METAL ION - DRUG INTERACTIONS IN SOLUTIONS

By

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IN SOLUTIONS

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CHAPTER I

INTRODUCTION

In the broad sense, alkaloids are the nitrogenous bases which occur naturally in plants. In addition, the alkaloids usually show specific pharmacological activity. A particular alkaloid is usually restricted to certain genera and families of the plant kingdom, rarely being present in a large groups of plants. However, there is no very sharp distinction between alkaloids and many naturally occuring nitrogenous compounds (1). There are approximately two thousand alkaloids which have been isolated and the molecular structure of most of them are known (2). Opium alkaloids or the alkaloids of the morphine group, have been subjected to more study than those of any other group, partly because of the remarkable analgesic properties of morphine and many of its derivatives, and partly because the alkaloids of this group undergo a wide variety of molecular transformations. The group consists of five closely related bases (3,4), morphine, codeine, neopine, thebaine and oripavine, and two bases of somewhat different type, sinominine and hubanonine.

Morphine and codeine are among the principal alkaloids in opium. Morphine was the first vegetable base to be

isolated from opium by Saturner in 1805, and is by far the major narcotic constituent accounting for 10 - 20 % of opium dry weight. Codeine by comparison is present in opium to the extent of only 0.2 - 0.8 %. The methods of isolation, structure determination, and synthesis of morphine were reported by Bently (5).

Morphine and most of its related compounds are classified as illegal drugs. The identification and determination of such drugs has been a problem of great interest for the pharmaceutical, clinical, and forensic analyst. The methods available for the identification of drugs are classified as biological, chemical and physical. It is generally accepted among narcotic analysts that a single method, either chemical or physical is not sufficient as a basis for the determination of identity. A combination of a physical method and a chemical test usually can lead to a definite result. The chemical and physical methods most commonly used for drug analysis include microcrystal tests (6,11), color tests (6,8), and immunoassay techniques (7). Many instrumental methods such as IR-UV-Visible spectroscopy (10,12,13), mass spectrometry (14), nuclear magnetic resonance spectroscopy (8,9), gas-liquid, HPLC, thin-layer chromatography (15,16,17) and circular dichroism (18,19) have been used in drug analysis so far.

The use of microcrystal and color tests in qualitative identification of drugs seems to have begun between 1820 and 1830. The introduction of new microcrystal and color tests

continued for many years to coincide fairly well with the discovery and characterization of new alkaloids, but the systematic identification of the groups of alkaloids by these methods began to emerge only in the later half of the 19^{th} century. In 1868, Dragendorff (6) introduced the first systematic approach to the separation and identification of alkaloids and other drugs by the color tests which were then extensively developed for the identificatin of drugs in toxicology by Umberger (6). Nitric acid and sulfuric acid reagents were among the earliest to have been observed to produce color when applied to the opium alkaloids and to be utilized in toxicology for their detection.

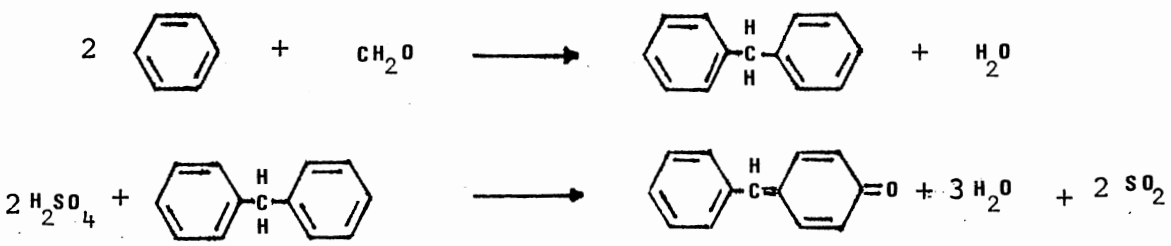
Color tests are widely recognized as the logical first step in a drug analysis scheme. The tests are entirely empirical and not very specific. They are used for quick screening to identify drug group rather than individual drug. More than fifty reagents have been suggested for the qualitative and quantitative color reactions with opium alkaloids (5,8). Six reagents which have been called "the alkaloidal color reagents" were listed by Umberger (6). All use concentrated sulfuric acid as solvent. The descriptions of the color produced from reactions are reported in the literature (20,21). Several color tests involve a metal ion as part of the reagent. Chemical reactions which involve metal ions include both complex formation and oxidation-reduction reactions. The reaction media may be different for each chemical reaction. Most color test reagents for opium

alkaloids require concentrated sulfuric acid as a reaction solvent (5,20,21) but some reactions do take place in aqueous media.

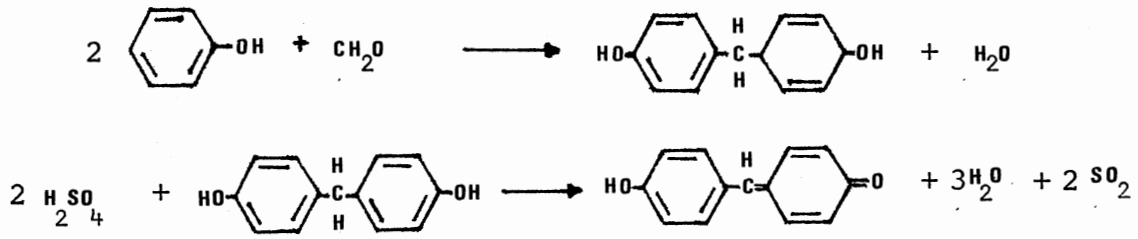
color reactions of morphine and its derivatives with the same color test reagent always produce similar colors. The studies on color reactions of drugs were begun when the Marquis reagent (formaldehyde + concentrated sulfuric acid) was first discovered to be a test reagent for morphine in 1896. Le Rosen et al. (22) applied it to a number of classes of aromatic compound types besides phenols. The products of the reactions were either colored precipitates or remained in solution producing intense colors. However, the composition of the colored product were not known.

A mechanism proposed by Feigl (23) for the reaction of benzene and phenol with the Marquis reagent suggests that the reaction involves the condensation of one mole of formaldehyde with two moles of aromatic compound. Following the condensation step, oxidation by sulfuric acid occurs to produce unidentified colored quinoidal products. The proposed reaction can be shown by the following chemical equations:

A) Benzene reaction



B) Phenol reaction



On the basis of this proposal mechanism, only those aromatic compounds with a free para-position or a para-OH group can produce p-quinoids.

Farmilo and Lucas (24) proposed another mechanism for the aromatic aldehyde-acid-phenol condensation reaction. In this mechanism the formation of a leuco base of a di- or tri-arylmethane derivative is required. Aromatic compounds with either a blocked or a free para-position may undergo the condensation step. Farmilo's hypothesis seems to be more reasonable than Feigl's proposed mechanism to explain the origin of the color in reactions involving the opiates morphine or codeine because the para- position of these drugs is definitely blocked.

No further information about color reactions of drugs were reported until 1964, when Schieser (25) employed the ESR (electron spin resonance) technique to study the colors produced in the reactions of alkaloids with the test

reagents. Free radicals were reported to be present in the colored mixture produced by color tests in which concentrated sulfuric acid was the reaction solvent, and the aromatic group was suggested to be the site of radical formation in alkaloids. No proposal for a chemical mechanism was made but it was suggested that a common mechanism is involved when aromatic compounds are treated with the Marquis reagent. At this time, no appropriate chemical equations have been written for the color reactions of drugs and the source of color has not been fully explained.

Physical data of morphine, codeine and 3,6 - diacetyl morphine (heroin) have been reported in the lilterature (26,27,28). All three drugs are optically active compounds which possess five different asymmetric centers and all contain the same light absorbing chromophores. Therefore the optical activity can be studied either by ORD (optical rotatory dispersion) or CD (circular dichroism). The structure of morphine, codeine and 3,6 - diacetylmorphine are shown below (Figure 1).

Morphine is a weak acid in aqueous solution because of the presence of the phenolic functional group at C(3). The pK_a was reported to be 9.85, a value first determined by Kolthoff (29) from pH colorimetric measurement in 1925. Spectrophotometry and potentiometry titrations had been used to determine the dissociation constants of the hydrochloride or sulfate salt of morphine (30,31). It is likely that morphine can undergo complexation with metal ions because of

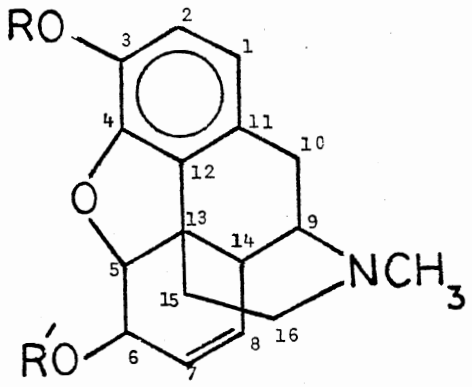


Figure 1. Structure of Drugs: Morphine: R = R' = H, Codeine: R = CH_3, R' = H, Heroin: R = R' = $-\overset{\overset{O}{\|}}{C}-CH_3$

the hydroxyl groups on C(3), C(6) and the oxygen in the dihydrofuran ring. Evidence for the complexation between morphine and Ca^{2+} or Mg^{2+} ions in aqueous solution from fluorescence studies was reported by Lin, Sutherland, and Way in 1975 (32). The binding site for metal ions was suggested to be either the ether linkage or phenolic or alcoholic group of the morphine molecule. Since the changes in CD spectra are due to the conformational reorientations of molecules (19), it is possible to investigate the complexation of morphine with metal ions by circular dichroism.

Statement of the Problem

This research was undertaken to study the interaction of metal ions with drug molecules from the opiate group, particularly morphine, codeine and heroin (3,6-diacetylmorphine). Interactions range from simple complex formation equilibria to irreversible oxidation-reduction reactions. Aqueous solution and concentrated sulfuric acid were the two reaction media used in this study. Dissociation constants for morphine and metal complexation reactions were to be investigated by CD in order to correlate conformational changes with changes in acidity and metal concentration.

Most color reactions of drugs take place in concentrated sulfuric acid. Circular dichroism (CD) and electron spin resonance (ESR) spectroscopy are the main physical methods which will be applied to examine the reactions. Results from drug molecules will be compared with results for the

same reactions on simple molecules which contain the same organic functional groups. Details on the CD and ESR techniques will be discussed in the following chapter.

CHAPTER II

BACKGROUND AND THEORY

Circular Dichroism

Circular Dichroism (CD) is the direct measurement of the difference in absorbance between the left and right components of an incident circularly polarized light beam by certain compounds as a function of wavelength. In order to observe the effect, two criteria must be met: 1) the light must be circularly polarized light; 2) the compound to be studied must absorb light and exhibit optical activity.

Origin of Light Polarization

Ordinary, natural, unreflected light behaves as though it consists of a large number of electromagnetic waves vibrating in all possible orientations around the direction of propagation. Plane polarized radiation is characterized by the electric vector being oriented in one plane only. Plane polarized light is produced when a beam of unpolarized monochromatic light is passed through a polarizing device made from crystals which exhibit a uniaxial optical axis. In uniaxial crystals the optical properties are identical along two axial directions, but differ from the same properties measured along the third crystal axis.

Birefringent polarizers such as the Rochon prism and the Glan-Thompson prism (33) are commonly used to resolve an unpolarized light beam into two plane polarized beams. The beam dependent on the polarizability along the X and Y axes is referred to as the ordinary ray, and the beam dependent on the polarizability along the Z axis is called the extraordinary ray (Figure 2).

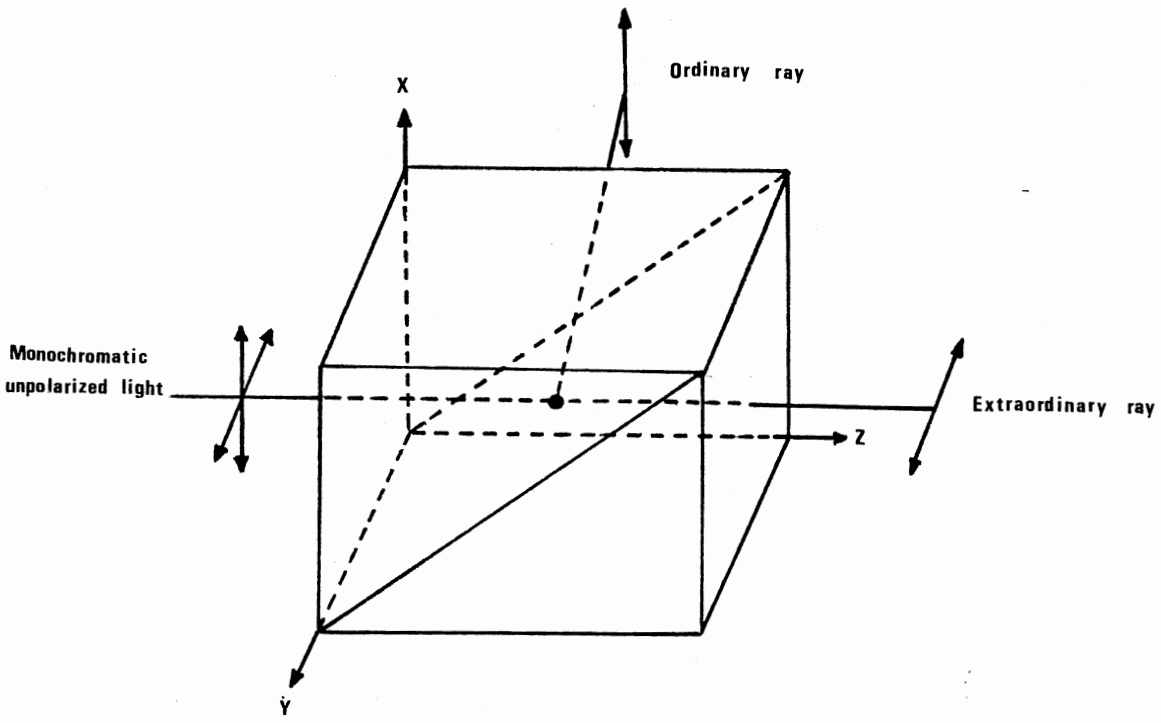


Figure 2. The Glan-Thompson Prism, Showing the Two Resolved Plane Polarized Beams of Light

The extraordinary ray is plane polarized in the plane containing the direction of propagation and the Z- axis, and its electric vector is parallel to the optic axis (Z-axis). Uniaxial crystals of ammonium or potassium dihydrogen phosphate ($NH_4H_2PO_4$ or KH_2PO_4) are commonly used to produce polarizers which function efficiently down to a wavelength of 185 nm (34).

At any point along the direction of propagation the amplitude of the electric vector of plane polarized radiation oscillates in a sinusoidal fashion. This oscillation can also be described as being equivalent to that for the vector sum of two angular components of equal and constant magnitude, whose orientations are changing by rotation in opposite directions at a constant angular velocity (Figure 3). The two vectors characterize right and left circularly polarized radiation which rotate in clockwise and counterclockwise directions about the axis of propagation.

Optically active compounds have different polarizabilities for right and left circularly polarized radiation. This means that the refractive indices of the compound for the circular components of given wavelength are different. As a result the two components are transmitted through the optically active medium with unequal velocities, which causes a phase difference for the two components and the plane of polarization transmitted will be rotated by an angle α from its original incident plane (Figure 4a). The angle α is defined by the following relationship:

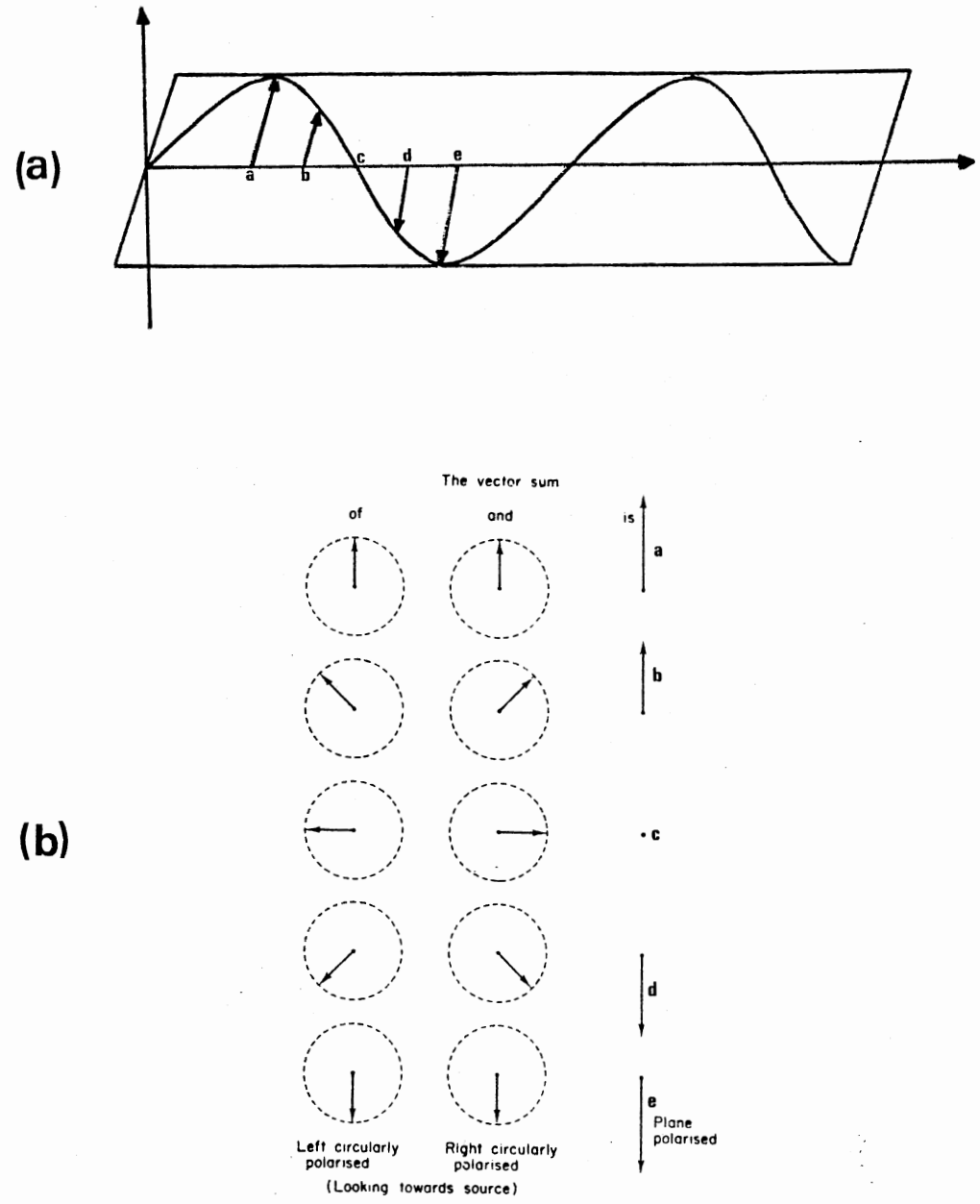


Figure 3. Plane Polarized Light Which Results From the Summation of the Two Circularly Polarized Components. The Circular Vectors and Their Summations at Any Point in (b) Are Represented By the Sine Wave In (a)

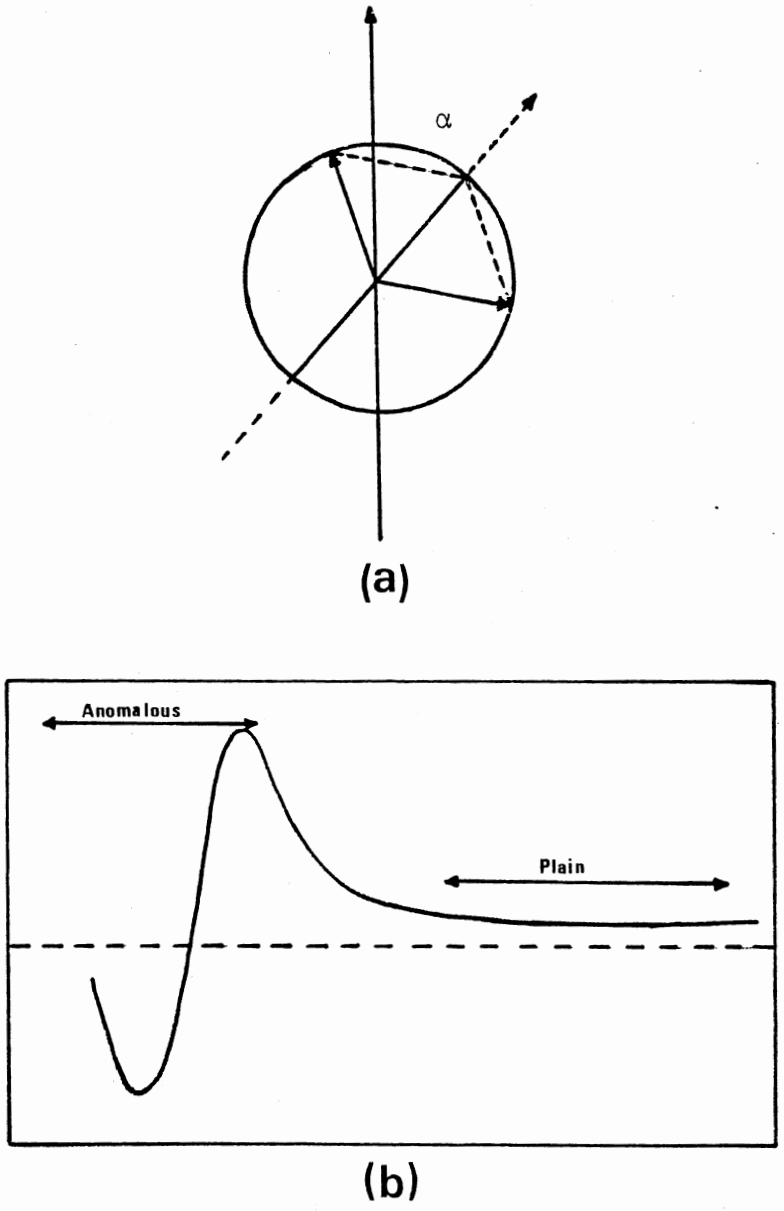


Figure 4. Diagram (a) Illustrating the Angle of Rotation (α) Caused by Unequal Speed of Propagation for the Two Circularly Polarized Components Through a Circularly Birefringent Medium. Diagram (b) Showing the "Plain" and the "Anomalous" ORD Curve

$$\alpha = \frac{\pi}{\lambda} (n_L - n_R) \qquad (2.1)$$

where α is the rotation of the plane of polarization in radian per unit length, λ is the wavelength of the incident light in cm., n_L and n_R are the indices of the refraction for the left and right circularly polarized light respectively. The variation of α with the wavelength is called optical rotatory dispersion (ORD). For a compound which is optically active, but which contains no chromophore, the optical activity decreases as the wavelength increases and a "plain" curve is obtained. If any compound possesses one or several optically active absorption bands within the spectral range being investigated, an "anomalous" curve is obtained (Figure4b). Anomalous curves are also called Cotton Effect curves, after their discoverer (35).

When the two circularly polarized components of light propagate through the optically active medium at unequal speeds and also undergo unequal absorption ($\varepsilon_L = \varepsilon_R$, where ε_L and ε_R are the respective molar extinction coefficient for the left and the right circularly polarized light), then the plane of polarization of the transmitted beam is not only rotated by an angle α but also is elliptically polarized. The medium is said to exhibit circular dichroism (CD). If an optically active medium absorbs left circularly polarized light, the L component, more strongly than the right circularly polarized light, the R component, that is $\varepsilon_L > \varepsilon_R$, then the amplitude of the R component will be greater than the L. Furthermore, if one assumes

that $n_L > n_R$, then the L component will be retarded more than the R component. The resulting elliptically polarized light is represented in Figure 5.

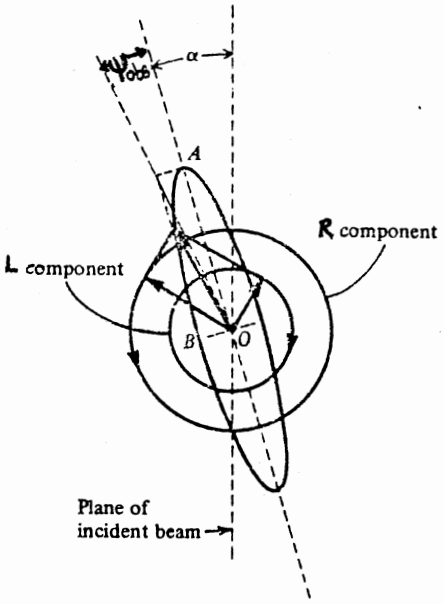


Figure 5. Elliptically Polarized Light Produced When $n_L > n_R$ and $\varepsilon_L > \varepsilon_R$

The differential dichroic absorption $\Delta\varepsilon$ is expressed by equation 2.2:

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R \qquad (2.2)$$

The angle between the major axis of the ellipse and the plane of the original radiation is the angle of rotation, α. The ellipticity, that is the angle whose tangent is the ratio of the minor axis of the ellipse, OB, to the major

axis, OA, is referred to as ψ_{obs} (observed or experimental ellipticity), that is:

$$\psi_{obs} = \tan \frac{OB}{OA} \qquad (2.3)$$

The variation of ellipticity with wavelength is called circular dichroism (CD). The molecular ellipticity [θ] is defined by the following relation (36):

$$[\ \theta\ ] = \frac{\psi_{obs} . MW}{100 .\ b .\ C} = 3300\ \Delta\varepsilon \qquad (2.4)$$

where b is the pathlength in dm., C is the concentration of the absorbing medium in g/l, MW is the molecular weight of the absorbing species, $\Delta\varepsilon$ is the difference in molar extinction coefficient between the left and the right circularly polarized light components. In this work the definition of [θ] in equation 2.4 has been changed to one that is more in accordance with the Beer-Lambert law for absorption spectroscopy, viz:

$$[\ \theta\ ] = \frac{\psi_{obs}}{M\ .\ b} \qquad (2.5)$$

where M is the molar concentration of the absorbing species, and b is the pathlength in cm. (37).

Circular dichroic absorption curves have a simpler form and are more easily analyzed than rotatory dispersion curves because in the regions where there is no absorption, the difference in molar extinction coefficients of the two circularly polarized components is zero, that is ε_L =

$\varepsilon_R = 0$, therefore $\psi_{obs} = 0$ (equation 2.5).

CD is particularly useful in the identification of molecules and in the assignment of structural conformations. It also has a great capability of resolving small overlapping polarized transitions. The spectra are characterized by their elllipticity maxima which can be either positive or negative ($^{+}\lambda_{max}, ^{-}\lambda_{max}$), their minima ($^{+}\lambda_{min}, ^{-}\lambda_{min}$), and the crossover points of zero ellipticity. Since CD is no more than a modified form of absorption spectrophotometry, the method should be quantitative according to Beer's Law which applied at all wavelength. This has been experimentally demonstrated (38).

CD technique can be applied to any optically active compound containing a chromophore which can give rise to a measurable Cotton Effect. The Cotton Effect is a consequence of either the chromophore itself being chiral or the presence of an achiral chromophore in a asymmetric environment in which chirality is induced. Chromophores can be divided into three main types (39):

1. The inherently disymmetric chromophores, such as non-planar aromatic substances and twisted conjugated systems.
2. Coupled oscillators formed by two non-conjugated chromophores in a disymmetric molecule.
3. Perturbed symmetrical chromophores, such as a double bond, carbonyl, carboxyl and aromatic ring, etc. In this case the optical activity is induced

into the chromophore by its environment e.g. those having an asymmetric carbon close to it.

