Project Description

a) Institutional Background

The University of Puerto Rico - Humacao Campus (UPRH) is a four year Institution located in Humacao, in the southeastern region of Puerto Rico. Its full accreditation was reconfirmed by the Middle States Association of Colleges and Secondary Schools in 1994, and in 2000 by the Council of Higher Education of Puerto Rico. All programs requiring individual accreditation by professional organizations or national associations are also fully accredited. The student population is 4,450 and about 99% are Puerto Rican. They typically come from a rural, non farming area, and from a household of four or five. Eighty six per cent (86%) of the student body comes from the public school system and commutes mainly from the thirteen (13) municipalities in its target service area. Over 68% the of enrollees are women. Seventy-two percent (72%) are 17 to 21 years of age (only 10% are over 25). Sixty-nine percent (69%) receive federal financial aid and 12% hold jobs in order to pay for their tuition. About twenty seven percent (27%) of the total students enrolled in UPRH are pursuing a career in Natural Science and Mathematics.

The Institutional goal is to increase the graduate pool of scientists poised to make a substantial contribution to the Nation's scientific and technological growth. To achieve this goal, the Institution aim to: (1) increase the number of Puerto Rican scientists and mathematicians to support industrial development of Puerto Rico and to sustain the technological base of the country through increased education and research training opportunities at the baccalaureate level, and through future graduate programs at UPRH; (2) develop, nurture and encourage talented Science and Mathematics undergraduates to compete to doctorate degrees in their respective fields; (3) increase the number of high school applicants in areas of Natural Science and Mathematics; and (4) enhance the educational achievements of the Natural Sciences and Mathematic departments at UPRH by offering to the student body a wide range of activities that lead to the full educational and professional development of the students. At the present time, the UPRH has the fastest growing and developing Science and Mathematics Programs for any four year college or university in Puerto Rico. The UPRH offers technical programs leading to an associate degree in the areas of Chemical Technology and Electronics, and baccalaureate degree programs in Industrial Chemistry, Applied Mathematics, Applied Physics (major in Electronics), Coastal Marine Biology, General Biology, Microbiology, and Wild Life Management. It also has transfer programs in Social and Natural Sciences, and Allied Health programs offered within the University of Puerto Rico system, and an Extension and Continuing Education Program which provides non-credit courses geared at the professional and personal development of the young and adult population of the region.

The UPRH has also a strong commitment to enhance the educational and undergraduate research training in Natural Science. This is demonstrated by its participation in federal and local programs for the improvement of the education for undergraduate students and minorities. The faculty members have been successful in obtaining funds for upgrading the teaching laboratories with computer technology (DE-MSIP), for research (NIH-MBRS, NIH-AREA, ACS-PRF, EPA and NSF-RUI), and research training grants (NIH-MARC, ONR, NASA and NSF). About 46% of the science and mathematics faculty holds a Ph.D. degree, and many have Post Doctoral Studies. The number of students who are presently majoring in chemistry (B.S. Program) is 263. The Department has graduated more than 180 students from our Industrial Chemistry B.S. program during the last 6 years, of which 17% have entered graduate schools in Puerto Rico and in universities located in the United States. Most
of them either have finished or are completing their requisites for a graduate degree (Ph.D., M.D. or D.M.D.). Near 80% of the total graduates are employed, mostly in the chemical/pharmaceutical industry.

The Physics, Chemistry, Biology and Mathematics Department at UPRH are distinguished in Puerto Rico and in the international academic arena for their achievements in undergraduate research. More than 20% of our science students continued their studies in graduate or in medical schools, and more than 90% of the students majoring in Chemistry, who pursued graduate studies, have had research experience at UPRH. One of the most important academic goals of the UPR central administration in recent years has been to establish new innovative and technological oriented graduate programs in Physics, Mathematics and Chemistry. Therefore, the UPRH has been driven to initiate a MS program in Material Sciences in the Physics Department for the next academic year, and the Chemistry and Mathematics Departments are presently developing similar programs. For more than a decade, Physics and Chemistry faculty have been involved in research collaborations with the Río Piedras Campus, in which MS and Ph.D. graduate students from the Physics and Chemistry Departments carry out their research work at UPRH, and they graduate with a UPRH professor as their thesis advisor. Several MS and Ph.D degrees have been awarded through these extended programs. Currently, there are 2 MS and 2 Ph. D candidates in this type of collaboration in the Chemistry Department. To strengthen the existing collaboration of the UPRH with the Chemistry Graduate Program at UPR-Río Piedras, a proposal to NIH- BRIDGES program was submitted recently by Dr. Ana Guadalupe, Dean of Graduate Studies at UPR-Río Piedras. However, the success of our programs depends on the ability to provide undergraduate and graduate students with a challenging education, combining both, the theoretical as well as the experimental perspectives of science. To continue providing students with a highly competitive baccalaureate degrees with a strong instrumental hand-on experience, the chemistry department must keep up with the recent advances in science, and it must also impact not only Chemistry majors, but Biology, Physics and Mathematics students as well. Therefore, the department has undertaken a major curriculum revision to incorporate new technology and experimental techniques to most of the laboratories.

b) Research Activities

The goal of this project is to foster a productive research environment for graduate students and to enrich the undergraduate curriculum to continue training highly competitive students for a science career in industry or graduate school.

The following objectives will address this goal:

I. To upgrade the existing research capabilities of the Chemistry Department by the acquisition of an 300 MHz NMR Spectrometer which will satisfy the existing needs to further develop and strengthen the present undergraduate and graduate research projects.

