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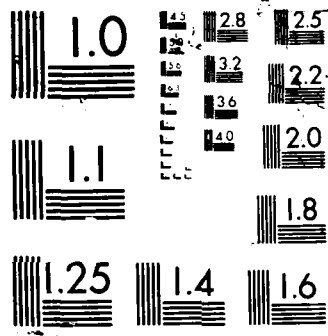
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**NORTH CAROLINA BIOMOLECULAR ENGINEERING AND
MATERIALS APPLICATIONS CENTER (NC-BEMAC)**

**FINAL REPORT
TO
OFFICE OF NAVAL RESEARCH
DECEMBER, 1987**



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1.0 EXECUTIVE SUMMARY

1.1 Overall Objectives

The overall objective of this program was to initiate a multi-disciplinary and multi-institutional research program involving the innovative application of biotechnology to materials science problems.

1.2 Significant Accomplishments

Fundamental research on the activation of CO_2 by carbonic anhydrase modified by the substitution of cobalt(II) for zinc in the enzyme demonstrates that rapid conversion of CO_2 to HCO_3^- occurs in the active site adjacent to the metal and that the catalytically active HCO_3^- is coordinated to the cobalt.

Techniques for the immobilization and activity characterization of bovine carbonic anhydrase on porous silica beads and graphite rods have been developed. The enzyme immobilized on porous silica beads maintains catalytic activity in nearly anhydrous organic solvents. We have also produced polyclonal (rabbit) and monoclonal (murine) antibodies to carbonic anhydrase for enzyme immobilization applications.

We have successfully prepared and characterized several low molecular weight complexes containing low valent transition metals which have potential for binding and activating small, biologically, relevant molecules. Multinuclear variable temperature NMR has been successfully utilized to study the solution dynamics of these complexes.

Tetraaza[14]annulene metal complexes were designed and prepared which could be electropolymerized on an electrode surface. Nickel(II) complexes of these polymerized macrocycles were used to electronically reduce CO_2 to HCO_3^- .

The nickel-containing cofactor (F_430) of the enzyme involved in the final step of CO_2 reduction in methanogenic bacteria has been characterized electrochemically and looked at for interaction with its substrate (CH_3CoM) and substrate analogs. The analysis indicated no specific interaction during electro-catalytic turnover.

A polyanionic sulfated glycoprotein was isolated from a molluscan shell, combined with various polymeric substrates, and found to induce mineralization at the interface with an aqueous solution. The minerals defined included various forms of calcium carbonate and of calcium phosphate. In order to induce bulk mineralization, strong hydrogels were prepared and seeded with a calcium phosphate microphase and further extensive mineralization was achieved throughout the material.

2.0 BIOCATALYTIC ACTIVATION OF CARBON DIOXIDE.

2.1 Original Objectives

An original objective was to study the binding and activation of CO_2 by Co(II) substituted carbonic anhydrase. This is a modified enzyme in which the zinc in the enzyme has been replaced with cobalt(II). A further objective was to investigate CO_2 activation by low molecular weight transition metal complexes as model systems for biocatalytic CO_2 activation.

2.2 Progress Made

2.2.1 Activation of CO_2 by a Modified Enzyme

NMR results on cobalt(II) substituted carbonic anhydrase (CA) suggest that catalytically active HCO_3^- is associated with tetracoordinate Co(II) in the active site and that the dissociation rate constant of HCO_3^- is only about 2.5 times the overall $\text{CO}_2 \rightleftharpoons \text{HCO}_3^-$ exchange rate constant, $k_{\text{cat ex}}$. We have also determined changes in catalysis and metal binding during protein folding and measured intracellular rates of CO_2 hydration using native CA.

2.2.2 Preparation and Characterization of Low Molecular Weight Metal Complexes as Potential Models for Bio-Catalytic Processes.

A number of transition metal complexes have been prepared and characterized which can act as active site models for enzyme catalyzed bioreactions. Carbon dioxide activation studies were initiated with a series of rhodium(I) phosphine complexes. Evidence for CO_2 activation by bis(cycloocta-1,5-diene)- μ_2 -bis[1-(3,4-dimethyl-3-phospholenyl)-3,4-dimethyl-3-phospholene] dirhodium(I) tetrafluoroborate $[\text{Rh}(\text{C}_8\text{H}_{12})\mu_2-(\text{C}_{12}\text{H}_{20}\text{P}_2)]_2(\text{BF}_4)_2$ was obtained, but the nature of the activation and intermediates formed were not completely elucidated. During the course of this investigation several new rhodium(I) phosphine complexes were synthesized and their solution dynamics investigated.

The solid state structure of bis(cycloocta-1,5-diene)- μ_2 -bis[1-(3,4-dimethyl-3-phospholenyl)-3,4-dimethyl-3-phospholene] dirhodium(I) tetrafluoroborate (1) $[\text{Rh}(\text{C}_8\text{H}_{12})\mu_2-(\text{C}_{12}\text{H}_{20}\text{P}_2)]_2(\text{BF}_4)_2$, was determined by x-ray diffraction. Two diphosphine ligands bridge two Rh(I) atoms to form a six-member Rh_2P_4 ring structure in a chair conformation. Solution studies (^1H and $^{31}\text{P}\{^1\text{H}\}$ variable temperature NMR spectra) exhibit characteristics of a ring inversion equilibrium with $G_{271}^\ddagger = 14.0(0.2)$ kcal/mole. Computer simulation of the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum was used to analyze the internuclear couplings of the AA'A'A'XX' pattern (A = ^{31}P , X = ^{13}CH). The bridging diphosphine ligands exhibit a deshielding effect on the trans olefinic carbon atoms of C_8H_{12} in (1) which is in the range normally found for phosphine ligands.

Conformational barriers in cis-phosphine complexes of two pairs of isomeric ligands $\text{Rh}(\text{COD})(\text{L})_2$, $\text{L} = \text{anti- or syn-9-phenyl-phosphabicyclo}[4.2.1]\text{nona-2,4,7-triene}$ (1 or 2) or $\text{L} = \text{anti- or syn-9-phenyl-phosphatricyclo}[4.2.1.0^{2,5}]\text{nona-7-ene}$ (3 or 4) were studied by variable temperature ^{13}C , ^{31}P and ^1H NMR spectroscopy. Conformational barriers result from interligand steric interactions encountered during Rh-P bond rotations. These barriers are quite sensitive to individual ligand structure, with $\text{L} = \text{1 or 3}$ exhibiting no conformational preference within the temperature range studied. The symmetries of the low-energy conformations for $\text{L} = \text{2 or 4}$ were assigned by considering the change in their chemical environments. Their conformer populations were determined by their ^{31}P and ^1H NMR spectra ($\text{L} = \text{4}$) and ^{13}C NMR spectra ($\text{L} = \text{2}$). Differences between the low-energy conformations for complexes with $\text{L} = \text{2}$ and $\text{L} = \text{4}$ are due to different ligand dimensions.

The hydrogenation and isomerization of 1-hexene homogeneously catalyzed by $\text{Rh}(\text{COD})\text{L}_2$, ($\text{L} = \text{P}(\text{Ph})_3$, 3, 4) were investigated in acetone solvent in order to assess the influence of inner coordination shell steric crowding on chemical reactivity. The relative initial rates of catalyzed hydrogenation of 1-hexene are 1:7.5:11 for $\text{L} = \text{P}(\text{Ph})_3$, 3, and 4, respectively. The rate of isomerization of 1-hexene is faster for a $\text{Rh}(\text{COD})(\text{3})_2^+$ catalyzed reaction than for a $\text{Rh}(\text{COD})(\text{4})_2^+$ catalyzed reaction. The difference in hydrogenation and isomerization rates for $\text{Rh}(\text{COD})(\text{3})_2^+$ and $\text{Rh}(\text{COD})(\text{4})_2^+$ catalyzed reactions are discussed in terms of the greater steric crowding imposed on the inner coordination shell of the catalyst in the case of syn phosphine ligand isomer 4.