CD spectra produced by compounds which are described by these three classifications are referred to as intrinsic CD because the compounds themselves exhibit the phenomenon. Optically inactive compounds containing absorption chromophores exposed to asymmetric environments, often produce CD spectra which are referred to as extrinsic CD. The most popular method of introducing an asymmetric environment to induce CD activity has been to place the sample in a magnetic field. The resultant spectrum is referred to as magnetic circular dichroism (MCD) (39). Another method of producing the asymmetric environment is to use either a solvent or co-solute molecules which of themselves are asymmetric or which aggregrate in an asymmetric manner (37). These might commonly be referred to as either solvent or solute-induced CD.

Electron Spin Resonance

Electron Spin Resonance (ESR) is a branch of spectroscopy in which absorption of radiation at microwave frequencies induces transitions between magnetic energy levels of unpaired electrons. The technique is applicable only to systems having one or more unpaired electrons.

The origin of ESR spectroscopy is based on the electron spin, its associated magnetic moment, and the orientation of magnetic dipoles in the presence of an applied magnetic

field. The magnetic dipoles associated with electrons may arise from both "spin" and "orbital" angular momenta. In the majority of cases it is assumed to arise from spin angular momentum only.

The electron has two spin states differing in spin quantum number, M_s, which are described by convention as follows:

$$M_s = +\frac{1}{2} \quad : \text{spin up } (\uparrow)$$

$$M_s = -\frac{1}{2} \quad : \text{spin down } (\downarrow)$$

In the absence of an external magnetic field, the two spin states, $M_s = \pm 1/2$ are degenerate, i.e., they have the same energy. A magnetic field, H, applied along the Z-axis, interacts with the magnetic moment of the electron, and the spin states, M_s, are no longer degenerate (Figure 6). This is the Zeeman effect. The energy of interaction, E, is given by the equation:

$$E = M_s g \beta H \tag{2.6}$$

where M_s is the spin quantum number, g is the dimensionless proportionality constant, ß is the Bohr magneton, 9.2741 $x10^{-21}$ erg/gauss, and H in gauss is the applied magnetic field. Since the difference in M_s is unity, the energy difference between the two spin states, ΔE, is simply:

$$\Delta E = E_2 - E_1 = g \beta H \tag{2.7}$$

that is ΔE is directly proportional to H.

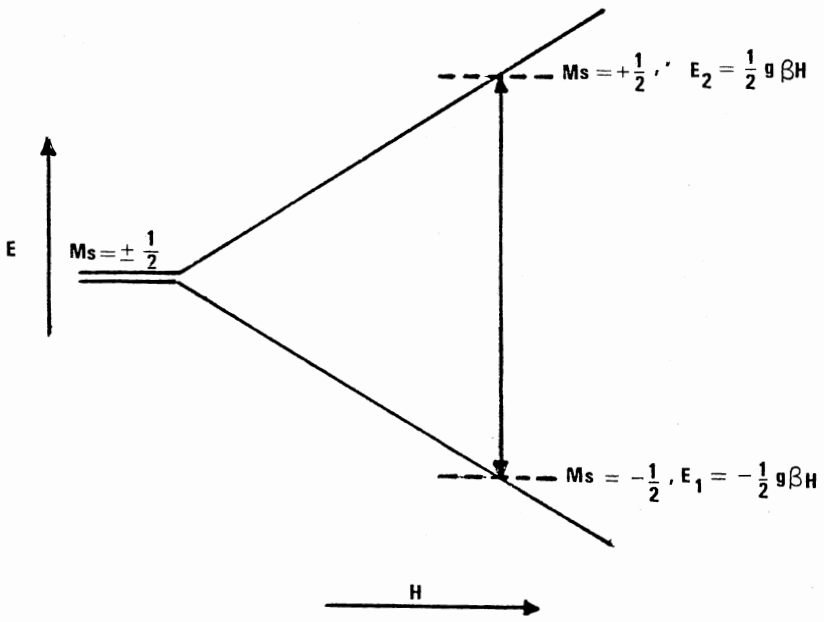


Figure 6. Splitting of Energy Levels of an Electron in an Applied Magnetic Field, H . E_1 and E_2 are the Energies of the Spin States in Which the Magnetic Dipoles are Aligned Parallel and Anti-parallel to the Applied Magnetic Field Respectively

When the system is exposed to electromagnetic radiation with suitable frequency, ν , this will cause a re-orientation of the electron magnetic moment and transition between two states occurs. It is this transition which is studied

by ESR. The condition to be satisfied in order to observe an ESR absorption is that

$$h\nu = g \beta H \qquad (2.8)$$

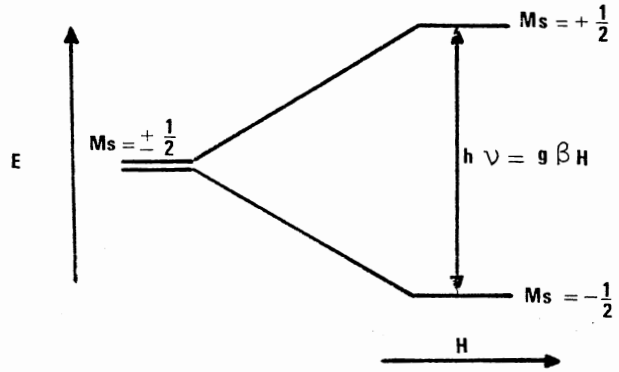
where h is Planck's constant (6.6262×10^{-27} erg.sec), and ν is the resonance frequency, in Hertz (Hz).

At resonance, transitions can occur either from $E_1 \rightarrow E_2$ or $E_2 \rightarrow E_1$ which signify energy absorption and emission respectively. These two transitions occur with equal probability. Whether the system undergoes absorption or emission, depends entirely upon the relative populations of the two Zeeman levels. Absorption can be observed only when the population, n_1, of the lower level is greater than the population, n_2, of the upper level. Substituting for ΔE in Boltzmann's distribution law

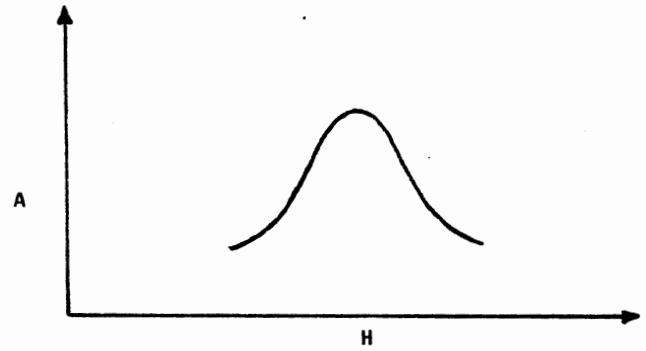
$$\frac{n_2}{n_1} = \exp[- \Delta E/kT] = \exp [-g\beta H/kT] \qquad (2.9)$$

where k is the Boltzmann constant (1.3805×10^{-16} erg/deg), and T is temperature, °K. In the presence of a magnetic field, the population of the two levels are equal, $n_1 = n_2$. The application of a magnetic field at constant T can bring about the difference in population, specified by equation 2.9.

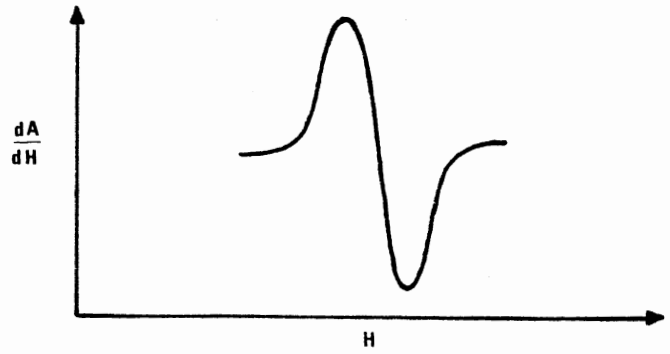
ESR absorption signals are recorded in a first derivative form of absorption spectra as shown in Figure 7. At a particular microwave frequency and constant T, the population difference between the two spin states is a



a) Splitting of the Spin Level of an Electron in the Presence of Applied Magnetic Field, H.



b) ESR Absorption Spectrum Obtained at Constant Frequency and Variable Field Strength, H.



c) First Derivative of Absorption Intensity, A, with Respect to H, as a Function of H.

Figure 7. Diagrams Showing Energy Levels, ESR Absorption Spectrum and the First Derivative of Absorption Intensity

constant proportional to the total number of unpaired electrons present. It is therefore possible to relate the integrated intensity of the ESR derivative curves to the number of unpaired electrons. The minimum number of unpaired electrons, N_{min}, which can be detected by an ESR spectrometer is referred to as the ultimate sensitivity of that spectrometer. For the ESR spectrometer at a particular frequency and particular field strength, the sensitivity at room temperature is often expressed as (40)

$$N_{min} = \frac{B.\ \Delta H}{\sqrt{\tau}} \qquad (2.10)$$

where B is a constant calculated from instrumental factors at optimal condition, ΔH is the linewidth measured in gauss, and τ is the time constant of the detection system. The value of N_{min} in terms of solution concentration for a typical commercial X-band spectrometer is in the range of 10^{-7}- 10^{-8}M (41,42).

Hyperfine Structure

If the interaction of an electron with a magnetic field were the only effect operative, then all ESR spectra would consist of one line. The only useful informaiton obtained from the spectra would be the g-factor (which will be discussed later), and the ESR technique would thus provide rather limited information.

Radical electrons are usually delocalized over the

whole molecule or at least part of it. Therefore the unpaired electron comes into contact-interaction with many nuclei with non-zero spin quantum numbers, I (Table I). These interactions cause further splittings of the electron resonance lines into a number of lines referred to as hyperfine structure (Figure 8 and 9).

The energy of the coupled levels are given by

$$E = g \beta M_s H + a_I h M_I \qquad (2.11)$$

where a_I is the hyperfine coupling constant and M_I is the spin quantum number of the coupling nucleus. The selection rules for ESR transitions: $\Delta M_s = \pm 1$ and $\Delta M_I = 0$ state that only those transitions are allowed which occur between spin states with the same quantum number M_I.

In general, n equivalent nuclei with spin quantum number I give rise to (2 nI + 1) equidistant hyperfine lines. When the spin configuration of the equivalent nuclei are P-fold degenerate, the transition between the attending levels have the same energy and result in a hyperfine line with a relative intensity P. The total number of lines is greatly reduced in this manner. The general rules for the number of lines and line intensity of nuclei with spin, I = 1/2 are summarized as follows:

1. n equivalent nuclei will give (n + 1) equally spaced hyperfine lines.
2. The relative intensities of these hyperfine lines

TABLE I

THE SPIN AND NATURAL ABUNDANCE OF SOME COMMON NUCLEI

Nucleus	Spin	Natural Abundance (%)
^{1}H	1/2	99.98
^{2}D	1	0.02
^{6}Li	1	7.42
^{7}Li	3/2	92.58
^{13}C	1/2	1.11
^{14}N	1	99.63
^{15}N	1/2	0.37
^{17}O	5/2	0.04
^{19}F	1/2	100
^{23}Na	3/2	100
^{29}Si	1/2	4.70
^{31}P	1/2	100
^{33}S	32	0.76
^{35}Cl	3/2	75.53
^{37}Cl	3/2	24.47
^{39}K	3/3	93.10

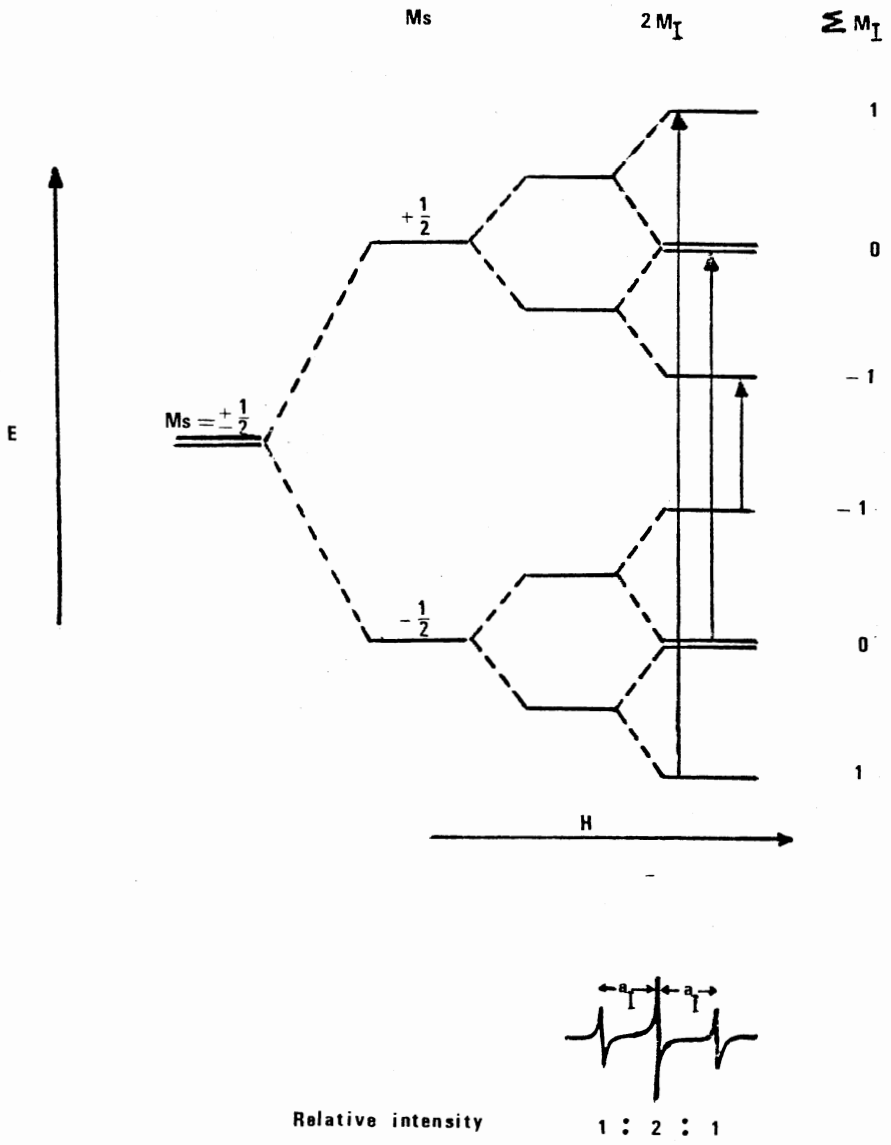


Figure 8. Diagram Drawing Energy Levels, Transition, Hyperfine Structures and Relative Intensity of Lines for Two Equivalent Nuclei with Nuclear Spin, I = 1/2

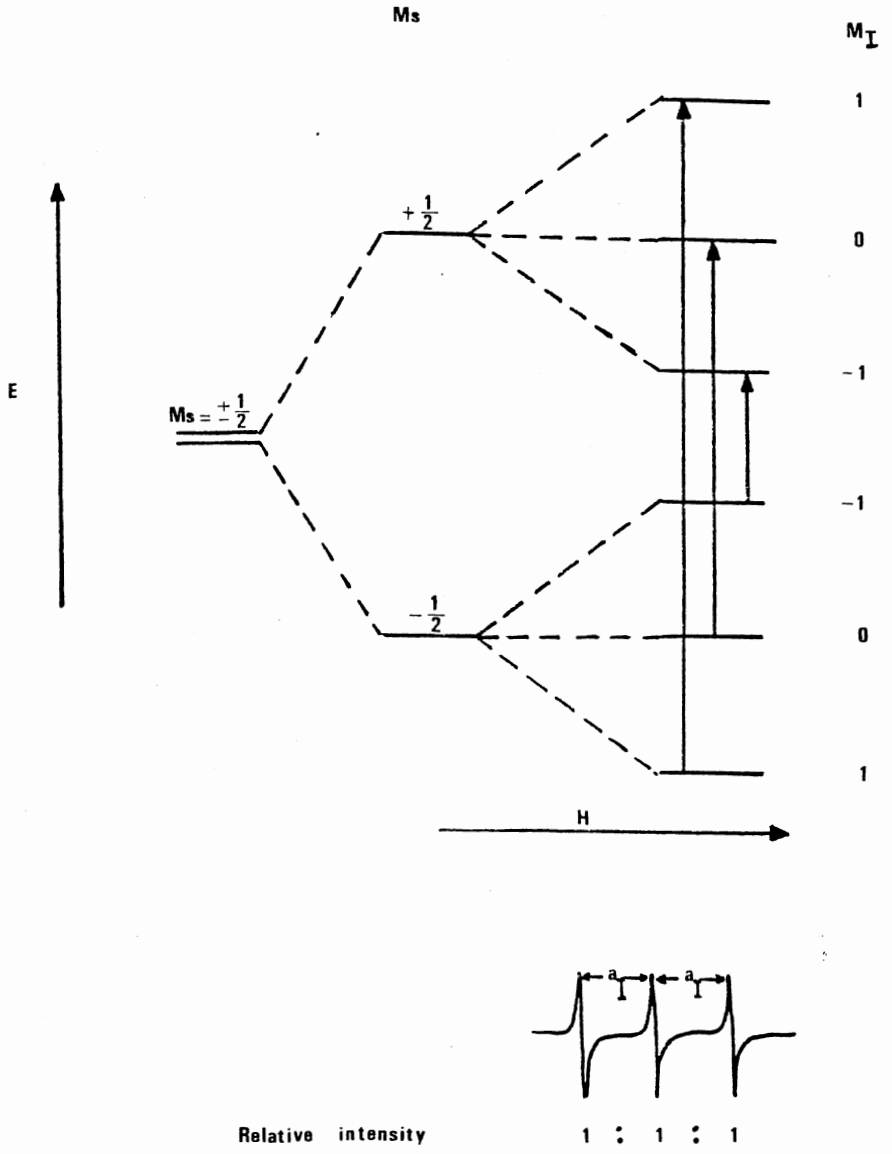


Figure 9. Diagram Drawing Energy Levels, Transitions, Hyperfine Structures and Relative Intensity of Lines for a Nucleus With Nuclear Spin, I = 1

will be proportional to the coefficient of the binoimal expansion of order n. The relative intensities are readily found from the appropriate Pascal's triangle(Figure 10).

n	Relative intensity of ESR absorptions	Number of energy levels for each value of M_s
0	1	1
1	1 1	2
2	1 2 1	4
3	1 3 3 1	8
4	1 4 6 4 1	16
5	1 5 10 10 5 1	32
6	1 6 15 20 15 6 1	64

Figure 10. The Relative Intensity of ESR Lines From the Pascal's Triangle

If a radical contains 1,2,3.....k sets, each consisting on n_1, n_2, n_3.....n_k equivalent nuclei with spin quantum numbers I_1, I_2, I_3.....I_k then the possible total number, N, of the lines is given by

$$N = (2n_1I_1 + 1)\ (2n_2I_2 + 1)\ (2n_3I_3 + 1)\ \ldots\ (2n_kI_k + 1) \qquad (2.12)$$

from the number and intensity distribution of the spectral lines, one can tell how many nuclei interact with the radical electron.

g-Factor

The g-factor is a number characteristic of the particular radical and is defined by equation 2.8 as the proportionality constant which relates the frequency to the field strength at which resonance occurs. The g-factor is a dimensionless constant and equal to 2.00232 (41) for a single, isolated free electron. The value calculated from equation 2.8 may deviate from 2.00232, particularly when heteroatoms are involved because the orbital angular momentum of the unpaired electron depends upon its chemical environment in an atom, molecule or crystal. In many organic free radicals, the g-factor of an unpaired electron is close to that of a free electron but in metal ions, g-factors are often greatly different from the free electron value.

The absolute determination of g-factors and hyperfine-splitting constants requires accurate measurement of the external field which envelops the sample. To measure the g-factor for free radicals, it is convenient to measure the field separation between the center of the sample spectrum and that of a reference substance whose g-value is accurately known. The use of a dual-sample cavity simplifies the measurement. Two signals will be observed simultaneously

with a field separation of ΔH_s. The g-factor for the sample is given by

$$g = g_{std}\left(1 - \frac{\Delta H_s}{H}\right) \qquad (2.13)$$

where H is the magnetic field at the center of the standard ESR spectrum, g is the g-factor of the sample, g_{std} is the g-factor of standard and ΔH_s is the field difference between sample and standard compound.

Interpretation of ESR Spectra

The g-factor, the separation of the hyperfine lines and their relative intensities are the experimental parameters obtained from ESR spectra. An ESR spectrum is assigned by determining the magnitude of the hyperfine coupling constants and correctly counting the lines. Frequently the the measured spectrum is complicated because of overlapping lines. Resolution of the spectrum is difficult and the number of lines is probably less than the possible total lines expected. In some cases, when many equivalent nuclei interact, relative peak heights become very large and it is difficult to observe the smallest peaks. These smallest peaks are especially important because the outer portions of a spectrum are invariably the simplest, and the correct interpretation of these lines may provide the key to resolve the more complex spectra is to assign a value to the coupling constant and by computer simulation, construct a spectrum for comparison with the experimental spectrum.

ESR is a very powerful technique useful in the study of any system containing one or more unpaired electrons. It is not without difficulty in interpretation of the resultant spectra. Systems which are amenable to study include (41):

1. Free radicals (molecules containing one unpaired electron) in solid, liquid or gaseous states.
2. Point defects (localized crystal imperfections) in solids.
3. Biradicals or molecules which contain two unpaired electrons but interactions between the electrons are very weak. The molecule behaves as two slightly interacting free radicals.
4. System in the triplet state (two unpaired electrons). Some of these molecules have a triplet ground state: others require excitation.
5. System with three or more unpaired electrons including most transition metal ions and rare-earth ions.

CD and ESR techniques have been used in this study in order to obtain some information about the role of metal ions in the color reactions with drugs, the chemicl species responsible for colors and the possible structure of the colored chemical species.

CHAPTER III

EXPERIMENTAL

Instrumental

Circular dichroism measurements were made on either a CARY model 61 of JASCO model J-500A spectropolarimeter. The wavelength range for both instruments is from 185 to 800 nm. Sensitivity of the CARY 61 is variable from 0.01 to 2.00 degrees of ellipticity full range over a 25.4 cm. (10 in.) chart, which theoretically allows ellipticities as low as 10^{-4} to 2 degrees to be observed. The JASCO spectropolarimeter has a sensitivity range from 0.1 to 50 millidegree per cm. which is equal to a theoretical ellipticity range for the 26 cm. chart of 10^{-5} to 1.3 degree.

Both instruments use a 450 W high pressure Xe arc lamp as the light source. In order to prevent the production of ozone from oxygen by UV irradiation, the instruments are purged with dry nitrogen gas during operation.

Wavelength calibration on CARY 61 and JASCO spectropolarimeters is performed by measuring the emission lines of a standard fluorescent lamp and neodymium doped glass respectively. The ellipticity scale is calibrated by using a 0.1 % (W/V) solution of anhydrous D-10 camphorsulfonic acid in distilled water. The recommended ellipticity value from the

literature is + 0.31 degree measured at the 290 nm maximum for a sample of 1.0 cm. pathlength.

The sensitivity range of the JASCO model can be extended to lower values by one order of magnitude using the data processor model number DP-500N. The microprocessor has additional capabilities for signal improvement such as baseline control, repeat scan, smoothing and spectral difference functions.

Where necessary the temperature of the solution for the CD studies was maintained constant to 25$\pm$ 0.1°C. Control was provided by a Haake constant temperature bath Model F, which utilized circulating water from an internal reservoir as the heat exchange fluid. The cells were thermostated by way of water circulating temperature controller through a CARY accessory water jacket cell holder when the equilibrium experiments were performed.

UV-Visible spectral measurements were performed on a Perkin-Elmer UV-Visible spectrophotometer Model 552. This instrument has a wavelength range from 190 to 800 nm. The instrument is equipped with an automatic baseline adjustment and has an absorbance range from - 0.300 to 3.00 absorbance unit (A).

ESR spectra were measured on an X-band Varian 4502 ESR spectrometer. Microwave power was supplied by a VA 153C klystron and the microwave frequency was stabilized by an automatic control system in which the klystron was locked to the sample cavity. The V-4531 sample cavity is rectangular

in shape. A self-locking HP 5340A frequency counter was used to accurately measure the microwave frequency. Magnetic field strengths were measured using a self-tracking Varian E-500 digital Gaussmeter. ESR signals from the detector were recorded on a Houston Instruments (Model 2000) X-Y recorder. Sample cells used in this work were cylindrical quartz tubes which had a 2 mm. inside diameter and were approximately 6 in. in length.

Sample weighings were made on two balances, a Sartorius balance model 2403 and a CAHN model 2000 RG electrobalance. The former was used for routine weighings in excess of 10 mg., the latter whose minimum weight of 0.01 mg. can be measured accurately was used for smaller weight measurements.

^{13}C NMR measurements of drugs and products of rections in different media were made on a Varian XL-100-15 NMR spectrometer. Standard proton NMR tubes with a 5 mm. o.d. were used as sample tubes.

Chemicals

The primary drugs used in this work were pure standards and were used without further purification. Morphine sulfate and codeine free base were obtained from Mallinckrodt Inc.. Morphine free base and 3,6-diacetylmorphine (heroin) hydrochloride were provided by the National Institute for Drug Abuse (NIDA) via the Research Triangle Laboratories (RTI). Distilled water was used as solvent for the

equilibrium experiments, while sulfuric acid, 96.74 % (Mallinckrodt, Inc.) was used as the reaction solvent for the color reactions of drugs. Anhydrous ferric chloride (Matheson Coleman and Bell), calcium perchlorate (G. Frederick Smith Chemical Company), calcium chloride and silver nitrate (Baker Chemical Company) were all reagent grade quality and were used without further purification.

Experimental Proceudres

CD and UV-Visible Measurements

Acid and base titrations of morphine in aqueous solution. Equal aliquots of a standardized morphine free base solution were mixed with a series of measuring volumes of standard acid or base solutions which would titrate the morphine through stage of partial to total neutralization. The final volumes of each mixture was adjusted to 10.00 ml.. The solution temperature was maintained at 25 $\pm$ 0.1°C. An equilibrium time of 5 minutes was allowed before recording the spectra. CD spectra were measured for each mixture and the spectral variations were carefully measured at two wavelengths.

Metal ion - drug interactions in aqueous solution. The metal ions used in this work were the Fe^{3+}, Ca^{2+}, and Ag^{+} ions. Salts of those metal ions were dissolved in distilled water. Solutions were prepared in which the mole ratio of cation to morphine or codeine was vaired from 1:1

to 10:1. CD spectral data were collected for all solutions.In the case of the Ca^{2+}- morphine interaction, solutions were also prepared using the method reported by Lin, Sutherland, and Way (32).

Reactions of drugs with concentrated sulfuric acid. Each drug was dissolved in 96.74 % sulfuric acid, and the spectral measurements were made within 20 minutes after preparation.

Pseudomorphine solutions. Pseudomorphine was prepared by following the procedure reported by Bently (43).The spectral measurements were made on the solution of pseudomorphine in distilled water, 1.0 M hydrochloric acid and 1.0 M sodium hydroxide.

ESR Measurements

All experiments described in this section are strictly qualitative in nature. The compositions of the color test reagents, and the color developed for each drug are described in Table II.