II. To expand the research projects to new areas in organic synthesis, enzyme catalyst, material science, environmental sciences, biological chemistry, photobiology, and photophysics through this new capability and promote the teaching and training of students in these important relevant areas.

III. To improve the competitive capabilities of the College of Natural Sciences for recruiting new investigators interested in areas akin to these new resources.
IV. To incorporate state of the art NMR techniques used in research and in industry to our undergraduate curriculum.

Initially, there will be seven major users of the requested Bruker Avance-300Hz NMR spectrometer: Dr. Antonio Alegría, Dr. Carmelo García, Dr. Margarita Ortiz-Marciales, Dr. Ileana Nieves, Dr. Gabriel Barletta and Dr. Mirna Rivera all from the Department of Chemistry and Dr. Nicolas Pinto from the Physics Department at UPRH. They and their students will be using this instrument as an integral part of their research efforts. In addition, several other faculty (minor users) in our Science College will use the instrument in teaching and research courses: Dr. Juan Suarez (Spectroscopy and Organic Chem.), Prof. Jorge Castillo (Analytical and Instrumental Chem.), Prof. Sonia Vázquez (Environmental Chem.), Dr. Carmen López (Biochemistry), and Dr. Esther Vega (Biology) and from other campuses of the University of Puerto Rico and private Colleges will also be able to use the facilities. Among them are: Dr. Osvaldo Cox from Metropolitan University, who work in collaboration on heterocyclic chemistry with Prof. Marisol Cordero from UPRH.

Beside the collaborative graduate research program, the Science Departments have a strong undergraduate research program oriented to enhance the scientific and the rational design of the students in their research work. Students are fully exposed to an array of instrumentation and laboratory experiences necessary for their scientific formation. There are three to eight undergraduate students involved in each research project. The research program is one of the main activities involved in the current research program at our institution that not only serves graduates and undergraduates but also includes summer training for precollege and two year college students (NIH-BRIDGES) interested in Natural Science and Mathematics.

In the following paragraphs there is a list of the investigators (from UPRH-Department of Chemistry and Physics), followed by a brief description of their research interests and justification for the acquisition of this spectrometer.

**Dr. Antonio E. Alegría**

I. **Membrane/Buffer Partition of Semiquinones:** Semiquinones, are postulated as important intermediates in the cytotoxic activity of quinones. Some of these quinones, specially anthraquinone containing quinones, are postulated to initiate their cytotoxic action upon interacting with cell membranes. However, with the exception of two of our manuscripts, very little is known regarding the partition or interaction of semiquinones with phospholipid membranes. The localization of semiquinones within the phosphatidylcholine bilayers has been determined using EPR spectroscopy. However, the use of spin labels (5-, 12-, and 16-doxyl stearic acids) to determine preferential sites of semiquinone localization within the membrane is subject to the problem of the spin-label being reduced by the semiquinone and to the inherent reactivity of each doxyl moiety as a function of membrane localization.

**Instrument justification:** We will be using $^{31}$P NMR and $^{13}$C NMR spectroscopy of phospholipid small unilamellar vesicles (SUVs), for which well-resolved NMR peaks are detected for both nuclei. Addition of semiquinone solutions to suspensions of these SUVs should produce changes in line widths and $T_1$ values in the resonance peak of the phosphate group ($^{31}$P NMR) and in the carbon resonance peaks of the acyl chain, terminal methyl group and the methylene and methyl carbons of the choline site, depending on the semiquinone preferential
localization. The rate of change in $T_1$ and line width values as a function of bulk semiquinone concentration should indicate preferential sites of semiquinones within the phospholipid bilayer and/or the interphase.

II. Photosensitized membrane degradation by quinones. Quinones are currently being used in the development of artificial sun light energy storing devices. Phospholipid membranes are used to minimize back electron transfer from the reduced quinone (semiquinone/hydroquinone) to the chlorophyll cation radical. However, although quinones absorb in the sun light region, very little is known regarding the damaging effects that these light-absorbing species could cause to membranes. These damaging processes could pose a serious limitation to the efficiency of the artificial sun light storing devices. In our laboratory we have found that OH radicals, superoxide ions and singlet oxygen are formed upon UVA or visible light absorption by quinones. These species will promote lipid peroxidation and eventual degradation.

Instrument justification: The binding constant of quinones to SUVs can be obtained from $^1$H NMR spectroscopy by measuring $T_1$ values of quinone proton resonance peaks as a function of SUV concentration. A fast exchange binding process is expected where binding constants can be obtained from $T_1$, free, $T_1$, bound, and $T_1$ obs. The ability of membrane degradation as a function of quinone structure could be probed using 2D NMR spectroscopy of deuterium-enriched fatty acids embeded within phospholipids SUVs. Using this technique, the order parameter, S, could be measured as a function of irradiation extent in the sun-light region. Also, $^7$Li NMR spectroscopy could be used as a probe of membrane damage by measuring the change in either line width or $T_1$ of this nucleus when going from a highly concentrated Li$^+$ site inside SUVs to a dilute Li$^+$ site outside the membrane.

