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A comparison of free radical detection methods

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A COMPARISON OF FREE RADICAL DETECTION METHODS

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

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CHAPTER I

GENERAL INTRODUCTION

Free Radicals

Free Radical is the term given to any chemical element or compound which has an unpaired electron in an atomic or molecular orbital. Species which have two unpaired electrons sufficiently remote from one another so the interaction between them is weak are called biradicals. Transition metal ions and their salts are not classified as free radicals, although they do have one or more unpaired electrons in their inner, unfilled orbitals, as they do not undergo the types of reactions free radicals usually do.

Gomberg, one of the first to study free radicals (1), discovered the triphenyl methyl radical and prepared the first crystalline organic radical in 1901. Since then large numbers of stable radicals have been prepared. Later Nernst, Wurtz and Bunsen tried unsuccessfully to isolate free atomic intermediates in such chemical reactions as the reaction between hydrogen gas and chlorine gas to produce hydrogen chloride gas. Paneth (2) in 1929 used the mirror technique to demonstrate the existence of free radicals. In 1937 Hey and Waters (3) investigated a number of

reactions which involve free radical intermediates, and by the late 1930's free radical intermediate involvement in certain chemical reactions had been confirmed.

Most of the early free radical studies were done with relatively stable organic radicals. Studies of inorganic free radicals started after the matrix method of isolation and electron spin resonance method of detection had been developed shortly after World War II.

Preparation of Free Radicals

Free radicals can be prepared by both chemical and physical methods.

Chemical reactions

Many stable free radicals such as O_2, NO_2, NO, nitrosyl disulfonate, $(ON(SO_3)_2^{2-}$, Fremy's ion), and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) can be prepared by simple chemical reactions.

Gas-phase dissociation method

Rather unstable free radicals can be prepared by thermal, photochemical, and electron discharge methods in the gas phase. Radford (4) made HS, HSe and HTe radicals by flowing a stream of H_2 through an electron discharge and down a tube coated with

appropriate elements. Other free radicals which have been prepared by this method are H, N and NF_2.

Matrix isolation method

Some free radicals can be formed in solution or gas phase and then trapped in an inert media at low temperature for preservation and later study. Bass and Broida (5) give a detailed description of this method. A photosensitive material may be trapped in a frozen matrix and then irradiated to produce free radicals. Another method is to mix two reactive species and then trap them rapidly. Intermediate stages of the reaction may be so preserved.

Irradiation of solids

Crystals can be irradiated with high energy gamma or X-rays to produce free radicals. The radicals formed will remain trapped in the lattice of the crystals. Visible, ultraviolet and infrared light are also used to remove electrons and break bonds.

Methods in solution

Some free radicals such as OH and HO_2 have been generated from solutions by photolysis and electrolytic processes. Stop flow techniques using two solutions mixed just prior to entering the detection cell have been used to produce short-lived free radicals. This method is also useful for kinetic studies of free radicals.

Transmutation method

This method has been used to generate free radicals which had never been made by any of the conventional methods. Now free carbon atoms may be generated by nuclear reactions:

$$^{14}N + n = {}^{14}C + p$$

$$^{12}C + \gamma = {}^{11}C + n$$

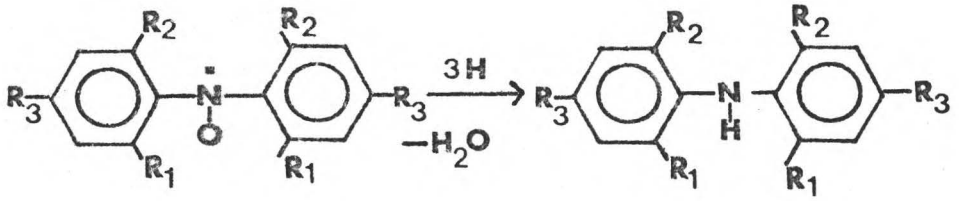
Other physical methods for free radical formation are ultrasonic vibration and rapid stirring. These are mainly used to produce free radicals from large polymeric compounds.

Free Radical Reactions

Free radicals may undergo a number of reactions resulting in pairing of the electrons and the elimination of the radical character of the sample. In an oxidation reaction the oxidation state of one of the atoms in the molecule is increased by one unit. A typical example of this type of reaction is:

$$2NO\cdot + X_2 = 2XNO$$

where the oxidation state of N changes from +2 to +3. A free radical can also undergo a reduction reaction in which the oxidation state of one of the atoms in the molecule is decreased by one unit, as in the hydrogenation of aromatic nitroxide on Raney nickel (6).



In a disproportionation reaction, changes in the oxidation state of two atoms are involved, as in the reaction of nitrogen dioxide with water:

$$2\dot{N}O_2 + H_2O = HNO_3 + NHO_2$$

in which the oxidation number of nitrogen goes from +4 to +5 and +3 states.

It is very common for two radicals to combine and lose their radical character, as in the formation of a chlorine molecule from two chlorine atoms:

$$2\dot{C}l \longrightarrow Cl_2$$

Decomposition and substitution are two reactions in which the free radical character may be perpetuated. Decomposition involves the breaking apart of a large size radical into smaller species, one of which has an unpaired electron. The decomposition of nitrosyl disulfonate radical ion is a multi-step process (7), the last of which is a radical combination reaction:

$$\dot{O}N(SO_3)_2^{2-} \longrightarrow \dot{O}NSO_3 + SO_3^{=}$$

$$\dot{O}NSO_3 \longrightarrow NO^{+} + .SO_3^{-}$$

$$\dot{O}N(SO_3)_2^{2-} + SO_3^{=} \longrightarrow ON(SO_3)_2^{-3} + .SO_3^{-}$$

$$\dot{O}N(SO_3)_2^{2-} + .SO_3^{-} \longrightarrow ON(SO_3)_3^{3-}$$

In a substitution reaction, a free radical reacts with a molecule and generates a new radical as in:

$$Cl\cdot + H_2 \longrightarrow HCl + H.$$

Interests in Free Radicals

Free radicals have become increasingly important expecially in areas of human health. Investigators in the United States, Russia and Japan have discovered a change of ESR signals during cancer growth which strongly suggests a free radical involvement in cancer development. It is presently believed that one of the causes of cancer involves radical reactions (8). In the body free radicals can be produced by radiation and chemical processes. The radicals can then react rapidly with cell membrane RNA and DNA. Because free radical scavengers are effective in reducing the number of free radicals, they are being used in cancer chemotherapy and also may prevent cancer (9).

Aging is another area in which free radicals may be important. Several authors have postulated the role of free radical

in the aging process. A few experimental results from Harmon and Piette (10) suggested that this may indeed be the case.

Arthritis has been suggested by McCord (11) as being due to the depolymerization of the synovial fluid which acts as a lubricant for the joints, the depolymerization agent being the hydroxy radical which comes from the reaction of superoxide (O_2^-) radical with hydrogen peroxide in the body.

$$HOOH + O_2^- \longrightarrow \dot{O}H + OH^- + O_2$$

Many of these free radical involvements in human health are complex processes and exact mechanisms at this time are not known.

Another area in which free radicals are of increasing interest is air pollution. Emission of free radical species such as NO and NO_2 from industry and transportation sources intensify air pollution problems.

Free radicals play important roles in organic and nitrogen chemistry. In many organic reactions free radicals exist as intermediates and the understanding of these reactions is based on our understanding of the free radicals.

CHAPTER II

DETECTION AND DETERMINATION OF FREE RADICALS

Lead Mirror Experiment

A classical demonstration of the existence of free radicals was performed by Paneth in 1929 (2). He prepared methyl radicals by decomposing gaseous tetramethyl lead which was passed through a glass tube heated at the far end. A lead mirror was left in the tube at the spot of heating. The heat decomposed the gaseous tetramethyl lead and produced the lead mirror, but as he moved the heat upstream, a second mirror was observed at the spot of heating while the first mirror gradually disappeared. This led him to the conclusion that when tetramethyl lead decomposed, metallic lead and methyl radicals were formed, and the methyl radicals reacted with the first mirror causing it to disappear. The gaseous products from the tube were found to be tetramethyl lead and ethane. Paneth then found that if he used a long enough tube, the first mirror would not disappear while the second one was being formed. This was due to the combination of methyl radicals to form ethane before they reach

the first lead mirror. From this experiment Paneth was able to calculate the rate constant for the combination of methyl radicals. This removal of mirror technique is particularly useful for studying volatile organic radicals. Labelling methods can be used to increase the sensitivity. The products caught in a trap are studied to determine what free radicals were present.

Chemical Titration Method

Chemical methods can be used for the detection and determination of free radicals. The purity of commercial DPPH has been determined by reaction with thiosalicylic acid (12). DPPH may also be determined by titration with quinol, hydrazobenzene, sodium iodide, ferrous salt in aqueous alcoholic solution and O-mercaptobenzonic acid. Another method is to use a stable free radical which has been well characterized to react with another radical of unknown concentration, as in the reaction between DPPH and Fremy's salt. The radicals decolorize each other when combined.

Gouy Method

Free radicals, due to the presence of the unpaired electrons, are paramagnetic and will interact with an external magnetic field. A Gouy balance, consisting of an analytical balance and a magnet, can be used to determine the magnetic properties of a radical. The

sample is weighed with and without the external field and the weight difference is used to calculate the magnetic moment, also known as the magnetic susceptibility of the sample. The equation used for the calculation is:

$$10^6 \chi_m = \frac{\alpha + \beta F'}{W}$$

Where

χ_m = Mass susceptibility of the substance

α = 0.029 x specimen volume (a constant allowing for displaced air)

β = Tube calibration constant

F' = The force on specimen i.e. F-δ, F being the observed force, δ is the force due to the tube alone.

W = Weight of the sample.

The tube constant is determined for each field strength using a sample of known susceptibility. From Gouy balance measurements it is possible to detect the presence of radicals and calculate the number of unpaired electrons in a paramagnetic molecule.

Faraday Method

Another method which is closely related to the Gouy method is the Faraday method, which uses a nonuniform field. The force experienced by the sample is directly measured.

$$F = m\chi H \frac{\partial H}{\partial X}$$

A small specimen is used in a region of constant $\frac{\partial H}{\partial X}$. Since regions of constant $\frac{\partial H}{\partial X}$ are difficult to obtain the displacement of the specimen is kept as small as possible. This method is particularly suitable for solids which may be powdered and compressed into tablets and placed at the region of maximum $\frac{\partial H}{\partial X}$ in the magnetic field. The force F may be measured with a torsion arm suspended from a fiber. A few milligrams of sample is required and a high degree of accuracy within $\pm$ 0.1% may be obtained in the measurement of mass susceptibility.

Quincke Method

There are some other methods which are similar to the Gouy balance method in principle. One of these is known as the Quincke method (Figure 1), which is strictly applicable to liquids and solutions and with some modification to gasses. The magnetic

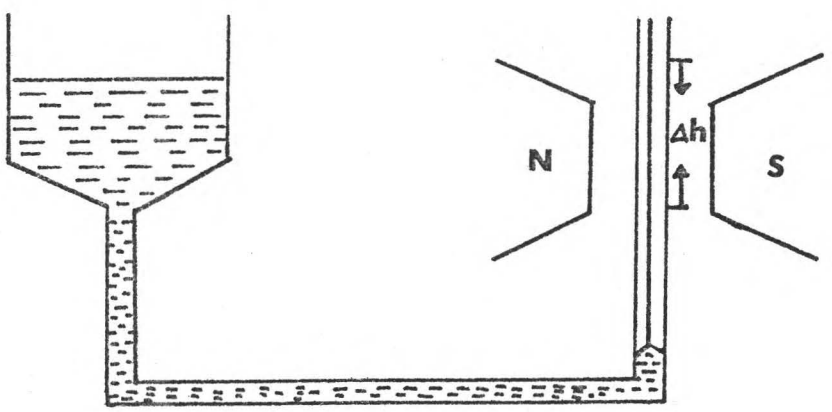


Figure 1. Quincke Method

force acting on the sample in a capillary tube is measured in terms of the hydrostatic pressure. The hydrostatic pressure being $g(p - p_0)\Delta h$ and the magnetic force is $1/2\ H^2(k - k_0)$ so the magnetic susceptibility is

$$\chi = \frac{k}{p} = \frac{2g\Delta h}{H^2} + \chi_0 \frac{p_0}{p}$$

In those equations

k_0 = χ of gas over the meniscus

k = χ of the liquid

χ = susceptibility per gram of liquid

χ_0 = χ of gas over meniscus

p_0 = density of gas over meniscus

p = density of liquid

H = applied magnetic field

NMR Method of Measuring Magnetic Susceptibility

Determination of the paramagnetism of a substance in solution by nuclear magnetic resonance was first mentioned by Evans (13) and was later referred as the Evan's method. Measuring of magnetic susceptibility by this NMR method has several advantages over the classical method of using a Gouy balance. A smaller sample is required; less than 0.03 ml of a dilute solution can be studied. The temperature is easily contolled and varied and measurements can be made with greater speed and simplicity.

