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Selection and Evaluation of Experiments in Instrumental Analysis for Schools with Limited Instrumental Resources Utilizing the Inquiry Oriented Approach

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ABSTRACT

Selection and Evaluation of Experiments in Instrumental Analysis for Schools with Limited Instrumental Resources Utilizing the Inquiry Oriented Approach

by Penny L. Mauldin

The purpose of this study was to develop and evaluate a selection of experiments in instrumental analysis for schools with limited instrumental resources. The primary intent behind the selection of experiments was to develop them in such a manner that students would have the opportunity to acquire physical skills in working with laboratory instrumentation, study the scientific method, develop critical thinking, and initiate investigation in the chemical laboratory. Six experiments for ultraviolet/visible and gas chromatography were selected for field-testing and evaluation. The instrument chosen for the content analysis was the Laboratory Structure and Task Analysis (LAI) by Tamir, Lunetta, Novick, and Fuhrman. The LAI was designed as a tool to measure inquiry-oriented laboratory curricula. Qualitative open-ended questions designed by the investigator were also included as part of the evaluation.

The investigator initially performed a content analysis of the experiments using the LAI. The field-testing by students was accomplished at Lee College, Cleveland, Tennessee during the spring semester of 1996, utilizing an instrumental analysis laboratory which consisted of junior and senior chemistry majors. In addition, professors from several large public institutions and smaller private schools in Tennessee, Kentucky, and Texas were asked to evaluate the experiments.

Results from the evaluation by the professors and the field-testing by the students indicated an average inter-coder agreement of 86% for professors and 90% for students signifying an acceptable consistency for the coders' assessment. Data from a *t*- test indicated the student and professor results were not significantly different from the investigator's results thus confirming the conclusions of the investigator's original content analysis of the selection of experiments. Another part of the evaluation was a test of preference to determine if there would be a significant difference between types (traditional, combination, or investigative) chosen by students and professors. The problem statement was written in the null form and rejected in all cases. Therefore, the conclusion was established that both students and professors preferred the combination type experiment with 92% of the professors and 69% of the students selecting the combination experiment. The selection of experiments is included in Appendix IV of the dissertation.

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CHAPTER I

INTRODUCTION

Significance of the Study

The combination of the qualitative chemical and spectroscopic analysis of a compound makes the identification and quantitation of an unknown possible (1). Many of these analyses are accomplished today employing instruments. The use of instrumentation in chemistry has ultimately become of such importance in the process of the qualitative and quantitative identification of unknowns that it has become a well organized and consolidated branch in analytical chemistry (2). In fact, a 1992 survey by Jones (3) indicates that 93% of the responding colleges or universities offer a course in instrumental analysis.

Courses in instrumental analysis generally offer a lecture and laboratory component at the junior/senior level for chemistry majors (4). A review of two current textbooks in instrumental analysis indicates the lecture component usually covers the basic theoretical principles upon which contemporary instrumentation and measuring devices are founded (5, 6). Specifically, Skoog and Leary (5) state their goal is to "provide the student with an introduction to the principles of spectroscopic, electrometric, and chromatographic methods of analysis, as well as to engender an appreciation of the kinds of instruments that are currently available and the strengths and limitations of these instruments."

One of the most important aspects of the undergraduate instrumental analysis course is the opportunity students have to work with various instruments available in the laboratory. Whether going on to graduate school or seeking a career in industry, graduates in chemistry are expected to acquire some experience with the important instrumental methods of analysis. Surveys of industry indicate that students are expected to have acquired experience with methods such as: gas chromatography (GC) and infrared (IR) and ultraviolet-visible (UV-VIS) spectrophotometry (4). Because of the importance placed on the learning of instrumental techniques, it is evident that a well designed laboratory experience must be provided for chemistry students.

In spite of the accepted importance of acquiring experience with instrumental methods of analysis, this investigator found only one instrumental analysis laboratory manual currently in print: *Chemistry Experiments for Instrumental Methods,* copyright 1984, by Sawyer, Heineman, and Beebe (7). With seventy-three experiments and additional supplemental exercises, this is an excellent manual for schools that have access to many instruments. Unfortunately, schools with limited instrumental resources could only use a modest fraction of the experiments in the manual. In many cases, instructors of instrumental analysis laboratories are faced with the challenge of developing their own laboratory manuals. Few chemistry professors involved in research have the time to embark upon such a major task.

Having limited instrumentation also creates additional laboratory time within a semester. This necessitates the supplementation of the student's experience with experiments designed not only to gain manipulative skills on the instruments but to

improve their ability in other areas as well. In investigating other areas or skills to include in the experiments, several articles in the science education literature proposed that a distinctive characteristic of the laboratory was to provide the students with an opportunity to involve themselves in the process of investigation and inquiry (8-12). J. J. Lagowski (13), editor of the *Journal of Chemical Education*, states, "But, the key to understanding has always been found in the laboratory, in the well-designed experiment that completes a cycle or starts a new cycle of scientific inquiry."

The investigative experiment, which allows the students to formulate their own hypothesis, design and execute the investigation, and analyze and interpret the corresponding data, is devised so that they are given the opportunity to improve their skills in the areas of analytical and scientific thinking. Studies support the conclusion that inquiry-oriented experiments, such as an investigative or combination (open-ended) experiment, increase student gains significantly in areas such as analytical and scientific thinking skills, abstract thinking ability, and content achievement above that of the traditional experiment (14, 15). These particular positive aspects of investigative experiments are beneficial for schools that are unable to provide the student with experience on a wide variety of instrumentation.

Purpose of the Study

The purpose of this study was to select, develop, and evaluate a selection of experiments in instrumental analysis for schools with limited instrumental resources which incorporates the traditional, combination, and investigative approaches as defined beginning on page 5.

Statement of the Problem

Development of the Selected Experiments

One aspect of the problem was to obtain a selection of experiments from the literature and then develop them into a series that increase in inquiry-oriented nature from traditional through combination to the investigative experiment utilizing IR, UV-VIS, and GC instrumentation.

Evaluation of the Selection of Experiments

The following items were used in the evaluation of the selection of experiments:

1. When possible, junior and senior chemistry majors in special topics courses at Lee College (Cleveland, Tennessee) performed the experiments to ensure that reasonable results could be obtained, and any problems could be noted and corrected.

2. For instruments unavailable at Lee College, the investigator performed the experiments at other institutions to ensure that reasonable results could be obtained, and any problems could be noted and corrected. This was done for all the experiments except the investigative.

3. The Laboratory Structure and Task Analysis Inventory (LAI) was used by the investigator to perform a content analysis on the individual experiments. The LAI is an instrument developed by Tamir, Lunetta, and Fuhrman to analyze and evaluate laboratory manuals of modern science curricula. Its specific categories in the task analysis area are geared toward determining the relative degree to which the experiment is inquiry oriented or investigative in nature (16).

4. Six experiments were selected from the ultraviolet-visible and gas chromatography sections to be field-tested in a laboratory setting by students and were sent to professors in the field to be evaluated. These students and professionals completed the LAI for each experiment and answered a set of questions developed by the investigator.

5. A *t*-test was performed between the investigator's and the students' results as well as the investigator's and the professors' results. This was performed on the LAI Part II to determine whether the investigator's analysis varied significantly from the analyses of the students and professors. A calculation of the percentage agreement between professors and students in each category was also completed and included in the appropriate tables.

6. A chi square analysis was performed on the results of a question proposed to the students and professors who evaluated the six field-tested experiments to determine whether there was a significant difference among the students and professors in their preference of the three experimental types written for the selection of experiments.

Definition of Terms

Combination experiment. Experiments that include an initial section written in a traditional approach with subsequent sections that require student modification in some respect to incorporate various investigative skills. These are sometimes termed open-ended experiments in the literature. Generally, these will include a select set of the skills mentioned for investigative experiments but not the full range of skills expected in an investigative experiment (17).

Deductive approach. The experiment involves the testing of phenomena or relationships that have been predicted based upon principles or processes that have previously been stated (18).

Demonstration approach. The instructor demonstrates the experiment rather than allowing the students to manipulate the chemicals or equipment. The students compile the data from the results of the instructor's demonstration (19).

Inductive approach. The experiment involves developing generalizations from data or from individual cases (18).

Inquiry experiment. Experiments in which students are not given a theoretical introduction or method of data analysis. However, they are given a specific problem to investigate and explicit experimental instructions. Students are required to generate their own analysis and explanation of the data. If the experiment consists of a series of questions that enables the student to explain the data, it is termed guided inquiry. Some literature sources also call this type a discovery experiment (20, 21).

Investigative experiment. Experiments in which students are allowed to choose or define the problem for investigation within the framework and limitations of the laboratory. Students are expected to formulate their own hypotheses, design and execute investigations, and analyze and interpret the corresponding data. There is time to replicate the work, and a written and/or oral report is required. The inductive approach is the major method employed in the lab. This has also been termed open-inquiry in the chemical education literature (14, 17, 22-24).

Open-induction experiment. Experiments that are similar to the investigative experiment except there is not an initial series of activities and experiments to prepare the students for the investigation. Students are given minimal direction as they design and carry out their own independent investigations. The work is completed almost entirely uninstructed (17, 24). This type of experiment is most similar to graduate research.

Traditional experiment. Experiments in which the emphasis is placed on memorization and verification of the known. They are primarily used to confirm facts and principles previously presented in the lecture. They are generally written in a "cookbook" fashion, and the deductive approach is the major method employed in the lab (25).

Limitations of the Study

The student evaluation of the selection of experiments was limited to a field-study done at Lee College by students that used experiments from the UV-Vis and GC sections. This was conducted in one existing laboratory section that was composed of 16 students. Lee College is a liberal arts Christian college in Tennessee with approximately 2,500 students. The science department has an average of 200 majors. Therefore, the sample may or may not be representative of students nationwide. The professors enlisted to evaluate the selection of experiments were from the following public institutions in Tennessee: Middle Tennessee State University, University of Tennessee at Knoxville, and the University of Tennessee at Chattanooga. Also included in the evaluation process were professors from private colleges in Tennessee, Kentucky, and Texas. This sampling of professors may or may not be representative of professors nationwide; however, it does include professors from small private institutions through larger public institutions.

CHAPTER II

SURVEY OF THE LITERATURE

Historical Overview

Although there was previous scientific activity in the United States, the initiation of American science education is commonly regarded to have occurred after the War of 1812. During this early period, the Baconian philosophy of gathering facts for inductive generalization was the method in which an inquiry was conducted. The current methods of scientific inquiry based on obtaining empirical data were actually resisted until the mid-1800s because of religious convictions. Faith, which was often a blending of Christian theology and tradition, held as much sway with the gentlemen-amateur scientists, who were often ministers of religion, as empirical data. This limited an inquiry-oriented approach in the laboratories of institutions of higher education because higher order classification (the continuous observation, collection, and categorization of information) rather than results based upon empirical data was seen as the ultimate attainment. Scientists of the time felt they could determine a scientific law by collecting and classifying enough information (26, 27).

Dr. Carlton Stedman (27) analyzed 80 texts dated from 1822 to 1910 for evidence of inquiry-oriented teaching strategies. These texts, in some cases, contain instructions for laboratory activities. The texts were evaluated using Addison Lee's taxonomy of teaching style examples, which are organized into the following nine inquiry stages:

(a) traditional lecturing, (b) experiments with set methods and observations with prescribed results, (c) lecturing with some inquiry questioning, (d) experimental data provided with students guided to interpretation, (e) teacher demonstration as a focus for inquiry questioning, (f) experiment with set methods to test an hypothesis in which the student must find the answer to the key question, (g) student-designed experiments to test the hypothesis, (h) experiments with set methods which lead to open-ended outcomes and hypothesis formulation, and (i) problem-centered activities in which the student defines the problem, variables, hypothesis, and designs the experiment to test the hypothesis. In the field of chemistry, Dr. Stedman found that from 1822-1880: 57% of the texts examined for inquiry attained a stage (a) level, 14% a stage (b), and 14% a stage (c). No texts were found that were inquiry oriented above the stage (c) level. And, he found in the 1881-1910 chemistry texts: 14% represented stage (a), 14% a stage (b), and 0% a stage (c). In this era, there were no chemistry texts reviewed that contained inquiry above the stage (b) level. Other fields of scientific study faired similarly.

Of course, Stedman's conclusion that there was very limited opportunity for inquiry for students in this early era comes as no surprise. A quote in Coleman's *New Lab Manual for Physics* (27) from the early 1900s echoes this conclusion: "These experiments should be regarded as limited inquiry into the facts at first hand, not as a source of adequate data for generalizations by the pupils...what he [the student] really does is to perform an experiment which, within a fair degree of accuracy, illustrates or exemplifies the law, and he does this in order that he may understand it better."

Although the overall result indicates very little inquiry-oriented experimentation was occurring, there were some educators who did incorporate varying levels of inquiry into their classrooms and laboratories during this period before 1910. Louis Agassiz, a zoologist from Harvard, was one such educator. His methodology was similar to European schools that modeled a professor with his proteges working beside him. On one occasion, it was noted that Agassiz handed a student a fish and told him to come back when he knew everything there was to know about it (26). Another example was Liebig whose undergraduate chemistry laboratories initially began in 1820 at Giessen. This early laboratory of Leibig was similar to the graduate research group of today in that the approach was open induction. In America, Ira Remsen was instrumental in the implementation of the teaching laboratory in chemistry. Remsen states, "The only way to learn about it was to see its results, to experiment, to work in the laboratory" (28).

In the early part of the twentieth century, John Dewey of the progressive education movement also supported an investigative approach that he termed "learning by doing". Although the progressive education movement did begin to gain momentum, there were still arguments on the role of laboratories in science education. However, after World War I, laboratories became more traditional in the sense that specific experiments were done to verify known facts and principles. In the 1930s, there were those who advocated the demonstration approach over the traditional laboratory method. In many cases, the advocation of traditional laboratories or demonstration approaches was due to large increases in the student population. This large increase in student participation in laboratories would necessitate a tremendous increase in funding for the

labs. At this point, administrators desired to know the value of the laboratory as part of the learning experience of the student (16, 19, 29).

In reaction against the traditional and demonstration approaches found in laboratories of the 1930s, the Woods Hole science conference was held which acquired the support of some science educators for inquiry-oriented experiments. This eventually led to the "new" science curricula of the 1960s which emphasized the importance of the processes of science and cognitive skills over verification of the known. The new science curricula also placed marked emphasis on providing the students with the opportunities to investigate, inquire, and find things out by themselves. Federal funding for science curriculum projects also increased during this time period due largely to the race for technological advancement in space travel (16, 19, 27, 30). The Commission on Undergraduate Education in the Biological Sciences (CUEBS) met in several workshops and symposia in 1969 and 1970 and proposed what they termed: the investigative laboratory. They defined all the characteristics of this type of laboratory, and it was instituted in several undergraduate programs (24).

In a 1993 article, Dr. Marshall Sundberg (29) summarized the responses to a questionnaire distributed concerning current trends in biological laboratory science education, including questions concerning investigative laboratories. The questionnaire was sent to 118 public and private institutions in the United States which included the top 50 institutions as rated by their ability to obtain federal grant money, as well as 68 additional regional schools. Seventy-three of the schools responded. Sundburg noted a trend toward increased interest in the investigative types of laboratories, especially in the

small liberal arts colleges where they appeared to be especially successful. Only three of the larger institutions were fully committed to investigative laboratories: Stanford University, Arizona State University, and the University of Missouri in St. Louis, and only four schools used the investigative approach for their entire freshman laboratory program. However, half of the schools responding made an effort to incorporate the investigative approach in some of their experiments. Of those that did, 89% used experiments that were developed at the institution for this purpose and others used modified versions of commercial laboratory manual exercises.

In this investigator's review of the literature in *Journal of Chemical Education* and *Educational Resources Information Clearinghouse (ERIC)*, the following individual chemistry experiments and curricula were found to incorporate an inquiry-oriented approach. This data is summarized briefly in Table I below.

TABLE I. SUMMARY OF INQUIRY-ORIENTED CHEMISTRY LABORATORIES

Author(s)	Reference	Date	Orientation	Type	Laboratory
Jay Young	31	1957	Open-ended (Combination)	Curriculum	General Chemistry
Wilmer Fife	32	1968	Investigative	Curriculum	Organic
Lauren Wilson	33	1969	Open-ended (Combination	Curriculum	General Chemistry
C.R. Barr J.L. Mackey	34	1969	Investigative	Curriculum	Organic and Inorganic
Richardson J.Renner	20	1970	Guided Inquiry (Discovery)	Curriculum	General Chemistry

Table I. Continued					
Author(s)	**Reference**	**Date**	**Orientation**	**Type**	**Laboratory**
G.Hiegel R.Belloli	35	1971	Investigative	Curriculum	Organic
E.Wehry	36	1970	Open-ended (Combination)	Curriculum	Analytical
J.Buono J.Fasching	37	1973	Investigative	Curriculum	Analytical
C.Venkata-chelam R.Rudolph	38	1974	Open-ended (Combination)	Curriculum	General Chemistry
M.Parsons G.Bentley	39	1975	Investigative	Curriculum	Instrument-al Analysis
L.Bowman C. Shull	40	1975	Guided Inquiry (Discovery)	Experiment	General Chemistry
A.Rhein-gold	41	1976	Investigative	Curriculum	Inorganic
J.Cody D.Treagust	15	1977	Investigative	Curriculum	Biochemist-ry
M.Pavelich M.Abraham	14	1979	Investigative	Curriculum	General Chemistry
D.Driscoll	42	1979	Guided Inquiry (Discovery)	Experiment	General Chemistry
I.Cohen R. Ben-Zvi	43	1982	Guided Inquiry (Discovery)	Experiment	General Chemistry
T.Mulder A.Verdonk	44	1984	Guided Inquiry (Discovery)	Experiment	General Chemistry
J.Allen et al.	21	1986	Guided Inquiry (Discovery)	Curriculum	General Chemistry
J.DeMoura J.Marcello	45	1987	Guided Inquiry (Discovery)	Experiment	General Chemistry

Table I. Continued					
Author(s)	**Reference**	**Date**	**Orientation**	**Type**	**Laboratory**
R.Schibeci C.Carlsen	46	1988	Investigative	Experiment	General Chemistry
J.Wherley	47	1989	Open-Ended (Combination)	Experiment	General Chemistry
J.Cooley	48	1991	Guided Inquiry (Discovery)	Curriculum	Organic
M.Pickering	49	1991	Guided Inquiry (Discovery)	Curriculum	Organic
R. Ricci M. Ditzler	50	1991	Guided Inquiry (Discovery).	Curriculum	General Chemistry
P.Mahaffy et al.	51	1993	Investigative	Curriculum	General Chemistry
M.Ditzler Ricci et al.	52 53	1994	Guided Inquiry (Discovery)	Curriculum	Organic
R.Jarret et al.	54	1995	Guided Inquiry (Discovery)	Experiment	Organic
M.Pearsall et al.	55	1995	Guided Inquiry (Discovery)	Experiment	Organic
Gary Slough	56	1995	Guided Inquiry (Discovery	Experiment	Polymer Science
D. Elderd et al.	57	1996	Guided Inquiry (Discovery)	Experiment (forensic)	General Chemistry

A review of the literature for different types of general chemistry laboratory curricula and individual experiments was done by Dr. E. K. Mellon (58) from Florida State University using: *Journal of Chemical Education* (50 articles), *Journal of College Science Teaching* (4 articles), and *Education in Chemistry* (8 articles) from 1926 to 1977.

Four criteria are referenced in his article relating to the inquiry-oriented laboratory. Criterion A generally represents open-ended (combination) type experiments. Criteria B and G indicate the experiments are developed along the guided inquiry (discovery) format. Criterion F denotes the investigative experiment. It can be seen from this analysis that there were 8 combination, 20 guided inquiry, and 27 investigative experiments found in his review.

Review of Educational Studies on the Laboratory

Traditional Laboratories

As noted earlier, the place of the laboratory in science education has been highly debated, particularly when large influxes of students require an increase in funding. All of this debate has led to studies to determine the value and effectiveness of the laboratory (59). Studies were conducted comparing the traditional laboratory method with other instructional methods for student achievement, critical thinking, and knowledge of the processes of science. Coulter (60) compared laboratory experiments with demonstrations in biology on tests of factual knowledge, application of principles, and critical thinking. Yager (30) correlated three groups (a laboratory group, a demonstration group, and a discussion group) in biology on tests of critical thinking, understanding of science, and knowledge of science content area. Ben-Zvi (61) compared a laboratory group to a group observing filmed experiments in chemistry on tests of achievement in chemistry and specific knowledge and understanding of the use of experimental techniques. None of these studies showed significant differences in the results of different modes of

instruction except for an increase in laboratory manipulative skills for the students performing the experiments.

Studies were also conducted in the affective domain to measure the interest in and attitude toward science that the laboratory elicits from students. Several studies found that laboratories generally increased interest in and developed positive attitudes toward science: Smith, Walberg, Poorman, and Schagrin (62) in physics; Selmens, Ashton, Meredith, and Newal (63) in biology; Bybee in earth science (64); Ben-Zvi, Hofstein, Samuel, and Kempa (65) in chemistry. All reported positive student responses. In the Ben-Zvi et al. (65) study, students reported that personal laboratory work was the most effective educational method for encouraging their interest in chemistry and education when compared with teacher demonstrations, group discussions, filmed experiments, and lectures.

Inquiry-Oriented and Investigative Laboratories

Various studies support the fact that investigative laboratories can increase student performance in scientific thinking. A validated test constructed by Burmester has been used to measure scientific and analytical thinking. Within the area of scientific thinking, Burmester includes ability to recognize problems, understand experimental methods, organize and interpret data, understand the relation of facts to the solution of problems, plan experiments to test hypotheses, and make generalizations and assumptions (66).

According to Burmester's Inventory, laboratory-related tasks require students to use analytical thinking to: delimit problems; analyze experimental methods; organize data; articulate the relationship of facts to the solution of the problem; interpret data and

plan experiments to test the hypothesis; evaluate conclusions in terms of reasonableness, sufficiency, and pertinent data; make generalizations and assumptions; apply the underlying theory (of an experiment) to solve a similar problem involving a different physical situation; formulate hypotheses; recognize and make assumptions; and design and execute original experiments (66, 67). In 1967, Kaplan (66) used a laboratory manual in biology designed to teach aspects of scientific thinking. Students in this study showed pretest-posttest gains in comprehension and use of the scientific method as measured by Burmester's Inventory.

The same improvement in analytical thought processes was seen utilizing a biological study by Wheatley (68) in which students were provided with special laboratory activities. After this study, students performed at higher cognitive levels on Bloom's taxonomy. Bloom's taxonomy, which is a hierarchical classification of educational objectives, includes these major levels for the cognitive domain: knowledge, comprehension, application, analysis, synthesis, and evaluation (69). Similarly, a study in physics by Reif and St. John (70) indicated that students in an inquiry-oriented laboratory scored better on a practical laboratory test designed to test analytical higher-order skills than those in the traditional physics laboratory. This laboratory was designed to teach general intellectual skills such as: "(a) the ability to apply the underlying theory of an experiment to solve a similar problem involving a different physical situation; (b) the ability to modify the experiment to find a different quantity, or to find the same quantity by using different methods; (c) the ability to predict the effect of an error in an experimental procedure or measurement."

A study in biological laboratories by Leonard (71) in 1983 generated support for the idea that an inquiry-oriented laboratory approach increased student cognitive factors (biological concepts) when compared to a traditional approach. A meta-analysis, which is defined as the process of analyzing the results of a collection of studies on one topic, was performed by Shymansky (72) in 1982 on the effectiveness of the BSCS (Biological Sciences Curriculum Study) inquiry type laboratories. Upon analyzing the effectiveness of 302 studies found in the literature, he reported that students in inquiry type laboratories performed better in achievement, attitude, process, and analytic skills than those students in traditional laboratories. Another meta-analysis by Wise and Okey confirms these results (73).

Hall and McCurdy (74) in 1988 conducted a comparison of the BSCS laboratory inquiry curriculum with a traditional curriculum. The experimental group that used the inquiry-oriented curriculum scored significantly higher in levels of performance on a test measuring biological content achievement. In 1994, Sundberg and Moncada (17) evaluated an investigative laboratory course developed in freshman biology at Louisiana State University. The course itself was designed by Sundberg to address common misconceptions in biology such as population growth and evolution. The assessment tool used was a 36-item multiple choice test that addressed the major concepts studied during the laboratory. Reliability is the consistency with which a test measures whatever it is measuring. The measurement of reliability produces a correlation coefficient of 1.00 when the correlation is perfect. The internal reliability of Sundberg's test was high with a Kuder-Richardson-20 index >0.95. The authors found a significant increase in student

understanding of the major concepts for the investigative group using a *t*-test at the 0.001 probability level which indicates that there is a 99.9% chance that the difference resulted from manipulation of the independent variable, not chance.

In a carefully designed study, Raghubir (22) compared students in a control group with those using a laboratory investigative approach in biology. The students were given a Test of Academic Progress from the BSCS to test for cognitive factors that had a reliability estimate of 0.68 and a validity estimate of 0.73. Validity is the degree to which a test measures what it is supposed to measure, and it generates a coefficient of 1.00 when the correlation is perfect. Raghubir defined the cognitive factors as: formulating hypotheses, making assumptions, designing and executing investigations, understanding variables, observing carefully, recording data, analyzing and interpreting results, and synthesizing new knowledge. Also, the students were tested for their affective response (curiosity, openness, responsibility, and satisfaction) using the Thurstone Scale developed by Iowa's Department of Public Instruction which had a reliability estimate of 0.74 and a validity estimate of 0.75. It was determined from the analysis of a *t*-test that students using the investigative approach had a significant (at the 0.01 probability level) increase in cognitive factors and affective responses. Raghbuir stated, "Students using the laboratory-investigative approach acquired a greater understanding of science, greater information retention, and better ability to think scientifically. A very important aspect of this methodology is that the gains that students make in the affective domain seem to have a positive effect on their achievement."

A study in the affective area in chemistry was conducted by Charen (75). He found that students' attitudes toward the learning of chemistry were enhanced when using open-ended (combination) laboratories. Another area that has been tested in inquiry-oriented experiments in chemistry is creativity. Creativity is defined as being able to combine ideas, techniques, or approaches in a new way. Hill (76) assessed the improvement in creativity in a college chemistry laboratory using the Minnesota Test of Creative Thinking. She found that creative thinking was significantly improved by the chemistry laboratory experience.

In a documented study by Cody and Treagust (15), several aspects of student learning were tested while using the investigative methodology in a biochemistry laboratory. Students were pretested-posttested with the Inorganic-Organic-Biological-Chemistry test from the American Chemical Society (ACS) to measure subject matter competency. Reliabilities for the tests themselves were 0.90, 0.68, and 0.85 respectively. The Methods and Procedures of Science test by Woodburn was used to evaluate gain in the understanding of various aspects of science and tools used in the pursuit of science. The test had three parts: meaning of words used in the pursuit of science, plan and design of scientific experiments, and student's ability to draw conclusions from scientific data. A reliability value for theWoodburn test had been established at 0.775. Students showed significant gains using a *t*-test at the 0.01 level of probability on the ACS tests for the areas of organic, biochemistry, and inorganic chemistry. There was also a significant increase at the 0.05 level of probability for the students on the Woodburn Methods and Procedures of Science test.