As a part of our work to use ^{31}P NMR to characterize the dynamic solution properties of metal phosphine complexes we have developed a model for stereochemically dependent ^{31}P values which relies on the identity of the chelating/bridging substituent and stereochemistry. In this model we show that complexing agents, chelating agents, and lone electron pairs can all be treated similarly as different substituents in developing an understanding of their influence on ^{31}P . This model will enhance our understanding of P-C coupling phenomena and our ability to use NMR as a tool for dynamic solution structural work with organophosphines and their metal complexes.

In parallel work with iron, dimers of phosphole (2,5-phosphaborabenzene) with μ_2 -anti configuration at the bridging phosphorus were found to react with $\text{Fe}(\text{CO})_5$ to give complexes, $\text{Fe}_2(\text{CO})_4$, with complete retention of configuration, contrary to a report in the literature, isomers of 9-phenylphosphole, 4,7,1]nona-2,5, also formed iron(CO) complexes with complete retention of configuration.

3.0 ENZYME IMMOBILIZATION

3.1 Original Objectives

1) To immobilize a model enzyme (carbonic anhydrase) on various supports and determine surface coverage, stability, and activity of immobilized enzyme in both aqueous and non-aqueous media.

2) To develop antibodies to carbonic anhydrase for use in enzyme immobilization.

3.2 Progress Made

3.2.1 Preparation and Activity of Bovine Carbonic Anhydrase Immobilized on Porous Silica Beads (CPG) and Graphite

Techniques for the immobilization of bovine carbonic anhydrase (BCA) on porous silica beads and graphite have been developed. Surface coverage on porous silica beads was found to be 1.5×10^{-5} mmol BCA/meter² and on graphite 1.7×10^{-3} mmol BCA/meter² nominal surface area. Greater than 97% (silica support) and 85% (graphite support) enzyme activity was maintained upon storage of the immobilized enzyme for 50 days in pH 8 buffer at 4°C. After 500 days storage, the porous silica bead immobilized enzyme exhibited over 70% activity. Operational stability of the enzyme on silica at 23°C and pH 8 was found to be 50% after 30 days.

Catalytic activity, expressed as an apparent second order rate constant, k_{app} , for the hydrolysis of p-nitrophenyl acetate (p-NPA) catalyzed by BCA immobilized on silica beads and graphite at pH 8 and 25°C is 1.2×10^4 and 5.6×10^4 M⁻¹ s⁻¹, respectively. The corresponding k_{app} value for the free enzyme is 0.1×10^4 M⁻¹ s⁻¹. Activity of the immobilized enzyme was found to vary with pH in a manner that the active site pK_a of the porous silica bead support is 6.25 and on graphite is 7.41. Possible reasons for a microenvironmental influence on carbonic anhydrase pK_a are currently being investigated. Current experimentation involves changing the microenvironment of the immobilized enzyme by means of an applied potential.

The enzyme storage on CPG beads obtained in our laboratory is similar to previously reported data for enzyme immobilized in various matrices, and comparable to enzyme activity in solution. Although directly comparable literature data are difficult to obtain, BCA immobilized in our laboratory on graphite and silica are comparable or greater activity to previous reports for immobilized BCA.

3.2.2 Catalytic Activity of Immobilized Bovine Carbonic Anhydrase in Mixed Aqueous/Organic Media

The catalytic activity of bovine carbonic anhydrase (BCA) immobilized on porous silica beads was determined in aqueous mixtures of ethanediol, acetonitrile, and dichloromethane. Enzyme activity was determined in each solvent system as an apparent second-order rate constant, k'_{ENZ} , for the hydrolysis of p-nitrophenyl acetate (p-NPA) at pH 8, 25°C. Immobilization was found to enhance the stability and activity of BCA in mixed solvent media relative to the free enzyme.

3.2.3 Anti-Enzyme Antibodies

To obtain monoclonal anti-carbonic anhydrase (CA) antibodies, fusions were performed using spleen cells from immunized mice of the BALB/c and of the RBF/Dn inbred strains. In both instances hybridomas producing anti-CA antibodies were identified and subsequently cloned and subcloned by limiting dilution. Most of the anti-CA monoclonal antibodies produced by these clones were of the IgM class although mice were repeatedly injected with immunogen and gave high titers of serum antibody as measured by enzyme immunoassay. Parent cell lines as well as first- and second-generation cloned cell lines were stored frozen and are being maintained at -135°C.

4.0 USE OF BIOMOLECULAR MODELS AS CATALYTIC MEDIATORS ON ELECTRODE SURFACES

4.1 Original Objectives

The overall objectives were to prepare and study inorganic macrocyclic compounds that were layered on electrode surfaces. Furthermore, experiments were planned to evaluate the utility of these modified electrodes as engineered catalysts, coatings, sensors, and a source for molecular level devices.

4.2 Progress Made

4.2.1 Synthetic Studies of Dibenzo[10]annulene Macrocycles and Their Transition Metal Compounds

A number of new dibenzo[10]annulene macrocycles and their transition metal compounds have been synthesized and characterized.

The "internally-bridged" macrocyclic compounds, $[10]_{1,2,3,4}$, tetraphenyl[10]annulene, $[10]_{1,3,4,7}$, and tetramethyl[10]annulene have been synthesized and it is expected that complexes containing the component parts taken together and in mixtures, spectroscopically and/or by x-ray were utilized to confirm the structure.

The attempted preparation of the tetraphenyl macrocycle via reaction conditions normally used in the synthesis of dibenzotetraaza[14]annulenes resulted in the formation of a diamine adduct of bis(1,3-diphenyl-1,3-propanedionato)-nickel(II). An earlier report of the synthesis of the tetraphenyl macrocycle as its iron(III) complex has been shown to be incorrect. The "iron(III) macrocycle" is a diamine adduct of an iron(III), 1,3-diketonate. The chemistry of bis(1,3-diketonato)nickel(II)bis(1,2-diamino-benzene) adducts was briefly investigated.

The use of an unsymmetric 1,3-diketone in the synthesis of dibenzotetraaza[14]annulenes raises the possibility of an isomeric mixture. If one of the diketone substituents is large, then this substituent may have a controlling influence on the distribution of the isomers. Ligands prepared from 1-phenyl-1,3-butanedione and some of its derivatives, as well as a ligand using 5-methyl-2,4-hexanedione, have been prepared, characterized and the effect of the bulky substituent examined.

4.2.2. New Macrocyclic Synthesis - Investigations of Template Mechanisms

The "sterically-crowded" ligand, 6, 8, 15, 17-tetra-phenyldibenzo[b,i][1,4,8,11]tetraazacyclotetradecene has been prepared and isolated as its nickel(II) complex. The reaction is low yield but a useful amount of product can be obtained on a reasonable (20 mmol) reaction scale. The template reaction proceeds through an [N,N"-(1,3-propane-diylidene)bis(1,2-benzenediaminato)]nickel(II) intermediate and this species has been isolated and characterized. The electrochemistry of the nickel(II) macrocycle indicates that it does not polymerize on the electrode surface. The second irreversible oxidation, the formation of the cationic π -diradical, is at a higher potential than that observed in other ligands and thus, if desired, the polymerization reaction can be avoided. The free ligand has been isolated and the copper(II) complex of Ph_2BzO_2 -[14]tetraene N_4 prepared by remetalation indicating that the coordination chemistry of this macrocycle is not limited to its nickel(II) complex. The preparation of the copper(II) macrocycle suggests that, like other dibenzotetraaza[14]annulenes, a number of transition metal complexes containing Ph_2BzO_2 -[14]tetraene N_4 can be synthesized.

