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Dipeptide Complexes Of Palladium(II) And Platinum(II)

Lewis Enos Nance
University of the Pacific

DIPEPTIDE COMPLEXES OF Pd(II) AND Pt(II)

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Lewis Enos Nance

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ABSTRACT

Due to recent interest in the role of heavy metals in enzymes, oxygen-carrying molecules, and anticarcinogenic compounds, the study of metal-protein bonding has assumed a new importance. Studies have been made utilizing platinum compounds as anticarcinogenic agents in several different types of cancerous activity. No study of the mechanism by which Pd(II) and Pt(II) compounds exhibit anticarcinogenic properties has been made to date.

The purpose of this research was to investigate the nature of bonding of Pd(II) and Pt(II) with various dipeptides, which were chosen in this study as a basic unit of protein-like material. These compounds have the advantage of having the bonding sites of larger peptides. An amino group, peptide-linkage carbonyl and amide group, and carboxyl group are available for bonding with metal ions to form complexes. The position of the donor atoms on dipeptides allow sterically for the formation of two five-membered rings in a complex when other conditions are suitable.

When $PdBr_2$ was refluxed in acetone with a dipeptide, $PdBr_2(XX)_2$ (where XX is a dipeptide) resulted. Bonding of the dipeptide to Pd(II) was through the amino nitrogen atom. Analogous compounds were found to result when $PtCl_2$ was refluxed in acetone with a dipeptide.

K_2PdCl_4 afforded a source of Pd(II) in which the dipeptide could function as a bidentate or tridentate ligand. At a pH near 13.0 the dipeptide was bound to Pd(II) through the amino and depro-

tonated nitrogen atoms. At a pH near 7.0 the carboxylate oxygen of the dipeptide was also bonded to Pd(II). Infrared measurement showed that the Amide I band of the dipeptide had shifted at least 50 cm^{-1} to lower energy upon coordination of the peptide nitrogen to Pd(II). Electronic studies indicated that the λ(max) was at a minimum when the dipeptide was acting in a tridentate mode. There was a red shift when the hydroxide ion replaced the carboxylate group in the coordination sphere at pH values near 13.0. Nuclear magnetic resonance spectra indicated tridentate behavior of glycylvaline with Pd(II) at pD 7.11 but showed detachment of the carboxylate group from the coordination sphere at pD 13.06.

Infrared analysis showed that there was no reaction of Pt(II) with the peptide linkage when K_2PtCl_4 was used at the Pt(II) source.

Zeise's salt was used as a third source of Pt(II). Infrared analysis, molecular weight determination, and elemental analysis indicated fission of the peptide bond and formation of compounds of the formula $(C_2H_4PtClX)_2$, where X is an amino acid residue from the dipeptide reacted with Zeise's salt. Both residues of each dipeptide, ValVal, ValLeu, and LeuVal, were incorporated in the product of Zeise's salt and the dipeptide. Infrared analysis indicated N-trans—O-trans of the donor atoms of the amino acid residues with respect to ethylene in the ValVal and LeuVal products, whereas Val-Leu gave an N-trans—N-trans product.

Dedicated to Jann, my best half.

ACKNOWLEDGMENTS

I would like to express my appreciation to Dr. Herschel G. Frye for his assistance in the preparation of this research and for his understanding and confidence given to me by telephone when this project was not being resolved optimally. I would also like to thank Dr. Anton F. Schreiner of N. C. State University for his help in obtaining circular dichroism and nuclear magnetic resonance spectra.

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CHAPTER I

INTRODUCTION

Due to a recent interest in the role of heavy metals in enzymes, oxygen-carrying molecules, and anticarcinogenic compounds, the study of metal-protein bonding has assumed a new importance.[1,2,3] Studies have been made utilizing platinum compounds as anticarcinogenic agents in several different areas. These researches with platinum have been done on a pragmatic basis with no mechanistic studies performed to date.

This research was conducted in order to investigate the nature of bonding of Pd(II) and Pt(II) with various dipeptides, which were chosen in this study as a basic unit of protein-like material. These compounds have the advantage of having the bonding sites of larger peptides. An amino group, peptide linkage carbonyl and amide group, and carboxyl group oxygen are available for bonding with metal ions to form complexes. The position of the donor atoms on dipeptides allow sterically for the formation of two 5-membered rings in a complex when other conditions are suitable. The conformation of a dipeptide will allow it to act as a ligand. Of significant interest is the position of bonding by the metal to the peptide linkage —CO—NH—. Of the commercially available dipeptides, only L-amino acid residues were chosen. In this study dipeptides are written in abbreviated form. For example, glycylglycine, L-leucyl-L-leucine, and L-valyl-L-valine are referred to as GlyGly, LeuLeu,

and ValVal, respectively, with no reference to spatial configuration. Compounds made from Zeise's salt and a particular dipeptide are given labels such as ZS-VV on spectra listed in figures in the appendix. Pd(II) forms a series of complexes with dipeptides that are pH dependent. These are designated in tables and figures in a form such as "Pd(II)-GV at pH 7.11". This refers to the particular species present when K_2PdCl_4 is reacted with glycyl-L-valine at pH 7.11.

The conformation of Pd(II)-dipeptide complexes at acidic and basic pH values as well as the various donor atoms to Pd(II) were investigated in this research. The conformations of Pd(II)-dipeptides at neutral pH values and Pd(II)-tripeptides at neutral and basic pH values has already been investigated by use of circular dichroism spectroscopy.[4,5] However, circular dichroism spectra run in the research presented here on Pd(II)-dipeptides at basic pH values were fundamentally different from those obtained at basic pH values from Pd(II)-tripeptide complexes by Pitner, Wilson, and Martin.[5]

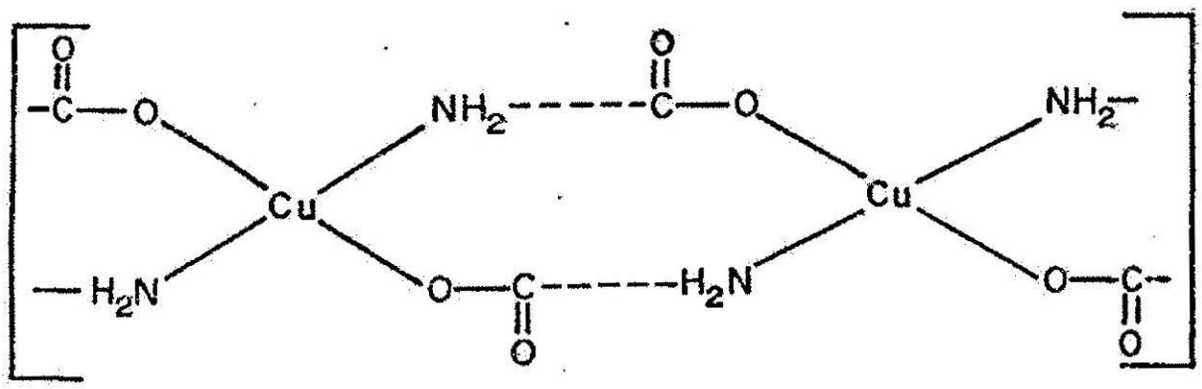
Novel dimer compounds in which Pt(II) of Zeise's salt, $K\left[(C_2H_4)Cl_3Pt\right]$, fissioned the peptide linkage of ValVal, ValLeu, and LeuVal were synthesized and characterized in this work. Two new compounds, $PdBr_2(ValVal)_2$ and $PtCl_2(ValLeu)_2$, in which the dipeptides were bonded to Pd(II) and Pt(II) in a monodentate mode were synthesized. No compounds resulting from a reaction of Pt(II) and a dipeptide were found in a research of the literature.

Infrared analysis was used to determine donor atom attachment to Pd(II) and Pt(II). In some Pt(II) compounds infrared

analysis was also used to determine relative cis- trans- structures. Molecular weight determinations were made on Pt(II)-dipeptide compounds that were formed from Zeise's salt and a dipeptide. Nuclear magnetic resonance spectra, ultraviolet-visible spectra, and circular dichroism spectra were run on solutions of K_2PdCl_4 and various dipeptides to investigate which bonding atoms were coordinated to Pd(II) and which particular conformations were present at various pH values. Elemental analysis was performed on Pt(II) and Pd(II) compounds to help determine structures and to indicate alternate methods of synthesis.

HISTORICAL BACKGROUND

In 1950 Klotz, et al.[6] suggested that dipeptides coordinate with Cu(II) only with terminal carboxylate and amino groups, giving rise to polymers as indicated by formula I:



I

Manyak, et al.[7] pictured the complexes of glycylglycine with Cu(II), Co(III), and Ni(II) as involving interactions at the amino nitrogen, peptide nitrogen, and the carboxylate group.

