INTEGRATION OF HANDS-ON NMR SPECTROSCOPY IN THE UNDERGRADUATE CHEMISTRY LABORATORY CURRICULUM AND RESEARCH PROGRAM AT DePAUL UNIVERSITY

Working knowledge of NMR spectroscopy is essential for any student of the chemical sciences, particularly those intending to pursue careers in research or academe. The primary objective of this proposal is to obtain a modern, high-field nuclear magnetic resonance (NMR) spectrometer for the integration of fundamental, hands-on techniques of NMR spectroscopy into the undergraduate Chemistry laboratory curriculum and research program at DePaul University. A pedagogical plan is presented which involves application of NMR techniques in conventional laboratory instruction, in individual research projects as part of the Organic laboratory course, and in implementation of new experiments in Instrumental Analysis, Biochemistry and Physical Chemistry laboratory courses. The new NMR instrument will benefit students at all levels of study, both majors and non-majors of the Chemistry, Biology and Environmental Sciences Departments, and will strongly impact the undergraduate research program. Advances in NMR technology will be incorporated into the laboratory curriculum through well-designed, experimentally tested exercises, as well as through new experiments intended to expose undergraduate students to a "discovery" approach to structure determination. Experiments in Organic, Analytical, Physical Chemistry and Biochemistry will demonstrate the power of NMR technology through two-dimensional techniques.

1. Project Description

Founded in 1898, DePaul University is a private, comprehensive university situated in Chicago, committed to providing outstanding educational opportunities to a diverse population of predominantly first-generation undergraduate students. The University's curriculum, based on a Liberal Studies Program, emphasizes reflective intellectual activity, value-consciousness and critical inquiry as well as active participation in the exploration and discovery of knowledge. The total enrollment of undergraduate students in 2000 is 11,776, with 42 % minority and 59 % women.

The Department of Chemistry, which is part of the College of Liberal Arts and Sciences, offers a standard concentration in chemistry as well as a concentration accredited by the American Chemical Society (ACS). The department also contributes to interdisciplinary concentrations in Biochemistry and Environmental Chemistry, and administers annually to about 350 science majors and 700 non-science majors. A Chemistry/Education program is offered in cooperation with the School of Education to prepare students for careers in teaching science. The department also offers a Pre-Engineering program in chemical engineering. The Master of Science degree in Chemistry is designed to prepare students for advanced work in chemistry, biochemistry or polymer and coatings technology, and for further graduate study.

The common core of the departmental program requirements includes introductory courses in general, organic and physical chemistry, biochemistry and instrumental analysis. Essential components of these courses are laboratory experiments, through which students learn the techniques of preparative organic chemistry, biochemistry, instrumental and analytical methods, and identification and structure determination of organic compounds. The eight full-time faculty members in the department regard undergraduate laboratory instruction as a major component in the preparation of students for success in many professional fields and graduate studies.

As part of the University's efforts to meet the challenges of undergraduate curriculum reform and in response to the rapid evolution of new technologies, the Chemistry Department is actively involved in the search for opportunities to enhance the learning process. In order to develop undergraduate laboratory courses to improve student understanding of principles and applications of chemistry, and to provide students with hands-on experience with state-of-the-art equipment, the department has acquired several new instruments (listed separately; annual equipment budget is $75,000). The faculty gives special attention to the development of innovative methods for improving students' understanding of basic principles through laboratory experiments that stress active and interactive instrumental analysis.

The application of nuclear magnetic resonance (NMR) spectroscopy in chemistry and related fields has become so great that a thorough understanding of its principles and capabilities is critical to the success of contemporary scientists. NMR spectroscopy is a fundamental tool, not only for determining the structure of complex compounds and polymers, but also for solving physical problems regarding kinetics, conformation and molecular complexation. Detailed descriptions of principles and applications of ^{1}H NMR and ^{13}C NMR techniques are found in every introductory course textbook in organic chemistry,[1] organic laboratory techniques[2-4] and instrumental analysis.[5] These methods are used routinely for analysis in industry, government and academic laboratories.