The NMR method is based on the principle that the position of a line in the spectrum of a molecule is dependent on the bulk susceptibility of the medium in which the molecule is found. The shift of a proton resonance line of an inert substance due to the presence of a paramagnetic ion is given by the theoretical expression:

$$\frac{\delta\nu}{\nu_0} = \frac{2\pi}{3}(\chi_v - \chi_{v'})$$

Where $\delta\nu$ is the shift, ν_0 is the applied field, χ_v is the volume susceptibility of the solution containing the paramagnetic ion and $\chi_{v'}$ is the volume susceptibility of the reference solution. The mass susceptibility of the dissolved substance is given by the equation:

$$\chi_m = \frac{3\,\Delta f}{2\,fm} + \chi_o + \chi_o\left(\frac{do - ds}{m}\right)$$

Where

χ_m = mass susceptibility of the dissolved substance

Δf = frequency separation between the two lines

f = frequency of the proton resonance

m = mass of the substance contained in 1 ml of solution

d_o = density of the solvent

d_s = density of the solution

χ_o = mass susceptibility of the solvent

Ortho-Para Hydrogen Conversion Method

Ordinary hydrogen gas at room temperature is a tautometric mixture of 3 volumes of ortho hydrogen (nuclear spins parallel) and 1 volume of para hydrogen (nuclear spins antiparallel). If hydrogen gas is cooled to very low temperature, the molecule tends to enter the lowest energy state possible. For the molecule of para hydrogen this state corresponds to J=0 and for the ortho hydrogen this state corresponds to J=1. Para hydrogen is therefore more stable at low temperature and molecules of ortho hydrogen tend to change to para hydrogen. The conversion from ortho to para hydrogen is exothermic, ΔH being -337.17 cal/mole. Bonhoeffer and Korteck (14) had confirmed through experiments that the conversion is extremely slow; it takes about a month for normal 25% para hydrogen to be converted to about 90% para hydrogen, and the energy released by the conversion is sufficient to evaporate 64% of the original liquid. In order for the transformation of one form of hydrogen into another to take place at faster rate, the molecules must be activated. One way to activate these molecules is to bring these molecules in contact with a catalyst. It has been observed that paramagnetic substances are efficient catalysts and the rate of conversion is related to the concentration of the paramagnetic ions. The theory of the conversion using a solid free radical has been given by Wingner (15), Kalckar and Teller (16).

Harrison and McDowell (17) studied the kinetics of the ortho-para hydrogen conversion and the effect of temperature and pressure on the rate of conversion using DPPH as the catalyst. They concluded that the free radical catalysis is due to the interaction between hydrogen molecules in a physically absorbed layer and the inhomogenous magnetic field at the surface of the catalyst which enables the otherwise forbidden ortho-para transition to take place Turkevich and Selwood (18) in their study of solid free radicals as a catalyst for the conversion concluded that the mixture of DPPH and zinc oxide was far more effective for the conversion at liquid air temperature than the zinc oxide or the free radical alone.

During the ortho-para hydrogen conversion process for determination of radical concentration, normal hydrogen is first cooled to a liquid in the presence of a catalyst to form the para species. It is then allowed to warm up while still in para form and is bubbled through a solution containing a radical of unknown concentration. The percent of ortho and para hydrogen then present is determined with a gas chromatograph. The greater the amount of ortho form the more concentrated was the radical in the solution. Sensitivity to about 10^{-4} mole is usually attained. This method has seldom been used since the 1940's, however, as more convenient methods have been developed.

Spectrophotometric Method

Ultraviolet spectroscopy is an important technique for studying the structure of molecules that can be prepared in gaseous phase. From the interpretation of the vibrational and rotational fine structure, the electronic structure of the radical can be derived. A number of simple radicals such as NO_2 and ClO_2, had been studied by this method and many other radicals could be studied in the solid state. Infrared and Raman spectroscopy are also used for the electronic structure studies but have relatively low sensitivity.

UV and visible spectra of a radical differ from the spectra of the parent molecule due to the presence of the unpaired electron. This brings about a rearrangement of the electronic energy levels of the radical as compared to those of the parent molecule and therefore a shift in the electronic absorption. The infrared spectrum of a radical also differs from its parent molecule due to the disappearance of one bond and therefore a change in its vibrational frequency.

Many free radicals are intensely colored and their concentrations can be determined from spectrophotometric data. The absorptivity of DPPH is around $10^4 M^{-1} cm^{-1}$ in benzene, about 5 times the absorptivity of potassium permanganate in water.

Flash photolysis is another technique useful in studying very short-lived species, such as NO_3 is aqueous solution. In this

technique a powerful flash of light is used to produce the radicals. Then a second flash is used as a photographic source, triggered at short intervals after the first to photograph the absorption spectrum. Concentration, reaction mechanism and kinetic data can be obtained from the spectrum.

The stop-flow method is relatively new in producing unstable radicals and studying the kinetics of radical reactions. Two cylinders are filled with solutions which will react when mixed. Aliquots of the two solutions are rapidly forced through the mixing jet into the spectrophotometric observation chamber. The flow is rapidly stopped by a stop plunger and the reaction is followed spectrophotometrically and a storage oscilloscope or a computer is used to store the data.

Mass spectrometry is another technique for free radical studies. It has been used to detect and identify free radicals species, measure radical concentrations and for the determination of radical ionization potentials and bond dissociation energies. Free radicals which have been studied by mass spectrometer include HO_2, N, NH_2, N_2H_3, and $NH_2(CH_3)_2$ (19).

Electron Spin Resonance (ESR)

Electron spin resonance is another physical technique for detecting species with unpaired electrons. This method was first

used in Russia and at Oxford in 1945. Most atoms or molecules do not give ESR signals, for in these substances electrons are paired, that is, for every electron in $M_s = -1/2$ there is another in $M_s = +1/2$ state. A transition from -1/2 to +1/2 never happens in these substances according to Pauli's Exclusion Principle, therefore paired electrons do not give ESR signals.

If an electron is placed in an external magnetic field there are two possible orientations for the magnetic moment of the electron which corresponds to two energy levels. Microwaves whose quanta have the energy of gBH will induce a transition between the two energy levels.

$$E = h\nu = gBH$$

g = g factor ν = microwave frequency

B = Bohr Magneton h = Plank's Constant

H = Magnetic field strength around 3500 gauss

The above equation is called the fundamental equation for ESR spectroscopy. Like most forms of spectroscopy, ESR spectroscopy records the net absorption of energy.

The unpaired electron interacts with nuclei in the radical which have non-zero spin to give hyperfine structure. This interaction between electron and nuclear spin of the same atom or molecule causes the ESR spectrum to consist of a number of lines instead of a single line. From the number of lines and their relative

intensities it is possible to deduce the spin of the nuclei with which the electron is interacting. The separation between the lines are determined by the magnetic moment of the nuclei concerned and the strength of the interaction between the electron spin and nuclear spin. In single crystal studies information such as the wave function of the electron and other quantum mechanical data are available.

Using ^{14}N as an example, the nuclear spin for N is 1 and the magnetic moment is 2 + 1 which is equal to 3 so it has 3 magnetic quantum numbers namely, +1, 0, -1, and the following diagram shows the possibility of the transition between the energy levels. The possibility of transition between the energy levels is governed by the ESR selection rules:

1) ΔM_s (electron spin quantum number) = 1

2) ΔM_i (magnetic quantum number) = 0

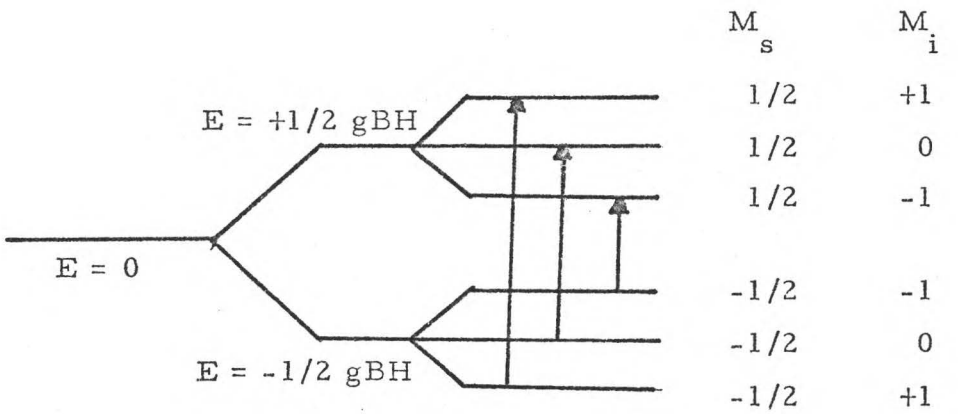


Figure 2. Hyperfine Splitting by the ^{14}N Nucleus

In ESR the microwave frequency applied to the sample is kept constant at about 9.5 G Hz, but the magnetic field is varied. Experimently, it is easier to vary the magnetic field than the frequency. The position of the line will thus be indicated by the value of the magnetic field where the absorption takes place. However, a function of the ratio of frequency to the field at resonance is used to determine the line position, that is

$$g = k\frac{\nu}{H} \qquad k = \frac{h}{B}$$

ν is microwave frequency
h is Plank's Constant
B is Bohr magenton

The g factor thus can be considered as a quantity characteristic of the molecule in which the unpaired electron is located. The value of g depends on the orientation of the molecule containing the unpaired electron with respect to the external magnetic field. In a

solution or in a gas g is the average over all the orientations because of the freedom of moment in those phases. But in a crystal the moment is restricted. In isotropic crystals the g value is independent of the orientation of the crystal in the magnetic field, and is called an isotropic g value. In less symmetrical atoms, the g value depends on the orientation and is called an anisotropic g value.

Since the discovery of ESR, this method has given more information about the structure of free radicals than any other method. It often indicates on which atom the unpaired electron is localized, which orbital it is in, the concentration of the unpaired electron, and can often detect a radical amount as low as 10^{-12} mole. But the handling of the experimental data is more difficult than any other method.

Quantitative measurements of radical concentration using ESR requires great care if reasonable accuracy is required. Usually a symmetrical dual cavity is used. The area under the absorption curve is proportional to the radical concentration so the procedure is to compare the area under the absorption curve of an unknown sample with that of a standard. Two widely spaced modulation frequencies have to be used in order to separate the signals from the two samples. Average values are obtained by interchanging the samples to eliminate possible different magnetic

and microwave fields at the samples. It is important to know that no comparison is valid unless the microwave power is low enough to avoid saturating the system.

A single rectangular cavity can be used to do quantitative work with some minor modification of the cavity. If the single cavity cell is used identical spectrometer setting such as microwave power, modulation amplitude, magnetic field, etc. are required for both the unknown and the standard. If C_1 and C_2 are the number of unpaired electron in two samples then

$$\frac{C_1}{C_2} = \frac{h_1}{h_2}$$

where h_1/h_2 is the ratio of absorption peak heights. This is true for species with the same spectral line width and shape. If the line shape is both Gaussian but their widths differ

$$C_1/C_2 = h_1/h_2 \frac{\Delta H_1^2}{\Delta H_2^2}$$

is used for comparison. ΔH_1 and ΔH_2 are the line widths.

ESR is by far the most useful method in studying free radicals. But since we did not have ready access to this instrument alternative methods were required to continue our work with free radicals.

In studies involving free radicals seldom have two or more methods been employed to determine the concentration of each specific free radical involved. No such study has been found

comparing the sensitivity and accuracy of two or more methods using a common radical. This is the first such study to do so. This thesis describes the magnetic measurements using the Gouy method and NMR, chemical methods and spectrophotometric techniques of detection and determination, and compares and contrasts such aspects as sensitivity, accuracy and ease to use.