Another study was performed in the field of chemistry on "inquiry-discovery" (terminology used by authors) laboratories developed by Richardson and Renner (20) for general chemistry. This study was conducted over a period of several years and data compiled for the entire period on control and experimental groups. Students were pretested-posttested on individual laboratory experiments and on the final laboratory examination. The experimental group showed a significant increase in test performance over the control groups for all three years studied at the 0.05 level of probability using a *t*-test. In a student questionnaire developed by Allen, Barker and Ramsden (21) at West Point, students enrolled in guided-inquiry and open-inquiry (investigative) laboratories responded with the statement that the inquiry-oriented laboratories "stimulated their powers of observation, enhanced their understanding of chemical concepts, and more experiments/laboratories should be offered in this format."

A study was accomplished by Pavelich and Abraham (14) at the University of Oklahoma in general chemistry laboratories for students using the investigative (open-inquiry) approach. A Piagetian-type test generated by the Cognitive Analysis Project was used to measure intellectual development. This was administered to both the control and experimental groups at the beginning and ending of the first semester. At the end of the first semester, the experimental (investigative) group showed significant gains in abstract thinking abilities at the 0.001 probability level. The other test administered to both groups was the Laboratory Programs Variables Inventory. The inventory allows students to rank 25 statements in order concerning their laboratory experiment. Eight of the statements deal with scientific inquiry such as: students are asked to design their own

experiments, and students usually know the general outcome of an experiment before doing the experiment. The experimental (investigative) group would be expected to rank the former statement higher than the latter statement. As was expected, the inquiry format was ranked better at promoting scientific inquiry.

A study was reported by Venkatachelam and Rudolph (38) from the University of Michigan on open-ended (combination) laboratories used in general chemistry. Both the control and the experimental group received a survey measuring attitudes toward chemistry at the beginning and ending of the semester. They also were given a laboratory final exam at the end of the semester. The experimental group achieved significantly better results on the laboratory final exam at the 0.05 level of probability. Attitudinal results showed the experimental group found their laboratory experience more rewarding. The students in the combination laboratories viewed their experience as being more interesting and containing more creative work.

In addition, Robert Allison (77) from the University of Northern Colorado used a control and experimental group in an introductory college chemistry course to determine the differences exhibited when the experimental group used inquiry-oriented laboratory experiments. Tests used in the appraisal were: the Anderson-Fisk Chemistry Test, a self-evaluation inventory, a laboratory performance examination, the Scientific Attitude Inventory, and the Watson-Glaser Critical Thinking Appraisal. The *t*-test was used to test for group differences at the 0.05 probability level. The inquiry approach was significantly more effective in increasing laboratory performance skills, improvement of intellectual attitudes toward science, and improvement in critical thinking skills.

Descriptive Aspects of Various Inquiry-Oriented Laboratory Curricula

Biology

Because the investigative laboratory was first described in the biological literature, a few descriptive biological curricula will be detailed as a basis for comparison with the chemistry curricula presented. These curricula are reviewed as a basis for the development of the selection of experiments by the investigator.

Marshall Sundburg (17) summarized an introductory botany course at Louisiana State University that incorporated the investigative approach. The first two weeks included an introduction to problem solving, basic scientific methodology, statistics, and the metric system. During the next few weeks, selected topics of investigation were discussed and the class generated a general plan of action for each selected topic. Student teams then chose the area they wished to investigate and completed a flow chart of their proposed research. In some cases, a new piece of equipment had to be demonstrated or some other technique described, since the participants were freshmen.

Verne Mills (78) described an investigative introductory biology course at Kalamazoo Valley Community College. He suggested that a good strategy in introductory classes was to use standard laboratory exercises as a foundation for investigation. The first four weeks consisted of the usual mixture of reading assignments, pre-lab discussions, standard laboratory exercises, and quizzes. At the end of the fourth week, students were required to choose problems for investigation. The topics came from a selection of a six subject-matter module and had to be approved by the instructor. The student then developed the hypothesis and planned the experimental procedures.

An investigative laboratory for microbiology was developed by Daniel Burke (79) at Mercer University. This program consisted of a series of four programmed research projects in microbiology offering guided experiences of increasing complexity. The guided experience ensured that students were trained at a suitable pace. The first step was the introduction of the scientific method. The program then progressed in levels. First, all segments of the experimental design were presented as in traditional experiments. Second, students were allowed a choice of experiment and were required to add details to the experimental procedure, to design controls, and to interpret results independently. In the final level, students chose the problem, designed, performed, and interpreted the experiment.

John Thornton (24) from Oklahoma State University reported an investigative cell biology laboratory. In order to save valuable time, Dr. Thornton first developed the cellular system upon which the investigations were conducted. The beginning of the course was composed of illustrated lectures on basic technique, and a handbook was provided for the students to practice these basic techniques. Around the mid-point of the semester, students chose a topic from abstracts made available by the instructor. The remainder of the semester consisted of the students defining their problem, setting-up and executing the experiment, interpreting the results, and presenting a formal report.

Chemistry

Ditzier and Ricci (52) and Ricci et al. (53) from the College of the Holy Cross developed a guided-inquiry (discovery) approach for their general and organic chemistry laboratories. Their program concentrated on the rediscovery of fundamental principles.

In a prelaboratory discussion, a question was introduced, and students made hypotheses and predictions concerning the question, discussed various experimental set-ups, and proposed a trend that the data might exhibit. A student or group of students was then assigned a variation of the experimental question. The students were given experimental details to follow. In a postlaboratory session, the students pooled their data. They were then required to examine the data and discover the general concept or trend.

Allen et al. (21) at West Point developed a guided-inquiry (discovery) laboratory for their basic general chemistry course and open-inquiry (investigative) laboratories for their advanced general chemistry course. Their guidelines for the guided-inquiry laboratories were as follows: principal concepts were never taught before the laboratory, procedural steps as presented to the student were reduced to ensure that students would have to ponder the collection and analysis of the data, a verification step was incorporated into the procedure, and short discussion questions were included in the laboratory report. Another entirely inquiry-oriented curricula in chemistry is illustrated by Richardson and Renner's (20) "inquiry-discovery" laboratory for freshman chemistry at Central State College in Oklahoma. The authors state, "The experiments used in this study were written to assist the student in discovering some of the important laws of chemistry for himself."

Cody and Treagust (15) at the University of Iowa produced an investigative laboratory program in the field of biochemistry. Initially experiments consisted of many biochemical techniques such as extraction, purification, identification, and assay procedures that were new to the students. Eventually students in the laboratory course

selected their own investigative project by identifying a problem, reviewing the relevant literature, designing the experiment, and defending their results orally. The role of the library and the review of the relevant literature in the investigative laboratory is discussed by Kirk (80). He suggests the literature review will uncover various aspects of the nature of science, allow the students to see the progress of a topic over a period of time, and help the students to gather information that will either agree or disagree with their specific data.

Pavelich and Abraham (14) described a general chemistry laboratory course for University of Oklahoma science majors that was designed in the open-inquiry format. This program could also be called an investigative laboratory program. The students chose the problem for investigation, designed their own experiments, and formulated the analysis and explanation of the data. They also termed this a mini-research experience for the student, and the laboratory had time built in to perform additional work on the experiment if necessary. A formal report was due at the end of the experiment.

Wehry (36) from Indiana University reported an open-ended (combination) laboratory course for analytical chemistry. The laboratory began with traditional experiments and graduated to the open-ended experiment. Generally the students were allowed five to six weeks to complete the open-ended experiments and were expected to submit a written report at the end of the semester. Students chose from a selection of eight open-ended experiments such as:

> Glucose in aqueous solution can be determined by measuring the rate of its oxidation to gluconic acid, as catalyzed by the enzyme glucose oxidase

glucose + O_2 +H_2O ---------> H_2O_2 + gluconic acid

This reaction is most conveniently followed by coupling the production of H_2O_2 to a second, much faster, enzyme-catalyzed reaction which consumes the H_2O_2 and produces a colored product. Evaluate the precision and sensitivity of this method, and the influence of the presence of (a) other sugars and (b) reducing agents. In the process of performing this experiment, determine the glucose content of an unknown supplied by the assistant. From your study of this method, comment on its applicability to the determination of glucose in blood samples.

In an investigative program in general chemistry, Mahaffy, Newman, and Bestman (51) from King's College described the following laboratory curriculum. The first part of the semester consisted of traditional experiments to introduce techniques and concepts. The remainder consisted of the investigative project. Students selected from a list of possible topics or suggested their own ideas. They searched the literature and produced a research plan which was approved by the faculty that incorporated cost, availability, and safety considerations. The experiments were then completed, and a formal report following ACS journal formatting was submitted two weeks after the conclusion of the project.

Buono and Fashing (37) from the University of Rhode Island related the ensuing investigative laboratory program in analytical chemistry. The first half of the semester was devoted to traditional laboratory experiments in quantitative analysis. In the remaining time, students selected their own problems or chose from a list provided, developed the procedures from a literature search, consulted the instructor for final approval, performed the experimental procedure in the laboratory, and composed a final report.

Finally, Parsons and Bentley (39) from Arizona State University developed an investigative laboratory curriculum for instrumental analysis. The entire semester involved the completion of two research oriented problems with the only limitation being the second project must incorporate different instrumentation. The criteria for the investigative problems were as follows: (a) The problem selected must have "real world" applicability. (b) A complete literature search was required with three suitable approaches to the problem proposed. (c) Student proposals were approved by the faculty, and the students were instructed in the use of instruments and techniques chosen if necessary. (d) Data collected must have suitable use of statistical methods, and an unknown was given for evaluation where possible. (e) A formal report was due at the end of each project which was written in a scientific journal style.

Laboratory Structure and Task Analysis Inventory

A critical component in the study of inquiry-oriented experiments is the selection and utilization of an applicable and reliable tool for measuring the investigative nature of the experiments being considered. An instrument that satisfies this criterion is the Laboratory Structure and Task Analysis Inventory (LAI). The initial version of the LAI was proposed by Tamir and Lunetta (81).

Tamir and Lunetta (81) began the development of the instrument by looking at the work of Pella, Schwab, and Herron. The content analysis scheme for laboratory manuals proposed by Schwab in 1962 was elaborated upon by Herron in 1971. Schwab's scheme proposed 3 levels of manuals: (a) The manual proposes the problems and gives

experimental detail for their solution. (b) The problems are proposed by the manual, but the experimental procedure is left open. (c) The problem as well as the experimental procedure are left open (82). Pella (83) also proposed a slightly more elaborate scheme that checked to see whether the particular categories were teacher or student directed: statement of problem, hypothesis, working plan, performance, data gathering, and conclusion. Although the idea to determine the degree of the inquiry-oriented nature of the laboratory manual is similar, the LAI as developed by Tamir and Lunetta (81) is a more refined scheme to analyze content than those previously proposed.

Content analysis is a "systematic replicable quantitation in analysis and description of content with a particular focus" (84). One of the many ways content analysis is beneficial is in providing the evaluator with information concerning the extent of specific modes of presentation (inquiry-oriented) and the kinds of activities the learner performs using the material under evaluation (82). Content analysis permits the investigator to determine the relationship of the actual materials and the stated objectives of the developer (16). Specifically, Tamir and Lunetta (16) indicate the LAI can be used to: "identify specific strengths and deficiencies of curricula in laboratory organization and tasks, determine the practical nature of the curriculum and the role of laboratory work as represented by the student laboratory manual, provide a basis for the assessment of goals regarding student development of inquiry skills, and guide instructors in planning laboratory experiences for their students."

Several educators have emphasized the need for content analysis. Grobman (85) judged the need for content analysis to be a predominant aspect of formative curriculum

evaluation. Other educators such as Anderson regard the thorough characterization of various curricula to be an integral necessity in the development of all new programs (84). Hurd (86) agrees with the need for content analysis and research in the area of curriculum and states:

> If I were to choose the one area of science education in the most need of serious research it would be curriculum, an issue the majority of the national reports on science failed to consider. Cognitive scientists are interested in how knowledge is acquired, but they do not directly consider the curriculum or its context. Science educators have been restating the goals of science teaching but have neglected to identify the supporting subject matter changes essential for achieving the goals. Currently, the most crucial and most neglected issue in science is a well structured and analyzed curriculum that recognizes new instructional goals.

The LAI is referred to as an excellent tool for the content analysis and evaluation of laboratory manuals by Hofstein (19) and Tamir (82). It has been used for evaluations by Tamir and Lunetta (16, 81, 87, 88); Fuhrman, Novick, and Lunetta (89); the New York Public School System (90); and a comprehensive study of science education and curriculum research and evaluation in Canada (84). In New York state, public teachers use the LAI to make decisions on the laboratory manuals to adopt for their classes. The manual as a whole must cover all the skills as listed in the categories of the LAI. As part of the Canadian study, 50 science curricula were studied by 18 science teaching specialists. Two of the themes studied, scientific skills and the image of science which are related to inquiry, were evaluated by the use of the LAI (84).

CHAPTER III

METHODS AND PROCEDURES

Development of the Selection of Experiments

The first part of the project was to select and assemble with appropriate modification a set of experiments based on the investigation and review of the available literature. The selection of experiments was designed to graduate in the level of inquiry-oriented skills required so the students could culminate the end of the semester experience with one or more investigative laboratories. The rationale to graduate in complexity was due to the demanding nature of an inquiry-oriented experiment. The student must develop a functional knowledge of the content of the chemistry under investigation, proficiency in laboratory techniques, inquiry skills such as formulating a hypothesis, and a knowledge of the scientific content of the literature reviewed for the experiment prior to attempting the investigative experiments.

The investigative laboratory curricula reviewed in the previous chapter allowed for basic techniques to be acquired by the students in initial traditional style experiments or in individual sessions with the instructors at a later time (15, 17, 24, 32, 34, 35, 37, 39, 41, 51, 78, 79). This rationale is also supported by Johnstone (91), Kozma (92), Nakhleh (93), Pendley (94), and Friedler and Tamir (95). The first one or two experiments in all instrumental categories was developed in the traditional style format. The remainder in each category were developed to be combination or investigative experiments with the

final experiment(s) designed to be investigative utilizing skills learned in previous experiments. This choice of experimental types also allows each instructor to adapt the selection of experiments to suit particular instructional needs and objectives.

The general educational objectives in the selection and development of the experiments were: (a) to elicit and increase a positive interest, attitude, and curiosity in science; (b) to develop analytical thinking and problem-solving ability; (c) to develop ability and manual dexterity in dealing with complex instruments, including safe working practices; (d) to promote scientific thinking abilities such as designing and executing investigations, making observations, recording data, formulating hypotheses, and analyzing and interpreting results; and (e) to apply facts and principles to different situations, including "real world concerns", using appropriate analytical and critical thinking skills (4, 19). Since inquiry-oriented laboratories have been extensively tested in the literature as disscussed in the previous chapter and proven to be effective in allowing the students the opportunity to meet the above objectives, these objectives are not specifically tested in this study. Rather, the investigator undertook a content analysis of a selection of experiments with a rating instrument, the LAI, which is designed to measure the inquiry-oriented nature of an experiment. The content analysis by the investigator was also confirmed in evaluations of selected experiments using the LAI by other teaching professionals and students.

The first experiment in the selection is a "dry-lab" entitled, "The Scientific Method." This experiment was designed to review terminology such as hypothesis, statement of the problem, and conclusions and to give a specific example of each from an

article in the analytical literature. A study in biology by Tamir and Amir concludes that scientific process skills do not just happen as a result of manipulation of experimental apparatus, but effort must be spent in teaching these skills (96). Also, Tamir (73) and Kuyper (97) advocate the idea of critiquing current research papers. And, Friedler and Tamir (95) developed a module to teach the scientific method for students using the inquiry approach in laboratories.

The selection of experiments was designed for schools with limited instrumental resources, so the following major instrumental categories were chosen: Ultraviolet-Visible (UV-VIS) (98-121), Gas Chromatography (GC) (122-139), and Infrared spectrophotometry (IR) (140-159). Surveys of techniques for instrumental analysis courses indicate these categories are in the top five instrumental techniques most commonly offered for the college chemistry departments surveyed (3, 4). An experiment in thin-layer chromatography (160) was also included because of its theoretical similarity to liquid chromatographic separations for those schools without instrumentation for liquid chromatography. The experiments were based on articles from the *Journal of Chemical Education*, *Analytical Chemistry*, and other scientific journals. Permission was granted by the ACS and *Journal of Chemical Education* (See Appendix I).

The following design for a typical semester is suggested for those using a curriculum similar to the proposed selection of experiments. The first laboratory session should include the scientific method experiment. The next seven laboratory sessions should include one traditional experiment for each instrument, one open-ended (combination) experiment on two different instruments, and two experiments that teach

special techniques such as internal standards and standard addition. In the remaining six weeks of the semester, the student chooses a problem to investigate. This represents the investigative experiment or project. The students are allowed to select their own problem or one from the instructor's listing. Two weeks before the investigative experiment begins and after an intensive literature search in the library, the students are required to seek approval from the instructor with a brief explanation of their experiment and a list of the materials required.

One week before the investigation begins, the students are required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, and bibliography from the library literature search. A photocopy of the literature upon which the experimental procedure is based should be included to help in the professor's evaluation. For the combination (open-ended) and investigative experiments, no literature references should be given to the students by the instructor. References included in Appendix IV in this dissertation are for the purpose of appropriately crediting sources and are not meant to be provided by the instructor for students performing these experiments. However, if students obtain these during their literature search, permission should be granted for their use. At the end of the semester, a formal report is due that is patterned after the scientific literature, such as *Analytical Chemistry*. The writing of this type of report requires high-level thinking skills and the drawing of specific conclusions (161). This type of student writing is also addressed and supported in other articles in the *Journal of Chemical Education* (162-171). The overall

sequencing of experiments is similar to other programs for investigative laboratories described in the literature (14, 15, 17, 24, 37, 39, 51, 78, 79).

Field-Testing of the Selection of Experiments

Initial Testing

Experiments selected to be a part of the course were first performed by junior or senior chemistry majors at Lee College in special topics courses or by the investigator. This was done to ascertain the feasibility of using the particular experiment in the selection. The investigator was also able to ensure that reasonable results could be obtained, and any problems could be noted and corrected. This part of the study was very similar to a preliminary field-test or a pilot study. In some cases, a few of the experiments that were initially chosen were discarded for reasons such as: use of dangerous or expensive chemicals, length of experiment, focus of experiment on chemical preparation such as a synthesis rather than the use of instrumental procedures, and failure to obtain reliable or successful results. Overall, the initial testing proved to be very successful in directing the investigator in the selection of the final experiments.

The Evaluation Instrument for the Experiments

The Laboratory Structure and Task Analysis Inventory (LAI) reached its present form in a work reported by Fuhrman (18). The first section (Part I) examines the laboratory organization and consists of the structure category (high-low, inductive, or deductive). The second section (Part II) consists of laboratory tasks and consists of 22 categories in four sub-sections as follows: (A) Planning and Design (formulates a

question, predicts results, formulates hypothesis, designs observation, and designs experiment); (B) Performance (carries out qualitative observation, manipulates apparatus, records results, performs calculations, selects experimental technique, and works according to own design; (C) Analysis (transforms results into standard form, graphs data, determines qualitative relationship, determines quantitative relationship, determines accuracy and/or precision, defines or discusses limitations and/or assumptions, generalizes, and explains a relationship; (D) Application (predicts on the basis of results, formulates hypothesis based on results, and applies experimental techniques to new problems). A detailed explanation for each category is included in Appendix II.

The student behaviors (interprets technique, works according to own design, defines limitations, formulates a generalization, explains a relationship, and all of the planning and design, and application sections) are considered to be higher order inquiry activities by the authors of the LAI. The other seven categories are generally present in traditional experiments. Consequently, the higher the number of total frequency counts in Part II an experiment receives, the more it tends to be inquiry oriented or investigative in nature (16, 18, 89).

The reliability of the LAI was documented by Fuhrman in a master's thesis at the University of Iowa. Reliability in this case means the measurement which "represents the various sources of error in the repeated measurements of a single phenomenon by different coders or the consistency in which an individual performs the same task over a period of time" (18). The Scott Coefficient π was the statistical measure used in the

determination. It is suggested as an appropriate index of inter-coder agreement when the research is composed of a nominal scale and is "comparable with the percentage agreement figure" (172).

Using the Scott Coefficient π, values of 0.57 to 0.81 with an overall average of 0.71 were obtained between coders. The coders were six individuals (three of whom were the developers of the LAI) involved in coding the same laboratory investigations to establish a quantitative measure of the reliability when each individual coder was compared to a master code (18).

The mathematical formula to obtain the Scott coefficient is detailed below.

$$\pi = \frac{(P_o - P_e)}{(1 - P_e)}$$

In the above formula, P_o is the percent agreement found by calculating the total proportion of agreement between a coder and the master code (or between two coders). P_e is the percent agreement expected by chance and is found by squaring the proportion of agreement between the coder and the master code and summing over all categories. So, the value of π is the amount that two coders exceed chance agreement divided by the amount that perfect agreement exceeds chance (172). The overall average of 0.71 indicates fairly high inter-coder agreement and therefore a good reliability measure for this instrument (18).

This investigator chose the LAI inventory to analyze the content of each experiment to determine the degree of the investigative nature of the experiment. This evaluation was completed on each of the six experiments to be field-tested immediately

after they were developed. This was done by the investigator before the field-test occurred for the students or the evaluation by the professors. Later, a content analysis was conducted by this investigator for the remainder of the experiments selected. The results are included in Tables III, IV and V in Chapter IV.

Field Test Conducted for Students

Six of the 15 experiments in the selection of experiments were designated from the UV-VIS and GC sections to be used as a part of the field-testing and evaluation procedure by students and in an evaluation by the professors. Part of the analysis of this evaluation included a percentage agreement comparison of the results in each category among students to obtain a measure of intercoder agreement. This was also evaluated for the professors. A *t*-test was used to compare the results of the investigator with those of the students and professors on Part II of the LAI to determine if there was a significant difference between the results.

The student participants of the study were Lee College junior and senior chemistry students. Lee College is a small liberal arts college in Cleveland, Tennessee, that is comprised of approximately 2500 students. Their selection for the project was based on the fact that they chanced to be enrolled in the laboratory for instrumental analysis being taught by the investigator during the Spring semester, January through May 1996. The sixteen laboratory students participating in the study were advised of the research project and were allowed to choose to participate (See Appendix III for Human Subjects Approval Form). All the students enrolled in the laboratory chose to participate in the study and signed the appropriate approval forms. They filled out an LAI, along

with the additional qualitative questions, following each of the six selected experiments comprising the study. The UV-VIS experiments were also tested at a college in Texas, but some students were unable to complete the experiments so their LAI data was not used in the analysis of the results.

A concerted effort was made to promote the confidentiality of all the results obtained in the study. After completion of the LAI for each experiment, the LAI was collected unsigned by a laboratory assistant and placed in a file to be analyzed by the investigator at the end of the semester. During the first laboratory session for the semester, the following items were covered with the students: an explanation of the study, a review of the scientific method, an explanation of the LAI and applicable terminology, an orientation to the laboratory, and the completion of "The Scientific Method" from the selection of experiments.

The qualitative questions included at the end of the LAI were generally open-ended questions of the type: What could be done to improve this experiment? However, one of the questions was: Which type of experiment do you prefer: traditional, combination, or investigative? This question was used in a chi square analysis to determine if there was one experimental type significantly chosen over the other types.

Evaluation Conducted for Professors

The six experiments that were tested by the students were also mailed to forty-seven professors in the states of Tennessee, Kentucky, and Texas. These professors included those from state public institutions such as Middle Tennessee State University, the University of Tennessee at Chattanooga, and the University of Tennessee at

Knoxville. The evaluation also included professors from small private schools such as from the Appalachian College Association and others in which Middle Tennessee State University D. A. students or graduates in chemistry work. The packet of materials sent to the professors included the following: a letter of explanation of the study, a copy of each of the six experiments to be evaluated, a copy of the LAI for each experiment with the definition of the terms and qualitative questions, and an offer of a copy of the selection of experiments for perusal upon its completion. Again, the confidentiality of the results was stressed, and all materials were sent back unsigned by the responders. Sixteen completed responses were received by the investigator. This is an approximately 34% rate of response which is considered acceptable for this type of study (23). All responses were then opened and filed by the laboratory assistant until the investigator began the process of data analysis.

CHAPTER IV

DATA ANALYSIS AND RESULTS

Presentation and Evaluation of the Selection of Experiments

The 16 experiments assembled for the selection with their inquiry orientation as determined by the investigator are listed in Table II below. The experiments that contain procedural portions are patterned after a specific procedure from the literature, but the overall design of the experiment was modified to exhibit a specific type of inquiry orientation by the investigator except in the case of the traditional experiments. From this listing, six of the experiments, which are starred, were selected to be used in a classroom field-test at Lee College and to be evaluated by professors at other institutions. The remaining experiments were scored with the LAI only by this investigator after the correlation of results between the students, professors, and this investigator was found to be satisfactory.

TABLE II. SELECTION OF EXPERIMENTS UTILIZED FOR THE STUDY

Number	Title	Inquiry Orientation	Ref. #
1*	Determination of Iron(II) and Total Iron with 1,10 Phenanthroline	Traditional	99
2*	The Analysis of the Iron Content of Soap	Combination	100
3*	Analysis of Copper Using Differential or Expanded Scale Spectroscopy	Combination	101

Table II. Continued			
Number	**Title**	**Orientation**	**Ref.**
4	Determination of the Formula for An Iron(III) Sulfosalicylate Complex Using The Method of Continuous Variations	Traditional	102
5*	Analysis of a Hydrocarbon Mixture	Traditional	----
6*	Analysis of Gasoline by Gas Chromatography	Combination	122
7*	Analysis of Fatty Acid Composition of Common Fats and Oils	Investigative	129
8	Determination of Congeners in Whiskey Using the Internal Standard Method	Combination	131
9	Determination of Methyl Salicylate in Rubbing Alcohol Using the Method of Standard Additions	Combination	135
10	Qualitative Spectral Analysis	Traditional	----
11	Quantitative Analysis of a Xylene Mixture Using the Internal Standards Method	Combination	140
12	Determination of the Vinylacetate Content of Packaging Films	Traditional	152 153
13	Separation and Identification of Derivatives of 2,4-Dinitrophenylhydrazine	Combination	160
14	Thin-Layer Chromatography	Investigative	----
15	Determination of Metals in Local Water Supplies	Investigative	----
16	Analysis of Alkaloids in Plants	Investigative	----

* Six Experiments that were selected for the field-test and evaluation.

The Laboratory Structure and Task Analysis Inventory (LAI) was used by the investigator to perform a content analysis on each experiment in Table II. Tables III, IV and V summarize the detailed results from that analysis. A score of zero indicates the

experiment is not designed in this manner or does not call for this student behavior. A score of one indicates the experiment was designed in this manner or calls for this student behavior at least once. At the bottom of each table is the total score (for Part II of the LAI) which relates the degree of investigative nature of the experiment.