In order to better understand the range of synthetically modified electrodes that are possible, the nickel(II) template coordination of 1,2-diaminobenzenediol and 1-phenyl-1,3-butanedione was investigated. The published structural assignment of $\text{Me}_2\text{Ph}_2\text{BzO}_2$ -[14]tetraene N_4 as the cis isomer is incorrect. This ligand was prepared by two different synthetic methods with each route producing only the cis isomer as determined by spectroscopy and x-ray crystallography. Holmes, et al., based the structural assignment on a nickel(II) complex that was incorrectly believed to be the reaction intermediate. The evidence obtained in this study

indicates that the template reaction intermediate is a $[N,N'-(1,3\text{-propanediylidene})bis(1,2\text{-benzenediaminato})]nickel(II)$ cation analogous to the intermediate observed in the synthesis of $Me_4\text{-}$ and $Ph_4\text{-Bzo}_2[14]tetraeneN_4Ni$. Two substituted derivatives of $Me_2Ph_2\text{-Bzo}_2[14]tetraeneN_4Ni$ were prepared and in each case the only isomer observed was the cis-macrocycle indicating that para-substituents on derivatives of 1-phenyl-1,3-butane-dione have no effect on the template condensation or the isomers formed in this reaction. To date, no evidence of trans- $Me_2Ph_2\text{-Bzo}_2[14]tetraeneN_4$ has been observed.

The nickel(II) template condensation of 1,2-diamino-benzene and 5-methyl-2,4-hexanedione was also investigated and this reaction yields a mixture of isomers. There are no structural features of the macrocyclic ligand that would allow steric interactions between the diamino-phenyl rings and the isopropyl groups to be eliminated thus, there is no sterically preferred isomer of $Me_2(i\text{-Pr})_2\text{-Bzo}_2[14]\text{-tetraeneN}_4$.

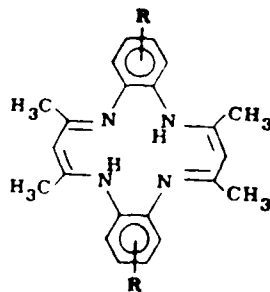
The attempted preparation of the sterically-crowded macrocycle, $Ph_4\text{-Bzo}_2[14]tetraeneN_4Ni$, via a template condensation results in the isolation of the bis(1,2-diaminobenzene)adduct of bis(1,3-diphenyl-1,3-propane-dionato)nickel(II). Extended reflux of this adduct does not induce macrocycle formation and the crystal structure illustrates the steric problems that might be encountered during macrocycle formation.

When solutions of bis(2,4-pentanedionato)nickel(II)-bis(1,2-diaminobenzene) were refluxed under different conditions, the macrocyclic yield varied greatly. Although the yield was greatest in refluxing 1-butanol, the standard literature procedures use ethanol or methanol. However, in order to achieve a reasonable yield using 1-butanol, it was necessary to add nickel acetate to the reaction mixture. It appears that the added nickel(II) is necessary to prevent oligermination of the nickel(II) 1,3-diketonate once the labile diamine ligands have dissociated. The macrocyclic yield is therefore enhanced if the formation of the dimeric compound, (1,3-diaminobenzene)-tetrakis-(2,4-pentanedionato)-dinickel(II), is minimized.

Bis(1,2-diaminobenzene) adducts of nickel(II) 1,3-diketonates are not macrocyclic intermediates but rather competing products and they do not provide an attractive alternative route to dibenzotetraaza[14]annulene macrocycles. Little or no adduct formation is observed in the literature procedures employed in macrocycle preparation and any that is observed dissociates and the component pieces reassemble. Adduct formation should be observed in situations where the condensation reaction is slow or, as in the case of 1,3-diphenyl 1,3-propanedione, completely hindered.

4.2.3 Transition metal complexes of Tetraazaannulene Ligands - Characterizations

A series of nickel(II) tetraazaannulenes complexes of the ligand $[\text{Me}_4(\text{RBzo})_2[14]\text{tetraeneN}_4]$ shown below, where $\text{R} = \text{H}, \text{CH}_3, \text{Cl}, \text{CO}_2\text{CH}_3, \text{CO}_2\text{C}_3\text{H}_7, \text{and NO}_2$, were investigated. The sensitivity of the complexes to substituents, in terms of their redox and spectral properties, was quantitated using the Hammett equation.



The resulting values have been discussed and analogies have been made between similar studies involving macrocyclic complexes and metalloporphyrins.

The copper(II) and cobalt(II) complexes, where $\text{R} = \text{H}$ and $\text{R} = \text{CO}_2\text{CH}_3$, were also examined and comparisons made between the electrochemical and spectral properties of the corresponding nickel(II) analogues. The products formed upon electrochemical oxidation and reduction were studied and identified using ESR and optical spectroscopy. The interaction of these complexes with superoxide was examined and the results have been compared to similar systems including metalloporphyrin superoxide adducts.

In a related study, it was found that nickel(II) and copper(II) tetraazaannulenes from electroactive polymer films on electrode surfaces following electrochemical oxidation. A systematic study of the physical and chemical properties of the films formed the nickel(II) series was carried out using XPS, AA, and optical spectroscopy. These data indicated that polymeric films were formed via coupling of ligand radicals at the carbon of the diiminate framework. The catalytic nature of the nickel(II) film formed from the $\text{R} = \text{H}$ derivative was investigated by following reduction of CO_2 to HCO_2^- (formate), utilizing cyclic voltammetric and ^{13}C NMR techniques. The analogous copper(II) film was also electroactive and catalyzed the reduction of O_2 to O_2^{2-} . Possible mechanisms have been discussed, based on similar catalytic studies involving surface modified electrodes.

4.2.4 Electrochemical Properties of Nickel Macrocycles

The preparation, spectral properties, and redox characteristics of a series of nickel(II) complexes was undertaken. The series consisted of the $[\text{Ni}(\text{L})]^{2+}$ complexes of the $[\text{Me}_4(\text{RBzo})_2[14]\text{tetraeneN}_4]$ ligand, where $\text{R} = \text{CH}_3, \text{H}, \text{Cl}$.

CO_2CH_3 , $\text{CO}_2\text{CH}_2\text{CH}_2\text{CH}_3$, NO_2 . Two irreversible oxidations, $E_{p(1)}$ and $E_{p(2)}$, were observed that ranged from 0.39 to 0.82 V and from 0.97 to 1.34 V vs. SSCE, respectively. A reversible reduction, $E_{1/2}$, was also observed that varied from -1.51 to -1.79 V. The oxidations compared favorably to the redox behavior of a copper(II) analogue and the free base ligand, and hence, were assigned as a ligand based oxidations. The nickel(II) complex ($R = \text{CO}_2\text{CH}_3$) was coulometrically reduced by one electron and an ESR spectrum obtained at 77 K. Two g values, $g_{\parallel} = 2.119 (\pm 0.002)$ and $g_{\perp} = 2.022 (\pm 0.002)$, characteristic of a nickel(I) derivative were obtained. Several intense uv-visible absorptions characteristic of charge transfer processes were observed. The bands in the near uv were assigned as intraligand transitions. The single band in the visible region was unique to the metal complexes and the energy maximum, ν_{max} , of the band ranged from 17.4 to 16.6 kK. Hammett plots of $E_{p(1)}$, $E_{p(2)}$, $E_{1/2}$, and ν_{max} (kK) vs. 2σ were linear and had slopes of 0.20, 0.19, and 0.23, and -0.36, respectively. Plots of ν_{max} (e.V.) vs. $E_{p(1)}$ and $E_{1/2}$ were also linear. On this basis and on the basis of the redox assignments of $E_{p(1)}$ and $E_{1/2}$, the visible absorption band was assigned as ligand to metal charge transfer.

