A Study of Diphenylpyrenylphosphine: A Fluorescent Molecule With Many Uses

An Honors Thesis (HONRS 499)

By

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Abstract

Diphenylpyrenylphosphine (DPPP) is a molecule that with a myriad of potential uses. Current published research on the molecule extols it uses in the food industry as a highly sensitive toxicity indicator. The research presented shows a preliminary framework for the characterization of DPPP and its oxide (DPPP=O). Thus far, no suitable crystals have been isolated to present the X-ray analysis of the molecules; therefore, no accurate data on cone angle and spatial arrangement of the molecule can be reported. DPPP=O recrystallization should be obtained in the near future by using a large non-polar organic solvent, such as mesitylene, in dilute conditions. DPPP has a chemical shift at -13.34 ppm in the ^{31}P NMR spectrum, while its oxide has a shift at 33.39 ppm. Nickel carbonyl experiments using infrared spectroscopy yield a successive decrease in the $\nu(CO)A_1$ stretching frequency as the number of pyrenyl groups substituted on triphenylphosphine increases from 0 – 2. The respective frequencies observed are: 2069.35 cm^{-1}, 2068.96 cm^{-1}, and 2068.45 cm^{-1}. This data indicates increasing donor ability of the phosphorus lone pair as the number of pyrenyl groups increases.

Rationale

Lipid hydroperoxides are a known cause of the lowering of food quality through toxicity and flavor alteration. Recent research confirms the plausibility of detecting the presence of the oxides of unsaturated fatty acids and their esters through the use of a phosphine molecule that fluoresces upon oxidation.

General Nature of Phosphines

Phosphines are trivalent phosphorus derivatives that are highly reactive. These molecules are active as Lewis and Brönsted bases and are frequently used to form transition metal complexes due to the availability of the lone pair on the phosphorus for donation to the metal atom [1]. Similar to metal-phosphine complexes, the sensitivity of the molecule to oxidation is also dependant upon the availability of the phosphorus lone pair.

The effect of electronic effects experienced by phosphorus center, due to the nature of its functional groups, on the donor ability of the lone pair has long had an established acceptance among the scientific community. However, according to data gathered by Tolman, the size of the functional groups also affects the ability of the phosphorus to act as a Lewis base. Tolman's "cone angle" concept correlates the sizes and condensability of the various species that comprise the trivalent structure of the phosphine with the steric strain within the phosphorus ligand and availability of the lone pair. Therefore, a phosphine with three methyl groups attached would have a smaller cone angle than a phosphine with three phenyl rings and the electron pair would be more susceptible to creating a bond with a Lewis acid species [2].

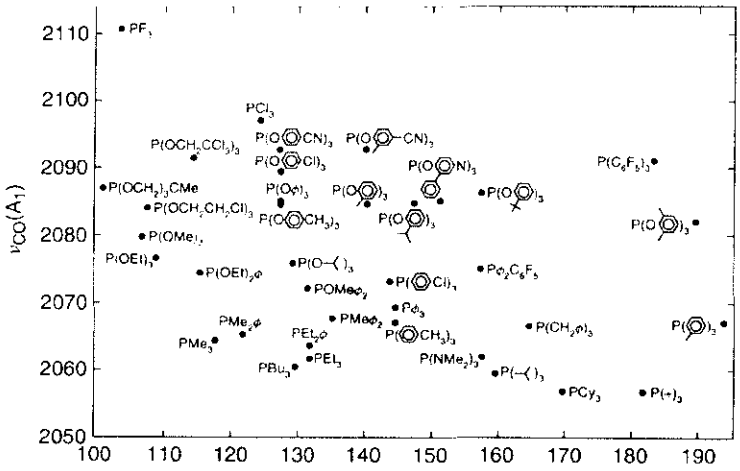


Figure 1: Electronic and steric effects of common phosphorus ligands plotted by Tolman [2]

Tolman extended his experiment by creating a comparison between the steric effects and electronic effects. He used a nickel carbonyl complex, $R_3PNi(CO)_3$, with the phosphorus ligands to determine the electronic effects via infrared spectroscopy (IR). The protocol uses the A_1 stretching frequency of the carbonyl ligands as a marker of the donor ability of the phosphorus lone pair. A phosphorus ligand with a more highly accessible lone pair will decrease the $\nu(CO)A_1$ by decreasing the bond order of the carbonyl ligand. The cause of this observation is the back bonding effects of the nickel carbonyl complex

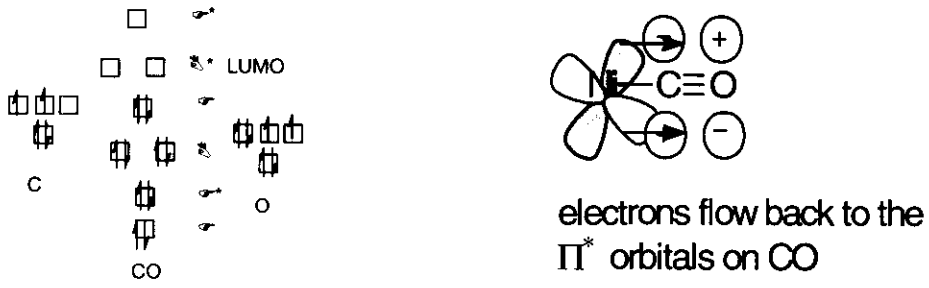


Figure 2: Backbonding in metal-carbonyl complexes.

The $d\pi$ orbitals of the nickel atom interact with the π orbitals of the carbon. Figure 2 shows a schematic of the back bonding as well as the molecular orbital diagram that results from the phenomenon. The back bonding weakens the carbon-oxygen bond because the extra electrons contributed by the nickel atom go into an anti-bonding π^* orbital. The ability of the phosphorus ligand to donate electron density to the nickel center of the metal complex increases the ability of the nickel to back bond with the carbonyl. Increasing the back bonding strength correlates with a decrease in the bond order of the carbonyl group; the decrease in bond order (weakening of the bond) corresponds with a decrease in $\nu(CO)A_1$ in an IR spectrum [1]. In summary, phosphorus ligands with increasing ability to donate the electron pair will display $\nu(CO)A_1$ that decrease accordingly. The nickel carbonyl experiment was used in this research to investigate the effect of the pyrenyl group on the electron pair donor ability of the trivalent complex and compare the data obtained with the broad spectrum of known phosphines.

General Description of DPPP

DPPP has been synthesized by the reaction of chlorodiphenylphosphine with a Grignard donor containing the aromatic, fluorescent pyrenyl group [3-8]. As shown in Figure 3, Ball State University chemistry researchers have recently devised an improved method that produces DPPP in ca 70% yield from commercially available reagents. The phosphorus center of the molecule has three single bonds, one each to the phenyl rings and the pyrenyl group, and a lone pair of electrons.

