The students were agreeable to performing 5 to 6-hour dissolution steps despite only being scheduled for four hours. Some initial GC-MS data was obtained, but was not conclusive with respect to content

and concentrations of the amino acids in the sample. The LC analyses did not provide useful data. The students were only graded on participation for this special project. After this initial "failure", the course was officially over. More than half the class was completely dissatisfied with their results and truly felt as if they had let Joyce Zucker down because they had not produced anything. In other words, this group of students began to feel a sense of ownership in a short time period for their project. A couple of students actually worked past the end of the term through the reading days (2 days at RPI that are class-free prior to finals) and into finals week to retry the GC-MS analysis. The results improved, but most likely the complex samples as well as the inexperience of the students working with such complex chemistry may have caused additional problems.

One student (Mr. Dan Vissani) had a very strong desire to bring the project to completion and inquired if he could do undergraduate research on this project. He quickly produced in less than a week the two chromatograms shown in Figures 3 and 4. Figure 3 shows the GC-TIC (total ion current) chromatogram for the amino acid standards. Figure 4 shows the GC-TIC for a paint sample from the Buffalo Room. Furthermore, he wanted to see this project extended to students that would take Instrumental Analysis in subsequent years. Mr. Vissani had changed majors and thus was finishing in 4.5 years. He regretted finishing in the December term since he had an interest in being an undergraduate student TA for the course in the spring so as to continue this project.



Lessons learned using this initial PBL approach.

1. More time than a few weeks is required for the analysis. The students need additional time to formulate their plan as well as time to actually experience failure in the laboratory with the opportunity to try again. Too often students experience failure (no product or erroneous results), yet there is not time in the current laboratory courses for them to try to learn from their mistakes. For most of our students, they only learn from their mistakes if they participate in undergraduate research. This initial project showed that some students are motivated to bring the analysis to completion.

1a) Additionally, newer faculty may be faced with completely revamping the curriculum. For those in a research university (such as Rensselaer) this is time consuming and given the current research funding climate could potentially be disastrous for new faculty seeking tenure.

2. The quality of the lecture was improved due to student participation and questions that would arise about how a particular instrument might be used for the analysis. In addition to the guest lecture by Joyce Zucker, the instructor spent time in the lecture course discussing art analysis applications when different instruments were discussed. In addition to the lecture notes, two separate brainstorming sessions were included where the students and instructor talked about the different component parts that may be in the samples and how to address analyzing each of these sections.

3. The students were concerned about not properly treating the samples and thus wasting a precious sample. This is a legitimate concern. For this reason, while the students will still analyze the original art sample, a greater emphasis in the future will focus on developing "model" chemical systems with student input and participation. These model systems will be used to validate and test analysis methods prior to the analysis of the real art sample. Additionally, these model systems will give the students an opportunity to repeat an analysis that gives questionable results.

4. The course instructor must provide greater oversight to the problem development and implementation plan. This is particularly important at research institutions. My experience suggests that instructors who plan to bring forth project based learning at a research institution should either develop it slowly over many years time or ask for a teaching load reduction so as to spend the necessary time to be able to develop an effective and well-organized laboratory course.

5. The graduate student TAs needed more time to become familiar with the complexities involved with these types of analyses. The TAs were not familiar with the derivatization procedures and the instrumentation prior to the start of the course.

6. Undergraduate students who have completed the course are your best resource for the next class. The more senior students would be consultants to the junior students. These students can be encouraged to be TAs for the course if they are either paid or given credit. These undergraduate students will provide more continuity than graduate course TAs which will undergo significant turnover due to the available graduate student TA pool. The undergraduates then develop leadership and communication skills that are useful for positions either in graduate school (where they would have to TA as well) or industrial positions where they will have to learn principles of project management. {As an aside, Mr. Vissani has obtained a challenging position at General Electric's Research & Development site in Niskayuna NY in their Analytical Chemistry core.}

I have used this option of giving a student credit for being a TA in a special case a few years ago where a double-major student got out of sequence and was not able to take Quantitative Analysis until his senior year. I offered him an undergraduate TA position for credit and he was responsible for knowing the chemistry and explaining it to the sophomores. This student is now a successful graduate student at the University of California, Santa Barbara. He repeatedly emailed me in his first semester to thank me since he felt very well prepared and confident as a TA in graduate school for Organic Chemistry and knew some of the problems, particularly safety and lack of student preparation, that can arise during a laboratory course and he had developed skills and strategies to effectively deal with these problems.