The electrochemical, spectral, and chemical properties of a somewhat more limited series of Cu(II) and Co(II) tetraazaannulene complexes of the ligand $[\text{Me}_4(\text{RBzo})_2[14]\text{tetraeneN}_4]^{2-}$ ($R = \text{H}, \text{CO}_2\text{CH}_3$) were also investigated. The copper complexes exhibited two irreversible oxidation peaks centered at 0.37 and 0.87 V for $R = \text{H}$ and at 0.54 and 1.05 V for $R = \text{CO}_2\text{CH}_3$, whereas the cobalt complexes exhibited three reversible oxidations at $E_{1/2} = 0.001, 0.55$ and 1.04 V for $R = \text{H}$ and at 0.05, 0.67, and 1.21 V for $R = \text{CO}_2\text{CH}_3$. The $E_{1/2}$ values for reduction of the copper complexes were -1.39 V for $R = \text{H}$ and -1.17 V for $R = \text{CO}_2\text{CH}_3$, while the analogous cobalt complexes exhibited reduction peaks at -1.65 V and -1.45 V, respectively. A plot of the differences in redox potentials between the first ligand oxidation and metal centered reduction vs. the energy of the first visible transition was linear, verifying the LMCT transition assignment. Oxidation of the cobalt complex ($R = \text{H}$) by controlled potential electrolysis resulted in a blue absorbance shift from 16.8 kK to 18.4 kK after removal of the first electron and to 18.5 kK after removal of the second electron. The second oxidation product exhibited an isotropic ESR spectrum with $g = 2.0027 (\pm 0.0003)$. Oxidation of the copper complex ($R = \text{H}$) resulted in formation of a copper(II) dimer which exhibited an ESR signal with $g_{\parallel} = 2.149 (\pm 0.002)$ and $g_{\perp} = 2.045 (\pm 0.002)$. Electrolytic reduction of the copper complex ($R = \text{H}$) resulted in loss of the visible absorption band at 16.6 kK, whereas reduction of the cobalt analogue resulted in a red shift from 16.3 to 15.9 kK. The five coordinate $\text{Co}[\text{Me}_4(\text{CO}_2\text{CH}_3, \text{Bzo})_2[14]\text{tetraeneN}_4]\text{py}$ complex formed in the presence of pyridine and gave magnetic tensor parameters of $g_{\parallel} = 2.202 (\pm 0.002)$, $g_{\perp} = 2.0021 (\pm 0.0002)$, and $A_{\parallel} = 74.80 (\pm 0.5) \times 10^{-4} \text{ cm}^{-1}$; the superoxide adduct formed in the presence of O_2 and

pyridine and gave ESR parameters of $g_{\parallel} = 1.998 (\pm 0.002)$, $g_{\perp} = 2.086 (\pm 0.002)$, $A_{\parallel} = 8.95 (\pm 0.5) \times 10^{-4} \text{ cm}^{-1}$ and $A_{\perp} = 18.68 (\pm 0.5) \times 10^{-4} \text{ cm}^{-1}$. The interaction of the copper complex ($R = H$) with O_2^- was noted by the fact that the molar absorptivity doubled at 15.6 kK upon stoichiometric addition of O_2^- . In addition, O_2 was reduced at a more favorable potential at a surface modified electrode containing a polymer film of $Cu[Me_4Bzo_2[tetraeneN_4]]$, which was shifted 70 mV from the observed for a bare electrode.

4.2.5 Electrochemically Induced Polymerization-Surface Modified Electrodes

A series of surface modified electrodes based on the oxidative electropolymerization of the monomeric complexes, characterized earlier, $Ni[Me_4(RBzo)_2[14]tetraeneN_4]$ ($R = CH_3$, H , Cl , CO_2CH_3 , $CO_2C_3H_7$, and NO_2), were examined. The monomers polymerized to give polymers of the form $(Ni[Me_4(RBzo)_2[14]tetraeneN_4])_n$ which formed thin films on electrode surfaces. These films formed on the electrode by constant potential electrolysis at +1.4 V, cycling from +1.4 to -2.0 V, and cycling from 0 to +1.4 V vs. SSCE. Films formed on Pt, Au, glassy carbon and SnO_2 surfaces, in acetonitrile, methylene chloride, and propylene carbonate solutions containing various tetraalkylammonium salts as electrolytes. The magnitude of film growth depended on the method of deposition, the solvent, and the rate of cycling; i.e. five cycles from +1.4 V to -2.0 V vs. SSCE (at a scanning rate of 200 mV/s) resulted in 124 "monolayers" forming on a Pt disk electrode in an acetonitrile solution which was 0.1 M TEAP, 184 "monolayers" at 50 mv/s, and 12 "monolayers" in propylene carbonate at 200 mV/s. Film growth was also dependent on the electrolyte and the electrode surface. The modified electrodes, when transferred to neat electrolyte solutions, continued to exhibit three surface waves. For various R derivatives, $E_o'(1)$ ranged from 0.51 to 0.70 V, $E_o'(2)$ ranged from 0.92 to 1.12 V, and $E_o'(3)$ ranged from -1.25 to -1.84 V vs. SSCE. $E_o'(1)$ and $E_o'(2)$ were assigned as ligand oxidations, $E_o'(3)$ as a metal centered reduction. The surface of the film appeared smooth; however, at higher SEM magnification the surface exhibited a ridged appearance. Visible and infrared spectra suggested that the nickel macrocycle remained in the film; XPS data indicated that the Ni to N ratio was 1:4.

4.2.6 Electrocatalytic CO_2 Reduction

Oxidative electropolymerization of $Ni[Me_4Bzo_2[14]tetraeneN_4]$ resulted in the formation of stable, electrochemically active films on Glassy carbon (GC) and platinum (Pt) electrodes. Modified electrodes exhibited surface-bound redox active $Ni(II/I)$ centers which were catalytically active towards the reduction of CO_2 to $HCOO^-$ (formate). In cyclic voltammetric experiments, the catalytic reduction was carried out with a GC electrode in CO_2 -

saturated solutions. In the absence of $\{Ni[Me_4Bzo_2[14]tetraeneN_4]\}_n$, negligible current was observed when scanning from 0.0 to -2.0 V. However, when the same range was scanned using a GC electrode modified with $\{Ni[Me_4Bzo_2[14]tetraeneN_4]\}_n$, and a 30 mV shift in the potential of the Ni(II/I) surface wave were observed. Formate was identified, using ^{13}C nuclear magnetic resonance spectroscopy (NMR), as the main reduction product in controlled potential electrolysis experiments involving reduction of $^{13}CO_2$ at -1.85 V at a Pt electrode modified with $\{Ni[Me_4Bzo_2[14]tetraeneN_4]\}_n$.

5.0 METHANOGENIC BACTERIA

5.1 Original Objectives

To characterize certain aspects of the mechanism of reduction of CO_2 to CH_4 by methanogenic bacteria in order to better develop biomaterials for use in the catalytic reduction of CO_2 .