Color test reagents (0.10 ml.) were added to a few milligrams of the dry solid forms of the drugs. Mixing was done immediately before physical measurements were made. Almost all the experiments were performed at room temperature. Exceptions had to be made for the reactions of codeine with Frohde, Mecke, Mandelin and Erdman reagents which by necessity had to be conducted at 10°C. In ESR

TABLE II

COMPOSITIONS OF COLOR TEST REAGENTS AND THE COLOR DEVELOPED IN EACH REACTION

REAGENTS	COMPOSITIONS	COLOR DEVELOPED
Marquis	10 drops of 37 % formaldehyde (Fisher Scientific Company) in 10 ml. of conc. H_2SO_4, freshly prepared.	morphine : red purple codeine : blue purple heroin : red purple
Erdman	10 drops of 70 % nitric acid (Mallinckrodt) in 20 ml. of conc. H_2SO_4, freshly prepared.	morphine : yellow codeine : yellow brown heroin : yellow
Frohde	500mg of ammonium molybdate (Mallinckrodt) in 100 ml. of conc. H_2SO_4.	morphine : purple to green codeine : green heroin : purple to green
Mecke	0.5 g of selenious acid (Aldrich Chemical Company) in 100 ml. of conc. H_2SO_4.	morphine : blue codeine : blue green heroin : blue
Mandelin	1 g of ammonium vanadate (Fisher Scientific Company) in 100 ml. of conc. H_2SO_4.	morphine : violet yellow codeine : rose yellow heroin : violet yellow

work precise measurements of the magnetic field require that a standard compound be recorded everytime. The standard used in this work was a chromium doped MgO crystal. Comparative measurements were made immediately following each sample keeping the resonance conditions the same.

NMR Measurements

^{13}C NMR spectra were obtained from solution of morphine sulfate in a) D_2O b) concentrated sulfuric acid. The sample were rotated in 5 mm. o.d. tubes. D_2O in a sealed capillary tube was used as an external lock signal. Sodium-3-trimethylsilyl-propionate-2,2,3,3,-d_4-(Merck Sharp and Dohme) was used as internal reference standard. All measurements were run overnight in order to obtain a high signal to noise ratio.

All measurements reported in this work were performed at room temperature unless specified otherwise.

CHAPTER IV

EXPERIMENTAL RESULTS

Isotropic CD Spectra

The aromatic ring(s) in morphine or morphine-like compounds are the principal absorption chromophores responsbile for the electronic transitions observed in the UV spectral region. The transition in the wavelength range 270-290 nm. has been attributed to a $^{*}\Pi \leftarrow \Pi$ transition of type $^{1}L_{b}$ and a second $^{*}\Pi \leftarrow \Pi$ transition which occurs at 220-260 nm. has been designated as $^{1}L_{a}$. Generally, for the isotropic solution of opiates in aqueous solution, the Cotton bands for $^{1}L_{b}$ transitions are negative and the Cotton bands for the $^{1}L_{a}$ transition is positive.

Acid and Base Titrations of Morphine Free Base in Aqueous Solution

The CD spectrum of morphine free base in distilled water shows two positive bands with maxima at 305 and 245 nm, and one negative band with maximum at 285 nm (Figure 11). The ellipticity of the positive band maximum at 305 nm increases in magnitude while the negative band at 285 nm decreases in magnitude when the pH of solution is increased. Base titration of morphine free base in aqueous solution was

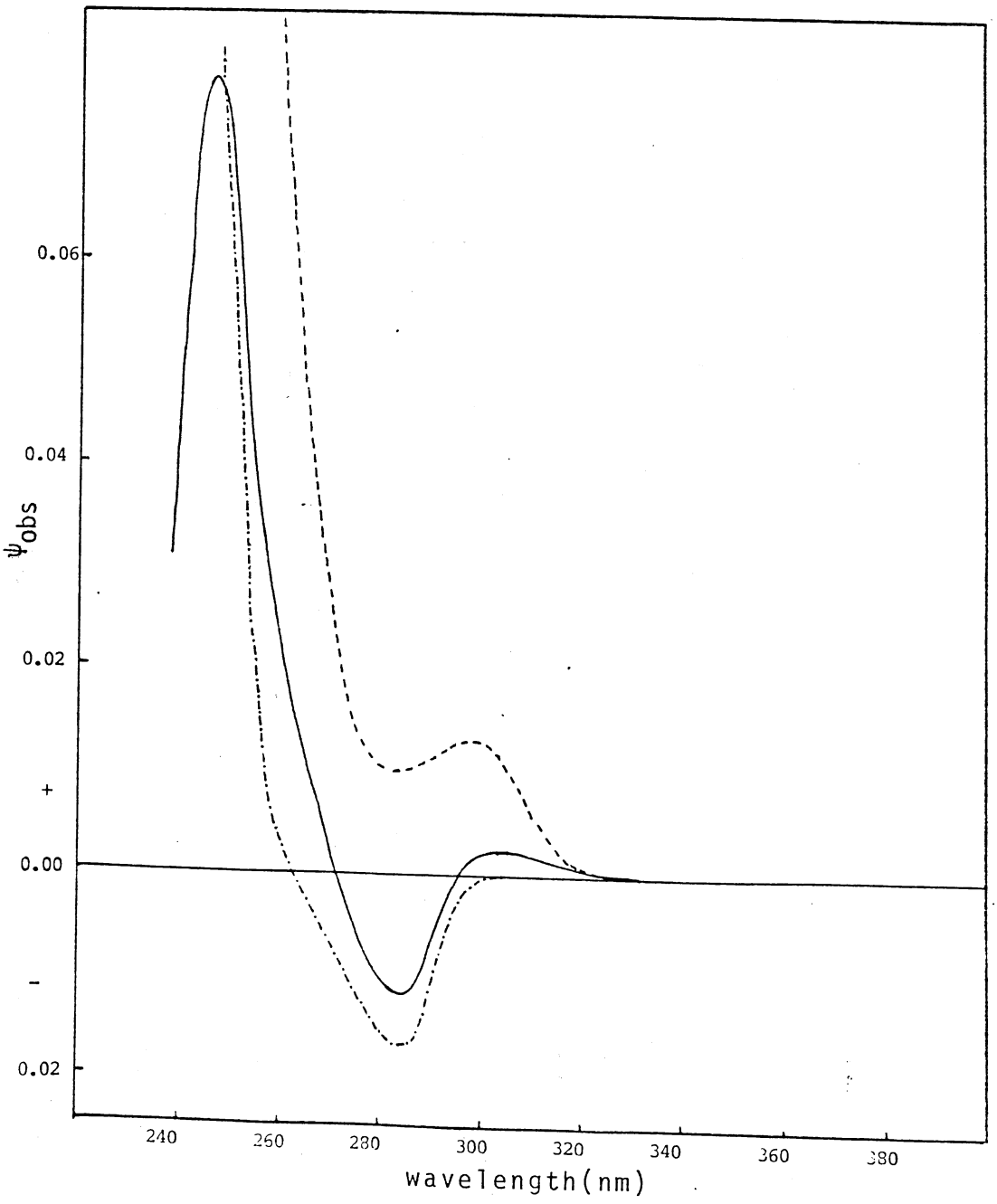


Figure 11. Isotropic CD Spectra of 2.5113×10^{-4} M Morphine Free Base Solution, (——) Morphine Free Base in Distilled Water, (---) Morphine Free Base in 1 M NaOH, (-•-•-), Morphine Free Base in 1 M HCl

performed in order to determine the second dissociation constant (the ionization of the phenolic proton) of morphine. The observed ellipticities were measured at two different wavelengths, that is 300 and 285 nm, for each increment of base added in the titration. The observed ellipticity at each wavelength, ψ_{obs}, is assumed to arise from contributions from two species present in solution, the protonated form, HM, and the ionized form, or phenolate ion, M^-, according to equation (4.1)

$$\psi_{obs} = \theta_{HM}[HM] + \theta_{M^-}[M^-] \qquad (4.1)$$

where θ_{HM} is the molar ellipticity of the protonated form and θ_{M^-} is the molar ellipticity of the ionized form both of which are assumed to be constant with degree of ionization at each wavelength.

When morphine free base is dissolved in 1 M NaOH solution the only species present in the solution is the ionized form, M^-. The plot of ψ_{obs} VS $[M^-]$ will give a straight line with slope equal to θ_{M^-}. In this work the values of θ_{M^-} at 300 and 285 nm were measured to be +50 and +41 respectively. A value for θ_{HM} could not be obtained from a similar plot because of incomplete ionization equilibrium. It was included as an unknown to be calculated from an iterative calculation.

The base titration of morphine free base is mathematically described by the following equations:

$$HM + OH^- \rightleftharpoons H_2O + M^- \qquad (4.2)$$

and $$\frac{1}{K_b} = [M^-] / [HM]\,[OH^-] \qquad (4.3)$$

where K_b is the hydrolysis constant for the ionized species, M^-. In the base titration, mass and charge balance equations can be expressed in term of the equilibrium concentrations of the species present in solution, as described by equation 4.4 and 4.5.

$$T_m = [HM] + [M^-] \qquad (4.4)$$

$$[Na^+] + [H^+] = [OH^-] + [M^-] \qquad (4.5)$$

$[H^+]$ is assumed to be negligible in the base titration, therefore

$$T_{OH^-} = [Na^+] = [OH^-] + [M^-]$$

and $$[OH^-] = T_{OH^-} - [M^-]$$

From equation 4.4, $[HM] = T_m - [M^-]$

$[M^-]$ can be calculated by subsititution of the expression for [HM] into equation 4.1, that is:

$$\psi_{obs} = \theta_{HM} (T_m - [M^-]) + \theta_{M^-}[M^-] \qquad (4.6)$$

and $$[M^-] = \frac{\psi_{obs} - \Theta_{HM} T_m}{\Theta_{M^-} - \Theta_{HM}} \qquad (4.7)$$

[HM] and [OH^-] can be calculated from equation 4.4, 4.5 respectively, the resultant expression for K_b is

$$K_b = \frac{\left[T_m - \frac{\psi_{obs} - \Theta_{HM}T_m}{\Theta_{M^-} - \Theta_{HM}}\right]\left[T_{OH^-} - \frac{\psi_{obs} - \Theta_{HM}T_m}{\Theta_{M^-} - \Theta_{HM}}\right]}{\left[\frac{\psi_{obs} - \Theta_{HM}T_m}{\Theta_{M^-} - \Theta_{HM}}\right]} \quad (4.8)$$

where T_m = total concentration of morphine free base and

T_{OH^-} = incremental concentration of total amount of 0.0200 M NaOH added.

The equation which express K_2, the second ionization constant, in term of concentrations of species is given for the equilibrium reaction

$$HM \rightleftharpoons H^+ + M^- \quad (4.9)$$

by $$K_2 = [H^+]\,[M^-]/\,[HM] \quad (4.10)$$

K_2 is related to K_b in the relationship:

$$K_2 = K_w/K_b \quad (4.11)$$

or $$pK_2 = 14 - pK_b \quad (4.12)$$

The values calculated from the CD-base titration of morphine free base in aqueous solution, at two different wavelengths are given in Table III. The values of K_2 are not the exact thermodynamic values since no correction was made

TABLE III

RESULTS FROM THE CD-BASE TITRATIONS OF MORPHINE FREE BASE AT 285 AND 300 nm

T_{OH}-X 10^4	*At 285 nm		**At 300 nm	
	ψ_{obs}	pK_2	ψ_{obs}	pK_2
0.62	-0.009125	9.24	0.003	9.78
0.94	-0.008	9.84	0.004	9.82
1.24	-0.0065	9.92	0.00475	9.88
1.56	-0.005	9.95	0.0053	9.97
1.88	-0.0036	9.97	0.006	9.98
2.20	-0.00245	9.99	0.007	9.89
2.52	0.00	9.86	0.00775	9.85
		average pK_2= 9.82		average pK_2= 9.88

*θ_{HM} at 285 nm = -59; **θ_{HM} at 300 nm = +3; T_m = 2.5113 X 10^{-4} M

for the ionic strength variation on adding titrant. However at the low concentrations used the activity coefficient corrections are minimal and the values are close to unity. A comparison with K_2 values from potentiometric titrations is made later in this chapter.

The acid titration of morphine free base in aqueous solution was performed to determine the first dissociation constant, K_1, (for the protonation of the tertiary nitrogen atom) of morphine. In this experiment, 0.0200 N HCl was used and the observed ellipticity at each step of the titration was measured only at 285 nm since the change in ellipticity observed at 300 nm is too small to measure with any accuracy.

In the acid titration, two species, the diprotonated form, H_2M^+ and the monoprotonated form, HM, are assumed to be present in solution. Therefore, the observed ellipticity can be expressed as a sum of two contributions by equation

$$\psi_{obs} = \theta_{HM}[HM] + \theta_{H_2M^+}[H_2M^+] \qquad (4.13)$$

where $\theta_{H_2M^+}$ is the molar ellipticity of H_2M^+. This value was determined by a method analogous to that used for θ_{M^-} determination: that is, a calibration curve was prepared using 1 M HCl as solvent. It was assumed that only the H_2M^+ species was present in this solution. The slope of the calibration curve is -69 at 285 nm.

From the chemical equation for the acid titration of

morphine free base,

$$HM + H^+ \rightleftharpoons H_2M^+ \qquad (4.14)$$

the expression for K_1 is

$$K_1 = [HM]\,[H^+]/\,[H_2M^+] \qquad (4.15)$$

The appropriate mass and charge balance equations are

$$T_m = [HM] + [H_2M^+] \qquad (4.16)$$

$$[Cl^-] + [OH^-] = [H^+] + [H_2M^+] \qquad (4.17)$$

In highly acidic solutions $[OH^-]$ is assumed to be neglible therefore equation 4.17 reduces to

$$T_{Cl^-} = [H^+] + [H_2M^+] \qquad (4.18)$$

and $$[H^+] = T_{Cl^-} - [H_2M^+]$$

where T_{Cl^-} is the incremental concentration of the total amount of 0.0200 M HCl added.

By making the appropriate substitutions, $[H_2M^+]$ can be calculated from the final expression

$$[H_2M^+] = \frac{\psi_{obs} - \theta_{HM}T_m}{\theta_{H_2M^+} - \theta_{HM}} \qquad (4.19)$$

Substituting this expression into equation 4.15, K_1 can be calculated from equation 4.20.

$$K_1 = \frac{\left[T_m - \frac{\psi_{obs} - \theta_{HM}T_m}{\theta_{H_2M^+} - \theta_{HM}}\right]\left[T_{Cl^-} - \frac{\psi_{obs} - \theta_{HM}T_m}{\theta_{H_2M^+} - \theta_{HM}}\right]}{\left[\frac{\psi_{obs} - \theta_{HM}T_m}{\theta_{H_2M^+} - \theta_{HM}}\right]} \qquad (4.20)$$

A θ_{HM} value of -59 taken from the K_2 analysis was used in the calculation of K_1. The values of K_1 from this work are shown in Table IV. Those values are about 2 order of magnitude different from the data reported in the literature, which had been obtained from potentiometric and colorimetric measurements, but they are close to the value obtained by conductance measurements (52) (Table V). The difference may be due to the limitation of the technique used since the very small changes in ψ_{obs} observed at each step of the titration were assumed to be directly proportional to the amount of H_2M^+ produced. Therefore the error in measuring a very small change in ψ_{obs} will lead to a larger variation in the calculated K_1.

Metal Ion - Drug Interactions in Aqueous Solutions

Ca^{2+} - Morphine Interaction

CD spectra of solutions containing different mole ratio band which maximizes at 285 nm., compared to the CD spectrum of morphine. One small quantitative change was observed

TABLE IV

CD-ACID TITRATION DATA OF MORPHINE FREE BASE AT 285 nm

ψ_{obs}	$T_{Cl^-} \times 10^4$	pK_1
-0.0160	1.24	5.20
-0.016325	1.56	5.46
-0.01665	1.88	5.76
-0.016925	2.20	5.76
-0.017125	2.52	5.73

average $pK_1 = 5.58 \pm 0.25$

TABLE V

COMPARISON OF pK'S

Compound	This Research, CD-Titration		Literature Values		Methods and References
	pK_1	pK_2	pK_1	pK_2	
morphine free base	5.58	9.85	7.87	9.85	colorimetry (29)
morphine			5.79		conductivity (52)
morphine sulfate			8.02	9.76	potentiometric titration (31)

namely a decrease in the observed ellipticity of the positive band maximum at 300-305 nm. as the molar concentration of Ca^{2+} ion was increased. The same change was observed when the pH of morphine free base solutions are lowered. The effect, therefore, may not be due to increasing Ca^{2+} ion concentration.

Lin and coworkers (32) reported evidence for Ca^{2+} complexation with morphine sulfate on incubation in a KRP solution buffered at pH 7.4 at 37°C for 3 hours. The CD spectrum measured after an identical experiment showed no evidence of changes in the observed maxima and ellipticities.

Fe^{3+} - Drug Interactions

Five percents ferric chloride in aqueous solution is a commonly used color test reagent for a quick qualitative determination of morphine as well as its derivatives which contain a phenolic opiate present in the sample. In this work, mixtures of morphine sulfate and Fe^{3+} in aqueous solution were made with both reagents in the concentration range 10^{-5} - 10^{-4} M. Low concentrations were required to reduce the noise from excessive absorption of the incident light by the fairly intensely yellow color present when Fe^{3+} ion is used in excess. The dark blue confirmatory color commonly observed in high concentrations, such as when solid morphine sulfate is added to an equilavent mole percent of ferric chloride solution, was not observed in these

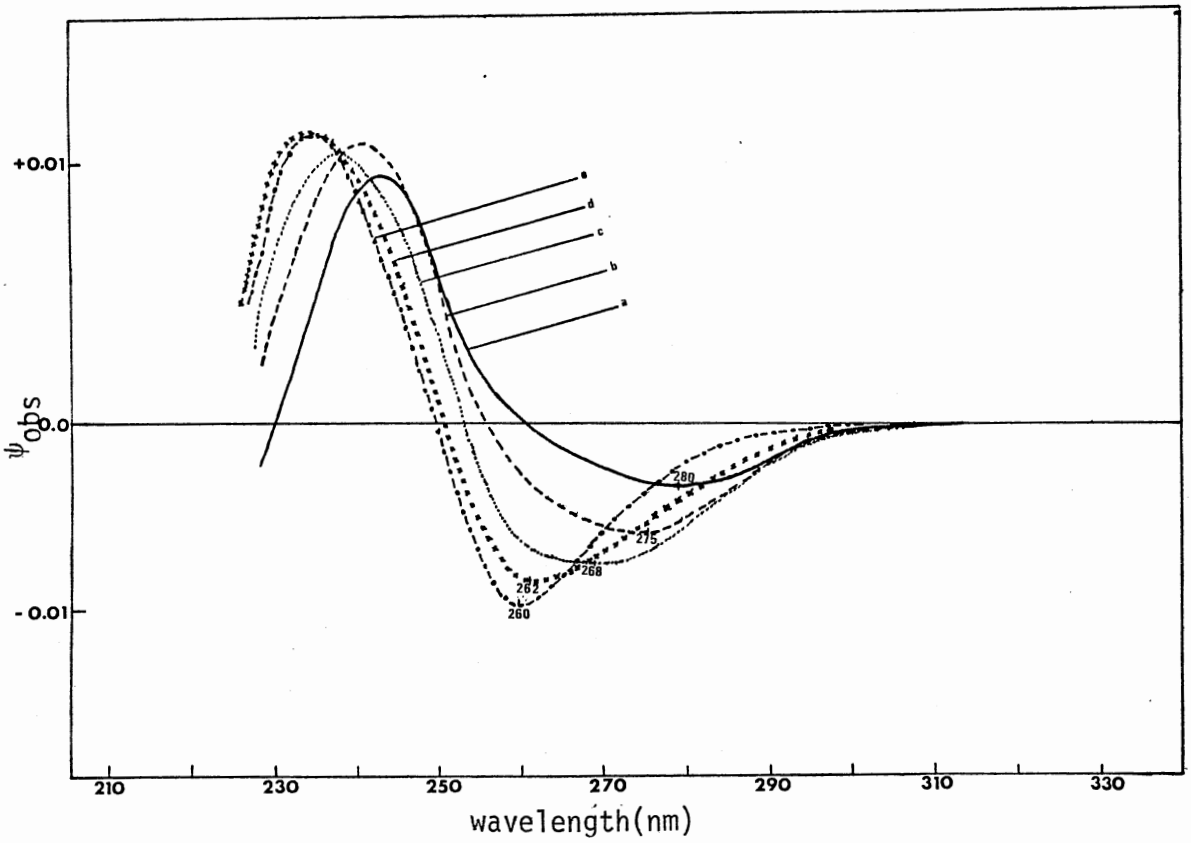
very dilute solutions.

CD spectra of solutions containing different mole ratios of morphine to Fe^{3+} ion (Figure 12) are different from the CD spectrum of morphine solution itself. The negative maximum progressively changes from 285 nm. to 260 nm. and the ellipticity increases as the mole ratio of Fe^{3+} to morphine is increased. This may be caused by the emergence of a new band which increases as the original band decreases or it may be caused by the blue shift of the original band. Simultaneously the positive band is blue-shifted by 15 nm. as the Fe^{3+} ion is increased. This trend is not a consequence of a pH change.

The same experiment performed for codeine showed no changes in either color or CD spectra. This is to be expected if the reactive site is a phenolic group since codeine has an aromatic methoxy substituent.

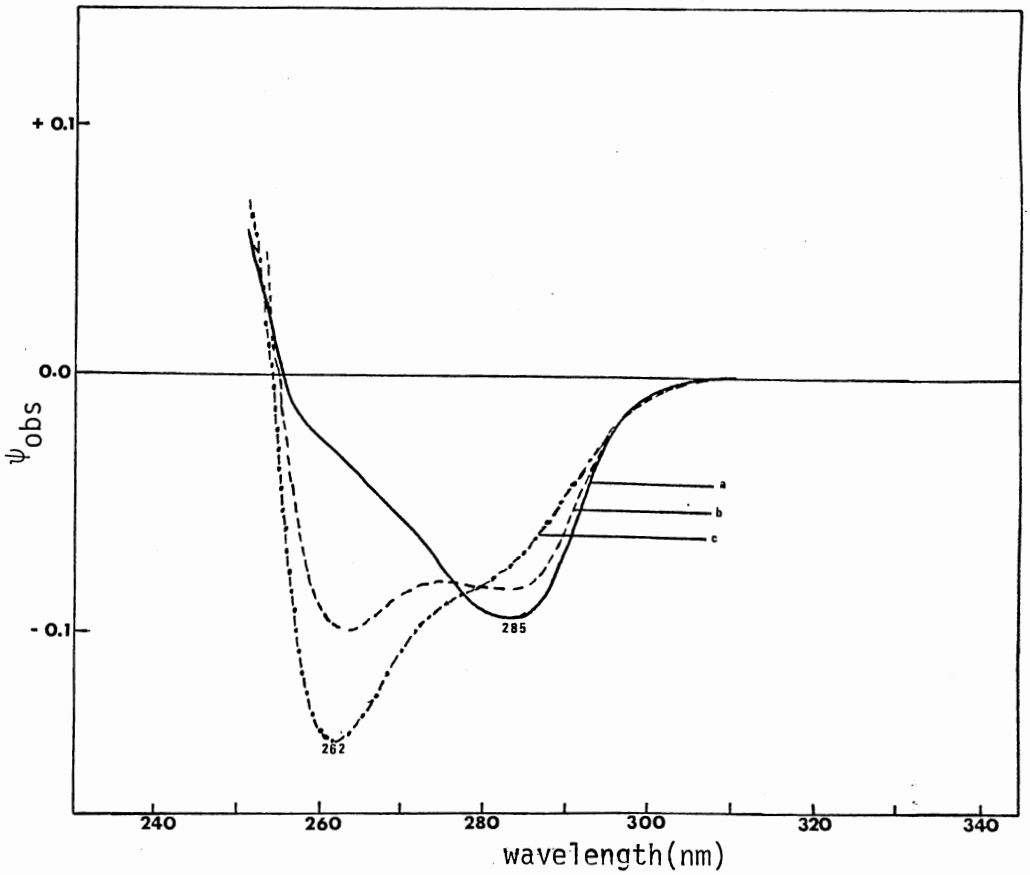
Ag^{+} - Morphine Interaction

Addition of an equivalent molar amount of solid morphine sulfate to an aliquiot of a 1 % silver nitrate aqueous solution produced a cloudy precipitate which increased with time over a few hours. The cloudy solution was centrifuged before performing the CD experiment. After centrifugation the CD spectrum of the solution showed the development of a negative band with maximum at 260 nm. (Figure 13). When a solution of morphine free base was used instead of morphine sulfate to prepare a 1:1 mixture with silver nitrate,



a) 1:1.5 b) 1:3 c) 1:5 d) 1:7 e) 1:10

Figure 12. CD Spectra of Solutions From Fe^{3+} - Morphine Sulfate Reaction at Different Mole Ratios of Morphine to Fe^{3+}



a) 1 hour b) 1 night c) 1 week

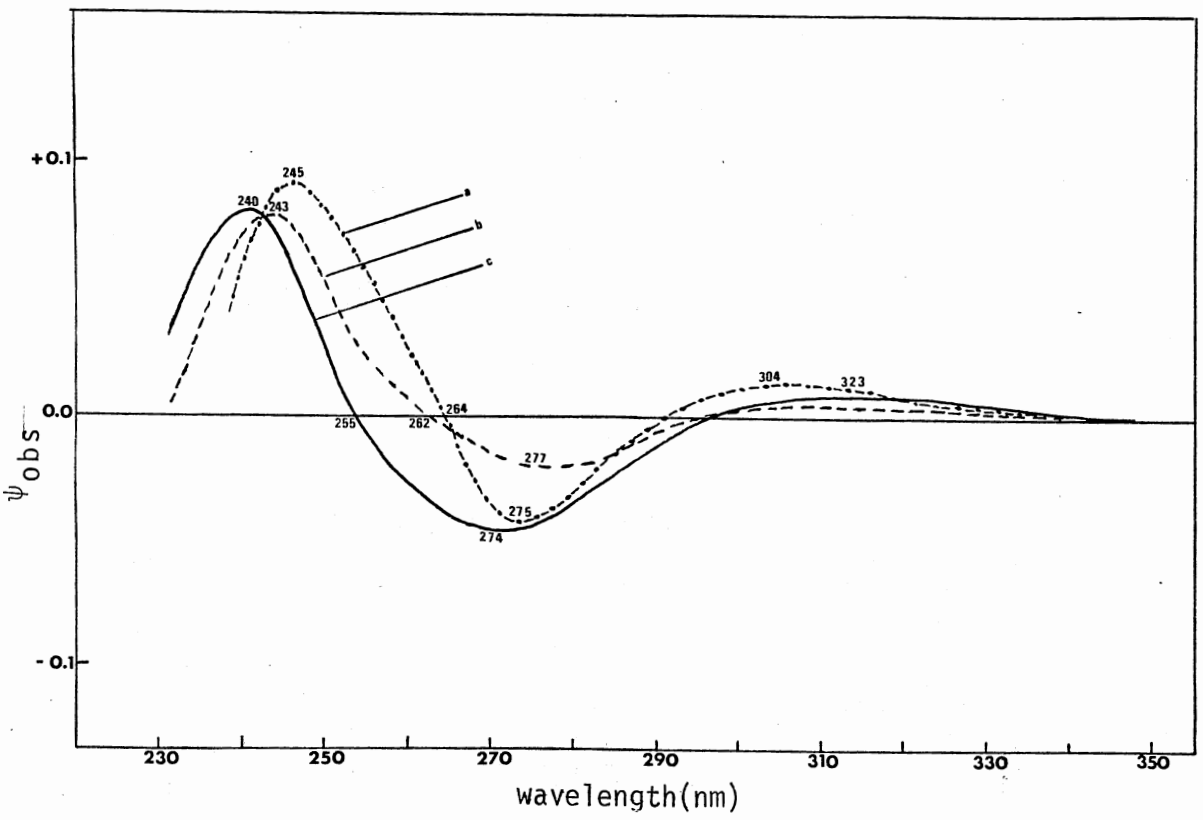
Figure 13. CD Spectra of Solutions Containing 1:1 Mole Ratio of Solid Morphine Sulfate to 1% $AgNO_3$ Solution Recorded at Different Time Intervals

significant changes in the CD spectra were observed to occur in less than 1 hour (Figure 14). The spectra of the morphine free base in excess 1 % silver nitrate solution showed two positive bands with maxima at 323 and 240 nm. and one negative band at 274 nm. The band at 274 and 240 nm. shifted to longer wavelength while the band at 323 nm. shifted to shorter wavelength when the pH of solution was increased. Changing pH is commonly done to probe for acidic groups in the molecule.