Summary.

The chemical analysis of paintings provides a unique opportunity for problem-based learning in the instrumental analysis laboratory. The important concepts of sampling, sample handling, sample preparation, and choice of instrumental method are required to be able to effectively work with art samples. Additionally, the chemical analysis of these samples also brings in fields of proteomics (binder analysis) and nanocomposites or nanotechnology (heterogeneity in the paint structure) which are concepts that students have a great interest in since they hear about these terms quite often. The PBL approach piques student interest in the material. However, instructors should be aware that a successful PBL-approach requires an extensive amount of planning and preparation. My experience has been that using the PBL-approach was worth the effort since it provided an intellectually challenging chemical analysis problem and for some students it was their first real positive research experience.

Project Update. Spring 2006 (Feb 2006).

During the Spring of 2005, I was not assigned to teach Instrumental Analysis due to a heavy service load in the fall and spring of academic year '04-'05. I am the instructor for the lecture course this spring (2006). While I technically am not responsible for the laboratory, the laboratory instructor, Professor Bailey has been convinced to give the students an entire month towards the end of the semester to develop the project. A class of 15 students has been asked to self-assemble themselves into three separate groups. The students are now finalizing how they wish to approach their individually assigned problems and are performing the necessary literature searches and are coming up with research plans in consultation with the instructors. Each group has been assigned the task of coming up with a realistic "model" system for their analysis.

LITERATURE CITED

- 1.a) P.T. Jackson and J.P. Walters, Role-playing in analytical chemistry: the alumni speak, *Journal of Chemical Education*, 2000, **77**, 1019-1025.
- b) J.P. Walters, Role-playing analytical chemistry laboratories. Part 1: structural and pedagogical ideas, *Analytical Chemistry*, 1991, **63**, 977A-985A.