Dobbie and Kermack[8] studied the general features of the reaction of Cu(II) with peptides in aqueous solution by means of potentiometric and visible spectrophotometric measurements. They indicated the sites of coordination to be the amino nitrogen and the deprotonated peptide nitrogen.

Datta, Leberman, and Rabin[9] made potentiometric studies of complexes of Ni(II), Co(II), Cd(II), and Zn(II) with dipeptides and related compounds. Coordination was postulated to be at the amino nitrogen and peptide oxygen.

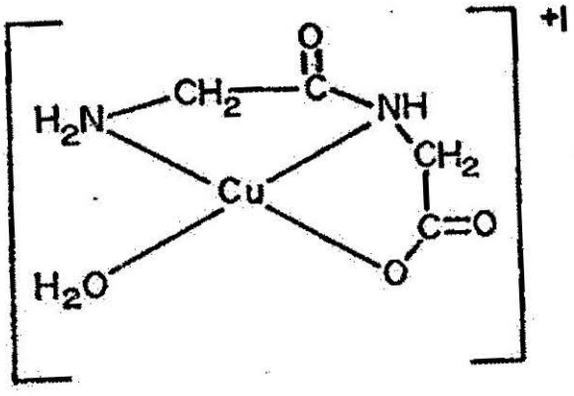
Li, et al.[10] stated that the coordination sites of glycylglycine, glycylglycylglycine, and glycylglycylglycylglycine toward Co(II) are probably the terminal amino group and the immediately adjacent peptide oxygen. Infrared spectra were taken in ethanol. The frequency of the Amide I band, due mainly to carbonyl stretch of the peptide linkage, was lowered. They proposed that the Amide I band would occur at higher frequency if coordination had taken place at the peptide nitrogen. The formation constants of Co(II) complexes with the three glycyl peptides are about the same. This would seem to indicate that the carboxyl group is not involved in bonding since the complexes would be 8, 11, and 14-membered, respectively. Therefore, the formation constants would not be in the same order.

Penland, et al.[11] analyzed the infrared spectra of urea with several metals. Their analysis indicated metal-to-nitrogen bonding with Pt(II) and Pd(II), and metal-to-oxygen with Cr(III), Fe(III), Zn(II), Cu(II). Similarities between Zn(II) and Co(II) led Li and co-workers to believe that the peptide oxygen and not the peptide

nitrogen was used in the cocrdination of peptides to Co(II).

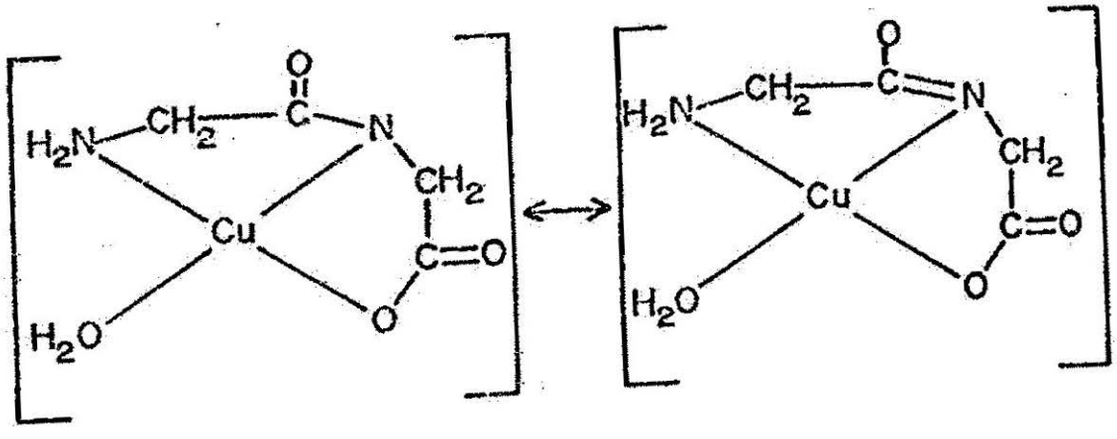
Koltun, et al.[12] reported that $Cu(GlyGly)^+$ is probably associated with the amino nitrogen and with the peptide oxygen.

Kim and Martell[13] studied Cu(II) complexes of glycylglycine at various pD values by means of infrared and visible spectroscopies and by potentiometric titration. They postulated structure I at low pD values:



I

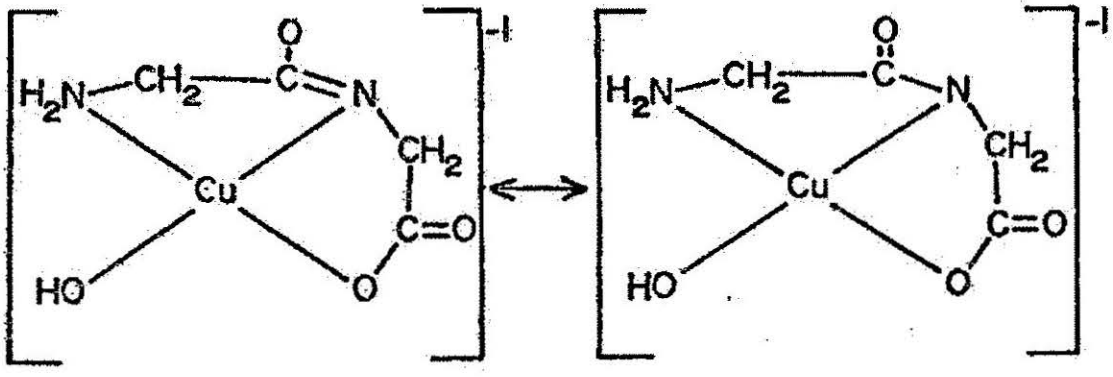
At higher pD values, structure II was indicated:



II

Since the bond order of the peptide carbonyl is lowered due to resonance, a lowering of the frequency of absorption of the Amide I band was postulated and found in structure II.

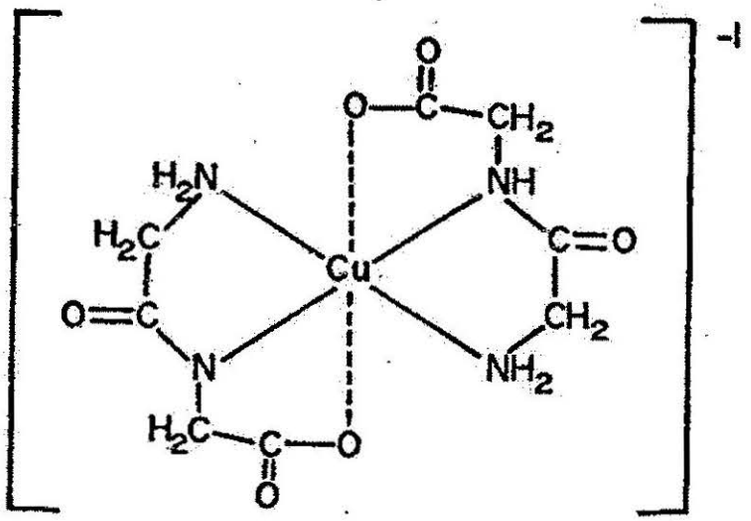
At still higher pD values, structure III was indicated:



III

The infrared absorption showed no frequency changes in the carbonyl region of the spectrum with the loss of a proton from structure II to form structure III.

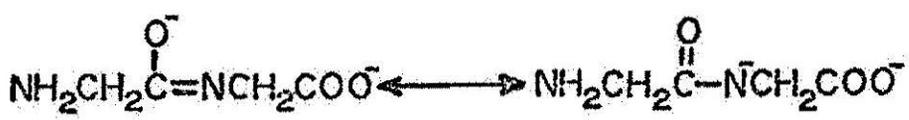
When the ligand-to-Cu(II) ratio is 2:1, other structures can be drawn. One of these utilizes the peptide nitrogen and the amino nitrogen as the principal donor atoms with carboxylate oxygen atoms forming long bonds along the apical positions. This is shown in structure IV:



IV

Datta, et al.[9] made potentiometric studies on some Cu(II) complexes of sarcosyl and leucyl ligands where the ligand-to-metal ratio is 2:1. Two hydrogen ions per Cu(II) were found for sarcosyl-L-leucine, leucylglycine, and leucylglycylglycine. When the ligand-to-metal ratio was 1:1, two acid ionizations per metal occured from the complexes of the above peptides. Sarcosylsarcosine lost only one hydrogen ion in the 1:1 combination, thereby giving credence to the postulate that the second hydrogen ion comes from the peptide nitrogen.