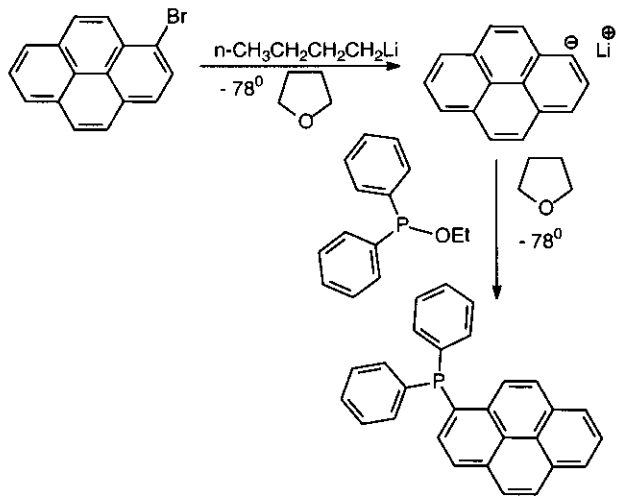


Figure 3: Synthesis of DPPP using commercially available reagents, from unpublished research provided by Dr. Bruce N. Storhoff, Department of Chemistry, Ball State University.

An important characteristic of DPPP is its strong reducing power. Since the molecule has a lone pair, it is forced into an unstable, three-coordinate structure. Reaction with an oxidizer, a lipid hydroperoxide, enables the molecule to take a more stable penta-coordinate structure.

DPPP was chosen as the ideal fluorescent indicator for research through a study by Akasaka in which compared the sensitivity, reactivity, and ease of preparation between phosphorus (III) compounds [3-8]. Triphenylphosphine (TPP) was a well-known reducing agent for lipid hydroperoxides. TPP is a naturally fluorescing molecule and oxidized TPP fluoresces at approximately 10 times stronger at 260nm than when unoxidized. Desiring higher sensitivity and selectivity than that offered by TPP, Akasaka conducted a study of phosphorus (III) compounds with one or more fluorophores (fluorescent groups) replacing the phenyl rings. Each phosphine synthesized displayed an unexpected ideal characteristic of fluorescing only when oxidized. The cause of the quenching phenomenon is the interaction of lone pair on phosphorus with the extended

π system of the fluorophore. This increased the sensitivity of the molecules for use as probes; it eliminated the need to factor out an inherent fluorescence from the unreacted molecules that may remain in a sample. The experimental conclusions labeled DPPP as the ideal molecule according to sensitivity, higher rate of reactivity, selectivity of reduction to lipid hydroperoxides, and ease in preparation [3-8].

Mechanism of Fluorescence

Fluorescence and phosphorescence result from the propensity of a molecule to lower its electrons from excited state energy levels to those at the lowest possible, or ground, state. In the process of the relaxation, a photon of energy is emitted; the wavelength of which does not necessarily correspond with the energy that was introduced into the system. Discrepancies between energy absorption and energy emission are the result of several alternative modes of relaxation that exist for excited molecules. These alternative modes, to be addressed later, are often more kinetically favored than photon emission. Molecules favor relaxation processes that are the quickest means from the higher to the lower energy state; often, these mechanisms do not involve the emission of a photon. Therefore, a majority of molecules do not fluoresce [9].

Photoluminescence, the emission of a photon as the means of lowering the energy of the molecule, is limited to a small number of systems that meet the structural and environmental conditions to make photon emission a kinetically favored, or competitive, mechanism. Examples of the favorable conditions for photoluminescence are: aromatic or conjugated systems with delocalized π electrons, rigid structure, low temperature, and high viscosity of the system [9].

Aromatic systems, such as the phenyl rings and the pyrenyl system present in DPPP, are called chromophores based on their ability to fluoresce via $\pi^* \rightarrow \pi$ transitions. These transitions are lower in energy than other possible transitions. Therefore, aromatic molecules readily absorb and emit photons of wavelengths in the ultraviolet and visible spectra. Chromophores do not require a source other than radiant energy to initiate transitions detectable within the common instrumental parameters available through Ultraviolet-Visible spectroscopy and fluorometry [9].

Quenching Fluorescence

Although most aromatic groups are fluorescent, most molecules do not possess the ability to emit photons in the visible spectrum due to the kinetic preference of other means to reduce the overall energy of the system. Internal and external sources for energy release are available to an excited molecule and these methods are often a quicker means of regaining the stability of the ground state electron configuration. External methods include the translation of energy onto other atoms and molecules in the surrounding environment via collisions in the matrix. Internally, molecules have a means of relaxation other than fluorescence [9].

Each electronic energy level of a molecule contains a number of vibrational energy levels through which an excited electron can pass to lower its energy. Vibrational relaxation is observed in fluorescent molecules as well; the electron undergoes a series of steps down in vibrational energy levels to the lowest available at the electronic level and then emits a photon to return to the ground state [9].

Recent research observes that the DPPP molecule fluoresces only when oxidized, making it a useful probe for analysis of lipid hydroperoxides [3-8]. However, the

explanation for why the molecule lacks fluorescence when unoxidized has not been thoroughly investigated. Two potential reasons exist to explain the inability of the molecule to fluoresce while unoxidized: Intramolecular Vibrational Redistribution (IVR) and lone pair interactions with a highly dispersed π system. IVR, as discussed by Pate, involves the dense upper regions of the anharmonic model for the vibrational motion of molecules [10,11]. When the rovibrational state density reaches a distribution of about 10-100 states/cm the IVR process readily operates. Pate also maintains that the density appropriate for IVR is easily attained in molecules comprised of over 10 atoms. The high density of the overall rovibrational states of the molecule allow for overlaps in energy levels between the individual bonds of the molecule. IVR allows the molecule to descend down a chain of small energy drops due to the ability of the electron to transfer from the energy states of one bond to another in a molecule [10,11]. Therefore, through IVR, an electron in the excited state can quickly pass down a rovibrational cascade rather than employing the much slower photon emission decay process.

IVR is a likely explanation for the quenching phenomenon in the DPPP molecule. Another possibility involves the structural positioning of the molecule. An initial glance at the structure of the molecule would indicate that it should fluoresce due to the attachment of the three aromatic groups. The molecule is similar to triphenylphosphine, a molecule that fluoresces when unoxidized. The steric bulk of the pyrenyl ring could force the aromatic structures around the phosphorus into an almost planar conformation. A planar arrangement would increase the possibility that the lone pair on phosphorus could bridge the π systems of the individual constituents of the molecule, thus dispersing the electron density over the entire molecule (Figure 4).

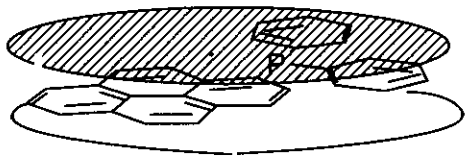


Figure 4: The possible behavior of DPPP when unoxidized. The phosphorus lone pair acts as a bridge to disperse the p system over the entire molecule. This is a potential means of quenching fluorescence.

The result of this dispersion would be a marked decrease in the energy of the system; therefore, any radiation from the system would be at a wavelength undetectable in the fluorescent spectrum. The breaking of the lone pair bridge would cause the appearance of fluorescence after oxidation from phosphorus. The double bonded oxygen would eliminate the presence of the lone pair to allow for dispersion of the π system.

An experiment to determine the presence of IVR quenching within the system could be conducted by measuring dispersed fluorescence using a monochrometer. If IVR is present, the spectrum would contain data gathered in lower frequencies than would be expected from the molecule. It is noted that Ball State University does not have the necessary instrument to carry out this step.

A potential outcome of the research presented in this paper could be the identification of the quenching mechanism. By recrystallizing DPPP and DPPP=O into a suitable means for X-ray crystallography the structure and spatial orientation of the molecules would be determined. In addition, there are suitable computer-based calculations that could be attempted to determine the feasibility of this argument. Such determinations would clarify the role of steric effects in quenching.