Dr. Gabriel Barletta

Enzyme Catalysis in Organic Solvents: An increasingly important consideration when planning the synthesis of biologically active organic molecules is the introduction of chirality. One of the methods to prepare enantiomerically enriched compounds is to use a chiral catalyst, such as an enzyme suspended in organic solvents. This approach has several advantages, for example, enzymes are highly enantioselective and specific catalysts, properties which can be controlled by the organic solvents used as the reaction medium. In addition, enzymes are inexpensive, they can be “recycled” and are non toxic. Unfortunately, the mechanism by which organic solvents influence an enzyme stereoselectivity, and thereby enable the rational design of stereoselective systems in non-aqueous media is still not well understood. Elucidating this mechanism is the general goal of this project.

There are several hypothesis for the chiral preferences of some enzymes suspended in organic solvents: (a) the solvent dependence of enzyme enantioselectivity is related to the enzyme flexibility in each of these solvents, (b) the enzyme enantioselectivity is governed by the desolvating properties of the portion of the substrate exposed to the solvent (during the formation of the tetrahedral intermediate), and (c) enzymes are generally not structurally defined in organic solvents, which in fact is our current hypothesis. To determine if (and how) organic solvents partially “denature” enzymes (and thereby change their tertiary structure), we study the selectivity and enantioselectivity of transesterification reactions between chiral alcohols and an activated ester, of several enzymes suspended in different solvents. This project was first funded in 1997 by two NIH proposals, and since
them the results obtained yielded three publications and two manuscripts ready to be submitted for publication.

**Instrument justification:** It is important to note that most of the substrates used in this study are synthesized in our laboratory, and as with any new compound reported, high field NMR data is required. Unfortunately, obtaining such data from Rio Piedras is not very feasible for reasons discussed elsewhere in this proposal. The instrument here requested will allow us and other laboratories from the department to obtain this routine, but extremely important data. In addition, we (as well as others) will be able to implement new NMR techniques to expand the scope of our research efforts, allowing us to be more competitive and innovative. Of the several new studies we will be able to conduct with the requested 300 MHz instrument, two experiments are on top of the list, and will be implemented as soon as the new facility is set-up. These are: (a) to use NMR techniques to determine the hydration of a protein suspended in organic solvents, a method which is more sensitive than Carl-Fisher titration, and (b) to determine the binding conformation of different substrates onto the enzyme’s binding pocket. These measurements (REDOR NMR) can be obtained in the solid or aggregated state (note that it has recently been reported the direct identification of an enzyme active site residues by solid-state REDOR NMR, with 300 MHz instrument). These studies will help us understand the mechanism of enzyme catalysis in non-aqueous media. The last one in particular, will be a valuable tool to elucidate the steric constrains and the conformational changes of the enzyme’s active site as a function of the solvents and of the structures of the substrates.

Dr. Carmelo García

**Photophysics and Photochemistry of Trycyclic Neuroleptic Drugs:** Our ongoing MBRS project on the deals with the synthesis, the steady-state photoconversion and the laser flash spectroscopy of promazine and imipramine derivatives. Antipsychotic or neuroleptic drugs are used primarily for the treatment of schizophrenia, but also have applications in mania, anxiety, dementia and drug abuse. The major used neuroleptics belong to the general trycyclic antidepressants family (phenothiazines, dibenzazepines and dibenzodiazepines). Most of the derivatives of these drugs do also produce serious side effects, including extra pyramidal syndrome (EPS), tardative dyskinesia, parkinsonism, allergy and photosensitization. Small changes in the structure of the derivatives, change the mode of action of the drugs, the potency and the spectrum and severity of the side effects. The molecular photochemical mechanisms for their photosensitizing ability are still unknown, even through they are actually used in the United States to treat thousands and thousands of psychiatric patients annually. Recent studies on the laser flash photolysis of some phenothiazine derivatives showed that the triplet state of chlorinated phenothiazine derivatives can be efficiently quenched by the protons in the solution. The effectiveness of the quenching is very sensitive to the structure of the drug and seems to correlate with their phototoxicity.

The goal of ours project is to measure the photophysical properties of a selected group of trycyclic neuroleptics and to study their short-lived transients, especially the cation radical and the triplet. Basic UV-Vis and luminescence techniques will be employed to study their absorption/emission properties. The transients will be characterized using optical absorption measurements with a Nd-YAG laser set-up. For the triplet state of these compounds, the extinction coefficient and the quantum yield will be determined using a comparative method and the triplet-triplet energy transfer principle, respectively. Combined MM+/PM3/RHF theoretical calculations will be performed with HyperChem™ on the whole set of photophysical parameters. The experimental and
theoretical values will be correlated with the phototoxicity of the drugs to find out if the triplet transient is directly involved in the phototoxic activity of this drug family. The major goal of this project is to find a molecular/photophysical descriptor for the phototoxic side effect of tricyclic antipsychotics.

Instrument justification: The oldest and most widely used method of synthesis is the reaction of diarylamines with sulfur (Thionation), catalyzed with iodine. The main disadvantage of this method is that two isomeric phenothiazines are obtained for meta-substituted amines (2- and 4-isomers). The isomers are separated by means of column chromatography. The presence of the corresponding substituent (R = OCH$_3$, CH$_3$, COCH$_3$, SCH$_3$, and CF$_3$) is demonstrated with IR spectroscopy. The position of each of this substituent can be assigned using the characteristic bands for ortho and asymmetrical substituted benzenes. The infrared of asymmetrical trisubstituted benzenes (2-isomer) show a characteristic deep band in the region 12.0 - 12.5 μ, while vicinal trisubstituted benzenes (4-isomer) have the band in the region 12.5 - 13.15 μ. Nevertheless a high field NMR study is a better method to differentiate the isomers. The substituted aromatic carbon can be distinguished by the decrease in the $^{13}$C-NMR peak height. Since it lacks a proton, it suffers from a longer T$_1$ and diminished NOE.

