10^4 s^{-1} for the covalent complex. Similar relative decreases in both k_4 and k_5 from the electrostatic to the covalent complex probably reflect some small difference in protein orientation or in conformational fluctuation[43] between these complexes. Remarkably, both reactions k_4 and k_5 within the covalent complex remain fast. The same increase in the driving force, from 0.10 to 1.2 eV, is accompanied by different increases in the rate constant from k_2 to k_4, from 1300 to 2.5×10^5 s^{-1} in the electrostatic diprotein complex, but from virtually 0 to 2.2×10^4 s^{-1} in the covalent diprotein complex. The last two numbers indicate that the electron-transfer pathway via the remote patch in plastocyanin, to which each donor is cross-linked, is unfavorable for the reaction k_2 and favorable for the reaction k_4.

Comparison of electrostatic precursor complexes (k_1 vs k_3) and comparison of reactions in covalent complexes (k_2 vs k_4) consistently indicate that ferrocytochrome *c* does not, whereas zinc cytochrome *c* does, reduce cupriplastocyanin when bound at the remote patch. Although the two reductants have the same topography and electrostatic properties, perhaps the stronger one (the latter) can overcome the long distance and small electronic coupling,[44] whereas the weaker one (the former) must seek more favorable conditions, presumably at the proximate patch. Perhaps thermodynamic driving force should be considered when analyzing pathways for electron transfer in proteins.

Acknowledgment. Ames Laboratory, U.S. Department of Energy is run by Iowa State University under Contract W-7405-Eng-82. This research was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences.

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Fiber Optic Attenuated Reflection Spectroscopy (FO-ATR) for Investigation of Organometallic Polymeric Films

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Received December 27, 1990
Revised Manuscript Received July 5, 1991

The use of optical fibers in spectroscopy has been demonstrated for both optical[1] and fluorescence[2] spectra. Infrared has received considerably less attention.[3] Attenuated reflection spectroscopy (ATR) has provided a very sensitive spectroscopic method[4] and in fact FT-IR-ATR has been used in the investigation of organic monolayers and assemblies on substrates such as glass Ge, ZnSe, and Si.[5] In this communication we report a novel method for performing sensitive infrared spectroscopic measurements on organometallic thin films. This method combines the use of optical chalcogenide IR glass fibers and ATR (FO-ATR). The characterization of thin films of this type is difficult, and the reports that do exist[6] include a need for new and improved techniques.

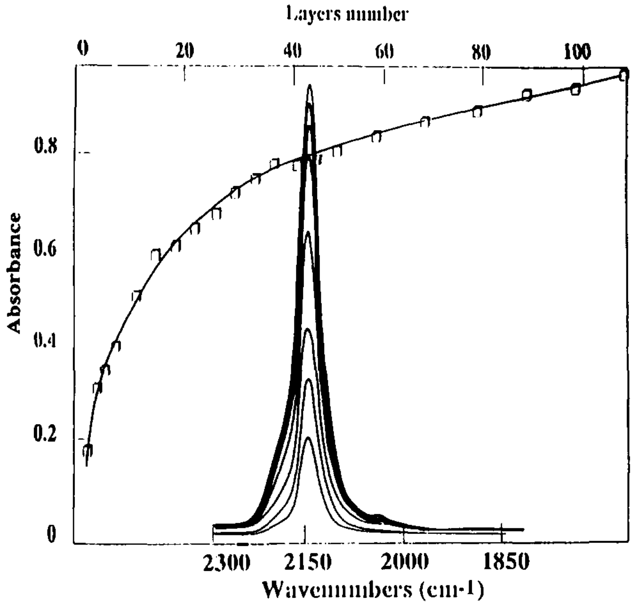

Figure 1. Absorbance bands $\nu(C\equiv N)$ as increasing number of layers are casted. In ascending order, 2, 4, 8, 20, 40, 60, 80, 100 layers (lower *x* axis). Upper axis: graphic representation of above bands as function of number of casted layers.