CHAPTER III

SOME STABLE FREE RADICALS

Free radicals can be divided into two groups according to their stability and reactivity. The first group consists of large, thermodynamically stable, primarily organic compounds which owe their existence to resonance stabilization. The unstable group consists of atoms and molecules produced under energy-rich conditions, such as NH, NH_2, and OH. A few inorganic free radicals of intermediate stability and reactivity are NF_2, SO_3F, SF_3, etc. In our work we have chosen one moderately stable inorganic free radical, Fremy's salt and five organic free radicals, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidin -1-yloxy (CTM-pyrrolidin), DPPH, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy (CTM-pyrrolin), galvinoxyl and 4-acetamido-2,2,6,6-tetramethylpiperidino -1-oxyl (ATMP).*

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*Note: CTM-pyrrolidin, CTM-pyrrolin and ATMP are the author's own way of abbreviation.

Nitrosyl Disulfonate Ion Free Radical

Nitrosyl disulfonate ion (Figure 3) was first prepared by E. Fremy in 1843 (20). He obtained the violet-colored ion by oxidizing hydroxylamine disulfonate ion (Figure 4) with lead dioxide. The radical ion was then precipitated as the golden-yellow salt with potassium chloride. More recently, electrochemical (21) and radiolysis (22) methods have been used to prepare this salt.

Two crystalline forms of this salt are known. At temperatures below 25°C monoclinic crystals of the salt will precipitate from solution as golden yellow diamagnetic crystals. At higher temperatures a golden-brown, weakly paramagnetic triclinic form crystallizes. In aqueous solution both forms give a bright violet paramagnetic solution which has a maximum absorbance at 540 nm with an absorptivity of 20.8 $M^{-1}cm^{-1}$.

Nitrosyl disulfonate salt is a dimer in the solid state. Its structure was studied by Yamada and Tschida (23). On the basis of UV and IR studies they favored the peroxide structure. In 1968 X-Ray crystallography (24) studies confirmed this structure.

Magnetic measurements of the solution have shown that there is one unpaired electron per molecule (25), and ESR studies suggest that the unpaired electron is located primarily on the nitrogen.

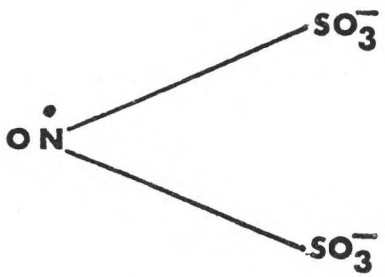


Figure 3. Nitrosyl Disulfonate Free Radical Ion

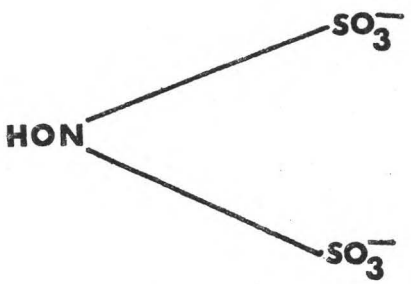


Figure 4. Hyroxylamine Disulfonate Ion

Several moderately stable salts of nitrosyl disulfonate have been synthesized and their magnetic properties were reported by Fillmore and Wilson (26).

In organic chemistry, Fremy's salt is often used as a mild, selective oxidizing agent, capable of oxidizing phenols and aromatic amines. It will oxidize hydrazobenzene to azobenzene and isobutuanol to 2-methyl-2-propene-1-al. Raschig(27) used

nitrosyl disulfonate ion to convert aniline to nitrobenzene and Teuber (28) studied the reaction mechanism of this conversion.

Nitrosyl disulfonate ion is a common standard for ESR. The ESR spectrum of the anion is a triplet with equidistant signals of equal intensity, indicating the electron is primarily located around nitrogen. The distance between the signals is 13 gauss and the width of each is 0.5 gauss. Physical chemists have used this radical in studying various magnetic effects such as the Overhauser Effect. Fremy's salt is also a working substance for high-sensitivity nuclear precision magnetometers and Maser-type quantum generators (6).

Various studies on the reactions and decomposition of nitrosyl disulfonate ion had been made. Gehlen (29) reported that nitrosyl disulfonate ion reacts with nitric oxide to form ON-$ON(SO_3)_2^{-2}$. Wilson and Hayes (7) studied the rapid reaction of nitrosyl disulfonate ion and DPPH in different solvents and reported the second-order rate constant of the reaction to range from $1.06 \times 10^{+3} M^{-1} sec^{-1}$ to $1.59 \times 10^{+3} M^{-1} sec^{-1}$.

Murib and Ritter (30) studied the decomposition of nitrosyl disulfonate ion in acid solution. They proposed that the decomposition proceeded by two routes. The first route was a reaction first order both in nitrosyl disulfonate ion and hydronium ion and the other route was a chain process propagated by nitrous

acid. Li and Ritter (31) also studied the decomposition of the Fremy's salt ion in nitrous acid and the reaction of the ion with sulfite ion. Before that Haga (32) had reported that the sulfite ion would react with nitrosyl disulfonate ion in a mildly alkaline solution and the decomposition was of first order. Hayes and Wilson (7) studied the decomposition of Fremy's salt ion in DMSO, acetonitrile and dichloromethane. A multi-step mechanism was found involving sulfite ion, sulfite ion radical and nitrosyl monsulfonate radical as intermediates. The products of the decomposition were nitrosonium ion, hydroxylamine disulfonate ion and hydroxyamine trisulfonate ion.

Fillmore and Wilson (33) studied the decomposition of nitrosyl disulfonate ion in strongly alkaline solution. They found the decomposition to be first order in both hydroxide and nitrosyl disulfonate ions. A multi-step mechanism was proposed with 2nd order rate constants of the initiating step ranging from 1.2×10^{-7} to $2.25 \times 10^{-5} M^{-1} sec.^{-1}$. The products of the decomposition reaction were hydroxylamine disulfonate ion, hydroxylamine trisulfonate ion and nitrite ion.

2, 2-Diphenyl-1-picryl Hydrazyl (DPPH)

DPPH (Figure 5) is another moderately stable free radical, first prepared by Goldschmidt (34) in 1929 by oxidizing 1, 1-diphenyl-2-picryl hydrazine (DPPH-H) (Figure 6) with lead dioxide.

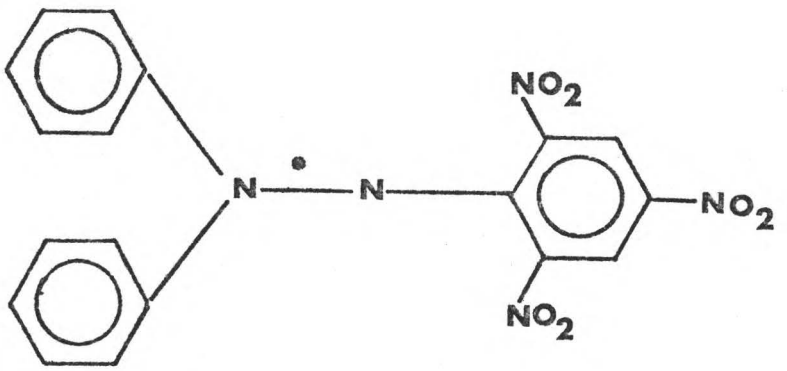


Figure 5. 2, 2, -Diphenyl-1-picryl Hydrazyl (DPPH)

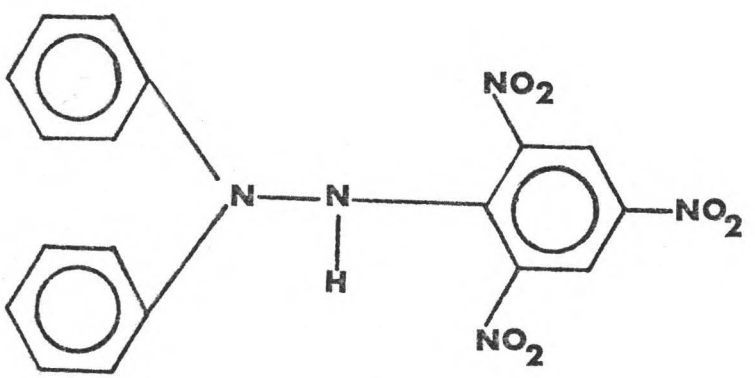


Figure 6. 1, 1-Diphenyl-2-picryl Hydrazine (DPPH-H)

Because of its several applications, interest in hydrazyl chemistry has been focused mainly on this radical. DPPH has been used as a standard in ESR work, for spin concentration, line spacing and field scale determinations. It has a g value of 2.0037 Bohr magnetons. The ESR spectrum of DPPH in benzene shows five absorption lines with separation of 2.8 gauss, indicating that the unpaired electron is localized near the N-N bond. A solution of DPPH in benzene has a deep purple color, with maximum absorption band at 525 nm and at 332 nm. DPPH is also soluble in DMSO, chloroform, and acetone. At 525 nm the absorptivity of DPPH in DMSO is 8580 $M^{-1}cm^{-1}$ (7).

The reactions of DPPH are many. Solomon and Swift (35) studied the reaction of DPPH with various halogens. Ionic attack on DPPH was the proposed mechanism. Weil and Anderson (12) studied the reaction of DPPH with thiosalicylic acid. This was found to be an accurate method for DPPH analysis. The product of this reaction was 1, 1-thiodibenzonic acid. The abstraction of hydrogen atom from mercaptans by DPPH was investigated by Russell in 1954 (36). The activation energy is approximately constant at 15.0 Kcal for a number of mercaptans. However, the most frequently reported reactions of DPPH as a radical scavenger are in polymer chemistry. Since Bartlett and Kwart (37) observed that DPPH strongly inhibits the polymerization of vinyl acetate,

DPPH has become a well-known and generally useful radical scavenger in polymer chemistry. DPPH has been used to determine the rate of formation of free radicals by following its spectrophotometrically during the course of the reaction. This kind of reaction may not be quantitive as reaction products have been shown to also react with DPPH.

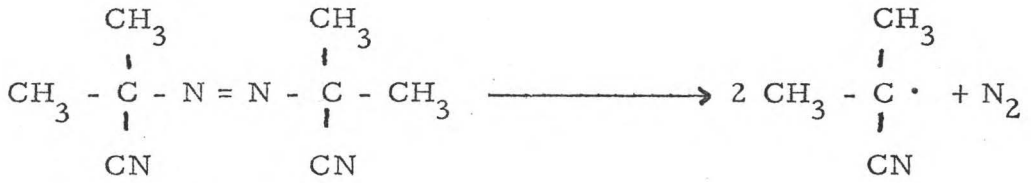
In 1966 Singh, Bhaskar and Rao (38) studied the kinetics of hydrogen abstraction from proton donors by DPPH using ESR spectroscopy. They found some relations between kinetic data and hydrogen bonding. Nemcova and his co-worker (39) obtained some kinetic data during the reactions between DPPH and oxidizing agents such as copper (II) perchlorate and iron (III) perchlorate. The oxidation reactions with copper (II) and iron (III) were shown to follow a second-order rate law, with rate constants $(2.73 \pm 0.15)\ 10^4 M^{-1} sec^{-1}$ and $(4.50 \pm 0.33) 10^4 M^{-1} sec^{-1}$ respectively. The reactions of DPPH with Cu (II) and Fe (III) are:

$$DPPH + Cu\ (II) = DP^+PH + Cu\ (I)$$

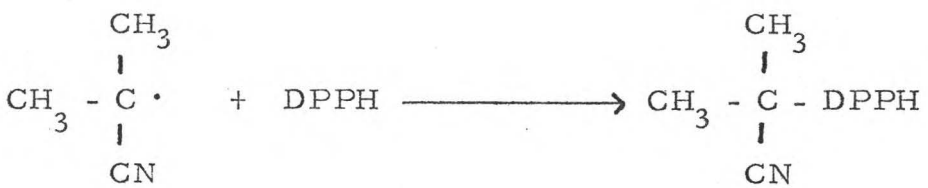
$$DPPH + Fe\ (III) = DP^+PH + Fe\ (II)$$

DPPH has been used in reactions with other radicals. Weil, Sane and Kinkade (40) studied the products resulted from reaction between DPPH and NO_2. DPPH has also been shown to react with triphenylmethyl radical in benzene solution in the absence of air to form adducts sufficiently stable to be isolated.