Doran, et al.[14] constructed a molecular model based on the following resonance structure:



They found that the peptide nitrogen and the carboxylate oxygen could be used as coordination sites along with the amino nitrogen. However, if the amino nitrogen and the peptide oxygen were used, the carboxylate oxygen could not be used due to steric hindrance.

Dissociation constants of glycylglycine, glycyl-L-alanine, glycyl-L-phenylalanine, glycyl-D-phenylalanine, and L-phenylalanylglycine and stability constants of the Co(II) and Cu(II) complexes of these ligands have been determined by potentiometric titration by Biester and Ruoff.[15] Variations in the stability constants were discussed in relation to the structural differences in the peptides. Substitution on the carbon atom adjacent to the peptide nitrogen produced small changes, whereas substitution on the carbon atom adjacent to the amino group led to more pronounced changes in the stability constant. Both electronic and steric effects were produced by the structural changes. The variation in stability constants of complexes can to some extent be ascribed to the electron-releasing or withdrawing character of the group attached to the alpha-carbon; but steric interference may be more significant when large side chains are present, especially when they are adjacent to the peptide bond.

Martin, Chamberlain, and Edsall[16] found that solutions of dipeptides such as glycylglycine in a ligand-to-metal ratio greater than 2:1 with Ni(II) when titrated gave two equivalents of acid in addition to the hydrogen ion that could be neutralized from the ligand alone. Solutions of glycylsarcosine with Ni(II) gave no indication of induced ionization caused by complexation. This

indicates the loss of a proton from the amide nitrogen in dipeptides not containing a sarcosyl carboxy-terminal residue. Blue complexes of Ni(II) with triglycine and tetraglycine turn yellow upon titration of the amide hydrogens. Tetraglycine forms a 1:1 complex with Ni(II) which probably has adopted the square planar configuration upon induced ionization of the amide hydrogens. The deprotonated amide nitrogen falls further to the right in the spectrochemical series than does the ordinary amide nitrogen.

Freeman, et al.[17] reported the structure of bis(biureto) cuprate(II) tetrahydrate, $K_2\left[Cu(NHCONHCONH)_2\right] \cdot 4H_2O$. The copper atom in this complex is square-coordinated by four amide nitrogen atoms of the two biuret ligands. Two nitrogen atoms belonging to neighboring complexes complete the usual elongated bonds above and below the plane of the molecule.

Bryce and Gurd[18] reported formation and ionization constants for 1:1 Cu(II) complexes of glycylglycyl-L-alanine, glycyl-L-alanylglycine, L-alanylglycylglycine, and L-alanyl-L-alanyl-L-alanine. They also reported visible absorption characteristics of intermediate species.

Koltun, Roth, and Gurd[12] studied equilibria between Cu(II) ions and diglycylsarcosine, triglycine, and glycylsarcosylglycine by potentiometric titration. All the peptides except glycylsarcosylglycine, which behaves like glycylsarcosine, form complexes with Cu(II) in which the first peptide bond hydrogen is displaced. The potentiometric results, coupled with spectral and kinetic measurements on the rate of hydrolysis of p-nitrophenyl acetate by complexes of the type $CuGGOH^{-1}$ with triglycine and tetraglycine

indicate that the addition of a third mole of alkali to 1:1 mixtures of Cu(II) with either of these peptides causes the ionization of the second peptide bond hydrogen and not of H_2O from the coordination sphere. A fourth mole of alkali added to tetraglycine releases a hydrogen ion from the third peptide bond.

Blount, et al.[19] crystallized (glycyl-L-histidinato)Cu(II) sesquihydrate from solution at pH 6.5. The copper atom has coordination number 5. The four closest donor atoms, which have an approximately square planar arrangement about the copper, are the amino-, peptide-, and imidazole-1-nitrogen atoms of one peptide molecule and a carboxyl oxygen atom of another. The fifth donor atom of the square-pyramidal arrangement is the oxygen of a water molecule. The copper also interacts weakly with the second oxygen atom of the carboxyl group to which it is bonded. The additional semi-molecule of water participates in the hydrogen-bond network.

Michailides and Martin[20] reported that in the absence of oxygen, glycylglycine undergoes a Co(II)-promoted amide hydrogen ionization near pH 10. For the 2:1 complex the two amide hydrogen ionizations occur in a cooperative manner, yielding a light blue solution with a magnetic susceptibility of 4.1 BM. These results and the absorption spectrum of the light blue solution suggest an equilibrium between high and low spin states in an octahedral complex. Admission of oxygen to pink or blue solutions of octahedral Co(II) complexes rapidly yields yellow or, at higher concentrations, brown solutions of binuclear oxygenated complexes. Depending on the ligands, the complexes decompose at various rates to red mononuclear Co(II) complexes. A minimum of three nitrogen donors seems

to be required for the formation of oxygenated complexes which are necessary intermediates in the oxidation of Co(II) complexes by molecular oxygen. The product Co(II) chelates appear to be derived from the binuclear oxygenated complexes by filling the coordination position voided by the departing peroxo group with an additional chelating group, if available. Otherwise, a hydroxo group will substitute in the coordination sphere.

Tsangaris and Martin[21] showed that the magnitude of CD in Cu(II)-dipeptide complexes, Cu(X-X), may be accurately estimated by adding the magnitudes of corresponding glycyl dipeptide complexes, Cu(XG) and Cu(GX), indicating that the CD is an additive function of independent contributions from amino and carboxyl terminal amino acid residues. They found that hexadecant rule accounts for the sign identity and magnitude of these complexes. Octant and quadrant rules were not applicable.

Morris and Martin[22] made Co(II) complexes of 18 dipeptides which yielded four d-d transitions: 1250, 1000, 610, and 480 nm. The first and third bands were assigned to the low-spin and the second and fourth to the high-spin components of octahedral complexes. Magnetic susceptibility and extensive titration results were consistent with high-spin—low-spin equilibrium. Large side chains on the carboxy terminal residue provided steric inhibition to amide hydrogen ionization and oxygenation of the Co(II) complexes.

Wilson and Martin[4] reported the complexation of Pd(II) with dipeptides with subsequent amide deprotonization. The spectra were taken at about pH 6. They postulated bonding at the amino nitrogen,

peptide nitrogen, and the carboxylate oxygen. The sum of the CD of $Pd(GX)^{-1}$ and $Pd(XG)^{-1}$ were generally additive at 375 and at 320 nm. Better addition results were obtained at the longer wavelength band.

Wilson and Martin[4] identified four spin-allowed ligand field bands in the solution circular dichroism of 2:1 N-methyl-L-alanine complexes of Cu(II) and Pd(II). Two spin-forbidden transitions were also reported in the Pd(II) complex. Both vicinal effects of substituents and chelate ring conformation were stated to contribute to the optical activity in the ligand field bands of N-methyl-amino acids.

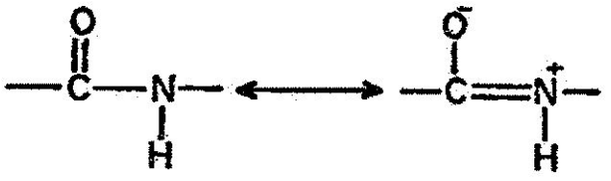
Pitner, Wilson, and Martin[5] showed a new circular dichroism sign change from negative to positive upon addition of base to the quadridentate Pd(II) complex of glycylglycyl-L-alanine. This was identified as replacement of bound carboxylate by hydroxide in the tetragonal coordination plane. Nuclear magnetic resonance chemical shift nonequivalence of glycyl methylene protons observed in the zwitterion form of L-alanylglycine does not occur in the terdentate Pd(II) complex, but appears upon displacement of the bound carboxylate group by hydroxide in Pd(en)(L-alanylglycine) where there are four nitrogen donors around Pd(II).

GENERAL STUDIES BACKGROUND

Infrared, circular dichroism, electronic, and nuclear magnetic resonance studies were performed on different compounds in order to establish bonding sites and conformations. Background material for each of these areas is presented in this section.

Infrared Studies of Dipeptides

Studies show that primary and secondary amides exist in the keto form and therefore show carbonyl absorption in the infrared spectrum. The frequency of this carbonyl absorption will depend on the dipolar structure with which it can resonate:



R groups attached to C and N will affect the absorption frequency to some extent. This absorption is called Amide I and is a vibration of the whole peptide linkage. It is composed mainly of a carbonyl stretching mode; however, it contains a contribution from C-N stretch and a small contribution from N-H deformation. The frequency of the Amide I band is dependent on the physical state of the compound. Since it is affected by hydrogen bonding, there are frequency shifts when passing from liquid to solid states.