The advantages in using chalcogenide IR glass optical fibers in this way are numerous. They are cheap, compact, and easily implemented. The fibers provide an inert surface for deposition of nonfree-standing films. In addition to providing a proper deposition media the fiber then is easily able to efficiently guide the "signal" to and from the test sample. Changing the fiber diameter per length unit allows one to obtain different sensitivities.[7] Experiments are able to be monitored on line as we demonstrate here.[8] Therefore the combination of ATR spectroscopy[4,9] with its significant sensitivity and the use of chalcogenide optical fibers considering their technological implications may serve to make this method a powerful tool for both basic and applied research in this area.

This short article attempts to exemplify just how this FO-ATR method may serve in the characterization of thin organometallic films. For this purpose two types of experiments are described. The first deals with casting of a thin organometallic film onto a chalcogenide IR glass fiber from a ready solution of the polymer. This serves to test the relationship between the sensor response and number of polymer layers. Provided that the index of refraction of these films are known, this experiment enables one to calculate film thickness. The second deals with preparation of

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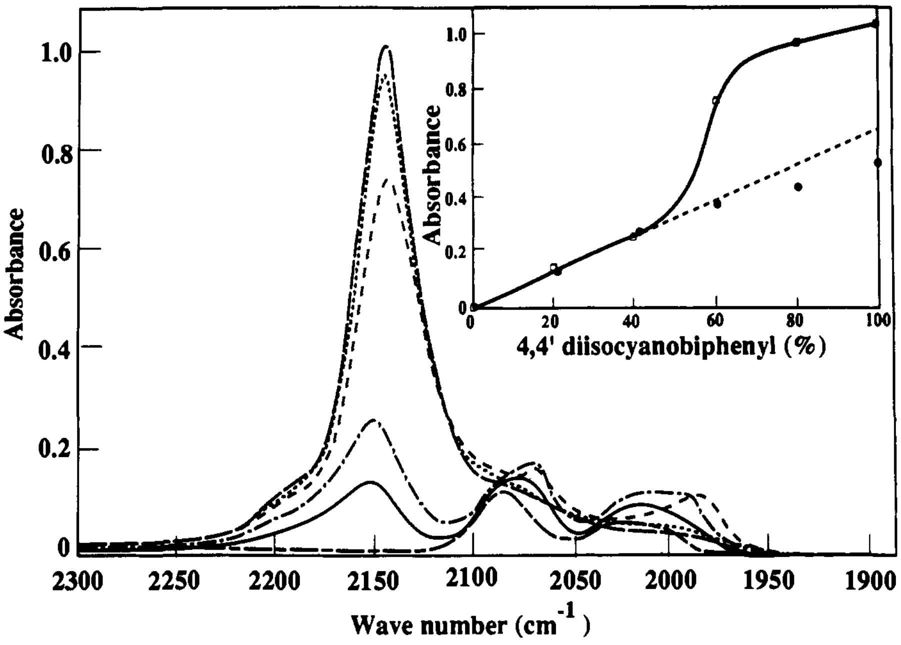

Figure 2. In situ preparation of Rh–B polymeric solution from $[Rh(CO)_2Cl]_2$ and 4,4′-diisocyanobiphenyl starting materials; (– – – –), $\nu(CO)$ starting rhodium dimer; (—), addition of 20% molar equivalent of B; (– —), addition of 40% B;(– –); addition of 60% B;(···); addition of 80% B; (— — —) addition of 100% B. Inset: graphic representation of $\nu(C{\equiv}N)$ abosorbance band as a function of % B added (unbroken curve). $\nu(C{\equiv}N)$ absorbance band as function of % B added in direct experiment without the optical fiber.

the polymer in solution in the presence of a glass fiber in order to investigate the preparation mechanism and characterize the ensuing polymers in solution.

Over the last decade we have been using bifunctional aryldiisocyanide ligands[10] to prepare numerous novel organometallic polymeric systems.[11] In particular, the rhodium aryldiisocyanide polymers, $[Rh(aryldiisocyanide)_2{}^+Cl^-]_n$ have been extensively investigated.[11,12] Thin films prepared from dilute solutions of these polymers have been previously reported.[12d]