5.2 Progress Made

5.2.1 Summary of Nickel(II) Tetraazaannulene Film Electrochemistry With Methyl-Coenzyme M (CH_3COM)

In an effort to understand the role of F_{430} in the last step of CH_3COM reduction to CoM and CH_4 , the electrochemical reduction of CH_3COM was attempted using a nickel(II) tetraazaannulene film surface modified electrode as a model for F_{430} . The complex $Ni[Me_4Bzo_2[14]tetraeneN_4]$ (NiN_4) was synthesized and used to form stable electrochemically active films on glassy carbon electrodes by an oxidative electro-polymerization mechanism. In preparation for the proposed experiments with CH_3COM , these film electrodes were characterized in 2% v/v CH_3OH in 0.1 M TEAP/ CH_3CN with the added CH_3OH both solubilizing the CH_3COM and also providing the system with a proton donor for the reduction of CH_3COM to CH_4 . Film behavior in 2% v/v CH_3OH in 0.1 M TEAP/ CH_3CN was quite similar to that in 0.1 M TEAP/ CH_3CN alone with the Ni(II/I) reduction couple present.

Cyclic voltammetry control experiments in 2% v/v CH_3OH in 0.1 M TEAP/ CH_3CN indicated that neither CH_3COM or CoM were reduced at a naked glassy carbon electrode in the region +0.5 to -2.0 V (Ag/AgCl). To determine if CH_3COM and/or CoM would be reduced at a NiN_4 film electrode, a NiN_4 film electrode was first cycled in 0.1 M TEAP/ CH_3CN to establish its "background" scan and then either 1 mM CH_3COM or CoM in CH_3OH was added to the cell and the +0.5 to -2.0 V (Ag/AgCl) region scanned again. Slight differences in the scan appearances were observed; however, the peak current for the Ni(II/I) couple at -1.7 V (Ag/AgCl) did not increase and no catalytic wave was present. An increase in the Ni(II/I) peak current or a catalytic wave would be expected if interaction and electron transfer had occurred between the nickel center and the sulfur atom of CH_3COM . Experiments done at slower

scan speeds (5 mV/sec), with the NiN_4 complex and CH_3CoM both free in solution, and the same experiments in DMF with a negative potential limit of -3.0 V (Ag/AgCl) also indicated that on the time scale of the CV experiments no interaction was occurring between CH_3CoM and the NiN_4 film electrode.

To further explore the possibility of a Ni-S interaction on a slower time scale, a series of controlled potential electrolysis experiments were done involving NiN_4 film electrodes alone and with CH_3CoM in both 0.1 M TEAP/DMF and 2% v/v CH_3OH in 0.1 M TEAP/DMF. The electrolysis experiments were monitored for the presence of CH_3CoM , CoM and also disulfide by both current and total charge measurements, and also by TLC of aliquots removed from the electrolysis cell at various times during the electrolysis. These experiments also showed that CH_3CoM was not reduced to either CoM or $(-\text{SCoM})_2$ in 0.1 M TEAP/DMF or 2% v/v CH_3OH in 0.1 M TEAP/DMF by either a naked glassy carbon electrode or a NiN_4 film electrode. The electrolysis of CH_3CoM in 2% v/v CH_3OH in 0.1 M TEAP/DMF with NiN_4 complex in solution using a naked glassy carbon electrode also gave negative results.

It appears that although NiN_4 does share many similar structural features with F_{430} , the pronounced difference in axial electrophilicity between F_{430} and NiN_4 has prevented the observation of any interaction between the NiN_4 surface modified film electrode and CH_3CoM .

5.2.2 Summary of F_{430} Solution Electrochemistry, Alone and With CH_3CoM and Various Mediators.

F_{430} in the free acid form has been successfully isolated and purified from *Methanobacterium thermoautotrophicum*, YT strain. This isolated F_{430} has been lyophilized and studied electrochemically in 0.1 M TEAP/DMF. At a freshly polished Pt button electrode, a well resolved Ni(II/I) couple was observed with $E_{1/2} = 0.756$ V (Ag/AgCl) and $E^{\text{pp}} = 150$ mV @ a scan rate 20 mV/sec. The appearance of the couple was such that a scan rate dependence study could be done and the diffusion coefficient, D_0 , was found to be 3.6×10^{-6} cm²/s and the heterogeneous rate constant, k_s to be 6.0×10^{-4} cm/s. The observed $E_{1/2}$ value is in agreement with the published $E_{1/2}$ for the esterified form of F_{430} . At glassy carbon and gold electrodes, the electrochemistry of F_{430} was marginal in 0.1 M TEAP/DMF.

The solution electrochemistry of F_{430} in the presence of CH_3CoM , CoM and their two corresponding alcohols ($\text{CH}_3\text{SCH}_2\text{CH}_2\text{OH}$ and $\text{HSCH}_2\text{CH}_2\text{OH}$) was also studied at a freshly polished Pt electrode in 0.1 M TEAP/DMF. None of the added species exhibited any electrochemistry over the +0.25 to -1.1 V (Ag/AgCl) region examined. Some differences were seen in the appearance of the F_{430} couple with these added compounds, but there was no strong indication of a definite Ni-S interaction (i.e., no current increase in the Ni(II/I) couple and no catalytic wave observed). Experiments in which these same compounds were used to modify the

Pt surface before performing a cyclic voltammetry experiment with F_{430} were also done, but resulted in no strong evidence for Ni-S interaction.

The use of an additional reducing agent or redox mediator in the $F_{430}CH_3CoM$ system has also been studied. Dithiothreitol addition gave no effect in the system. FAD was also introduced as a mediator both free in solution and through the use of a FAD surface modified glassy carbon electrode, but again no current increase was observed in the F_{430} nickel couple in the presence of CH_3CoM and the added FAD.

6.0 A NEW CLASS OF MATERIALS: SYNTHETIC ORGANIC/CERAMIC COMPOSITES

6.1 Original Objectives.

- 1) To determine the particular components of biological matrices that promote mineralization.
- 2) To determine the influence of the bulk solution on crystal formation.
- 3) To fabricate hydrogels that will serve as neutral supports for the biologically active matrix components.
- 4) To mineralize these hydrogels and determine physical and mechanical properties.

6.2 Progress Made.

6.2.1 Studies of Biological Nucleating Agents

Our first goal was to determine which component(s) of the macromolecular matrix promotes mineralization in biological systems so that we could fabricate organic/ceramic materials by mineralizing special synthetic polymers. Because of our previous experience and the relatively less complex nature of the system, we used molluscan shell matrix as the biological model.

From our previous research, we knew that molluscan shell matrix is divided into two fractions; a larger, water insoluble, hydrophobic fraction and a smaller, water-soluble fraction that specifically binds calcium. The water-soluble fraction is a polyanionic, sulfated glycoprotein located at the site of initial crystal formation. This polyanionic site is localized in the hydrophobic domain of the insoluble matrix. It seemed likely that this polyanion has a central role in inducing mineral formation. For the polyanionic fraction to function in this manner, it must be fixed to a surface. In solution, the polyanion inhibits crystal growth. A similar model of mineral induction in which ethylene diamine tetraacetic acid (EDTA) was the anion has been described. When EDTA was absorbed onto

Formvar it induced mineral formation. When EDTA was free in solution it inhibited mineralization.

We began studies to determine whether the polyanion would induce mineralization from solutions having a calcium-carbonate activity product and pH like those of molluscan body fluids and which did not spontaneously precipitate.

The initial studies were conducted using the matrix from the nautiloid septal nacre. We decalcified the nacre with low ionic strength EDTA and 0.5% cetyl pyridinium chloride. From our previous work we knew that this procedure would fix the polyanion on the insoluble matrix. The insoluble matrix alone was used as a control.