Pseudomorphine Solutions

Pseudomorphine is a dimolecular morphine compound produced from the mild oxidation of morphine in alkaline solution. Many successful oxidizing agents have been reported in the literature (5). The popular concept of the structure of pseudomorphine is that two morphine molecules are oxidatively linked to each other via the C(2) and C(2') positions (Figure 15) (43) with the elimination of two hydrogen atoms. This structure has not been confirmed.

In this work pseudomorphine was synthesized by the oxidation of morphine sulfate with alkaline potassium ferricyanide (43). The white solid obtained after purification was divided and dissolved in distilled water, 1.0 N sodium hydroxide, and 1.0 N hydrochloric acid. CD and UV spectra of those solutions are presented in Figure 16 and Figure 17 respectively. The principle CD band maxima for the three solutions are a) positive around 240-250 nm. and b) negative



a) Excess Ag^+ Ions and a Few Drops of 1 N NaOH
b) 1:1 Mole Ratio of Ag^+ to Morphine Free Base
c) Excess Ag^+ Ion

Figure 14. CD Spectra of Solutions Obtained from Ag^+ - Morphine Free Base Reactions After 1 Hour

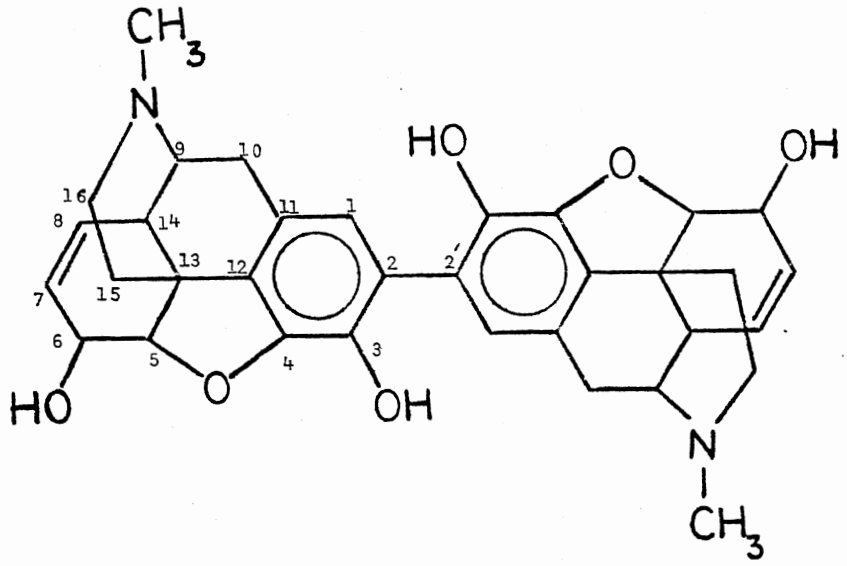
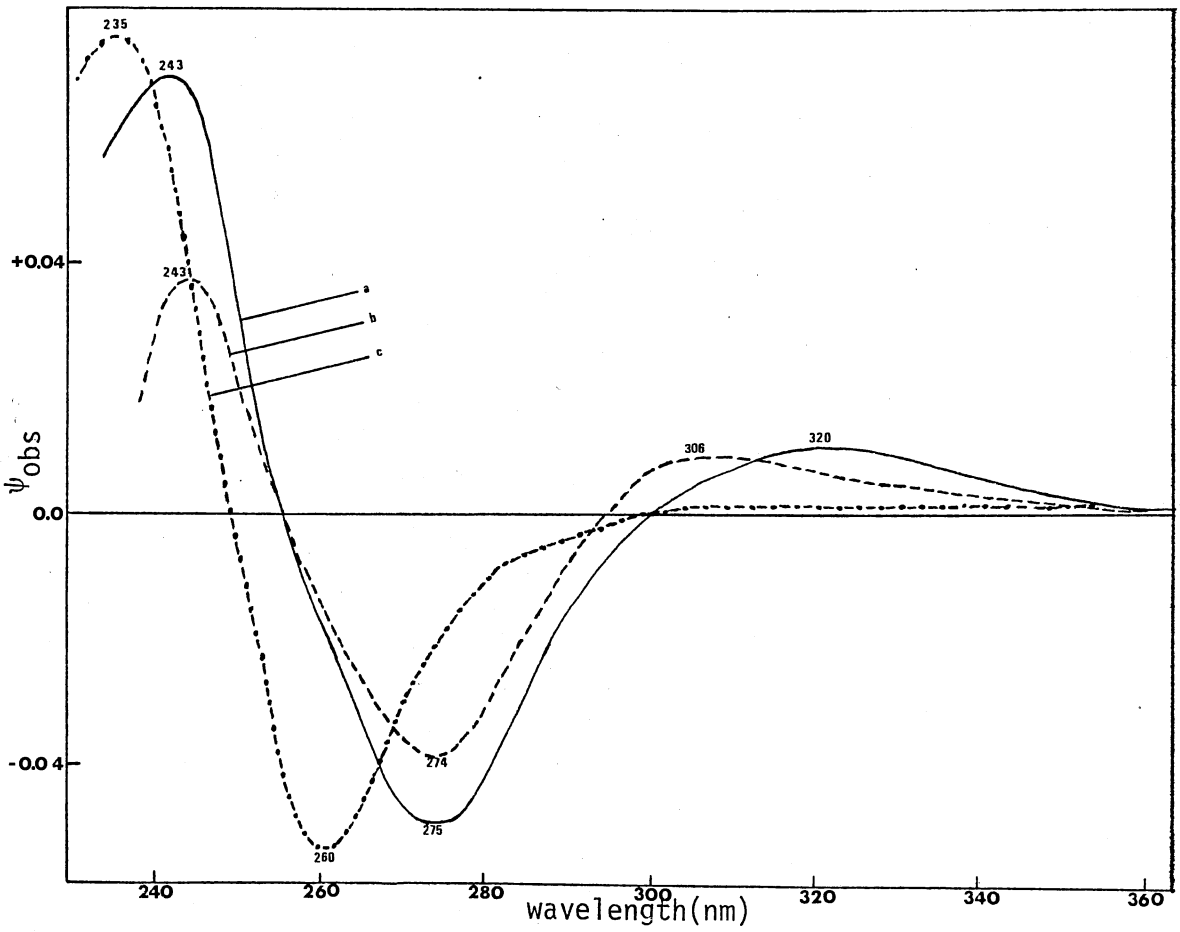
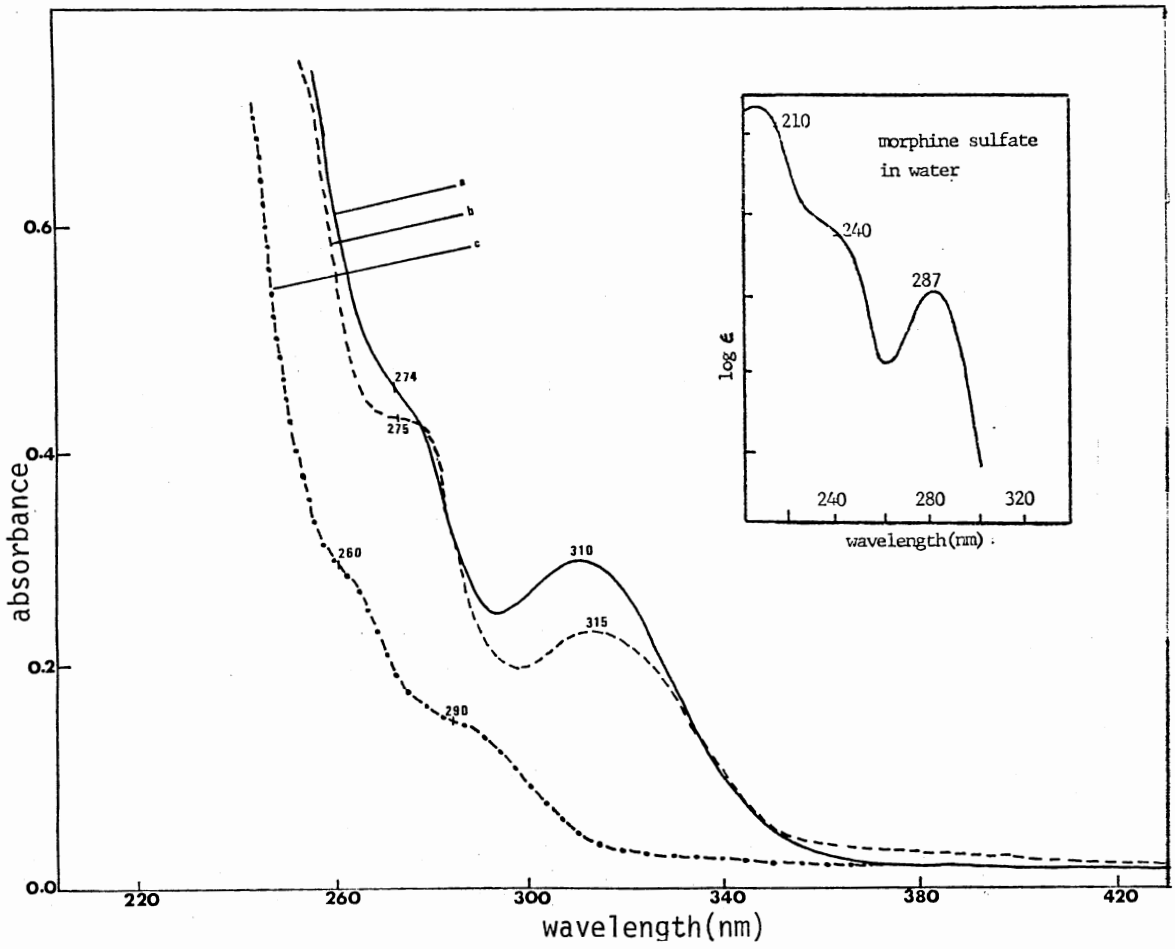


Figure 15. The proposed molecular structure of pseudomorphine



a) In Distilled Water b) In 1 N NaOH c) In 1 N HCl

Figure 16. CD Spectra of Pseudomorphine



a) In 1 N NaOH b) In Distilled Water c) In 1 N HCl

Figure 17. UV Spectra of Pseudomorphine

in the wavelength range 260-275 nm. A second positive band with maximum in the range 306-323 nm. is also observed but only for the aqueous and basic solutions.

UV spectra of the same three solutions show the absorption maxima which correspond to the peak maxima and minima in the CD spectra (Figure 17). An inset spectra for morphine sulfate in water is included for comparison.

It is of interest that the CD spectra obtained from Fe^{3+} - morphine, Ag^{+}- morphine and pseudomorphine solutions are similar in band patterns, i.e. CD maxima ($^{+}\lambda_{max}$, $^{-}\lambda_{max}$), and crossover points (λ_{o}). The data are collected for easier comparison in Table VI. Ca^{2+}- morphine solution spectra on the other hand are dissimilar and typical only of morphine.

Two solutions a) pseudomorphine in water with an excess of Ca^{2+} b) pseudomorphine in water with an excess of Fe^{3+} were prepared. The CD spectrum of each solution was recorded in order to investigate any possible interactions between metal ions and pseudomorphine. No significant changes in the CD spectra of these solutions were observed upon comparison with pseudomorphine in water (Table VI).

Reactions of Drugs in Concentrated Sulfuric Acid

Colorless solutions are obtained when small quantities of drugs are used. UV and CD spectra of the dilute solutions for each drug (Figure 18 and 19) are different from the

TABLE VI

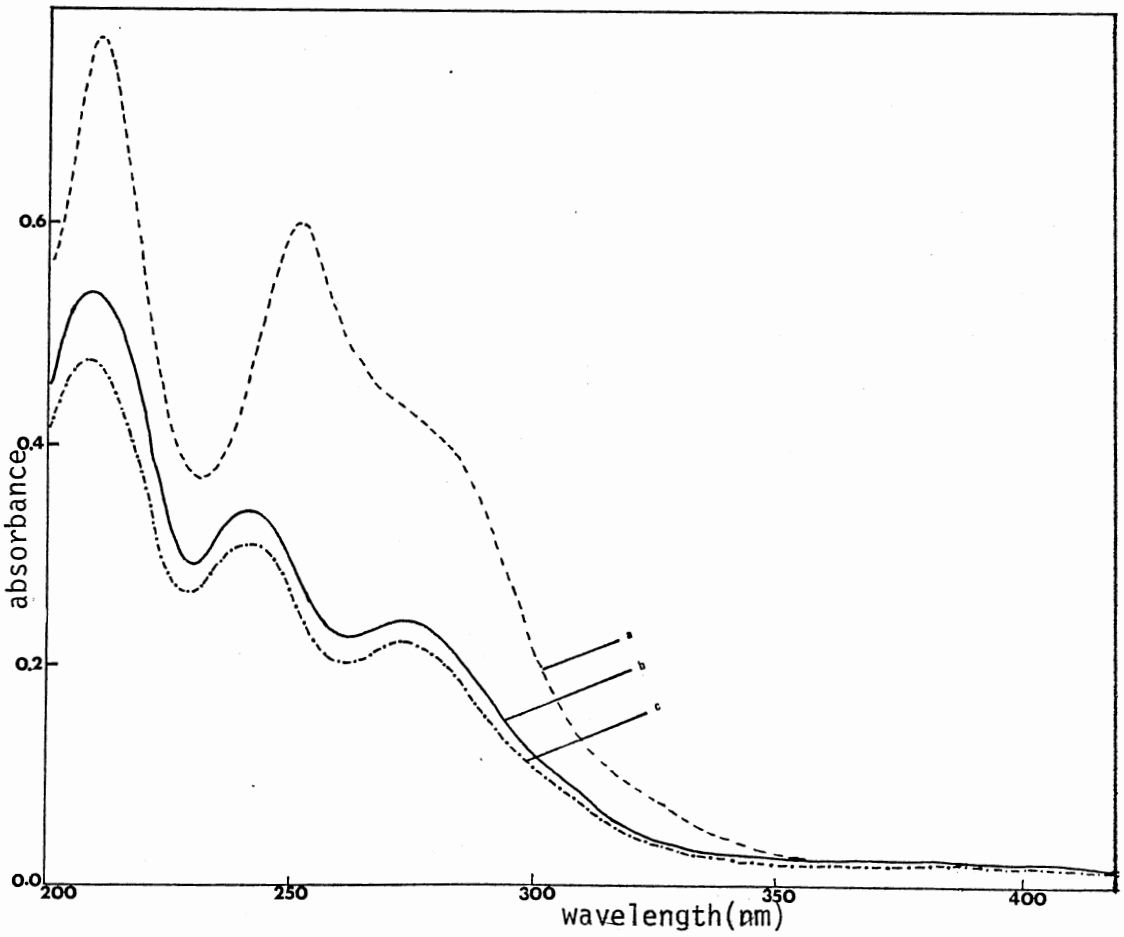
CD DATA OF MORPHINE AND THE METAL IONS INTERACTIONS IN AQUEOUS SOLUTION

SOLUTES	SOLVENTS	$^{+}\lambda_{max}$(nm)	λ_o(nm)	$^{-}\lambda_{max}$ (nm)
morphine free base	H_2O	303, 245	272	285
morphine free base	1 N HCl	240	262	285
morphine sulfate	H_2O	245	262	285
morphine sulfate	1 N NaOH	300, 252	-	-
Fe^{3+}-morphine sulfate (ratio 10:1)	H_2O	230	251	260
*Ag^{+}- morphine free base	H_2O	323, 240	255	274
**Ag^{+}-morphine free base	H_2O	304, 245	264	275
Pseudomorphine	H_2O	321, 243	259	275
Pseudomorphine	1 N HCl	235	252	260
Pseudomorphine	1 N NaOH	306, 243	258	274
Ca^{2+} or Fe^{3+}-pseudomorphine	H_2O	320, 240	255	273

TABLE VI (Continued)

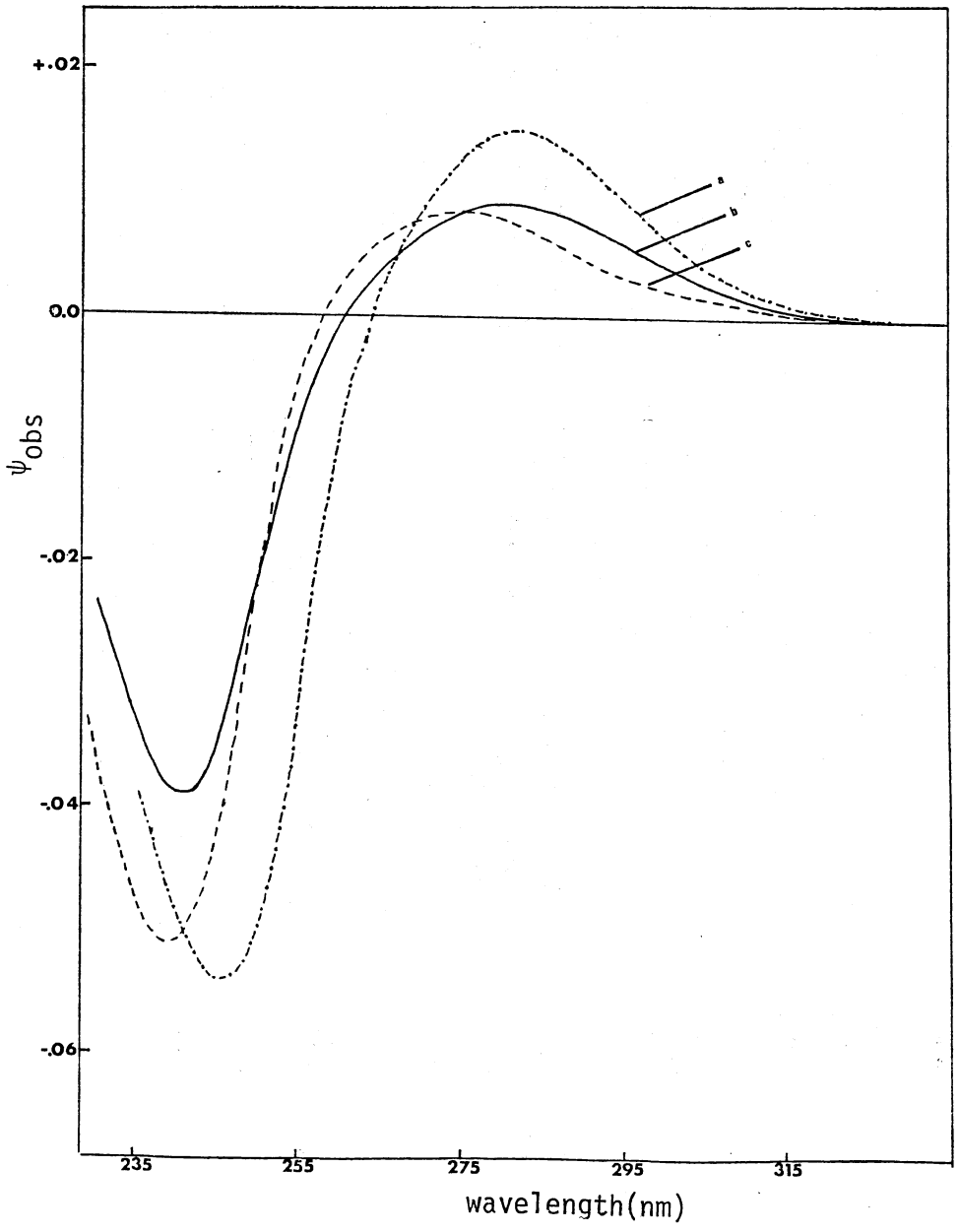
SOLUTES	SOLVENTS	$^{+}\lambda_{max}$ (nm)	λ_{o} (nm)	$^{-}\lambda_{max}$ (nm)
*Ca^{2+}- morphine free base	H_2O	240	262	285
Ca^{2+}- morphine sulfate	KRP buffer	245	262	285

* = metal ion is in excess, ** = metal ion is in excess and solution is basic



a) Codeine b) Morphine Sulfate c) Heroin HCl

Figure 18. UV Spectra of Drugs in Conc. H_2SO_4



a) Heroin HCl b) Morphine Sulfate c) Codeine

Figure 19. CD Spectra of Drugs in Conc. H_2SO_4

spectra of the same drugs in aqueous solution. Almost identical UV absorption bands are obtained from morphine sulfate and heroin HCl solutions, i.e. three absorption bands with maxima at 275, 240 and 210 nm. Codeine solutions by comparison show three absorption bands one a shoulder at 280 nm and two with maxima at 252 and 215 nm. On the other hand, the CD spectra of all three solutions appear to consist of only one principle negative band with a maximum in the range of 240-252 nm and one positive band with a maximum around 275-280 nm. These two bands are of opposite sign with respect to ellipticity compared to the spectra observed when water is used as solvent. This could indicate a solvent induced conformational change or a reaction. A further experiment was performed by the dilution of the original solution with distilled water ($[H^+] \simeq 0.5$ M). The CD spectra of the dilute solutions were recorded and showed almost identical spectra to those of the original concentrated solutions.

Reactions of Drugs with Color Test Reagents in Concentrated Sulfuric Acid

The color reagents used in this study were the Marquis, Frohde, Erdman, Mecke and Mandelin. These were prepared according to the recommended procedures (Table III). The first experiments done were to measure the absorption and CD spectra of the reagents by themselves. These spectra were then substracted from the spectra of the colored products

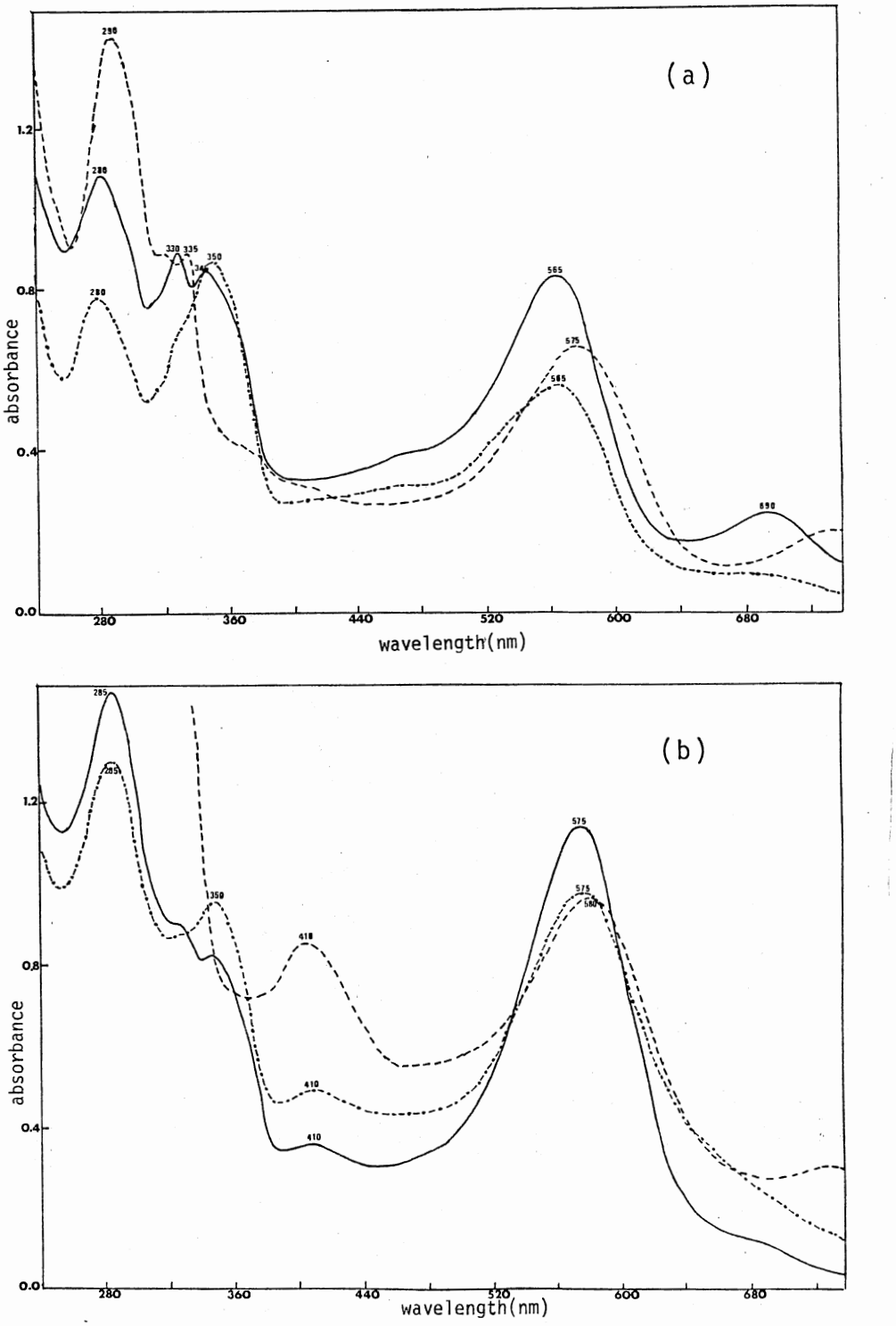
formed on the addition of drugs. Drugs were added to produce concentration on the order of 10^{-4} M. Color reagents were present in large excess and no changes in their spectra were assumed to occur on reaction with the drugs.

UV-Visible Studies

Since the colors change with time, the spectral data were collected after different time intervals: a) immediately upon mixing, b) 15 minutes later and c) after one night. UV-Visible spectra of the colored mixtures (Figure 20, 21, 22, 23) show absorption bands which range from 220 to 800 nm. Among the spectral changes which were observed to occur with time were an increase or decrease in absorbances, shifts in the positions of absorption maxima and the emergence of new bands. Morphine sulfate and heroin.HCl color reactions show the same trends in spectral variations (Table VII, VIII). Overnight, the spectra of the colored mixtures obtained from any of the drugs with the same color test reagent appear to be similar in band pattern and absorption maxima. The data are presented in Figure 24, 25, 26, 27 and Table IX.