Dr. Ileana Nieves

$^{51}$V and $^{31}$P NMR studies of the Na$^+$-K$^+$ATPase in the presence of vanadate and ouabain inhibitors:

Na$^+$ - K$^+$ ATPase exchanges Na$^+$ and K$^+$ across the cellular membrane against electrochemical gradients and controls muscle contraction. It is an E$_1$- E$_2$ enzyme oscillation between two conformations in the transport cycle producing inorganic phosphate (P$_i$) after the cycle is completed. Ionic vanadium inhibits plasma membrane Na$^+$ - K$^+$ ATPase binding at the aspartyl residue that is phosphorylated during the normal catalytic cycle at the site from which the phosphate is normally released. Studies by Hensen and Myers demonstrated that Na$^+$ - K$^+$ ATPase treated with vanadate in combination with Mg$^{2+}$ promotes ouabain binding (well known enzyme inhibitor) but ouabain binding is antagonized by vanadium in the presence of K$^+$. They concluded that the inhibition by ouabain is simply added to an existing enzyme inhibition by intracellular vanadate. These results are in apparent contradiction to other studies where the inhibitory ability of ouabain and vanadate infused simultaneously, was completely abolished resulting in a massive vasodilation, opposite to what is expected for the inhibited enzyme. The contradictory experimental results suggest that the study of the interaction of the two inhibitors simultaneously and in situ with the enzyme is essential.

The main objective of this work is to determine the effects of both vanadate and ouabain on the Na+-K+-ATPase activity according to the following specific aims characterizing: (1) the interaction between ouabain and vanadate by $^{51}$V NMR and $^{13}$C NMR; (2) the interaction vanadate species with Na$^+$-K$^+$-ATPase in solution and in proteoliposomes, with and without ouabain, using $^{51}$V NMR and using 2D $^{51}$V EXSY and variable temperature NMR spectroscopy to measure the rates of interconversion of the vanadate oligomers in solution. (3) the activity Na$^+$-K$^+$-ATPase, monitoring P$_i$ by $^{31}$P NMR in the presence of vanadate and ouabain individually and simultaneously.

$^{51}$V nucleus has very favorable NMR properties to study the interaction of vanadate with small and large biomolecules that provide good information about the vanadium coordination environment. Vanadium phosphate analog, responsible for the enzyme inhibition, may spontaneously esterify hydroxyl groups of small biological molecules (as ouabain) which may be accepted as enzyme substrates replacing the normally phosphorylated.
substrate. In this way the inhibition of both ouabain and vanadate will be abolished. $^{51}$V NMR will be very useful in the detailed and quantitative analysis of the vanadate esterification. Also, $^{51}$V NMR signals of the different complex geometries have been well characterized in terms of line width and chemical shifts, allowing the characterization of the postulated ouabain-vanadate complex. $^{31}$C NMR will be used as alternative tool to identify interactions with the ligand (ouabain) in aqueous unbuffered solutions.

In any enzyme study the aqueous vanadate chemistry taking place in the assay solutions could dictate the responses of the biological system under examination. $^{51}$V-NMR signal of vanadate species interacting with the enzyme will be monitored as a function of ouabain under controlled pH, concentration, ionic strength and buffer conditions because protonation, oligomerization and complexation of this oxyanion depend markedly on these conditions. Vanadate interactions with other enzymes are well documented in the literature. $^{51}$V NMR can also be used to determine the inhibiting monovanadate concentration in enzyme kinetics. 2D $^{51}$V EXSY and variable temperature NMR will be done to measure the effect of ouabain in the rates of interconversion of the vanadate oligomers that are important under the physiological conditions studied. The results will be correlated with the abolition of the inhibitory effect of vanadate in the presence of ouabain.

The enzyme activity with vanadate as a function of ouabain is determined by the P$_i$ liberated by the hydrolyzed ATP using $^{31}$P NMR. All these results will provide an explanation for the abolition of the inhibition properties of ouabain and vanadate.

**Instrument justification:** The instrumentation available at our Institution (JEOL FX-90Q) allows us to perform experiments only at relatively high vanadate concentration where the oligomeric species predominate. Inhibition of Na$^+$ - K$^+$-ATPase has been attributed to the monomer, therefore oligomer formation should be avoided. The instrument requested in this application can analyze samples at lower and physiologically relevant vanadate concentrations. Also, the experimental procedures had to be done for long periods of time in order do obtain reliable results due to the low sensitivity of the JEOL FX-90Q. The experiments we described above are less time consuming using the instrument we are requesting and also an adequate signal to noise ratio will allow us to determine the various species of vanadate involved in the inhibition mechanism.

**Dr. Margarita Ortiz**

1. **Synthesis of Chiral Amino Compounds.** New technology for the preparation of chiral compounds in organic chemistry is an area of intense developments due to the concern that many chiral drugs are consumed as racemic mixtures with the potential toxic effects caused by the other enantiomer. Chiral amino derivatives are important organic compounds used as building blocks for the synthesis of many pharmaceutical products, and auxiliaries in a variety of enantioselective organic preparations.