Calcium carbonate spherulites were formed on the matrix surface during the 8-hour incubation period. Infrared analysis showed that the first deposits were amorphous. With an additional time (ca 4 hr.) aragonite was present. When the calcium activity of the solution was tripled, more numerous acicular and blade-like crystallites were induced on the surface. Some typical calcite crystals were also deposited. Mineral induction was not observed in the absence of the matrix nor when the insoluble matrix alone was incubated in the solutions. These results show that the polyanionic glycoprote in from molluscan shell induces mineral formation, but only when it is attached to the insoluble matrix.

These initial studies were made only in the early stages of mineralization, and they were conducted under essentially constant composition conditions in that we did not detect a change in either the calcium or carbonate concentrations. This approach was adopted to ascertain the function of the polyanionic glycoprotein in the induction of mineral deposition.

Our next experimental approach was to determine if the molluscan glycoprotein could induce mineral formation when it was attached to other insoluble substrates, such as synthetic hydrogels. In these studies we coupled the polyanionic protein to well-defined synthetic polymers, commercially available hydrogels. We also used a phosphate rather than a carbonate system for these studies because the anion is easier to control, since it does not exchange with the atmosphere, and because phosphate ceramics will have greater applicability than carbonates.

For these experiments we coupled the polyanionic glycoprotein isolated from molluscan shell with glutaraldehyde to polyacrylamide beads. This modified matrix induced calcium phosphate formation onto the beads from solutions that do not spontaneously precipitate. With longer incubations and a continuous supply of calcium and phosphate a solid mass of mineral and beads is formed. In the early stages, the molar

calcium/phosphate ratio in the deposited mineral is 1.2. This ratio increases to 1.5 with further mineral deposition.

The studies described thus far were designed to achieve mineral formation on the surfaces of natural and synthetic matrices. The question arose as to whether a sufficient supply of lattice ions could diffuse into the internal domains of hydrogels to form highly mineralized composites. This question was addressed directly by determining whether a hydrogel containing a ceramic filler can induce further mineralization. For these experiments polyhydroxyethyl methacrylate (polyHEMA) containing a fine calcium phosphate filler (40%) was incubated in the mineralizing solution. Additional mineral (up to 80%) was deposited in these filled hydrogels. These experiments indicate that a sufficient supply of lattice ions can diffuse into the hydrogels to form highly mineralized composites.

In summary, we have identified the soluble, calcium binding, sulfated glycoprotein as the matrix component that induced mineral formation when it is immobilized. This mineralization is effected whether the glycoprotein is attached to the insoluble fraction of the shell matrix or to synthetic hydrogels. We have also shown that lattice ions can diffuse into hydrogels at a rate sufficient for the mineralization of the hydrogel.

6.2.2 Hydrogels

Polyvinyl pyrrolidone (PVP) is biocompatible and provides gels which contain as much as several thousand percent water. Therefore, these gels are good candidates as matrices for biological mineralizing agents. In the limit of high mineralization, they might provide ceramic material with small amounts of residual polymer sufficient to limit crack growth and, thereby, brittle fracture. However, a disadvantage of PVP hydrogels is their mechanical weakness which makes handling difficult or impractical. For this reason, a major objective was to make highly swollen PVP hydrogels of increased strength. Some improvement can be affected by covalent crosslinking, but this is limited by increasing brittleness with increasing concentrations of crosslinks. In some other hydrogels it has been found that mechanical properties can be improved by control of chemical (covalent) and physical (especially hydrophobic) crosslinking. It was pointed out that small clusters of hydrophobic groups in a predominantly hydrophilic network may serve to increase load bearing yet with avoidance of the embrittlement caused by carbon-carbon crosslinks.

The hydrophobic monomers chosen for copolymerization with vinyl pyrrolidone (VP) were methyl methacrylate and methyl acrylate; tetraethylene glycol dimethacrylate (TEGMD) was introduced as a covalent crosslinker.

Hydrogels were prepared by gamma-irradiation of aqueous solutions. TEGMD had only a low crosslinking efficiency but

served to prevent phase separation, giving hydrogels of improved optical clarity and mechanical strength. As expected, inclusion of the hydrophobic monomers did improve strength over hydrogels prepared from just VP and TEGMD. A marked difference from ordinary hydrogels was in the occurrence of a yield strength. Hydrogels generally conform simply to the theory of rubber elasticity without a yielding phenomenon. Presumably in the VP copolymer hydrogels the stress is concentrated on physical (hydrophobic domain) crosslinks which eventually yield instead of giving rise to large unrelieved stress concentrations which result in fracture.

Information about the structure of VP copolymer hydrogels was deduced from measurements of glass transition temperatures, T_g , using a differential scanning calorimeter (DSC). Microphase separation could be detected by the occurrence of two values of T_g in a single sample and compositions analyzed quantitatively using a copolymer equation. By this means it was demonstrated that the best limit, microphase separation. A fuller account of work on VP copolymer hydrogels is given in an accompanying reprint.

For some purposes it would be desirable to be able to prepare VP copolymer hydrogels by methods other than by gamma-irradiation. A wide range of other free radical techniques were tried but without success. At present it appears that high energy irradiation is unique in yielding satisfactory products. This distinction is potentially important but is being checked further. In the past there have been too many premature reports of results unique to high energy irradiation.

Hydrogels made from VP present some problems in respect of efficient entrapment of nucleators of mineralization. These problems may be illustrated by analogous problems reported in previous studies of enzyme immobilization. When immobilization was effected by physical entrapment, i.e. without covalent binding, the enzyme suffered loss of activity during radiation polymerization. When covalent binding was used it was necessary to introduce suitably reactive functional groups by copolymerization plus post-polymerization chemical modifications.

A simple approach to the introduction of potential covalent binding sites is to copolymerize VP with various monomers which also yield biocompatible polymers, such as hydroxyethyl methacrylate (HEMA). Poly-HEMA hydrogels are well known, especially from their use in contact lens. In the present work studies were made of hydrogels prepared either from HEMA alone or with TEGMD as crosslinker. The hydrogels were made by gamma-irradiation of aqueous solutions (4% water). DSC studies of these hydrogels showed part of the water to be free and part to be bound, in agreement with previous work. This type of analysis should be useful in diagnosing whether mineralization begins in the free water zones.

One of the methods to be used to monitor the effect of mineralization of hydrogels on mechanical properties is by dynamic mechanical analysis (DMA). Preliminary analyses of unmineralized hydrogel controls (made from HEMA: 43% water) revealed a glass transition near -140°C . In contrast, dehydrated specimens (xerogels) had no transition near this temperature. The transition was attributed to vitrification of water, by comparison with reports that, from extrapolation of data on aqueous solutions, $T_g = -137 \pm 1^{\circ}\text{C}$ for water.

Although the crystallization of water can be prevented in very confined spaces on cooling, e.g. between sheets of glass separated at $<1 \mu\text{m}$, it is believed that vitrification of water is only to be expected at extremely high rates of cooling. Therefore, the present claim of vitrification is surprising. Other workers have interpreted similar observations on protein/water systems as an interfacial water phenomenon. An important factor in any interpretation is in a correct assignment of a value of T_g . Most workers in DMA characterization assign a value by reference to the temperature at which there is a maximal mechanical loss ($\tan \delta$). However a case has been made, in studies of hydrophobic crosslinked networks, that it is more appropriate to assign T_g by reference to the thermal dependence of moduli of elasticity. The main justification for this procedure is that it gives T_g values in better agreement with values obtained by more conventional assignments of the temperature dependence of thermal expansion.