Failures of Previous Assay Methods

Traditionally, it has been difficult to determine quantitatively the degree of lipid hydroperoxidation in biological materials and foodstuff due to the low concentration

levels, inherent instability, and molecular diversity [3-8]. A variety of assay techniques were used to some measure of success; however, no one technique encompassed the needs for both quantitative and qualitative analysis.

Iodometry, extensively used for identification of lipid hydroperoxides in foodstuff, presents neither a sensitive nor a simple procedure for use with biomolecules. Thiobarbituric acid assays are used for the detection of biomolecules; however, the methods are insufficient for quantization and selective identification. Highly selective mechanisms, such as those employing enzymes, can determine the total degree of lipid hydoperoxidation in biomolecules with a great deal of sensitivity, but are also dependant upon the availability of the required enzymes and a complex identification procedure. Previous methods using High-Performance Liquid Chromatography (HPLC) with ultraviolet detection at 235nm (targeting conjugated diene systems) reported success at determining lipid hydroperoxides at molecular levels. Akasaka believed, however, that improvements to the HPLC assay could increase selectivity and sensitivity in the procedure [3-8].

Newly Established Methods

Akasaka's proposed change to previous HPLC studies includes the use of a fluorescent probe molecule for detection [3-8]. Fluorometry allows for the accurate calculation of lipid hydroperoxide concentration without the dependence upon sophisticated procedures and multiple enzymes. Two methods are available for study using fluorescence, including the batch method and High Performance Liquid Chromatography (HPLC) post-column procedures.

The batch method is the more general, less selective examination for lipid hydroperoxide concentration; DPPP is added to the sample being examined, allowed to react, and the percent transmittance read by a fluorometer. Although not specific, the batch method gives an overall picture of the amount of peroxidation in the sample [3].

HPLC allows for the separation of a complex sample of lipids by polarity and molecular weight. Treatment of the analyte with DPPP occurs after separation in the column; therefore, the degree of peroxidation in each type of lipid in the overall sample may be determined by fluorometry. Another advantage to the process is the ability of DPPP to reduce the analyte in wide range of solvents; therefore, the HPLC method is not limited in efficiency by limitations on type of solvent or isocratic elution. DPPP allows the use of these methods to substitute for earlier procedures by creating two standard, sensitive, and efficient methods that are useful in the analysis of both biological materials and foodstuff [3-8].

Real World Uses

The presence of lipid hydroperoxides in foodstuff is a common cause of lowering of food quality due to flavor change and, in some cases, toxicity. The study of food is the most common research in the literature available. Akasaka examined various oils and foods in route to developing his protocol for using DPPP in highly sensitive HPLC-fluorometric detection [3-8]. Hartvigsen used size exclusion HPLC in combination with fluorometric detection to analyze fish oil enriched mayonnaises (12). Both groups found comparable success in accurately measuring the degree of peroxidation of the lipids in the samples.

There are a myriad of potential negative results from the presence of oxidized unsaturated fatty acids and fatty acid esters that have yet to be thoroughly explored in the scientific community. Noguchi's study used DPPP as mode of lipid peroxidation detection in the cellular membranes of polymorphonuclear leukocytes (PMNs) using a spectrofluorophotometer and fluorescent microscopy [13]. DPPP proved to be a useful indicator of the degree of oxidation in the PMNs due to its stoichiometric reactivity with lipid hydroperoxides and ability to integrate within, and stain, the cell membrane. He also found an overwhelming preference for reaction with a lipid hydroperoxide as opposed to those involving aqueous peroxides. Noguchi also quantified the sensitivity of the DPPP → DPPP=O system as having the possible detection range down to 1 femto mol of peroxide [13].

The Noguchi study is a significant link between lipid hydroperoxide detection and an ability to study their role in the onset of atherosclerosis. Low density lipoprotein (LDL) levels are generally used as an indicator of risk for heart disease and oxidized LDL particles, which occur naturally in vivo, are biologically active on the endothelial cell lined arterial walls [14]. In a study conducted by Chapman, it was determined that oxidation of the lipids on a low to medium density LDL caused the particle to be apoptosis-inducing in endothelial cells [14]. Furthermore, the extent of peroxidation and concentration of oxidized LDL particles correlated to cytotoxicity in endothelial cells. Apoptotic cells have been found in atherosclerotic plaques and are correlated with an elevated risk of heart disease [14]. Using DPPP to analyze LDL samples from patients at risk for heart disease may help indicate the stage of progression of disease and the chance that plaques have formed on the walls of the arteries.

In addition to heart related uses, DPPP has the potential to be used in an anti-tumor platinum complex. The lone pair on the phosphorus (III) center of the DPPP molecule can donate to a variety of transition metals upon which it is essentially oxidized to phosphorus (V). The extent of the donor ability will be addressed in the research included in this paper. Platinum, the transition metal used in a widely-used anti-tumor drug, *cis platin*, possesses cytotoxic properties when incorporated in the DNA of a cell. The molecule binds to the guanine bases of the DNA double helix causing irreversible damage and inducing cell death. The problem with *cis platin* is the tendency for the molecule to accumulate in the kidney and cause non-cancer related problems [15]. Ongoing research into the field of anti-tumor therapy is looking at modifying the platinum center by binding it to phosphines. DPPP, with its fluorescent capabilities and lipophilic nature, would be a novel agent to complex with the platinum and would provide a tracking mechanism for the drug's movement inside and outside the cell.

Purpose

The purposes of this study were to investigate the general nature of DPPP and its oxide. Nuclear magnetic resonance (NMR) spectra will be presented for both molecules as well as the sequences of attempts at recrystallation. A discussion of the ability of the phosphorus to donate its lone pair is also included.

Methods and Results

Solvents	Dissolving Temperature	Recrystallization Temperature	Result
3ml dichloromethane. 2ml abs. ethanol, 2ml benzene	boil 5 min	Refrigerator (stoppered, open arm)	Microcrystals formed
2ml dichloromethane. 3ml abs ethanol	heat, but never reached boiling	Refrigerator (stoppered, open arm) Next day freezer	White crystals formed in solution – "fluffy" – solvent adhered
Solution reheated (mostly ethanol with small amount ethanol and benzene) to dissolve, 1ml ethanol, 1.5ml heptane	Cooled to room temp	Refrigerator (stoppered, open arm)	White crystals formed in solution - solvent adhered
0.5ml dichloromethane	heat, but never reached boiling	Refrigerator (stoppered, open arm) Freezer	White crystals formed in solution – solvent adhered

Table 1: Descriptions of the various recrystallization attempts of DPPP. All attempts were made in a single arm Erlenmeyer flask using a variety of solvents and temperature variations. As can be seen, the results of the attempts were not the quality of crystal required for X-ray analysis.

Recrystallization Attempts of DPPP

Dichloromethane, Ethanol, Benzene

Diphenyl-1-pyrenylphosphine (DPPP) was placed in a single arm flask with 3 ml dichloromethane, 3 ml absolute ethanol, and 2 ml benzene. The solution was heated to dissolve and allowed to boil for 5 minutes. After cooling to room temperature, the flask was stoppered and cooled to 4°C in a refrigerator and then transferred to a freezer with the arm stoppered to induce crystallization. This crystallization attempt yielded microcrystals, which were unsuitable for X-ray analysis.