The refurbished 90 MHz JEOL NMR spectrometer (originally manufactured in 1979), which was purchased by the department in 1996, is an outdated, unreliable instrument. After many repairs, the magnet has very low field and temperature stability and is unsuitable for hands-on undergraduate instruction and research. Presently, though students are instructed on principles and applications of NMR spectroscopy as required by the curriculum, they do not have an opportunity to perform NMR analysis in the laboratory due to the lack of an appropriate instrument. Advances over the last 20 years in NMR design have led to the development of user-friendly NMR spectrometers and to new techniques that facilitate structural analysis of organic compounds. The development of new, user-friendly software makes these instruments and techniques accessible to undergraduate students and invaluable instructional tools for laboratory exercises and research.

The primary objective of this proposal is to obtain a modern, high-field NMR spectrometer for the integration of fundamental, hands-on techniques of NMR spectroscopy into the undergraduate laboratory courses and research programs in organic, analytical, physical chemistry and biochemistry. Students in the Chemistry, Biology and

Environmental Science Departments, will benefit from the proposed curriculum enhancement. Several course sequences will be affected (DePaul's calendar follows the quarter system):

- Organic Chemistry (CHE 171, 173, 175)
- Instrumental Analysis (CHE 261)
- Experimental Biochemistry (CHE 341)
- Physical Chemistry (CHE 211, 215)
- Laboratory Projects in Polymer Science (CHE 251)

Ongoing research programs in the laboratories of Associate Professor Gregory Kharas (Organic and Polymer Chemistry), Assistant Professor Matthew Dintzner (Synthetic Organic Chemistry), Assistant Professor Kathleen Helm-Bychowski (Biochemistry), and Assistant Professor Richard Niedziela (Physical Chemistry) will incorporate NMR technology not currently available in the department.

2. Project Plan and Requirements

The Chemistry Department at DePaul University will integrate hands-on, modern NMR technology into its laboratory curriculum to better serve the current needs of its students and faculty. A pedagogical plan has been developed to make maximum use of the requested instrument for each of the affected laboratory courses and research programs. Choice of instrument, based on requirements as specified by the proposed curriculum improvements, is described.

2.1 Organic Chemistry

DePaul University operates on a quarter system, and the Organic Chemistry sequence is typically taken in the second year, over three quarters. In the first quarter students are introduced to classic synthetic, separation and purification laboratory techniques. If a new NMR spectrometer is available in the beginning of the first quarter, principles of NMR theory and interpretation will be presented to students in lecture, prior to organic laboratory experiments that include ^{1}H NMR analysis. Particular attention will be given to the relationship between resonance phenomena (such as chemical environment, diamagnetic shielding, anisotropy and spin-spin splitting) and quantitative measurements (such as chemical shift, integration and coupling). In the laboratory students will learn how to (a) prepare a sample for NMR analysis, (b) choose an appropriate solvent and standard, and (c) record a spectrum. Students will learn to interpret spectra and communicate the results of their NMR analysis in formal laboratory reports.

Application of NMR spectroscopy to structural analysis of products obtained in laboratory exercises will significantly enhance students' understanding of chemistry and the research process. Latest editions of textbooks for laboratory courses in organic chemistry provide experiments for which structure analysis of reaction products involves ^{1}H and ^{13}C NMR spectroscopy.[2-4] *Experimental Organic Chemistry* by Gilbert and Martin,[2] the text currently used in the department, provides a comprehensive background on theory and practice of NMR technology for incorporating this fundamental analytical technique as a routine part of experiments executed in the laboratory. Thus, the learning objectives for the first quarter will be to (a) record spectra of a reaction product and compare the observed data with those reported in the text, (b) use chemical shift tables to do peak assignments, and (c) use integration data for compositional analysis of a mixture of products.