Further evidence was given to the vitrification hypothesis by showing that gels prepared using glycerol, in place of water, vitrified at -80°C . This is the same value, as detected by DSC, for vitrification of glycerol alone.

Values of T_g Estimated for Glycerol Gels

Method	DMA		DSC
	E'	$\tan \delta$	
$T_g, ^{\circ}\text{C}$	-80	-60	-80

7.0. PUBLICATIONS

7.1. Journals

Redox and Spectral Properties of Ni(II) Macrocycles Containing Diaionic, Tetraazaannulene Ligands, C.L. Bailey, R.D. Bereman, D. Paul Rillema, and Robert Nowak, Inorg. Chem., **23**, 3956 (1984).

Surface Modified Electrodes: Oxidative Electropolymerization and Deposition of Ni-Tetraazaannulene Complexes, Cynthia L. Bailey, Robert D. Bereman, D. Paul Rillema, and N. Nowak, Inorg. Chem., **25**, 933 (1986)

Redox and Spectral Properties of Cobalt(II) and Copper(II) Complexes of $\{H_2(Me_4(RBzo)_2[14]tetraeneN_4)\}$ ($R=H, CO_2CH_3$). Evidence for Superoxide Ligation and Reduction, C.L. Bailey, R. D. Bereman and D.P. Rillema, Inorg. Chem., **25**, 3149 (1986).

The Reported Synthesis of the Tetraphenyl Tetraazaannulene Macrocycle: 6, 8, 15, 17-Tetraphenyldibenzo [b,i] [1,4,8,11] Tetraazacyclotetradecene, Richard P. Hotz, S. Purrington, and Robert D. Bereman, Inorg. Chim. Acta., **115**, L49 (1986).

Carbon Dioxide Reduction Mediated by Electropolymerized Electrodes of a Nickel Tetraazaannulene Complex, $Ni[Me_4Bzo_2[14]tetraeneN_4]$, C.L. Bailey, R.D. Bereman, D.P. Rillema, and R. Nowak, Inorg. Chim. Acta., **116**, L45 (1986).

1,2-Diaminobenzene Adducts of Nickel(II) 1,3-Diketonates: Their Role in Dibenzotetraaza[14]annulene Macrocycle Formation, Richard P. Hotz, Suzanne T. Purrington, Phirtu Singh, Robert Bereman and Ekkehard Sinn, Inorg. Chim. Acta., **130**, 195 (1987).

Synthesis of Ni(II) Complexes of the Elusive Ligand, 6,8,15,17-Tetraphenyldibenzo[b,i][1,4,8,11]Tetraazacyclotetradecene, R.P. Hotz, S.T. Purrington, and R.D. Bereman, Inorg. Chim. Acta., in press.

Synthesis and Electrochemical Properties of Pt(II) 5,7,12,14-Tetramethyldibenzo[b,i][1,4,8,11]Tetraza[14]annulene: Factors Affecting Electropolymerization Rates, Paul Huchgesand and R.D. Bereman, Inorg. Chim. Acta., submitted.

Ion transport nucleation of calcium carbonate by molluscan matrix. E.M. Greenfield, D.C. Wilson, and M.A. Crenshaw, American Zoologist, **24**, 925 (1984).

Electrically swollen hydrogels: Vinyl pyrrolidone copolymers, D.T. Hurst, A. Schwarz, S. H. Lee, R. Sugg, and J.L. Williams, Polymer, **27**, 615 (1986).

Mineral induction by the soluble matrix from molluscan shells, E.M. Greenfield, and M.A. Crenshaw, In R.E. Crick (ed.) Evolution of Ocean Biochemistry and Its Significance to Biomineralization. Elsevier, Amsterdam, in press, 1988.

In Vitro Mineral Induction by Soluble matrix from Molluscan Shells, E.M. Greenfield, E.M. Ph.D. Thesis in Marine Sciences, University of North Carolina at Chapel Hill, 1987.

The Synthesis, X-ray Crystal Structure and Solution Characterization of Bis(diphosphine) Bridged Di-Rhodium(I) Complex Containing a Rh_2P_4 Six-Membered Ring, A.L. Crumbliss, R.J. Topping, J. Szewczyk, A.T. McPhail and L.D. Quin, J. Chem Soc. Dalton Transactions, 1895 (1986).

Retention of Phosphorus Configuration on forming Irontetracarbonyl Complexes with Phosphines in the 9-Phosphabicyclo[4.2.1] nonatriene and 7-Phosphanorbornene Systems, A.L. Crumbliss, R.J. Topping and L.D. Quin, Tetrahedron Letters, 27, 889 (1986).

Preparation and Activity of Carbonic Anhydrase Immobilized on Porous Silica Beads and Graphite Rods, A.L. Crumbliss, K.L. McLachlan, J.P. O'Daly, and R.W. Henkens, Biotechnology and Bioengineering, accepted for publication.

The Activity of Carbonic Anhydrase Immobilized on Porous Silica Beads in Aqueous/Organic Solvents, J.P. O'Daly, A.L. Crumbliss, and R.W. Henkens, Biotechnology and Bioengineering, submitted for publication.

The Influence of Rigid Cyclic Phosphine Ligands In $\text{Cis-Rh}-(\text{COD})(\text{Phosphine})_2^+$ On Inner Coordination Shell Dynamics And Catalyzed Olefin Hydrogenation/Isomerization, R.J. Topping, L.D. Quin, and A.L. Crumbliss, Org. Chem., in preparation.

The Influence of Substituent Effects and Stereochemistry On the Magnitude of Internuclear Couplings Within Derivatized Organophosphines", R.J. Topping, L.D. Quin, and A.L. Crumbliss, J. Mag. Res., in preparation.

Dynamic ^{13}C NMR Investigations of Substrate Interaction and Catalysis by Cobalt(II) Human Carbonic Anhydrase I, T.J. Williams and R.W. Henkens, Biochemistry, 24, 2459 (1985).

Active Site Formation in the Last Stages of Folding of Carbonic Anhydrase, R.W. Henkens, F.H. Groover and R.A. Sheffey, Rev. Port. Quim., 27, 162 (1985).

The Rates of Fast Reactions of Carbon Dioxide and Bicarbonate in Human Erythrocytes Measured by Carbon-13 NMR, D.W. Hoffmann and R.W. Henkens, Biochim. Biophys. Res. Commun., 143, 67 (1987).

C-13 NMR Studies on The Active Site Structure and Kinetics of Human Carbonic Anhydrase I, R.W. Henkens, S.P. Merrill, and T.J. Williams, Ann. N.Y. Acad. Sci. 429, 143 (1984).

Mussel Glue Protein Has An Open Conformation, K. Marumo, H.H. Waite, R.W. Henkens, and T.J. Williams, Biochemistry, submitted.

Biologically Formed Composites, M. Crenshaw, Materials Research Society Symposium in Ceramics, Materials Research Soc. PA, 1988.

Phosphate Mineral Formation on Immobilized Matrix Proteins, A. Lussi, A. Linde, and M.A. Crenshaw, Calcified Tissue International, submitted for publication.

Induction and Inhibition of Hydroxyapatite Formation by Dentin Fossil Protein, A. Lussi, A. Linde, and M.A. Crenshaw, Arch Oral Biology, submitted for publication.

Mineral Induction by Immobilized Polyanionic Matrix Proteins, M.A. Crenshaw, A. Lussi, and A. Linde, Nature, Submitted for publication.

7.2 Oral Presentations

Coordination Chemistry of Ni(II) Tetraazaannulenes, Cynthia L. Bailey and R.D. Bereman, National ACS Meeting, August, 1984.