Dichloromethane, Ethanol

2 ml dichloromethane was added to the remaining mixture from above (mostly ethanol, some dichloromethane and benzene). The mixture was heated until just before boiling and then 3 ml absolute ethanol was added. The final mixture was stoppered and placed in the refrigerator. The next day the solution was placed in the freezer. The

resultant precipitate was highly adsorbed with the solvent system; the crystals appeared fluffy, white, and fragile.

Ethanol, Heptane

The above solution was heated to dissolve the precipitated DPPP. 1 ml ethanol was added along with 1.5 ml heptane. The solution should be mostly ethanol with small amounts of dichloromethane and benzene. The warm solution was then stoppered and allowed to cool to room temperature. The precipitated yield was white crystals with solvent adsorbed (unsuitable for analysis).

Dichloromethane

The above solution was reheated to dissolve the DPPP and 0.5 ml dichloromethane was added. The solution was allowed to cool, stoppered overnight to room temperature, then was placed into a refrigerator. After approximately a week, the solution was transferred to a freezer. White crystals precipitated out of solution with solvent adsorbed (unsuitable for analysis).

Unsuccessful Oxidation of DPPP

0.75g of DPPP was dissolved into a solution of 18ml dichloromethane. 3 drops of 30% hydrogen peroxide was added as an oxidant along with a miniscule amount of concentrated sulfuric acid as a catalyst. After allowing the solution to stir for 30 minutes, it was added to a separatory funnel and extracted with 25ml of a 10% sodium thiosulfate solution. The bottom layer was saved and 10ml of ethanol was added to clear. After the solvent evaporated off, the remaining crystals were yellow-orange in color, asymmetric

and varied in shape and thickness. There were no crystals isolated that were suitable for X-ray analysis due to their non-uniform shape and fragility.

Successful Oxidation of DPPP

0.75g of DPPP was dissolved in a solution of 3ml methanol, 3ml dichloromethane. 4 drops of 30% hydrogen peroxide was added as an oxidant with a spatula tip of tetrabutylammonium hydrogen to catalyze the reaction. The solution was allowed to stir for three days. The resultant fine, tan particles from the evaporated solution were redissolved in 10ml dichloromethane to make a dark yellow-brown solution. The solution was transferred to a 125ml seperatory funnel and extracted with 25ml 10% sodium thiosulfate solution. The aqueous layer was saved and 10ml ethanol added to the dark brown solution. The single arm flask was stoppered and allowed to sit at room temperature.

Since the solution appeared to be a dark brown color it was determined that decolorization was needed. The solids were redissolved in 7ml dichloromethane over heat to boil. A spatula tip of decolorizing carbon was added and the solution boiled for 5 minutes. The solution was then hot vacuum filtered through diatomaceous earth and washed with 2ml dichloromethane. A noticeable amount of decolorization was noticed; the solution changed from dark brown to a lighter yellow-brown.

Solvents	Dissolving Temperature	Recrystallization Temperature	Result
9ml dichloromethane (decolorizing step), 3ml abs. ethanol	Boiled with dichloromethane 5 minutes	Refrigerator (10 days, stoppered, open arm) Freezer (7 days)	Dark, brown granular crystals formed (mostly large ~3-4mm in length?)
18ml ethanol	Boiled ~10 minutes to concentrate	Room temperature (stoppered, open arm)	Fluffy white-tan crystals
~5ml toluene	Boiled to concentrate, ~0.5-1ml taken off	Room temperature (1 day, stoppered, open arm)	Spherical crystals, some promising needle shaped crystals on walls of flask
~5ml toluene	Boiled to dissolve	Freezer (1 day)	Small crystals, brown, seemingly less adsorbed
~7ml toluene then decolorized and added ~3 ml additional	Boiled to dissolve	Cooled to room temperature, Freezer (? days, stoppered, open arm)	Mixture of cream and amber crystals, fragile

Table 2: Descriptions of the various attempts at recrystallization of DPPP=O. Smaller, less adsorbed crystal formations were apparent in these attempts; however, there were no X-ray quality samples yielded.

Recrystallization of DPPP=O

Dichloromethane, Ethanol

Following the decolorization step, the oxidized DPPP product remained dissolved in approximately 7 ml dichloromethane. 3 ml absolute ethanol was added to the single arm flask; the vessel was stoppered and placed in the refrigerator with the side arm open. After 10 days the solution was moved to the freezer for 7 days, over which the precipitate formed. The resulting precipitate was dark, brown granular crystals, many as large as approximately 3-4 mm in length, yet still unsuitable for X-ray crystallographic analysis.

Pure Ethanol

Solid DPPP=0 was dissolved into 18 ml boiling absolute ethanol. After complete dissolution, the mixture was allowed to boil 10 minutes to concentrate. The single arm flask was stoppered and allowed to cool to room temperature with an open arm. After approximately 1 week, fluffy, white to tan precipitate formed in solution. These were unsuitable for analysis due to the apparent absorption of the solvent.

Toluene

- Room Temperature

Solid DPPP=O was dissolved in 5 ml boiling toluene and allowed to concentrate; approximately 0.5 ml were taken off. The solution was removed from the heat and stoppered. The liquid was yellow-brown, dark, and almost cloudy in appearance. The resultant precipitate after one day was almost perfectly spherical of crystals with solvent adsorbed. A small amount of needle-like crystals formed on the solvent-air line of the flask; however, seemed too fragile for analysis.

Toluene

- Freezer

The oxidized DPPP was redissolved in boiling toluene, removed from the heat, and placed directly into the freezer. Overnight, small brown crystals precipitated out of the solvent; they began small, but by afternoon seemed to be adsorbing the solvent.

Toluene

- After Decolorization, Freezer

The oxidized DPPP was redissolved in 7 ml of toluene and then decolorized due to the observation of a significant amount of black residue accumulated on the normally white boiling chip. The solution was boiled for 7 minutes with a spatula tip of decolorizing carbon. The solution was then filtered through diatomaceous earth and the resultant fluid was amber in color – a noticeable change from the previous solution. Approximately 3 ml of additional toluene was added to the solution. The decolorized

solution was placed in the freezer, stoppered, with the arm open. After 3 days, a fine tan precipitate formed a think cake on the bottom of the flask.

Toluene

- More Dilute, Freezer

Since the last trial seemed to precipitated too quickly, the DPPP=O was redissolved into the existent toluene by heating to boiling. 3.5 ml additional toluene were added and brought to a boil. The solution was then stoppered and placed into the freezer with the arm open. These crystals showed the most promising results in both size and shape. The structure when examined under the stereoscope displayed the appearance of less solvent adsorption into the structure. Current trials, concluded after the deadline of this paper, explore the use of mesitylene as a solvent.

Nickel Carbonyl, Phosphorus Lone Pair Availability

This data was contributed by Dr. Bruce Storhoff, from his unpublished experimental results:

	$\nu(CO)A_1$ (cm^{-1})	Difference from TPP (cm^{-1})
TPP	2069.35	0
DPPP	2068.96	0.39
PDPP	2068.45	0.90

Table 3: $\nu(CO)A_1$ stretching frequencies for TPP, DPPP, and PDPP. The estimated error for this experiment is +/- 0.1 cm^{-1}.