The design of new methodologies for the enantioselective synthesis of enantiopure compounds with biological activity will be the main purpose of this project. Through our previous research work, we have developed new procedures for the synthesis of primary phenyl alkylamine with modest to good optical purity via the reduction of $N$-substituted organometallic imino derivatives such as, $N$-substituted silyl-, silyloxy- and borylimino compounds, using known chiral organoborane reagents. Presently, we envision the application of the developed methodologies for the preparation of polyfunctional amino derivatives. Additionally, the synthesis of novel chiral aminoborohydrides and organoboranes reagents generated by the reaction of 1,3,2-oxazaborolidines with
organolithiums, will be investigated for the reduction of imine and/or carbonyl groups. These reagents offer a great potential for a variety of organic applications. Representative prochiral imines and aromatic ketoimines will be reduced with the proposed chiral agents and their enantioselectivity studied under various reaction conditions. We will continue to study the chemistry of O-silylated oximes for the synthesis of heterocyclic compound and other amino derivatives. In addition, based on the results of the \( \alpha \)-alkylation and silylation of O-TBS aromatic ketoimines, asymmetric induction for the preparation of non-racemic products will be studied using chiral amides as bases, or chiral catalysts. The proposed synthetic strategies will be focused on the development of new methods for the preparation of homochiral compounds used as intermediaries or reagents for the synthesis of important pharmacological products.

**Instrument justification:** From 5 to 6 undergraduates, participate in this project per year, in which they carry out their own research project. A Ph. D student from the UPR -Río Piedras Graduate Program, work on the synthesis of organoborane reagents as her doctoral thesis project under the PI supervision and in collaboration with Dr. José A. Prieto from UPRR. The graduate and undergraduate students use advanced chromatographic and spectroscopic techniques such as: FT-IR, GC/MS, FT-\(^1\)H, \(^{13}\)C, \(^{11}\)B, \(^{29}\)S-NMR, and HPLC required for the structure elucidation of intermediates compounds, and racemic as well as enantiopure products. Yet, the research possibilities have been restricted to stable final products due to the limitations of the available 90 MHz NMR on campus. Attempts to use the higher resolution NMR at the main campus in Rio Piedras for unstable organoboranes have been unsuccessful. With the availability of a modern FT- 300 MHz, temperature control NMR spectrometer, more sophisticated structural studies of this highly reactive reagents, such as, 1,3,2-oxazaborolidines, can be carried out. These organoboranes are widely used by this group as catalysts for the enantioselective reduction of prochiral imines; therefore, their previous characterization by \(^{11}\)B-, \(^1\)H-, and \(^{13}\)C-NMR provide the necessary information to start up the reaction. During the stereoselective reduction and at different reaction conditions, an NMR study will enhance the knowledge and understanding of the catalytic role in the reduction process. The enantioselective \( \alpha \)-alkylation, silylation and functionalization of \textit{N}-silyloximes and \textit{N}-substituted imines is another proposed research area that will be advanced with a higher resolution multinuclear NMR spectrometer to allow the study of organometallic compounds at low temperature. 2D NMR experiments will be decisive to show the stereochemistry of prepared bicyclic substrates, and to elucidate the structure of more complex multi functional intermediates and products. The determination of the enantiomeric excess of amines is measured using an HPLC technique. However, a high resolution NMR offers a better alternative, since it provides faster, sensitive and more reliable methods of analysis to measure the optical purity of chiral compounds and determination of their absolute configuration. By the acquisition of the requested NMR, the research participants will benefit in advanced knowledge, skills and research productivity that will prepare them better to promote their scientific career goals. During the past ten years more than sixty student have benefit from the research experience in this group. Two have obtained a medical degree, one a MS and more than ten are presently in a graduate chemistry program. During the last five years, five papers have been published (see M. Ortiz C. Vita) in which undergraduate students are coauthors.

**II Polyaniline as an UV Radiation Sensor** -Polyaniline is an important conductive polymer that has been well studied by Dr. Alan G. MacDiarmid from UPENN. We have a collaboration with him (NSF-CIRE) to develop novel radiation sensors using this type of polymer. The polyaniline was synthesized and characterized by
Acquisition of a 300 MHz Nuclear Magnetic Resonance Spectrometer to Enhance Research and Education at UPR-Humacao

P.I.: M. Ortiz - Marciales

IR and UV. The NMR of polyaniline and its substituted analogs can better characterized by NMR techniques. The conducting properties of this polymer depend on the ratio of quinoide imines groups to the aniline units in the chain. In this project, the reaction of 2-nitrobenzaldehyde (NBA), exposed to UV light, was studied using the emeraldine base as a pH indicator. The product of this reaction is 2-nitrosobenzoic acid, which can dope the EB, changing the color solution from blue to green. The blue EB solution in NMP, without NBA, showed an absorption band at $\lambda_{\text{max}} = 637$ nm. When a solution of $4.4 \times 10^{-7}$ mmol of basic polymeric units of EB and 0.106 mmol of NBA in NMP was exposed to UV light for 15 minutes, it changed its deep blue color to green, and showed an absorption band at $\lambda_{\text{max}} = 740$ nm, characteristic of the protonated emeraldine base. This changes can be studied by proton and carbon NMR spectroscopy. One undergraduate student work on this project.