Dichloromethane solutions of Rh–B (B = 4,4′-diisocyanobiphenyl) polymers (2.0×10^{-3} M) have been cast onto uncladded IR glass chalcogenide optical fibers.[13] Layers can be added as desired, by additional casting, allowing the fiber to dry in between newly coated layers. The Rh–B polymeric solutions show strong infrared absorbances due to the isocyano moiety at 2150 cm^{-1}. The $\nu(C{\equiv}N)$ absorbance grows nearly linearly for the first four layers. Thereafter it begins to slowly plateau. Between 40 and 100 layers, the changes in absorbance as a function of additional cast layers are minimal (Figure 1). These observations are in keeping with the hypothesis that when the layers coated on the fiber reach a thickness value which is equal to that of the defined depth of penetration[14] further coating of the fiber will not measureably increase the total absorbance. In accordance with this, and knowing the refractive index of the test material, one could calculate precisely the thickness (Å) of the film coated onto the optical fiber. In such a manner we have estimated that each casted layer of the Rh–B polymer has a thickness of 160 ± 30 Å.[15] This method therefore allows for calculation of film depth, which, currently, other than ellipsometry is not easily obtained.

In addition, the fiber allows for monitoring of the substitution reaction of the $[Rh(CO)_2Cl]_2$ dimer with 4,4′-diisocyanobiphenyl to yield the Rh–B polymers. In order to accomplish this we used a simple cell in which the chalcogenide IR glass fiber was fixed inside. A solution of the $[Rh(CO)_2Cl]_2$ dimer was added, and subsequently 4,4′-diisocyanobiphenyl solution was added in equivalent portions. In order to obtain better responsitivity per unit length a biconical taper fiber is used in this experiment.[16] Figure 2 demonstrates the changes in the infrared spectrum during polymer preparation as a function of the addition of increasing amounts of 4,4′-diisocyanobiphenyl. The carbonyl peaks of the rhodium dimer (2025, 2075 cm^{-1}) disappear as they are substituted by 4,4′-diisocyanobiphenyl whose infrared peak grows in at 2150 cm^{-1}. These results support the previously reported reaction mechanism[17] for tetrakis(monoisocyanide) rhodium complexes.

Substitution at rhodium of more than one ligand by aryldiisocyanide allows for propagation via the diisocyanide moiety. Referring to the reported mechanistic scheme[17] it follows that the moiety wherein the aryldiisocyano ligands are trans to one another is the most probable for polymeric propagation and deposition onto the optical fiber. We have shown earlier that the addition of 1 equiv (50%) of aryldiisocyanide to the rhodium dimer leads to $[Rh(CO)Cl(aryldiisocyanide)]_n$ polymers.[18] Figure 2 (inset) shows that between 40% and 60% added 4,4′-diisocyanobiphenyl causes a significant increase in the absorbance $\nu(C{\equiv}N)$ band which does not follow the near linear increase witnessed up to 40% addition. This same experiment carried out without the presence of the fixed optical fiber has shown no increase in the absorbance

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[13] These sieves have been developed and commercially produced "in-house" and have a refractive index of 2.77. They are transparent from 1μ to 12μ and can be made with diameters of 30μ to 800μ. (See supplementary material.)

[14] Reference 4 p 27–33.

[15] As the refractive indices of these polymers have not been precisely measured, for the sake of calculation we have assumed them to be between 1.5 and 1.8.

[16] The biconical taper is made by pulling the heated 400 μm fiber diameter. The biconical taper length is about 20 mm, and the taper waist is about 80 μm and is nearly constant for 10 mm.

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ν(C≡N) band (Figure 2, inset). We believe the "jump" in absorbance is due to the deposition of Rh–B polymers from the preparative solution onto the fiber itself demonstrating the existence of "in situ" coating of the optical fiber.

Preliminary experiments have shown that these thin films coated on optical fibers adsorb water molecules when exposed to air. The polymers themselves have been shown to be hygroscopic.[12f] Other small molecules of interest may also be adsorbed by these films, undoubtedly due their network-like nature, and this point is now under investigation. Providing that interesting and relevent adsorbates be found one may envision using these and similar coated fibers as chemical sensors.

Acknowledgment. The authors wish to thank Prof. A. Katzir of the School of Physics and Astronomy of Tel-Aviv University for his helpful discussions. We extend our appreciation to Ms. Carmela Barash for her diligent technical aid and Ms. Lily Forte for typing this manuscript. Partial funding for this project was provided by the NCRD (Israel).

Registry No. ([Rh(CO)$_2$Cl]$_2$)(OCN(C$_6$H$_4$-*p*-)$_2$NCO) (copolymer), 135366-75-5.