Discussion

Availability of the Lone Pair on Phosphorus

Spectra 1, 2, and 3 are infrared spectra of TPP, DPPP, and phenyldipyrenylphosphine (PDPP), respectively. A noticeable shift of the $\nu(CO)A_1$ to a lower wavenumber occurs upon the sequential substitution of pyrenyl groups for phenyl rings. Table 3 shows the A_1 stretching frequencies for the three compounds and the difference between TPP and the molecules with substituted pyrenyl groups. DPPP causes the nickel carbonyls to stretch at a frequency 0.30 cm^{-1} lower than TPP, indicating that the lone pair is more readily donated. In addition, PDPP causes a change in stretch 0.90 cm^{-1} lower in frequency than TPP. This data indicates that the substitution of the pyrenyl group on the phosphine causes the phosphorus lone pairs to participate more actively in metal carbonyl backbonding. The backbonding weakens the carbon-oxygen double bond and decreases the stretching frequencies. Since the error in this experiment is estimated to be only ± 0.1 cm^{-1}, the 0.35 cm^{-1} should be considered significant.

In comparison with the nickel carbonyl $\nu(CO)A_1$ data published by Tolman [2], TPP, DPPP, and PDPP all lie in the middle of the range (2056 - 2110 cm^{-1}) for lone pair donor ability. The information garnered from this experiment suggests that DPPP could be a useful ligand for a platinum anti-tumor agent. Although it is readily apparent that the substitution of pyrenyl groups to the phosphine molecule increases the availability of the lone pair of electrons, the reasoning for this increase is not yet known.

DPPP Recrystallation

The attempts at recrystallizing DPPP from a variety of solvents and in a range of temperatures and concentrations failed. Each attempt yielded similar results; the precipitate formed were large, "fluffy", extremely fragile masses, which were unsuitable for X-ray analysis. Due to time constraints and difficulty, recrystallization attempts on DPPP were abandoned to pursue other aspects of this investigation.

Volatile, polar solvents at high concentrations comprised most of the trials for recrystallization and that could be reasoning behind much of the difficulty experienced. DPPP=O recrystallizations yielded smaller, more needle-like crystals when toluene, a relatively large, non-polar organic solvent, was used and the recrystallization took place in dilute solution. Adherence of the solvent appears to be unavoidable; therefore, pure crystals should not be expected. Although purity would be ideal, sharp, needle-like crystals obtained by any means would allow for X-ray analysis.

DPPP=O Recrystallization

Recrystallization from a dilute solution of a large aromatic solvent should prepare an adequate crystal for X-ray analysis. Small, polar, volatile solvents did not provide precipitate that had the sharp, thin appearance required. DPPP=O appears to respond to a solvent with opposite properties. It should be noted that due to the dilute nature of the system, as well as the low volatility of the solvent, the formation of crystals takes four or more days in the freezer to complete. The next trial for recrystallization will use mysitylene (1,3,5-trimethylbenzene) as the solvent.

Oxidation of DPPP to DPPP=O

The ^{31}P NMR spectra for DPPP and the two oxidation attempts to DPPP=O are Spectra 4 – 6. DPPP has a characteristic chemical shift at -13.34 ppm, while the oxide occurs downfield at 33.39 ppm. These chemical shifts are very sensible; the unoxidized phosphorus, bonded to only carbon is shifted toward a very low ppm, while the phosphorus double bonded to the electronegative, deshielding oxygen causes a large downfield shift.

Spectrum 5 is the NMR for the unsuccessful oxidation attempt on DPPP. The NMR has two peaks: -13.34 ppm, indicating the presence of DPPP, and 33.39 ppm, indicating the presence of DPPP=O. By comparing the magnitudes of the peaks, the estimated percent oxidation for the unsuccessful reaction was only about 28%. Spectrum 6, the NMR from the product of the second oxidation, shows only one peak at 33.39 ppm, corresponding with the presence of only oxidized product.

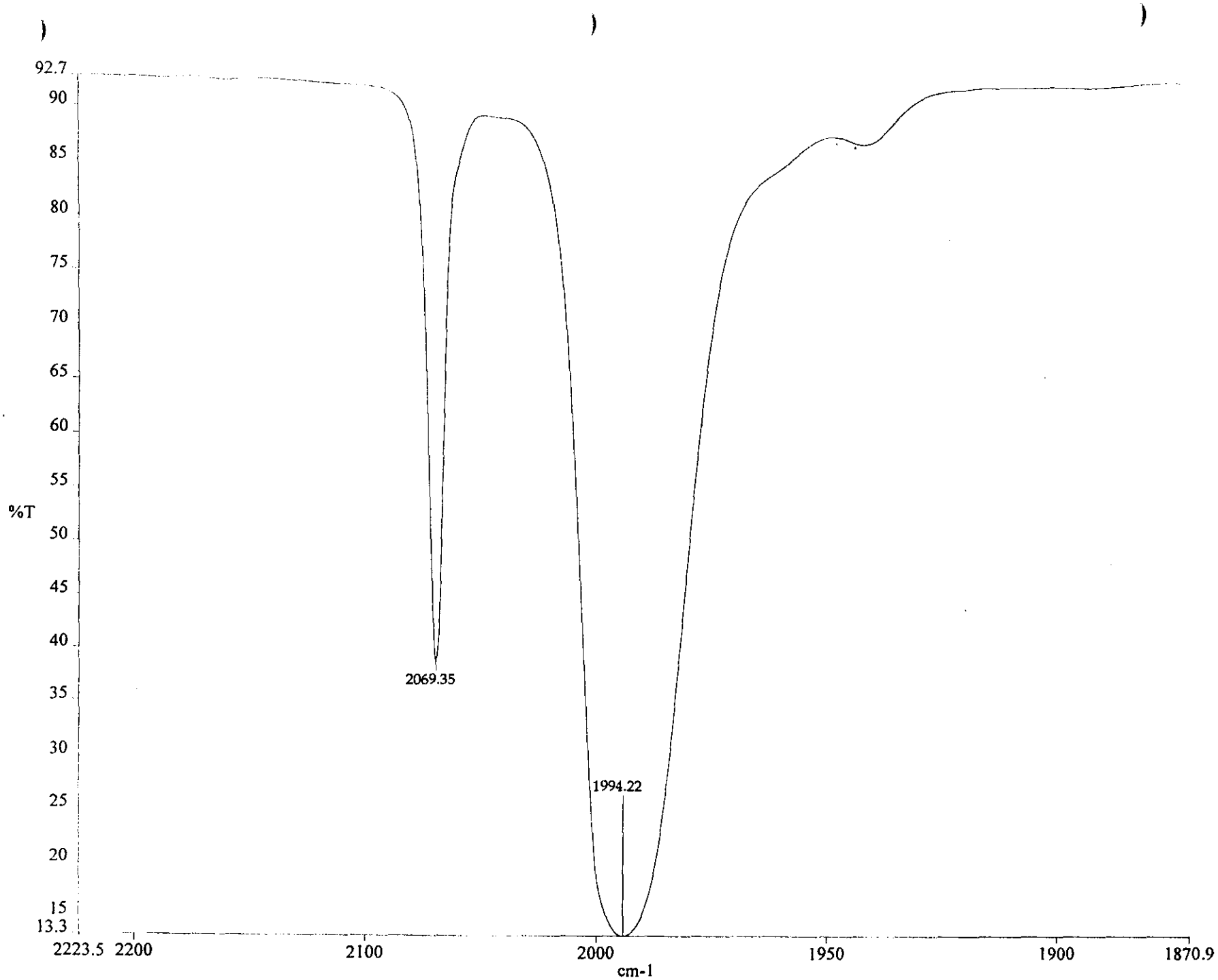
The actual required time frame is not known for the oxidation step. 24 hours allowed enough time to produce full oxidation; however, it may be possible to complete this step in a shorter amount of time. The mixed solvent system allowed the reaction to take place in a smaller volume of solvent, while the tetrabutylammonium hydrogen catalyzed the reaction efficiently at room temperature. It should be noted that the initial product of the second oxidation was a dark liquid requiring two decolorization steps to make it suitable for recrystallization.