Molecular oxygen reacts with DPPH and ends up with the reversible formation of a molecular complex (41). DPPH had been used to react with azo-bis-iso-butyronitrile (AIBN). When AIBN is exposed to the light, a radical species, iso-butyronitrile, is formed which reacts with DPPH. Work by Verdin (42) showed that the reaction is further complicated by the presence of oxygen.



AIBN iso-butyronitrile radical



Some Stable Organic Free Radicals

In the course of this work four other stable organic free radicals have been used. 3-carbamoyl-2,2,5,5-tetramethylpyrrolidin-1-yloxy (CTM-pyrrolidin), (Figure 7), and 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy (Figure 8). Both of these radicals are yellow crystals, soluble in methanol forming a pale yellow solution. 4-acetamido-2,2,6,6-tetramethylpiperidino-1-oxyl (ATMP) (Figure 9) is a red crystal also soluble in methanol. The synthesis of these three radicals has been reported by Rozantsev(6).

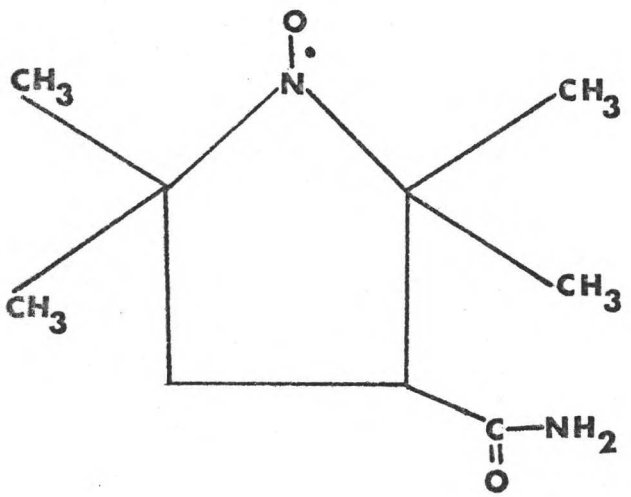


Figure 7. 3-Carbamoyl-2,2,5,5-tetramethyl-pyrrolidin-1-yloxy (CTM-pyrrolidin)

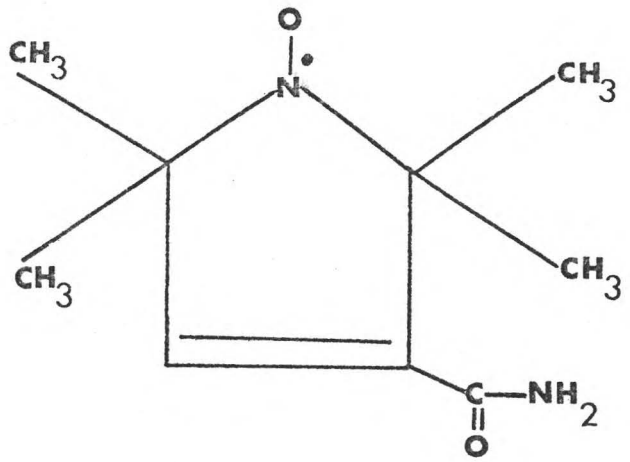


Figure 8. 3-Carbamoyl-2, 2, 5, 5-tetramethyl-3-pyrrolin-1-yloxy (CTM-pyrrolin)

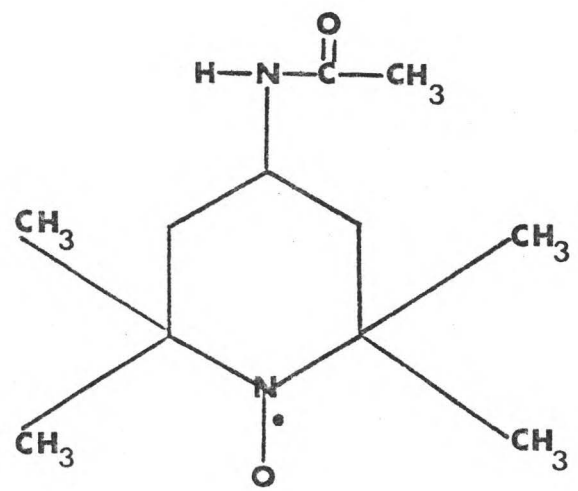


Figure 9. 4-Acetamindo-2, 2, 6, 6-tetramethyl-piperidino-1-oxyl (ATMP)

Galvinoxyl (Figure 10) in the solid state is a dark blue crystal. It is soluble in benzene, chloroform and in methanol. This compound can be prepared from 4, 4'-dihydroxy-3, 3', 5, 5'-tetra-t-butyldiphenyl methane and lead dioxide in anhydrous ether while being stirred for about four hours under nitrogen atmosphere (43). Galvinoxyl had been shown to have a very large absorptivity at 420 nm, around 200, 000 $M^{-1}cm^{-1}$ in benzene solution. At 407 nm the value is 30, 000 and at 772.5 nm a weak maxium occures with a = 697 $M^{-1}cm^{-1}$. Galvinoxyl can be tritrated iodometrically for its purity determination.

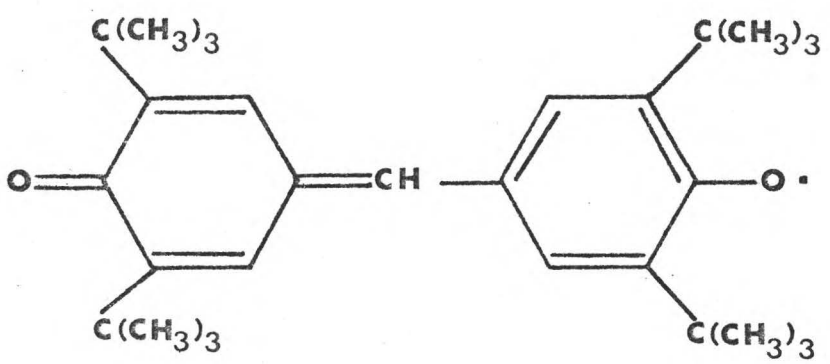


Figure 10. Galvinoxyl Free Radical

CHAPTER IV

EXPERIMENTAL AND RESULTS

Materials

Potassium nitrosyl disulfonate was prepared by the method of Raschig, as modified by Ritter and Murib (30), in which sodium nitrite reacted with sodium hydrogen sulfite in an aqueous solution at $0^{\circ}C$ to form hydroxylamine disulfonate ion,

$$NO_2^- + 2HSO_3^- \longrightarrow HON(SO_3)_2^{-2} + OH^-$$

which was then oxidized to nitrosyl disulfonate ion using potassium permanganate.

$$3HON(SO_3)_2^{-2} + H^+ + MnO_4^- \longrightarrow 3ON(SO_3)_2^{-2} + MnO_2 + 2H_2O$$

The solution was stirred for an hour at $0^{\circ}C$ in a brine bath and the solid manganese dioxide was filtered off, leaving a deep purple nitrosyl disulfonate ion solution. The potassium salt was then crystallized by adding solid KNO_3 to the solution at $0^{\circ}C$. The product was then collected by filtering. The golden yellow crystals were purified by recrystallization from a warm (45°-$50^{\circ}C$) 1 N potassium hydroxide solution, washed with ice water and methanol and stored in a vacuum dessicator.

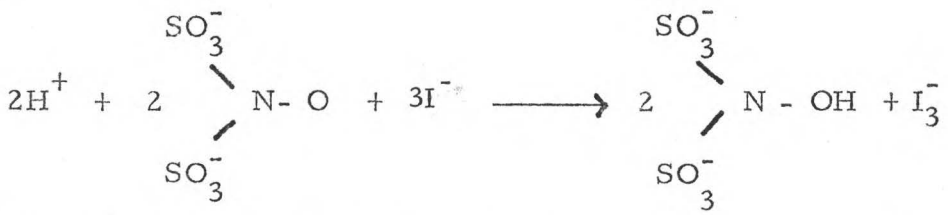
DPPH, CTM-pyrrolidin, CTM-pyrrolin and galvinoxyl were purchased from Aldrich Chemical Company and ATMP from Eastman Kodak Chemical Company. They were used as received.

Thiosulfate solution was prepared from reagent grade sodium thiosulfate and was standardized using analytical reagent grade potassium dichromate. All the samples were weighed on a Mettler H6T balance.

Chemical Titration

Fremy's salt

A weighed amount of Fremy's salt was dissolved in water and a freshly prepared acidic KI solution was added to the violet solution. The color of the solution changed to brown as the following reaction took place:



The solution was allowed to stay in the dark for five minutes and was then quickly titrated with thiosulfate solution to the starch endpoint. The results were tabulated in Table 1.

DPPH

The most common impurities in commercial DPPH is DPPH-H and solvent (44). DPPH-H will not affect our determination

since it does not have any radical character (12).

Ten ml of reagent grade acetic anhydride and an excess of sodium iodide were added to DPPH in a glass stoppered flask. The solution was allowed to stand in the dark for about ten minutes, then 50 ml of water and 15 ml of benzene were poured into the flask. The mixture was then saturated with sodium sulfate to clear the aqueous layer, and the liberated iodine was titrated with standard thiosulfate solution with vigorous stirring. Benzene was added to absorb colored organic products so that the endpoint of the titration would be clearly seen. See Table 2 for the results.

CTM-pyrrolidin and ATMP

A weighted amount of the radical was first dissolved in methanol. An excess amount of KI was then added to the solution. It was then acidified with 6 M HCl. After standing in the dark for ten minutes the solution was titrated with the thiosulfate solution. The results were tabulated in Table 3 and 4.

CTM-pyrrolin

The purity of this free radical was not determined by titration as a suitable solvent for this compound could not be found. The solvents that had been tried were ethanol, methanol, acetone, chloroform and benzene.

Galvinoxyl

Galvinoxyl was titrated by the method of Muller and Ley (45). About 0.05 gm of the radical was dissolved in 3-4 ml of benzene and a mixture of 25 ml of acetic acid, 20 ml of benzene and 1 gm of sodium iodide was poured into the radical solution with vigorous stirring. Small pieces of dry ice were added periodically during the analysis. After two minutes 20 ml of water was added to the mixture and the free iodine was titrated with the thiosulfate solution. See Table 5 for the results.

TABLE 1

TITRATION OF FREMY'S SALT

Weight of Sample	Vol. of $S_2O_3^=$	% Purity
0.0551 gm	1.67 ml (0.12001M)	97.7
0.1475 gm	4.46 ml (0.12001M)	97.4
0.1084 gm	6.36 ml (0.0618M)	97.2
0.1079 gm	6.35 ml (0.0618M)	97.5
0.1461 gm	8.34 ml (0.0618M)	97.7

Average purity: 97.5%

TABLE 2

TITRATION OF DPPH

Weight of Sample	Vol. of $S_2O_3^=$ (0.1236M)	% Purity
0.0400 gm	7.89 ml	96.1
0.0359 gm	7.08 ml	96.1
0.0430 gm	8.48 ml	96.1

Average purity: 96.1 %

TABLE 3

TITRATION OF CTM-PYRROLIDIN

Weight of Sample	Vol. of $S_2O_3^=$ (0.0191 M)	% Purity
0.0800 gm	22.1 ml	97.8
0.0601 gm	16.6 ml	97.7
0.0762 gm	21.0 ml	97.5

Average purity: 97.7%

TABLE 4

TITRATION OF ATMP

Weight of Sample	Vol. of $S_2O_3^{=}$ (0.0191M)	% Purity
0.0346 gm	7.96 ml	93.7
0.0369 gm	8.48 ml	93.6
0.0379 gm	8.72 ml	93.7

Average purity: 93.7%

TABLE 5

TITRATION OF GALVINOXYL

Weight of Sample	Vol. of $S_2O_3^{=}$ (0.0191M)	% Purity
0.0569 gm	6.52 ml	92.3
0.0593 gm	6.80 ml	92.4
0.0540 gm	6.18 ml	92.2

Average purity: 92.3%

Spectrophotometric Determination of Radical Concentration in Solution

Equipment

Beckman D. U. and Cary 15 spectrophotometers were used to determine the concentrations. Coleman 1.0 cm light path cells with stoppers were used. All the spectra were taken at room temperature (23 $\pm$ 1°C). The solvent used for the different radicals are listed below in Table 6.