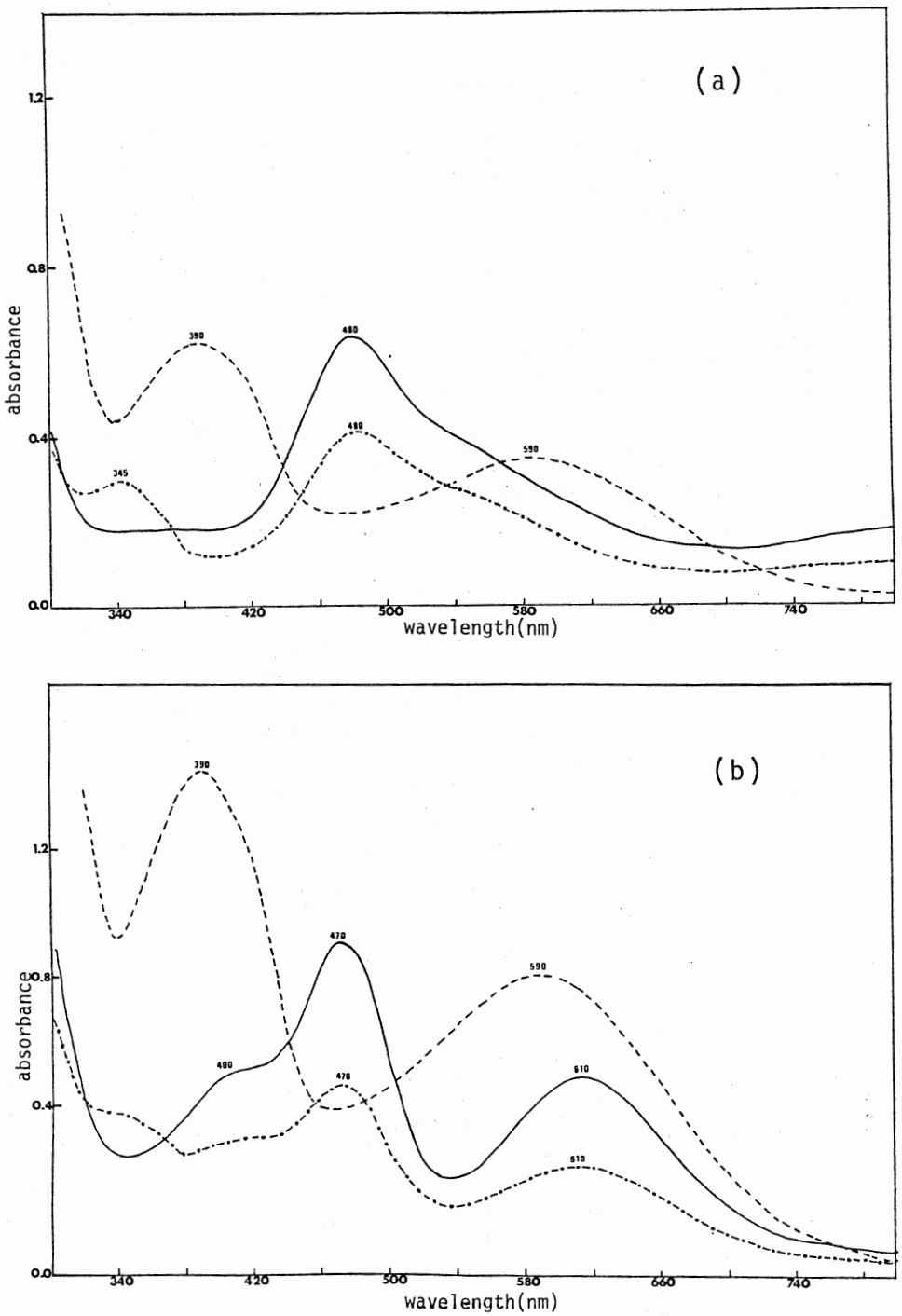
CD Studies

The first obvious result from the CD measurements on the color test reaction products are new bands which occur in the visible spectral range in addition to familiar bands in the UV range (Figure 28, 29, 30, 31). Most of the CD bands observed in the visible region are broader than the



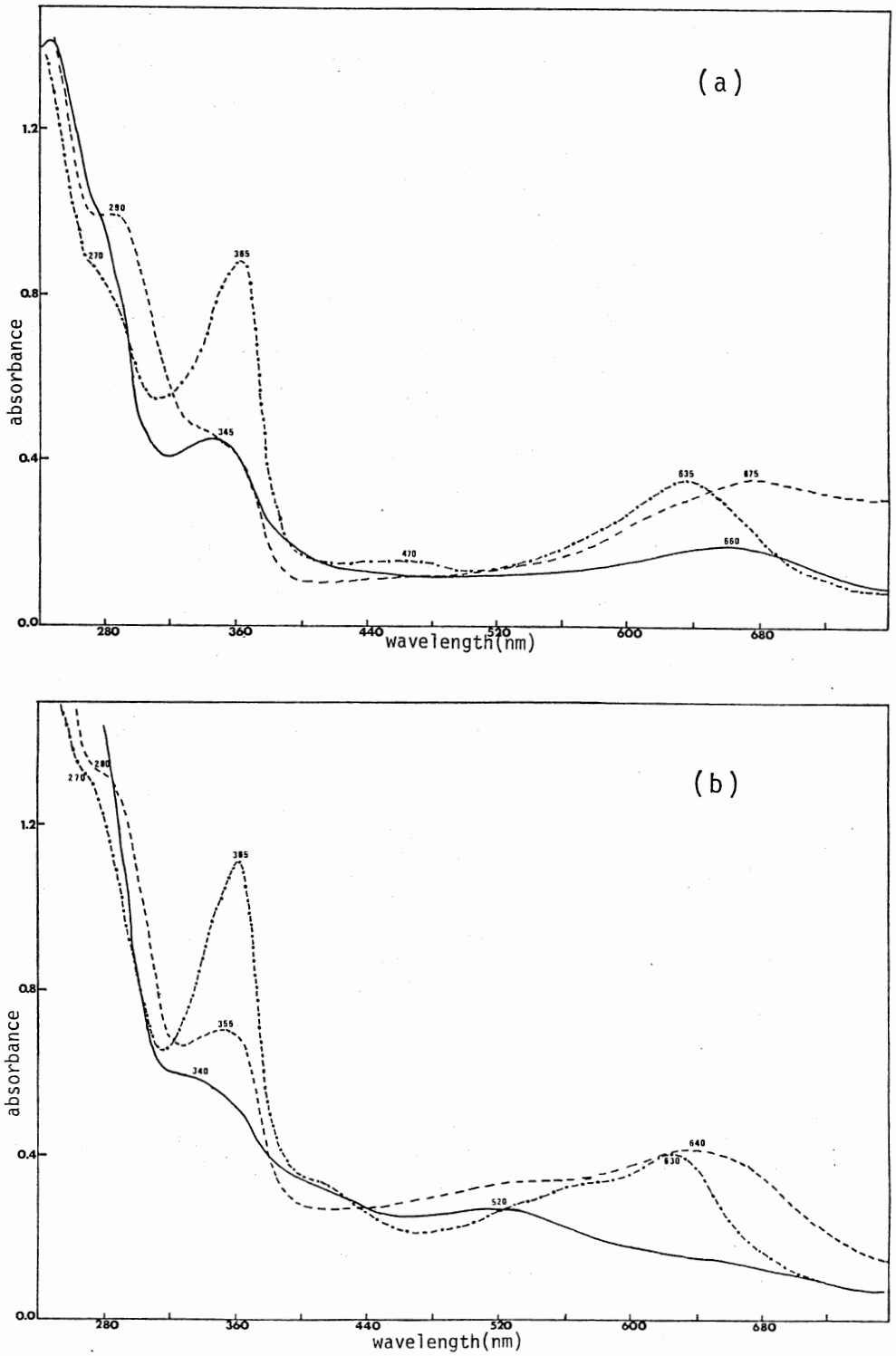
(a) Immediately After Mixing (b) 15 Minutes Later

Figure 20. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-·-·-) with Marquis Reagent



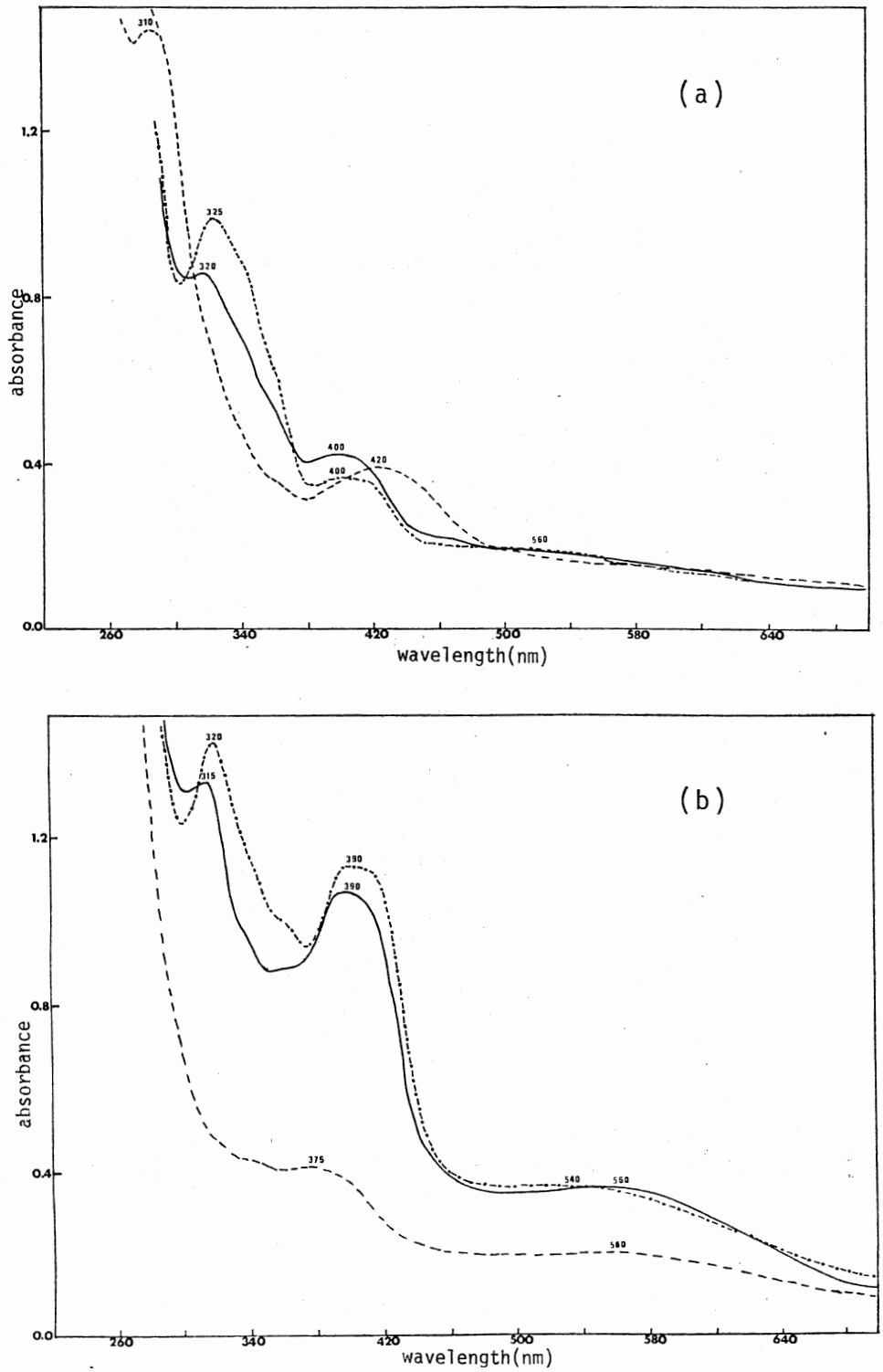
a) Immediately After Mixing b) 15 Minutes Later

Figure 21. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) with Frohde Reagent



a) Immediately After Mixing b) 15 Minutes Later

Figure 22. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mecke Reagent



a) Immediately After Mixing b) 15 Minutes Later

Figure 23. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mandelin Reagent

TABLE VII

UV-VISIBLE DATA OF DRUG-COLOR REACTIONS, IMMEDIATELY AFTER MIXING

Reagents	λ_{max} of mixtures		
	morphine sulfate	codeine	heroin HCl
Marquis	280, 330-345^{sp}, 565, 690	290, 330-335^{sp}, 575, 730	280, 330^{sh}, 350, 565, 690^{sh}
Frohde	480, 540^{sh}	390, 590	345, 480, 540^{sh}
Mecke	280^{sh}, 345, 660	290^{sh}, 350^{sh}, 675	270^{sh}, 365 635
Mandelin	320, 400, 560^{sh}	310, 420	325, 400, 560

Note: sh = shoulder, b = broad, sp = split

TABLE VIII

UV-VISIBLE DATA OF DRUG-COLOR REACTIONS,
AFTER 15 MINUTES

Reagents	λ_{max} of mixtures		
	morphine sulfate	codeine	heroin HCl
Marquis	285, 330-345^{sp}, 410, 575	410, 580	285, 350, 410, 575
Frohde	400^{sh}, 470, 610	390, 590	400^{sh}, 470, 610
Mecke	280^{sh}, 340, 520^{b}	280^{sh}, 355 520^{sh}, 640	270^{sh}, 365 560^{sh}, 630
Mandelin	315, 390, 560	375, 560	320, 390, 540

Note: sh = shoulder, b = broad, sp = split

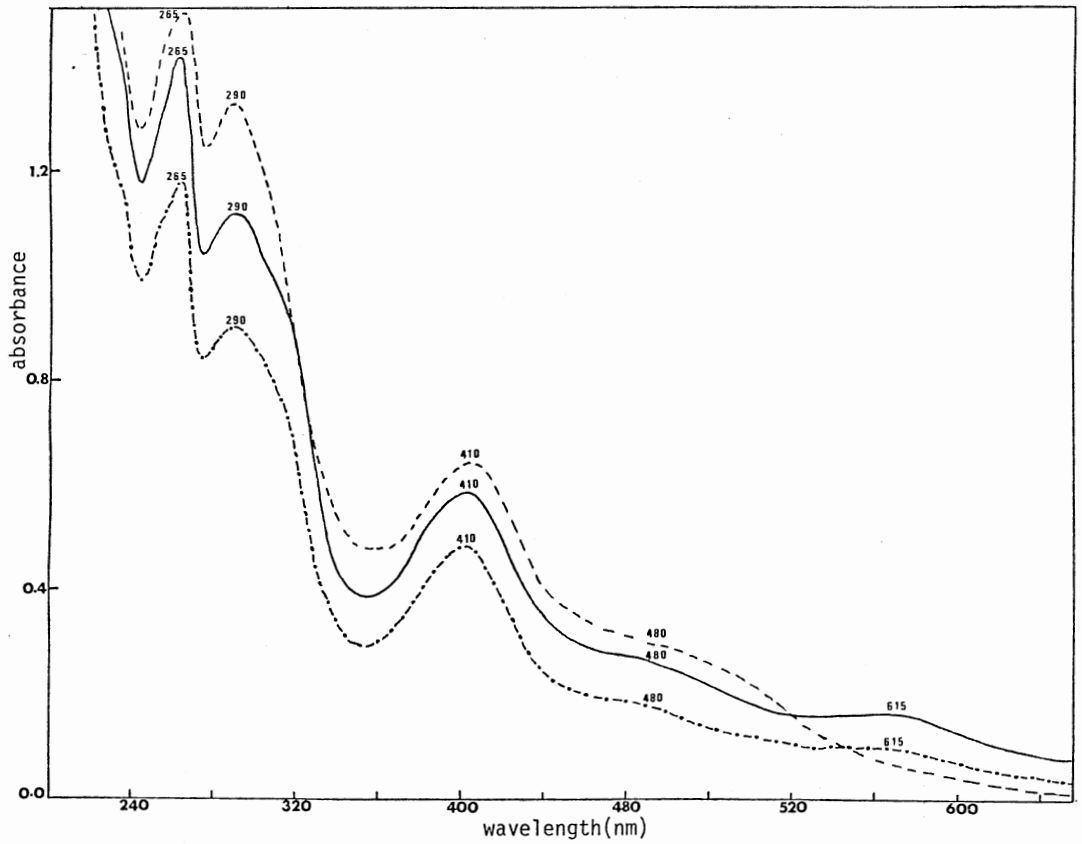


Figure 24. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Marquis Reagent Recorded After 24 Hours

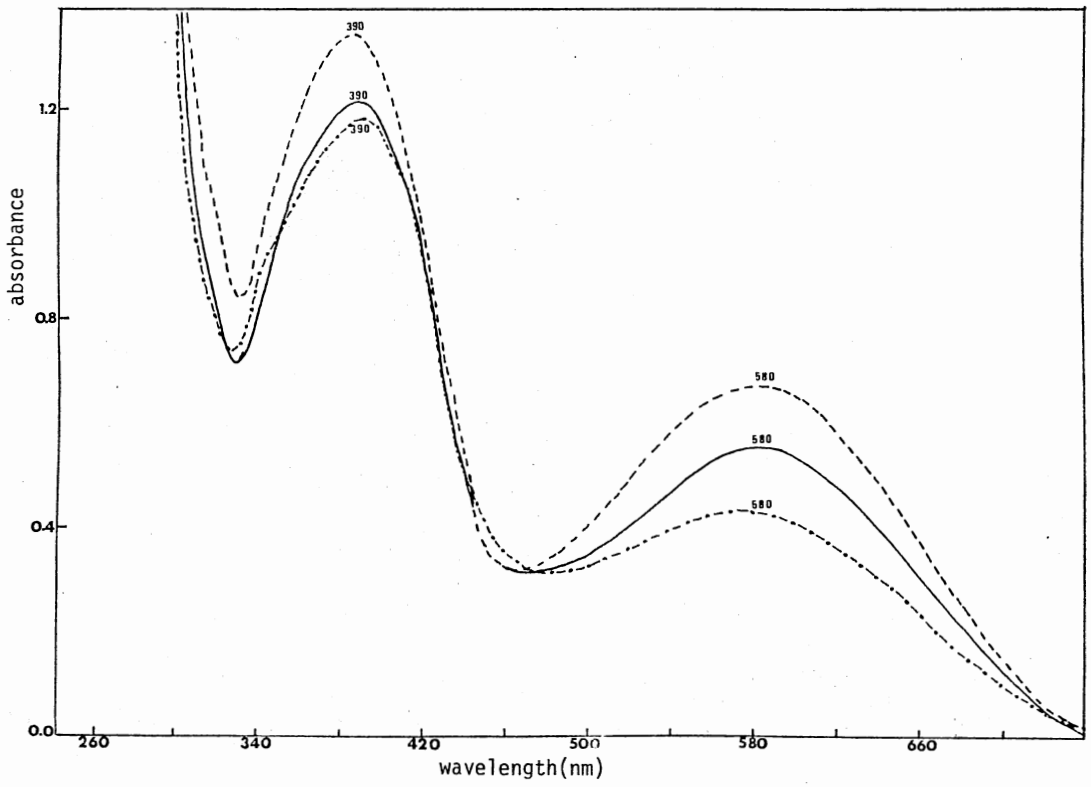


Figure 25. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Frohde Reagent Recorded After 24 Hours

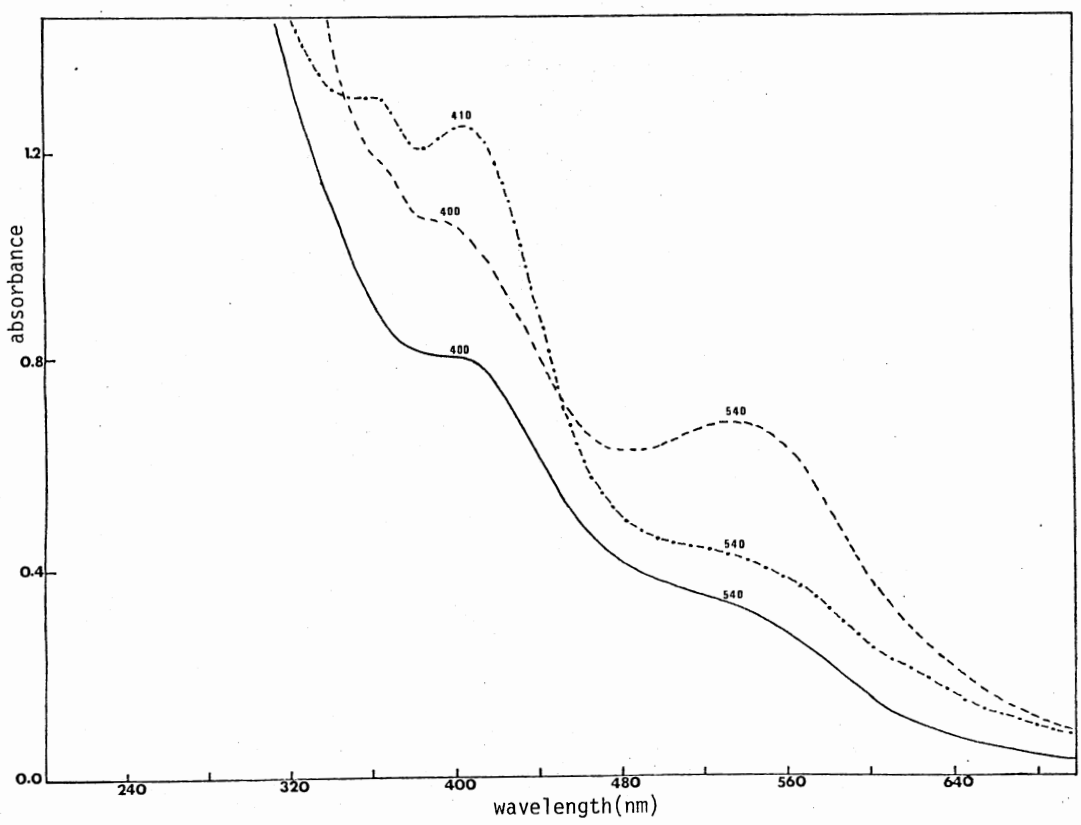


Figure 26. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mecke Reagent Recorded After 24 Hours

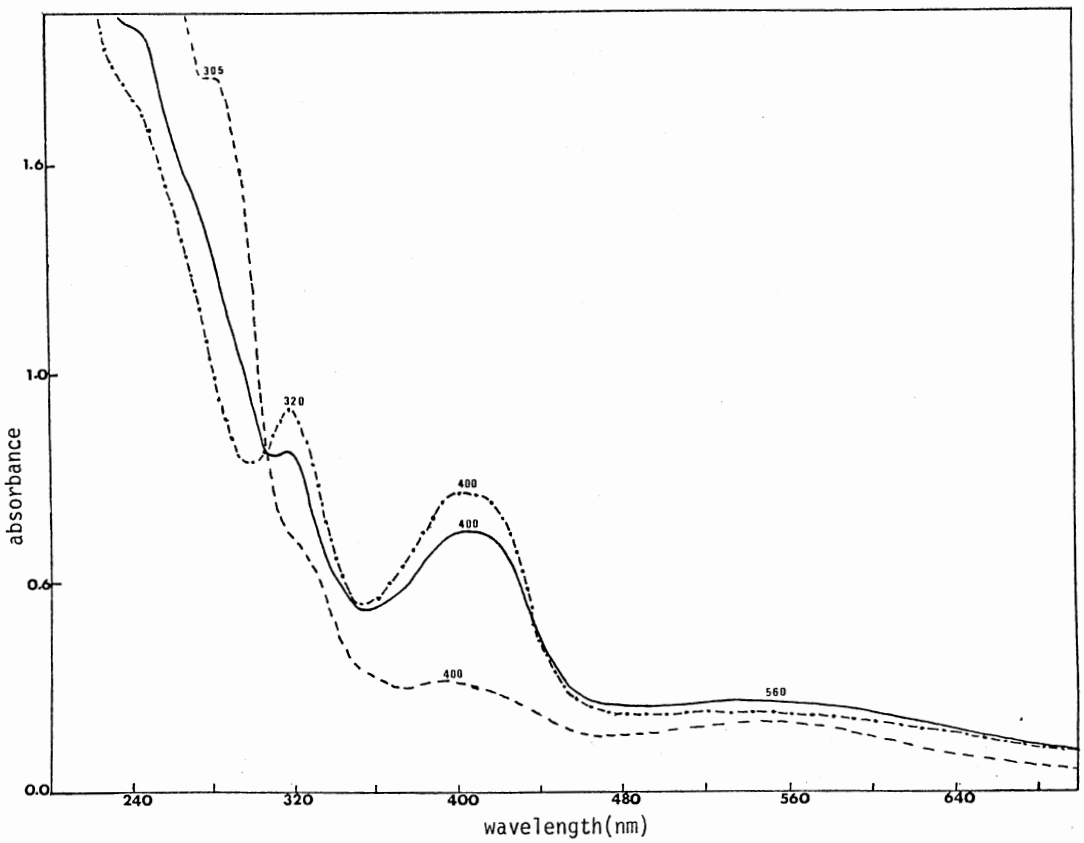


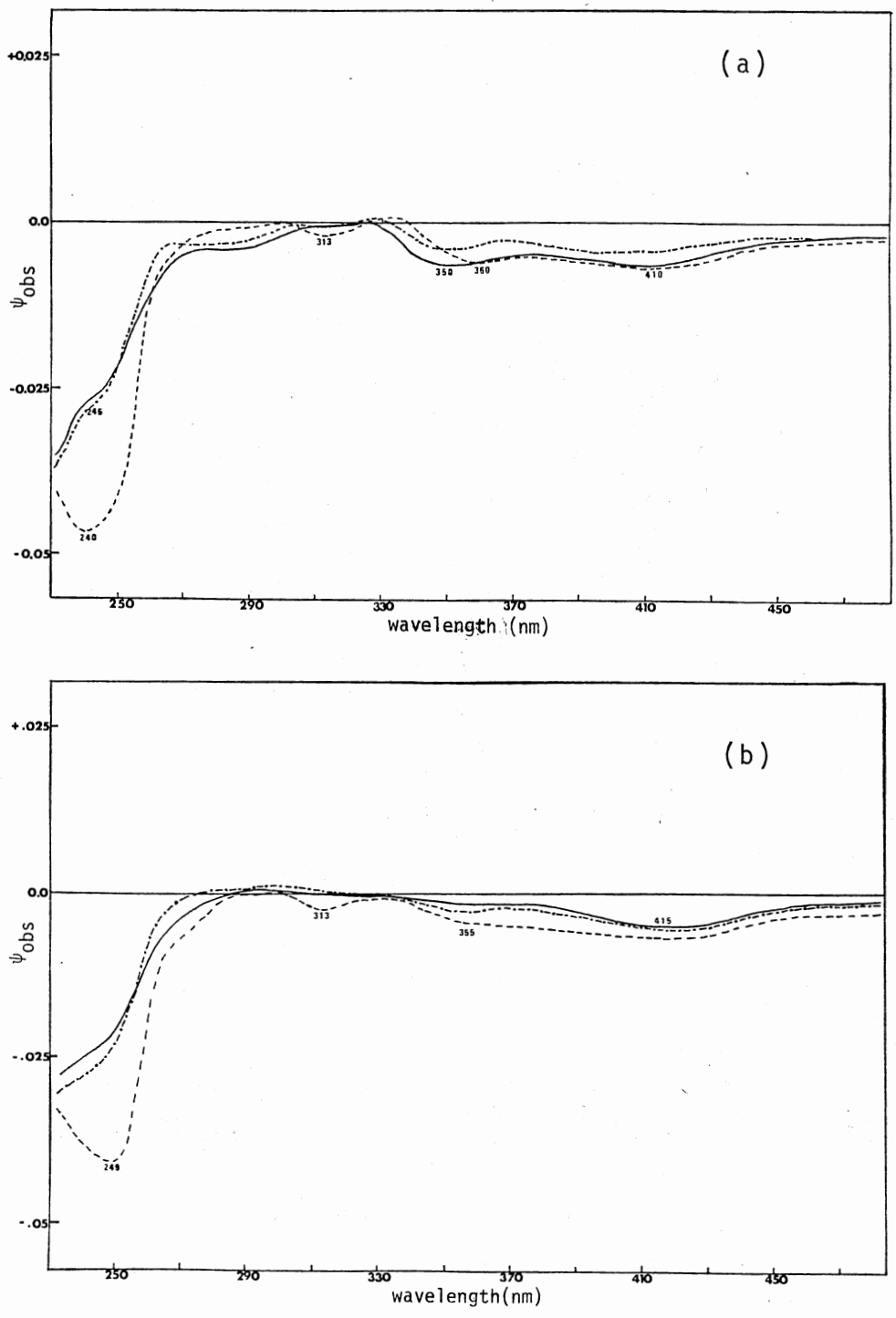
Figure 27. UV-Visible Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mandelin Reagent Recorded After 24 Hours