TABLE III. INVESTIGATOR'S CONTENT ANALYSIS UTILIZING THE LAI FOR EXPERIMENTS 1-6

I. Organizational Categories	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5	Exp. 6
A. **Structure**						
1. High-degree	1	0	0	1	1	0
2. Low-degree	0	1	1	0	0	1
3. Inductive approach	0	1	1	0	0	1
4. Deductive approach	1	0	0	1	1	0
II. ***Task Categories***						
A. **Planning and Design**						
1. Formulates a question or defines a problem	0	1	1	0	0	1
2. Predicts results	0	0	1	0	0	1
3. Formulates hypothesis	0	1	1	0	0	1
4. Designs observation	0	1	1	0	0	1
5. Designs experiment	0	1	1	0	0	1
B. **Performance**						
1. Carries out qualitative observation	1	1	1	1	1	1
2. Manipulates apparatus	1	1	1	1	1	1
3. Records results	1	1	1	1	1	1

Table III. Continued.	Exp.1	Exp.2	Exp.3	Exp.4	Exp.5	Exp.6
4. Performs numeric calculation	1	1	1	1	1	1
5. Explains or makes a decision about technique	1	1	1	0	1	1
6. Works according to own design	0	0	1	0	0	1
C. **Analysis and Interpretation**						
1. Transforms results into standard form	1	1	1	1	1	1
2. Graphs data	1	1	1	1	1	1
3. Determines qualitative relationship	0	0	0	0	1	1
4. Determines quantitative relationship	1	1	1	1	1	1
5. Determines accuracy and / or precision	1	1	1	0	1	1
6. Defines or discusses limitations/assumptions	1	1	1	1	0	1
7. Formulates or proposes a generalization or model	0	1	0	0	0	1
8. Explains a relationship	0	1	1	1	0	1
D. **Application**						
1.Predicts based upon results	0	1	0	0	0	0
2. Formulates hypothesis based upon results	0	1	0	0	0	0
3. Applies experimental technique to new problem or variable	0	1	1	0	0	0
Total Frequency Counts from Part II	10	19	18	9	10	19

TABLE IV. INVESTIGATOR'S CONTENT ANALYSIS UTILIZING THE LAI FOR EXPERIMENTS 7-12

I.*Organizational Categories*	Exp.7	Exp.8	Exp.9	Exp. 10	Exp. 11	Exp. 12
A. **Structure**						
1. High-degree	0	0	0	1	0	1
2. Low-degree	1	1	1	0	1	0
3. Inductive approach	1	1	1	0	1	0
4. Deductive approach	0	0	0	1	0	1
II. ***Task Categories***						
A. **Planning and Design**						
1. Formulates a question or defines a problem	1	1	1	0	1	0
2. Predicts results	1	1	1	0	1	0
3. Formulates hypothesis	1	1	1	0	0	0
4. Designs observation	1	1	1	0	1	0
5. Designs experiment	1	1	1	0	1	0
B. **Performance**						
1. Carries out qualitative observation	1	0	1	1	1	1
2. Manipulates apparatus	1	1	1	1	1	1
3. Records results	1	1	1	1	1	1
4. Performs numeric calculation	1	1	1	0	1	1
5. Explains or makes a decision about technique	1	1	1	1	1	1
6. Works according to own design	1	1	1	0	1	0

Table IV. Continued	Exp.7	Exp.8	Exp.9	Exp. 10	Exp. 11	Exp. 12
C. **Analysis and Interpretation**						
1. Transforms results into standard form	1	1	1	1	1	1
2. Graphs data	1	1	1	0	1	1
3. Determines qualitative relationship	1	1	1	1	1	1
4. Determines quantitative relationship	1	1	1	0	1	1
5. Determines accuracy and / or precision	1	1	1	0	1	1
6. Defines or discusses limitations and/or underlying assumptions	1	1	1	1	1	1
7. Formulates or proposes a generalization or model	1	0	0	1	0	0
8. Explains a relationship	1	1	0	1	1	0
D. **Application**						
1. Predicts based upon results	1	0	0	0	0	0
2. Formulates hypothesis based upon results	1	0	0	0	0	0
3. Applies experimental technique to new problem or variable	1	0	0	0	0	0
Total Frequency Counts from Part II	22	17	17	9	17	11

TABLE V. INVESTIGATOR'S CONTENT ANALYSIS UTILIZING THE LAI FOR EXPERIMENTS 13-16

I.Organizational Categories	Exp. 13	Exp. 14	Exp. 15	Exp. 16
A. **Structure**				
1. High-degree	0	0	0	0
2. Low-degree	1	1	1	1
3. Inductive approach	1	1	1	1
4. Deductive approach	0	0	0	0
II. ***Task Categories***				
A. **Planning and Design**				
1. Formulates a question or defines a problem	1	1	1	1
2. Predicts results	0	1	1	1
3. Formulates hypothesis	0	1	1	1
4. Designs observation	1	1	1	1
5. Designs experiment	1	1	1	1
B. **Performance**				
1. Carries out qualitative observation	1	1	1	1
2. Manipulates apparatus	1	1	1	1
3. Records results	1	1	1	1
4. Performs numeric calculation	1	1	1	1
5. Explains or makes a decision about technique	1	1	1	1
6. Works according to own design	1	1	1	1

Table V. Continued	Exp. 13	Exp. 14	Exp. 15	Exp. 16
C. **Analysis and Interpretation**				
1. Transforms results into standard form	1	1	1	1
2. Graphs data	1	1	1	1
3. Determines qualitative relationship	1	1	1	1
4. Determines quantitative relationship	1	1	1	1
5. Determines accuracy and / or precision	1	1	1	1
6. Defines or discusses limitations and/or underlying assumptions	1	1	1	1
7. Formulates or proposes a generalization or model	1	1	1	1
8. Explains a relationship	1	1	1	1
D. **Application**				
1. Predicts based upon results	0	1	1	1
2. Formulates hypothesis based upon results	0	1	1	1
3. Applies experimental technique to new problem or variable	0	1	1	1
Total Frequency Counts from Part II	17	22	22	22

Results and Conclusions from the Investigator's Evalulation

The original characterization of an experiment as traditional, combination, or investigative as defined from the relevant literature was determined by this investigator from the analysis of the overall design of the experiment. Afterwards, the following general range of results for each type was found for the summation of the frequency counts in Part II of the LAI: (a) traditional: 9-11; (b) combination (open-ended): 17-19; and (c) investigative: 22. This indicates the LAI is useful in distinguishing between varying levels of skills required in the inquiry-oriented experiments within the framework of this study. Certainly, the difference numerically between the traditional and open-ended (combination) experiments is large enough to characterize an experiment as traditional or combination as determined inferentially from the confidence intervals as a result of the *t*-test. The previous statement would also be true when comparing traditional and investigative experiments. However, the difference between the combination and investigative experiments was much less, and the higher scores for the investigative experiments should be interpreted as requiring more inquiry-oriented skills rather than as specifically categorizing an experiment as investigative rather than combination (See confidence intervals Table VIII).

The preceding evaluation was done by this investigator in an upper division college chemistry course (instrumental analysis), and the resulting scores on the LAI ranged from 9-22. In the opinion of this investigator, these upper division chemistry experiments generally have higher level analytical skills represented even in the traditional experiment. Note that the lowest score was a 9, even though a 0 is possible.

High school and freshman chemistry classes might see an even broader range among the different levels of inquiry-oriented experiments making a delineation easier.

This investigator also evaluated several types of inquiry-oriented experiments, which were classified as such by their authors from the literature, to have a basis for comparison. First, some individual guided-inquiry (discovery) experiments were examined for two organic and one polymer science laboratory. The experiments chosen were Jarrett's "Electrophilic Aromatic Substitution Discovery Lab" (54), Pearsall's "Discovery-Based Microscale Catalytic Decarbonylation of Aldehydes" (55), and Slough's "The Molecular Mass Determination of Polystyrene: A Simple, Discovery-Based Laboratory Exercise" (56). These experiments totaled a 14, 13, and 12 respectively on Part II of the LAI. Next, a curriculum by Wehry for analytical chemistry entitled, "Open-Ended Experiments for Undergraduate Analytical Chemistry" was evaluated (36). This article also included eight individual open-ended experiments in analytical chemistry which were ranked between 17 and 19 on Part II of the LAI. And finally, an investigative curriculum by Barr (34) for organic and inorganic chemistry, including three specific problems that students had investigated in the inorganic area, entitled, "The Chemistry Curriculum and the January Term" was evaluated. The results were all 22. The evaluations from the literature generally support the range of results obtained from the evaluations of the selection of experiments for this dissertation.

Presentation of Percentage Agreement Data

Because the preceding content analysis depended on the judgement of the individual investigator, confirming the results with the opinions of students actually performing the experiments and professionals in the field was deemed necessary. First, a percentage agreement determination among professors and students was calculated. This was calculated by dividing the number of professors or students agreeing by the total and multiplying by 100. An example calculation is shown below.

$$\% \ Agreement = \frac{15 \ (Number \ of \ professors \ ranking \ a \ category \ one)}{16 \ (Total \ Number \ of \ Professors)} \ X \ 100$$

$$= 94\%$$

Please note Table VI and Table VII below for results.

Results and Conclusion from Percentage Agreement Data

When comparing the total averages of % agreement between professors for the six experiments, the scores are 85, 83, 85, 86, 85 and 90%. The inter-coder agreement is quite consistent over the six experiments with a range of 83 to 90% and an overall average of 86%. The students show similar although somewhat higher results with scores of 90, 86, 89, 93, 89, and 94%. Again the inter-coder agreement is consistent with a range of 86 to 94% and an overall average of 90%. These inter-coder agreement percentages are deemed acceptable in the literature and contribute an important estimate of the consistency of the coders' assessments (23).

TABLE VI. PERCENTAGE AGREEMENT AMONG PROFESSORS FOR LAI EVALUATIONS

I.Organizational Categories	Exp.1	Exp.2	Exp.3	Exp.5	Exp.6	Exp. 7
A. **Structure**						
1. High-degree	81	100	94	94	100	100
2. Low-degree	75	100	94	94	100	100
3. Inductive approach	88	75	69	81	81	81
4. Deductive approach	88	75	69	81	81	81
II. ***Task Categories***						
A. **Planning and Design**						
1. Formulates a question or defines a problem	81	69	62	81	81	88
2. Predicts results	75	38	75	88	75	81
3. Formulates hypothesis	94	81	75	81	88	94
4. Designs observation	69	88	88	75	100	100
5. Designs experiment	94	75	81	94	75	88
B. **Performance**						
1. Carries out qualitative observation	81	88	81	88	88	88
2. Manipulates apparatus	94	100	100	94	100	100
3. Records results	100	100	100	100	100	100
4. Performs numeric calculation	100	100	100	100	94	100
5. Explains or makes a decision about technique	75	88	88	81	88	81
6. Works according to own design	94	88	94	94	100	100

Table VI. Continued	Exp.1	Exp.2	Exp.3	Exp.5	Exp.6	Exp.7
C. **Analysis and Interpretation**						
1. Transforms results into standard form	81	81	88	94	94	94
2. Graphs data	100	100	100	100	94	94
3. Determines qualitative relationship	69	69	69	81	81	88
4. Determines quantitative relationship	100	94	100	100	88	94
5. Determines accuracy and / or precision	88	94	100	94	81	75
6. Defines or discusses limitations and/or underlying assumptions	75	75	75	69	81	75
7. Formulates or proposes a generalization or model	88	69	81	94	62	69
8. Explains a relationship	56	81	75	50	94	88
D. **Application**						
1. Predicts based upon results	88	75	75	62	56	88
2. Formulates hypothesis based upon results	88	75	75	81	62	100
3. Applies experimental technique to new problem or variable	81	88	94	88	56	94
Average Percentage Agreement	85	83	85	86	85	90

TABLE VII. PERCENTAGE AGREEMENT AMONG STUDENTS FOR LAI EVALUATIONS

I.*Organizational Categories*	Exp.1	Exp.2	Exp.3	Exp.5	Exp.6	Exp. 7
A. **Structure**						
1. High-degree	94	75	69	100	81	94
2. Low-degree	94	75	69	100	81	94
3. Inductive approach	94	88	94	94	81	100
4. Deductive approach	94	88	94	94	81	100
II. ***Task Categories***						
A. **Planning and Design**						
1. Formulates a question or defines a problem	88	94	94	94	94	100
2. Predicts results	88	38	81	100	81	94
3. Formulates hypothesis	94	81	81	81	88	88
4. Designs observation	88	94	100	75	100	100
5. Designs experiment	94	69	62	94	69	81
B. **Performance**						
1. Carries out qualitative observation	100	100	100	100	100	100
2. Manipulates apparatus	94	94	100	100	100	100
3. Records results	94	100	100	100	100	100
4. Performs numeric calculation	100	100	100	100	100	100
5. Explains or makes a decision about technique	62	69	75	88	88	88
6. Works according to own design	100	81	100	100	100	100

Table VII. Continued	Exp.1	Exp.2	Exp.3	Exp.5	Exp.6	Exp.7
C. **Analysis and Interpretation**						
1. Transforms results into standard form	94	94	88	100	100	94
2. Graphs data	100	100	100	100	88	100
3. Determines qualitative relationship	75	75	88	81	100	94
4. Determines quantitative relationship	94	94	100	100	100	94
5. Determines accuracy and / or precision	94	94	100	94	94	88
6. Defines or discusses limitations and/or underlying assumptions	69	88	75	81	75	81
7. Formulates or proposes a generalization or model	88	88	88	88	88	88
8. Explains a relationship	81	94	88	81	94	88
D. **Application**						
1. Predicts based upon results	88	88	81	88	88	94
2. Formulates hypothesis based upon results	100	88	75	100	75	94
3. Applies experimental technique to new problem or variable	94	81	94	94	69	88
Overall Percentage Agreement	90	86	89	93	89	94

The overall average student inter-coder agreement is approximately 5% higher than that of the professors. This would be expected because of the student performance of the experiment and subsequent submission of a written report which gives them a more intimate view of the experiment.

Presentation of *t*-Test Data

Having ascertained an overall acceptable consistency of inter-coder agreement among both the professors and students, the next phase in the evaluation was to determine if the investigator's analysis varied significantly from the analyses of the students and professors. The *t*-test was the statistical procedure used in the determination. The total frequency counts in Part II of the LAI for the investigator were compared with the average value of the counts in Part II of the LAI for the professors and the students. The *t*-test can be used to compare a total number with an average as described in Glass and Hopkins (173).

The equation used is given below:

$$t = \frac{\bar{x} - \mu}{S_x}$$

In this equation, μ is equal to the total frequency count of the investigator in any particular experiment. The $\bar{x}$ is the mean frequency count for either the students or the professors. And, the s_x is the standard error of the mean which is equal to the standard deviation divided by the square root of the number of students or professors, minus one. See Table VIII following for the results.

TABLE VIII. RESULTS FOR THE *t*-TEST ON A COMPARISON OF FREQUENCY COUNTS BETWEEN THE INVESTIGATOR, STUDENTS, AND PROFESSORS

Exp. #	Total for Investigator	Student Average	Stud. 95 % C.I.*	Prof. Average	Prof. 95% C.I.*	Stud. *t*-Test Results	Prof. *t*-Test Results
1	10	10.2	7.4-13.2	11.2	8.6-13.8	0.15	0.78
2	19	18.2	16.7-19.7	17.2	15.8-18.8	0.92	2.03
3	18	17.1	14.9-19.2	16.8	14.9-18.6	0.76	1.16
5	10	10.9	7.8-14.0	11.8	9.0-14.4	0.49	1.13
6	19	18.2	16.4-20.1	17.9	16.6-19.2	0.72	1.64
7	22	20.5	19.1-21.8	19.8	17.1-22.4	1.88	1.50

* 95% Confidence Intervals - inferential statistical procedure defined so that the value of the parameter lies within the range at the defined percentage.

Results and Conclusion from *t*-Test Data

The value for α at the 0.01 probability level from Table C: Percentile Points of *t* Distribution in Glass and Hopkins (173) is 2.602. The statement of the research problem (also in null hypothesis form) is as follows: There is no significant difference between the professors' or the students' average scores on Part II of the LAI when compared with the total score of the investigator on Part II of the LAI. An observed *t*-value greater than 2.602 is required to reject the statement of the problem. From the above data, none of

the *t*-test results can be rejected and the statement of the research problem is true. Consequently, at the 0.01 probability level the professor and student results are not significantly different from the results of the investigator.

For this study, the confidence interval data confirms mathematically the qualitative observation that a numerical distinction can be made between the traditional and combination or investigative experiments but not between the combination and investigative experiments. The highest numerical range for a traditional experiment was in experiment 5 for the professors: 9.0-14.4. And, the lowest numerical range for a combination experiments was in experiment 3 for both the students: 14.9-19.2 and the professors: 14.9-18.6. Therefore, there is no numerical overlap between the traditional and combination experiments. However, there are overlaps in the ranges between the combination and investigative experiments. An example of overlap occurs in the student combination experiments 2, 3, and 6 with the investigative experiment 7. Experiment 2 has a confidence interval of 16.7-19.7, experiment 3 is 14.9-19.2, and experiment 6 is 16.4-20.1. Student investigative experiment 7 results are 19.1-21.8. This is an overlap of 1.0 when compared with experiment 6.

Presentation of Chi Square Analysis Data

Another part of the evaluation of the selection of experiments was to determine whether there was a preference among the professors and students as to type of experiment: traditional, combination, or investigative. The technique employed to examine this part of the evaluation was a chi square analysis (χ^2). Gay (23) defines the

chi square analysis as a "nonparametric test of significance appropriate when the data are in the form of frequency counts occurring in two or more mutually exclusive categories." The statement of the problem is that no significant difference is expected among the choices of experimental types. The statement of the problem was in the null form because it suited the statistical technique in determining whether the observed responses were chance or true. The question used in the chi square analysis was stated as follows in the analysis sent to the participants: "Which type of lab do you prefer: Traditional, combination, or investigative [Circle only one]."

The formula used for the chi square analysis was as follows

$$\chi^2 = \Sigma \frac{(f_o - f_e)^2}{f_e}$$

f_o = observed frequency f_e = expected frequency

The degrees of freedom, df, associated with a chi square analysis are determined by the number of categories which in this study were three. The formula for degrees of freedom in a chi square analysis is K-1; therefore, df for this study is 2. From Gay (23) Table A.6 "Distribution of χ^2", the numerical value associated with df = 2 at $\alpha = .05$ is 5.991. To reject the null hypothesis, all values obtained from the analysis must be greater than 5.991. The study contained 16 students and 16 professors for a total of 32 participants. The null hypothesis was rejected at the 0.05 probability level in all 3 areas evaluated (student, professor, and combined). Note tables IX, X, and XI below and a sample data calculation.

TABLE IX. CHI SQUARE ANALYSIS FOR STUDENT RESULTS

	Investigative Exps.	Combination Exps.	Traditional Exps.
Expected, f_e *	4.33	4.33	4.33
Observed, f_o	4	9	0

Thirteen of the sixteen students responded in answer to the question.
*f_e = Thirteen responding students divided by 3 categories.

Student calculation for chi square:

$$\chi^2 = \frac{(4 - 4.33)^2}{4.33} + \frac{(9 - 4.33)^2}{4.33} + \frac{(0 - 4.33)^2}{4.33}$$

$$= 9.392 \text{ which is} > 5.991$$

The problem statement is therefore rejected, and there is a statistically significant choice of experimental type which is the combination.

TABLE X. CHI SQUARE ANALYSIS FOR PROFESSOR RESULTS

	InvestigativeExps.	CombinationExps.	Traditional Exps.
Expected, f_e	4.33	4.33	4.33
Observed, f_o	0	12	1

*Fourteen of the sixteen professors responded in answer to the question; however, one responded by circling all three categories making the response invalid. Therefore, the calculation is made with 13 subjects.

The calculation for chi square for the results of the professors gives 20.481. This, of course, is much greater than 5.991, and therefore the statement of the problem is rejected. The preferred experimental type is combination.

TABLE XI. CHI SQUARE ANALYSIS FOR COMBINED RESULTS

	Investigative Exps.	**Combination Exps.**	**Traditional Exps.**
Expected, f_e	8.67	8.67	8.67
Observed, f_o	4	21	1

The calculation for chi square for the combined results of the students and professors gives a value equal to 26.83 which is much greater than 5.991, and again the statement of the problem is rejected. The experimental type of preference is therefore combination.

Results and Conclusions for the Chi Square Data Analysis

The rejection of the statement of the problem from the chi square analysis at the 0.05 probability level indicates that there is only a 5 out of 100 chance that a difference as large as the one found would occur as a result of sampling error. Therefore, it can be concluded that there is a high probability that the combination type experiments would be preferred by students and professors alike. For the students, 69% prefer the combination (open-ended) experiments, 31% the investigative, and 0% the traditional. For the professors, 92% prefer the combination experiments, 8% the traditional, and 0% the

investigative. When combining the results of both groups, 81% prefer the combination experiments, 15% prefer the investigative, and 4% the traditional.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to select, develop, and evaluate a selection of experiments in instrumental analysis for schools with limited instrumental resources. The primary intent behind the selection of experiments was to develop them in such a manner that students would have the opportunity to acquire physical skills with laboratory instrumentation and study the scientific method, develop critical thinking, and initiate investigation in the chemical laboratory. A set of specific educational objectives was written with this in mind (see Chapter III) plus the hope that students would develop an interest and appreciation for research. Previous research has indicated that inquiry-oriented experiments would allow the students the opportunity to meet these goals.

After extensive literature research in the areas of inquiry-oriented versus traditional laboratories and specific experiments for the instrumental lab, six experiments for ultraviolet/visible and gas chromatography were selected to be evaluated and field-tested. The instrument chosen to accomplish the content analysis for the evaluation was the Laboratory Structure and Task Analysis (LAI), because of its development as a tool to measure inquiry-oriented curricula. Qualitative open-ended questions developed by the investigator were also included as part of the field-test. The investigator initially performed a content analysis on the experiments using the LAI.

The field-testing and evaluation by students was accomplished at Lee College, Cleveland, Tennessee, during the spring semester 1996, utilizing an instrumental analysis laboratory which consisted of junior and senior chemistry majors. Professors from several large public institutions and smaller private schools in Tennessee, Kentucky, and Texas were also asked to evaluate the experiments in the laboratory manual.

Results from the evaluation by professors and field-testing and evaluation of the students indicated an average inter-coder agreement of 86% for professors and 90% for students signifying an acceptable consistency for the coders' assessment. Data from a *t*-test indicated the student and professor results were not significantly different from the investigator's results, which confirms the results of the investigator's original content analysis of the selection of experiments and provides the basis for the investigator's evaluation of the remaining experiments. Another part of the evaluation was a test of preference to determine if there would be a significant difference between types (traditional, combination, investigative) of experiments chosen by students and professors. The problem statement was written in the null form and rejected in all cases. Thereby, the conclusion was established that both students and professors preferred the combination type laboratory with 92% of the professors and 69% of the students selecting the combination experiment.

In analyzing the results for the test of preference, the professors overwhelmingly chose the combination experiment. In the remaining categories, one professor chose the traditional experiment and none the investigative. In the opinion of the investigator, this is due to the fear that the students would be academically unprepared to function at the

investigative level coupled with the greater difficulty in planning and executing the variety of possible student experimental designs. However, this investigator found that beginning with traditional experiments and graduating to the investigative experiment provided students with sufficient experience and expertise to complete the investigative experiments.

The majority of students preferred the combination experiments. Unlike the professors, four students preferred the investigative experiment and none the traditional. In their qualitative comments, many students expressed that they learned more in the investigative experiment, and they particularly liked the freedom to choose a topic that piqued their interest. Nonetheless, those students that chose the combination experiment remarked that they liked the security in having some background preparation. As students gain more confidence in their abilities, freedom to choose topics of interest could outweigh the need for security as they undertake experiments that are more investigative in nature.

After reviewing the data and qualitative comments, this investigator reached the conclusion that the investigative and combination experiment format is an excellent and viable option for schools that do not have a large variety of instruments to cover an entire semester's work. Rather than limiting the learning experience to several traditional experiments on one or two instruments, incorporating the investigative and combination experiments greatly enhanced the learning experience. It allowed the students the opportunity to meet the goals listed previously for the selection of experiments.

Suggestions for Further Research

During the course of this study, certain conclusions surfaced that might possibly serve as the foundation for further investigation or research in this area.

1. Further field-testing is definitely warranted for the all the experiments especially those that were not classroom field-tested.

For this selection of experiments

2. Additional research may be indicated concerning the differences between achievement among students who use these types of inquiry-oriented versus traditional experiments. This would require an experimental design with control and experimental groups (two or more laboratory sections).

3. In conjunction with measuring achievement as suggested in number 2, it might prove interesting to measure the attitude of the students using the investigative or combination approach versus the traditional approach. Would this create an increased interest in science and research?

For general science education study

4. The significance of the age of the students and cognitive ability of an individual to engage in higher-level thinking might prove to be an enlightening research topic if correlated to the results of the experimental study as outlined in number two above. Research to evaluate the maturity level of students in terms of degree and duration of day-to-day exposure to real-world work and other factors might prove helpful in determining at what stage students should be exposed to the investigative experiment.

5. It might prove beneficial to conduct research on the proper usage of an instructional

module on the philosophy of science and the scientific method. A study could be undertaken to determine the most advantageous length and appropriate method of presenting such a module. For example, should it be a one-time experiment as developed for this selection of experiments, a separate portion of the overall course, integrated throughout the course, or presented in three to four separate steps. This study could also examine the depth of the material presented relative to the method of presentation and educational preparedness of the students. Such a study might assist in the palatability of the module on the scientific method and consequently contribute to the overall classroom and laboratory instruction of the investigative method in science education.

5. A content analysis on different levels (high school, college) of science laboratory curricula using the LAI (or another appropriate instrument) to determine whether a specific numerical scale could be developed that would distinguish between levels of inquiry-oriented curricula. An analysis similar to this on textbooks might also prove beneficial to instructors in selecting appropriate curricular materials to meet their classroom goals.

APPENDICES

APPENDIX I

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"An Analytical Chemistry Experiment in Simultaneous Spectrophotometric Determination of Fe(III) and Cu(II) with Hexacyanoruthenate" by M. C. Mehra and J. Rioux. *Journal of Chemical Education*, **1982**, 59, 688-689.

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Full bibliographic reference to the original article in the *Journal* (as illustrated above) should be given.

Thank you for your continued interest in our *Journal*.

Sincerely,

J. J. Lagowski
Editor

jjm

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APPENDIX II

LABORATORY STRUCTURE AND TASK ANALYSIS

INVENTORY

DEFINTION AND DESCRIPTION OF TERMINOLOGY

Categories with Definitions from LAI

The following is from Fuhrman, Lunetta, Novick, and Tamir (18).

I.A.1 High-structure. Investigation in which the student is explicitly directed in all aspects of the experiment.