Conclusion

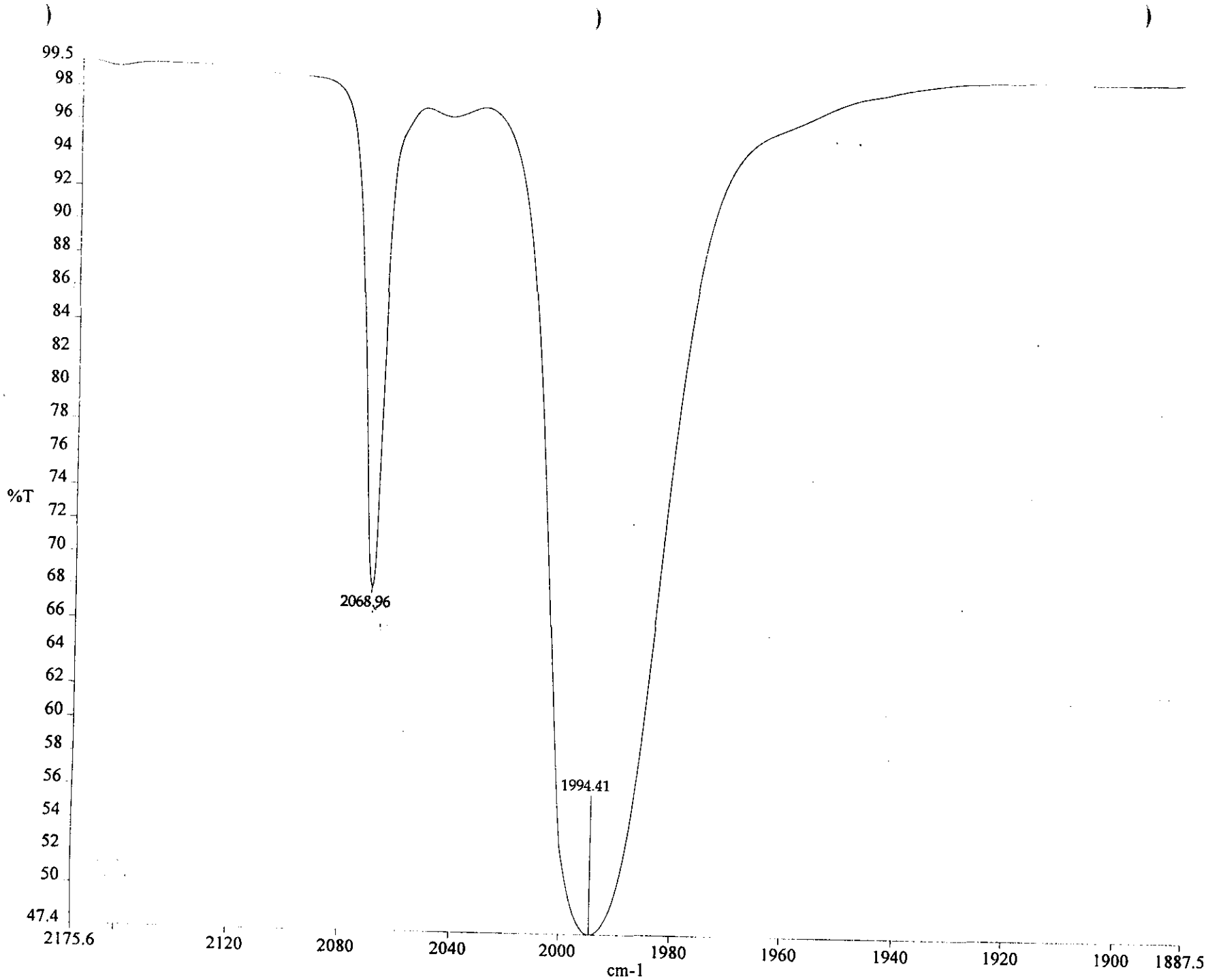
Recrystallization of DPPP=O to a product suitable for X-ray crystallography seems to be within grasp. The results of the X-ray analysis will allow for further characterization of the oxide cone angles, spatial arrangement, and, possibly, indicate a reason behind the quenching phenomenon observed in the unoxidized DPPP. Further trials are needed to explore recrystallizing DPPP; perhaps a solvent similar to that which is useful in the oxide recrystallization will be suitable for DPPP as well.

Although a viable electron donor, DPPP apparently has not been considered among those studied as possible phosphorus ligands for *cis platin* derivatives. DPPP could be ideal due to the fluorescent properties of its oxide; it could serve as both a component of an anti-tumor drug, as well as a fluorescent tag to determine the selectivity of the molecule for certain cells or regions of DNA.

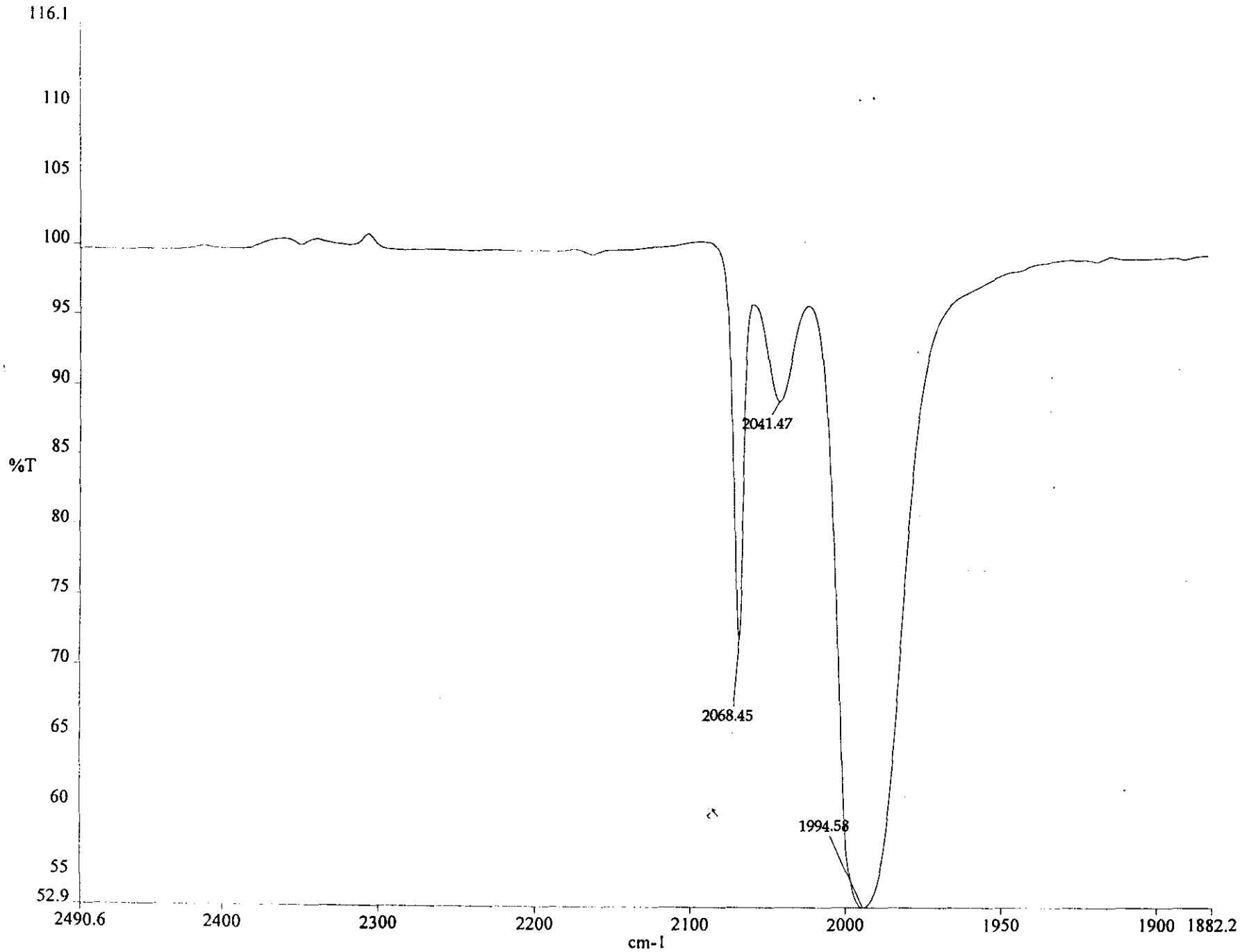
Future research conducted on this topic should include further recrystallizations of DPPP to enable the X-ray analysis. A dispersed fluorescence study of DPPP would indicate whether IVR is the mechanism for quenching within the unoxidized DPPP molecule. Biochemical research should not only explore the use of DPPP in both anti-cancer research, but also for use in a fluorescent DNA tag.