TABLE IX

UV-VISIBLE DATA OF DRUG-COLOR REACTIONS, AFTER 24 HOURS

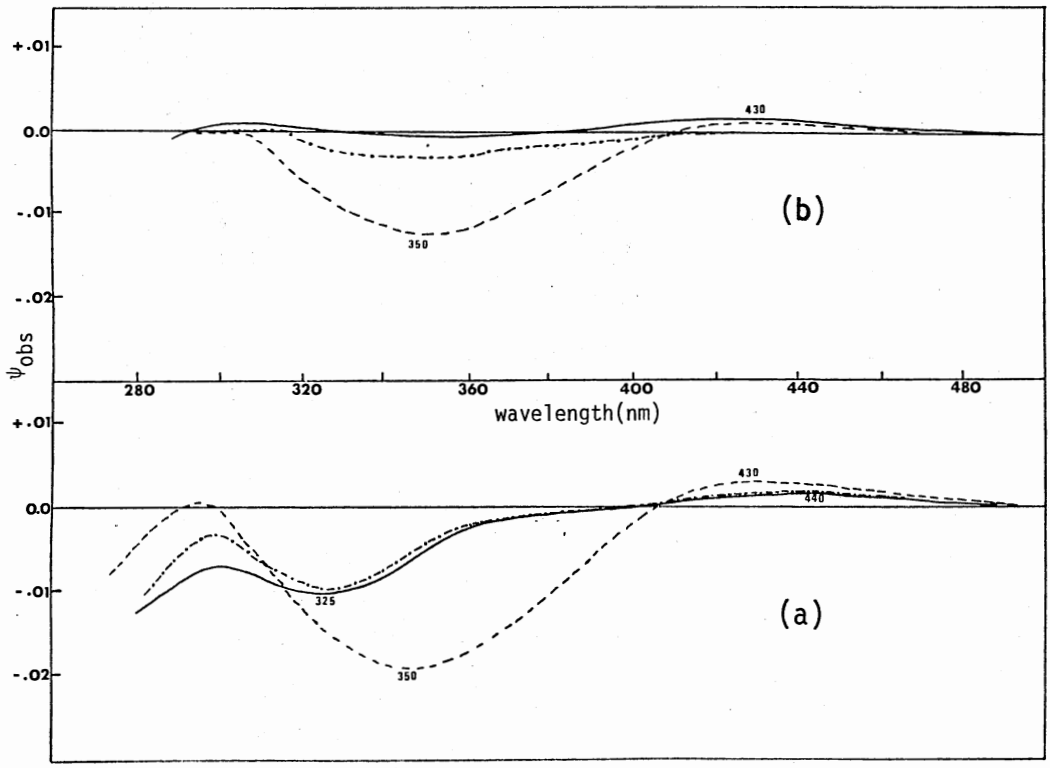
Reagents	λ_{max} of mixtures		
	morphine sulfate	codeine	heroin HCl
Marquis	265, 290, 410, 480^{sh}, 615^{b}	265, 290, 410, 480^{sh}	265, 290,410, 480^{sh}, 615^{b}
Frohde	390, 580	390, 580	390, 580
Mecke	400^{b}, 540^{b}	400^{b}, 540	410, 540^{b}
Mandelin	265^{sh}, 320, 400 560^{b}	305^{sh}, 320^{sh}, 560^{b}	260^{sh}, 320, 400, 560^{b}

Note: sh = shoulder, b = broad, sp = split



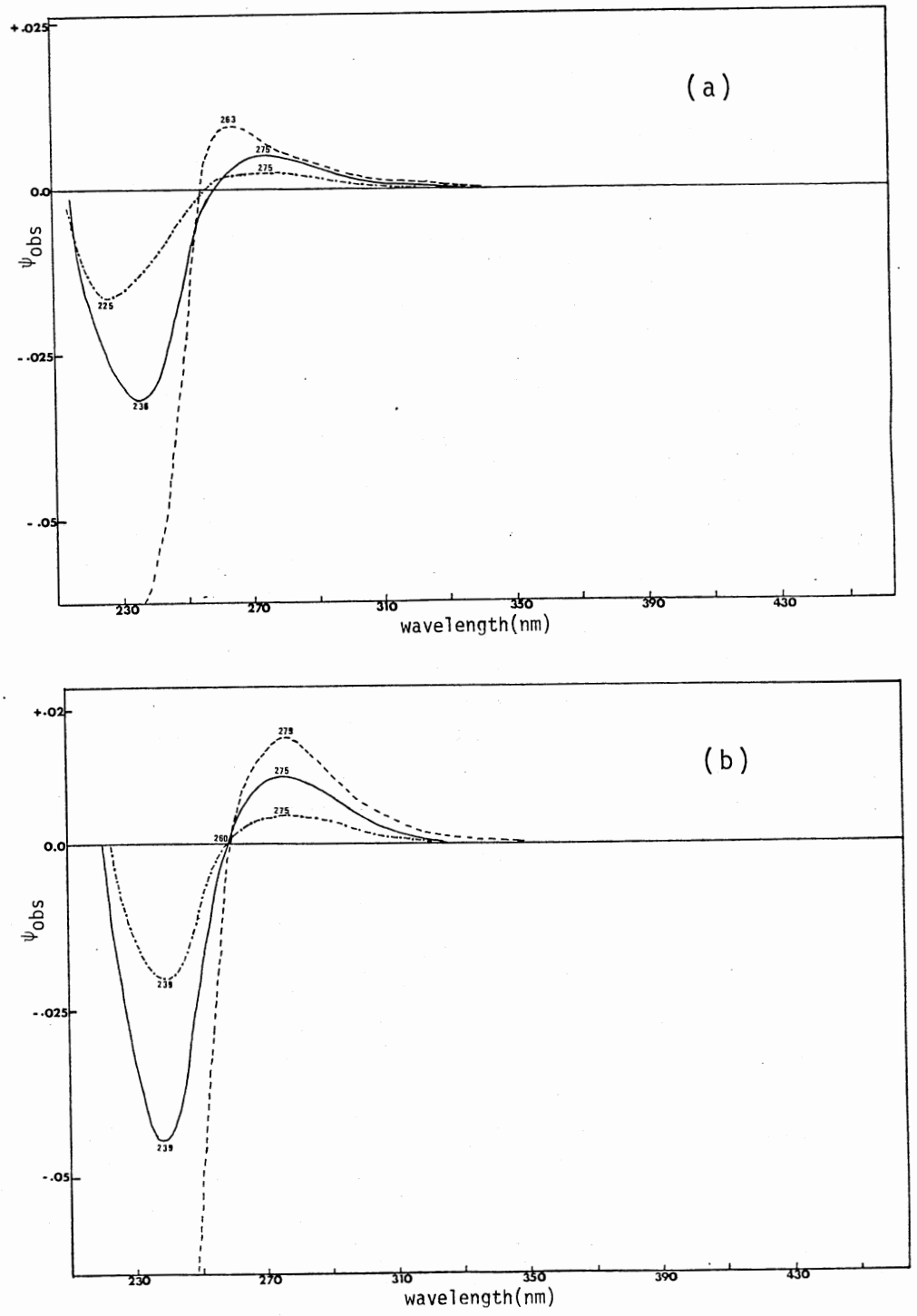
(a) Immediately After Mixing (b) 15 Minutes Later

Figure 28. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Marquis Reagent



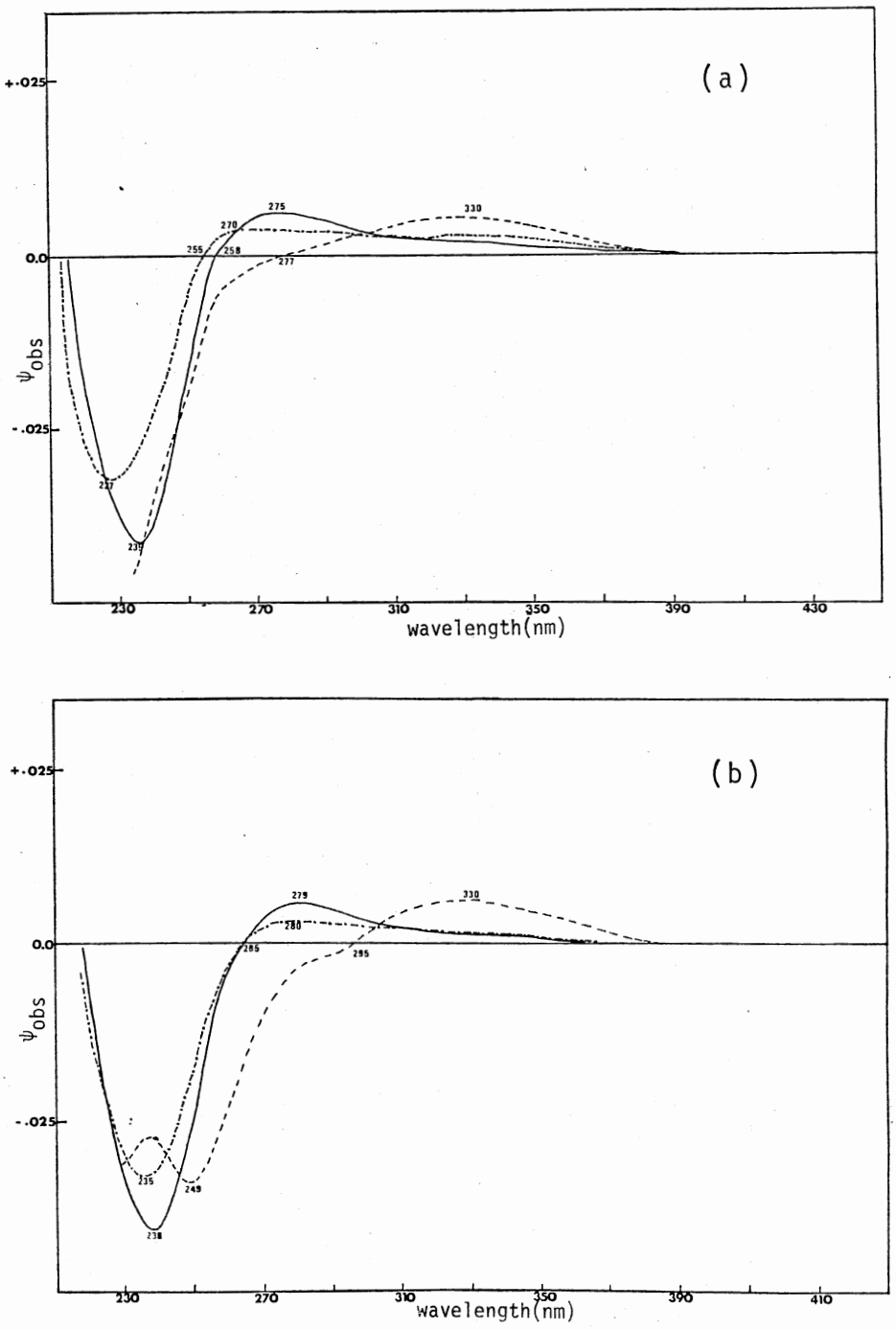
a) Immediately After Mixing b) 15 Minutes Later

Figure 29. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Erdman Reagent



a) Immediately After Mixing b) 15 Minutes Later

Figure 30. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•) With Mandelin Reagent



a) Immediately After Mixing b) 15 Minutes Later

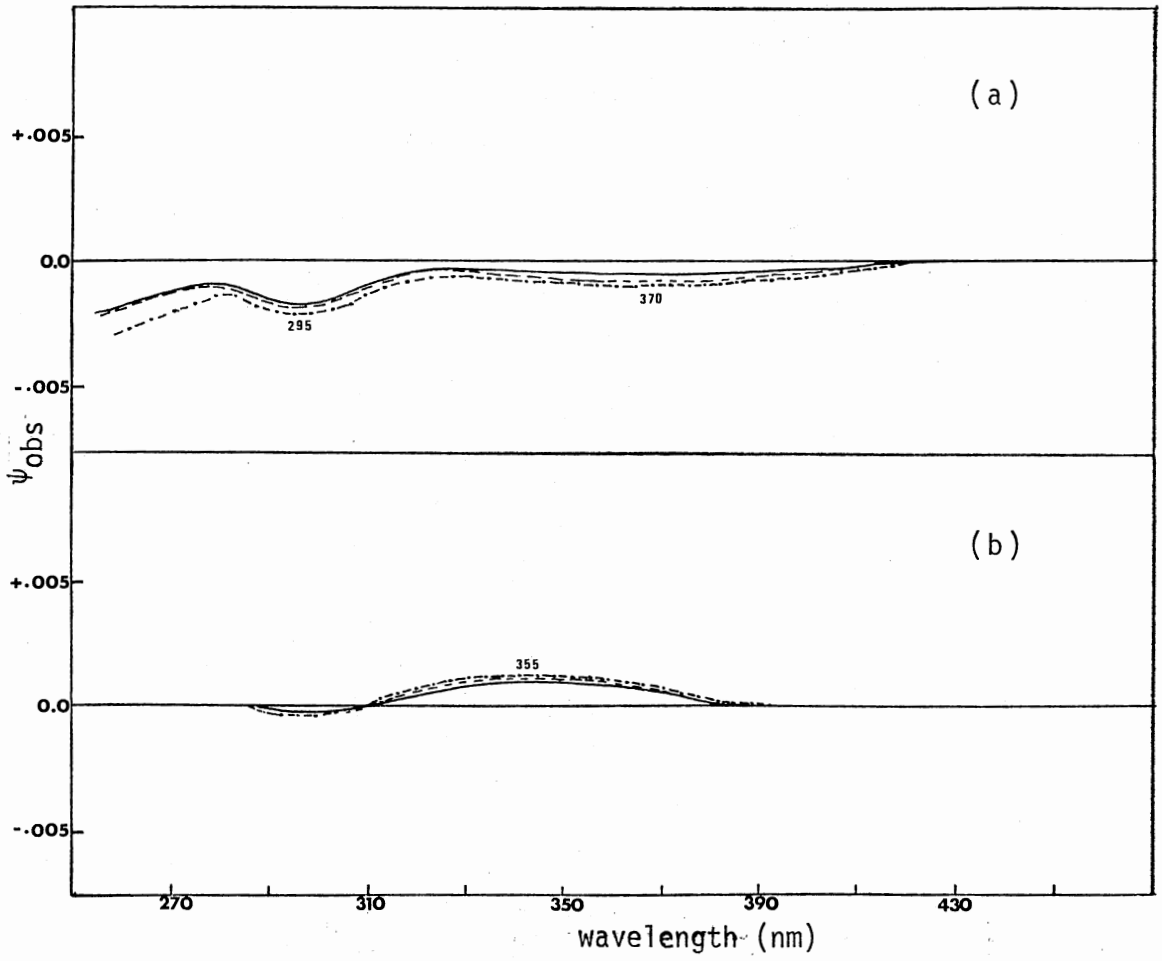
Figure 31. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•) With Mecke Reagent

band which occur at wavelengths below 300 nm. Shifts in CD maxima and decreases or increases in degrees of ellipticity were observed when the spectra were collected after different time intervals. Morphine sulfate and heroin HCl again show the same trends in CD spectral variations with time.

The spectra recorded after one night for the product solutions on drugs reactions with Marquis and Erdman reagents which do not contain metal ions, show decreases in the observed ellipticity when compared to the spectra obtained within a short period of time after mixing. The final spectra consist of weak and very broad CD bands (Figure 32). On the other hand, the CD spectra obtained when reagents which contain metal ions (Mandelin, Mecke) are used are completely different. The negative CD bands in the range of 230-260 nm and the positive CD bands at wavelengths around 300-350 nm (Figure 33, 34) are sharp and intense. As was observed in the UV studies the CD spectra of the products measured after one night were similar for all three drugs with any one color reagent. CD data on color reactions of drugs after different periods of time are summaried and presented in Table X, XI, XII.

ESR Measurements

The same color test reagents listed in Table III were used as reagents with solid forms of the drugs for ESR studies. To follow the time dependence of the color change



(a) Marquis Reagent (b) Erdman Reagent, Recorded After 24 Hours

Figure 32. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•)

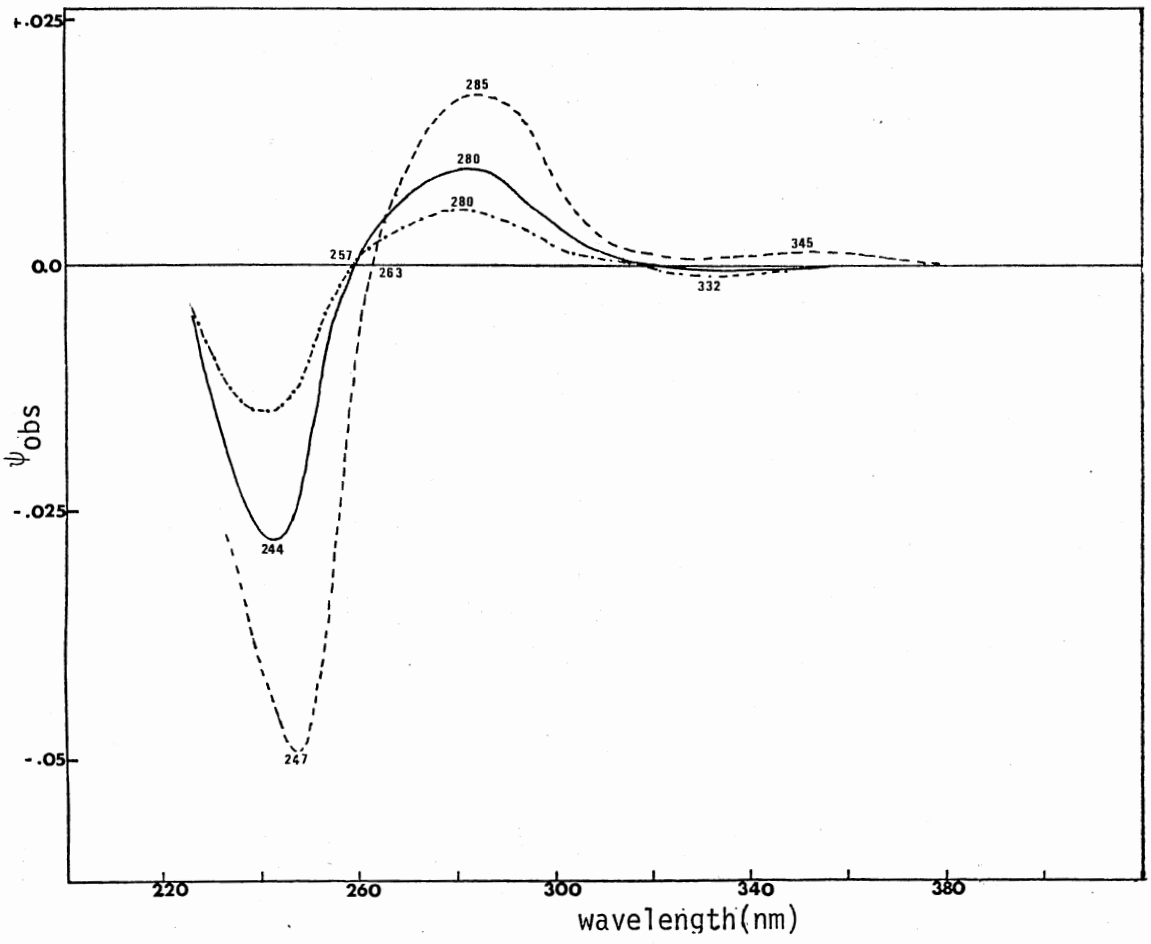


Figure 33. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mandelin Reagent, After 24 Hours

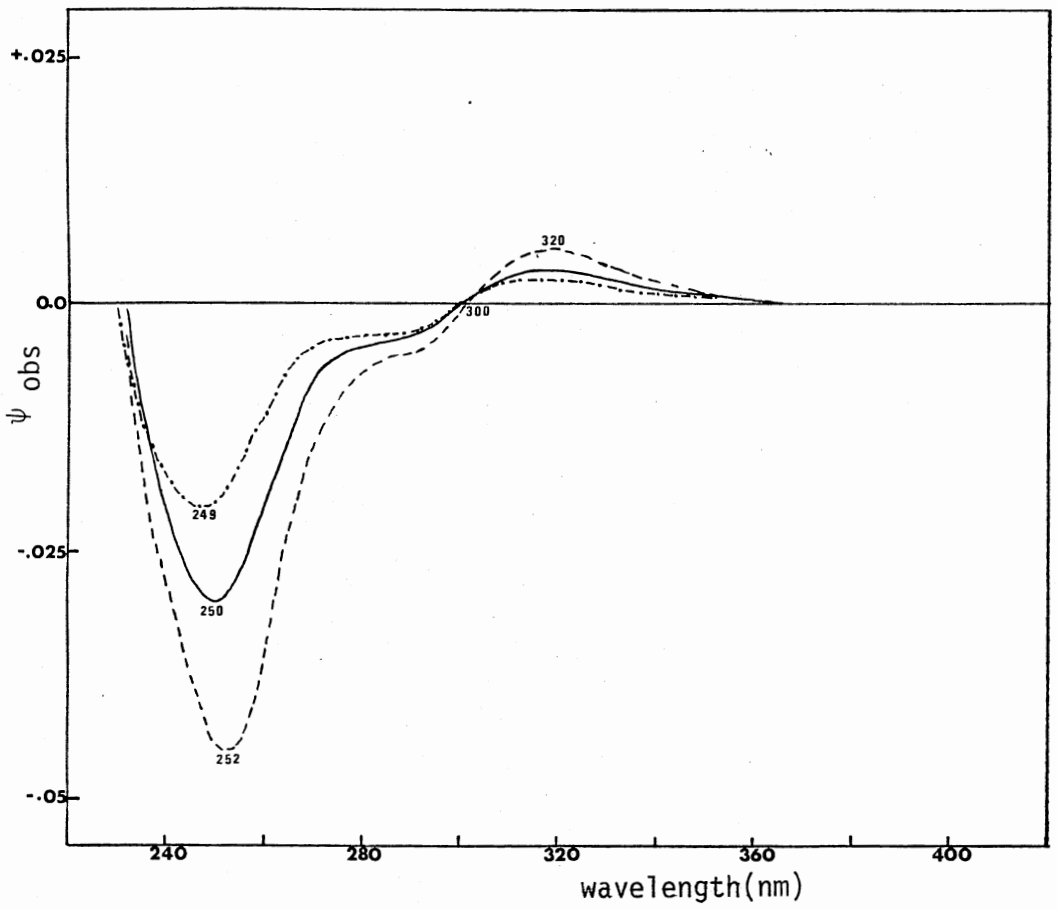


Figure 34. CD Spectra of Morphine Sulfate (——), Codeine (---), and Heroin HCl (-•-•-) With Mecke Reagent, After 24 Hours

TABLE X

CD DATA OF DRUG - COLOR REACTIONS, IMMEDIATELY AFTER MIXING

REAGENTS	CD maxima ($^{+}\lambda_{max}$, $^{-}\lambda_{max}$, λ_o)								
	morphine sulfate			codeine			heroin.HCl		
	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_o	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_o	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_o
Marquis	-	350 410	-	-	240 313 360 410	-	-	350 410	-
Erdman	440	325	400	430	350	405	440	325	400
Mandelin	275	236	257	263	235	256	275	225	258
Mecke	275	235	252	330	225	277	270	227	250

TABLE XI

CD DATA OF DRUG - COLOR REACTIONS, AFTER 15 MINUTES

REGENTS	CD maxima ($^{+}\lambda_{max}$, $^{-}\lambda_{max}$, λ_{o})								
	morphine sulfate			codeine			heroin.HCl		
	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}
Marquis	-	355 415	-	-	415 313 355 249	-	-	355 415	-
Erdman	440	350	380	440	350	410	-	350	410
Mandelin	275	239	258	279	243	262	275	239	259
Mecke	279	238	265	330	249	295	280	235	265

TABLE XII

CD DATA OF DRUG - COLOR REACTIONS, AFTER 24 HOURS

REAGENTS	CD maxima ($^{+}\lambda_{max}$, $^{-}\lambda_{max}$, λ_{o})								
	morphine sulfate			codeine			heroin.HCl		
	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}	$^{+}\lambda_{max}$	$^{-}\lambda_{max}$	λ_{o}
Marquis	-	295 370	-	-	295 370	-	-	295 370	-
Erdman	355	295	-	355	295	-	355	295	-
Mandelin	280	244 332	257	285 345	247	263	280	244 332	257
Mecke	320	250	300	320	252	300	320	249	300

was a little more difficult because 10 to 15 minutes was required to complete spectral measurements after a more significant delay in setting up the sample for observation. Immediately after mixing was probably 5 minutes later.

ESR spectra obtained from color reactions of morphine sulfate and heroin.HCl with any color test reagent are similar in terms of the pattern of line splitting and the total number of lines. Codeine by comparison has unique splitting patterns and a different number of resonance lines (Figure 35, 36, 37, 38, 39).

In order to calculate the g-factor and hyperfine splitting constant of radicals in the colored mixtures, the resonance magnetic field strength and microwave frequency at the center of each line must be accurately measured. Those values vaired from sample to sample.