Hydrogen bonding affects the N-H vibration; in concentrated solutions both free and hydrogen bonded vibrations can be observed. Diketopiperazine shows five bands in the solid state, all of which are believed to be associated with N-H stretching modes.[23]

The Amide I absorption is at an appreciably lower frequency than the carbonyl absorption of normal ketones due to the resonance effect that is possible in an amide. Contributions from form II of

the resonance structures above give the carbonyl group in an amide a lower bond order than that of the carbonyl group in a ketone. This lowers the stretching frequency of the band.

The vapors of some of these compounds absorb at a much higher frequency. The values suggest that the charged resonance form makes a smaller contribution in the vapor state than it does in the solid state.

Primary and secondary amides show another strong absorption in the 1600-1500 cm^{-1} region, the origins of which are not definitely established. Therefore, it is not conclusive that the cause of this band is the same for primary and secondary amides. This band is called Amide II.

The intensity of the Amide II band is about one-half to one-third that of the Amide I band. These bands fall close enough together in some cases for primary amides so that only one band is observed. N-H deformation is likely to be the predominant factor in the Amide II absorption for primary amides.

Research has been done on secondary amides which support the N-H deformation as the mode of vibration which is responsible for the Amide II absorption.[24] This includes the following:

1. Absence of the Amide II band in tertiary amides
2. Weakening of the band on deuteration
3. Directions of frequency shift upon change of state
4. Polarization studies that indicate an in-plane N-H deformation

Frazer and Price[25] assigned the Amide II band as a mixed vibration of an out-of-phase combination of OCN and NH. The

deformation of H is assigned to play a major part. They assigned the corresponding in-phase mode that is primarily C-N stretch as the Amide III band. This band occurs in secondary amides in the region 1305-1200 cm^{-1}. It is usually weaker than the Amide I and Amide II bands. The Amide III band has not been studied in great detail.

There are two modes, Amide IV and VI, that are of little use for characterization purposes. These are located at 620 cm^{-1} and at 600 cm^{-1}, respectively, in secondary amides and are skeletal modes of vibration.

Mizushima et al.[26] have termed the out-of-plane NH deformation that occurs near 700 cm^{-1} as the Amide V band. It is very broad in the spectra of solids and concentrated solutions.

Polypeptides exhibit a common pattern of bands, which are as follows:

1. 3300 cm^{-1}; hydrogen bonded N-H groups
2. 1680-1500 cm^{-1}; Amide I and Amide II bands
3. Bands related to characteristic R-group absorption.

The simple peptides have infrared structures that are composed of bands from the peptide linkage as well as from certain R groups, carboxyl and carboxylate groups, alpha-amino and protonated alpha-amino groups, and interactions from these groups. The zwitterion form is important in dipeptides, which makes the spectrum more complicated than in some polypeptides.

X-ray studies have shown that glycylglycine is known to exist in open-chain structure in the zwitterion form.[27] Thompson et al.[28] have shown that glycylglycine has NH absorptions at 3300

cm^{-1}, 3080 cm^{-1}, 1630 cm^{-1}, 1575 cm^{-1}, and 1240 cm^{-1}. These bands were indicated by deuteration studies. Thompson et al.[28] gave the first two as N-H stretching frequencies and the 1540 cm^{-1} band as the Amide II band. The 1630 cm^{-1} and 1575 cm^{-1} have parallels in the amino-acid I and II bands. (These bands are associated with deformation of the NH_3^+— group).

The normal absorption for the peptide carbonyl (Amide I) band is 1655 cm^{-1}. Strong bands are found at 1608 cm^{-1} and at 1400 cm^{-1}, which are given the carboxylate (antisymmetric and symmetric) stretching modes as a probable assignment.

The zwitterion structure is found to be less important in polyglycines. The 1400 cm^{-1} band is absent in higher members. There is a band at 3300 cm^{-1} that is assigned as an N-H stretching mode. All the polyglycines absorbed at 1015 $\pm$ 10 cm^{-1}.

Two groups of workers have studied mixed polypeptides.[30,31] The infrared spectra are similar but have interesting differences. Glycine-leucine peptides show absorptions at 3400 cm^{-1}, 3500 cm^{-1}, and 3380 cm^{-1}, which indicates the presence of unbonded NH_2 groups. This suggests that the packing of crystals is such that some of the NH_2 groups are not hydrogen bonded.

The spectra of LLL and DDD peptides are alike but differ from mixed forms. The differences mainly concern Amide I, Amide II, and N-H stretching modes.

General Circular Dichroism Studies

To have optical rotatory activity a molecule will be devoid of a center of inversion, a plane of symmetry, and an alternating rotation-reflection axis of symmetry.[29] The chromophore does not

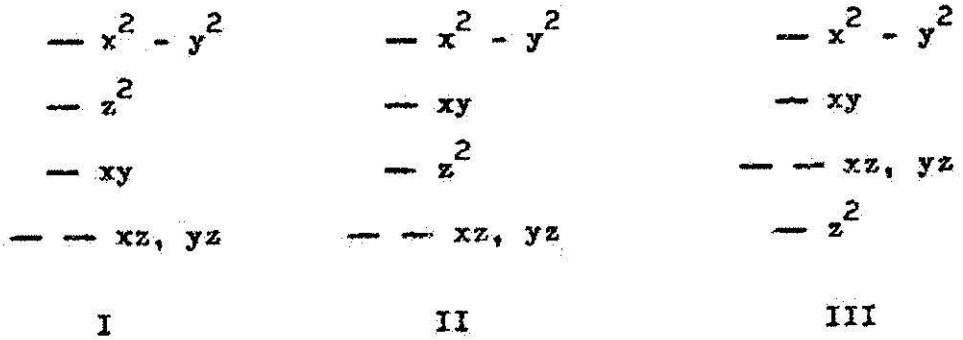
have to be a center of asymmetry in order that the molecule show optical rotatory activity. A carbonyl group will show optical activity in the ultraviolet region when located in a steroid that has one or more asymmetric carbons. A coordination complex that possesses the proper asymmetry may render ligand field bands optically active.

Bryce and Gurd[30] reported that all ionic species of glycyl-L-alanylglycine show no extrema above 210 nm. Urry and Eyring[31] found that the ORD spectrum of L-histidine can be resolved into contributions from a positive Cotton effect centered at 214 nm attributable to the $n \longrightarrow \pi^*$ electronic transition in the carboxyl group, and a negative Cotton effect centered at 190 nm due to some other electronic transition in the amino or carboxyl group.

Pd(II)-dipeptides show charge transfer in the electronic spectrum starting around 275 nm. The CD peaks nearest this wavelength are less reliable in terms of "pure" d-d electronic transitions.

General Electronic Studies

Pd(II) square-planar compounds do not usually yield a detailed electronic spectrum. One peak is ordinarily observed. Possible energy level diagrams for Pd(II) in D_{4h} symmetry follow:



I II III

The electronic structure will depend on the coordinating ligand. If the symmetry is less than D_{4h} the degeneracy of the dxz and dyz orbitals will be lifted. Three or four spin-allowed electronic transitions are expected in the square planar arrangement. The fourth electronic transition arises from the loss of degeneracy of the Eg level (dxz, dyz).

The electronic transitions are not assigned within the framework of LS coupling since this scheme does not hold very well for heavy metals. The singlet-singlet electronic transitions are best described by an orbital $\longrightarrow$ orbital method. For electronic structure III the order of orbitals follows: $z^2 < xz = yz < xy < x^2 - y^2$. The following is the symmetry of these orbitals: $a_{1g} < e_g < b_{2g} < b_{1g}$. The electron transitions are described in orbital form as follows:

$$b_{2g} \longrightarrow b_{1g}$$

$$e_g \longrightarrow b_{1g}$$

$$a_{1g} \longrightarrow b_{1g}$$

The symmetry of the total electronic wave function is as follows:

$$^1A_{1g} \longrightarrow {}^1A_{2g}$$

$$^1A_{1g} \longrightarrow {}^1E_g$$

$$^1A_{1g} \longrightarrow {}^1B_{1g}$$

These electronic transitions are usually energetically similar and form one peak of varying width.