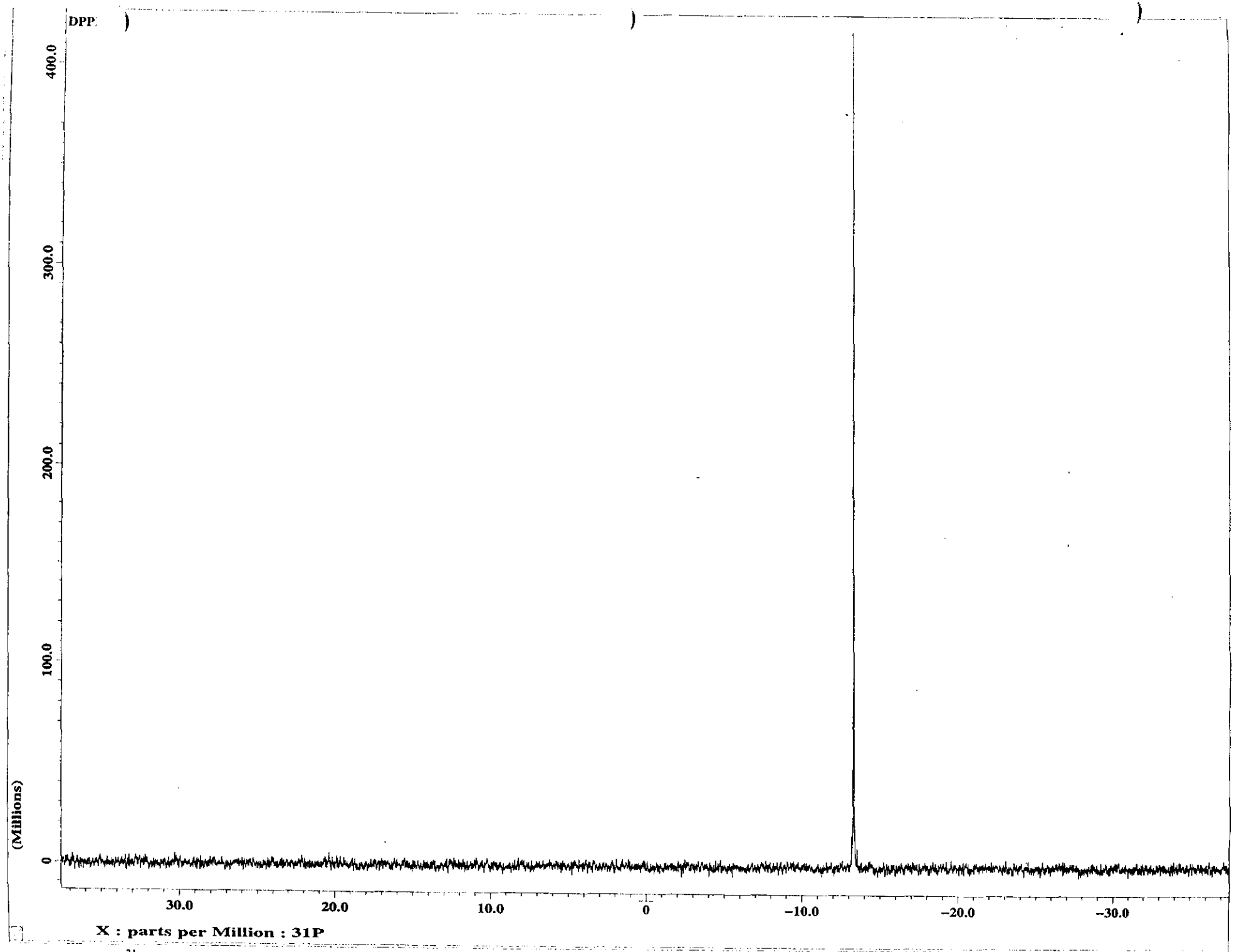
Spectrum 1: IR spectrum of TPP. The $\nu(CO)A_1$ stretching frequency for TPP is 2069.35 cm^{-1}.