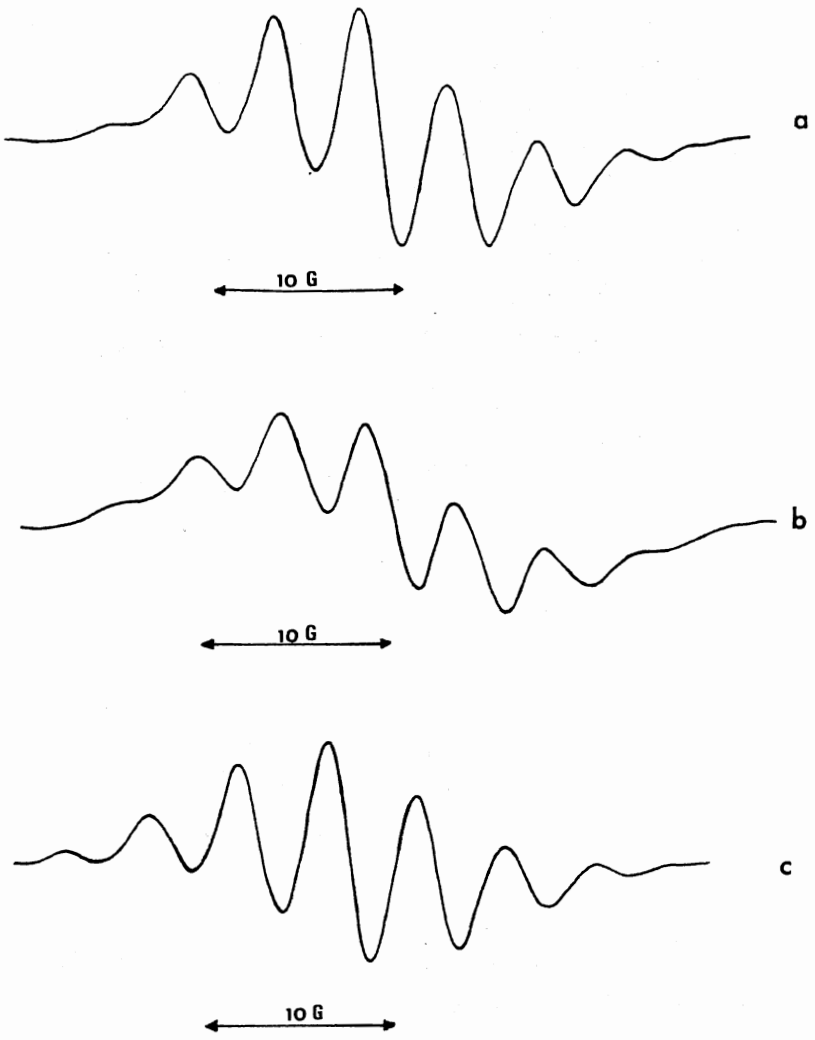
When the magnetic field strength and microwave frequency at the sample are known, one can calculate the g-factor value of each sample directly from the relationship:

$$g = \frac{h \nu_{res}}{\beta H_{res}} \qquad (4.21)$$

where ν_{res} = resonance microwave frequency (GHz), and

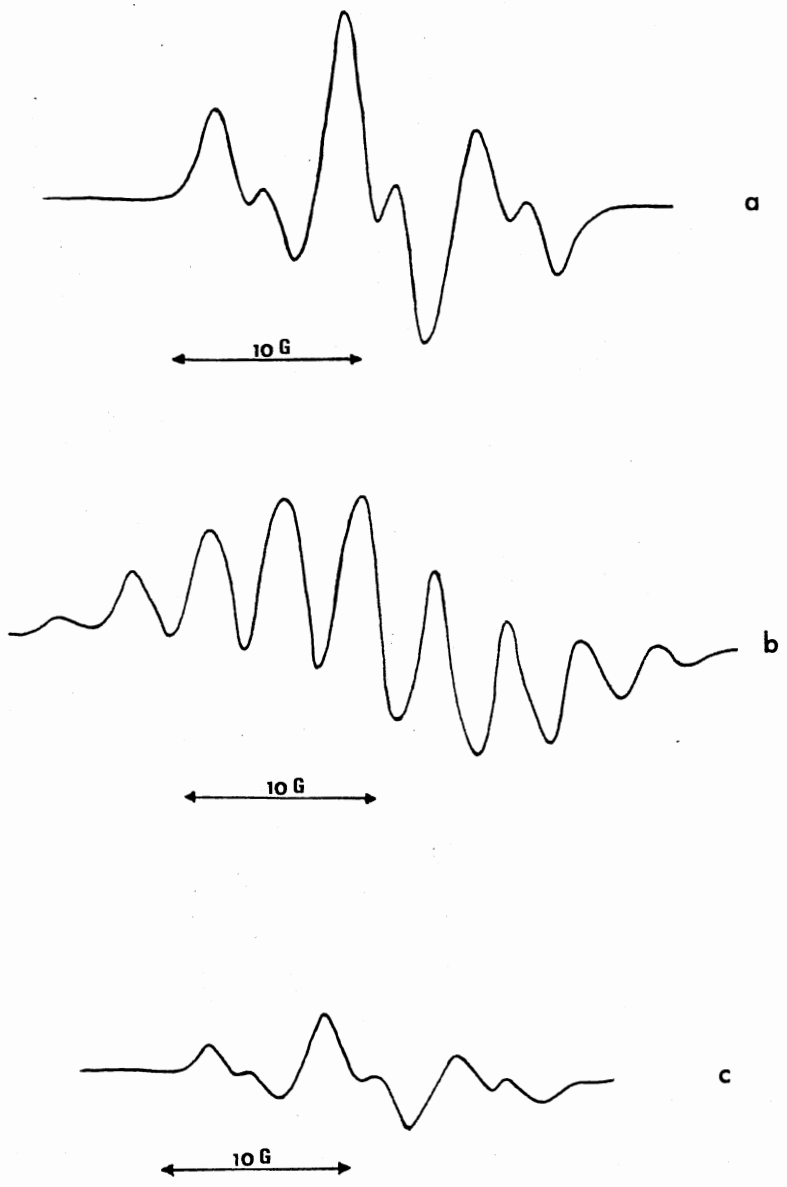
H_{res} = resonance magnetic field (G).

In this work the magnetic field strengths were measured at the NMR probe which was placed close to the sample cavity. Therefore the measured magnetic field (H_{mea}) has to be corrected by comparing those values with the magnetic field strength measured directly at the sample cavity



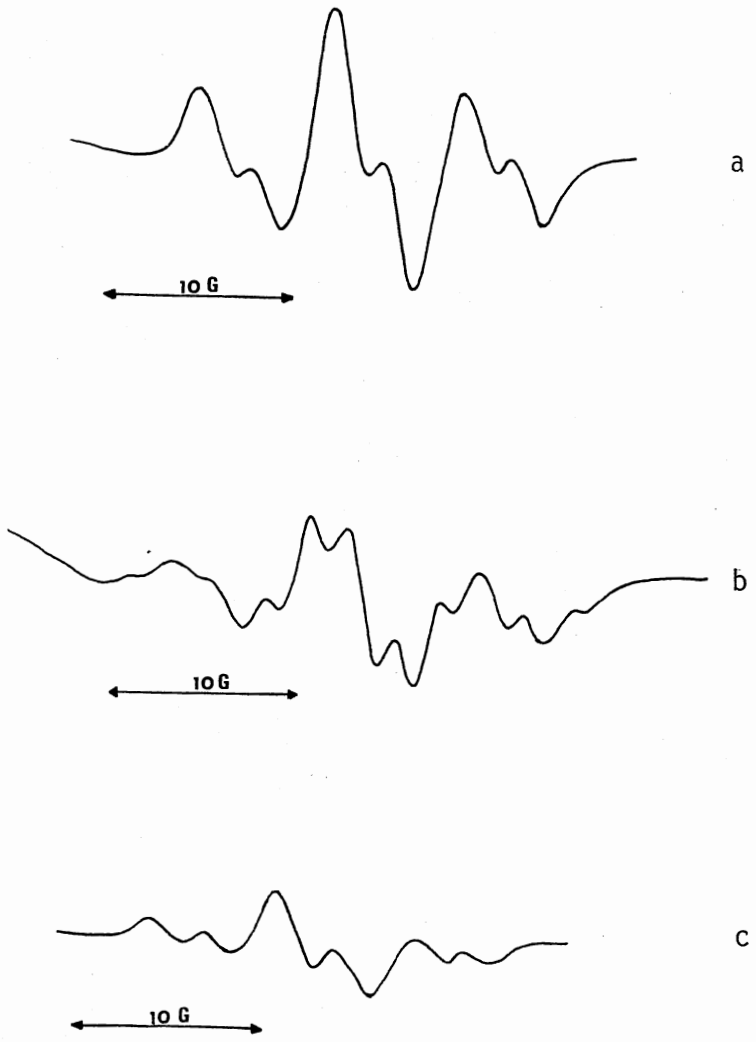
a) Morphine Sulfate b) codeine c) Heroin.HCl

Figure 35. ESR Spectra of Drugs - Marquis Color Reactions



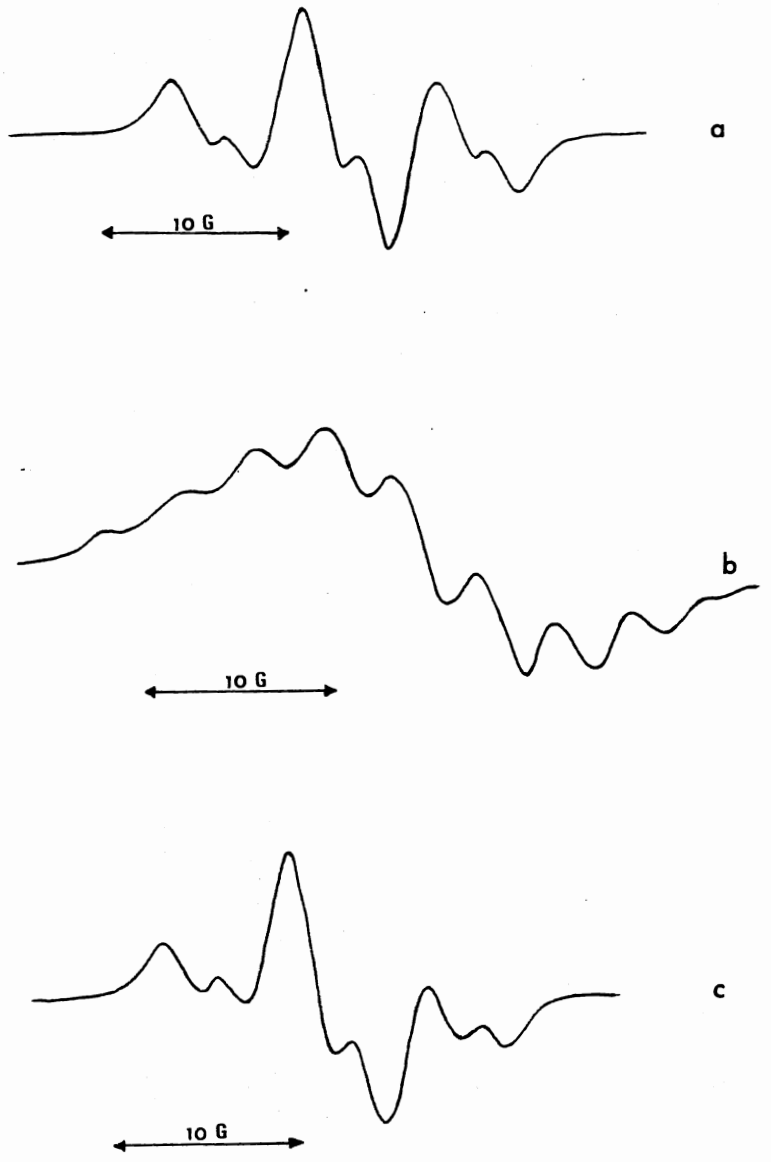
a) Morphine Sulfate b) codeine c) Heroin.HCl

Figure 36. ESR Spectra of Drugs - Erdman Color Reactions



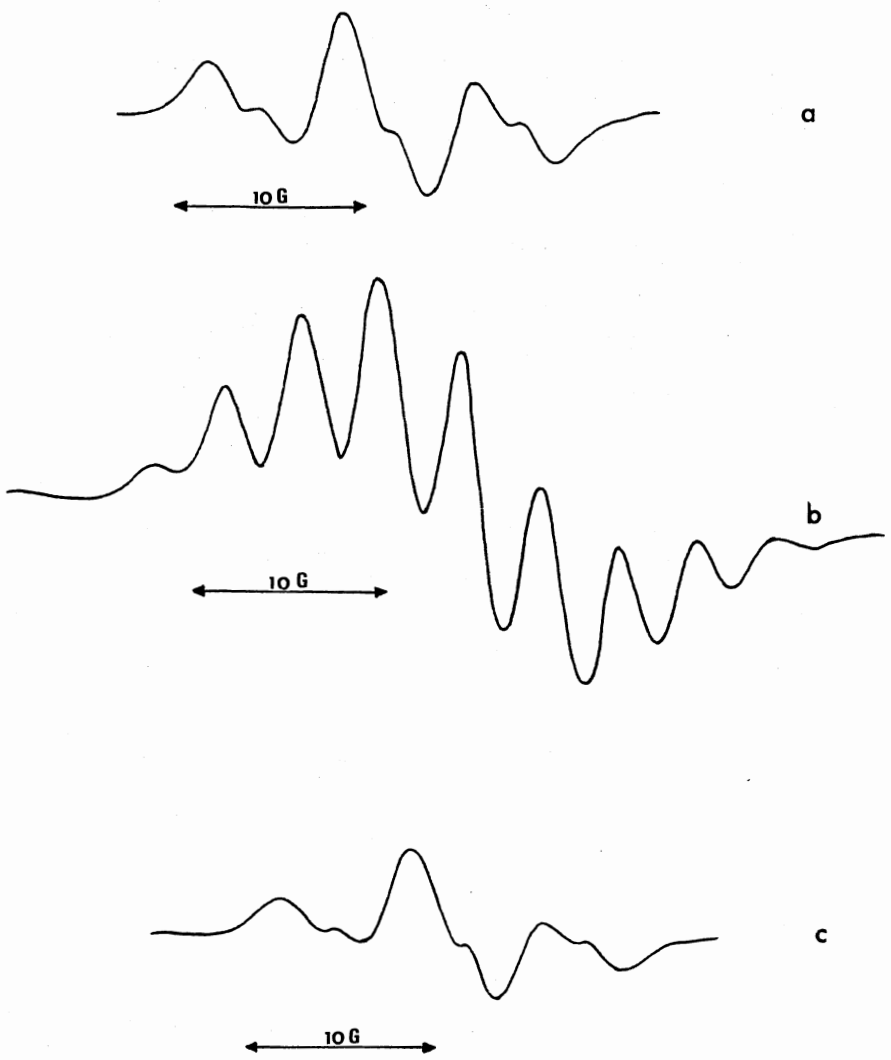
a) Morphine Sulfate b) codeine c) Heroin.HCl

Figure 37. ESR Spectra of Drugs - Mecke Color Reactions



a) Morphine Sulfate b) codeine c) Heroin.HCl

Figure 38. ESR Spectra of Drugs - Mandelin Color Reactions



a) Morphine Sulfate b) codeine c) Heroin.HCl

Figure 39. ESR Spectra of Drugs - Frohde Color Reactions

containing a standard compound (chromium doped MgO crystal) in which the g-value is accruately known and is equal to 1.9799 (g_{std}).

Since the g-value of the standard compound is constant and the microwave frequency at resonance is known, the calculated magnetic field strength of the standard compound (H_{std}) can be computed from the equation:

$$H_{std} = \frac{h \quad \nu_{res} \quad X\ 1000}{\beta \quad g_{std}} \qquad (4.22)$$

For each ESR experiment the measured magnetic field (H_{mea}) of the standard compound showed a small deviation from the value calculated from equation 4.22. This deviation (ΔH_d) can be expressed as follow:

$$\Delta H_d = H_{std} - H_{mea} \qquad (4.23)$$

Value of ΔH_d is used as the correction factor for all magnetic field strengths measured in each spectrum. ΔH_d can be either a negative or a positive number. In order to obtain the corrected magnetic field strength (H_{corr}), ΔH_d is added to the measured magnetic field, that is

$$H_{corr} = H_{mea} + \Delta H_d \qquad (4.24)$$

After the magnetic field is corrected and the microwave frequency is known, the g- value for each sample can be calculated from equation 4.21.

ESR spectra of these drug-color reaction products

consist of a set of lines with unequal intensity. The magnetic field separation between any two successive lines in a spectrum is the value of the hyperfine splitting constant (a). This value varies from spectrum to spectrum depending on the pattern of the line splitting.

The ESR spectra of the products from the color reactions of the three drugs with Marquis reagent consist of seven equally spaced lines (Figure 35). The ratios of the line intensities are 1:6:15:20:15:6:1. There is only one value for the hyperfine splitting constant for each spectrum. For other color reactions, the spectra obtained from morphine sulfate and heroin.HCl consist of three principle lines with 1:2:1 ratio in line intensities, then each line is split into a doublet resulting in a total of six lines. In this case, there are two different values for the hyperfine splitting constants, one is the field separatin between the two lines in a triplet set, and the other results from the field separation between the two lines in a doublet.

Spectra obtained from the color reactions of codeine free base with the color test reagents were recorded at low temperature (10°C). Each spectrum consists of nine equally spaced lines. At higher temperature the spectrum is changed to consist of nine lines with unequal spacing, i.e. a triplet of triplet splittings.

Color reactions of drugs with Mecke and Frohde reagents produced ESR spectra which are different from the others. The spectra obtained from Mecke-drug reactions show a

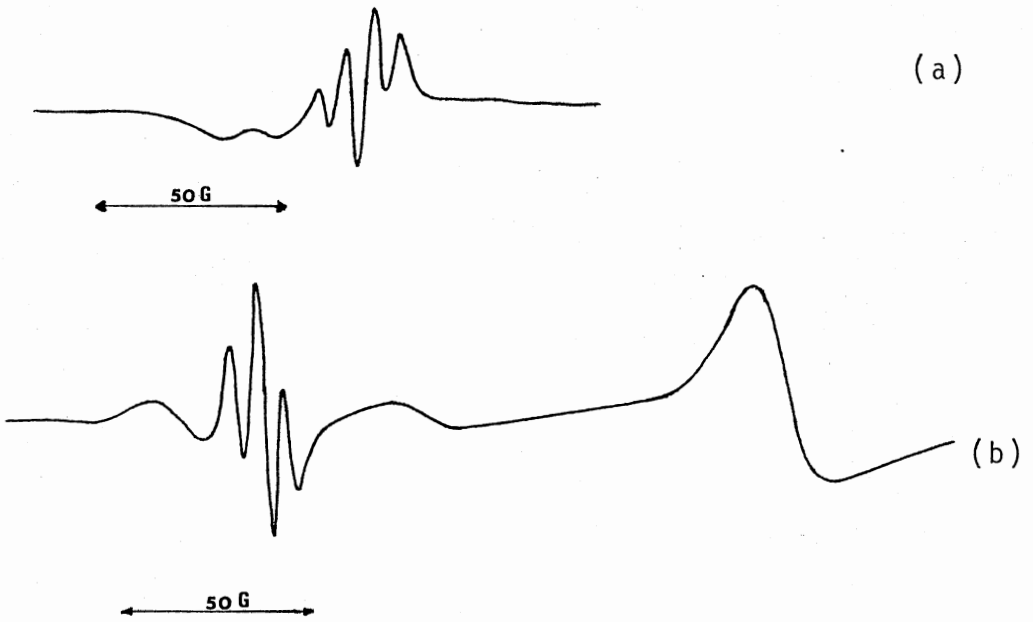
broad band signal at lower magnetic field and a well resolved signal at higher mangetic field (Figure 40a). From these the g-value was calculated. The spectra of the Frohde-drug reaction mixtures consist of two different signals, one is a resolved signal which appears at lower magnetic field strength and the other is a very intense signal which appears at an applied magnetic field of about 3400 gauss (Figure 40b). All the spectra for all colored mixtures change with time. Changes observed include a decrease in line intensity accompanied by line broadening.

The g-values calculated from the drug color reactions with the Marquis reagent are almost equal, but are different from those obtained for the reactions with other color test reagents. Small variations in g-values were observed among drugs with different color test reagents, other than the Marquis reagent

All data from ESR studies on color reactions of drugs are presented in Table XIII, XIV, XV.

It has been suggested in the literature (51) that apomorphine is a possible intermediate in the color reactions of morphine and derivatives. Therefore reactions of apomorphine in conc. H_2SO_4 and its reactions with the color reagents were also studied by ESR, UV-Visible and CD techniques in order to compare the results with those obtained from morphine color reactions.

ESR spectra of the colored mixtures obtained from apomorphine color reactions are structureless and of very low



a) Morphine Sulfate With Mecke Reagent b) Morphine Sulfate With Froehde Reagent

Figure 40. ESR Spectra of the Colored Mixtures

TABLE XIII

ESR DATA OF MORPHINE SULFATE - COLOR REACTIONS

REAGENTS	# lines	H_{res} (G)	ν_{res} (GHz)	g-factor	a_1 (G)	a_2 (G)
Marquis	7	3279.65	9.194662	2.0031 ± 0.0001	4.6 ± 0.2	-
Erdman	6	3278.94	9.194809	2.0036 ± 0.0001	7.0 ± 0.1	2.8 ± 0.1
Mecke	6	3258.99	9.138832	2.0036 ± 0.0001	7.0 ± 0.1	2.6 ± 0.1
Mandelin	6	3256.50	9.132682	2.0037 ± 0.0001	7.0 ± 0.1	2.3 ± 0.1
Frohde	6	3258.18	9.136098	2.0035 ± 0.0001	7.1 ± 0.2	2.4 ± 0.2

TABLE XIV

ESR DTA OF CODEINE - COLOR REACTIONS

REAGENTS	# lines	H_{res} (G)	ν_{res} (GHz)	g-factor	a_1 (G)	a_2 (G)
Marquis	7	3280.20	9.195934	2.0030 ± 0.0001	4.6 ± 0.1	-
Erdman	*9	3257.29	9.134652	2.0037 ± 0.0001	4.3 ± 0.2	-
	9	3258.58	9.138273	2.0037 ± 0.0001	4.8 ± 0.2	2.4± 0.2
Mecke	9	3258.87	9.138358	2.0035 ± 0.0001	4.6 ± 0.1	2.3 ± 0.1
Mandelin	*9	3258.83	9.136781	2.0037 ± 0.0001	4.3 ± 0.2	-
	9	3256.79	9.134078	2.0039 ± 0.0001	4.5 ± 0.2	2.3± 0.2
Frohde	*9	3256.70	9.132351	2.0035 ± 0.0001	4.2 ± 0.1	-

*means at 10 °C

TABLE XV

ESR DATA OF HEROIN.HCl - COLOR REACTIONS

REAGENTS	# lines	H_{res} (G)	ν_{res} (GHz)	g-factor	a_1 (G)	a_2 (G)
Marquis	7	3279.46	9.194018	2.0031 ± 0.0001	4.5 ± 0.1	-
Erdman	6	3258.59	9.137968	2.0036 ± 0.0001	7.1 ± 0.1	2.9 ± 0.1
Mecke	6	3257.41	9.134237	2.0035 ± 0.0001	7.0 ± 0.2	2.7 ± 0.2
Mandelin	6	3256.62	9.132848	2.0037 ± 0.0001	6.9 ± 0.1	2.4 ± 0.1
Frohde	6	3258.07	9.136278	2.0036 ± 0.0001	6.7 ± 0.2	2.6 ± 0.2

intensity and difficult to separate from the base line signals. The experiments were also conducted at 10°C to try to resovle the spectra but no change in the ESR signals was observed. This may result from either no radical having been produced in the apomoprhine reactions or the radical is formed but its life time is so short that the radical can not be detected.

The CD and UV-Visible spectra of freshly prepared apomorphine in conc. H_2SO_4 are similar to those for a morphine sulfate solution after 48 hours (Table XVI). At higher concentrations, freshly prepared solutions of morphine sulfate in conc. H_2SO_4 show a broad absorption band with maximum at 580 nm. This band is shifted to 510 nm after 48 hours. After one month, the product from the morphine reaction is dark green in color while the product from an apomorphine reaction is dark purple. The CD and UV-Visible spectra of these final solutions are different from each other (Table XVI). This is particularly obvious in the CD study where no CD spectrum is observed for the dark green solution of morphine.

CD spectra of freshly prepared colored mixtures of apomorphine with Erdman, Mandelin and Mecke reagents show a very close similarity to that of morphine sulfate colored mixtures obtained from the reactions with the same reagents after 24 hours (Table XVII, XVIII).

TABLE XVI

CD AND UV-VISIBLE DATA OF DRUGS IN CONC. H_2SO_4

SOLUTES	COLOR	CD maxima			UV-Visible absorption maxima
		$^{+}\lambda_{max}$	λ_o	$^{-}\lambda_{max}$	
*morphine sulfate	clear	280	260	242	275, 240, 212
*codeine	clear	285	264	246	280^{sh}, 252, 215
*heroin.HCl	clear	275	258	240	275, 240,210
apomorphine	clear	283	260	239	520^{b}, 370^{sh}, 275, 240
morphine sulfate (after 48 hours)	clear	283	260	242	510^{b}, 380^{sh}, 285, 249
morphine sulfate (after one month)	dark green	-	-	-	600, 385, 310^{sh}
apomorphine (after one month)	dark purple	285	258	233	540, 392, 275

* means dilute solution, b = broad, sh = shoulder

TABLE XVII

CD AND UV-VISIBLE DATA OF APOMORPHINE
WITH SOME COLOR TEST REAGENTS

REAGENTS	COLOR	CD Maxima $^{+}\lambda_{max}$	CD Maxima $^{-}\lambda_{max}$	CD Maxima λ_o	UV-Visible absorption maximum
Erdman	rose-brown	445	325	400	440, 310^{sh}, 265
Mandelin	clear	278	238	259	590^{sh}, 390^{sh}, 275, 240
Mecke	brown-green	325	240	300	560^{b}, 400, 355^{sh}

b = broad, sh = shoulder

TABLE XVIII

CD AND UV-VISIBLE DATA OF MORPHINE SULFATE
WITH SOME COLOR TEST REAGENTS

REAGENTS	CD Maxima $^{+}\lambda_{max}$	CD Maxima $^{-}\lambda_{max}$	CD Maxima λ_o	UV-Visible absorption maximum
Erdman	440	325	400	450, 310^{sh}, 265
Mandelin (after 24 hours)	280	244	257	560, 400, 275, 245^{sh}
Mecke	320	250	300	520, 400^{sh} 340^{sh}

b = broad, sh = shoulder

This is not the case for the UV-Visible spectra of those same colored mixtures (Table XVII and XVIII) which do not show as good a comparison as that observed for the CD data. After 24 hours, maxima in the CD spectra of the apomorphine-Erdman and apomorphine-Mecke colored mixtures have decreased in magnitude but the maximum ellipticity of the one obtained from the apomorphine-Mandelin reactions is constant with time when compared to that of the freshly prepared colored mixtures.

NMR Measurements

^{13}C NMR spectrum of morphine sulfate in D_2O shows the expected well resolved lines representative of the total number of carbon atoms in the molecule (Figure 41). The spectrum consists of 16 lines which have been assigned to the corresponding carbon atoms in the molecule (Table XIX). One line which should correspond to C(10) has not been observed in the spectrum. This may be caused by a very low intensity for the line, in which case it could not be separated from the noise level. This line should appear at about 20 ppm shifted from TMS (9).