General Nuclear Magnetic Resonance Studies

Morlino and Martin[32] studied 19 L-aminoacylglycine dipeptides. In most compounds the methylene hydrogens of the glycine residue absorbed as a singlet when in the cationic or anionic form. In the zwitterion form the methylene protons appeared magnetically nonequivalent and formed an AB quartet. They were unable to find any single unifying factor on the beta carbon that would account for the magnitude of the splitting. The two methylene protons are not related by a symmetry operation; therefore, they are not equivalent and even rotation around the planar amide bond would not make them so. The authors did not attach a great deal of significance to rotamers as a cause of magnetic nonequivalence.

Nakamura and Jardetzky[33] discussed the nonequivalence of the methylene protons in terms of the existence of preferred rotamers and of incomplete averaging of electric field gradients in the presence of free rotation. They concluded that the observed nonequivalence largely reflects the restriction of rotation and stated that this does not indicate a certain number of rotamers but rather "a range of probable and less probable rotamers". Nakamura and Jardetzky reported chemical shifts of glycine methylene protons (from DDS) in peptides as follows:

1.	glycylamino acid cations	231.8 CPS
2.	glycylamino acid zwitterion	228.8 CPS
3.	glycylamino acid anions	199.0 CPS
4.	aminoacylglycine cations	242.5 CPS
5.	aminoacylglycine zwitterions	227.2 CPS
6.	aminoacylglycine anions	225.0 CPS

CHAPTER II

EXPERIMENTAL

INSTRUMENTATION AND CHEMICALS

Infrared spectra were determined on Perkin-Elmer models 137 and 421 spectrophotometers. All samples were prepared for infrared analysis in the KBr pellet form. Models 137 and 421 scanned 4000—667 cm^{-1} and 4000—200 cm^{-1}, respectively.

Circular dichroism and ultraviolet spectra were run on a JASCO J-10B spectrophotometer.

All nuclear magnetic resonance spectra were recorded on a Varian HA-100 spectrometer.

A Fisher research model 320 Acumet pH meter was used to obtain pH and pD values. For nuclear magnetic resonance studies a microprobe combination electrode (cat. no. 13-639-92) was used for samples as small as 3 ml. For most other work a pH sensitive glass electrode (cat. no. 13-639-3) and a calomel reference electrode (cat. no. 13-639-51) were used. The electrodes were purchased from Fisher Scientific.

All dipeptides were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. $PtCl_2$ and $PdBr_2$ were supplied by K and K Laboratories, Inc., Plainview, New York. K_2PtCl_4, D_2O (99.75% pure), a 40% solution of NaOD, and $K\left[(C_2H_4)Cl_3Pt\right]$ were purchased from Alfa Inorganics, Beverly, Mass. K_2PdCl_4 was obtained from Research Organic/Inorganic Chemical Corporation,

Sun Valley, California. All these chemicals were used without further purification.

Elemental analyses and molecular weight determinations were done by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

INSTRUMENTAL METHODS

Circular Dichroism Spectra

Solutions 1.00×10^{-3} molar in each Pd(II)-dipeptide complex were prepared by dissolving the dipeptide in about 50 ml of water then adding K_2PdCl_4. The dipeptide and K_2PdCl_4 were added in equal amounts of 1.00×10^{-4} mole. The solution was allowed to sit for 1 hr to reach a minimum pH value near 3.0. The pH of each solution was then set with HCl or KOH solutions to values near 1.3, 7.15, 11.7, and 13.0. A fifth sample with a pH near 3.0 was made with no adjustment with acid or base. Spectra were scanned from 500 to about 280 nm for each Pd(II)-dipeptide combination at pH values near 1.3, 3.0, 7.15, 11.7, and 13.0.

All circular dichroism data are shown as λ versus $\Delta\epsilon$(nm. $\Delta\epsilon$ is $\epsilon_L - \epsilon_R$ and is calculated as a molar quantity by the following equation:

$$\Delta\epsilon = \frac{(H)(S)}{(L)(dm)}$$

S is the scale factor and was 0.002 for all determinations. The concentration is given in terms of decimoles per liter (dm). L is the length of the light path in cm (a 1-cm cuvette was used in all determinations). H is the distance in cm from the base line to the curve at any wavelength. A base line was run every few samples.

Stability of the base line depends upon the stability of the instrument and the Xe-lamp light source.

Electronic Spectra

The electronic spectra of 1:1 ratios of K_2PdCl_4 and of each dipeptide were run at pH values near 1.3, 3.0, 7.15, 11.7, and 13.0. These were taken in conjunction with the circular dichroism spectra.

Nuclear Magnetic Resonance Spectra

The nuclear magnetic resonance spectra of GlyVal in zwitterion form (pD 5.71) and of approximately 0.1M GlyVal-K_2PdCl_4 solutions at pD values of 2.96, 7.11, and 13.06 were determined. These values were chosen to correlate with the pH values used in the circular dichroism studies. D_2O was used as the solvent for all samples. Tetramethylsilane served as an external reference. A sweep width of 500 Hz with an offset of 80 Hz was employed.

pH was converted to pD by the following relationship: pD = pH + 0.41.[34] This relationship holds when the pH meter is standardized with a regular protonic buffer. If a deuterium buffer system is used, the pH meter readings will be pD values and need no correction. By using a microprobe combination electrode, pD values were determined for sample volumes as small as 2-3 ml.

GlyVal was prepared in zwitterion forms by saturating 3 ml of D_2O with GlyVal. Greenstein and Winitz[35] related that pH approaches pI (isoelectric pH) as the concentration of a simple dipeptide increases. The pI of GlyVal is 5.71. A saturated solution of GlyVal gave a pH of 5.30 and a pD of 5.71.

Solutions of K_2PdCl_4—GlyVal were prepared by adding GlyVal (0.0174 g, 0.0001 mole) and then K_2PdCl_4 (0.0327 g, 0.0001 mole) per ml of D_2O. A pD of 2.96 was obtained for a 0.1 M solution of the complex. Solutions were adjusted to pD values of 7.11 and 13.06 by addition of a solution of NaOD in D_2O.

PREPARATION OF COMPOUNDS

Dibromobis(valylvaline)paladium(II)

ValVal (0.108 g, 0.0005 mole) and $PdBr_2$ (0.133 g, 0.0005 mole) were refluxed in acetone for about 24 hr. A yellow-brown solution indicated that the reaction had gone to completion. The solution was filtered through fritted glass to remove excess ligand or $PdBr_2$ and then allowed to evaporate. It was extracted five times into the ethyl acetate layer of an ethyl acetate-water mixture. After drying, the precipitate was redissolved in ethyl acetate and reprecipitated by addition of petroleum ether. The compound was again dissolved, precipitated, and placed in a vacuum desiccator for 48 hr. Anal. Calcd for $PdBr_2(ValVal)_2$: C, 34.37; H, 5.78; Br, 22.86; N, 8.01; Pd, 15.23. Found: C, 34.50; H, 5.67; Br, 22.66; N, 7.51; Pd, 15.60. mp: 153-157°C. Yield: 19%.

Dichlorobis(valylleucine)platinum(II)

$PtCl_2$ (0.133 g, 0.0005 mole) and ValLeu (0.115 g, 0.0005 mole) were refluxed in acetone for about 24 hr, after which the solvent attained a yellow color, indicating that the reaction was essentially complete. The solution was slowly filtered to remove unreacted or altered starting materials, then was allowed to

evaporate to dryness and was dissolved in ethyl acetate. Small portions of the ethyl acetate solution were placed in a separatory funnel and extracted three times with water. The resulting ethyl acetate solution was allowed to evaporate to dryness and was redissolved in ethyl acetate. The compound was precipitated from this solution by dropwise addition of petroleum ether. It was dissolved and precipitated again before vacuum desiccation. Anal. Calcd for $PtCl_2(ValLeu)_2$: C, 35.32; H, 6.08; Cl, 9.76; N, 7.71; Pt, 29.72. Found: C, 35.32; H, 5.72; Cl, 10.51; N, 6.36; Pt, 29.72. mp: 174-180°C. Yield: 14%.