I.A.2 Low-structure. These investigations may make an initial suggestion or statement but otherwise the design and extent of the experiment is, for the most part, left to the student.

I.A.3 Inductive approach. The investigation involves developing generalizations from data or from individual cases.

I.A.4 Deductive approach. The investigation involves the testing of phenomena or relationships that have been predicted based upon principles or processes that have previously been stated.

I.B.1-3 This will not be evaluated for this laboratory manual.

I.C.1 Students work on a common task and pool results. It should be clear from the laboratory investigation being analyzed that students are to pool their results of the task.

I.C.2 Students work on different tasks and pool results.

It should be clear from the investigation that students are to pool their results on various tasks.

I.C.3 Postlab discussion required. Members of the class are explicitly directed to further process or compare data or results through group discussion after gathering experimental data.

II.A.1 Formulates question or defines problem to be investigated. The experimental problem is not stated or defined explicitly and the student is required to come up with his/her own formulation of the problem.

II.A.2 Predicts experimental result. The student is asked to predict an experimental result based on his/her previous knowledge; a prediction is a logical outcome and in this respect differs from hypothesis formulation, which implies alternative explanations.

II.A.3 Formulates hypothesis to be tested in this investigation. The student is asked to formulate an hypothesis, to offer a tentative explanation, or to propose a relationship. The explanation or relationship must be tested experimentally in order to decide among competing hypotheses. Hypotheses are commonly sought before engaging in laboratory activity, but students may be asked to generate them at many points in a laboratory investigation.

II.A.4 Designs observation, measurement or calculation procedure. The student must be asked to design and carry out or describe the procedure for part of a laboratory investigation.

II.A.5 Designs experiment. The student is asked to design an entire experimental procedure, not just part of it. The student need not be asked to actually perform the experiment in order for this category to be checked.

II.B.1 Carries out qualitative observation. The student does what can be termed observation.

II.B.2 Carries out quantitative observation or measurement. This category is checked only when the observation involves counting or numeric measurement.

II.B.3 Manipulates apparatus: develops technique. When a statement specifies or implies the use of a specific laboratory technique, this category applies.

II.B.4 Records result, describes observation. This category should be checked if subsequent treatment of the data clearly requires that it was recorded.

II.B.5 Performs numeric calculation. The student is directed to calculate a numerical quantity from data obtained in the laboratory investigation.

II.B.6 Interprets, explains or makes a decision about experimental technique. The student is required to make an analytical or critical statement concerning a technique used in the laboratory investigation.

II.B.7 Works according to own design. Student must be required during the course of the lab to actually perform the experiment or procedure that he/she designed.

II.C.1 Transforms result into standard form (other than graphs). Standard form refers to a standard ordering process, such as generating a table, a classification scheme, a diagram, a sketch (to the exclusion of diagrams or sketches or apparatus).

II.C.2 Graphs data. Student is required to graph quantitative measurements.

II.C.3 Determines qualitative relationships. The student is required to make an inference or draw a conclusion that does not involve mathematical manipulation.

II.C.4 Determines quantitative relationships. Student is required to formulate the inference or conclusion as a quantitative relationship that involves mathematical manipulation. The determination of an empirical or molecular formula from experimental data is a task in this category.

II.C.5 Determines accuracy/precision of experimental data. Student is required to determine a numeric value that describes the amount of error or deviation.

II.C.6 Defines or discusses variables, limitations or assumptions that underlie the experiment. The student must respond to a direction or a question by defining or discussing variables, limitations or assumptions that underlie the observation or the interpretation of experimental data. It is not checked when a variable, limitation or assumption is merely specified or implied in the text.

II.C.7 Formulates or proposes a generalization or model. This goes beyond data specification and asks the student to generalize to or about other instances outside the immediate lab results. In this respect, it differs from drawing conclusions that pertain only to the phenomena sampled in the lab.

II.C.8 Explains a relationship. This requires making relationships clear and intelligible. The explanation involves a discussion of relationships that fit the results of the experiment.

II.C.9 Formulates new questions or defines problem based upon result of investigation. The student is directed to come up with his/her own formulation of new questions or a problem for further investigation as a result of the lab work.

II.D.1 Predicts based upon result of this investigation. The student is required to make a prediction on the basis of his/her findings in the experiment or on the basis of previous knowledge combined with the findings. The prediction does not require testing for validation.

II.D.2 Formulates hypothesis based upon results of this investigation. The student is required to formulate an hypothesis on the basis of his/her findings in the experiment, or on the basis of previous knowledge combined with the findings.

II.D.3 Applies experimental technique to new problem or variable. The technique refers to one which the student has learned or used in the lab investigation.

APPENDIX III

RESEARCH APPROVAL FORM

MEMO

TO: Penny L. Mauldin

FROM: JAMES V. BALCH
Basic and Applied Sciences Representative
Institutional Review Committee

RE: Research Application
IRC Protocol No. 96-003
Submitted for expedited review
on September 1, 1995

DATE: September 14, 1995

This is to officially notify you that your research proposal titled "A Laboratory Manual in Instrumental Analysis for Spectroscopy and Chromatography" qualifies, in my opinion, for expedited review and is herewith approved by me as the representative from your college of the Institutional Review Committee. Good luck as you pursue this project.

James V. Balch

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REFERENCES

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171. Goodman, W. D.; Bean J. C. *J. Chem. Educ.* **1983**, *60*, 483.

172. Scott, W. A. *Public Opinion Quarterly* **1955**, *Fall*, 321-325.

173. Glass, G. V.; Hopkins, K. D. *Statistical Methods in Education and Psychology*, 3rd ed., Allyn and Bacon: Needham Heights, MA, 1996.

APPENDIX IV

A SELECTION OF EXPERIMENTS IN INSTRUMENTAL ANALYSIS FOR SCHOOLS WITH LIMITED INSTRUMENTAL RESOURCES UTILIZING AN INQUIRY ORIENTED APPROACH

A SELECTION OF EXPERIMENTS IN INSTRUMENTAL ANALYSIS FOR SCHOOLS WITH LIMITED INSTRUMENTAL RESOURCES UTILIZING AN INQUIRY ORIENTED APPROACH

By

Penny L. Mauldin

FOREWORD

The general educational objectives for this selection of experiments are: to elicit and increase a positive interest, attitude, and curiosity in science; to develop analytical thinking and problem solving ability; to develop capability and manual dexterity in dealing with complex instruments, including safe working practices; to promote scientific thinking abilities such as designing and executing investigations, making observations, recording data, formulating hypotheses, and analyzing and interpreting results; to apply facts and principles to novel situations including 'real' world concerns using appropriate analytical and critical thinking skills.

In helping to meet goals two and four, this selection contains experiments written in three styles, two of which incorporate an inquiry-oriented approach: traditional, combination (contains an optional open-ended portion), and investigative. The traditional experiments are written in a "cookbook" fashion in the sense that every necessary step for the experiment is detailed for the student. However, even the traditional experiments require data analysis portions that include critical thinking and higher level thought processes one would expect from a junior level chemistry course. The investigative experiments (or open-ended portion of a combination experiment) requires students to do such things as state their own problem, hypothesis, design their own experiment, and other steps involved in the scientific method. Note in the table of contents that each experiment is classified as to type. The instructor has the option of omitting the open-ended portion of a combined experiment; thus giving more flexibility as far as instructor preference and time allows.

To introduce students to the scientific method as it is employed in analytical chemistry, the first experiment is a "dry-lab" entitled, "The Scientific Method". The purpose of this experiment is not only to review the basic concepts of the scientific method, but to analyze a literature article using a proposed outline of the scientific method. The first experiment then becomes an integral introduction to the semester. The author has found that a good way to proceed with this experiment is to: (a) give a brief lecture on the scientific method and relevant details on an atomic absorption spectrophotometer with graphite furnace, (b) have students get into small collaborative groups to read the article and answer questions 1- 3, (c) finish with students from each group responding to answers to questions 1- 3, and (d) detail any other procedural expectations for the laboratory such as journal requirements.

In meeting goals one and five, the author has included many experiments that deal with consumer products such as gasoline and whiskey. Students are generally more interested when the results of an experiment apply directly to something in every day life.

The experiments in this manual are based on articles from *Journal of Chemical Education, Analytical Chemistry,* and various other journals. The experiments sometimes have been altered in procedural detail, but more often altered to meet the goals of increasing levels of critical thinking with the incorporation of the scientific method. Not only are the experiments based on articles from the literature (especially *Journal of Chemical Education*), but all the experiments have been performed successfully either by the author or various senior chemistry majors, and six have been classroom field tested.

At the end of the selection of experiments is an instructor's guide. This lists all the equipment, instruments, and chemicals (including amounts) required for each experiment. It contains the full bibliographic reference for the articles upon which the procedure was based. Also included in some cases are comments that the author has found helpful in dealing with the experiment. Most of the experiments have been written to be completed in one three-hour laboratory period. This may vary if the class is extremely large. Depending on the level of student ability, the investigative labs may take four to six weeks. Most of the combination labs are one week labs; however, if the instructor feels that his/her class will take longer, the open-ended portion may be omitted.

Design for the Semester

The following design for the semester is suggested for the selection of experiments. The first laboratory session should include the scientific method experiment. Then, the next seven laboratory sessions should include one traditional experiment for each instrument, one open-ended (combination) experiment on two different instruments, and two experiments that teach special techniques such as internal standards, and standard addition. In the remaining six weeks of the semester, the student chooses a problem to investigate. This represents the investigative experiment or project. The student is allowed to pick their own problem or select one from the instructor's listing. Two weeks before the investigative experiment begins after an intensive literature search in the library, the students are required to seek approval from the instructor with a brief explanation of their experiment and a list of the materials required.

One week before the investigation begins, the students are required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, and bibliography from the library literature search. A photocopy of the literature that the experimental procedure is based on should be included to help in the professor's evaluation. For the combination (open-ended) and investigative experiments, no literature references should be given to the students by the instructor. Any included in the following selection of experiments and instructor's guide are in the dissertation for the purpose of appropriately crediting sources and are not meant to be available for students performing these experiments unless the student obtains the specific article in their literature search. At the end of the semester a formal report that is patterned after the scientific literature, such as *Analytical Chemistry*, is due.

TABLE OF CONTENTS

THE SCIENTIFIC METHOD

Required Reading:

1. Skoog, D. A.; West, D. M. ; Holler, F. J. *Analytical Chemistry An Introduction*, 6th ed.; Harcourt Brace College Publishers: Philadelphia, 1994; Chapters 1 and 4. (Any basic quantitative analysis text would be acceptable. The two chapters cover: steps in a quantitative analysis, sampling, replicates, calibration and measurements, and statistics and errors in chemical analysis.)

2. Liang, L.; D'Haese, P.C.; Lamberts, L.V.; Van de Vyver, F.L.; De Broe, M. E. *Anal. Chem.* **1991**, *63*, 423-427. Please bring a copy of this article to your first laboratory session.

Definition

Pasteur considered the obtaining of knowledge by organized observation to be fundamanetal to science (3). This method of gaining knowledge has become known as the scientific method. The scientific method has played the major role in technological change and knowledge acquisition in the past two centuries. Differing authors offer various steps in the scientific method. For the purpose of this selection of experiments, the following includes the major steps that will be used in the laboratory. The following material is not presented to promote the idea that there is a "formula" for scientific investigation or that one can simply follow a precise sequence of steps. Instead, these are the basic elements that can be found in modern scientific work.

Major Steps in the Scientific Method

All the major steps are listed from the analysis of reference 2.

I. Development and Statement of a Problem

Generally, no research or experiment is done until and unless some difficulty or idea is found in a practical or theoretical situation. It is the difficulty, problem, or idea that initiates the search for an answer. Therefore, if a problem is the occasion for inquiry, the solution of the problem is the goal and function of the inquiry. A basic characteristic of the problem must be that it can be investigated through the collection and analysis of

data (4). A well-written and thought-out problem statement can prevent the experiment from becoming too broad and guide subsequent statistical considerations. An example of the problem statement might be: The problem to be investigated in this study is the determination of gadolinium in biological material using graphite furnace atomic absorption spectrometry (GFAAS).

II. Gathering Related Information from the Literature and Other Sources

Note that the article begins with an introduction that includes a review of the related literature. At this point, the researcher may have a tentative hypothesis concerning the problem solution that will help guide him/her in the review of the literature. The review of the literature is very important. Its purpose is to determine what has already been done relating to the problem. The problem may have already been answered and the review will prevent duplication. It also gives the researcher insight into pitfalls to avoid and procedures and instruments that work well in a given situation. One of the many things the authors learned from the article's review was that the GFAAS determination of lanthanoids is prone to severe matrix interference. (The matrix in this case would be the biological materials that contain the Gd).

III. Formulation of the Hypothesis

The function of a hypothesis is to direct the search. A hypothesis is a tentative explanation or solution to the problem. In the absence of knowledge concerning a subject, no well-founded hypothesis can be formulated. The formal hypothesis is generally developed following the review of the related literature and prior to the execution of the experiment. The following are characteristics of a good hypothesis from Gay (4): (a) consistent with previous research, (b) provides a reasonable explanation, (c) states as clearly and concisely as possible the expected results and defines any variables in measurable terms, and (d) must be testable by collecting and analyzing data.

A possible formal hypothesis could be stated as: The use of tantalum boats in the GFAAS determination of Gd in biological material will improve the sensitivity of the determination substantially as compared to the use of pyrolytically coated graphite tubes.

IV. Development of the Experimental Procedure and Statistical Design

The first things to consider in this step are the factors that might influence the outcome of the experiment. In Skoog et al. (1) Chapter 1, the basic steps in a typical quantitative analysis are listed as follows: (a) selecting a method of analysis, (b) sampling, (c) preparing a sample, (d) defining replicate samples, (e) preparing solutions of the samples, (f) eliminating interferences, (g) calibration and measurement, (h) calculating results, and (i) evaluating results and estimating their reliability. Skoog et al. includes a thorough definition of each of these. For most experiments in this class, this

format will be the basis for our experimental procedure because instrumental analysis is in the field of analytical chemistry. Other areas of science may well use differing parameters to define the steps appropriate for their experimental procedure. This can also vary in the field of analytical chemistry depending on the actual statement of the problem and hypothesis.

Replicate samples are employed because they can be used to determine the precision of the experiment. For our purposes, most sample measurements will be reported as an average value (mean). The precision is generally reported as the standard deviation (1, pg. 63), and if the true value of the sample is known, the error is reported as relative error (1, pg. 55). Note from Skoog et al. (1) there are many other ways of reporting these. These are the minimum requirements for the experiments in this manual. A calibration curve from standard solutions is used in most experimental designs in analytical chemistry. The sample concentrations are read from these curves. Other factors that can be determined from the calibration curve will be listed below under statistical analysis.

Note in the article that the authors assessed the precision and accuracy of their method by adding the Gd-complex to several blank tissue samples. After normal digestion and extraction, they measured the analyte concentration against the working standard curve (calibration curve) and found recovery values (accuracy) ranging from 92.0% to 99.3% and relative standard deviations ranging from 2.4 % to 10.3 %.

V. Performance of the Experiment and Data Collection

In most chemical journals, the details of the method and data collection are spelled out in the experimental section. The following steps were taken in the article: (a) development of extraction technique of Gd from biological tissue; (b) processing of standard solutions containing absolute amounts of 0, 2, 4, and 8 micrograms of Gd as if they were samples; (c) optimization of several factors using the above standards (use of tantalum boats in graphite furnace, selection of optimum pyrolysis and atomization temperatures, optimum gas flow determination and determination of optimal injection volume; (d) refinement of the extraction technique so that the sample being analyzed would contain only Gd in a 0.15 M hydrochloric acid solution to prevent matrix interferences; (e) development of calibration curves with the Gd standards undergoing the whole process of sample preparation to allow the Gd in tissues to be measured using direct standardization.

VI. Statistical Analysis

This along with the next category, interpretation of results, is often included in the chemical literature under the title: results and discussion. In the analytical literature, some common things assessed other than accuracy and precision are: limit of detection

(LOD) sometimes called detection limit (DL), sensitivity, and dynamic range. These are collectively called figures of merit. A well-developed experiment will plan to include these values so that the validity of the technique can be determined. The LOD is usually calculated as either 2 or 3 times the standard deviation (SD) of the blank. The sensitivity is equal to the slope of the calibration curve. And, the dynamic range is the concentration range over which the analytical curve is linear or the calibration slope is constant. The technique would not then be considered valid for samples that are below the LOD. Most authorities consider it good practice to measure a series of standards of decreasing concentration down to concentrations below the LOD to confirm that the calibration curve near the LOD is linear and known.

In the article, the LOD, which they defined as 2 SD, was found to be 2060 pg or 2.060 ng. Their calibration curve is linear down to a point well below the amount of standard they put in their tissue samples for the assessment of the accuracy and precision of the method. However, they defined their LOD as a concentration (amount); therefore, it would be best to extend their calibration curve down to 2 ng or further to ensure that the curve is linear at the point where they are actually defining the LOD (the slope should also be measured nearer the zero point). Do you agree or disagree with the last statement? Support your conclusions.

VII. Interpretation of Results

In this section, the researchers discuss their findings after all the data has been analyzed and any statistical analysis or figures of merit calculated. Usually, the data at this point will either support or reject the hypothesis. Does the new technique work, or work better than older methods? In the current article, the method for the determination of Gd has been proven to be more sensitive than older methods. In this case, the hypothesis has been proven true.

VIII. Application and Formulation of New Hypothesis (Conclusions)

In most chemical literature an ending section will be included that applies the work to current situations and projects the work to novel situations and/or formulates a new hypothesis. In other words, are there new questions that need to be answered that arise from this current work? The article suggests that the new procedure might be tried on lanthanoids other than Gd.

Conclusion

The steps in the scientific method and the statistical methods used vary tremendously depending on the discipline of study. The examples used above are very limited specifically to the field of analytical chemistry. And, even in a single field, there are many variations. The examples chosen above are geared to the type of experiments

presented in the following selection of experiments. This laboratory exercise is considered to be a very brief introduction to the scientific method and research as deemed necessary to introduce this selection of experiments. In many cases, serendipity and human ingenuity are the most vital tools.

I. Questions

1. Restate the problem statement from Reference 2 in your own words.

2. List two other facts the authors in Reference 2 learned from the review of the literature in the introduction.

3. How do the authors in Reference 2 meet Skoog's et al. basic steps 1-6 in a typical quantitative analysis? For example a. selecting a method of analysis: GFAAS in a tantalum boat.

4. Obtain Reference 5 listed below. For this article, analyze all eight steps (I-VIII) of the scientific method discussed above and determine these from Reference 5 as was done for the article above. Or, pick an article of your choice from *Analytical Chemistry* on which to perform the above analysis. A copy of your article must be included with the answers to your questions.

II. Additional References

3. Black, Max R. *Critical Thinking*, 1st edition; Prentice-Hall, Inc.: Englewood Cliffs, N.J., 1946.

4. Gay, L. R. *Educational Research*, 3rd edition; Merrill Publishing Company: Columbus Ohio, 1987.

5. Preininger, Claudia; Klimant, Ingo; Wolfbeis, Otto S. *Anal. Chem.* **1994**, *66*, 1841-1846.

ULTRAVIOLET/VISIBLE SPECTROSCOPY

Required Reading:

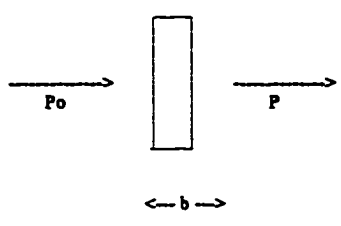
1. Skoog, D.A.; Leary, J. J. *Principles of Instrumental Analysis* , 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 7, "An Introduction to Molecular Ultraviolet/Visible Absorption Spectroscopy".

Introduction

Beer's Law

Spectroscopy is the science dealing with the interactions of matter and electromagnetic radiation (2). When irradiated with light, matter will selectively absorb incident light of some wavelengths but not of others. The wavelengths absorbed can be determined by exposing the matter to light of different wavelengths and obtaining a spectrum. The wavelength at which the absorbance is the highest (λ_{max}) is the wavelength usually used for analysis and is called the analytical wavelength.

Absorbance can be expressed by the following equation: $A = \log P_o/P$ where P_o represents the power of the beam directed at the solution and P is the power (intensity) of the beam transmitted by the solution. The pathlength of light or the cell size is represented by b. Sample concentration is represented by c.



The relationship between the three variables that influence the specific response of the solution is known as Beer's Law and is expressed as:

$$A = \epsilon bc$$

The absorptivity is a proportionality constant, the magnitude of which depends on the units for b and c. When c is expressed in moles per liter and b in centimeters, it is called the molar absorptivity and given the symbol, ϵ (1). The symbol, α, is used for absorptivity when the concentration is in grams per liter.

Operating a Spectrophotometer (Spectronic® 20)

An ultraviolet-visible (UV-Vis) spectrophotometer is the instrument that operates over the wavelength range typically including 185 to 780 nm. Either a scanning UV-Vis, or a Spectronic® 20 can be used when the analytical wavelength is in the visible range. The directions will be written for a Spectronic® 20, but can easily be adapted for a scanning instrument. The instructor will give specific directions for the scanning instrument.

A. Warming up the instrument.

Rotate the amplifier control knob (left-hand knob) clockwise. A red LED lights up on some models. Allow approximately 20 minutes warm up time to stabilize the source before taking readings.

B. Cleaning cuvettes.

Carefully wash and rinse all cuvettes with distilled water (or dichloromethane if using an organic solvent) and wipe with Kimwipes®. Then, rinse one of the cuvettes three times with the reference solution. In a second cuvette, do likewise with the solution to be measured. Do not handle the lower portion of the cuvettes because smudges will affect the light beam as it passes through the cuvette. It is good practice to wipe the cuvette with a Kimwipe® or lens paper before you place it in the instrument. For more detailed instructions of correct cuvette handling see Reference 3 (pp. 168-169).

C. Setting the wavelength

Turn the wavelength control knob (on the top of the instrument) until the appropriate wavelength setting appears on the scale at the top. Every time you change this setting to scan a new wavelength, you must reset the 0 % and 100 % transmittance as described below.

D. Setting 0 % Transmittance

With no cuvette in the holder and the lid closed, adjust the left hand knob so that the needle on the meter points to zero on the % transmittance scale.

E. Setting 100 % Transmittance

Turn the light control knob (right-hand knob) counterclockwise almost to its limit before inserting a cuvette into the sample holder. Insert the cuvette containing the reference solution into the sample holder. Match exactly the index line on the cuvette with the index line on the holder. Close the lid. Turn the right-hand knob clockwise until the needle points to 100 on the percent transmission scale. Immediately remove the cuvette. Remove the cuvettes with the sample solution as soon as the reading is taken to avoid fatiguing the phototube.

F. Taking the Readings

The instrument is now ready to take readings by simply inserting the cell containing the sample or the standard. Generally, use the absorbance scale for the measurements. However, higher absorbance measurements can not be read directly with accuracy. In these cases, read the % transmittance and convert to absorbance or dilute the sample.

Additional References

2. Ingle, J. D.; Crouch, Stanley R. *Spectrochemical Methods of Analysis*, 1st ed.; Prentice Hall: Englewood Cliffs, New Jersey, 1988.

3. Willard, H.H.; Merritt, L.L.; Dean, J.A.; Settle, F.A. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth Publishing Company: Belmont, California, 1988.

NOTE: If using a Spectronic® 20, it is strongly recommended that the instrument be converted to use square cells. The cost is minimal, and it is well worth the expense for experiments which require the simultaneous determination of several compounds because the pathlength is more reproducible.

EXPERIMENT 1: ULTRAVIOLET/VISIBLE

DETERMINATION OF IRON(II) AND TOTAL IRON WITH 1,10-PHENANTHROLINE

Required Reading:

1. Harvey, A. E.; Smart, J. A.; Amis, Edward S. *Anal. Chem.*, **1955**, *27*, 26-29.

Experimental*

The emphasis of this experiment is the simultaneous analysis of a two-component mixture. In some cases, there are two component systems [such as iron(II) and total iron] which do not react or interact with each other. The absorption of light by the components in a system such as this is additive. In other words, the total absorbance of the two-component system is the sum of the absorbances of the individual substances, just as if the substances had been measured separately under similar conditions in different containers and then added. In this experiment, the iron(II) and total iron can be measured, and then the iron(III) can be determined by difference. The two components have identical absorptivity at a particular wavelength (where total iron is measured), and the iron(II) has an absorbance maximum where there is little absorption by the iron(III) component. The following is modified from Harvey, Smart, and Amis (1).

I. Preparation of Solutions

Note: All dilutions to volume are made with distilled water.

A. 1,10-Phenanthroline: Prepare 300 mL of a 0.3 % solution. Boil and cool 350 mL of distilled water. Add 1 mL of 1,10-phenanthroline to 300 mL of the boiled, distilled water to obtain an approximately 0.3% reagent solution.

B. Buffer Solution: Make 150 mL of a 0.2 M solution of KHP, potassium biphthalate, to give a buffer of pH 3.98.

C. Standard Iron Solutions: Prepared by instructor.

* Abstracted with permission from Reference 1. Copyright 1955 American Chemical Society.

II. Determination of Analytical Wavelengths

NOTE: All measurements in the literature reference are given in ppm (parts per million) because of the age of the reference. Current usage is mg/L. Please use the more current format for your laboratory notebook.

Dilution of standard iron solutions (Make these solutions up one at a time. Finish taking absorbance measurements for one before making up the other): Pipet 1 mL of the iron(II) solution into a 100-mL volumetric flask. To this volumetric flask, add 20 mL buffer and 25 mL of 0.3 % 1,10-phenanthroline and dilute to volume. This will give a 10 mg/L solution of iron(II). Take absorbance measurements over the range of 380 to 600 nm at intervals of 5 nm. Remember to set the 0% and 100% transmittance at each wavelength. Follow the above procedure for the iron(III) standard solution. Graph wavelength versus absorbance for both complexes on the same graph and determine the appropriate analytical wavelengths from the graphs. If using a scanning instrument, include a copy of the spectrum in your laboratory notebook.

III. Standard Concentration Curves

NOTE: Be sure to perform this step only when you are ready to take readings because the solutions are stable for approximately 30 minutes.

Dilution of standard iron solutions: Follow the procedure as in Part II above and prepare a 10 mg/L dilution of iron(II). (If using a scanning instrument, it will be possible to use solutions from above unless there is a time lapse of over 30 minutes). From the iron(II) 10 mg/L dilution, prepare a 2, 4, 6, and 8 mg/L dilution. For example, add 2 mL of iron(II) complex to a 10-mL volumetric flask and dilute to volume to give the 2 mg/L solution of iron(II). Take absorbance readings of the above dilutions including the 10 mg/L dilution at the appropriate analytical wavelength determined in Part II.