In the second quarter, the plan is to build on the foundation that was established in the first quarter and to introduce the principles and practice of ^{13}C NMR spectroscopy, with emphasis on decoupling phenomenon. Laboratory exercises will stress the importance of chemical shift measurement. Techniques for calculating and predicting NMR resonances for some reaction products will be introduced, as well. Students will have the opportunity for active and interactive learning with computers in the department, using PC-based software (WindowCHem™, SoftShell C-13 module) for ^{1}H and ^{13}C NMR chemical shift prediction, as related to the structure characterization of organic compounds. The learning objectives for the second quarter will be to (a) record and provide peak assignments for the ^{13}C NMR spectrum of a reaction product using chemical shift tables and spectra of model compounds provided in the text, and (b) predict ^{1}H and ^{13}C NMR shifts by using computer calculations and correlate with the recorded spectra.

In the third quarter, students will apply techniques of ^{1}H and ^{13}C NMR spectroscopy to the analysis of products obtained in laboratory exercises. In addition, students will record and assign resonance signals to specific protons and carbons for structure determination of unknown compounds. Coupled with other instrumental (IR, UV-Vis, GC-MS) and wet chemistry techniques (preparation of derivatives), the direct assignment will stimulate and enhance students' learning experience and emphasize the tremendous power and utility of NMR spectroscopy in the research process.

In recent years Professor Kharas has developed an innovative approach to teaching the sophomore Organic laboratory course, with an objective of exposing students to the excitement of the research experience by assigning individual discovery projects in the third quarter.[6] After completing two quarters of lecture, instruction and laboratory experimentation, students have enough background to apply what they have learned to a supervised, independent research project in polymer synthesis. The discovery projects are structured to provide genuine research experience, including: manual and computer literature searches for information on targeted compounds; synthesis and characterization of a monomer; copolymerization and characterization of the copolymer; communication and interpretation of results in a formal (ACS publication format) report. The projects involve micro- and macroscale synthetic techniques based on two reactions, (1) synthesis of a family of trisubstituted ethylenes (TSE) via base-catalyzed Knovenagel condensation of substituted aldehydes with activated methylene compounds, followed by (2) copolymerization of a TSE monomer with styrene in the presence of a radical initiator.

The results of the discovery projects are checked subsequently by undergraduate and graduate students prior to submission for publication.[7] The availability of a high-field NMR spectrometer will give students the opportunity to get first-hand experience in the structure analysis of monomers and copolymers they have prepared. This will result in better integration and connection between the theory of organic chemistry learned in lecture, the analytical techniques learned in the laboratory, and the application of organic chemistry in the research of new materials (polymers). The learning objectives of the third quarter discovery projects, with regard to NMR analysis, are to (a) prepare monomer and copolymer NMR samples in an appropriate solvent, (b) perform NMR analysis of the samples, (c) interpret the spectra of individual products in terms of chemical shift, splitting pattern and integration, (d) discuss data and observations with other students involved in preparation of compounds in the same family, and (e) report results in a form appropriate for publication.

2.2 Instrumental/Analytical Chemistry

The Instrumental Analysis lecture and laboratory sequence, designed for chemistry and other science majors (typical enrollment is about 20), includes experiments based on applications of modern instrumentation. The Instrumental Analysis course usually involves a single NMR experiment since it incorporates a variety of other spectroscopic and electrochemical methods. In recent years, modern NMR techniques such as ^{13}C-distortionless enhancement by polarization transfer (DEPT) and two-dimensional (2D) ^{13}C-^{1}H correlated (HETCOR) experiments have been introduced in the lecture part of the course. Since most modern NMR spectrometers make these techniques accessible to novice users, the integration of a new instrument into our curriculum will significantly enhance the laboratory portion of the Instrumental Analysis course.

Students enrolled in Instrumental Analysis will perform DEPT and HETCOR analyses on specific copolymers, generated from the discovery projects of students enrolled in the third quarter of the Organic laboratory, with the objective to confirm ^{1}H and ^{13}C NMR assignments of corresponding structures. This experiment will afford students the opportunity to be involved in guided discovery, working collaboratively to solve structure elucidation problems using modern NMR techniques.