- c) J.P. Walters, Role-playing analytical chemistry laboratories. Part II: physical resources. *Analytical Chemistry*, 1991, **63**, 1077A-1084A.
- d) J.P. Walters, Role-playing analytical chemistry laboratories. Part III: experiment objectives and design, *Analytical Chemistry*, **63**, 1179A-1191A.
- e) K.D. Hughes, Marine microcosm - using an aquarium to teach undergraduate analytical chemistry, *Analytical Chemistry*, 1993, **65**, 883A-889A.
- f) G.S. Wilson, M.R. Anderson, C.E. Lunte, Instrumental analysis at the University of Kansas: An experiment in problem-based learning. *Analytical Chemistry*, 1999, **71**, 677A-681A
- g) W.W. Hope and L.P. Johnson, Urban air: Real samples for undergraduate analytical chemistry. Students learn analytical chemistry by addressing urban environmental issues, *Analytical Chemistry*, 2000, **72**, 460A-467A.
- h) T.C. Werner, P. Tobissen, and K. Lou, The water project. A "Real-World" experience for the Quantitative Analysis Laboratory, *Analytical Chemistry*, **73**, 84A-97A.
- i) T. J. Wenzel, Problem-based learning: in need of supporting materials, *Analytical Chemistry*, 2001, **73**, 501A-502A.
- j) I. C. Dorey, Problem-based learning exercise in the quantitative analysis laboratory. Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001
- k) J.F. Tyson, Collaborative learning through project work: The impact of two NSF awards on Chem 312, "analytical chemistry for non-chemistry majors". Abstracts of Papers, 222nd ACS National Meeting, Chicago, IL, United States, August 26-30, 2001
2. C.L. Nelson, J. Castro-Sanchez, and J.E. Pemberton, Materials characterization project: A student's view of problem-based learning. Abstracts of Papers, 221st ACS National Meeting, San Diego, CA, United States, April 1-5, 2001
3. C. K. Larive, Problem-based learning in the analytical chemistry laboratory course, *Analytical and Bioanalytical Chemistry*, 2004, **380**, 357-359.
4. S. M. Halpine, Introducing molecular visualization to primary schools in California: the STArt! teaching science through art program. *Journal of Chemical Education*, 2004, **81**, 1431-1436.
5. P. J. Ogren and D. L. Bunse, Interdisciplinary course in art and chemistry, *Journal of Chemical Education*, 1971, **48**, 681-682.
6. M. V. Orna, The molecular basis of form and color. A chemistry course for art majors, *Journal of Chemical Education*, 1976, **53**, 638-639.
7. www.amolf.nl (Go to Molecular Painting Research). Numerous PhD theses are available for download. (Accessed May 1, 2005).
8. M. V. Merritt, Problem-based Learning in Teaching Analytical Chemistry: An Integrative Approach. *Managing the Modern Laboratory*, 2000, 5 (1) 1-5.
9. R.J. Gettens and G.L. Stout, *Painting Materials, A short encyclopedia* Dover Publications, Inc., NY. 1966.
10. J.S. Mills and R. White, *The Organic Chemistry of Museum Objects, 2nd Edition* Butterworth-Heinemann, Oxford. 1994.
11. J. van der Weerd, Microspectroscopic Analysis of Traditional Oil Paint. 2002, PhD Thesis, AMOLF, Chapter 2, p. 19. Available at www.amolf.nl (accessed June 2004).

12. J. van der Weerd, M.K. van Veen, R.M.A. Heeren, and J.J. Boon, Identification of pigments in paint cross sections by reflection visible light imaging microspectroscopy. *Analytical Chemistry* 2003, **75**, 716-722.
13. E. Ciliberto and G. Spoto, *Modern Analytical Methods in Art and Archaeology*, Vol. 155, Chemical Analysis. A Series of Monographs on Analytical Chemistry and its Applications, John Wiley and Sons, New York, 2000.
14. S. L. Vallance, Applications of chromatography in art conservation: techniques used for the analysis and identification of proteinaceous and gum binding media, *Analyst*, 1997, **122**, 75R-81R.
15. M. P. Colombini and F. Modugno, Characterisation of proteinaceous binders in artistic paintings by chromatographic techniques, *Journal of Separation Science*, 2004, **27**, 147-160.
16. R. Aruga, P. Mirti, A. Casoli, and G. Palla, Classification of ancient proteinaceous painting media by the joint use of pattern recognition and factor analysis on GC/MS data, *Fresenius' Journal of Analytical Chemistry*, 1999, **365**, 559-566.
17. J. D. J. van den Berg, J. J. Boon, K. J. van den Berg, I. Fiedler, and M. A. Miller, Identification of an Original Non-Terpenoid Varnish from the Early 20th Century Oil Painting "The White Horse" (1929), by H. Menzel, *Analytical Chemistry*, 1998, **70**, 1823-1830.
18. M. P. Colombini, F. Modugno, M. Giacomelli, and S. Francesconi, Characterisation of proteinaceous binders and drying oils in wall painting samples by gas chromatography-mass spectrometry, *Journal of Chromatography, A*, 1999, **846**, 113-124.
19. G. Chiavari and S. Prati, Analytical pyrolysis as diagnostic tool in the investigation of works of art. *Chromatographia* 2003, **58(9/10)**, 543-554.
20. J. J. Boon, Analytical pyrolysis mass spectrometry: new vistas opened by temperature-resolved in-source PYMS, *International Journal of Mass Spectrometry and Ion Processes*, 1992, **118-119**, 755-787.
21. S. M. Halpine, HPLC applications in art conservation. *Chromatographic Science Series* (1998), 78(Handbook of HPLC), 903-927.
22. S. M. Halpine, Amino acid analysis of proteinaceous media from Cosimo Tura's 'The Annunciation with Saint Francis and Saint Louis of Toulouse', *Studies in Conservation*, 1992, **37**, 22-38.
23. S. Peulve, J. J. Boon, M. Duursma, O. van den Brink, P. O'Connor, and R. M. A. Heeren, Mass spectrometric studies of proteins in fresh egg and aged egg tempera paint films. Progress report I, *Advances in Mass Spectrometry*, 1998, **14**, C022050/1-C022050/15.
24. N. Shibayama, S. Q. Lomax, K. Sutherland, and E. R. De la Rie, Atmospheric pressure chemical ionization liquid chromatography mass spectrometry and its application to conservation: analysis of triacylglycerols, *Studies in Conservation*, 1999, **44**, 253-268.
25. M. Puchalska, K. Polec-Pawlak, I. Zadrozna, H. Hryszko, and M. Jarosz, Identification of indigoid dyes in natural organic pigments used in historical art objects by high-performance liquid chromatography coupled to electrospray ionization mass spectrometry, *Journal of Mass Spectrometry*, 2004, **39**, 1441-1449.
26. M. Odlyha and A. Burmester, Preliminary investigations of the binding media of paintings by differential thermal analysis, *Journal of Thermal Analysis*, 1988, **33**, 1041-52.