Prepare a 10 mg/L dilution of iron(III) as in Part II above. Using combinations of the 10 mg/L iron(II) and iron(III) solutions, prepare a 2, 4, 6, 8, and 10 mg/L dilution of the iron(II) and iron(III) solutions to read total iron. For example, add 1 mL of iron(II) and 1 mL of iron(III) complexes to a 10-mL volumetric flask and dilute to volume to give the 2 mg/L dilution of the total iron solution. Take absorbance readings of the above dilutions including the 10 mg/L solution at the appropriate analytical wavelength determined in graphically in Reference 1 above (396 nm). For both sets of data, graph concentration versus absorbance. From these two graphs, the concentrations of your unknown total iron and iron(II) will be read. The iron(III) concentration will be determined by difference (see Reference 1).

IV. Analysis of Unknowns

Obtain an unknown sample from the instructor. Be sure to record its number in your laboratory notebook. It will contain a specific amount of both the iron(II) and iron(III). If grinding is required, it must be carried out under a nitrogen atmosphere. Weigh out a 300 mg sample and make up to volume in a 250-mL volumetric flask that contains approximately 100 mL of distilled water and 1 mL of concentrated sulfuric acid. From this point, samples must be analyzed immediately. Pipet three 1-mL samples into separate 25-mL volumetric flasks. Next, add 5 mL of the buffer solution and 10 mL of the 0.3% 1,10-phenanthroline solution to each. Dilute to volume with distilled water. Take absorbance readings for each solution at the two analytical wavelengths determined above. Use your graphs from Part III to determine the mg/L iron(II) and total iron from each of the three samples. Determine the iron(III) by difference (see Reference 1). Make a table of the results.

V. Reporting Results

A. Record all the results in your lab notebook which should include the following: title, reagents required, equipment required, brief summary of procedure, calculations, data (all graphs and tables indicated), interpretation of results with a statistical analysis of the unknown sample including mean and standard deviation, and answers to the questions below.

VI. Questions

1. Given the following two equations which represent a more specific form of the general equation for the simultaneous analysis of a two-component mixture, solve the equations simultaneously for C_1 and C_2.

$$A_1 = k_1C_1 + k_2C_2$$
$$A_2 = k_3C_1 + k_4C_2$$

(k values represent the molar absorbptivities.) Note these equations give the concentration directly.

2. Describe an alternative to the graphical (slope) method used in this experiment to calculate k using the results of question one above. (Remember $k = \epsilon$ if cell thickness equals 1 cm.)

3. Were there any absorbance readings above 1.0 in this experiment? If so, what type of error would this introduce into the experiment? Explain how you could correct for this.

VII. Safety Information

A. Concentrated sulfuric acid is corrosive, highly toxic, and a severe irritant. It requires use of appropriate goggles, clothing, gloves, and fume hood.

B. Ferrous ammonium sulfate hexahydrate and ferric ammonium sulfate dodecahydrate are eye, mucous membrane, and skin irritants. They require use of appropriate goggles, and general ventilation. Use appropriate gloves if expected to have repeated or prolonged skin contact.

C. KHP and phenanthroline are mild eye, skin, mucous membrane and respiratory track irritants. They require use of appropriate goggles, gloves, and general ventilation.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing this experiment.

EXPERIMENT 2: ULTRAVIOLET/VISIBLE

ANALYSIS OF THE IRON CONTENT OF SOAP

Experimental*

I. Preparation of Solutions

All dilutions are made to volume with distilled water.

A. A standard solution of 10 mg/L Fe(III). This is obtained from the instructor.

B. An 8 % NH_4SCN (ammonium thiocyanate) solution. The basic experiment requires 100 mL. For each additional type of soap analyzed add 10 mL. This is a student preparation.

II. Determination of Analytical Wavelength

NOTE: Add all chemicals in the order as listed below:

To a 100-mL volumetric flask, add 15 mL of the standard iron solution, 4 mL of concentrated nitric acid, 10 mL of 8% ammonium thiocyanate, and dilute to volume with distilled water. A blank that includes everything above except the standard iron(III) solution should be made and used as the reference. Determine the wavelength of maximum absorption by scanning from 400 to 600 nm by 5 nm increments. Be sure to reset the 0% and 100% transmittance every time the wavelength is changed. Graph your results in terms of wavelength versus absorbance. If using a scanning UV-Vis, include a copy of the spectrum in your report.

III. Standard Concentration Curve

In each of four 100-mL volumetric flasks add: 5, 10, 20, and 30 mL of the iron standard respectively; 4 mL of concentrated nitric acid; 10 mL of 8% ammonium thiocyanate and dilute to volume. Readings should be obtained at the analytical wavelength determined above within 30 minutes of mixing. Graph the results as mg/L iron(III) versus absorbance. Show all the necessary calculations.

* This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

IV. Sample Preparation

In an appropriate container dissolve 20 grams of ordinary white bar soap in 150 mL of hot distilled water. Add 15 mL of concentrated hydrochloric acid and heat until a second layer is obtained. Place the container in an ice bath until the clear layer solidifies. Decant and filter the liquid into a 250-mL volumetric flask. To the solid add 30 mL of hot distilled water and 1 mL of concentrated hydrochloric acid. Heat until boiling. Cool in ice, filter the aqueous layer into the same 250-mL volumetric flask, and dilute to volume. At this point the solution should be clear. If it is not, filter again to remove any soapy residue remaining in the solution. Take an 80-mL sample from this and place in a 100-mL volumetric flask. Add 4 mL of concentrated nitric acid, 10 mL of 8 % ammonium thiocyanate and dilute to volume. Take the reading at the analytical wavelength within 30 minutes. Calculate the mg/L of Fe(III) in the soap solution.

V. Combination (Open-Ended) Experiment

The soap used above was white. There is available for use three soaps of various colors and one of a different consistency. Would this have an effect on the results? Design your own experiment to determine this. It should include: statement of problem, hypothesis, and overall experimental design. It can be set-up to be done simultaneously with Part IV. After completion of the experiment, predict any new questions or problems that might arise and formulate a new problem statement and hypothesis accordingly.

VI. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, introduction which explains the importance and relevance of the experiment, summary of procedure (includes any unusual or noteworthy occurrences), calculations, equation for acidification of soap, data (all tables and graphs as indicated), and answers to the questions below.

B. Combination (Open-ended) Experiment: It should be written up in the laboratory notebook and include all details as listed in Part V above plus all required calculations and interpretation of results with any feasible statistical analyses. If other students are setting up similar experiments, pool results and run comparisons with standard statistical methods such as mean and standard deviation. Reference 2 listed at the end of the experiment has a chapter on statistical analysis. Also include all information asked in Part V above.

VII. Questions

1. Why is the soap sample acidified twice under procedure Part IV?

2. What is contained in the clear layer that separates and solidifies upon the addition of hydrochloric acid and cooling in Part IV? What is contained in the other layer?

3. Copper and cobalt are two other possible metal contaminants in this process. What are some possible complexing agents that could be used to give a species that could be read in the visible region? List sources for your answers (3-5).

VIII. Safety Information

A. NH_4SCN is an eye, skin, and mucous membrane irritant. It is stable under normal conditions, but may release extremely toxic HCN gas upon thermal decomposition. Requires use of appropriate goggles, clothing, gloves, and local exhaust or process enclosure ventiliation.

B. Hydrochloric acid is a very corrosive acid. It is an extreme irritant causing burns upon contact with skin, eyes, or by ingestion. It is moderately toxic by inhalation. Use of appropriate goggles, clothing, gloves, and fume hood is required.

C. HNO_3 is a very corrosive acid and an oxidizer. It is an extreme irritant causing burns upon contact with skin, eyes or by ingestion. Use of appropriate goggles, clothing, gloves, and fume hood is required.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing this experiment.

IX. Additional References

2. Skoog, D.A.; West, D. M.; Holler, F. J. *Fundamentals of Analytical Chemistry*, 6th ed., Saunders College Publishing, Philadelphia, 1992.

3. Hargis, L. G.; Howell, J. A. *Anal. Chem.*, **1984**, *56*, 225R.

4. Howell, J. A.; Hargis, L. G. *Anal. Chem.*, **1982**, *54*, 171R.

5. Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*. 4th ed.; Harcourt Brace College Publishers: New York, 1992; Appendix 1.

EXPERIMENT 3: ULTRAVIOLET/VISIBLE

ANALYSIS OF COPPER USING DIFFERENTIAL OR

EXPANDED-SCALE SPECTROSCOPY

Experimental Procedure for High Absorbance Method*

NOTE: Dilutions to volume are made with distilled water.

I. Preparation of Standards and Sample Solutions

Place approximately 1.5, 1.6, 1.7 and 1.8 grams of Cu standard (weighed accurately to 4 decimal places) in 250-mL beakers under the hood. Prepare the assigned unknown sample simultaneously to save time. The unknown sample will usually contain only a certain percentage copper. The instructor will give an approximate amount to weigh out for the unknown. Do a triplicate analysis of the unknown. To each of the 250-mL beakers of standard copper and unknown, add 20 mL of concentrated HNO_3. DO THIS UNDER THE HOOD! After the fuming has stopped and the sample is cooled, pour the solution into a 100-mL volumetric flask containing 50 mL of water very slowly, and dilute to volume. If the sample is turbid, trace amounts of Sn are indicated and the sample must be filtered before absorbance is read. This could be a source of error. (Save standard solutions until total completion of the experiment).

II. Preliminary Evaluation of Cuvettes

To achieve the high accuracy inherent in differential spectroscopy, careful technique is required in every step. Because the sample and reference (blank) cells will be slightly different, a correction factor must be evaluated and applied. Using the subscript s for the sample cuvette and the subscript r for the reference cuvette gives:

$$\frac{A_r}{A_s} = \frac{\epsilon_r}{\epsilon_s} \cdot \frac{b_r}{b_s} \cdot \frac{C_r}{C_s}$$

* Abstracted with permission from Reference 1 (See Instructor's Guide). Copyright 1949 American Chemical Society.

Placing an identical sample in both cuvettes gives $C_s = C_r$ and $\epsilon_s = \epsilon_r$. Therefore concentration and molar absorptivity cancel and:

$$\frac{A_r}{A_s} = \frac{b_r}{b_s} = \beta$$

To correct sample absorbance readings, one must then multiply the absorbance readings by β which is calculated by dividing the absorbance reading of the reference by that of the sample. From the above equation, it is clear that any difference in the absorbance readings of the sample and reference would be due to a difference in pathlength and the glass itself because of variations in cuvettes.

Set the wavelength at 620 nm. Be sure to align the cuvette properly each time using the line on the instrument to match that on the cuvette. Zero the instrument and then, using distilled water as a blank, set the 100.% transmittance. Empty the cuvette and add the 1.5 gram Cu standard solution. Take an absorbance reading. Next, take a different cuvette and place more of the 1.5 gram Cu solution in it and take an absorbance reading. Do this several times rapidly for both cuvettes and take an average value for each. These average values will be equal to A_s. Use the cuvette with the higher absorbance value as the sample cuvette throughout the remainder of the experiment. This means it must be emptied between readings. During the remainder of the experiment, use the cuvette that has the lower absorbance reading as the reference (or blank) cuvette. Take several readings of the reference solution in this cuvette and average your values. This will be equal to A_r. Calculate your β factor from these absorbance readings. (The β factor must be redetermined every laboratory period if using different cuvettes).

III. Analytical Determinations

Setting the analytical wavelength at 620 nm, read all your standards and samples against the 1.5 gram copper standard set at 100% transmittance (or as the reference in a scanning instrument), and place the data in an appropriate table. Correct all absorbance readings using β. Draw the standard calibration curve (grams copper versus absorbance) and obtain the amount of Cu in your unknown by using the calibration curve. Do not forget to account for using 1.5 grams as a zero point. Then calculate the percent of copper in your unknown sample.

IV. Combination (Open-Ended) Experiment

The above experiment was designed for high percentages of copper. Consider the situation in which there are only trace amounts of copper in a sample. Design your own experiment to determine trace amounts of copper in a solution by the differential method.

An unknown copper solution will be given to you by the instructor (between 0.002 to 0.01 M copper). Your design should include: overall experimental design (steps in procedure, etc.), statement of problem, formulation of hypothesis, and prediction of results.

V. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, introduction which explains the importance and relevance of the experiment (required reading will be helpful), summary of procedure (includes anything unusual or noteworthy), calculations, relevant equations, data (all tables and graphs as indicated), a statistical analysis on the triplicate sample including the mean and standard deviation, and answer to the question below.

B. Combination (open-ended) Experiment: It should be written up in the laboratory notebook and include all details as listed in Parts IV and V above plus all required calculations and interpretation of results with any feasible statistical analysis, and conclusions. The same unknown may be given to all students. Check with the instructor. If this is the case, report your answer, compare results with other students, and do a group statistical analysis which includes the mean and standard deviation. The reference listed at the end of the experiment has a chapter on statistical analysis (2).

VI. Question

1. Describe in detail the maximum-precision method and its experimental set-up.

VII. Safety Information

A. HNO_3 is a very corrosive acid and an oxidizer. It is an extreme irritant causing burns on contact with skin, eyes or ingestion. It requires the use of appropriate goggles, clothing, gloves, and use in a fume hood.

B. When concentrated nitric acid is added to the copper, toxic vapors may result. This part of the experiment must be performed under an appropriate hood.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing this experiment.

VIII. Additional References

2. Skoog, D.A.; West, D. M.; Holler, F. J. *Fundamentals of Analytical Chemistry*, 6th ed., Saunders College Publishing, Philadelphia, 1992.

3. Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Appendix 1.

EXPERIMENT 4: ULTRAVIOLET/VISIBLE

DETERMINATION OF THE FORMULA FOR AN IRON(III) SULFOSALICYLATE COMPLEX USING THE METHOD OF CONTINUOUS VARIATIONS

Required Reading:

1. Foley, R. T.; Anderson, R. C. *J. Am. Chem. Soc.* **1948**, *70*, 1195-1197.
2. Voshburgh, W. C.; Cooper, G. R. *J. Am. Chem. Soc.* **1941**, *63*, 437-442.

Introduction

$$A + mB \rightharpoonup AB_m$$

To determine the ratio in which two substances form a complex, that is to determine m in the complex formed from A and B, Job developed a method of mixing solutions of A and B in varying proportions and measuring a suitable property. Voshburgh and Cooper (2) state that when plotting the difference between each value measured and the value of the property versus the composition, the resulting curve will have a maximum (or minimum) that bears a simple relationship to m. This method of determining m has become known as the method of continuous variations (or the Job method).

Experimental*

I. Preparation of Solutions

A. 0.1 M perchloric acid (This will be prepared in advance by the instructor.)

B. 100 mL of 0.0100 M ferric nitrate diluted to volume in 0.1 M perchloric acid

C. 50 mL of 0.0100 M sulfosalicylic acid diluted to volume in 0.1 M perchloric acid

* Abstracted with permission from Reference 1. Copyright 1948 American Chemical Society.

II. Procedure

A. Sample Preparation

Load the ferric nitrate solution into a clean buret and deliver it from the buret to prepare five solutions containing the following: A = 2.00 mL, B = 4.00 mL, C = 6.00 mL, D = 8.00 mL, E = 9.00 mL of the 0.0100 M ferric nitrate solution diluted to 50 mL in a volumetric flask with the 0.1 M perchloric acid. Next, load a buret with the 0.0100 M sulfosalicylic acid solution and prepare 5 solutions that contain a combination of the 0.0100 M ferric nitrate solution and 0.0100 M sulfosalicylic acid solution in 50 mL volumetric flasks as follows: F = 8.00 mL sulfosalicylic acid + 2.00 mL ferric nitrate, G = 6.00 mL sulfosalicylic acid + 4.00 mL ferric nitrate, H = 4.00 mL sulfosalicylic acid + 6.00 mL ferric nitrate, I = 2.00 mL sulfosalicylic acid + 8.00 mL ferric nitrate, J = 1.00 mL sulfosalicylic acid + 9.00 mL ferric nitrate. Dilute each solution (F - J) to volume with 0.1 M perchloric acid. Be sure to mix well. All solutions should stand for one hour before the absorbance is read. For the ferric nitrate and sulfosalicylic acid solutions, the total number of moles added is constant.

B. Determination of Analytical Wavelength

Using solution B (40 mol % iron), measure the absorption spectrum over the range from 350 to 625 nm. Put the data obtained in Table I. Plot the absorbance versus the wavelength and from this determine the λ_{max} to use for the remainder of the analysis (Graph I). If using a scanning instrument, obtain a print-out of the spectrum and the data to place in the laboratory notebook.

C. Sample and Data Analysis

Using the wavelength (λ_{max}) determined above, read the absorbance measurements for the remainder of the solutions (A - J) and place the data obtained in Table II. Next, correct the absorbances of the complex formed (F-J) for free iron(III) by subtracting the absorbances of the corresponding concentrations of ferric nitrate solution (A-E). Construct Graph II for corrected absorbance versus mol % ligand (sulfosalicylic acid). When plugged into equation 1 below, the mole fraction at the point of intersection on Graph II gives X from which the formula (m) for the complex may be calculated. (Note Figure 1 from Reference 2). If your curve in Graph II is more rounded, it will require extrapolation to a triangular shape. See Reference 3 page 179, Figure 7.9 (f). The legs of the triangle are extrapolated until they cross. At this point, the formula for the complex is AB_m

$$m = \frac{X}{1 - X} \quad [Equation\ 1]$$

where X is the mole fraction of the ligand (sulfosalicylic acid) in the complex.
*NOTE: Please see Instructor's Guide.

III. Reporting Results

Report all results in your laboratory notebook which should include all the following: title, reagents required, equipment required, introduction which explains importance and relevance of the experiment, summary of procedure (which includes any unusual or noteworthy occurrences), calculations, equation for the complex formation, data (all tables and graphs as indicated), and answers to the questions below.

IV. Questions

1. Is the method of continuous variations applicable when more than one complex is formed? Why or why not (2)?.

2. If more than one complex is formed, what are the results dependent upon (2)?

3. Derive Equation 1 above (2).

V. Safety Information

A. Perchloric acid is a fuming, volatile unstable liquid that is a strong oxidizer. It is very corrosive and toxic if inhaled. It burns skin on exposure and can destroy the cornea. It can react explosively with combustibles and organic matter. Use local exhaust or process enclosure ventilation, gloves, safety goggles, and a face shield.

B. Sulfosalicylic acid is corrosive to the skin and eyes and toxic by ingestion. Use local exhaust and general dilution ventilation, gloves and goggles.

C. Ferric nitrate is corrosive to the skin and eyes and is moderately toxic by ingestion (severe cases can lead to death). It is irritating to the respiratory tract and can cause burns to skin and eyes. Use local exhaust and general dilution ventilation, gloves, and goggles.

This is not a comprehensive listing of information. It is required by law that MSDS be available to students before beginning this experiment.

VII. Additional References

3. Willard, H. H.; Merritt, L. L.; Dean J. A., Settle, F. A. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth Publishing Company: Belmont, CA., 1988; pp 179-181.

GAS CHROMATOGRAPHY

Required Reading:

1. Skoog, D. A.; Leary J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 25, "Gas Chromatography".

Introduction

In general terms, chromatography is a process in which a mixture can be separated into its components due to the differing rates at which the components migrate through a stationary phase under the influence of a mobile phase. There are two basic types of gas chromatography (the mobile phase in both consists of gas): gas-liquid chromatography (GLC) and gas-solid chromatography (GSC). When the term gas chromatography is used, it generally implies gas-liquid chromatography. In liquid chromatography, the mobile phase is a liquid while the stationary phase can be either a liquid adsorbed onto a solid, an organic species bonded to a solid surface, or a solid. Thin-layer chromatography is a type of planar chromatography in which the mobile phase is a liquid and the stationary phase is a layer of finely divided particles such as silica gel coated on a thin glass or plastic plate.

In GLC, the stationary phase is a liquid coated onto a solid supporting material. The sample is vaporized as it is injected onto the head of a chromatographic column and is carried through by the carrier gas to the detector. The column usually consists of a tube made of glass or metal that is filled with an inert support material which is coated with the liquid phase. Common liquids for columns are high molecular weight paraffins that will have varying -R groups to either increase or decrease the polarity. The general rule for choosing the liquid phase is "like dissolves like". If the sample is polar, the best separation generally occurs when using a polar column thus ensuring a reasonable residence time in the column. If the match between sample and column is good, the order of elution is determined by the boiling points of the eluents (1). The mixture emerges separated from the column as peaks at characteristic retention times: t_{r1}, t_{r2}, ... t_{rn}. The resulting recorded peaks are termed a chromatogram. The area under each peak is proportional to the quantity of material present.

There are several methods to determine the area under a peak if your instrument does not have an integrator. They are planimetry (precision error: 4.06%), height times width at half height (precision error: 2.58 %), cut and weigh (precision error: 1.74%), and triangulation (precision error: 4.06%) (2). For those instruments that do not have an integrator, the cut and weigh method is preferred unless otherwise specified (extremely

narrow peaks) (3). The procedure is as follows: make a photocopy of the chromatogram, cut out the peaks, weigh the peaks on an analytical balance, and immediately record the weight in your laboratory notebook next to the appropriate sample name. It is very important to make sure that you do not write either on the original chart or the copy on or near the area that you will be cutting out for the peak weights. Also, use extreme care in cutting. Be sure the baseline is determined in the same manner for both the standard and sample. This is illustrated in the required textbook reading above.

A quantitative chromatographic analysis can be done by preparing a series of standard solutions that approximate the composition of the unknown. Chromatograms of the standards are obtained and then concentration versus peak weight (or height, area) can be plotted. The unknown is then analyzed and concentration is determined from the standard calibration curve.

Operating a Gas Chromatograph

The following is a listing of the general parameters that must be determined for the set-up and shutdown of a gas chromatograph (GC). Specific directions will vary according to the instrument available and the detector type. The laboratory instructor will distribute a set of specific directions. In some cases, the instrument may be set-up in advance to allow time for the various oven temperatures to stabilize. (Time must be allowed for gas flow and temperature to stabilize before beginning an experiment).

1. Turn on the carrier gas (in many cases this is helium or nitrogen) and adjust the flow rate as specified in the experiment. (Recheck the flow rate after all the ovens have reached their final temperature and make adjustments as necessary). If a double column is used, make sure the flow rate is the same through both. NOTE: If carrier gas is not flowing through the detector, irreparable detector damage will occur to a thermal conductivity detector!

2. Turn on the main power switch, injection port heater, and column heater. (Adjust these to temperatures as indicated in your experiment).

3. For a TCD: Turn on the bridge current using the current control and adjust according to specifications in your experiment. If this is not specified, use 150 mA. For a FID: Set the fuel gases (usually hydrogen and air) as specified in the experiment for a flame ionization detector.

4. Turn on the recorder (or computer) and set the attenuator according to specifications.

5. Allow 30 minutes to one hour for warm-up (depending on the specific instrument). Some require much longer. Recheck the carrier gas flow rate and make the necessary adjustments. This can be confirmed with a bubble flow meter.

6. For a TCD: Inject 10 µl of air to make sure the peak is positive. If it is negative, reverse polarity of the recorder input terminals (for a thermal conductivity detector only). Some instruments may have a switch to reverse the polarity.

In shutting down the instrument, one should reverse the above process, turning off the carrier gas ONLY AFTER THE OVENS/HEATERS HAVE COOLED and the thermal conductivity detector current is turned off for a TCD detector!

Injection Technique

Syringes can be easily damaged and must be handled with extreme care. Clean and dry the syringe by flushing it with acetone and drying with Kimwipes®. The plunger should never be touched by fingers or it may freeze in the syringe. Rinse the syringe several times with the solvent, pull up approximately 1/4 to 1/2 µL of solvent, then pull in air bubbles followed by the exact amount of sample. Record this amount. Insert the needle into the septum while maintaining pressure on the end of the plunger. The sample should be injected all at once into the column and vaporized immediately. As soon as the sample has been injected, withdraw the needle from the septum. Be sure to flush the syringe several times with acetone and then several times with the next sample before injecting a new sample.

Additional References

2. McNair, H. M.; Bonelli, E. J. *Basic Gas Chromatography;* Varian Instrument Division Offices: Palo Alto, CA.,1968; p 158.

3. Pacer, R. A. *J. Chem. Educ.* **1976,** *53,* 592-593.

EXPERIMENT 5: GAS CHROMATOGRAPHY

ANALYSIS OF A HYDROCARBON MIXTURE

Required Reading:

1. Skoog, D. A.; Leary J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 25, "Gas Chromatography".

Experimental

The purpose of this experiment is to make a quantitative determination of the following hydrocarbons in an unknown solution: toluene, ethyl benzene, and p-xylene.

I. GC Parameters:
Column Temperature: 90^0 C
Injection Port Temperature: 160^0 C
Detector Temperature: 120^0C
Attenuator: 16 (dependent on individual instrument)
Carrier Gas Flow Rate (Helium): 60 mL/min (will vary with column diameter)
Sample Size: Approximately 5 μL
Chart Speed: 1 inch/min.
Detector: FID * : Air (600 ml/min); Hydrogen (30 ml/min)
Column: 3' X 1/4" o.d., packed with 80 - 100 mesh Chromsorb-P coated with 2.5% SE-30 *

* Instructor: Please see instructor's guide.

II. Preparation of Solutions

A. Standard Mixture: Into a 10-mL volumetric flask, weigh exactly 0.865 grams of toluene, 0.866 grams of *p*-xylene, and 0.867 grams of ethyl benzene (standard solution A). Dilute to the mark with methyl acetate and mix thoroughly. Do the same for: (B) 0.432 grams of toluene, 0.433 grams of *p*-xylene, and 0.434 of ethyl benzene; (C) 1.298 grams of toluene, 1.299 grams of *p*-xylene, and 1.300 grams of ethyl benzene.

III. Determination of Retention Times

Draw approximately 1 μL of methyl acetate and inject onto the column. For each injection make a mark on the chart paper at the moment of injection (0 time). Allow the

peak to elute before the next injection. Repeat for toluene, ethyl benzene, and *p*-xylene. The retention time is then measured at the maximum of the eluted peak. Label each chromatogram appropriately, avoiding writing on or near the peaks. Rinse the syringe several times between injections to avoid contamination.

IV. Standard Calibration Curve

Inject an accurately known volume (approximately 5.0 μL) of each standard solution. Allow all peaks to be eluted. Repeat the procedure twice for each standard. If the sample size varies slightly, be sure to record the exact sample volume used. Do this for each standard mixture. Tabulate the data, calculate average peak area, and prepare a standard curve (concentration versus peak weight or area) for each standard.

V. Analysis of Unknowns

Obtain an unknown from the instructor. Make it up to volume in a 10.00 mL volumetric flask with methyl acetate. Make three separate injections of the unknown. Determine the concentration of the diluted unknown from your standard curves. The unknown can contain one, two or all of the hydrocarbon components.

VI. Reporting Results

Record all results in the laboratory notebook which should include the following: either a copy or the original of all chromatograms, title, reagents required, equipment required, brief summary of the procedure, calculations, data (all graphs and tables as indicated), and interpretation of results (to include identity and quantity of all peaks in the unknowns). Be sure to include a statistical analysis since samples are done in triplicate (mean and standard deviation). The included chromatograms should contain the following information: name, date, labeled and calibrated time axis, sample, sample size, column type, flow rate, injection port temperature, column temperature, detector temperature, and attenuation.