1.5 Biochemistry

Experimental Biochemistry is taught by Assistant Professor Kathleen Helm-Bychowski. The course provides students (typical enrollment is 10-12) with hands-on experience in the classical and modern techniques for isolating and characterizing biomolecules. Among the ever-evolving techniques that are explored in this laboratory are various methods of spectroscopy, electrophoresis and chromatography. Recent advances in NMR spectroscopy are making its applications in biotechnology routine. In this regard, the power of NMR spectroscopy will be demonstrated through an experimental study of the three-dimensional structure of small proteins.[8]

Students will observe the 1-D ^{1}H NMR spectrum of the 34-43 fragment of the protein *transforming growth factor*-α, which contains a disulfide bridge between residues 34 and 43. Two-dimensional correlation spectra (COSY, NOESY, ROESY) will be collected to determine amino acid "fingerprints" and distance constraints between atoms. These data will be compiled to ascertain the secondary and tertiary nature of the protein fragment. The disulfide bridge will then be reduced chemically, thereby disrupting the 3D configuration of the polypeptide, and NMR data (1D and 2D, as before) will be collected. Students will compare NMR spectra of the protein fragment before and after cleavage of the disulfide bond. A variety of commercially available, low-molecular weight bioactive peptides, including insulin, will be studied in subsequent experiments. These exercises will give students the opportunity to apply NMR spectroscopy to the study of biomolecules, using sophisticated techniques that are becoming routine in modern research.

2.4 Physical Chemistry

In Physical Chemistry, students study concepts in thermodynamics and kinetics, as well as electrochemistry, transport processes and crystal structure. NMR spectroscopy will be incorporated into this laboratory course for solving physical problems regarding reaction thermodynamics, kinetics, conformation and molecular complexation.

One experiment has been developed and tested which is related to equilibrium determinations of donor-acceptor π-complexes by NMR.[9] Students will prepare mixtures of a donor (e.g. styrene) and an acceptor compound (e.g., methyl 2-cyano-3-phenyl-2-propenoate) of different compositions and record the ^{1}H NMR chemical shift of the acceptor's vinylic hydrogen. The chemical shift data are then used to calculate an equilibrium constant of the complexation by application of the Benisi-Hildebrand method,[10] adapted for NMR data.[11,12] This experiment provides students with a good demonstration of NMR application to the kinetic studies of intermolecular interactions.

2.5 Undergraduate Research

A modern NMR spectrometer will tremendously enhance the Chemistry Department's undergraduate research program, which includes a number of projects requiring the routine characterization of organic compounds and polymers. Availability of a modern NMR spectrometer will facilitate the undertaking of more challenging research problems and provide the necessary foundation for personal and professional advancement for students and faculty. A new course, Laboratory Projects in Polymer Science (CHE 251), will be offered each Spring through the University's Experiential Learning program. Professor Kharas, who will serve as co-project director for the new NMR, will teach the course, which will involve individual research projects, synthesis and characterization of novel polymers. Students enrolled in CHE 251 will receive Experiential Learning credit (required by the University) toward their degrees.

In Kharas's laboratory students are working on the synthesis and characterization of novel monomers and polymers based on trisubstituted ethylenes.[13] Kharas's research interests also include the synthesis and characterization of resorbable polymers for biomedical applications.[14] Students involved in research projects with Kharas will greatly benefit by having the opportunity to perform NMR analyses on new compounds synthesized in the laboratory. Professor Kharas has supervised the research of more than 50 students since arriving at DePaul in 1992.

In the laboratory of Assistant Professor Matthew Dintzner, a new faculty member and the NMR project director, students will synthesize haptens (protein-conjugated molecules capable of stimulating a specific immune response) for the development of potential catalytic antibodies. Dintzner's haptens will be submitted to the hybridoma facilities at The Scripps Research Institute, where they will be used to immunize mice. Antibodies generated in response to the haptens will be isolated and purified at Scripps and sent back to Dintzner's laboratory. Antibodies found to be catalytic will be employed in the synthesis of physiologically active natural products. Dintzner's research involves multi-step synthesis of small molecules, which necessitates routine structure determination. Dintzner and his students will use NMR spectroscopy as their primary method for characterization of new compounds.