27. S. Felder-Casagrande and M. Odlyha, Development of standard paint films based on artists' materials, *Journal of Thermal Analysis*, 1997, **49**, 1585-1591.
28. M. P. Colombini, F. Modugno, R. Fuoco, and A. Tognazzi, A GC-MS study on the deterioration of lipidic paint binders, *Microchemical Journal*, 2002, **73**, 175-185.
29. R. J. Meilunas, J. G. Bentsen, and A. Steinberg, Analysis of aged paint binders by FTIR spectroscopy, *Studies in Conservation*, 1990, **35**, 33-51.
30. L. Masschelein-Kleiner, Improved method for the thin-layer chromatography of media in tempera paintings, *Studies in Conservation*, 1974, **19**, 207-11.
31. B. Ramirez-Barat and S. de la Vina, Characterization of proteins in paint media by immunofluorescence. A note on methodological aspects, *Studies in Conservation*, 2001, **46**, 282-288.
32. I. Zadrozna, K. Polec-Pawlak, I. Gluch, M. A. Ackacha, M. Mojski, J. Witowska-Jarosz, and M. Jarosz, Old master paintings - a fruitful field of activity for analysts: targets, methods, outlook, *Journal of Separation Science*, 2003, **26**, 996-1004.
33. R. Hynek, S. Kuckova, J. Hradilova, and M. Kodicek, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as a tool for fast identification of protein binders in color layers of paintings, *Rapid Communications in Mass Spectrometry*, 2004, **18**, 1896-1900.
34. V. F. Hanson, Quantitative elemental analysis of art objects by energy-dispersive x-ray fluorescence spectroscopy, *Applied Spectroscopy*, 1973, **27**, 309-33.
35. <http://www.plantingfields.org/PhotoHouse/01.cfm> (accessed, May 16, 2005)
36. W.S. Taft and J.W. Mayer, *The Science of Paintings*. Springer-Verlag, NY, 2000, p. 28.
37. A. Casolli, P. Musini, G. Palla, Gas chromatographic-mass spectrometric approach to the problem of characterizing binding media in paintings, *Journal of Chromatography A*, 1996 **731**, 1996, 237-246.
38. B.A. Bidlingmeyer, S.A. Cohen, and T. L. Tarvin, Rapid analysis of amino acids using pre-column derivatization, *Journal of Chromatography*, 1984, **336**, 93-104.