Dr. Mirna Rivera

**Synthesis of Asymmetric-Disubstituted Ferrocenes:** Our research group studies the synthesis of asymmetric 1,2-disubstituted ferrocenes to evaluate their potential use as anticancer drugs. The ferrocenyl moiety is a versatile one with a wide range of applications in medicine, asymmetric catalysis, asymmetric synthesis, and synthesis of polymeric materials among others. Spectroscopy is the best tool to identify new ferrocenyl derivatives. It is necessary to fully characterize these compounds by $^1$H, $^{13}$C NMR, and other nucleus as $^{29}$Si and $^{31}$P that may be present in these compounds. For the ferrocenes with planar chirality, we plan to determine the diastereoselectivity and enantioselectivity using $^1$H NMR. This work can be done only using a high resolution NMR as the 300 MHz NMR that we are requesting in order to obtain the corresponding $^1$H and $^{13}$C NMR data. The students involved in the research course will have hands-on experience in the characterization and interpretation of the data. We have a weekly group meeting in which we discuss deeply the spectroscopic information and other topics. That experience will strengthen their skill and knowledge in chemistry.

Dr. Nicolas Pinto

**NMR as a Tool to Investigate Charge Dynamics in Conducting Polymers and Proton Glasses:** This research group will use the NMR equipment to study the dynamics of charge motion in conducting polymers and look for evidence of phase segregation. Polyaniline, an organic conducting polymer will be the focus of his investigation. It has been reported that only a small fraction of the charges are responsible for the observed bulk dc conductivity in polyaniline. This implies that the majority of the charge carriers present are trapped and unable to contribute toward charge transport.

**Instrument justification:** By deuterating selective sites on the polymer chain it will be possible via deuteron NMR to be able to study local charge dynamics together with the activation energies corresponding to barrier potentials that may be the source of the trapping. Further, the emeraldine base form of polyaniline in solution with 1-methyl-2-pyrrololidinone (NMP) is suggested to comprise of a rapidly changing diblock copolymer. Once the solvent is evaporated there appears to be a phase separation in the polymer. These include the benzenoid and quinoid repeat units found in the emeraldine base form of polyaniline. Since only an alternating benzenoid and quinoid sequence can be doped into the conducting state any phase separation of the two copolymer blocks which cannot be doped into the conducting form will introduce disorder and reduce conductivity. NMR will be used in this instance to probe any phase separation since phase separation will result in different relaxation times for the
same kind of nucleus as a result of different chemical surroundings. Spin-lattice and spin-spin relaxation times will be measured to determine chemical changes in the immediate environment of the nuclei under study. Prof. Pinto will also use the NMR instrument to study charge dynamics in proton glasses. These are systems that are partly ferroelectric and partly antiferroelectric leading to frustration in the electric dipole moment ordering upon cooling. Such glassy behavior has many potential applications in slow switching ferroelectric capacitors due to large relaxation times upon electrical stimulus. NMR on selected nuclei mostly associated with the glass transition will yield useful information on how one can best modify the system say by chemical substitution for enhanced performance as capacitors.

Undergraduate Education

The Department of Chemistry proposes to implement (in Organic Chemistry I/II, Instrumental Analysis, and Physical Chem. I/II Laboratories) a series of modern experiments to expose students to the newest instruments and techniques. These curricular improvements will be in the following areas: computational skills, practice in the interpretation of spectra (FT IR, NMR, GC, UV/Vis, Fluorescence, MS), and in the use of modern instrumentation currently used in industry and graduate schools. The department of chemistry has already begun to address some of these deficiencies by modifying existing laboratories and implementing new experiments.

The UPRH and the Department of Chemistry are devoted to provide students with a highly competitive undergraduate program in Industrial Chemistry. The Department believes that, in order to be successful, the education offered must have two inseparable parts: the theoretical and the experimental components. Therefore, the experimental activities in the department are devoted to hands-on experiences for students to complement the theoretical concepts learn during the lectures. To fulfill this commitment the Department of Chemistry has undertaken the endeavor of upgrading and acquiring a great variety of essential instruments, through various educational and research instrumentation proposals. Among these essential instruments, the department acquired eleven years ago, a broad-band, computer up-graded NMR JEOL FX-90Q to support the research undergraduate training program. Routine $^1$H and $^{13}$C NMR for structure elucidation of simple molecules and other nucleus (i.e., $^{51}$V and $^{31}$P NMR) are currently run in our facilities to provide limited support to the undergraduate research program.

c) Description of the Research Instrumentation and Needs

A Bruker-Avance 300 MHz NMR (att.: Fred J. Haberle III-Sales Manager -Tel.: (978) 667-9580) is requested to upgrade the current NMR facilities at UPRH with a total cost of $200,000. After a serious consideration of all NMR instrument available in the market, the Bruker-Avance-300 MHz NMR was the choice based-on the (1) greater capabilities of the instrument not only to cover our deficiencies but also will allow the development of our research environment in the future, (2) the experience of other NMR users in other institutions, and (3) the available services at lower cost since there are other Bruker NMR spectrometers, and (4) there are personnel at UPR-Rio Piedras that can assist us with the training and maintenance of the facilities.

The whole basic system Bruker-300 MHz NMR will allow the implementation of novel ID (NOE, DEPT,) and 2D experiments (COSY, HETCOR, NOESY), time dependent phenomena, and kinetic studies. All this will be supported by a user friendly software of the Bruker 300 MHz NMR for undergraduate students and faculty. The 300 MHz superconductor magnet will allow us to enhance the academic and research NMR capabilities providing:
Acquisition of a 300 MHz Nuclear Magnetic Resonance Spectrometer to Enhance Research and Education at UPR-Humacao
P.I.: M. Ortiz - Marciales

(1) higher and mandatory sensitivity for today's routine experiments, (2) great increase in the resolution for analysis of more complex molecules and, (3) less time consuming experiments that will allow us to serve a greater number of students in chemistry and in other departments. The Variable Temperature system is necessary for the organometallic, physical chemistry and biorganic projects. The state-of-the-art Silicon Graphic workstation provides access to advanced data processing in an easy-to-use environment for the students. The multi nuclear broadband probe is necessary to observe other nucleus besides $^1H$ and $^{13}C$ for research and for future experiments. The Broadband system does not limit; (1) the faculty to implement new experiments in the future, and (2) the education of the undergraduate students to the increasing number of applications of multinuclear NMR techniques in research and industry.