TABLE 6

SOLVENTS USED FOR SPECTRO PHOTOMETRIC DETERMINATIONS

Radical	Solvent
Fremy's salt	water buffered at pH7
DPPH	benzene
CTM-pyrrolin	methanol
CTM-pyrrolidin	methanol
ATMP	methanol
Galvinoxyl	benzene

Spectrophotometric Results

DPPH

A solution of known concentration: 5.57×10^{-5} M (prepared from a solid sample whose purity had previously been determined by titration) was used to determine the absorptivity at 520 nm. The absorbance was 0.663 and the calculated absorptivity was $1.20 \times 10^{4} M^{-1} cm^{-1}$. See Figure 11.

Fremy's salt

The concentration of aqueous Fremy's salt solution could easily be determined since its absorptivity at 540 nm (Figure 12) is well known (20.8 $M^{-1} cm^{-1}$). The purity of the salt we prepared, as was determined from this method, was 97.9% pure.

CTM-pyrrolidin

This radical in methanol has a maximum absorption band at 408 nm (Figure 13). The calculated absorptivity was $6.76 M^{-1} cm^{-1}$.

CTM-pyrrolin

This radical, in methanol had no absorption in the visible region. It has a very strong band at 205 nm ; near the cut-off region of the Cary 15 spectrometer. No reliable spectrophotometric data was obtained.

ATMP

This radical formed a reddish-brown solution in methanol, and absorbed rather strongly at 450 nm with an absorptivity of 11.20 $M^{-1}cm^{-1}$. See Figure 14.

Galvinoxyl

The visible spectrum of this radical in benzene showed maxima absorption at 408 nm (a = 29400 $M^{-1}cm^{-1}$), 435 nm (a = 166000 $M^{-1}cm^{-1}$) and a weaker maximum at 773 nm (a = 607 $M^{-1}cm^{-1}$). The radical was characterized by the extinction coefficient at 773 nm (Figure 15) using a = 607 $M^{-1}cm^{-1}$ for the purest sample (100%).

Summary of spectrophotometric results is given in Table 7.