Potassium hydroxo(valylvalinato)pallidate(II) (Structure, p 27)

K_2PdCl_4 (0.1633 g, 0.0005 mole) was added to 100 ml of H_2O. ValVal (0.108 g, 0.0005 mole) was added to this solution. The reaction mixture was titrated with 0.1M KOH to pH 10.5, evaporated to dryness in a "rotovapor" apparatus, then placed in a vacuum desiccator for 24 hr. Approximately 50 ml of 95% ethanol was added to the flask to dissolve the complex and leave the KCl that is displaced by the ligand in the reaction. The solution was filtered through a fritted glass funnel, evaporated to dryness, then treated again with about 50 ml of 95% ethanol. This procedure was repeated until the product gave a negative chloride test when treated with 0.1M of $AgNO_3$ solution. At this point it was assumed that the ligands in the coordination sphere of Pd(II) were hydroxo and the dipeptide. The compound was precipitated by adding dropwise an ethanolic solution of product into petroleum ether. The product was filtered, dissolved in 95% ethanol, and precipitated again by

adding dropwise to petroleum ether. It was then vacuum desiccated for seven days before elemental analysis was performed. Anal. Calcd for K[OH(ValVal)Pd]: C, 31.89; H, 5.96; K, 10.3; N, 7.43; Pd, 28.26. Found: C, 37.82; H, 6.32; K, 6.28; N, 7.19; Pd, 25.19. Dec: 205°C. Yield: 25%.

Potassium hydroxo(valylleucinato)pallidate(II) (Structure, p 27)

The procedure for making this compound follows exactly that for potassium hydroxo(valylvalinato)pallidate(II). K_2PdCl_4 (0.1633 g, 0.0005 mole) and ValLeu (0.115 g, 0.0005 mole) were used in this synthesis. Anal. Calcd for K[OH(ValLeu)Pd]: C, 33.8; H, 5.43; K, 10.01; N, 7.17; Pd, 27.25. Found: C, 35.69; H, 6.08; K, 6.65; N, 7.22; Pd, 24.97. Dec: 205°C. Yield: 53%.

Potassium hydroxo(leucylvalinato)pallidate(II) (Structure, p 27)

The procedure for making this compound follows exactly that for potassium hydroxo(valylvalinato)pallidate(II). K_2PdCl_4 (0.1633 g, 0.0005 mole) and LeuVal (0.115 g, 0.0005 mole) were used in this synthesis. Anal. Calcd for K[OH(LeuVal)Pd]: C, 33.8; H, 5.43; K, 10.01; N, 7.17; Pd, 27.25. Found: C, 35.68; H, 5.99; K, 5.63; N, 8.67; Pd, 25.40. Dec: 220°C. Yield: 25%.

Potassium hydroxo(leucylleucinato)pallidate(II) (Structure, p 27)

The procedure for making this compound follows exactly that for potassium hydroxo(valylvalinato)pallidate(II). K_2PdCl_4 (0.1633 g, 0.0005 mole) and LeuLeu (0.122 g, 0.0005 mole) were used in this synthesis. Anal. Calcd for K[OH(LeuLeu)Pd]: C, 35.6; H, 6.20;

K, 9.67; N, 6.92, Pd, 26.3. Found: C, 36.12; H, 5.43; K, 6.24; N, 6.27; Pd, 24.12. Dec: 195°C. Yield: 42%.

μ-(N-trans-O-trans-valinato)-μ-(O-cis-N-cis-valinato)chloro (ethene)platinum(II) (Structure, p 36)

Zeise's salt, $K[(C_2H_4)Cl_3Pt]$, (0.0714 g, 0.000185 mole) was added to 200 ml of stirred water. Zeise's salt was allowed to dissolve before ValVal (0.0401 g, 0.000185 mole) was added. Needle-like crystals began to form over a 24-36 hr period at room temperature. These crystals were filtered and washed with distilled water five times before vacuum desiccation for 48 hr. Anal. Calcd for above cmpd: C, 22.43; H, 3.77; Cl, 9.46; N, 3.76; Pt, 52.06; Mol wt, 749.5. Found: C, 25.22, 22.02; H, 3.71, 3.75; Cl, 9.03, 9.06; N, 3.91; Pt, 56.64; Mol wt, 846, 827. Yield: 19.5%. Dec: 170°C.

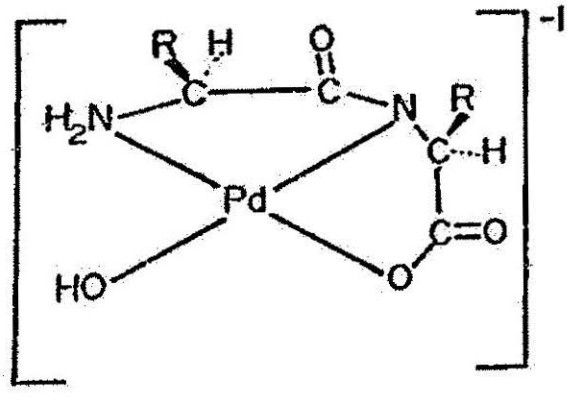
μ-(N-trans-O-trans-leucinato)-μ-(O-cis-N-cis-valinato)chloro (ethene)platinum(II) (Structure, p 37)

Zeise's salt (0.0328 g, 0.00085 mole) was added to 200 ml of stirred water. Zeise's salt was allowed to dissolve before Leu-Val (0.0197 g, 0.00085 mole) was added. Needle-like crystals began to form over a 24-36 hr period at room temperature. These crystals were filtered and washed with distilled water five times before being vacuum desiccated for 48 hr. Anal. Calcd for above cmpd: C, 23.59; H, 3.97; Cl, 9.28; N, 3.67; Pt, 51.11; Mol wt, 763.4. Found: C, 24.14, 23.22; H, 4.09, 3.93; Cl, 5.94, 8.97; N, 3.89; Pt, 52.51; Mol wt, 839. Dec: 190°C. Yield: 9.8%.

μ-(N-trans-O-cis-valinato)-μ-(O-cis-N-trans-leucinato)chloro (ethene)platinum(II) (Structure, p 38)

Zeise's salt (0.0639 g, 0.000162 mole) was added to 200 ml of stirred water. Zeise's salt was allowed to dissolve before Val-Leu was added. Feather-like crystals began to form over a 24-36 hr period at room temperature. These crystals were filtered and washed with distilled water five times before being vacuum desiccated for 48 hr. Anal. Calcd for above cmpd: C, 23.59; H, 3.97; Cl, 9.28; N, 3.67; Pt, 51.11; Mol wt, 763.4. Found: C, 24.62, 24.13; H, 4.04, 3.98; Cl, 8.56, 8.6; N, 3.67; Pt, 54.04, 51.82; Mol wt, 763. Dec: 188°C. Yield: 19.5%.

R is isopropyl and isobutyl for valyl and leucyl residues, respectively, in K[OH(XX)Pd] compounds (pp 24, 25). The general structure is as follows:



CHAPTER III

RESULTS AND DISCUSSION

There is sufficient difference between Pt(II)- and Pd(II)-dipeptide studies to warrant their inclusion in separate sections of this chapter. Certain Zeise's salt-dipeptide procedures are also included here rather than in the preceding chapter since no products resulted or since only an intermediate of doubtful purity was isolated.

Pt(II)-DIPEPTIDE STUDIES

The first compounds that were used as a source of Pt(II) were $PtCl_2$ and K_2PtCl_4. Both of these starting materials, when combined with a dipeptide, gave no infrared evidence for reaction in the peptide region. There were no significant shifts of the Amide I band in any of the products, whereas the K_2PdCl_4-dipeptide complexes had a shift of at least 50 cm^{-1} toward lower energy.

Infrared analysis (Figure 35) indicated that the amino nitrogen of ValLeu was attached to Pt(II) in $PtCl_2(ValLeu)_2$ but not to the peptide linkage nor to the carboxylate group. There was a new peak at 1720 cm^{-1} due to the formation of COOH from COO^{-1} after migration of an "ammonium" hydrogen upon coordination of the ligand. There was an N-H stretching frequency at about 3150 cm^{-1} in both ValLeu and $PtCl_2(ValLeu)_2$. Usually ν(N-H) is greater when the nitrogen atom is bonded to Pt(II) than when bonded to a proton.

The Amide I band at 1680 cm^{-1} was essentially the same in the complex and in the ligand. Elemental analysis (page 24) and infrared analysis indicate $PtCl_2(ValLeu)_2$ as the formula for this compound.

K_2PtCl_4 was used in a second attempt to react Pt(II) with the peptide linkage. There was again no indication of bonding at the peptide linkage of the ligand.

1 mmole each of LeuLeu (0.244 g) and K_2PtCl_4 (0.431 g) were dissolved in 125 ml of water in sequence. The pH of the LeuLeu solution was 5.60. After adding K_2PtCl_4 the pH was essentially the same. This solution was titrated with 1.00 M KOH. There was an equivalence point when 1.00 meq of KOH had been added, indicating the titration of the "ammonium" hydrogen of the amino group. 3 meq of KOH were added, which raised the pH to 11.95. This is essentially the pH of 3 meq of KOH in 125 ml of H_2O. There apparently had been little reaction of hydroxide with K_2PtCl_4 or with LeuLeu after the "ammonium" hydrogen had been neutralized. Infrared analysis (Figure 36) showed no appreciable shift of the Amide I band before or after extraction of the residue with ethanol.