VII. Questions

1. Why was the injection port temperature set much higher than the column temperature?

2. How could the elution time of a nonretained sample such as air be used to help in the calculation of retention times to make them more applicable in differing conditions?

3. From the type of compounds used in this experiment, what type of liquid phase should be used?

VIII. Safety Information

A. Toluene is a highly flammable fire and explosion hazard. It is a severe irritant. It is toxic by inhalation, skin contact, or ingestion. Use only in adequately ventilated hood and with appropriate goggles, gloves, and clothing.

B. Xylene is a fire and explosion hazard. It is an irritant by inhalation or upon contact with eyes and skin. Local exhaust or general ventilation is required with appropriate goggles, gloves, and clothing.

C. Ethylbenzene is a flammable irritant. Local exhaust or general ventilation is required with appropriate goggles, gloves, and clothing.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing this experiment.

IX. Additional References

2. Pacer, R. A. *J. Chem. Educ.* **1976**, *53*, 592-593.
3. Hanrahan, E. S. *J. Chem. Educ.* **1966**, *43*, 321-322.
4. Zlatkis, A.; Ling, Su-Yu; Kaufman, H. R. *Anal. Chem.* **1959**, *31*, 945-947.

EXPERIMENT 6: GAS CHROMATOGRAPHY

ANALYSIS OF GASOLINE BY GAS CHROMATOGRAPHY

Gasoline is composed of a complex mixture of aromatic and straight and branched chained aliphatic hydrocarbons. The initial portion of this experiment is to qualitatively identify some of the common hydrocarbons comprising gasoline using a temperature programmed sequence. The remainder of the experiment consists of a combination (open-ended) experiment to determine the amount of a popular blending agent in gasoline.

Experimental*

I. GC Parameters
Carrier Gas Flow Rate (Helium): 45 mL/min
Sample Size: 1μL
Injection Port Temperature: 190° C
Column Temperature: 3.0 minutes at 50°C rising to 150°C at 10° per minute
Detector Temperature: 190°C
Chart Speed: 1 inch/min
Attenuator: 8 (Depends on the instrument)
Detector: FID ** : Air (600 mL/min); Hydrogen (30 mL/min)
Column:** 2 meters X 1/8" packed with 10% Carbowax 20 M on 80/100 mesh Chromosorb W solid support

** Instructor: Please see instructors guide for additional options. This column is to be used for the basic and combination experiment. If performing the basic experiment only, a nonpolar column may be substituted.

II. Sample Analysis

Obtain a gasoline sample from the instructor. Inject 1μL sample. Mark the injection point on the chart paper. Do not inject another sample until the completion of the temperature program and the temperature is stabilized at 50°C. Number the individual peaks. Add one drop of a known component of gasoline (see table below) to a 2 mL sample of gasoline. Mix and inject 1 μL into the GC. Identify and label the peak with increased relative size. Repeat the procedure with the number of components the

*Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

instructor has available. The run time is approximately 15 minutes per sample. Typically, students will need to work in groups. The instructor may assign 3 or 4 hydrocarbons to each student group and let the class pool these results. Construct a table that includes peak number and compound name (from the table below) that corresponds to that number with a t_r (retention time) for each peak.

Typical Hydrocarbons in Gasoline

isopentane	*n*-octane
n-pentane	ethylbenzene
2-methylpentane	toluene
n-hexane	*o*-xylene
n-heptane	*p*-cymene
n-nonane	durene
2,2 -dimethylhexane	methylcyclohexane

III. Combination (open-ended) Experiment

The addition of oxygen-containing organic compounds such as alcohols as additives to gasolines improves their octane ratings. Design your own experiment to identify and quantitatively determine the amount of ethanol plus three additional oxygenates in a gasoline sample. It should include: statement of problem, hypothesis, prediction of results, overall experimental design, and conclusions. The precision of the method should also be determined.

IV. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences), calculations, tables as indicated, chromatograms appropriately labeled, and answers to the questions below.

B. Combination (open-ended) Experiment: It should be written up in the laboratory notebook and include all details as listed in Parts III and IV above plus an introduction which explains the importance and relevance of results, standard graphs for oxygenates, chromatograms appropriately labeled, and interpretation of results (with statistical analysis).

V. Questions

1. Why was a temperature-programmed sequence required for the experiment?

2. What factors might interfere with the quantitation of the oxygenates in gasoline? How could these be controlled?

VI. Safety Information

A. Gasoline and its components are highly flammable are and skin, eye, and inhalation irritants. They are also toxic upon ingestion and narcotic on inhalation. Use of appropriate goggles, gloves, clothing, and local exhaust ventilation are required.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing the experiment.

EXPERIMENT 7: GAS CHROMATOGRAPHY

ANALYSIS OF FATTY ACID COMPOSITION OF COMMON FATS AND OILS

Experimental *

I. GC Parameters:
Carrier Gas Flow Rate (Helium): 90 mL/min
Sample Size: 2-3 μL range
Injection Port Temperature: 228°C
Column Temperature: 190°C
Detector Temperature: 200°C
Detector Type: FID** : Air (600 ml/min); Hydrogen (30 ml/min)
Column Type:** 10' X 1/4" packed with 60/80 mesh Chromosorb W coated with 15% DEGS (bisethyleneglycolsuccinate polyester)

**Instructors: Please see the instructor's guide for additional options.

II. Standard Identification and Quantitation (Preliminary Exercise)

Obtain the mixtures that consist of known fatty acid methyl esters from your instructor. There will be two sets which will be injected separately, a saturated and unsaturated series. Inject a 3 μL sample of each series separately into the GC. Be sure to record the initial injection point and other GC parameters. If the known mixtures contain methyl heptadecanoate, use it as an internal standard and report relative retention times (divide the retention time of the ester peak by the retention time of the methyl heptadecanoate). If the relative retention time is not used, correct for the time it takes an unretained species to reach the detector (use air for a TCD and methane for an FID as the reference). From the data obtained, prepare a plot of the relative retention time values of the saturated methyl esters versus their carbon numbers. This may be done using semilog paper or a spreadsheet and graphing software program. The equivalent chain length (ECL) values for the unsaturated methyl esters can then be determined as follows: (a) Plot the value obtained for the relative retention for the unsaturated methyl esters on the graph prepared for the saturated methyl esters. (b) The ECL value for the unsaturated ester can be read from the X-axis. Devise a table containing the fatty acid methyl esters (both saturated and unsaturated), relative retention time, and the ECL for the unsaturated esters.

*Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

III. Investigative

In much of the nutritional literature, the benefits of unsaturated and monounsaturated oils for many nutritionally related diseases are stressed. Design an experiment to measure and compare saturated versus unsaturated fatty acid content for three common fats and oils. In designing the experiment, consider the structural differences between a fatty acid methyl ester and a fat or oil.

V. Reporting Results

Investigative experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, chromatograms labeled appropriately, summary of procedure (include any unusual or noteworthy occurrences) with the overall experimental design for the investigative portion, calculations, tables, graphs, and other details as indicated.

Two weeks before the investigative experiment begins, the student is required to seek approval from the instructor with a brief explanation of the experiment and a list of the materials required after an intensive literature search in the library. One week before the investigation begins, the students are required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, safety information on all chemicals from the MSDS, and bibliography from a thorough library literature search. A photocopy of the literature upon which the experimental procedure is based upon should be included to help in the professor's evaluation. At the end of the semester a formal typewritten report that is patterned after the scientific literature, such as *Analytical Chemistry*, is due. Also include the answers to the questions below.

V. Questions

1. Why are unsaturated and monounsaturated fatty acids considered nutritionally better for human consumption than saturated? This should be explained in relationship to the difference in chemical structure between them.

2. What is the significance of having the methyl heptadecanoate as an internal standard in part II?

EXPERIMENT 8: GAS CHROMATOGRAPHY

DETERMINATION OF CONGENERS IN WHISKEY USING THE INTERNAL STANDARD METHOD

Experimental*

The purpose of this experiment is to make a quantitative determination of 5 common natural ingredients in whiskey using the internal standard technique.

I. GC Parameters:
Column Temperature: 60 to 100°C @ 8°C/min
Injection Port Temperature: 200°C
Detector Temperature: 200°C
Attenuator: 10 X 32 (Varies according to instrument)
Carrier Gas Flow Rate (Helium): 30 mL/min
Sample Size: 1 μL
Chart Speed: 1 cm/min
Detector: FID: Air (600 mL/min); Hydrogen (30 mL/min)
Column:** 5% Carbowax 20M on 80/120 Carbopak B (2 m X 2 mm)

**Instructor: Please see instructor's guide for additional options.

II. Preparation of Solutions

A. Ethanol/water mixture: Dilute 80 mL of ethanol to volume in a 200-mL flask with distilled water to approximate a whiskey matrix (40% or 80 proof).

B. Whiskey Sample: In a clean 200-mL volumetric flask, place 100 mL of the whiskey sample supplied by your instructor. Add 100 μL of 1-butanol as the internal standard, dilute to volume with more of the whiskey sample, and mix thoroughly.

C. Standard Solution: In a clean 200-mL volumetric flask, place approximately 100 mL of the prepared ethanol/water mixture and add 100 μL of each of the following:

* Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

1-butanol (internal standard), ethyl acetate, 1-propranol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, and 3-methyl-1-butanol. Dilute to volume with more of the ethanol/water mixture and mix thoroughly.

III. Standard and Sample Analysis

Make duplicate 1 μL injections of each of the following: ethyl acetate, 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethanol. Determine the retention times of each of these components. Next, make triplicate 1 μL injections of the standard solution and the whiskey sample. The average peak areas of each component obtained from the standard solution and whiskey sample will be used to calculate the response factors.

IV. Data Analysis

Place all data obtained into appropriate tables. Be sure to retain the original chromatograms (or a photocopy). From either the peak areas (or heights if necessary), calculate the response factors for each component using the 1-butanol as the internal standard. Using the response factors, calculate the ppm (v/v%) of each of the five components found in the whiskey. When using multiple injections calculate this as an average with the standard deviation. Place the ppm data obtained in an appropriate table. Use the equations below for calculations:

Response Factor (F)

$$F_c = \frac{(A/C)_c}{(A/C)_{is}}$$

$(A/C)_c$ is the area and concentration for a given component.
$(A/C)_{is}$ is the area and concentration for 1-butanol.
Note that $C_c = C_{is} = 500$ ppm for the standard.

ppm Concentration of Components (C_c)

$$C_c = \frac{C_{is} X A_c}{F_C X A_{is}}$$

A_c and A_{is} are the areas of the compound and internal standard. C_{is} is the concentration of the internal standard (500 ppm). F_c is the response factor for the compound.

Obtain an appropriate literature reference and compare your results. Include a copy of the reference used with your laboratory notebook.

V. Combination (open-ended) Experiment

There are other common congeners found in whiskey. Design your own experiment to identify and quantitatively determine two additional congeners in whiskey. The samples will be supplied by the instructor. Compare various brands of American and Scotch whiskeys. This should include: statement of problem, hypothesis, prediction of results, overall experimental design, conclusions, and precision of the method.

VI. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences), calculations, tables as indicated, chromatograms appropriately labeled, and answers to the questions below.

B. Combination (Open-ended) Experiment: It should be written up in the laboratory notebook and include all details as listed in V above plus an introduction which explains the importance and relevance of results, chromatograms appropriately labeled, and interpretation of results (with statistical analysis).

VII. Questions

1. Explain in detail the internal standard method. When is its use indicated? How does its use improve data analysis? (1)

2. List at least three problems previously encountered in the analysis of components of whiskeys from appropriate literature references. Be sure to cite the complete reference for each source listed.

VIII. Safety Information

A. Ethyl acetate is an irritant to skin, nose and throat with narcotic action. Prevent skin and eye contact. Wear appropriate gloves, goggles, and clothing. Use local exhaust or general ventilation.

B. Ethanol is an irritant to eyes, skin and nose. It has a narcotic action which can cause liver damage. Prevent skin and eye contact. Wear appropriate gloves, goggles, and clothing. Use local exhaust or general ventilation.

C. Methanol is an extreme irritant to eyes, skin and nose. Toxic upon ingestion and flammable. Can cause mucous membrane burns and dermatitis. Prevent skin and eye contact. Wear appropriate gloves, goggles and clothing. Use local exhaust or general ventilation.

D. 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, and 3-methyl-1-butanol are all flammable liquids that are irritants to eyes, skin, and nose and are toxic upon ingestion. Prevent eye and skin contact. Wear appropriate gloves, goggles and clothing. Use local exhaust or general ventilation.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing this experiment.

IX. Additional References

2. Skoog, D.A.; Leary, J.J. *Principles of Instrumental Analysis*, 4th ed.; Saunders College Publishing: New York, 1992; pp. 600-601.

EXPERIMENT 9: GAS CHROMATOGRAPHY

DETERMINATION OF METHYL SALICYLATE IN RUBBING ALCOHOL USING THE METHOD OF STANDARD ADDITION

Required Reading:

2. Skoog, D. A.; Leary J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; pp 162-164.

Experimental*

The purpose of this experiment is to make a quantitative determination of methyl salicylate in rubbing alcohol using the important quantitative technique of standard addition.

I. GC Parameters:
Column Temperature: 190° C
Injector Port Temperature: 280° C
Detector Temperature: 230° C
Attenuator: 8 (Varies according to instrument)
Carrier Gas Flow Rate (Helium): 60 mL/min
Sample Size: 2 μL
Chart Speed: 2 cm/min
Detector: FID: Air (600 mL/min); Hydrogen (30 mL/min)
Column:** 4 ft X 1/4 in. 15 % Carbowax 20M on Chromsorb P 80/100 mesh column

**Instructor: Please see instructor's guide for additional options.

II. Preparation of Solutions

Each student (or group of students) should analyze one commercial wintergreen rubbing alcohol and one unknown prepared alcohol. For each solution analyzed, pipet 20.0 mL of the commercial alcohol (or unknown solution) into four separate 25-mL volumetric flasks. Pipet 0.20, 0.30, and 0.50 mL of methyl salicylate respectively into three of the four flasks in each determination. Dilute all flasks to volume with solvent grade isopropyl alcohol and mix well.

* Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

III. Procedure

Inject 2 μL samples of each of the solutions prepared into the GC. Be sure to record all parameters. If time permits, each analysis may be repeated one or more times, and the values averaged for better precision. Initially, the recorder pen will go off scale due to the large alcohol/water peak. The methyl salicylate peak will appear afterwards. Construct a standard addition graph using peak height versus concentration of the added methyl salicylate. See the equation below to determine volume % of added ester. The resulting straight line graph can be extrapolated to its y-intercept and the concentration of the ester in the unknown or commercial product determined.

$$Volume\ \% = \frac{mL\ ester\ added}{25.0\ mL\ solution} X\ 100$$

IV. Combination (Open-ended) Experiment

Methyl salicylate is also a common component in fragrances and after shave lotions. Design an experiment to identify and quantitate an over the counter brand of after shave or fragrance that contains methyl salicylate (or another essential oil of preference). Students should obtain the product(s) for sampling. If another essential oil has been selected for analysis, the instructor must be notified at least one week in advance to ensure a standard is available. The laboratory notebook should contain statement of the problem, hypothesis, prediction of results, experimental design, bibliography (if necessary), conclusions, and precision of the method.

V. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences), calculations, chromatograms appropriately labeled, and mean and standard deviation of triplicate analysis. If different students are analyzing various commercial products, results can be shared and pooled. In that case, construct a table that compares the amount of methyl salicylate in the various brands. Include answers to the questions below.

B. Combination (Open-ended) Experiment: It should be written up the in laboratory notebook and include all the details as listed in Part VA above plus an introduction which explains the importance and relevance of results as detailed in Part IV.

V. Questions

1. List some examples of error in the experiment that would have prevented the standard addition graph from being linear.

VI. Safety Information

A. Isopropyl alcohol is a volatile liquid that is harmful if inhaled, and can cause severe irritation on contact with skin or if swallowed. Use appropriate gloves, and goggles and use only in a chemical fume hood.

B. Methyl salicylate is harmful if swallowed, inhaled or aborbed through the skin. Vapor or mist is irritating to the eyes, mucous membranes, and upper respiratory tract. It can also cause skin irritation. Use appropriate gloves and goggles. Use adequate ventilation so as not to exceed published exposure limits.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be available to students before performing the experiment.

VII. Additional Reference

3. Willard, H. H.; Merritt, L. L.; Dean J. A.; Settle, F. A. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth Publishing Company: Belmont, CA., 1988; pp. 33-34.

INFRARED SPECTROSCOPY

Required Reading:

1. Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 12, "Infrared Absorption Spectroscopy".

Introduction

The infrared region covers the range 12,800 cm^{-1} to 10 cm^{-1}; however, the mid region, 4000 cm^{-1} to 400 cm^{-1}, is most frequently studied. By far the most widespread usage is in the identification of organic unknowns in the unique "fingerprint" region 1300 cm^{-1} to 650 cm^{-1}.

When a molecule absorbs infrared radiation, it undergoes several types of fundamental molecular vibrations such as stretching, bending, and twisting resulting in the peaks of a spectrum. Each vibration is associated with a characteristic frequency. For example, the C-H stretch of the aldehyde group -CHO occurs around 2720 cm^{-1}. Infrared spectra can be used to give information on the functional groups in a molecule as well as the molecular structure as a whole.

As mentioned above, infrared techniques are used in qualitative analysis for specific chemical substances. The infrared spectrum is one of the specific molecular properties known for a compound. This is true because the vibrational frequencies of a molecule depend on the weight, number, and geometrical arrangement of the atoms along with the force constant of the interatomic bonds. However, in the spectra of certain compounds such as nonane and decane, the added methylene groups do not add any bands that are not already present nor do they change the geometry of the molecule thus making identification by infrared alone difficult. Therefore if two compounds have identical infrared spectra they can be considered to be identical within certain limitations. When reporting the synthesis of a new compound in the literature, several instrumental methods are usually used along with the infrared spectrum to elucidate the structure of the compound.

Quantitative analysis can also be carried out on compounds using an infrared spectrum. Theoretically, Beer's Law applies in this region also. It does have some limitations in comparison to the ultraviolet range. Several instrumental limitations are as follows: limited output by source and detector resulting in the usage of a large slit width and wide spectral bandwidth, absorbance is an integrated response over the range of absorbance expected using a number of points to define the curve across the wavelengths

passed, the natural widths of the absorbance peaks are relatively narrow, and the presence of large amounts of stray radiation. Therefore, calibration curves must be prepared over the range of absorbances expected using a number of points to define the curve. Experimentally, problems can occur if the pathlength is not fixed. This can be overcome by using a fixed pathlength cell or the internal standard method. The required reading assignment above covers all the necessary background information to have the thorough understanding necessary to complete the experiments in this section successfully.

Infrared Sample Technique and Preparation

Liquid Sample Cells

A liquid sample is often placed between two transparent windows in a cell mount (consisting of a cell body and cell cap). Covalently bonded materials (including glass) are not suitable for window material because they absorb in the infrared region. The material used must be transparent in the infrared region; therefore, ionic materials such as NaCl, KBr, and AgCl are used. The commonly used window materials are described below.

1. NaCl. This is the most common material. It is the cheapest and it works. The disadvantage is that it is hygroscopic. It will pick up moisture from the air and dissolve. The relative humidity at which it is kept must be below 50% so storage generally occurs in a desiccator.

2. KBr. It is hygroscopic and must be stored in a desiccator. It is soft and can be easily compressed to make windows. Both NaCl and KBr are available for fixed pathlength cells for quantitiative work although these are more expensive than the regular cells.

3. AgCl. This is not hygroscopic but it is photosensitive. It turns black in ultraviolet or visible light. Silver chloride windows are generally stored in a black felt box and are relatively expensive.

4. KRS-5. This is a mixture of thallium iodide and thallium bromide. It is very expensive and toxic. It is not hygroscopic or photosensitive, and is generally used in reflectance methods.

5. CsI. This is expensive and hygroscopic, but its advantage is that is won't crack at low temperatures. Therefore, it is generally used when low temperature methods are necessary. It also gives acess to longer wavelength ranges than KBr or NaCl.

Cells must be handled with care because of their composition. First, never allow water to come into contact with the cells. All hygrosocopic cells must be washed with a

solvent such as dicloromethane or chloroform, dried with a slow flow of nitrogen or a Kimwipe®, and immediately returned to a dessicator. (Note that both of these solvents are toxic and must be used according to specifications on MSDS). Cells should be handled with solvent resistant gloves and kept in the constant humidity instrumental room. If it is suspected that the material to be analyzed has any moisture content, AgCl windows should be used. AgCl cells must be handled carefully because they bend easily and should be returned to their containers immediately after use.

Sample preparation techniques can be divided into categories depending on the state of the material such as solid, liquid, or gas. The major techniques for solids and liquids will be reviewed below.

Liquids

A. Neat. This consists of running 100% straight sample in a thin cell of approximately 0.01 cm (or less) thickness. NaCl windows can be used with and without spacers. For NaCl, perfect thickness can be achieved by using a spacer between the windows (or using cells of fixed pathlength), but if the application is qualitative this is generally omitted and sample thickness is estimated. AgCl windows are beveled so that a spacer is not necessary for correct thickness. AgCl windows can be placed so that both beveled sides are facing each other or so that one beveled side is facing a flat side resulting in different path lengths. To use a spacer, place one window in the cell body and place the spacer on the window. Put several drops of the liquid sample on the window to fill the spacer area using a clean dry syringe or a microcapillary pipet. Place one edge of a second window on an edge of the bottom window, holding the second window at a slant with respect to the bottom window. Then lower the raised edge of the second window until it contacts the entire surface of the bottom window. Screw the cell cap firmly onto the cell body.

B. Solutions. These are handled in the same manner as pure liquids except a thicker sample must be used resulting in a longer pathlength. Larger spacers or larger cells of fixed pathlength can be obtained for these. There are cells designed especially for solutions that contain a space permanently sealed between two windows to prevent leakage. Leur-lock fittings are provided for filling the cell with a syringe. Unfortunately, there is no nonabsorbing solvent in the infrared so extra peaks will be obtained. The best solvents are nonpolar, nonhydrogen liquids such as CS_2 or CCl_4.

C. 3 M Cards. These are cards with a piece of polymer stretched across a hole in the card which is designed to fit within the cell holder of the instrument. A drop or two of liquid is placed on the polymeric material and is absorbed into the polymer. These are used with FTIR.

II. Solids

A. Thin film. Samples can be either cut with a microtome (polymers), melted and allowed to dry as a film or evaporated from solution. For example, a solid sample could be dissolved in acetone or other solvent and evaporated on a AgCl cell. This would leave a thin layer of the sample on the window.

B. Mulls. The sample is suspended in a thick liquid such as mineral oil. Generally the sample is ground with the mulling agent in a mortar and pestle. A mixture of about 10:1 (mineral oil to sample) is used, and the mixture is ground to a fine paste. If the sample is very fine-grained and quite soft, it can be placed on the face of a window and one or two drops of the oil added. Rub and distribute the mixture with the second window. However, if the solid is coarse or hard, it will be necessary to pre-grind the sample. After grinding in a mortar and pestle, mix in the mineral oil and regrind to a paste (similar to vaseline) and place on a suitable window. A disadvantage of this method is the difficulty in controlling cell thickness and the spectral contribution of the mineral oil. Other agents available that do not give spectra are hexachlorobutadiene and perfluorocarbon oil.

C. KBr Pellet. Solid samples can be mixed with KBr and pressed into a disk or pellet. Conventionally dried infrared-quality potassium bromide is used in pellet formation since it requires no grinding and provides a uniform matrix for the sample. Thoroughly grind the sample (0.5 to 1.0 mg) in an agate mortar and pestle. Add approximately 100 mg of the KBr powder, and mix until uniform. Do not grind the KBr because of moisture absorption. To form the pellet, place one bolt in the barrel of the mini-press and rotate it approximately five turns. Deposit 50 to 100 mg of the sample-matrix mixture on the polished surface of the bolt inside the barrel. Tap the press gently to spread the mixture uniformly over the bolt. Insert the second bolt in the barrel and rotate it until it is fingertight. Using two wrenches, gradually exert pressure on each bolt. This may also be done with a bench vise and a wrench. Apply pressure for about one minute. Use a wrench to loosen one of the bolts. Remove both bolts. The pellet inside will appear more or less translucent. The ability to obtain a translucent pellet depends in part on the amount of the sample, the presence of moisture, and the nature of the sample. Several attempts may be necessary to obtain a good pellet. The barrel containing the pellet can be mounted directly in the instrument on the cell holder. When finished, the barrel and bolts are first washed with water and then acetone to remove the KBr.

D. 3 M Cards. A solution of the sample in a volatile solvent is placed upon the card and the solvent is allowed to evaporate leaving the solid residue on the polymeric surface.

E. Reflectance. This is used for polymeric materials or fibers and papers that won't dissolve or grind easily. It is generally used as a last resort. It requires a special sample holder attachment to which the sample as a whole solid can be attached.

F. FTIR- microscope attachment. In some labs, a microscope has been added to the FTIR to analyze extremely small samples. In this case, the small sample is rolled to an extremely thin film so that the light can pass directly through the sample rather than use reflectance. Results from this are generally much better than the reflectance method.

Instrumentation

The following procedure includes directions for using a double beam spectrophotometer such as the Perkin-Elmer 710B. This may vary slightly between instruments, but will generally be similar.

A. Install chart paper.

With the carriage at the center, press open the clamp and slip the pad under the pen carriage and under the clamp. Align the 2000 cm^{-1} mark on the top sheet with the 2000 cm^{-1} mark on the frequency scale on the top of the instrument above the chart paper. Be sure the bottom of the paper is flush against the lip on the bottom of the carriage. The drive mechanism is delicate. Always make sure SCAN is off before moving the carriage slowly and deliberately. Push carriage back to the right before beginning the scan. If the instrument is computer controlled or has other type of print-out simply turn this on.

B. Install Pen

Screw pen into pen holder. Do not leave the pen in prolonged contact with the chart paper due to seepage. Also, be sure to cap the pen when it is no longer in use to prevent drying out.

C. Set Gain

1. Press the POWER button to turn on the instrument. Allow ten minutes to warm up.

2. With nothing in either beam, move the carriage (SCAN light off) to the 3000 cm^{-1} position. Use the 100% control to set the pen at 90% transmission.

3. Press the Gain Auto-Check Knob. The pen should move down scale 10% (+ or - 1 %). If the pen moves either lower or higher than this, adjust the gain knob. The knob must be depressed while adjustments are being made. (Clockwise rotation if the gain is greater than 11% or counterclockwise rotation if the gain is less than 9%.)

D. Set 100 % Transmission

With nothing in either beam, set the pen at 100% transmission with the 100% control.

E. Record a Spectrum

1. Insert the polystyrene test film card in the sample beam cell holder.

2. With the SCAN light off, move the carriage to the extreme right (4000 cm^{1}).

3. Choose either the instrument NORMAL or FAST scan speed.

4. Press the SCAN button. The carriage will move to the left as the pen records the sample transmission on the chart. At the end of the frequency range, the pen is lifted automatically from the chart paper, the instrument stops scanning, and the SCAN light goes off. Repeat procedure E with other samples to be analyzed.