Other research groups that will benefit from a new NMR spectrometer include the laboratories of Assistant Professors Kathleen Helm-Bychowski, and Richard Niedziela. Helm-Bychowski is a biochemist working on the molecular evolution of mitochondrial and nuclear DNA sequences in birds and mammals. Neidziela's research involves the development of spectroscopic methods for the characterization of organic and inorganic aerosols.

2.6 Equipment

Funding is requested for a modern NMR instrument to successfully implement the proposed development of the undergraduate laboratory curriculum and research programs at DePaul. Our requirements include: a magnet that provides high stability and low operation cost; a user-friendly operating system with high-throughput capabilities for walk-on use; sophisticated software for advanced chemistry courses and undergraduate research. The ideal instrument to meet our department's needs is Varian's Mercury™ (300 MHz), with a four-nucleus probe and a Sun Microsystems host computer (with Glide™ user interface). The Mercury™ is well suited for routine, high-throughput and research experiments. The Mercury comes with a variety of preprogrammed 2D experiments (HETCOR, COSY, ROESY, TOCSY, HMQC), a one-year warranty and excellent service contract options for subsequent years. We think this is the best package currently available for the money.

In addition, funding is requested for a variable temperature module, which allows for automatically controlled temperature scans without additional tuning. Since some of our experiments involve NMR spectra of polymers, the ability to acquire spectra at higher-than-ambient temperatures will increase resolution and permit faster acquisition. Variable temperature capabilities will also enable us to implement physical chemistry experiments involving kinetics and determine equilibrium constants of complexation reactions requiring data as a function of temperature.

To ensure maximum use of the NMR spectrometer, the project directors will coordinate training, development and implementation of laboratory experiments adopted by faculty members assigned to specific laboratory courses. Dintzner will serve as project director; Kharas will act as co-project director. The University is fully committed to the service and maintenance of the instrument (even if there is a magnet-quench) beyond funding of the proposal. An appropriate space for the instrument is available, an annual contract for supply of liquid helium and nitrogen will be obtained by the Chemistry Department, and indefinite service contracts for the instrument will be purchased from the manufacturer. The project directors will assume responsibility for training, routine operation, scheduling, maintenance and service of the instrument.

3. Experience and Capabilities of the Principal Investigators

Faculty involved in teaching laboratory chemistry courses are strongly committed to curriculum improvement through integration of modern NMR techniques. The project directors have considerable experience in the application of NMR spectroscopy for the characterization of organic compounds, and are proficient with the routine upkeep and operation of the requested instrument. Professor Tom Murphy, who is the Director of the Environmental Science Department and Full Professor of Chemistry, has considerable experience in teaching NMR spectroscopy, Organic chemistry and Instrumental Analysis, and is fully committed to integrating hands-on NMR technology in these laboratory courses. Kharas, who teaches

Organic, Polymer Chemistry and Instrumental Analysis, has almost twenty years of experience in the application of NMR spectroscopy in organic, physical and polymer chemistry, including the use of high resolution techniques (DEPT, HETCOR, COSY) for the analysis of microstructure of polymers.[12] Dintzner, who will teach Organic and Advanced Organic Chemistry, is a synthetic chemist with considerable experience in the NMR analysis of organic compounds and a strong commitment to the development of undergraduate research at DePaul.[15,16]

4. Evaluation Plan and Dissemination of Results

The NMR experiments of the proposed project will be evaluated for reproducibility

and educational value, and results submitted by participating faculty to appropriate journals. The project directors will coordinate collection of data on the impact of curriculum improvement through specifically designed questionnaires, which will be distributed among students in the affected laboratory courses. The evaluation forms will reflect the number of students, ethnicity, gender, education level, grade-point-average, years of study, future career plans and allow for student feedback and suggestions for improvements. Results of student discovery projects and independent research will continue to be reported in presentations at ACS meetings and in peer-reviewed scientific journals. The Chemistry Department is confident that a modern NMR spectrometer will significantly enhance the quality of education it provides for the growing number of students involved in studying chemistry and related fields at DePaul University.