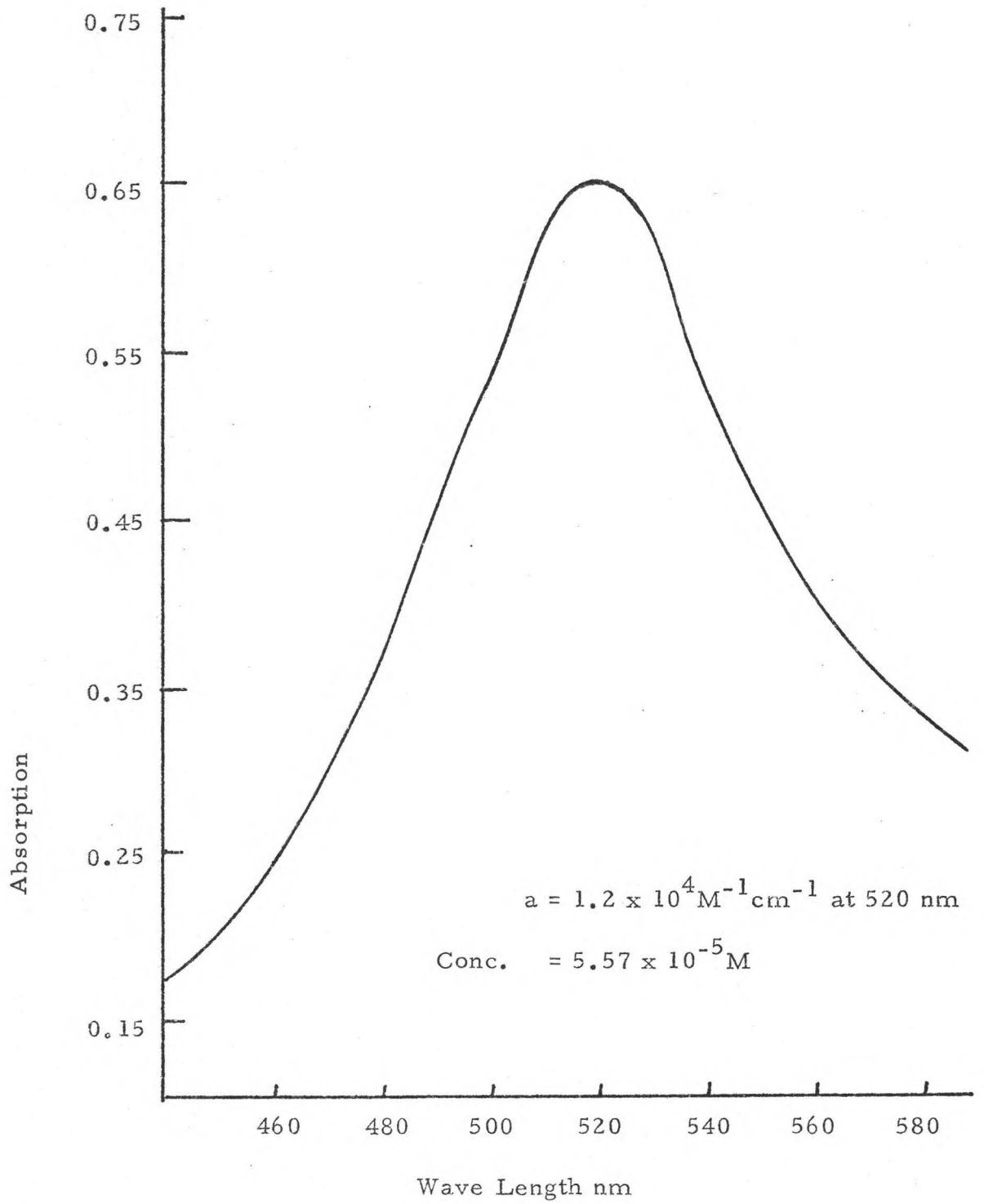


Figure 11. Absorption Spectrum of DPPH in Benzene

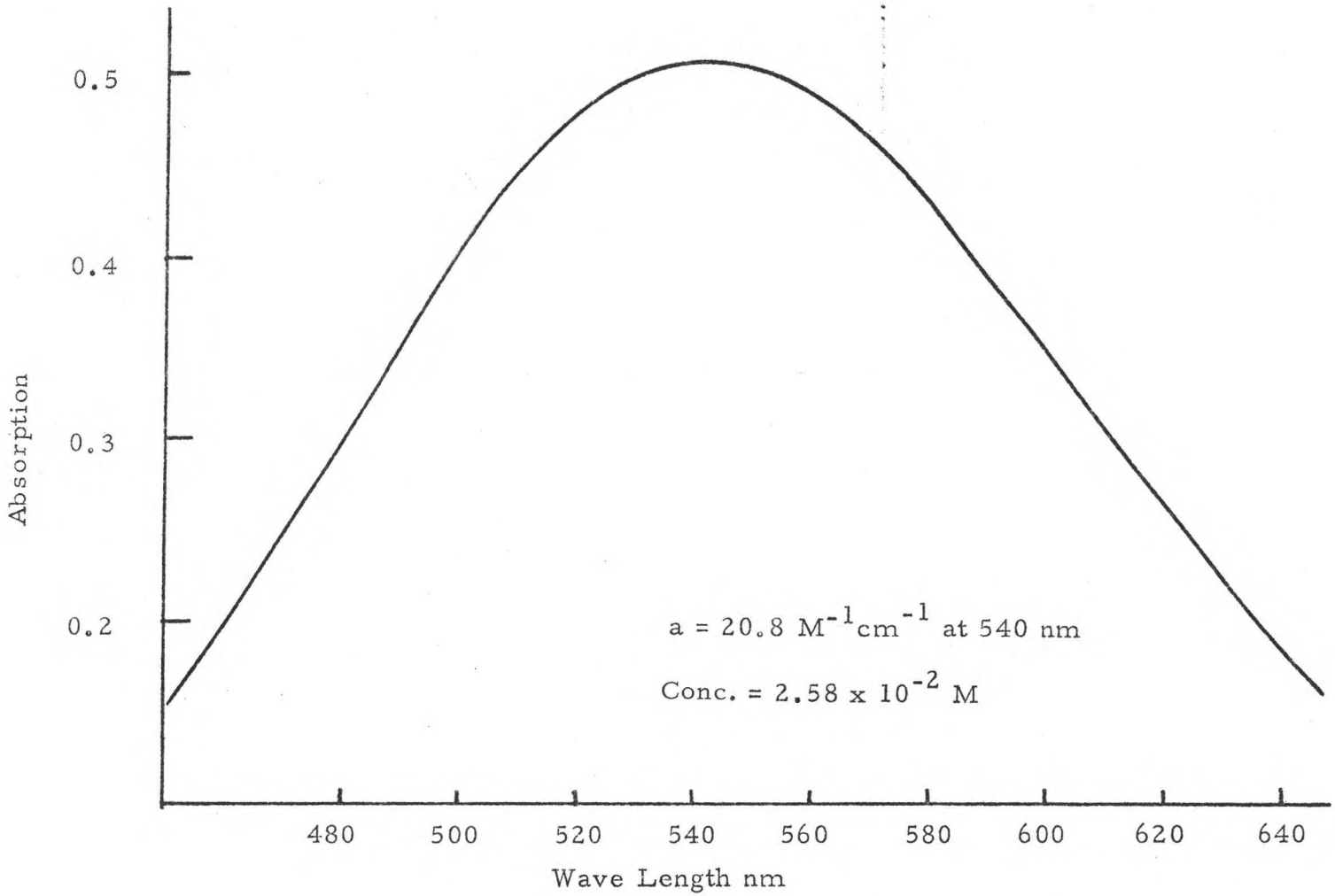


Figure 12. Absorption Spectrum of Fremy's Salt in Aqueous Solution

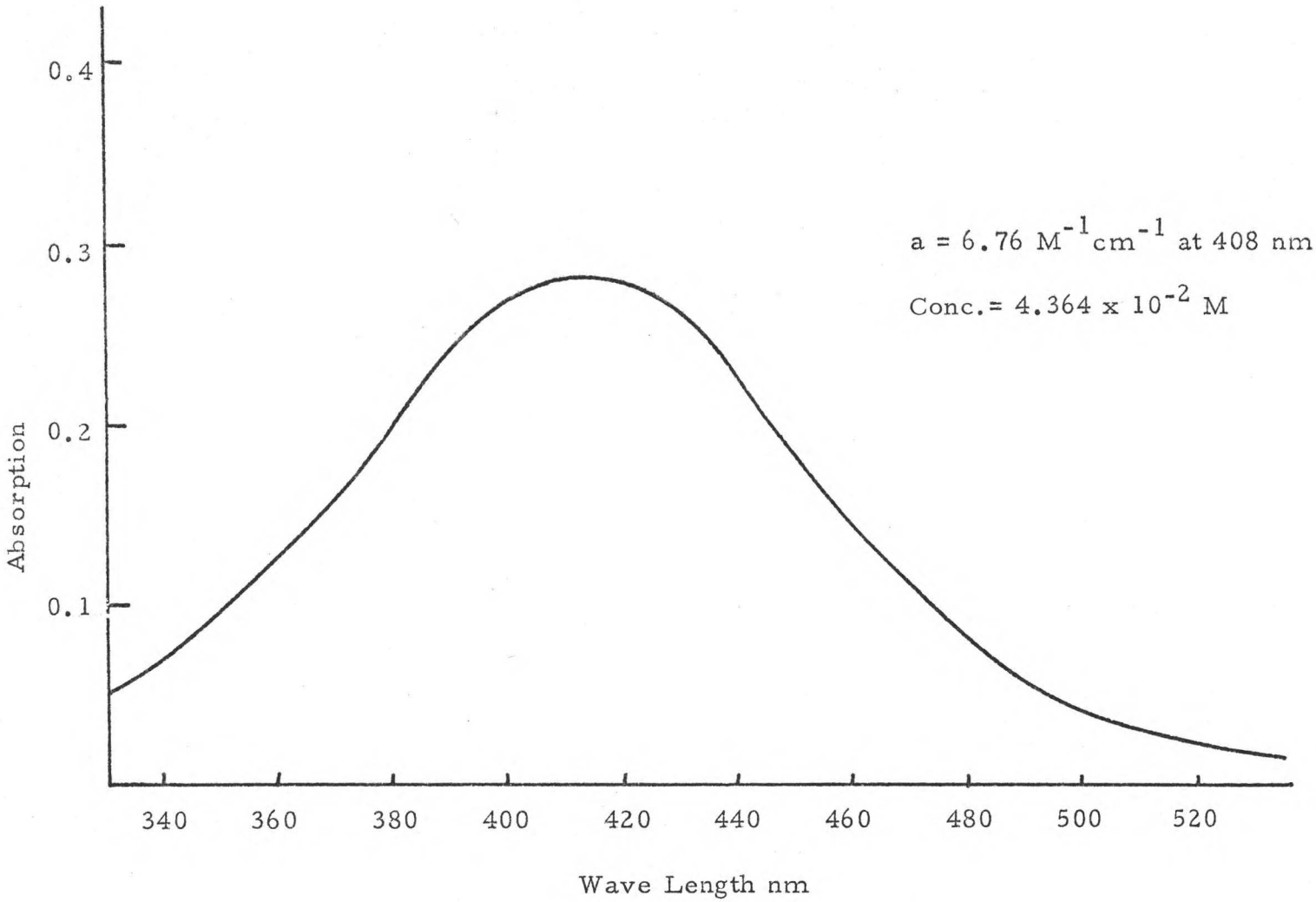


Figure 13. Absorption Spectrum of CTM-pyrrolidin in Methanol

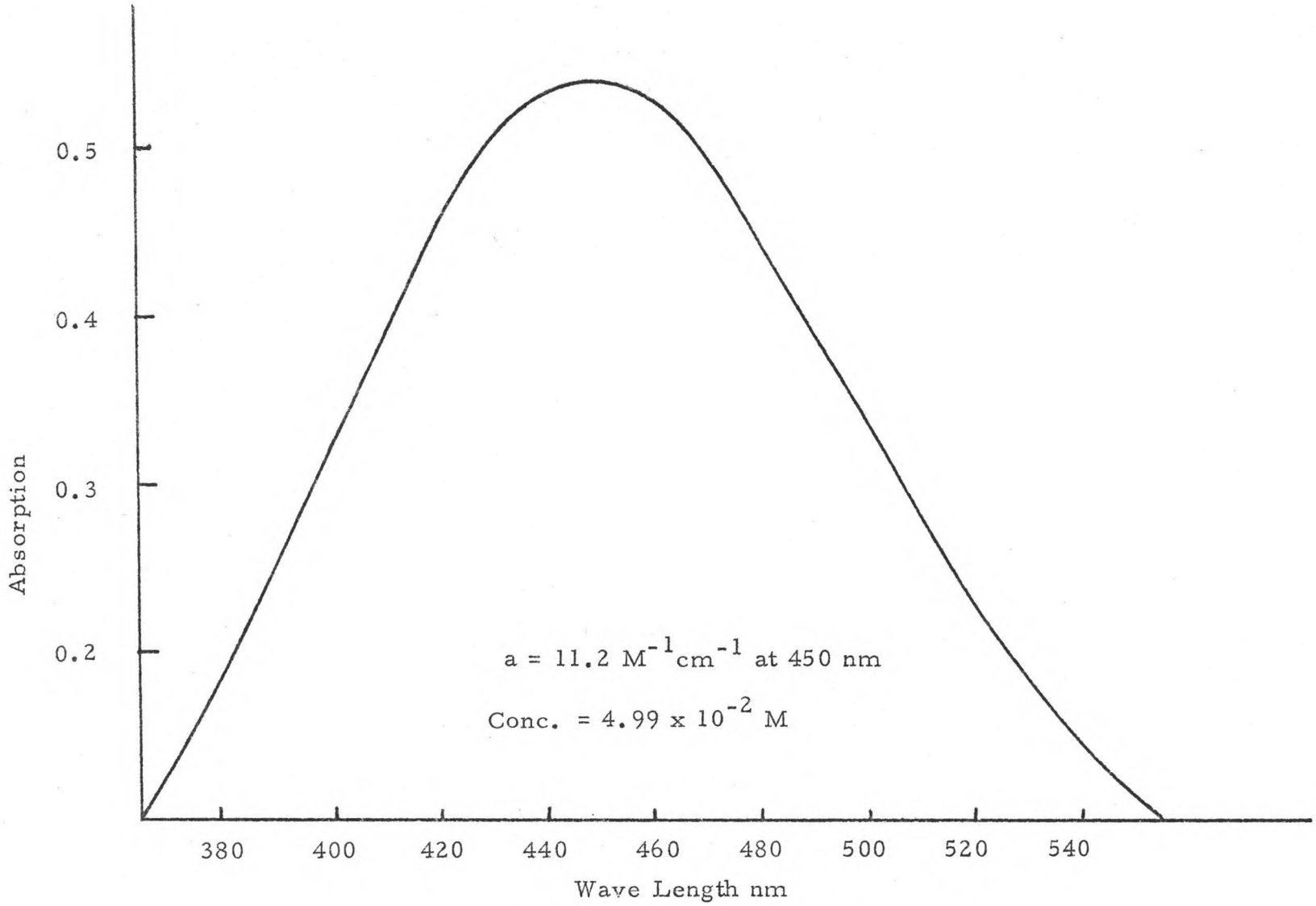


Figure 14. Absorption Spectrum of ATMP in Methanol

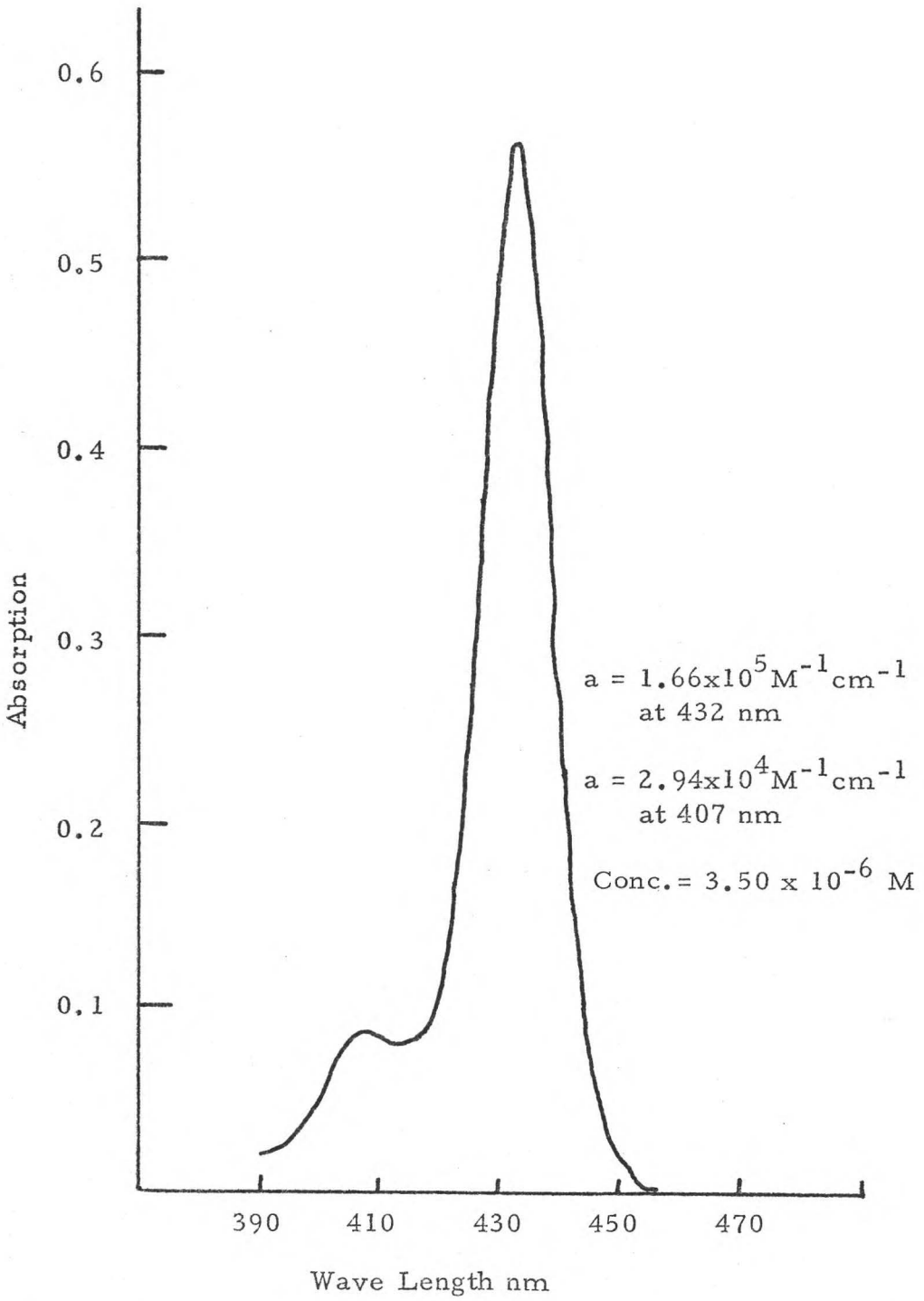


Figure 15. Absorption Spectrum of Galvinoxyl in Benzene

TABLE 7

SUMMARY OF SPECTROPHOTOMETRIC RESULTS

Radical	Solvent	Wave Length at Max. Absorption	a ($M^{-1}cm^{-1}$)	Lit. Ref. for a
DPPH	benzene	520 nm	1.20×10^4	14450 (46) 8580 (7)
Fremy's salt	water, pH 7	540 nm	20.8	20.8 (7)
CTM-pyrrolidin	methanol	408 nm	6.76	
CTM-pyrrolin	methanol			
ATMP	methanol	450 nm	11.2	
Galvinoxyl	benzene	432 nm 407 nm	1.66×10^5 2.94×10^4	154000 (43) 30000 (43)

Gouy Method

Equipment

A Model 7500 electromagnet with Model 7500 PS filtered power supply and Model 7500 R Series current regulator, all from Alpha Scientific Lab., were used to assemble the electromagnet. A Mettler H20T balance was used for weighing the samples. The sample tubes were made of pyrex glass. Wooden or rubber stoppers were used in preference to ground glass stoppers. Parafilm was used to cover the stoppers to ensure that the solvent was not evaporating.

Tube constant calibration

Degasified distilled water was used for the tube constant calibration using -0.72×10^{-6} cgs unit as the susceptibility for water. The density of water at a given temperature was taken from the Handbook of Chemistry and Physics. The volume of the sample used was calculated from its density and mass. Two different magnetic fields were used for each sample. The field was measured with a Model 600A gaussmeter from Bell, Inc.

Illustration of tube constant calculation at 8400 gauss

Data:

weight of Gouy tube, magnetic field off 41.22673 gm

weight of Gouy tube, magnetic field on 41.21742 gm

Data (continued)

room temperature $22.5^{\circ}C$

weight of tube filled with water with field off 44.32984 gm

weight of tube filled with water with field on 44.31136 gm

volume of the tube 3.11077 ml

$\alpha = 0.029 \times 10^{-6} \times 3.11077$ $\qquad$ $\delta = -9.31$ mg

$\chi_{m(H_2O)} = -0.72 \times 10^{-6}$ $\qquad$ $F' = -9.17$ mg

$W = 3.10311$ gm

$F = -18.48$ mg

$$\chi_m = \frac{\alpha + \beta F'}{W}$$

$$(-0.72 \times 10^{-6}) = \frac{0.029 \times 10^{-6} \times 3.11007 + \beta(-9.17)}{3.10311}$$

$$\beta = 0.25348 \times 10^{-6}$$

(Notice the convention in units, α, F' & δ are in mg and W in gm.)

So the formula for use of this Gouy tube under the conditions employed is:

$$10^6 \chi_m = \frac{(0.029 \times 3.11077) + 0.025348\ F'}{W}$$

At 1400 gauss the tube constant was calculated to be 0.11623×10^{-6} c.g.s. unit.