At 75°C. a mixture of 1 mmole of ValVal and 1 mmole of K_2PtCl_4 was rendered black in 45 min. The pure compounds K_2PtCl_4 and K_2PdCl_4 also decomposed when heated in water. An aqueous solution of K_2PtCl_4 decomposed after about 5 days at room temperature. K_2PtCl_4 was not a promising Pt(II) source for reaction with dipeptides.

Since there had been no indication of the bonding of Pt(II) with the peptide region from $PtCl_2$ and K_2PtCl_4, a third source of Pt(II) was selected. Zeise's salt, $K[(C_2H_4)PtCl_3]$, was chosen due

to the unusual reactivities of the cis- and trans-chloro groups. The chloro group trans to ethylene is much more reactive toward substitution than are the cis-chloro groups. It was postulated that the trans position would react with the amino nitrogen, leaving the peptide region in near proximity of the cis-chloro group for a second reaction.

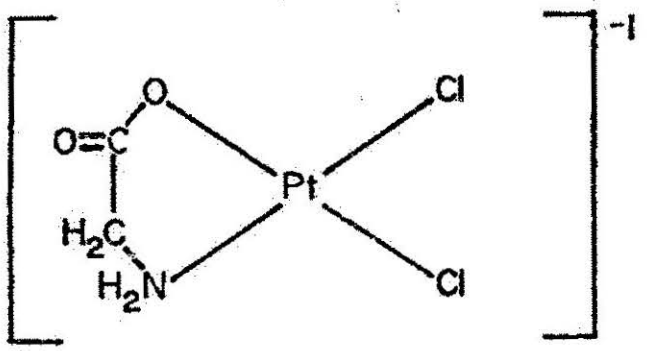
Zeise's salt gave crystalline precipitates with ValVal, LeuVal, and ValLeu (procedures on pp. 26, 27). ValGly, LeuGly, GlyLeu, GlyVal, and GlyGly did not give a precipitate with Zeise's salt. With the exception of GlyGly, there was decomposition resulting in a dark color of all solutions in which the dipeptide had glycine as the first residue. Reaction mixtures of GlyGly, LeuGly, and ValGly remained for weeks with no change in color from the original yellow solution.

Solutions of Zeise's salt and ValVal, LeuVal, or ValLeu (the dipeptides that did yield precipitates) began to develop a dark color after about 24-36 hr. The solution of the Zeise's salt with ValVal was especially prone to decomposition. In some cases decomposition began to occur about the same time that the maximum yield took place; therefore, the crystals had to be removed before darkening of the solution began.

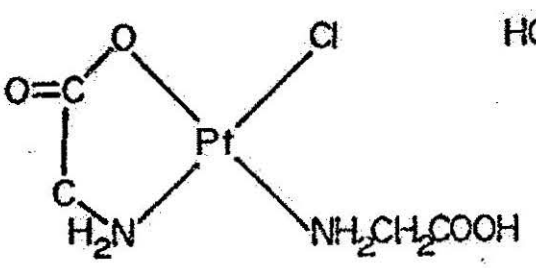
Attempts to dissolve the crystals in acetone or ethanol to reprecipitate with addition of water led to powdery products of doubtful purity. Since the crystals were formed very slowly from dilute solutions they were not recrystallized, but were filtered from solution and washed five times with demineralized water before desiccation.

The crystals obtained from ValVal and LeuVal were needle-like and in some cases were about an inch in length. The product of Zeise's salt and ValLeu was feather-like. All three crystalline materials were soluble in acetone. The product of Zeise's salt and ValVal was also soluble in ethanol. All three compounds were insoluble in water and nonpolar solvents. Molecular weight determinations and elemental analyses (pp. 26, 27) indicated that these products were dimers with the peptide linkage fissioned.

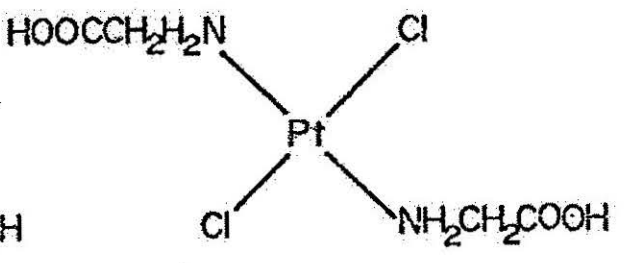
Several compounds of interest have been synthesized and subjected to infrared analysis.[36,37] They are as follows:



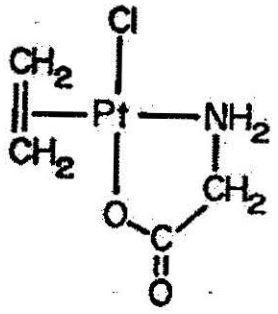
I



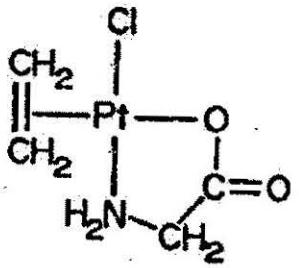
II



III



IV



V

The infrared spectra of ZS-VV and ZS-LV (the products of Zeise's salt and ValVal and Zeise's salt and LeuVal) are similar to those of compounds IV and V as published by Fujita, Konya, and Nakamoto.[37] This similarity would be expected of a dimer that is related to both compounds IV and V, being both O-*trans* and N-*trans* with respect to ethylene.

Compound I has bands at 1643 and 1575 cm^{-1} that are similar to the absorptions at 1660 and 1560 cm^{-1}; 1660 and 1560 cm^{-1}; and 1660 and 1565 cm^{-1} for ZS-VV, ZS-VL, and ZS-LV, respectively (Figures 38, 39, 40). Compounds IV and V exhibit these two absorptions at essentially the same frequencies as the compounds synthesized in this work. Condrate and Nakamoto[38] give 1643, 1642, 1593, and 1589 cm^{-1}, respectively, for the *trans* compounds $Pt(Gly)_2$, $Pd(Gly)_2$, $Cu(Gly)_2$, and $Ni(Gly)_2$ as due to ν(C=O) of COO-M. Nakamoto *et al.*[39] have made extensive measurements of COO stretching frequencies of metal-amino acid complexes in D_2O solution and in the hydrated and anhydrous crystalline state. In general the antisymmetric frequencies increase, the symmetric frequencies decrease,

and the separation between the two frequencies increases in the following order of metals:

$$Ni(II) < Zn(II) < Cu(II) < Co(II) < Pd(II) \approx Pt(II) < Cr(III)$$

This is explained by assuming that the covalent character of the M-X bond increases along the series leading to a more asymmetrical carboxylate group. This results in an increased frequency separation of the two bands.

$Pt(Val)_2$ and $Pt(Leu)_2$ have frequency separations for the symmetric and antisymmetric COO modes of vibration of 285 and 261 cm^{-1}, respectively.[40, 41] ZS-VV, ZS-VL, and ZS-LV each have frequency separations of 295 cm^{-1}. Both the compounds in this work and compounds IV and V have ν(C=O) and $\delta(NH_2)$ at higher frequencies than compound I. This is commensurate with stronger bonding of the amino acid to Pt(II) when Pt(II) is also coordinated with ethylene.

Glycine may also coordinate as a monodentate ligand with Pt(II) with its amino nitrogen, leaving a free carboxyl group. In compounds II and III the carboxyl-carbonyl group absorbs at 1730 and 1708 cm^{-1}, respectively. Compounds I, IV, V, and those prepared in this work have no bands in this area, implying that the carboxylate group is coordinated with Pt(II). In trans-$[Pt(Gly)_2(NH_3)_2]$ the antisymmetric stretching frequency of ionized COO^- is near 1610 cm^{-1}.[36] $Pt(Gly)_2$ has a band at 1610 cm^{-1} that is assigned to $\delta(NH_2)$.[38] This corresponds to 1560, 1560, 1565 cm^{-1} for ZS-VV, ZS-VL, and ZS-LV, respectively. Compounds I, II, and III have this absorption at 1575, 1570, and 1576 cm^{-1}. Fujita,

Konya, and Nakamoto[37] did not assign this band in compounds IV and V (they aasign only ν (Pt-Cl) as proof of structure).