The spectrum obtained from the solution of morphine sulfate in conc. H_2SO_4 (orange solution) (Figure 42a) is different from the previous spectrum, these differences being a higher noise level and more lines in the aromatic region. After one month this solution changed from an orange color to dark green. ^{13}C NMR spectrum of the dark

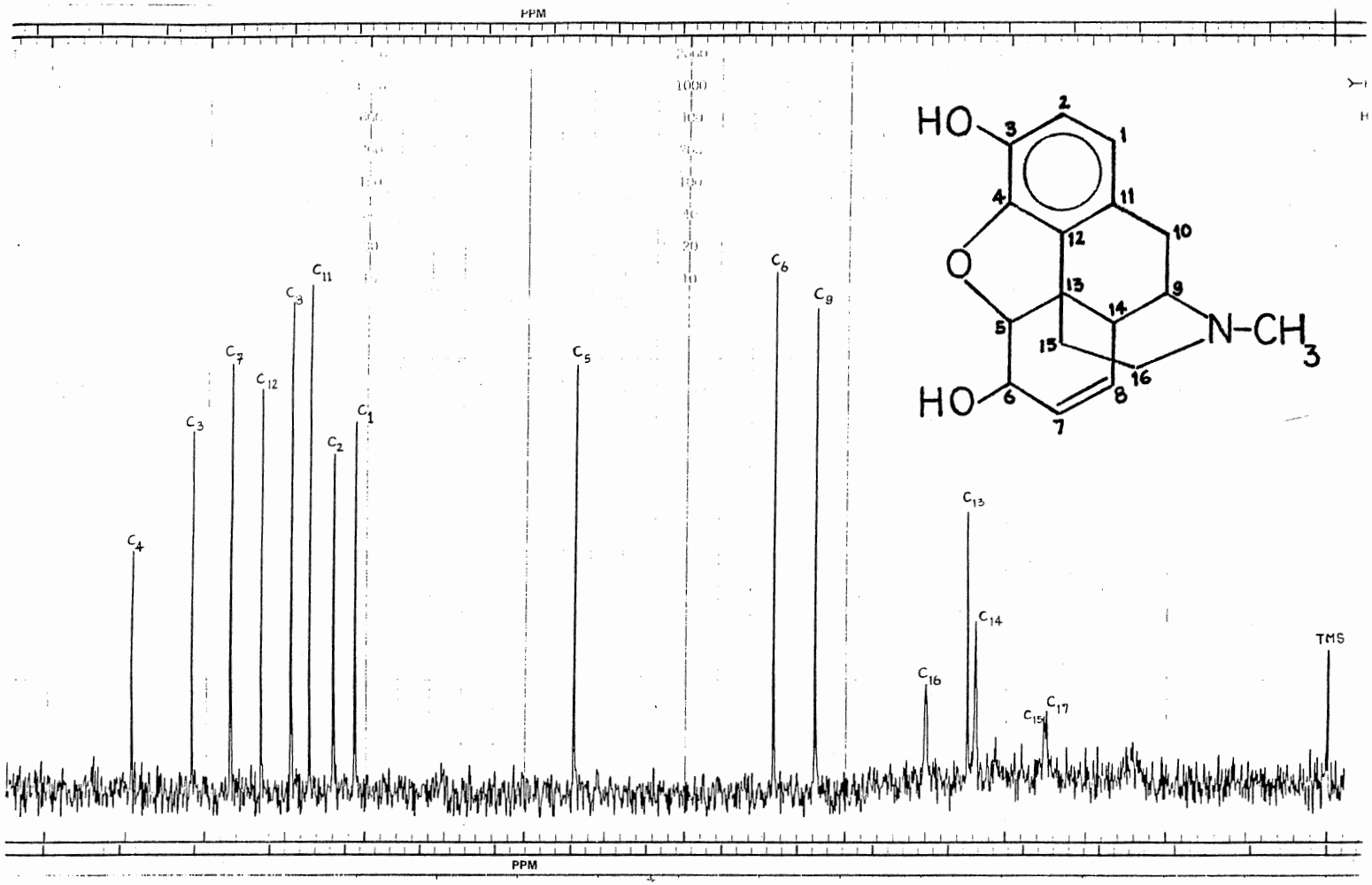
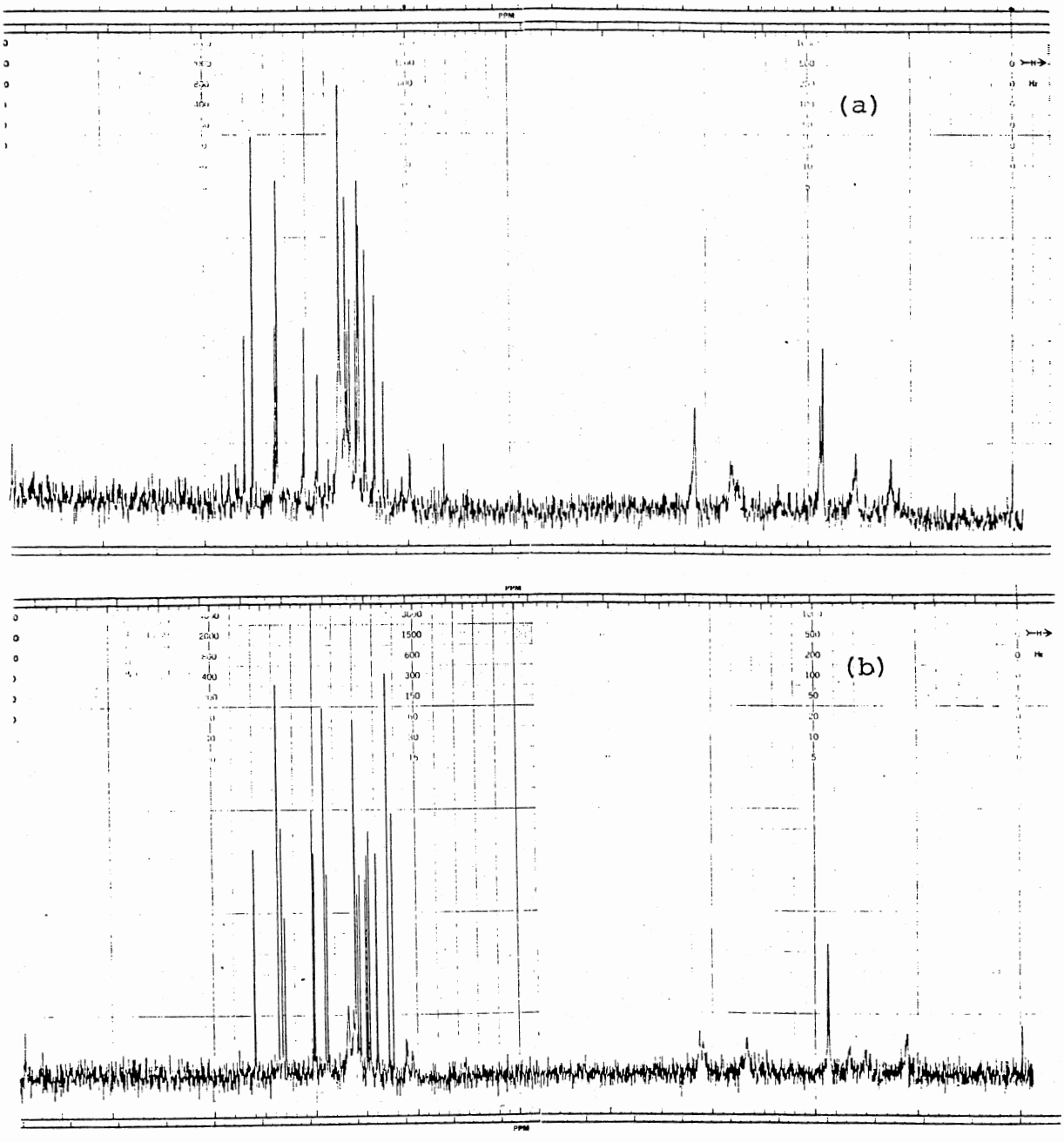


Figure 41. ^{13}C NMR Spectrum of Morphine Sulfate in D_2O



a) Freshly Prepared b) After One Month

Figure 42. ^{13}C NMR Spectra of Morphine Sulfate in Concentrated Sulfuric Acid

green solution (Figure 42b) shows differences in number of lines and their intensities and also in the noise level which is lower than that of the freshly prepared solution.

TABLE XIX

^{13}C NMR CHEMICAL SHIFTS OF MORPHINE SULFATE IN D_2O

Identification Carbon	Chemical Shifts (ppm) from TMS
1	120.31
2	123.01
3	140.63
4	148.13
5	93.04
6	68.41
7	135.76
8	128.26
9	63.30
10	-
11	125.98
12	131.96
13	44.43
14	43.48
15	35.05
16	49.62
$N-CH_3$	34.73

CHAPTER V

DISCUSSION

The thrust of this research was to examine the details of interactions of metal ions and metal complexes with drug molecules of the opium alkaloids, to better understanding the processes which have been described in the chemical literature. These processes include acid dissociation and metal complexation and oxidation-reduction reactions. Results from each of the studies are discussed in turn.

Acid Dissociation of Morphine

Previously calculated equilibrium constants for this process in aqueous solution reported in the literature differ considerably for the first ionization step, K_1, but compare favorably for the second ionization equilibrium K_2.

Circular dichroism spectropolarimetry is a quantitative technique with fewer limitations than absorption spectrophotometry. There are no precedents in the literature for the application of CD to the determination of acid dissociation constants calculated from titration data in which the experimental ellipticity was measured as a function of the concentration of strong acid and strong base added.

Base-Titration of Morphine Free Base

The average pK_2 calculated at two different wavelengths are very comparable (Table III), and also compare favorably with the values reported in the literature, 9.85. Results from replicate experiments agree to within $\pm$7 %. CD therefore can effectively be used as a technique to calculate the dissociation constant of a weak acid or base provided the molecule is chiral.

Acid-Titration of Morphine Free Base

The average pK_1 value calculated at 285 nm (Table IV) is in good agreement and compares favorably with the results from the conductivity method (52). These differ considerably from values calculated from colorimetric (29) and potentiometric (31) titrations. Variation in the pK_1 values in this work is greater than that observed for the pK_2 calculations. This is in part due to the much smaller changes in the ψ_{obs} at 285 nm with increasing acid concentration, which leads to greater error in successive measurements. The value of θ_{HM} calculated in the determination of pK_2 was used in the calculation of pK_1, reducing the number of unknowns in the second calculation. Any error in θ_{HM} in the pK_2 analysis is proliferated in the calculation of pK_1.

Metal Ion - Drug Interactions in Aqueous Solution

Ca^{2+} - Morphine Interaction

Ca^{2+} ion has been reported to have many functions involving the nervous system, particularly in release of neurotransmitters and neuronal excitability. Several literature reports indicate that Ca^{2+} ion may have a direct role in the analgesia produced by narcotic drugs. Excessive morphine injection was found to reduce the Ca^{2+} level in the brain (44). Complex formation between Ca^{2+} ion and the morphine molecule may be implicated in the pharmacological mechanism of these phenomena, and may affect the lipid-aqueous distribution of the drug.

Since Ca^{2+} ion prefers to coordinate to oxygen atoms (45), oxygen on C(3), in the dihydorfuran ring, and on C(6) are the most likely binding sites. The only other Lewis base donor in the molecule is the tertiary amine nitrogen. From an X-ray study (46), the morphine molecule has been shown to have a T-shaped structure and the aromatic ring is not perfectly planar which may be due to the strain in the molecular skeleton. The chiral centers in the molecule are at C(5), C(6), C(9), C(13) and C(14). If the complex formation takes place through oxygen, this might be expected to produce a conformational rearrangement in the morphine molecule which could be followed by CD.

No changes in the fundamental CD spectrum of morphine

in aqueous acid were observed when $Ca(ClO_4)_2$ was added in excess. At low pH on the basic side, the morphine spectrum was again unchanged on the addition of Ca^{2+}. At pH in excess of 8, calcium was precipitated as the hydroxide. The results would seem to suggest that complexation does not take place, if a rearrangement is a prerequisite. The evidence can not exclude possible monodentate complexation via the nitrogen atom, however unlikely this may be.

Pseudomorphine

Pseudomorphine has attracted the attention of analysts in the pharmaceutical, clinical and forensic science fields, because it is a highly fluorescent species. This property is employed in determinations of trace quantities of morphine in biological systems of medical importance (47, 48). The oxidative dimerization process of morphine by alkaline potassium ferricyanide is most likely a one electron transfer process involving radical formation on the aromatic ring (49). Codeine and heroin.HCl do not undergo the dimerization process. This result implicates the involvement of the O^- or phenolate functional group on C(3) in the reaction mechanism. It is believed that the C(2) position of morphine is ortho-activated by the presence of the C(3) phenolic group through a tautomeric rearrangement to a quinone-like structure which may then undergo the coupling reaction. X-ray evidence for the C(2)-C(2') linkage is not available. Attempts to prepare a single crystal are in

progress in this laboratory.

Pseudomorphine has two phenolic groups on the C(3) and C(3') positions (Figure 15). In basic solutions, the phenolate ion(s) formed from the proton dissociation of the two phenolic groups produce a 13 nm red shift for the positive CD band maximum. This same red shift is also observed for morphine free base in basic solutions. The negative CD band at 275 nm remains the same within experimental error. On the other hand, the negative CD band at 285 nm of the morphine free base basic solution is changed to a positive CD band minimum at that wavelength. This suggests that there is a conformational change which may be a result of the aromatic ring becoming planar because of aromatic delocalization of the negative charge on the phenolate oxygen. In the case of pseudomorphine, which is a dimer of two morphine molecules, the conformational changes in each part of the molecule may occur in such a way that they compensate each other and no change in $\Delta\varepsilon$ is observed.

Fe^{3+} and Ag^{+} - Morphine Interactions

Reactions of morphine with either Fe^{3+} or Ag^{+} ion in aqueous solution are also oxidative reactions because of the following evidence:

1) With the Fe^{3+}, the color of the mixture is blue, Fe^{3+} solutions are yellow.
2) Fe^{3+} ion is present in the final solution (detected by the familiar red Fe^{2+}- o - phenanthroline

complex).

3) Ag metal is precipitated.

4) CD spectra which are comparable to that from alkaline ferricyanide reaction solution.

The CD spectra of both product solutions are completely different from that of morphine solutions, but they are similar to each other. Therefore the same product is apparently formed in both reactions. The product is believed to be the dimolecular morphine compound pseudomorphine.

The CD spectra of Fe^{3+} - morphine sulfate solution when Fe^{3+} ion is used in excess are similar to the CD spectra of pseudomorphine in acid. The oxidation is relatively slow and a gradual change from the spectrum of morphine to the spectrum of pseudomorphine can be observed to occur over several hours.

Rates of reaction depend on both cation concentration and the pH of the reaction medium. The rate increases as the pH of the reaction medium is increased because at higher pH morphine is in the form of the phenolate ion which easily undergoes the dimerization process. The mole ratio of oxidizing agent to morphine should be at least 1:1 because it requires two moles of morphine to form one mole of pseudomorphine.

Since all reagents are mild oxidizing agents, the mechanism might be the same for all. No reactions between Fe^{3+} or Ag^{+} ion with codeine and heroin.HCl were observed, which again emphasizes the role of the phenolate

functional group in the oxidative mechanism. On transferring electrons from morphine two hydrogen atoms are lost from C(2) and C(2') in the coupling mechanism.

Reaction of Drugs in Concentrated Sulfuric Acid

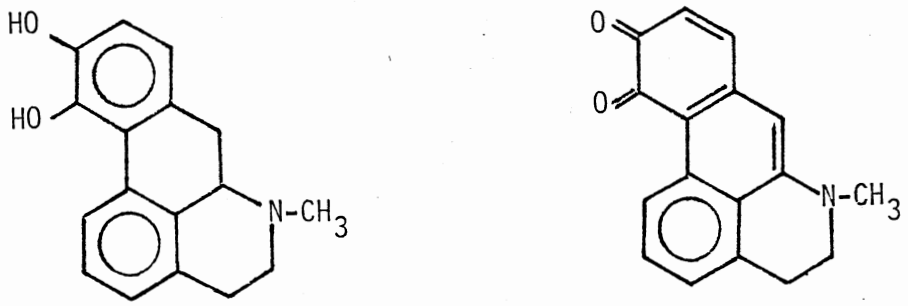
Many irreversible reactions such as acid hydrolysis, dehydration, isomerization and rearrangements are possible in sulfuric acid media (50). Hydrolysis and dehydration reactions are commonly found to occur for various substrates dissolved in conc. H_2SO_4. The evidence from spectral changes in the CD and UV studies of drugs in conc. H_2SO_4 compared to aqueous media suggests that some kind of reaction has taken place. It is not a simple protonation of the drug molecules that causes a reversal in sign in the CD spectra. If only protonation reactions had taken place the changes in CD spectra would be due to the conformational change in the drug molecule, and the spectra should convert back to that of the drugs in dilute aqueous acid when the solution of drug in conc. H_2SO_4 is diluted with distilled water to lower the $[H^+]$.

Since the quantities of each drug used are not equal, the magnitude of absorbances or ellipticities can not be used in any comparisons. However the UV and CD spectra of morphine sulfate and heroin.HCl in conc. H_2SO_4 are identical in pattern and absorption maxima. This suggests that heroin.HCl may undergo ester-hydrolysis to form morphine.

The spectra of codeine are uniquely different because codeine contains an ether functional group which is remarkably resistant to hydrolysis.

Ahlers and Auterhoff (51) reported that the solution of morphine.HCl in conc. H_2SO_4 after 24 hours produced apomorphine-o-quinone as a final product (Figure 43b). Morphine has undergone dehydration to make the C-ring aromatic, and considerable rearrangement in which the dihydrofuran ring and the nitrogen containing ring have been opened. The latter ring is closed again on C(8). The UV-Visible spectra of the product consist of three absorption bands with maxima at 572, 410 and 315 nm. In this work, the freshly prepared dilute solution of morphine sulfate in conc. H_2SO_4 was colorless and the absorption spectra were observed only in the UV region. The dark green solution obtained when the solution was kept in the air at room temperature for more than one month shows two absorption bands with maxima t 600,385 nm and a shoulder at 310 nm. No CD signals were observed for this solution. This is consistent with the achiral quinone product, but the spectral maxima do not correspond with those observed by Ahlers and Auterhoff.

The ^{13}C NMR spectrum of the dark green solution appears to be different from that of the freshly prepared solution (orange color). If the product in the dark green solution is the quinone of apomorphine the two lines which correspond to the two carbonyl carbons should appear at



(a) (b)

Figure 43. Molecular Structure of (a) Apomorphine and (b) Apomorphine-o-quinone

about 200 ppm shifted from TSP (internal standard). From the ^{13}C NMR spectra, only one line with very low intensity is observed in that range and there are too many lines in the region where one expects to find the aromatic carbons. The number of lines observed is greater than what would be expected from the number of carbon atoms in the molecular structure of apomorphine-o-quinone. Therefore the colored mixture probably consists of a mixture of products part of which may be a quinone compound. A separation of the components of this final green mixture is under way.

Dilute solutions of morphine sulfate in conc. H_2SO_4 after 48 hours are colorless and show UV and CD spectra which are similar to the UV and CD spectra obtained from solutions of apomorphine in conc. H_2SO_4. Therefore apomorphine, which is a chiral compound (Figure 43a), is a possible intermediate product which undergoes further oxidative reaction to form an achiral product.

Color Reactions of Drugs in Concentrated Sulfuric Acid

In the absence of color reagents, a number of irreversible reactions are possible with the solvent. The only oxidation possible was due to oxygen in the air. All of the color reagents are potentially oxidizing agents with more favorable oxidation potentials than oxygen gas. The products may or may not be the same for both cases. Color differences between solutions with and without color

reagents added are in part due to the color of the reduced forms of the reagents and may also indicate the formation of different products. UV and CD spectra were corrected for the reagents by substracting the spectra of the reagents themselves. All reagents were present in large excess over the drug concentrations making the substraction of the spectra valid.

Colors developed in reactions of morphine, heroin.HCl and codeine with different color test reagents are distinguishable from one another, while almost the same shade of color is formed for all these drugs with any one of the color test reagents. In each reaction, more than one product is formed with time because many shades of colors were observed. Since the UV-Visible spectra are representative of all the chromophoric species present in the colored mixtures, the spectra are quite complex. The colored mixtures also produced CD signals, some appear in the visible region, for example with the Marquis and Erdman reagents, but the intense CD signals mostly occur in the UV region. This means that some products formed during the reactions still retain chirality because the CD technique focuses only on the chiral products.

It should be pointed out that the differences in the absorbances or in the degrees of ellipticity among drugs with the same or different color reagents can not be used for comparative identifications because the concentrations of drugs in each mixture are not exactly equal. In

preparing the solutions, 0.3 to 0.5 mg of each drug were dissolved in 10.0 ml. of each color test reagent. The concentration range was 8 X 10^{-5} to 1.3 X 10^{-4}M for morphine sulfate and heroin.HCl, and 1.4 X 10^{-4} to 2.3 X 10^{-4}M for codeine. The concentrations of the color reagents were at least 0.03 M.

The similarity in pattern and wavelength maxima of the overnight UV-Visible and CD spectra for all three drugs with any one color test reagent is very interesting. The evidence suggests that the same product is formed even although the functional groups are different. This is expected for morphine and heroin because of ester hydrolysis of the latter. The result for codeine may be coincidence.

In the case of the Marquis and Erdman reagents, the product chirality is lost as the reaction progresses, evidenced by the decrease in the observed ellipticities (Figure 32). These results are different from those of the Madelin and Mecke-drug color reactions. That is, the overnight spectra of the color mixtures show intense CD bands in the UV region which means that the products are chiral. This may simply be a result of different reaction rates or significant differences in the oxidation potentials of the reagents in conc. H_2SO_4. It is not yet possible to distinguish between these possibilities because oxidation potential data are not available.

It was found from ESR studies on the color reactions of drugs that radicals are formed as preliminary intermediate

products in the colored mixtures. ESR spectra were obtained immediately on mixing and showed a gradual decay with time, particularly in the case of codeine. Neither the drugs alone in sulfuric acid nor the reagents alone in sulfuric acid gave any evidence of an ESR spectrum. The radical is presumably formed by electron transfer (oxidation-reduction) from the drug to the color test reagent.

The ESR of the colored mixtures obtained from the reactions of drugs with Marquis reagent, each consist of seven equally spaced lines and the ratio of the line intensities are 1:6:15:20:15:6:1. This corresponds to the interaction of unpaired electron with six equivalent nuclei with nuclear spin, I = 1/2, possibly H atoms. The g-factors and hyperfine splitting constant of the radicals formed are almost equal. That means the color reactions of all three drugs may produce the same radical as one of the intermediate products. It is difficult to interpret these data as evidence that the radical is formed on the starting material because it is difficult to identify six equivalent H atoms. A more likely prospect is an apomorphine-like structure, once its structure has been clarified.

With the other color test reagents the ESR spectra for codeine are completely different from those of morphine sulfate and heroin.HCl which are similar to each other but they are different from those with the Marquis reagent. For codeine the ESR spectra had to be measured at low temperature because the radical is quite unstable. The spectra

consist of nine almost equally spaced lines and the line intensity ratios are close to the value expected for eight equivalent nuclei with I = 1/2. At higher temperature, equal splitting of the nine lines is changed to a triplet of triplets which possibly correspond to the interaction of an unpaired electron with two sets of non-equivalent nuclei of I = 1/2, each set of which contains two equivalent nuclei. The splitting can not be interpreted in terms of the parent codeine molecule and must be associated with a more symmetrical intermediate of unknown structure.

Colored mixtures of morphine sulfate and heroin.HCl with any of the color test reagents produce ESR spectra that are identical in pattern, i.e. a triplet of doublets, which are possibly due to the interaction between an unpaired electron with two sets of non-equivalent nuclei. One set contains two equivalent nuclei and the other set has only one nucleus. The values of the g-factors and hyperfine splitting constants from these two drugs with the same color test reagent are almost equal. This is further evidence that heroin.HCl has been hydrolysed to morphine by strong acid. The radical formed may be another product which is different in molecular structure from the starting material. It is not likely to be formed on the apomorphine molecule per se which has been suggested to be one of the intermediate products in the color reactions, because no ESR spectra were observed for apomorphine-color reactions.

The Frohde and Mecke reagents produce colored mixtures

that may contain more than one radical because asymmetrical ESR signals were observed in each spectrum. Those signals that were used to calculate the g-factors are believed to be produced by radicals formed from the organic molecules. The intense unresolved signals in the Frohde drug-color reactions which appear at higher magnetic field strengths are possibly due to the metal complex ion itself in which the metal is paramagnetic.

The simple organic molecules phenol and anisole which contain the -OH and $-OCH_3$ functional groups were used as model organic compounds in the color reactions to probe the nature of the radicals. Each colored mixture obtianed from those two compounds produced only one broad ESR line instead of a fine structure. One can safely say that the complex ESR spectra of the drug-reagent solutions must involve the whole molecular structure.

Some ESR spectra are not perfectly symmetrical and in most cases lines are broad. This may be caused by the high viscosity of the reaction medium conc. H_2SO_4, that makes the system anisotropic.

The possible intermediate products formed in the drug - color reactions at different time intervals can be summarized as a flow chart in Figure 44.

Conclusions and Suggestions for Further Work

From the evidence from UV and CD studies on the

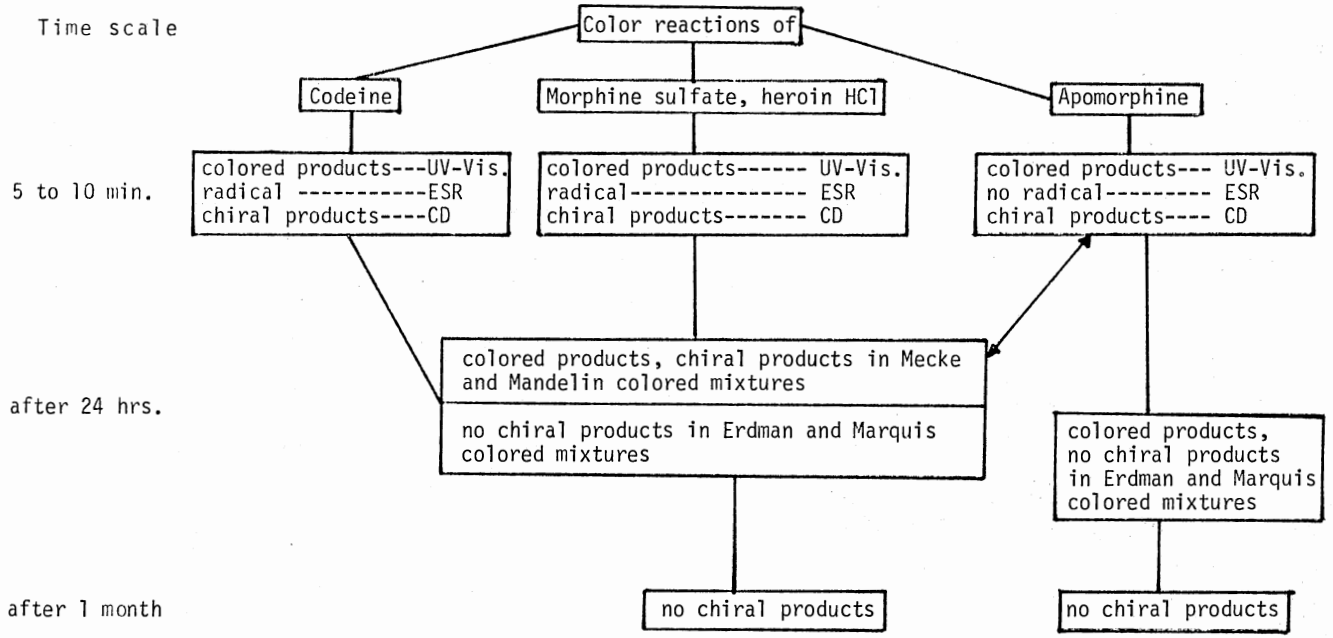


Figure 44. The Flow Chart Showing the Possible Intermediates Formed in the Drug-Color Reactions

interaction of morphine with mild oxidizing agents, i.e. 5% $FeCl_3$ aqueous solution, 1% $AgNO_3$ aqueous solution and alkaline solution of $K_3Fe(CN)_6$, the same product, pseudomorphine is formed. The reaction is believed to invovle one electron transfer from the morphine molecule to the metal ion and takes place only on the aromatic ring, the rest of the morphine molecule remains intact. On the other hand, reactions of drugs in strong acid are very complicated and many products are formed because the strong acid, conc. H_2SO_4, can initiate numerous reactions e.g. dehydration, protonation, rearrangement etc. and the acid itself is a strong oxidizing agent.

The color reactions of drugs are also oxidation reactions. The product is not pseudomorphine probably because the conditions are not mild and because conc. H_2SO_4 is a very reactive solvent.

It was found that radicals are formed in every color reaction mixture containing a drug and that this radical is stabilized in conc. H_2SO_4 for a period of time. The values of the g-factor and hyperfine splitting constants for the radical formed from any one drug with all of color test reagent except the Marquis reagent are almost equal. This means that the same radical is formed and that the radical may not be the only species responsible for color in the mixture since the colors and CD and UV-Visible spectra of all colored mixtures are different. Many colored products are formed in each color reaction and the shading varies

with time as the reaction progresses at its own kinetically controlled rate.

Evidence from the CD and UV-Visible studies on color reactions of drugs in comparison with apomorphine color reactions strongly suggest that apomorphine is one of the intermediate products formed but it is not the first. The radicals which have been detected by ESR technique may form first and then gradually convert to the more stable product. The products in the colored mixtures obtained from apomorphine with the Erdman and Mecke reagents are not optically active compounds and may possibly be a quinone of apomorphine because the CD studies on those colored mixtures show a decrease in magnitude of ellipticities with time. A knowledge of the oxidation potentials of the reagents is necessary for a fuller understanding of the multiple process.

All color test reagents are strong oxidizing agents. The rate of reaction depends on the oxidizing power of each color test reagent. It is very likely that the Erdman reagent may have a higher oxidizing power than either the Mecke or Mandelin reagents, since the CD spectra of colored mixtures obtained from morphine sulfate or apomorphine-Erdman color reactions show the significant decrease in magnitude of ellipticities within the comparable time.

It is known that all color reactions of drugs are oxidation-reduction reactions, but the numbers of electrons involved in each reaction have not been reported. Electrochemical studies on drug- color reactions are planned, in

order to determine the number of electrons transferred in each of those reacitons. Polarography or cyclic voltammetry will be used in those studies. The isolation of product at different time intervals is also planned and the structural determination of the products will be performed by using the physical technique available, such as X-ray crystallography and the usual spectroscopic methods.

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