Illustration of the calculation of magnetic measurements of a radical

radical: DPPH gm of radical per gm of solution = 0.0127gm

magnetic field: 14000 gauss

weight of empty tube no field: 39.47107 gm

weight of empty tube with field: 39.45094 gm

weight of tube + sample no field: 42.28218 gm

weight of tube + sample with field: 42.24579 gm

weight of sample: 2.6999 gm

volume of sample: 3.1069 ml

solvent: benzene ($\chi_m = -0.702 \times 10^{-6}$)

tube constant: 0.11623×10^{-6}

$$10^{+6}\chi_m = \frac{(0.029 \times 3.1069) + 0.11623(-16.26)}{2.6999}$$

$$= -0.66662$$

$$\text{net } 10^{+6}\chi_m = -0.66662 - (-0.702)$$

= 0.03538 c.g.s. unit (mass susceptibility for the radical in 1 gm of solution)

$$\chi_{gm} = 0.03538/0.0127 \text{ gm}$$

$$= 2.79 \times 10^{-6} \text{ c.g.s. unit}$$

Table 8 gives a summary of the magnetic measurements from the Gouy method.

TABLE 8

MAGNETIC MEASUREMENTS OF THE RADICALS - GOUY METHOD

Radical	Solvent ($10^6\chi_m$)	Field (gauss)	$10^6\chi_m$ (temp.)	Literature Val.
DPPH	benzene (-0.702)	8400 14000	2.75 (23°C) 2.79 (23°C)	2.07 (13), 2.75 (13) 2.7 (13)
Fremy's salt	2%t-butanol 98% water pH7 (-0.72)	8400 14000	4.65 (23°C) 4.69 (23°C)	4.34 (25), 3.87 (25) 4.11 (25)
CTM-pyrrolidin	methanol (-0.688)	8400 14000	6.71 (23°C) 6.70 (23°C)	
CTM-pyrrolin	methanol (-0.688)	8400 14000	6.79 (23°C) 6.81 (23°C)	
ATMP	methanol (-0.688)	8400 14000	5.77 (22°C) 5.80 (22°C)	
galvinoxyl	chloroform (-0.497)	8400 14000	3.63 (23°C) 3.61 (23°C)	

Nuclear Magnetic Resonance Measurements

Equipment

All the NMR spectra were obtained on a Varian A 60 A spectrometer, and concentric cavity tubes were from Wilmad Glass Co. (catalogue #517). The NMR probe temperature was determined from the difference in chemical shift of signals in the ethylene glycol spectrum. The density of the radical solution at 39°C (the temperature of the NMR probe) was measured with a pycnometer. Table 9 gives the list of the solvents used for the NMR method.

TABLE 9

SOLVENTS USED FOR NMR MEASUREMENTS

Radical	Solvent
DPPH	10% cyclohexane 90% benzene
Fremy's salt	2% T-butyl alcohol 98% water pH7
CTM-pyrrolidin	methanol
CTM-pyrrolin	methanol
ATMP	methanol
Galvinoxyl	chloroform

The density of methanol and chloroform at various temperatures were calculated from equations given in the International Table of Critical Values. The density of 10% cyclohexane and 90% benzene solution, and 2% t-butyl alcohol and 98% water solution were determined in our laboratory.

Procedure

The external standard (which was simply the solvent system without the paramagnetic substance), was placed in the outer tube. The sample was introduced into the inner tube using a syringe and a fine teflon tube. The NMR tube was then capped tightly. Two resonance lines would normally be obtained from the protons of the reference substance owing to the difference in its volume susceptibility (see Figure 16).

Illustration of the calculation of mass susceptibility of a chloroform solution containing 0.01122 gm/ml of galvinoxyl radical at 39°C

$$\chi_{gm} = \frac{3\Delta f}{2\ fm} + \chi_0 + \frac{\chi_0(d_0 - d_s)}{m}$$

Δf = 3.8 Hz

m = 0.01122 gm/ml of solution

f = 60×10^6 Hz

χ_0 = -0.497×10^{-6} gm^{-1}

$d_0(39^\circ C) = 1.4430$ gm/ml

$d_s(39^\circ C) = 1.43628$ gm/ml

$$\chi_{gm} = \frac{3\ (3.8)}{2 \times 3.14159 \times (60 \times 10^6) \times 0.01122} + 0.497 \times 10^{-6} + \frac{0.479\ (1.4430 - 1.43628)}{0.01122} \times 10^{-6}$$

$$10^6\ \chi_{gm} = 3.48$$

Table 10 gives a summary of the magnetic measurements from this NMR technique.

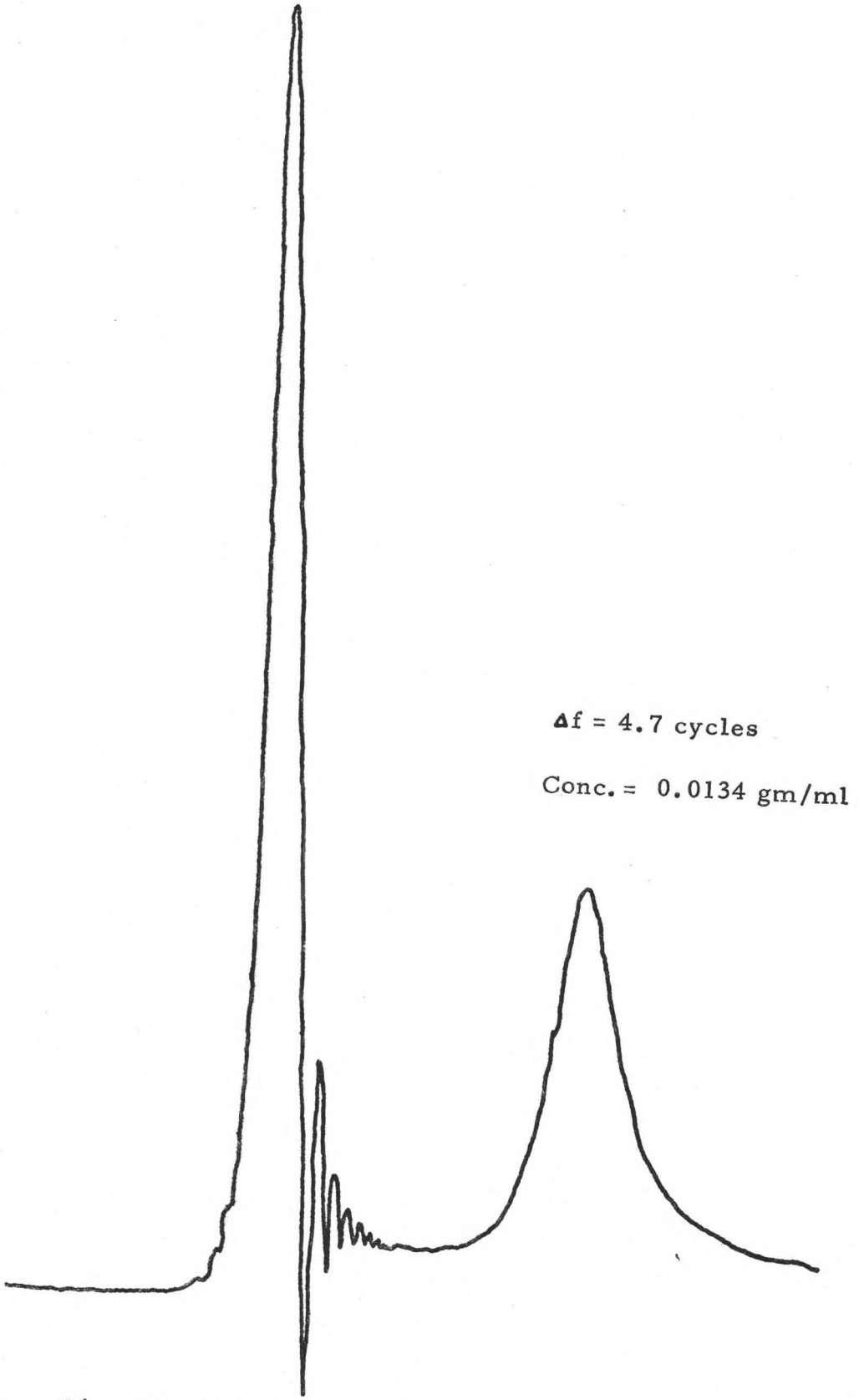


Figure 16. NMR Splitting by Galvinoxyl Radical in Chloroform

TABLE 10

MAGNETIC SUSCEPTIBILITIES OF THE RADICALS AS DETERMINED FROM NMR METHOD

Radical	Conc. gm/ml (corrected)	Δf (cycles/sec.)	Calculated $10^6 \chi_{gm}^{39^\circ C}$
DPPH	0.00954	3.3	2.7
Fremy's salt	0.01340	7.0	4.39
CTM-pyrrolidin	0.00793	6.1	6.55
CTM-pyrrolin	0.01048	8.1	6.65
ATMP	0.00944	6.1	5.70
Galvinoxyl	0.01122	3.8	3.48

CHAPTER V

DISCUSSION

Chemical Titration

During the iodometric titration of a radical the radical is reduced by capturing a hydrogen atom. The products from the titration of DPPH had been studied spectrophotometrically. The spectrum of DPPH after reduction with sodium iodide was exactly the same as that of DPPH-H, showing a maximum absorption at 320 nm. The origin of the hydrogen transferred to the hyrazyl is not clear. The following equation has been proposed for the reaction between DPPH and iodide ion:

$$2DPPH + 2I^- + 2CH_3COOH \longrightarrow 2DPPH\text{-}H + 2CH_3COO^- + I_2$$

Or in more general terms:

$$2R\cdot + 2I^- + 2H^+ \longrightarrow 2R - H + I_2$$

It seems possible to determine the purity of all the stable solid free radicals by this titration method provided that a suitable solvent for the radical can be found.

The sources of error in this iodometric method are few. Air oxidation of iodide ion is believed to be a problem.

$$4I^- + O_2 + 4H^+ \longrightarrow 2H_2O + 2I_2$$

An inert atomsphere over the titration mixture is preferred. This can be attained by periodically adding small pieces of dry ice or small portions of sodium bicarbonate during the titration. Small errors may also come from volatilization of liberated iodine. This can be prevented by using stoppered containers and by placing the solution in the dark when the solution must stand. A good excess of iodide ion and a low temperature will also minimize this error. Other sources of error, such as premature addition of starch indicator solution and decomposition of thiosulfate solution, should not be neglected.

The sensitivity of this method depends on the sensitivity of the starch indicator. Under normal conditions iodine concentration as low as 2×10^{-7} F can be detected with the starch solution and the intensity of the blue starch-iodine color offers a real advantage.

Sometimes choosing a suitable solvent for the titration could present a problem. In this study we could not find a solvent for the CTM-pyrrolin. For both DPPH and galvinoxyl a mixture of two solvents was used.

During the chemical titration we assumed that no other parts of the radical were being reduced other than the radical site. We had no evidence in any case that such occurred.

Spectrophotometric Method

The absorptivities of the radicals studied range from 6.76 $M^{-1}cm^{-1}$ for CTM-pyrrolidin to 1.66×10^5 $M^{-1}cm^{-1}$ for galvinoxyl. For most UV and visible spectrophotometers, the absorbance can easily go from 0.1 - 0.9. The lowest detectable limit calculated for each radical is tabulated in Table 11.

TABLE 11

SPECTROPHOTOMETRIC DETECTABLE LIMITS FOR THE RADICALS

Radical	Lowest Detectable Range (1.0 cm cell)
DPPH	8.33×10^{-6} M
Fremy's Salt	4.8×10^{-3} M
CTM-pyrrolidin	1.48×10^{-2} M
CTM-pyrrolin	?
ATMP	8.93×10^{-3} M
Galvinoxyl	6.03×10^{-7} M (435 nm)
	3.4×10^{-6} M (407 nm)

If a 10.0 cm cell is used the detectable range indicated above can further be reduced to one tenth of the concentration

DPPH and Fremy's salt have been used to determined the concentration of other radicals in solution by following their

decrease in absorption as they react in a radical - radical combination reaction.

CTM-pyrrolin, due to the presence of the double bond, absorbs very strongly in the UV region with no absorbance in the visible region. It seems impossible to obtain any useful spectrophotometric data for this radical.

Gouy Method

In calibrating the tube constant for the Gouy Method, several materials can be used. Water is very often used as its susceptibility is very accurately known. The use of benzene as a reference has also been suggested, but its susceptibility depends on whether it is saturated with air ($\chi_{gm} = -0.702 \times 10^{-6}$ cgs unit) or with nitrogen ($\chi_{gm} = -7.08 \times 10^{-6}$ cgs unit). Nickel chloride solutions had been suggested by several investigators (47) as a reference for magnetic work.