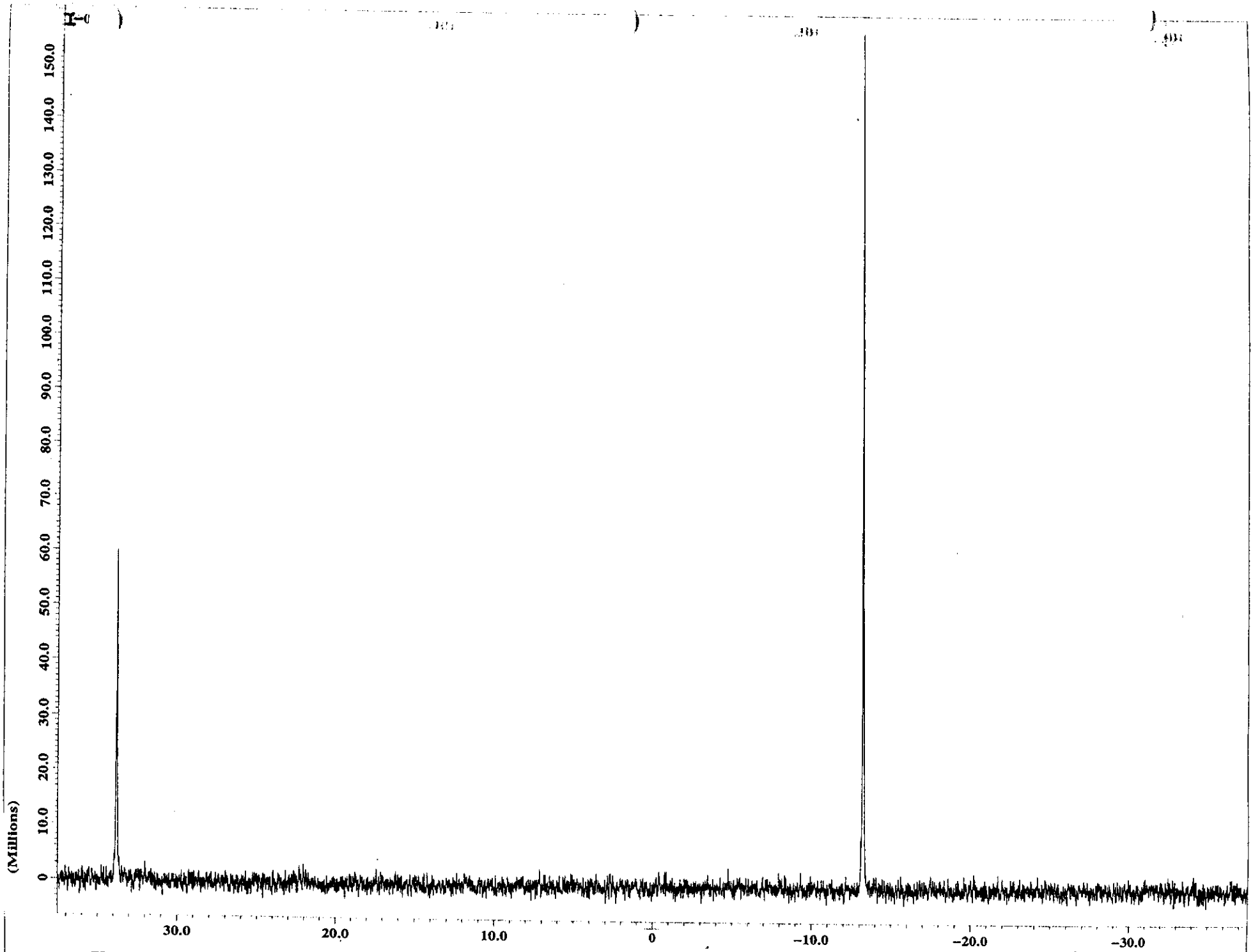
Spectrum 2: IR spectrum of DPPP. The $\nu(CO)A_1$ stretching frequency for DPPP is 2068.96 cm^{-1}.



Spectrum 3: IR spectrum of PDPP. The $\nu(CO)A_1$ stretching frequency for PDPP is 2068.45 cm^{-1}.



Spectrum 4: ^{31}P NMR spectrum of DPPP. DPPP has a characteristic chemical shift of -13.34 ppm.



Spectrum 5: ^{31}P NMR spectrum of DPPP attempted oxidation product . There are two peaks, -13.34 ppm and 33.39 ppm. This sample was only approximately 28% oxidized.

JEOL

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Sample ID          = S$322205
Content            = Single Pulse with Broa
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Revision Date      =  1-APR-2003 09:13:17
Spec Site          = Eclipse+ 400

Spec Type          = DELTA_NMR
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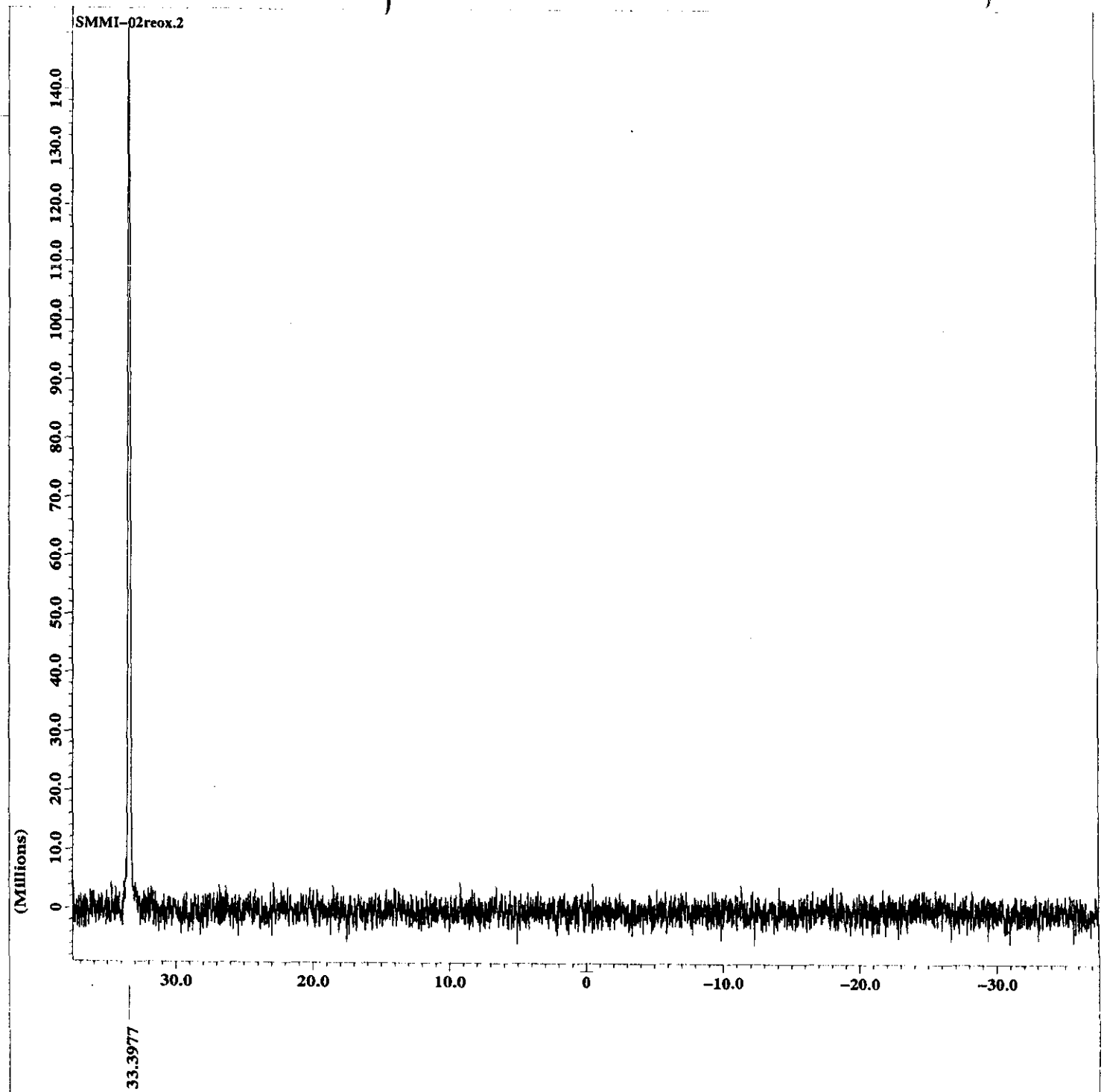

Spectrum 6: ^{31}P NMR spectrum of DPPP=O. The single peak at 33.39 ppm indicates complete oxidation of DPPP to DPPP=O.

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