Supplementary Material Available: Relevent experimental and measurement procedures including two diagrams of experimental systems (4 pages). Ordering information is given on any current masthead page.

Pulsed ENDOR and ESEEM Spectroscopic Evidence for Unusual Nitrogen Coordination to the Novel H$_2$-Activating Fe–S Center in Hydrogenase

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Received September 10, 1990

Hydrogenases catalyze the activation of molecular hydrogen: $H_2 = 2H^+ + 2e^-$. The proposed site for H_2 oxidation and production in the Fe-containing hydrogenases is a novel Fe–S center, termed the hydrogenase or H cluster.[1-4] The H cluster of hydrogenase I from the anaerobic N_2-fixing bacterium, *Clostridium pasteurianum*, studied by a variety of spectroscopic techniques[5] has an unknown structure comprised of at least three, and possibly, six, Fe atoms.[5d]

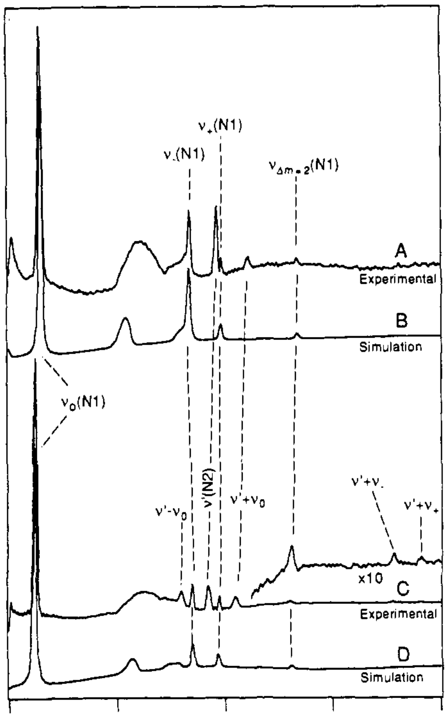

Figure 1. Cosine Fourier transforms of the stimulated electron spin–echo waveforms for experimental data (A and C) and for numerical simulations (B and D). Experimental conditions for A and C: $T = 1.7$ K; (A) 7.930 GHz, 2706 G, $\tau = 0.14$ μs; (B) 9.087 GHz, 3101 G, $\tau = 0.18$ μs. Simulation parameters for (B and D): $e^2qQ/h = 4.85$ MHz, $\eta = 0.20$, $A_{\text{iso}} = -1.2$ MHz, $r_{\text{eff}} = 2.8$ Å, $\theta = 90$, $\phi = 90$, $\alpha = \gamma = 0$, $\beta = 10$.

We have studied the coordination structure of the H cluster using a unique combination of pulsed ENDOR spectroscopy, which is the first application of this technique to a metalloenzyme, and electron spin–echo envelope modulation (ESEEM) spectroscopy. The H cluster is coordinated by two nitrogens. One of the two nitrogen atoms has an unusually large quadrupole coupling for a protein nitrogen donor ligand, suggesting a unique coordination structure for the H cluster.

Hydrogenase I was purified and oxidized as previously described.[5a,6] ESEEM and ENDOR data were recorded at several *g* values and at several microwave excitation frequencies. ESEEM spectra, obtained by cosine Fourier transformation of the three pulse stimulated echo waveforms, recorded at 7.930 and 9.087 GHz, are shown in Figure 1. These spectra were recorded near the extrema, $g_{\text{max}} = 2.10$, of the EPR spectrum[1,5a] to take advantage of the enhanced resolution afforded by *g*-matrix orientation selectivity.[7,8] The ESEEM frequencies, the frequency shifts between the two microwave excitation frequencies, and the depth of the ESEEM intensities are indicative of covalently coordinated nitrogen.[9]

Quantitative analysis of the ESEEM spectra is possible by tracking the ESEEM frequencies in spectra recorded at multiple microwave excitation frequencies.[10-12] Pure nuclear quadrupole resonance (NQR) transitions can be observed in ESEEM spectroscopy when the local magnetic field vanishes in one of the electron spin manifolds. This occurs when the nuclear Zeeman field is cancelled by the hyperfine field.[10] The three ESEEM transitions in this manifold are pure ^{14}N NQR transitions: ν_0,

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