Comparative Redox Properties of Cobalt(II), Nickel(II), and Copper(II) Tetraazaannulene Complexes. Cynthia Bailey and R.D. Bereman, Southeastern Regional ACS Meeting, 1984.

Coordination of Diamines to Bis-(1,3-Diphenyl-1,3-Propanato)Nickel(II), R.P. Hotz, S.T. Purrington, and R.D. Bereman, Southeastern Regional ACS Meeting, 1984.

Oxidation Electropolymerization of Nickel Tetraazaannulene Complexes: Properties of Surface Modified Electrodes, C.L. Bailey, R.D. Bereman, D.P. Rillema, and E. Nowak, 1984 IES Symposium, Purdue University.

Synthesis of the Nickel(II) Complex of the Flexible Ligand: 6,8,15,17-Tetraphenyl-[1,1]-(1,4,8,11)-tetraazaannulene. R.P. Hotz, S.T. Purrington, and R.D. Bereman, ACS Meeting in Miniature, Duke University, April 11, 1984.

Synthesis and Characterization of Pt(II) Complexes of 1,7,11,14-tetramethylbenzo[b,1]-(1,4,8,11)-tetraazaannulene. P. Hochgesand and R. Bereman, Southeastern Regional ACS Meeting, November, 1984.

Molluscan Shell Formation, M.A. Crenshaw Symposium on Invertebrates, M.A. Crenshaw, Calcification, Toledo, June 1984.

In Vitro Crystal Induction by Molluscan Nacreous Matrix, M.A. Crenshaw, and E.M. Greenfield, Invited paper. Carnegie Institution of Washington Conference on Biomineralization Processes and the Fossil Record, Arlie, Virginia, April, 1985.

Biomolecular Engineering Materials Applications. M.A. Crenshaw, Invited paper, Carolina Conference on Marine Biotechnology, Rougemont, NC, April 1985.

The Molluscan Nacreous Layer as a Model for Biomineralization, M.A. Crenshaw, Invited paper, Gordon Research Conference on Bones and Teeth, Meriden, NH, July, 1985.

Macromolecular Matrix Control of Biomineralization, M.A. Crenshaw, and D.T. Turner, Invited paper, Symposium on the Chemistry of Contemporary Technological Problems. Control of Chemical Processes by Molecular Morphology. American Chemical Society Annual Meeting, New York, NY, April, 1986 .

Crystal Initiation by Molluscan Shell Matrix Proteins, M.A. Crenshaw, and E.M. Greenfield, Invited paper, Symposium on Autocatalysis in Physical, Chemical and Biological Systems. American Chemical Society Annual Meeting, New York, NY, April, 1986.

Mineral Induction by Molluscan Shell Soluble Matrix from Solutions Containing Ion Activities Similar to Extrapallial Fluid, E.M. Greenfield, and E.M. Crenshaw, Invited paper, Fifth International Symposium on Biomineralization, University of Texas at Arlington, Texas, May, 1986 .

The Synthesis and Characterization of a Bis-Diphosphine Bridged Dirhodium(I) Complex Containing a Rh_2P_4 Ring System, R.J. Topping, A.L. Crumbliss, A.T. McPhail, L.D. Quin, and J. Szerwcyk, 36th Southeastern Regional ACS Meeting, October, 1984.

Strained Heterocyclic Phosphine Complexes of the Type $Rh(COD)L_2^+$ and $Fe(COD)_2$, R.J. Topping, A.L. Crumbliss and L.D. Quin, 191st National Meeting, American Chemical Society, April, 1986.

Bioelectronics and Selectivity, A.L. Crumbliss, First North Carolina Workshop on Bioelectronics, Quail Roost Conference Center, Mount, N.C., October, 1986

Preparation and Activity of Carbonic Anhydrase Immobilized on Inorganic Polymer IV Support Materials, A.L. Crumbliss, R.L. McLachlan, M.L. O'Daly, and R.W. Henkens, 38th Southeast Regional Meeting, American Chemical Society, November, 1986.

Activity of Immobilized Carbonic Anhydrase in Aqueous/Organic media, A.L. Crumbliss, J.P. O'Daly and R.W. Henkens, 38th Southeast Regional Meeting, American Chemical Society, November 1986.

Rates of Fast Reactions of CO_2 and HCO_3^- in Human Erythrocytes Measured by C-13 NMR, D.W. Hoffman and R.W. Henkens, Third International Symposium on Structure and Dynamics in Biomolecules, Riva Del Giarda, Italy, 1986.

Dynamic C-13 NMR of Catalysis by Cobalt(II) Carbonic Anhydrase, R.W. Henkens and T.J. Williams, Second International Symposium on Structure and Dynamics in Biomolecules, Rome, Italy, 1984.

Active Site Formation in the Last Stages of Folding of Carbonic Anhydrase, R.W. Henkens, K.H. Grover, R.A. Sheffey and T.J. Williams, Second International Conference on Bioinorganic Chemistry, Algrave, Portugal, 1985.

Characterization of "Randomly Coiled" Denatured Carbonic Anhydrase by ^1H NMR, T.P. Oleksiak and R.W. Henkens, S.E. Magnetic Resonance Conference, Research Triangle Park, N.C. 1987.

Carbon-13 NMR Study of CO_2 and HCO_3^- Dynamics in Human and Llama Erythrocytes, D.W. Henkens, S.E. Magnetic Resonance Conference, Research Triangle Park, N.C. 1987.

Evidence that Mussel Glue Protein Has An Open Conformation, T.J. Williams, H.H. Waite, R.W. Henkens, and K. Marumo, FASEB Conference, 1988.

8.0 LIST OF NORTH CAROLINA BIOMOLECULAR ENGINEERING AND MATERIAL APPLICATIONS CENTER (NC-BEMAC) PRINCIPAL INVESTIGATORS

Dr. Robert D. Bereman, Professor of Chemistry, North Carolina State University; bioinorganic chemistry, electrochemistry, synthetic chemistry and model systems.

Dr. Miles A. Crenshaw, Professor of Pedodontics and Marine Sciences, University of North Carolina at Chapel Hill; biochemistry of marine organisms, principally extracellular macromolecules, biomineralization and biocomposites.

Dr. Alvin L. Crumbliss, Professor of Chemistry, Duke University; bioinorganic chemistry, kinetics and mechanisms of transition metal complex reactions, metal-specific chelators, redox proteins and metal-containing plasma polymers.

Dr. Robert W. Henkens, Associate Professor of Chemistry and Associate Professor of Biochemical Engineering, Duke University; Chairman, Enzyme Technology Research Group, Inc.; biophysical chemistry of proteins, including technological development of immobilized enzyme systems.

Dr. D. Paul Rillema, Professor of Chemistry, University of North Carolina at Charlotte; inorganic chemistry, electrochemistry and electrocatalysis, kinetics and mechanisms, photochemistry, and photoelectron transfer processes.

Dr. James N. Siedow, Professor of Botany, Duke University; plant biochemistry, enzyme kinetics and redox proteins.

Dr. Derek T. Turner, Professor of Oral Biology, Biomaterials Group, University of North Carolina at Chapel Hill; materials science, polymer chemistry and physics, and composite materials.

Dr. Carol C. Whisnant, Research Immunologist, Research Triangle Institute; immunology, macromolecular interactions with surfaces, and monoclonal antibodies.

9.0 GRADUATE STUDENTS

Cynthia Bailey, Ph.D., 1986, NCSU

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Rod Davis, (summer, 1987). Minority Student

Edward M. Greenfield, Ph.D., May 1987, UNC-CH, Marine Sciences

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