<table>
<thead>
<tr>
<th>Budget Description</th>
<th>DoD</th>
<th>UPRH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Equipment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A High Performance Digital Bruker- Avance-300 High Resolution FT-NMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The Basic System includes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Superconducting magnetic/ Shim System (bore diameter-52mm) operating at 7.05 T</td>
<td>198,900</td>
<td></td>
</tr>
<tr>
<td>b. Blaxh 100/50 Transmitter system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Quatro-nuclear (5 mm sample diameter) probe for proton, $^{13}C$, $^{31}P$, and $^{19}F$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. proton and carbon homo/hetero multi-mode gated decoupler with spin lock capability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Digital Acquisition Control System</td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Industry Standard Host Workstation with software including 1, 2 and 3D capabilities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. installation of the instrument</td>
<td>$4,500</td>
<td></td>
</tr>
<tr>
<td>•Printer and other accessories</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **B. Maintenance** |     |      |
| •Service contract/cryogenics liquids ($35,000 per year) | $35,000 | $3,020 |

| **C. Personnel** |     |      |
| •Release Time for Prof. Castillo ($8,500 per year) | $8,500 |      |
| •Full time technician ($30,000 per year) | $30,000 |      |

| Total Amount Requested to UPR-H |     | $41,520 |
| Total Amount Requested to DoD | $238,400 |      |

*The UPRH will contribute to the maintenance and the full time technician budget beyond the duration of the grant period.
The present NMR JEOL FX-90Q has serious deficiencies; (1) low resolution and limitations for today's NMR analysis of more complex and challenging structures and nuclei, (2) low sensitivity and therefore, even simple experiments take a long time to complete, forcing the faculty to carry out their experiments at the Center of Material Characterization at UPR-Río Piedras campus, where the UPRH faculty does not have priority. Besides, the 50 mile trips can take more than an hour and without parking space in this crowded campus, the student and the professors have to spend the day, without the security to run the experiments. Many times, the researchers have been forced to redo the experiments or to modified the objectives, (3) a mandatory use of high sample concentration, which is in the opposite direction of the currently microscale approach required in research laboratories, (4) reliable 2D $^1$H (and other nucleus) coupling experiments can not be run due to the low sensitivity of the instrument, (5) performance (sensitivity and stability) has been badly deteriorated, as expected, by the continuous use during these years, (6) the technology is 20 years old and it is not compatible with most of the modern routine NMR experiments, (7) no service contract and parts are available, and (8) the spectra data obtained are not acceptable for publication. Due to all these limitation, the NMR techniques taught at UPRH are far behind today's expected teaching level, and a mayor improvement in our curriculum is not possible with the current facilities.

d) Impact on the Quality and Quantity of Research Projects and Curriculum Improvement at UPRH Benefitted from the Requested Instrument.

The upgrade of the current instrumental facilities with a superconductor high resolution 300 MHz NMR instrument will: (1) strengthen the quality of the education offered to graduate and undergraduate students; (2) increase the number of Puerto Rican students trained in NMR that are pursuing a carrier in science at UPRH. The resolution of the new instrument will introduce the students to a more comprehensive analysis of complex molecules using 1D and 2D NMR techniques. This will give the department the opportunity to implement contemporary experiments (i.e., 2D $^1$H/other nucleus correlation experiments) currently excluded from the curriculum. In addition, the high resolution and high sensitivity of the new 300MHz instrument will allow to perform these experiments in less time, giving students the opportunity to use the NMR instrument during some of the laboratory courses. The new instrument will support the departmental efforts to increase the number of highly educated Puerto Rican students in Science, and to provide more and better research training opportunities for precollege and undergraduate students. The new instrument will provide a contemporary and more dependable "state of the art" tool for the faculty and it will keep open the "arena" for the implementation of innovative strategies for research projects.

The upgrade of the NMR facilities with a 300 MHz NMR will help to achieve the institutional and departmental goals for higher quality of education, and research training for a greater number of students interested in science at UPRH. The new instrument will also complement the departmental efforts to attract potential freshmen undergraduate students from the local high schools. The chemistry curriculum will be enriched through the implementation of new experiments that can only be included in the undergraduate courses if a high resolution 300 MHZ NMR is available. The laboratory courses that will be improved are (1) Organic (CHEM 3033/3034), (2) Spectroscopy (CHEM 3035), (3) Biochemistry (CHEM 4055), (4) Instrumental Analysis
Acquisition of a 300 MHz Nuclear Magnetic Resonance Spectrometer to Enhance Research and Education at UPR-Humacao

P.I.: M. Ortiz-Virgilio

P.I.: M. Ortiz-Virgilio

(4) Chemistry (CHEM 4016), and (5) Physical Chemistry (CHEM 4051/4052). A new (6) NMR course is proposed by the P.I. (to be taught prof. J. Castillo) which will be ready for evaluation by the Academic Affairs Committee in the near future. The 300 MHz instrument will also help to expand and improve the on-going research on campus, allowing for the implementation of new experiments - (7) Undergraduate Research Course (CHEM 4086). Students will be introduced to the basic concepts and NMR techniques in the introductory courses (CHEM 3033/3034). Further applications of NMR will be presented in a step wise manner throughout the advanced courses. About 260 students in chemistry and 540 students of related science disciplines will benefit from these curricular improvements. In addition, to stimulate the students’ rational design to solve a particular problem using NMR techniques, they will be exposed, by the end of their undergraduate career, to a series of experiments in NMR spectroscopy. This can only be accomplished with an ungraded - higher field NMR instrument and it may promote the cooperation of UPRH faculty with nearby research and development pharmaceutical industries.