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Appendix 2: Course Descriptions

CHE 171 – Organic Chemistry I

First in a sequence of courses that investigate concepts in organic chemistry, including stereochemistry, free radical substitution and electrophilic addition. (Prerequisites: CHE 115, General Chemistry II)

CHE 173 – Organic Chemistry II

Aromaticity and electrophilic and nucleophilic substitution. (Prerequisites: CHE 171)

CHE 175 – Organic Chemistry III

Carbanions and the preparation and reactions of many organic compounds, including those of biological interest. (Preqequisites: CHE 173)

CHE 211 – Physical Chemistry II

Thermodynamics continued; electrochemistry, transport processes and crystal structure. (Prerequisites: CHE 147, Analytical Techniques or CHE 127 Quantitative Analysis,; CHE 210, Physical Chemistry I)

CHE 215 – Physical Chemistry III

Surface chemistry, statistical thermodynamics, kinetics. (Prerequisites: CHE 211)

CHE 251 – Laboratory Projects in Polymer Science

Individual research projects, syntheses and characterizations of novel polymers. (Prerequisite: CHE 175)

CHE 261 – Instrumental Analysis

Lecture and laboratory course dealing with the use of modern instrumentation in chemical analysis. (Prerequisites: CHE 215)

CHE 341 – Experimental Biochemistry

Covers classical and modern techniques for isolating and characterizing proteins, nucleic acids and carbohydrates. Two quarter hours. (Corequisite: CHE 340, Biochemisry I)

CHE 343 – Experimental Biochemistry II

Selected experiments in enzymology, cell membrane structure, and in molecular, viral, bacterial and animal genetics. Two quarter hours. (Prerequisites: CHE 341)

CHE 397 – Research

Acquiring skills in library and laboratory chemical research techniques. Variable credit. (Prerequisites: consent)

CHE 399 – Independent Study

Expanding one’s knowledge in chemistry on an informal basis by individual consultation with department faculty. Variable credit. (Prerequisites: consent)

Appendix 3: Chemistry Department Graduates List

1999	Bachelor's Degree	11	Master's Degree	15	Total	26
1998	Bachelor's Degree	7	Master's Degree	3	Total	10
1997	Bachelor's Degree	13	Master's Degree	3	Total	16
1996	Bachelor's Degree	18	Master's Degree	4	Total	22
1995	Bachelor's Degree	10	Master's Degree	4	Total	14
1994	Bachelor's Degree	8	Master's Degree	4	Total	12
1993	Bachelor's Degree	10	Master's Degree	12	Total	22
1992	Bachelor's Degree	8	Master's Degree	7	Total	15
1991	Bachelor's Degree	9	Master's Degree	8	Total	17
1990	Bachelor's Degree	2	Master's Degree	13	Total	15

Appendix 4: Undergraduate Student Research as Part of a Laboratory Course

Beginning in 1993, the curriculum has included a program developed to involve research projects for the nine laboratories of the Spring quarter of a three quarter introductory organic chemistry course.[6] These projects integrate research and learning experiences for the students via interdisciplinary approaches of sophomore organic and polymer chemistry. The individual research projects carried out during the last seven years in the organic laboratory have involved syntheses of various trisubstituted ethylene (TSE) compounds, and their radical polymerization and copolymerization with styrene. Each student was assigned a target TSE compound with a particular combination of functional groups. Over the years more than 150 new compounds (methyl α-cyanocinnamates, benzylidenemalononitriles and α-cyanocinnamides) have been prepared. None of the prepared TSE-sytrene copolymers had been reported in the chemical literature, thus making each individual research project a genuine discovery experience. Some of these projects were repeated, expanded, and subsequently presented and published by undergraduate and graduate students.[7]

The individual research projects idea has worked very well in the Organic laboratory curriculum here at DePaul. We have found that the element of discovery is both stimulating and motivating for students. The concept, research in undergraduate instruction via individual reserarch projects, is effective in training students, as is evidenced by enrollment, student evaluations and the success of students in subsequent summer research and post-baccalaureate careers.