EXPERIMENT 10: INFRARED SPECTROSCOPY

QUALITATIVE SPECTRAL ANALYSIS

Required Reference:

1. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; John Wiley & Sons, Inc.: New York, 1991.

Required Reading:

2. Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 12, "Infrared Absorption Spectroscopy."

Experimental

The purpose of this experiment is to obtain spectra from several different known compounds that contain different functional groups. Different sampling techniques will be used. An unknown compound will be analyzed and a spectrum obtained. The student will then have the opportunity to determine its identity by functional group analysis and identification from a spectral library.

Unknown Compounds

benzophenone	acetamide
benzaldehyde	acetanilide
benzoic acid	dimethyl formamide
p-cresol	ethyl malonate
iso-butyraldehyde	acetic anhydride
acetone	ethyl acetate
cyclohexanone	propionic anhydride
acetophenone	phthalic anhydride
n-butyl alcohol	benzonitrile
benzyl alcohol	nitrobenzene
sec-butyl alcohol	*p*-nitrobenzene
tert-butyl alcohol	cyclohexanol

I. Sample Analysis

Obtain spectra of the following compounds: methyl ethyl ketone, *n*-butyl alcohol, salicylic acid, *m*-nitrotoluene, ethyl benzoate, the polystyrene standard, and Nujol®. Be sure to follow the sample handling and preparation technique as described in the introduction to Infrared Spectroscopy in this section. For the liquid samples, use the neat liquid sample technique and for the solids use both the mull and the KBr pellet technique. Each solid sample will, therefore, have two spectra. From a standard text such as Reference 1 above, obtain frequencies of the functional groups and label the major peaks in each of the spectra from the known compounds above accordingly. Make notations on the spectra of the solids that show the difference between the mull and the pellet technique. This could be done by shading in the part of the spectra that is from the Nujol® oil.

II. Unknown Analysis

For this analysis, you are not allowed to use the computer search systems of your instrument if it is available. Obtain the IR spectrum for the unknown sample. If the unknown is a liquid, use the neat liquid technique. For solids, use the KBr pellet technique. Check the spectrum immediately to see if the absorption is either too great (peaks off scale) or too small (extremely small peaks). If peaks are too large and off scale, either dilute a liquid sample or decrease the pathlength, or make a new pellet with less sample. If peaks are too small, increase path length (use a larger spacer) or make a new pellet with more sample, then rerun your unknown. Upon completion, be sure to clean the windows or pellet press with dichloromethane and dry with Kimwipes® or a slow flow of nitrogen gas, then return the windows to a desiccator.

For the unknown compound, make a table that lists all the peaks (cm^{-1}) of significant absorbance and with References 1 and 2 above or a reference of choice, attempt to deduce the principal structural or functional group. Narrow the number of possible compounds down to a few whose names are given in the listing above. Confirm the identification by comparing the relevant spectra in the listing with ones obtained of the same compound from a standard source such as Reference 3 below or the *Sadtler Standard Spectral Index*. Obtain the standard compound and run a standard spectrum to support your conclusion.

III. Reporting Results

Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, brief summary of procedure, calculations, data (all spectra appropriately labeled, and table), analysis of unknown with major functional groups labeled on the spectrum and a comparison made with the

spectrum from the standard source, standard spectrum, identity of the unknown, and answers to the questions below.

IV. Questions

1. Discuss the limitations of using correlation charts alone to establish the identity or the structure of a compound. (2) [Currently, IR by itself is seldom used alone in the literature to determine the identity of an unknown, but in conjunction with several other methods such as NMR, etc.]

2. In what areas of chemistry does the use of FTIR prove to be particularly beneficial? (2).

V. Safety Information

A. Nujol® oil (mineral or paraffin oil) is an irritant. Use appropriate gloves, goggles and general dilution ventilation.

B. KBr is a solid hygroscopic irritant. Use appropriate gloves, goggles and general dilution ventilation.

C. Dichloromethane is a toxic irritant that is combustible. It is known to cause tumors in animals. Use of appropriate gloves, goggles and clothing is required. Use local or process enclosure ventilation.

D. 2-butanone is a flammable liquid irritant. Use appropriate gloves, goggles, local or process enclosure ventilation.

E. Salicylic acid is an irritant to skin and eyes and toxic if ingested. Use appropriate gloves and goggles. Local exhaust or general dilution ventilation is required.

F. *n*-Butyl alcohol is a flammable liquid irritant. Use appropriate gloves and goggles. Local or process enclosure ventilation is required.

G. Ethyl benzoate is an irritant. Prevent skin and eye contact by using gloves and goggles. Local exhaust or general dilution ventilation is required.

H. *m*-Nitrotoluene is a toxic solid irritant. Use appropriate gloves and goggles. Use local or process enclosure ventilation.

I. Unknowns. The instructor will provide any necessary specific information for the ones assigned. It is always best to treat unknowns as flammable highly toxic irritants and possible carcinogenic agents.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be made available (including listings for all unknowns in table above) to students before performing this experiment.

VI. Additional References

3. Pouchert, C. J. *The Aldrich Library of Infrared Spectra*, 2nd ed; Aldrich Chemical Company: Milwaukee, WI, 1975.

EXPERIMENT 11: INFRARED

QUANTITATIVE ANALYSIS OF XYLENE MIXTURE USING INTERNAL STANDARDS METHOD

Experimental *

Even though infrared spectroscopy is generally used for qualitative analysis, it can also be used for quantitative analysis. Beer's law also applies to quantitative measurements in the infrared region of the spectrum.

$$A = \epsilon bc \quad \text{Beer's Law} \quad [\text{Equation 1}]$$

A = absorbance; ϵ = molar absorptivity in moles per liter; b = path length and c = concentration. In ultraviolet and visible spectrophotometers, the sample cells are of a fixed pathlength. However, in the infrared, it is more difficult to determine the exact path length unless special cells for this purpose are used. If these cells are unavailable, this can be overcome by adding an internal standard whose concentration is known to the solution. The internal standard, I , must absorb at a wavelength different than the component being measured, s. Thus it is possible to eliminate b from Beer's law. The expression derived is

$$\frac{A_s}{A_I} = kc_s \quad [\textit{Equation 2}]$$

where k is equal to the sample molar absorbtivity divided by the internal standard absorbtivity times the concentration of the internal standard. This results in obtaining a calibration curve by plotting the ratio of A_s/A_I against the concentration of the component being measured, s, in known solutions which will yield a straight line from which the concentration of the unknown can be obtained.

I. Preparation of Standard Solutions

For this experiment, the samples to be analyzed are *para*- and *meta*-xylene. The internal standard is *ortho*-xylene. Obtain twelve clean 10-mL volumetric flasks. To

* Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

each, pipet in 2.0 mL of ortho-xylene. Thus, each will contain the internal standard at a constant 20% by volume. To the first six flasks pipet (or load and use buret) 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL of *para*-xylene. Calculate the % by volume added. To the remaining six flasks pipet (or load and use buret) 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL of *meta*-xylene. Calculate the % by volume added. Dilute each flask to the mark with cyclohexane and mix well.

II. Spectral Analysis

Obtain the unknown sample from the instructor. It will contain 2.0 mL of the *ortho*-xylene and an unknown mixture of *meta*- and *para*-xylene diluted to 10 mL with cyclohexane. Prepare the samples for spectral analysis by mixing five drops of sample with five drops of Nujol® and placing a small quantity of the mixture between NaCl windows. Clamp or screw the windows in the cell body and insert in the sample compartment of the infrared. Be careful to follow procedure for handling the windows correctly as outlined in the introduction to the infrared section. Record each of the following infrared spectra from 4000 to 650 cm^{-1}: Nujol®, Nujol® plus cyclohexane, Nujol® plus *ortho*-xylene, Nujol® plus *meta*-xylene, Nujol® plus *para*-xylene, the twelve prepared samples, and three spectra of the unknown using separate portions. Record and label all spectra appropriately.

III. Data Analysis

Calculate the percent transmittance for each analytically useful band using the baseline method as detailed in Reference 2. Recommended bands for each compound are: 12.6 μm for *para*-xylene, 13.0 or 14.5 μm for *meta*-xylene, and 13.5 μm for *ortho*-xylene. Calculate the absorbance for each band using the following equation:

$$A = 2.00 - \log \%T \qquad \text{[Equation 3]}$$

Calculate the ratio of absorbances A_s/A_I for each sample. Recall that *ortho*-xylene is the internal standard. Place all of the above data in an appropriately prepared table for each of the twelve samples analyzed. Prepare calibration plots of A_s/A_I vs C_s (% by volume) for the *meta*- and *para*-xylene. Calculate the absorbance ratios for the unknown and determine the percentage by volume of *meta*- and *para*-xylene using the standard plots. Report a mean and standard deviation for the three separate portions of unknown analyzed.

IV. Combination (Open-ended) Experiment

An alternative method in analyzing a mixture with overlapping absorption bands is mentioned in Reference 1. Design an experiment to determine the percent by volume of *meta*- and *para*-xylene in the assigned unknown using the alternative method.

This should include: statement of problem, overall experimental design, conclusions, and precision of the method. A comparison of the results from both methods should be incorporated with a justification for the method chosen to be most accurate.

V. Reporting Results

A. Basic Experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, brief summary of procedure, calculations, data (all spectra appropriately labeled), table, mean concentration of unknown and standard deviation, plus the answer to the question below.

B. Combination (Open-ended) Experiment: It should be written up in the laboratory notebook and include all details as listed in Part VA above plus an introduction which explains the importance and relevance of the results and interpretation of the results with statistical analysis.

VI. Question:

1. Discuss the major reasons for deviation from Beer's law in the infrared region of the spectrum and what is used in quantitative work to compensate for this.

VII. Safety Information

A. Nujol® is an irritant. Use appropriate gloves, goggles and general dilution ventilation.

B. *o*-Xylene is a flammable liquid irritant. Prevent skin and eye contact by using appropriate gloves and goggles. Local or process enclosure ventilation is required.

C. *m*-Xylene: see *ortho*-xylene

D. *p*-Xylene: see *ortho*-xylene

E. Cyclohexane is a flammable liquid irritant. Prevent skin and eye contact by using appropriate gloves, goggles and local or process enclosure ventilation.

F. Dichloromethane is a toxic combustible irritant. Use appropriate gloves and goggles and local or process enclosure ventilation.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be made available to students before performing this experiment.

VIII. Additional Reference

2. Skoog, D. A.; Leary J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 12, "Infrared Absorption Spectroscopy".

EXPERIMENT 12: INFRARED

QUANTITATIVE DETERMINATION OF THE VINYLACETATE CONTENT OF PACKAGING FILMS

Required Reading:

1. Allpress, K. N.; Cowell, B. J.; Herd, A. C. *J. Chem. Educ.* **1981**, *58*, 741-742.
2. Mathias, L. J.; Hankins, M. G., Bertolucci, C. M.; Grubb, T. L.; Muthiah, J. *J. Chem. Educ.* **1992**, *69*, A217-A219.

Experimental*

Among analytical techniques, infrared spectroscopy is well-established for qualitative analysis of polymers. The Beer-Lambert law is valid in the infrared, and quantitative determinations can also be made provided the pathlength can be accurately measured. This experiment measures the vinyl acetate content of packaging films composed of a copolymer of polyethylene/vinyl acetate by the following methods: (a) use of a micrometer to measure film thickness, (b) measurement of interference (fringe) peaks to determine pathlength, and (c) use of a polyethylene peak as an internal standard to establish pathlength. A comparison of the three methods is then made.

I. Procedure

If one is using a dispersive instrument instead of an FTIR, slow scans of a smaller range will produce better results. Samples of packaging film of known vinyl acetate content can be obtained from the instructor to be used to obtain a calibration curve. Also obtain an unknown sample and prepare it simultaneously. This may vary from 2 to 40 % vinyl acetate content. The film can be cut to size and mounted in 35 mm slide holders, or cardboard strips (old manila files) can be cut to fit the sample holder of the IR with a hole, through which the beam passes, to which the film may be taped. The cell sample holder can be used as a template in both these procedures. Samples that are too thick for analysis may be placed on foil on a hotplate, warmed and then pressed or stretched. Be sure to have the hotplate under a hood and not to heat the sample for over a minute at a time to prevent degradation of the sample. After sample preparation is complete, use a micrometer to measure the film thickness. Measure several areas of the film to ensure uniform thickness. If it is not uniform, it will need to be repressed and stretched. Obtain three separate spectra for each standard and the unknown from 4000 to 650 cm^{-1}.

*Note: This procedure is abstracted with permission from References 1 and 2.

II. Data Analysis

The three methods used to obtain the calibration curves are as follows: (a) absorbance at 1020 cm^{-1}/film thickness (measured by micrometer) plotted versus % vinyl acetate content. When using this method method, the absorbance at 1020 cm^{-1} divided by the film thickness is proportional to the vinyl acetate content (also true for method 3). (b) A_{1020}/A_{720} plotted versus % vinyl acetate content. These are two good peaks to analyze. The peak at 1020 cm^{-1} is due to the vinyl acetate and the peak at 720 cm^{-1} is due to polyethylene. (c) Absorbance at 1020 cm^{-1}/film thickness (measured by interference fringes) plotted versus % vinyl acetate content. In the use of interference peaks, recall that

$$b = \frac{\Delta N}{2(\nabla_1 - \nabla_2)}$$

The baseline method for determining absorbance is illustrated in both References 1 and 3.

Construct calibration curves using the Beer-Lambert Law for each of the three methods used and determine the vinyl acetate content of the unknown. Use the average (mean) value of the absorbances from the spectra run for each determination. Present the data in a table including the standard deviations for each calculated mean value.

III. Reporting Results

Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, brief summary of procedure, calculations, data (all spectra appropriately labeled and table), and statistical analysis. Report the calculations of the unknown composition to your instructor and obtain the actual value. Compare the actual content to the value obtained from each of the three methods. Comment on the results, calculate the % error, and discuss the method deemed to be more accurate (see Reference 3, another textbook, or a literature source).

IV. Questions

1. What functional groups are causing the 1020 cm^{-1} and 720 cm^{-1} peaks ?

2. Spectra could be taken by the internal-reflection method without having to do sample preparation by thinning. Describe this method including its limitations and advantages (3).

V. Safety Information

If heating the polymers be sure to do so under an appropriate safety hood.

VI. Additional Reference

3. Skoog, D. A.; Leary J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992.

THIN-LAYER CHROMATOGRAPHY

Required Reading:

Skoog, D. A.; Leary, J. J. *Principles of Instrumental Analysis*, 4th ed.; Harcourt Brace College Publishers: New York, 1992; Chapter 26, "High Performance Liquid Chromatography".

Introduction

Thin layer chromatography (TLC) is similar to High Performance Liquid Chromatography (HPLC) in theory. It involves the use of a flat thin layer of stationary phase that is coated on a glass, plastic or metal surface. This is called a thin layer plate. Often, the material on the plate is silica gel or a bonded type of silica gel as in HPLC. The mobile phase can be a variety of liquid solvents which move through the stationary phase by capillary action.

In this method, the solvent (mobile phase) is placed in the bottom of a development chamber. This is then capped so that the air in the chamber becomes saturated with the solvent. A plate which contains the stationary phase is spotted with sample and placed into the development chamber. The solvent migrates up the plate by capillary action and carries the sample components. The sample components are then separated by their differing affinities for the mobile and stationary phases.

Perhaps one of the most critical aspects of this process is the spotting of the plate. The spot of sample is applied by micropipet or hypodermic syringe 1 to 2 cm from the bottom edge of the plate and should have a diameter of 5 mm or less for best results. After the solvent has traveled approximately 3/4 the length of the plate, the plate is removed and dried. This is described in detail in the experiment on TLC.

Often the sample spots are not visible, and various reagents are sprayed on the plates to allow visualization of the spots. These include solutions of iodine, sulfuric acid, or more specific reagents such as ninhydrin. In some cases, a fluorescent material may be used in the stationary phase and UV light can then be used to help in the visualization of the spots.

In analyzing the results, most of the terms and relationships for other types of chromatography apply. Two common factors used in looking at the results are:

1. Retardation factor

$$R_F = \frac{d_R}{d_M}$$

where
d_R = the linear distance a sample species has moved from the origin.
d_M = the linear distance the solvent has moved from the origin.

2. Relative Retention factor

$$R_x = \frac{\textit{distance traveled by the analyte}}{\textit{distance traveled by the substance}}$$

EXPERIMENT 13: THIN-LAYER CHROMATOGRAPHY

SEPARATION AND IDENTIFICATION OF DERIVATIVES OF 2,4-DINITROPHENYLHYDRAZINE

Experimental*

There are many advantages to using thin-layer chromatography, among which are speed and low cost. Many chromatographers feel TLC should always precede HPLC or other types of column chromatography as a quick easy check on the ability of the phases to do the required separation. TLC is also used frequently in industry and clinical laboratories in a variety of applications as the analysis of choice. The purpose of this experiment is to introduce TLC with the preparation and separation of derivatives of 2,4-dinitrophenylhydrazine and to identify an unknown mixture of the derivatives.

DUE TO THE USE OF DIETHYL ETHER IN THIS EXPERIMENT, THERE SHOULD BE NO OPEN FLAMES AT ANY TIME!

I. Preparation of Derivatives

Label a series of 10-mL volumetric flasks 1 - 6. All carbonyl compounds should be added with glass syringes (except solid). The ethanol may be added with a clean 1-mL pipet. Be careful not to cross contaminate solutions through the use of the syringes. The following is a list of what each flask should contain: Flask 1 - blank, 1 mL 95% ethanol; Flask 2 - 100 μL of 3-heptanone, 1 mL 95% ethanol; Flask 3 - 100 μL of butanal, 1 mL 95% ethanol; Flask 4 - 0.08 grams of ethyl levulinate, 1 mL of 95% ethanol; Flask 5 - 100μL each of the unknown(s) chosen by the instructor, 1 mL 95% ethanol; Flask 6 (only needed if doing investigative portion of lab) - an amount of the unknown from 5 (in amounts varying from 50 to 90 μL for the liquids and 0.05 to 0.07 grams for the solid), 1 mL 95% ethanol. To each flask, add the 2,4-dinitrophenylhydrazine solution with disposable glass micropipet dropwise until no further reaction occurs. It is extremely important not to add an excess. Add 2 mL diethyl ether with a pipet and dilute to volume with distilled water, mixing well. Using a disposable micropipet, transfer the ether layer of each volumetric flask to a clean labeled test tube. Stopper each test tube to prevent evaporation.

* Note: This procedure is abstracted with permission from Reference 1 (See Instructor's Guide).

II. Sample Analysis

Obtain the development chambers (tanks or screw cap jars) and place a 9:1 mixture of petroleum ether:diethyl ether in the chamber and close the lid. There should be no contact between the spotted sample and the ether mixture; therefore, the plates should be placed so that the level of the solvent is below where the plates have been spotted. The amount of ether mixture added will vary depending on the specific chamber used. To ensure the addition of the correct amount of ether mixture, measure from the bottom of the chromatographic plate to the position of sample application. Make sure the level of the ether mixture is below this in the chamber. Using a pencil (not a pen) and ruler, draw a line lightly near the bottom of the plate approximately 2 cm from the edge with a single dot where each solution will be placed. Next, using a 1μL syringe, spot each derivative separately as follows: apply 1μL of the derivative making sure the diameter of the spot is less than 5 mm, allow the spot to dry, apply another 1μL amount of the derivative, and allow the spot to dry again. Continue to do this on the same spot until 5 μL have been applied. After all of the solutions and have been applied and dried, place the plate in the developing chamber. Watch the plates carefully. When the solvent front has moved approximately 3/4 up the plate, remove the plate and mark the position of the solvent front. The plate is allowed to dry and is then ready for further analysis. The samples should be easily located without further treatment because of their color. Colors of the spots are characteristic of each derivative and can be used to aid in identification.

III. Data Analysis

Determine the R_f value for each component in the five solutions and tabulate the data. From the data, provide the tentative identification of the components in solution 5 (qualitative unknown).

$$R_f = \frac{\textit{distance traveled by spot beginningfrom the origin}}{\textit{distance traveled by the solvent front beginningfrom the origin}}$$

The distance traveled by the solvent can be measured by the distance from the origin (sample line) to where the pencil mark was made after the plate was removed from development chamber. The distance traveled by the spot is measured from the origin to the center of the spot.

IV. Combination (Open-ended) Experiment

Develop a method using visible spectrophotometry to determine quantitatively the amount of unknown(s) in solution 6. Please include the following in the laboratory report: statement of problem, related information from other literature sources, experimental procedure and statistical design, performance of the experiment and data collected, and interpretation of results and conclusions.

V. Reporting Results

A. Basic Experiment: Please include a sketch of the chromatogram obtained (TLC plate) appropriately labeled. Record all results in the laboratory notebook which should include the following: title, reagents required, equipment required, brief summary of the procedure, calculations, data, table, and identity of the components of the unknown.

B. Combination (Open-ended) Experiment: Include all the information requested in IV plus the quantity of the unknown(s) in the solution.

VI. Safety Information

A. Diethyl ether is a highly flammable toxic liquid. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

B. Petroleum ether is a flammable toxic liquid. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

C. 2, 4-Dinitrophenylhydrazine is a flammable solid irritant. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

D. 3- Heptanone is a liquid irritant. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

E. Butanal is a flammable liquid corrosive. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

F. Ethyl levulinate is a solid irritant. Appropriate safety goggles, gloves, and process enclosure ventilation are required.

G. Sulfuric acid is corrosive, highly toxic, and a severe irritant. It may cause severe burns. It requires the use of appropriate goggles, gloves, and a fume hood.

H. Ethanol is a flammable liquid irritant. Appropriate safety goggles, gloves, and general dilution ventilation are required.

This is not a comprehensive listing of information. It is required by law that appropriate MSDS be made available to the students before performing the experiment.

VII. Questions

1. What derivative in this study was more strongly retained in the stationary phase?

2. Why is the qualitative identification considered tentative? List two confirmatory tests that could be used to prove the identification.

3. Why must the thin-layer plate be removed before the mobile phase (solvent) reaches the top of the plate?

EXPERIMENT 14: THIN-LAYER CHROMATOGRAPHY

INVESTIGATIVE EXPERIMENTS

I. Investigative

Amino Acids: The isolation and analysis of the amino acid content of various food products supplies important information in many nutritional studies and in the quality control of nutritional supplements currently on the market. Design a study that investigates the amino acid content of food and nutritional supplement products.

Alternative (Molecular Weights of Polymers): Thin-layer chromatography can also be used to determine the approximate molecular weights of polmers. Design a system that has the capability of approximating the weight of various polymers.

Alternative (Flavanoids): Recently nutritional scientists have proposed that chemicals particularly phytochemicals in plants have cancer preventative properties. Flavanoids are one of such groups that exist in most plants. Design a study to analyze the flavanoid composition of plant(s) using thin-layer chromatography.

II. Reporting Results

Two weeks before the investigative experiment begins, the student is required to seek approval from the instructor with a brief explanation of the experiment and a list of the materials required after an intensive literature search in the library. One week before the investigation begins, the student is required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, safety information on all chemicals from the MSDS, and bibliography from a thorough library literature search. A photocopy of the literature that the experimental procedure is based upon should be included to help in the professor's evaluation. At the end of the semester a formal typewritten report that is patterned after the scientific literature, such as *Analytical Chemistry*, is due.

Investigative experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences) with the overall experimental design for the investigative portion, calculations, tables, graphs, and other details as indicated.

EXPERIMENT 15: DETERMINATION OF METALS IN LOCAL WATER SUPPLIES

INVESTIGATIVE EXPERIMENT

I. Investigative

The possible toxics effect of metals may adversely affect the biological organisms of our area waters such as rivers, lakes, ponds, and well-waters. Eventually, this type of contamination can also pose a hazard to humans. Design a study that investigates metal levels from waters in the surrounding area.

II. Reporting Results

Two weeks before the investigative experiment begins, the student is required to seek approval from the instructor with a brief explanation of the experiment and a list of the materials required after an intensive literature search in the library. One week before the investigation begins, the student is required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, safety information on all chemicals from the MSDS, and bibliography from a thorough library literature search. A photocopy of the literature that the experimental procedure is based upon should be included to help in the professor's evaluation. At the end of the semester a formal typewritten report that is patterned after the scientific literature, such as *Analytical Chemistry*, is due.

Investigative experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences) with the overall experimental design for the investigative portion, calculations, tables, graphs, and other details as indicated.

EXPERIMENT 16: ANALYSIS OF ALKALOIDS IN PLANTS

INVESTIGATIVE EXPERIMENT

I. Investigative

Alkaloids are amines that have been isolated from various parts of a plant. Many alkaloids have positive physiological activity and are used in the treatment of disease. Others can be quite deadly. Examples are as follows: nicotine from tobacco; belladona from Jimson-weed; buxene from boxwood; euphorban from poinsettas; beta-phenylethylamine and tyramine from mistletoe; phytolaccotoxin and phytolaccine from pokeweed; caffeine from coffee and tea; and atropine, hyoscyamine, and solanine from sprouting potatoes. Select a plant and design a study to analyze the appropriate alkaloid(s).

II. Reporting Results

Two weeks before the investigative experiment begins, the student is required to seek approval from the instructor with a brief explanation of the experiment and a list of the materials required after an intensive literature search in the library. One week before the investigation begins, the student is required to turn in a report that includes: statement of the problem, hypothesis, experimental design, plan for data analysis, safety information on all chemicals from the MSDS, and bibliography from a thorough library literature search. A photocopy of the literature that the experimental procedure is based upon should be included to help in the professor's evaluation. At the end of the semester a formal typewritten report that is patterned after the scientific literature, such as *Analytical Chemistry*, is due.

Investigative experiment: Record all results in your laboratory notebook which should include the following: title, reagents required, equipment required, summary of procedure (include any unusual or noteworthy occurrences) with the overall experimental design for the investigative portion, calculations, tables, graphs, and other details as indicated.

APPENDIX V

INSTRUCTOR'S GUIDE TO

A SELECTION OF EXPERIMENTS IN INSTRUMENTAL ANALYSIS

FOR SCHOOLS WITH LIMITED INSTRUMENTAL RESOURCES

UTILIZING AN INQUIRY ORIENTED APPROACH

INSTRUCTOR'S GUIDE TO

A SELECTION OF EXPERIMENTS IN INSTRUMENTAL ANALYSIS

FOR SCHOOLS WITH LIMITED INSTRUMENTAL RESOURCES

UTILIZING AN INQUIRY ORIENTED APPROACH

By

Penny L. Mauldin

INSTRUCTOR'S GUIDE TO EXPERIMENTS

ULTRAVIOLET/VISIBLE

EXPERIMENT 1: DETERMINATION OF IRON(II) AND TOTAL IRON WITH 1, 10-PHENANTHROLINE

Instruments and Equipment: Scanning UV-Vis spectrophotometer or Spectronic® 20; cuvettes: glass for the Spectronic® 20 and visible range of the Scanning UV-Vis (quartz is also acceptable for the scanning UV-Vis); Volumetric flasks: 8 per student or group of students: 10, 25, 100, and 1000 mL; Pipets: 1, 2, 5, and 10 mL (other volumes can be done with combinations of the above if necessary). Most glassware required is one or two items per student or group of students working together unless otherwise specified.