Activities that will Impact Other Educational Groups and Natural Science Students

Recruiting talented students is an important feature of a successful Natural Science program. Open House activities are currently held in Natural Science at UPRH. The instrument requested will be available for activities to attract potential Natural Science students from high school and two year colleges and it may promote the cooperation of UPRH faculty with high school teachers to aid students interested in projects involved in science. It will also support the summer training program where precollege students participate in various activities at UPRH. As part of their summer training, the students are involved in short research projects where they are able to use the instrumentation available (including the NMR technique) according to the specific problem. The high resolution NMR instrument will enhance the chemistry B.S. educational program through the laboratory experiences described in the previous sections. The hand-on experiences proposed intend to increase the interest and motivation of the students to achieve a degree and pursue graduate degrees in their field. The use of the high resolution NMR instrument in the research course will allow the students involved, as well as the students of various minority educational programs (ie., ONR, MBRS, MARC), to be in contact with modern advanced NMR techniques. As described in the previous sections, NMR will be used as a tool to develop their research projects in many $^{1}H$, $^{13}C$ and multinuclear NMR application. The research students are more inclined to pursue graduate studies, therefore, these experiences will open new frontiers that are not available in our institution at the present time, to reach their goal.

In summary, the instrument will make a substantial contribution to the scientific and technological growth of the Chemistry, Biology and Physics departments by: (1) increasing the number of graduate students doing research at UPRH, (2) increase the capability of the faculty at Humacao to perform a meaningful and competitive research project, (3) promote the development of new graduate programs, (4) sustaining the technological base increasing science education and research training opportunities to undergraduate students and, encouraging talented students to pursue doctoral degree in their field.

e). Project and Management Plans

Qualification of the Key Personnel Dr. Gabriel Barletta (P.I.) and Professor Jorge Castillo are currently in charge of the operation, maintenance and operational policies of the NMR JEOL FX-90Q at UPRH. Dr.
Barletta and Professor Castillo have extensive experience in superconductor NMR instruments (200, 300, and 500 MHz with Bruker, Varian and JEOL instruments). Their experience include implementation and extensive use of 1D and 2D NMR techniques for elucidation of complex molecules structure. Dr. Barletta and Professor Castillo participated in courses about concepts and operational techniques in NMR and advance interpretation of 1D and 2D NMR given by a world expert in NMR; Dr. Daniel Traficante, at the University of Rhode Island. The PI has broad experience in elucidation of organic molecular structures using high resolution NMR (GN-300 MHz, Varian 300, 400 and 500 MHz) at other institutions, and she is currently an intensive user of the NMR FX-90Q. Dr. Nieves and Dr. Antonio Alegria were the P.I.’s of the NIH instrumentation proposal that funded the JEOL FX-90Q NMR. Their research interests involve magnetic resonance and Dr. Nieves participated in courses of high resolution NMR with Dr. Luis Echegoyen, as well as with Dr. José A. Prieto. She has extensive experience in 1D, $^{31}$P and, $^{51}$V NMR and is also a user of the FX-90Q. A full time specialized technician with at least a bachelors degree in chemistry is required to run the NMR facilities and assure a proper maintenance and operation of the instrument. The technician will be provided by UPRH.

**Equipment Implementation and Maintenance**  The requested instrument will occupy the Instrumentation Laboratory (20’ X 20’) facility currently used by the NMR JEOL FX-90Q. The room is accessible to teaching and research laboratories and located near other instrumentation facilities. The laboratory will be prepared with all the instrumental specifications required for a superconductor NMR prior to the arrival of the instrument. Prof. Castillo will be in charge of the supervision of the use and operation of the instrument and he will be assisted by a technician (provided by the institution) who, in addition to be in charge of the operation of the instrument, will be responsible for maintaining the cryogenic system. A committee integrated by the P.I., Dr. Barletta, and Professor Castillo will be in charge of the current and future implementation of operational policies. This committee will be in charge of implementing any changes and/or modifications to the operational policies of the instrument. In addition, this committee will coordinate the installation, start-up of the equipment, and the preparation and implementation of regular training sessions for the students and the faculty. In addition, Dr. José A. Prieto, a well known professor for his expertise on NMR techniques and operation of an NMR research facility from the UPRR Materials and Characterization Center, will offer assistance and seminars on the proper management and use of this instrument. The P.I. will record periodically the status of the operation of the instrument (including the performance of the new experiments), the number of users (students and faculty), the time distribution related to the use of the instrument, and the progress on the implementation of the proposed curriculum improvements. The P.I. will also provide relevant information to the university community and to the faculty about the capabilities of the instrument and the new teaching strategies available with the instrument. This information will be disseminated through the Internet system. The expected life of the new instrument is 12 years.