Figgs and Lewis (48) recommended mercury tetrathiocyanatocobaltate (11), $Hg[Co(CNS)_4]$ as an all around calibrant for solids. It has a susceptibility of 16.40×10^{-6} cgs unit. Tris (ethylenediamine) nickel (II) thiosulfate ($\chi_{gm} = 10.82 \times 10^{-6}$ cgs unit) had also been used (49).

The tube constant differs at different field settings. Hence it is necessary to determine the tube constant each time a different field strength is used.

The Gouy tube itself acts as a specimen and consequently develops a force which is always present and has to be subtracted from the observed force in order to obtain the force acting on the sample alone. This force is always negative due to the diamagnetic material present. Experimentally the measurements are always made in air and since air has a certain amount of magnetic susceptibility the amount displaced by the specimen must be allowed for.

An electromagnet will always leave a residual field even if the source of current is off. This should not cause any problem since this low residual field is fairly constant and is taken into consideration in the calibration for the tube constant.

The paramagnetic susceptibility of a sample is independent of the magnetic field. But if there is a trace amount of ferromagnetism present in the sample and since ferromagnetism is dependent on the magnetic field strength, the susceptibility of the sample will differ at different field strengths. For this reason at least two or more field strengths are used in determining the paramagnetic susceptibility.

In this method, accurate measurement of weight change is essential, for it can be shown that 0.01 mg difference in weight would cause 0.65% differ in calculated susceptibility. Since our balance is accurate to $\pm$ 0.01 mg the accuracy of this method is

close to ± 1%. The lowest detectable range for each radical using the Gouy method is listed in Table 12.

TABLE 12

THE LOWEST DETECTABLE AMOUNT FOR EACH OF THE RADICALS USING THE GOUY METHOD

Radical	Lowest Amount Detectable 14000 gauss	8000 gauss
DPPH	1.6×10^{-5} gm/ml	3.4×10^{-5} gm/ml
Fremy's Salt	1.5×10^{-5} gm/ml	3.4×10^{-5} gm/ml
CTM-pyrrolidin	1.5×10^{-5} gm/ml	3.3×10^{-5} gm/ml
CTM-pyrrolin	2.1×10^{-5} gm/ml	4.5×10^{-5} gm/ml
ATMP	1.3×10^{-5} gm/ml	3.2×10^{-5} gm/ml
Galvinoxyl	5.0×10^{-6} gm/ml	1.1×10^{-5} gm/ml

NMR Method

If the magnetic susceptibility of a solution needs to be determined NMR spectrometry provides a rapid and accurate means of determination. Accuracy up to ± 1% is easily obtained. Besides being rapid this method requires very little sample; less than 0.03ml of a dilute solution can be studied.

Several difficulties are related to this method which may limit its versatility. One of these is the line broadening effect.

This will occur if the sample is too concentrated in the paramagnetic species. A greater problem exists in studying paramagnetic transition metals. The difficulty arises if the paramagnetic transition metal is capable of a contact or pseudo contact interaction with the reference compound. This involves the transfer of some of the unpaired electron density to the reference compound, causing a contact or pseudo-contact shift of the compound. Furthermore, the presence of signals from solvent protons may overlap with reference signals.

For more accurate results, the temperature effect on solvent and solution density and concentration of paramagnetic substance is essential. For a solvent with a volume change about 10% for 100°C change in temperature, the density and concentration correction become important. An error of 20-25% in susceptibility may result if this temperature versus density and concentration is not taken into account. The concentration of the solute can be calculated at different temperatures using:

$$M_t = M_{rt} \frac{d_t}{d_{rt}}$$

where M_t and M_{rt} are the solute concentrations at temperature t and room temperature and d_t and d_{rt} are the solvent densities at temperature t and room temperature.

Magnetic susceptibilities are temperature dependent as shown by Curie's Law:

$$\chi_m = C/T$$

where C is the Curie constant; characteristic of the paramagnetic substance and T is the absolute temperature. If χ_m of a sample is measured at several temperatures, the plot of the reciprocal of χ_m against the absolute temperature yields a straight line of slope C and the line intersects the origion, (see Figure 17, line b). There are many substances that do show this behavior, but there are many others for which the line does not intersect at the origion. They may cut the temperature axis below (line a) or above (line c) the $0^{\circ}K$.

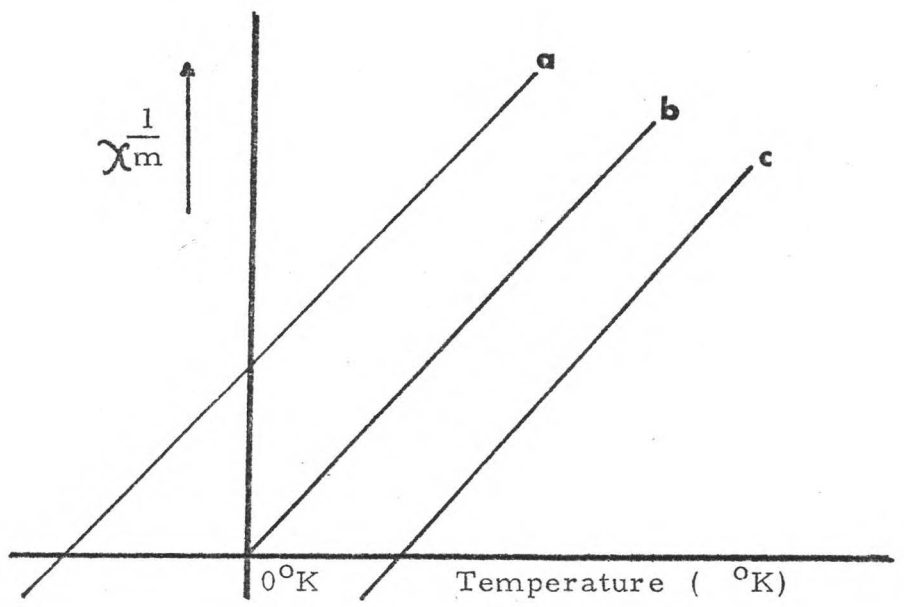


Figure 17. Curie-Weiss Law

Line a and c can be represented by a modified Curie's equation:

$$\chi_m = \frac{C}{T - \theta}$$

where θ is the temperature at which the line cuts the temperature axis. The modified equation is known as the Curie-Weiss Law.

In our determination of χ_m using the Gouy method the values were determined at room temperature and for the NMR method at 39°C. When converting the χ_m from Gouy method at room temperature to 39°C using the Curie's Law alone the result for each radical is tabulated in Table 13.

A substitution for the precision made coaxial NMR tube is available. A simple melting point tube can be used as the inner part of the coaxial cell and the outer part can be an ordinary NMR tube. The solution can be introduced into the melting point tube with a syringe and the tube is then sealed off. When the sample spins in the NMR spectrometer, the inner tube is automatically centered due to the buoyancy from the unfilled space in the upper part of the melting point tube. With this micro technique only 5×10^{-7} mole is required for a melting point tube of 0.025 ml volume (or 2.5×10^{-5} mole/ml).

The accuracy of this method depends on how accurately the splitting can be measured. With the Varian A60A spectrometer the lowest measurable splitting is 0.1 cycle with sweep width set on fifty cycles. With the new E M-390 MH_z spectrometer the lowest

measurable splitting is 0.04 cycle and hence increases the sensitivity by 2.75 fold.

TABLE 13

A COMPARISON OF MAGNETIC SUSCEPTIBILITIES DETERMINED FROM GOUY METHOD AND THE NMR METHOD

Radical	Mass Susceptibility (cgs unit) $\times 10^6$ from Gouy method corrected to 39° C by Curie's Law	$\times 10^6$ from NMR at 39°C
DPPH	2.61 (8400 gauss) 2.64 (14000 gauss)	2.7
Fremy's salt	4.41 4.45	4.39
CTM-pyrrolidin	6.34 6.33	6.55
CTM-pyrrolin	6.44 6.46	6.65
ATMP	5.47 5.50	5.70
Galvinoxyl	3.44 3.42	3.48

There are other applications of this NMR proton shifting method, such as doing temperature studies to determine the Curie constants of a sample and finding θ, the correction factor for the Curie-Weiss equation. NMR has been used to study the structure

equilibrium for certain transition complexes. Other more common applications include determining the magnetic moment of transition elements and hence their number of unpaired electrons.

With the six free radicals we have worked with we found that they all have similar molar splitting (see Table 14); close to 140 cycles. This value can serve as a quick check for the concentration of an unknown radical sample.

TABLE 14

NMR SPLITTINGS OF THE RADICALS AT 39°C

Radical (Mo. Weight)	Conc. (gm/ml)	Conc. (M)	Splitting (cycles)	Molar Splitting (cycles)
DPPH (394.53)	0.0954	0.0242	3.3	136
Fremy's salt (268.33)	0.01342	0.0500	7.0	140
CTM-pyrrolidin (185.25)	0.00793	0.0428	6.1	143
CTM-pyrrolin (183.23)	0.01048	0.0572	8.1	142
ATMP (213.30)	0.00944	0.0443	6.1	138
Galvinoxyl (421.65)	0.01122	0.0266	3.8	143
			Average	140

CHAPTER VI

SUMMARY

In studying free radicals ESR is generally used. This is an expensive method as not every laboratory is equipped with an ESR instrument. However, ESR is not readily suitable for radical concentration determinations. It is possible to determine the concentration, but not as easily as with some other methods. However ESR is the best technique for detecting very low amounts of radicals.

Chemical titration of a stable radical is feasible only if a suitable solvent for the radical can be found. The spectrophotometric method is convenient for concentration determination provided that the radical is colored in a solvent, as most are, and that the absorptivity at a certain wave length is known or can be determined.

The two methods we used which depend on the radical nature of the sample are the Gouy and NMR methods. A Gouy balance for the determination of paramagnetism has long been used. Both solid and liquid samples can be used, and the accuracy is close to 1%.

NMR is by far the easiest and most convenient way for concentration determination for a radical in a solvent. Only a minute amount of sample is required. The accuracy of this method is comparable to that of the Gouy method. Structure determination of a transition element complex and equilibrium constant determinations for certain system can also be done with the NMR method.

The lowest limits for the four methods are listed in Table 15.

TABLE 15

LOWEST LIMITS FOR DIFFERENT METHODS OF RADICAL CONCENTRATION DETERMINATION

Radical	Chemical Titration	Spectrophotometric	Gouy Method	NMR
DPPH	4.0×10^{-7} M	8.5×10^{-7} M	4.0×10^{-5} M	7.5×10^{-4} M
Fremy's salt	4.0×10^{-7} M	4.8×10^{-4} M	5.6×10^{-5} M	7.5×10^{-4} M
CTM-pyrrolidin	4.0×10^{-7} M	1.5×10^{-3} M	8.1×10^{-5} M	7.6×10^{-4} M
CTM-pyrrolin			1.2×10^{-4} M	7.2×10^{-4} M
ATMP	4.0×10^{-7} M	8.9×10^{-4} M	6.1×10^{-5} M	7.5×10^{-4} M
Galvinoxyl	4.0×10^{-7} M	6.0×10^{-8} M	1.2×10^{-5} M	9.8×10^{-4} M

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A COMPARISON OF FREE RADICAL DETECTION METHODS

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ABSTRACT

The methods used for free radical detection are chemical titration, spectrophotometry, Gouy magnetic measurements and NMR shift technique. The free radicals chosen for this study are Fremy's salt ($ON(SO_3)K_2$), 2, 2-diphenyl-1-picryl hydrazyl (DPPH) 3-carbamoyl-2, 2, 5, 5-tetramethylpyrrolidin-1-yloxy (CTM-pyrrolidin), 3-carbamoyl-2, 2, 5, 5-tetramethyl-3-pyrrolin-1-yloxy (CTM-pyrrolin), 4-acetamido-2, 2, 6, 6-tetramethylpiperidino-1-oxyl (ATMP) and galvinoxyl.

The detectable range for each method is given. The NMR method is a fast and convenient method for the magnetic susceptibility determination of a sample in solution, with accuracy up to $\pm$ 1%.

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