Chemicals: A. 7.0213 g of ferrous ammonium sulfate hexahydrate: Add 200-mL water, 3-mL concentrated sulfuric acid, and ferrous ammonium sulfate hexahydrate into a 1-L volumetric flask. Mix until sample dissolves, dilute to volume with distilled water, and mix well. This solution contains 1mg/mL Fe.

B. 8.6337 g of ferric ammonium sulfate dodecahydrate: Add 200-mL water, 3-mL concentrated sulfuric acid, and ferric ammonium sulfate dodecahydrate into a 1-L volumetric flask. Mix until sample dissolves, dilute to volume with distilled water, and mix well. This solution contains 1 mg/mL Fe.

One liter of A and B above should be enough for an entire class of 20 students. It is recommended that the instructor or a teaching assistant make this up immediately prior to class. If using a Spectronic® 20, this experiment may require two weeks. In that case, depending on the stopping point (if stopping after II), A and B must be remade for the following week because of possible oxidation on standing. For the Fe(II) solution, consider adding a reducing agent such as hydroxylammonium chloride to assure Fe(II) remains unoxidized.

Per student or group of students:

C. 1 mL of pure 1,10-phenanthroline
(to make 0.3 % solution)

D. Approximately 3.2 grams of KHP (Potassium biphthalate)
(to make 0.2 M solution pH = 3.98)

EXPERIMENT 1. Continued

E. 1 mL concentrated sulfuric acid

F. Unknown samples:
0.5 gram samples of varying weighed amounts of iron(II) and iron(III) in the ammonium sulfate forms as above.
If these samples cannot be made up immediately before lab, they should be sealed in a container under nitrogen. Students should grind them together and mix thoroughly to ensure a uniform sampling. Grind under nitrogen atmosphere.

Watch for oxidation of iron solutions, particularly iron(II). Take all readings within 30 minutes of mixing complexes or use reducing agent. Reference 1 uses 512 nm for the determination of iron(II) and 396 nm for total Fe.

EXPERIMENT 2: ANALYSIS OF THE IRON CONTENT OF SOAP

Instruments and Equipment: Scanning UV-Vis spectrophotometer or Spectronic 20; cuvettes: glass for the Spectronic 20 and visible range of the Scanning UV-Vis (quartz is also acceptable for the scanning UV-Vis); Volumetric flasks: 100 and 250 mL; Pipets: 5, 10, and 20 mL.

Per student or group of students:

Chemicals: A. 75 mL of Standard Iron(III) Solution containing 10 micrograms per mL (or something that can be easily diluted to this). Aldrich offers this particular standard for atomic absorption.

B. Approximately 200 mL of 8% ammonium thiocyanate solution. (1.6 grams per student) Either sodium or potassium thiocyanate may be substituted for this.

C. 50 mL of concentrated nitric acid

D. 80 mL of concentrated hydrochloric acid

If using a Spectronic® 20, the experiment may require two weeks. In that case, it is recommended that it be stopped after part III. Have the students use the same Spectronic® 20 the following week. All readings need to begin within 30 minutes of mixing the solutions with none to exceed one hour after mixing. Students need to remake a reference solution to use as a blank. It should contain all the components except the iron(III) standard.

In Part V, students may pick up on the fact that a coloring agent that is soluble in water may interfere with the analysis. If they do, they could use that as a new question and resulting hypothesis.

Literature

1. Grompone, M.A. *J. Chem. Educ.* **1987**, *64*, 1057-1058.

This reference should not be made available to students. However, if they find it in a literature search, it is permissible for them to use it.

EXPERIMENT 3: ANALYSIS OF COPPER USING DIFFERENTIAL OR EXPANDED-SCALE SPECTROSCOPY

Instruments and Equipment: Scanning UV-Vis spectrophotometer; Cuvettes: glass for the visible range of the scanning UV-Vis (quartz is also acceptable for the scanning UV-Vis); 100 mL volumetric flasks; Pipets and volumetric flasks according to student needs in combination (open-ended) experimental part.

Per student or group of students:

Chemicals:

A. 7 grams of copper (high grade copper wire)

B. 150 mL concentrated nitric acid

C. Unknown ore samples (brass). These may be obtained from Thorne with high copper content. Thorne also has ore samples with no Sn which should be obtained if possible.

CAUTION: If sample contains Sn, it must be filtered or treated in some manner to eliminate the Sn. The Sn precipitate or filter paper could absorb some copper, introducing a source of error.

D. Unknown sample for combination (open-ended) experiment. Prepare an appropriate dilution of copper (around 0.005 M works well) and give to the students in solution.

Address: Thorne Chemicals
P.O. Box 3029
Malvern, PA 19355

NOTE FOR SPECTRONIC® 20: This experiment may not work well on a Spectronic® 20 because of the scale expansion technique used. If using a Spectronic® 20, it would be best to have it modified to take square cuvettes. The linearity of the calibration curves for the Spectronic® 20 would need to be established first. If the laboratory has both a

EXPERIMENT 3. Continued

Spectronic® 20 and a scanning instrument, a variation on the experiment could be to assess the precision and accuracy of the method using both instruments.

Literature

1. Bastian, R. *Anal. Chem.* **1949**, *21*, 972-974.

2. {Combination (open-ended) Experiment}: Willard, H. H.; Merrit, L. L.; Dean, J. A.; Settle, F. A. *Instrumental Methods of Analysis*, 7th ed.; Wadsworth: Belmont, CA., 1988; pp 173-177.

These references should not be made available to students. However, if they find them in a literature search, it is permissible for them to use them.

EXPERIMENT 4: DETERMINATION OF THE FORMULA FOR AN IRON(III) SULFOSALICYLATE COMPLEX USING THE METHOD OF CONTINUOUS VARIATIONS

Instruments and Equipment: Scanning UV-Vis spectrophotometer or Spectronic® 20; Cuvettes: glass for the Spectronic® 20 and visible range of the scanning UV-Vis (quartz is also acceptable for the scanning UV-Vis); Volumetric flasks: ten 50-mL volumetric flasks per student or group of students, 2 burets per student or group of students.

Per student or group of students:

Chemicals:

A. 700 mL of 0.1 M perchloric acid: It is highly recommended that the instructor prepare this due to the hazardous nature of concentrated perchloric acid. Dilute approximately 8.6 mL of concentrated acid (71%) to 1 liter with water.

B. 50 mL of 0.0100 M sulfosalicylic acid: 0.1090 grams diluted to 50 mL with 0.1 M perchloric acid.

C. 100 mL of 0.0100 M ferric nitrate: 0.2418 grams of ferric nitrate diluted to 100 mL with 0.1 M perchloric acid.

Even with a Spectronic® 20, this experiment should only require one laboratory period. The complex is a 1:1 ratio. The maximum absorbance is approximately 500 nm.

*Note: The experimental data analysis has been set-up to work for a 1:1 complex as is typically the case in undergraduate laboratories. Because this is designed as a traditional experiment, the students are allowed to have the literature references and will need to use them to answer the questions in Part IV. Hopefully, reading the articles will help students gain understanding of some of the complexities involved such as the applicability of this method when more than one complex is formed.

GAS CHROMATOGRAPHY

EXPERIMENT 5: ANALYSIS OF A HYDROCARBON MIXTURE

Instruments and Equipment: GC (does not require one with programmable temperature); most standard nonpolar columns should work (3' X 1/4" packed with 80-100 mesh Chromosorb-P coated with 2.5% SE -30 was successfully used); Volumetric flasks: 10 mL; Pipets: 0.5, 1.0, and 1.5 mL; 10-μL GC syringe.

** It is also possible to use another column of similar polarity. The column size may also differ. Obviously, an 1/8 inch or capillary column would be even better. And, a TCD detector may also be used. If using a TCD, set bridge current to 150 mA. If using an FID, instructor should set and confirm the gas flow and determine that the flame is burning.

Per student or group of students:

Chemicals:

A. 4 mL of toluene
B. 4 mL of ethyl benzene
C. 4 mL of *p*-xylene
D. 100 mL of methyl acetate (solvent)

The unknown should be a combination from 0.3 mL to 1.3 mL of A-C above. For example, an unknown could be made that contains 1.0 mL of toluene, 1.0 mL of ethyl benzene, and 1.0 mL of *p*-xylene. This may be weighed out rather than pipetted for greater accuracy (Densities are: 0.865 g/mL for toluene, 0.866 g/mL for *p*-xylene, and 0.867 g/mL for ethyl benzene). The experiment is simple and straightforward. This makes a good first experiment. Take time to make sure that technique is good, especially use of the syringe. Give time at the end of the experiment to work on calculations especially if the instrument does not have an electronic integrator. If using peak weight, have them photocopy and do this at the end of the lab period. Monitor them carefully to make sure their technique is good. For sharp narrow peaks, Reference 3 (Pacer) gives an excellent method using peak height. Be sure they have mastered this data analysis before going on to the next experiment in the chromatographic sequence.

If there is concern about student contact with chemicals, the instructor can prepare the dilutions and place them and the pure chemicals in closed containers with crimped septum coverings. This would save on the amount of chemicals (the whole class would need only one set) and chemical contact. After the septa have been punctured, it may be necessary to recap with a new cap after each day of use to prevent changes in peak area with time.

EXPERIMENT 5: Continued

Special Note: Students should inject air into the septum bottle before withdrawing a sample; otherwise, after a few withdrawals a vacuum will be created inside. This creates difficulty in getting accurate sample measurements.

EXPERIMENT 6: ANALYSIS OF GASOLINE BY GAS CHROMATOGRAPHY

Instruments and Equipment: GC (temperature programming capabilities required); column (2 m X 1/8" with 10% Carbowax 20M on 80/100 mesh Chromosorb W); 10-μL GC syringe; assorted pipets and 100-mL volumetric flasks for the dilutions of methanol.

** It is also possible to use another column and a TCD detector. Reference 2 lists parameters for a TCD. Reference 1 lists another possible column for gasoline which is nonpolar, DC 200 6 % on Chromosorb G. Both of the columns are available from Supelco. The SPB-1 (15m X 0.20mm) from Supelco is a good choice for a capillary column. This column can also be used for two other experiments in this selection and works for the determination of the oxygenates in gasoline as well.

In addition, J&W Scientific has a capillary that works well for the experiment: 15 m X 0.53 mm DB-1. The following parameters have given successful results: Carrier Gas Flow Rate (Helium) - 35 cm/sec; Sample Size - 1 μL; Injection Port Temperature - 190°C; Column Temperature - 30°C for 1 min., 30-50° at 5°/min., 50-150° at 10°/min.; Detector Temperature - 190°C; Chart Speed - 1 inch/min.; Attenuator - 8 (Depends on the instrument)

If using a FID, the instructor should set and confirm the gas flows and determine that the flame is burning before allowing students to use the instrument.

Chemicals:

A. Approximately one gallon of unleaded gasoline per class of 20.

B. 10 mL per class of 20 students of each of the hydrocarbons listed as components in gasoline that the instructor wishes the students to analyze.

C. Approximately 500 mL of each oxygenate analyzed per class of twenty students if doing the combination (open-ended) experimental section. Some common oxygenates are the C_1-C_5 alcohols and tertiary butyl ether. Tennessee currently uses 5% ethanol. 1% methanol is also common. The instructor or laboratory assistant may add differing amounts of oxygenates to unknown gasoline samples for analysis.

Be sure to have the lab assistant or the instructor check the student's set-up for their combination (open-ended) experiment before they begin (especially the instrumental parameters). Their notebooks can be initialed. The parameters they should use are given in the two articles listed as reference and are very similar (basically identical) to the parameters for the preceding gasoline analysis.

EXPERIMENT 6. Continued

Special Note: The instructor may decide to place the chemicals for the experiment in bottles with a septum covering. If so, students should inject air into the septum bottle before withdrawing sample; otherwise, after a few withdrawals a vacuum will be created inside. This creates difficulty in getting accurate sample measurements. After the septa have been punctured, it may be necessary to recap with a new cap after each day of use to prevent changes in peak area with time.

Address: Supelco Inc. Phone: 1-800-247-6628
Supelco Park
Bellefonte, PA 16823

Address: J&W Scientific Phone: 1-800-223-3424
91 Blue Ravine Road
Folsom, CA 95630-4714

Literature:

1. Cassidy, R. F.; Schuerch, C. *J. Chem. Educ.* **1976**, *53*, 51-52.
2. Tackett, S. L. *J. Chem. Educ.* **1987**, *64*, 1059-1060.
3. Tackett, S. L. *Analyst* **1987**, *112*, 339-340.

These references should not be made available to students. However, if they find them in a literature search, it is permissible for them to use them.

EXPERIMENT 7: ANALYSIS OF THE FATTY ACID COMPOSITION OF COMMON FATS AND OILS

Because this is an investigative experiment, students will develop their own procedures from the literature. The standards listed below under "chemicals" are for the preliminary exercise. The preliminary exercise was included because of the unlikelihood that a student would have previously worked with fatty acid methyl esters. Of course, the students may obtain a literature source that incorporates different instrumental parameters, procedures, and chemicals. These are listed only as alternative references for the instructor.

Instruments and Equipment: GC; Column: 10' X 1/4" packed with 60/80 mesh Chromosorb W coated with 15 % DEGS is recommended (available from Supelco); 10-μL GC syringe.

**A good choice of capillary column to use is the SPB-1 (15m X 0.20mm) from Supelco. It can be used for this experiment and the gasoline experiment.

** It is possible to use a TCD. Reference 1 uses one and has the appropriate parameters. If using a FID, the instructor should set and confirm the gas flows and determine that the flame is burning.

Chemicals: For the Entire Class:

A. Two sets of standard fatty acid methyl ester samples that should include at least: 1. saturated fatty acids: myristic (14:0), palmitic (16:0), stearic (18:0), arachidic (20:0), behemic (22:0), 2. unsaturared fatty acids: palmitoleic (16:1), oleic (18:1), linoleic (18:2), linolenic(18:3), and eicosenic (20:1) with methyl heptadecanoate (17:0) [if possible, to use as an internal standard]. Sigma contains various sets of these standards. Give these to the students in sealed containers with a septum covering.

Special Note: The instructor may decide to place the chemicals for the experiment in bottles with a septum covering. If so, students should inject air into the septum bottle before withdrawing sample; otherwise, after a few withdrawals a vacuum will be created inside. This creates difficulty in getting accurate sample measurements. After the

EXPERIMENT 7. Continued

septa have been punctured, it may be necessary to recap with a new cap after each day of use to prevent changes in peak area with time.

Sigma/Aldrich	Supelco, Inc.
P.O. Box 355	Supelco Park
Milwaukee, WI 53201	Bellefonte, PA 16823
1-800-558-9160	1-800-247-6628

Literature:

1. Paulson, D.R.; Saranto, J. R.; Forman, W. A. *J. Chem. Educ.* **1974**, *51*, 406-408.
2. Metcalfe, L.D.; Schmitz, A. A. *Anal. Chem.* **1961**, *33*, 363-364.

EXPERIMENT 8: DETERMINATION OF CONGENERS IN WHISKEY USING THE INTERNAL STANDARD METHOD

Instruments and Equipment: GC (capable of temperature programming);
Column: 2 m X 2 mm of 5% Carbowax 20M on 80/120 Carbopak B. (This is the best column because of the various types of compounds to be separated and the large amount of water present.); 10-μL GC syringe; 200-mL volumetric flasks, 100-μL syringe.

A capillary column that would be appropriate for this experiment is the SPB-1 from Supelco which works for alcohols, gasolines and FAME's (fatty acid methyl esters). The only questionable separation would be the 2- and 3-methyl-1-butanol peaks. The other alcohols are illustrated as being separated by this column in the Supelco catalog.

Chemicals: For entire class (of 20 students):

A. 20 mL chromatographic grade of each of the following: 1-butanol, ethyl acetate, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and ethanol.

The instructor will be required to obtain additional congeners and whiskey samples for the combination (open-ended) experiment depending on the student proposals.

Per student or group of students:

A. 80 mL ethanol
B. 200 mL of each sample of whiskey to be analyzed

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Results of the students for the combination (open-ended) experiment can be compared with those obtained in reference 2. Students may also obtain additional references to cite from literature searches. These references should not be made available to students. However, if they find them in a literature search, it is permissible for them to use them.

Literature

1. Rice, G. W. *J. Chem. Educ.* **1987**, *64*, 1055-1056.
2. Wilson, L. A.; Ding, J. H.; Woods, A. E. *J. Assoc. Off. Anal. Chem.* **1991**, *74*, 248-256.
3. Martin, G. E.; Burggraff, J. M.; Dyer, R. H.; Buscemi P. C. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 186-190.

EXPERIMENT 9: DETERMINATION OF METHYL SALICYLATE IN RUBBING ALCOHOL USING THE METHOD OF STANDARD ADDITION

Instruments and Equipment: GC; Column**: 4 ft X 1/4 in 15 % Carbowax 20 M on Chromsorb P 80/100 mesh column (or similar); 10-μL GC syringe; 25-mL volumetric flasks. This column can also be used for Experiment 10.

** Obviously, other columns may be used successfully. A capillary column that would be appropriate for this experiment is the SPB-1 from Supelco which also works for alcohols, gasolines and FAME's (fatty acid methyl esters).

Chemicals: Per student or group of students:

A. 60 mL of each type of commercial alcohol analyzed
B. 3 mL of methyl salicylate
C. 20 mL solvent grade isopropyl alcohol

The instructor may be required to obtain other essential oils for the combination (open-ended) experiment.

This is a very safe, very cheap lab as far as chemicals are concerned.

Combination (open-ended): Reference to characterization of essential oils in food, flavors, and fragrances: Hewlett Packard Application Note Number 101.3kb; Hewlett Packard, Palo Alto, CA. World Wide Webb Address: http://www.dmo.hp.com/apg/literature/appnotes/59633426.html (updated July 15, 1996). This reference may prove helpful to the instructor in evaluating the open-ended portion of the student's experiments.

Literature

1. Van Atta, R. E.; Van Atta, R. L. *J. Chem. Educ.* **1980**, *57*, 230-231.

These references should not be made available to students. However, if they find them in a literature search, it is permissible for them to use them.

INFRARED SPECTROSCOPY

EXPERIMENT 10: QUALITATIVE SPECTRAL ANALYSIS

Instruments and Equipment: Double beam infrared or Fourier Transform infrared spectrophotometer; KBr pellet press; syringe or microcapillary pipet to fill cell, NaCl or appropriate windows, cell bodies, Kimwipes®

Chemicals: For class of 20:

A. 20 mL of "Nujol®" (mineral oil)
B. 100 mL of chloroform or dichloromethane: as solvent to clean windows. (Be sure students dispose of this in a waste container for halogenated organics.)
C. 20 grams of 200 mesh IR grade potassium bromide
D. 20 mL of *n*-butyl alcohol
E. 10 grams each of salicylic acid, ethyl benzoate, benzaldehyde, and *o*-nitrotoluene

For student or group of students:

F. Unknown: Approximately 0.5 grams of solid unknown or 2 mL of liquid unknown. This will give them a bit extra for running additional spectra if necessary. Choose any unknown from the list in the experiment.

EXPERIMENT 11: QUANTITATIVE ANALYSIS OF XYLENE MIXTURE USING THE INTERNAL STANDARD METHOD

Instruments and Equipment: Double beam infrared or Fourier Transform infrared spectrophotometer; syringe or microcapillary pipet to fill cell; NaCl windows; Kimwipes®; twelve 10-mL volumetric flasks per student or group of students; various pipets to deliver chemicals or 3 burets per student or group of students

Chemicals: For class of 20:

A. 100 mL of "Nujol®" (mineral oil)
B. 200 mL of chloroform or dichloromethane: as solvent to clean windows (Be sure students dispose of this in a waste container for halogenated organics.)
C. 520 mL of *o*-xylene
D. 250 mL of *m*-xylene
E. 250 mL of *p*-xylene
F. 1200 mL of cyclohexane

The baseline method for calculating percent transmission is reviewed in the textbook by Skoog and Leary (2) in the student experiment. The reference below upon which the experimental procedure was based also illustrates the baseline method and shows a figure of a sample calibration or standard curve plot.

The unknown mixtures should be made with percentages somewhere in the range of those in the standard curve (from 5 to 30 %). It is helpful to make these up in 10 mL volumetric flasks by adding for example, 1.20 mL *p*-xylene, 1.60 mL *m*-xylene, and 2.00 mL of *o*-xylene (from separate burets) diluted to volume with cylcohexane to give a 12% *p*-xylene, 16% *m*-xylene, and 20% *o*-xylene solution. Record this by number and then cap the flask and number it accordingly.

Literature

1. Veening, H. *J. Chem. Educ.* **1966**, *43*, 319-321.

This reference should not be made available to students. However, if they find it in a literature search, it is permissible for them to use.

EXPERIMENT 12: QUANTITATIVE DETERMINATION OF THE VINYLACETATE CONTENT OF PACKAGING FILMS

Instruments and Equipment: A double beam or Fourier Transform infrared spectrophotometer; micrometer.

Chemicals:

A. Samples of packaging film with known contents of vinyl acetate. These may be obtained from Dupont or other local packaging film manufacturers. Students may also bring in samples to analyze, but these would be of unknown content.

B. 35 mm slide holders or old manila folders to mount samples. Students may use the sample cell holder as a template to mark off and cut out the holders.

THIN-LAYER CHROMATOGRAPHY

EXPERIMENT 13: SEPARATION AND IDENTIFICATION OF DERIVATIVES OF 2,4-DINITROPHENYLHYDRAZINE

Instruments and Equipment: 10 cm X 20 cm thin-layer plate (Eastman® Chromatogram 13181 silica gel was successfully used - one per student); disposable micropipets, pipet bulbs; Six 100-mL volumetric flasks per student; glass syringes that deliver 1 and 100μL; tanks or wide-mouth screw top jars to act as a development chamber: one per student; pencils; rulers.

Chemicals: Per student:

A. 10 mL of 95% ethanol

B. 1 mL of butanal

C. 1 mL of 3-hepanone

D. 0.1 grams of ethyl levulinate

E. 0. 4 grams of 2, 4-dinitrophenylhydrazine*

F. 2 mL of concentrated sulfuric acid

G. 30 mL of ether

H. 100 mL of petroleum ether

If doing the combination (open-ended) experiment, it is recommended that only one of the compounds be placed in the unknown. The experiment can thus be completed in one three hour laboratory session.

* It is highly recommended that the instructor prepare this solution. The following preparation of 200 mL is adequate for 20 students.

Be sure to wear appropriate safety goggles, gloves, and clothing. In a 500 mL Erlenmeyer flask, add 27 mL of concentrated sulfuric acid to 5.3 grams of 2,4-dinitrophenylhydrazine slowly UNDER THE HOOD. Next add 40 mL of water dropwise and dilute with 133 mL of 95% ethanol.

EXPERIMENT 13: Continued

Combination (open-ended) experiment: This procedure is to be developed by the students. It will involve removing the analyte(s) from the plate by using a razor or spatula to scrape off the silica gel that contains the spot, using the ether mixture to dissolve the analytes, and filtering the solution to remove the silica gel. The analysis may be performed by visible spectrophotometry. The λ_{max} must be determined for samples of the pure substances identified qualitatively in the basic experiment.

Literature:

1. Jones, T. B.; Jones, T. H. *J. Chem. Educ.* **1985**, *62*, 813- 814.

This reference should not be made available to students. However, if they find it in a literature search, it is permissible for them to use.

EXPERIMENT 14: INVESTIGATIVE EXPERIMENTS USING THIN-LAYER CHROMATOGRAPHY

These are designed as investigative experiments; therefore, chemical needs will vary depending on the student's design of the study. The literature sources below are listed as alternative references for the benefit of the instructor only.

Literature

Amino acid content:

1. Gatto, K.; Borders, C. L. *J.Chem. Educ.* **1985**, *62*, 840.

This article gives an experimental design for the separation of leucine and isoleucine. It also lists three very extensive reference books.

Molecular Mass:

2. Slough, G. A. *J. Chem. Educ.* **1995**, *72*, 1031-1032.

Flavanoids:

3. Giannasi, D. *Botan. Rev.* **1978**, *44*, 399-429.

This article lists several good references for the thin-layer separation of flavanoids from plants.

4. Duke, J. A. *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants*; CRC Press: Boca Raton, FL., 1992.

This book lists the content of a wide variety of plants.

EXPERIMENT 15: DETERMINATION OF METALS IN LOCAL WATER SUPPLIES

This is designed as an investigative experiment; therefore, chemical needs will vary depending on the student's design of the study. The literature sources below are listed as alternative references for the benefit of the instructor only. Most schools with limited resources will have to use visible spectrometry rather than atomic absorption so that is what is described in the references.

Literature:

Specific Experiment: Simultaneous Determination of Fe(III) and Cu(II) with Hexacyanoruthenate(II) in Water.

1. Mehra, M. C.; Rioux, J. *J. Chem. Educ.* **1982**, *59*, 688-689.
2. Mehra, M. C.; Landry, J. C. *Talanta* **1980**, *27*, 445-447.

General Methods:

3. Greenberg, A. Ed. *Standard Methods for the Examination of Water and Wastewater*, 14th ed.; American Public Health Association: Washington, DC, 1976.

This book has atomic absorption methods along with visible spectrophotometric methods for the determination of various metals such as mercury using the dithizonate complex. Other editions in the 1970s of this book incorporates similar methods.

4. *Analytical Chemistry* has a biannual review on methods of analyses such as this. (Visible spectrophotometric methods for metals will be limited to older issues).

EXPERIMENT 16: ANALYSIS OF ALKALOIDS IN PLANTS

This is designed as an investigative experiment; therefore, chemical needs will vary depending on the student's design of the study. The literature source below is listed as an alternative reference for the benefit of the instructor only. This type of analysis could involve the use of infrared (qualitative identification) and either GC or UV-VIS for quantitative work. A single project might also involve muliple instruments.

General Methods:

Many methods contain simple acidic or basic extraction procedures into chloroform or dichloromethane. Some may require a preliminary distillation or soxhlet distillation extraction step.

Suggestions (would work for many alkaloids, but not necessarily all): Grind up the plant material (or outer peeling area of sprouting potatoes) in water and make the mixture basic with NaOH. Extract the mixture with several portions of dichloromethane (or ether) using a separatory funnel, and filter. This can be evaporated and diluted to volume for GC or extracted into 0.1 M HCl for UV.

Another suggestion for analysis is poppy seeds. Both morphine (soluble in acetone) and codeine can be extracted and identified. Students will need to select the plant, determine the alkaloid content and a method of extraction (solvent to use, strong or weak base, and other factors).

Literature

Specific Experiment: Determination of Nicotine in Tobacco.

1. Navari, R. M. *J. Chem. Educ.* **1974**, *51*, 748-750.