Bair[42] reports 1600-1622 cm^{-1} as $\delta(NH_2)$ in Cu(II) complexes of GlyGly, GlyLeu, LeuGly, and GlyGlyGly. As the strength of the M-N bond increases, the N-H stretching frequencies of $M(NH_3)_x$ decrease, and the bending and deformation frequencies are increased.[43] The compounds synthesized in this work and compounds IV and V have $\delta(NH_2)$ at higher frequencies than compound I. This is commensurate with stronger bonding of the amino acids in Zeise's salt type compounds.

NH_2 stretching frequencies (Table 3) are higher than those given for valine and leucine.[40,41] They are 3132 and 3108 cm^{-1}, respectively. This behavior is expected since the N-H bond order is lower in NH_3^+ than in M—NH_2—.

Chatt, et al.[44] give 2920 and 3010 cm^{-1} as ν(C-H) for the bound ethylene of Zeise's salt. 2922 and 3018 cm^{-1}; 2930 and 3010 cm^{-1}; 2930 and 3010 cm^{-1} are reported as ν (C-H) for the coordinated ethylene in ZS-VV, ZS-VL, and ZS-LV, respectively.

All ν (C-H) for $Pt(Val)_2$,[40] $Pt(Leu)_2$,[41] and ZS-VV, ZS-VL, and ZS-LV is reported in Table 3, along with frequencies for NH_2 stretching, COO^{-1} symmetric and antisymmetric stretch, NH_2 scissoring, CH_3-degenerate deformation, and CH_3-symmetric deformation. These series show the principal absorptions that relate to the structures of the compounds synthesized in this work.

Chatt, et al.[44] relate that C=C stretching bands cannot be observed even in Zeise's salt. Asymmetrical olefins exhibit C=C stretching bands near 1650 cm^{-1}. This band is shifted to

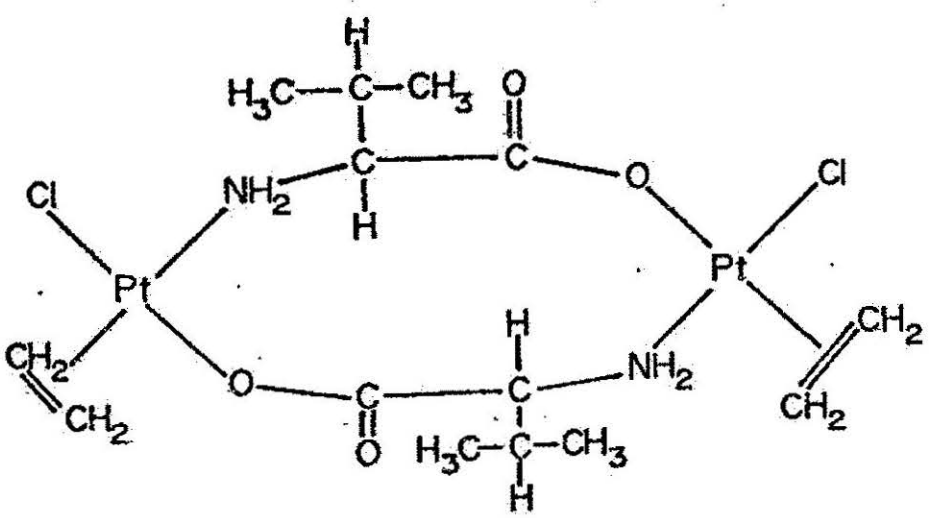
1504 cm^{-1} in $K[PtCl_3(C_3H_6)] \cdot H_2O$. Since the C=C axis is parallel to the plane of $PtCl_3$, Powell and Sheppard[45] attempted to assign all bands observed in Zeise's salt on the basis of C_{2v} symmetry. They concluded that the fundamental frequencies of ethylene change little upon coordination.

Nakagawa, et al.[40] assign the bands from 1620-1200 cm^{-1} in $Pt(Val)_2$ to CH_3 degenerate and symmetric deformation vibrations and to C-H bending vibrations corresponding to similar modes in DL-valine. In the region from 1200 to 800 cm^{-1} the fundamental modes for $Pt(Val)_2$ are NH_2 wagging and twisting, CH_3 rocking, and skeletal stretching vibrations. The NH_2 rocking frequency is given by Jackovitz and Walter[41] as 801 cm^{-1}. In ZS-VV and ZS-LV this corresponds to 795 cm^{-1}. In ZS-VL, assignment is uncertain since bands are at 770 and 820 cm^{-1}.

Absorptions occur as follows for ZS-VV, ZS-VL, and ZS-LV: 342 and 358 cm^{-1}; 340 cm^{-1}; 342 and 358 cm^{-1}. With the exception of ZS-VL, two frequencies for ν (Pt-Cl) are observed. Compound I shows doublet structure for ν (Pt-Cl) since the $PtCl_2$ moiety is in a cis configuration, whereas compound II whows a singlet peak. Compounds I, II, and III have ν (Pt-Cl) near 350 cm^{-1}.

Compound I shows ν (Pt-O) at 388 cm^{-1}. Compound III in which glycine is bonded with Pt(II) through only the amino nitrogen, shows no absorption between 470 and 350 cm^{-1}. Compound II shows ν (Pt-O) at 407 cm^{-1}. Compounds IV and V show peaks near 410 and 415 cm^{-1}, respectively. ZS-VV, ZS-VL, and ZS-LV have absorption peaks as follows: 387 and 399 cm^{-1}, 370 cm^{-1}, 384 and 399 cm^{-1}. These bands are assigned to ν (Pt-O).

There are strong absorption bands at 585, 605, and 589 cm^{-1} for compounds I, II, and III, respectively. Compounds IV and V have peaks near 590 and 610 cm^{-1}. Condrate and Nakamoto[38] have carried out normal coordinate analysis of bis(glycine) complexes of Pt(II), Pd(II), Cu(II), and Ni(II). They showed that C=O stretching, NH_2 rocking, M-N and M-O stretching are metal-sensitive and are shifted progressively to higher frequencies in the order Ni(II) < Cu(II) < Pd(II) < Pt(II). They listed ν (Pt-N) as 549 cm^{-1} in trans-$Pt(Gly)_2$. Peaks assigned to ν (Pt-N) in ZS-VV, ZS-VL, and ZS-LV are given as 575 and 585 cm^{-1}, 570 and 580 cm^{-1}, 575 cm^{-1}, respectively. The structure for ZS-VV is given as follows:



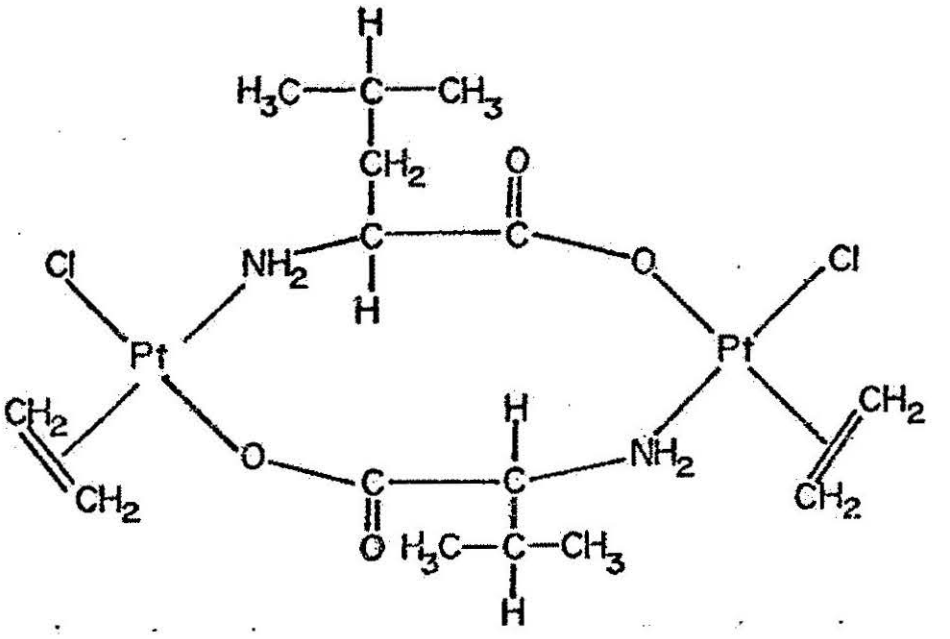
VI

This structure, μ-(N-trans-O-trans-valinato)-μ-(O-cis-N-cis-valinato)chloro(ethene)platinum(II), is consistent with cis-trans sensitive modes of vibration as seen with doublet bands for

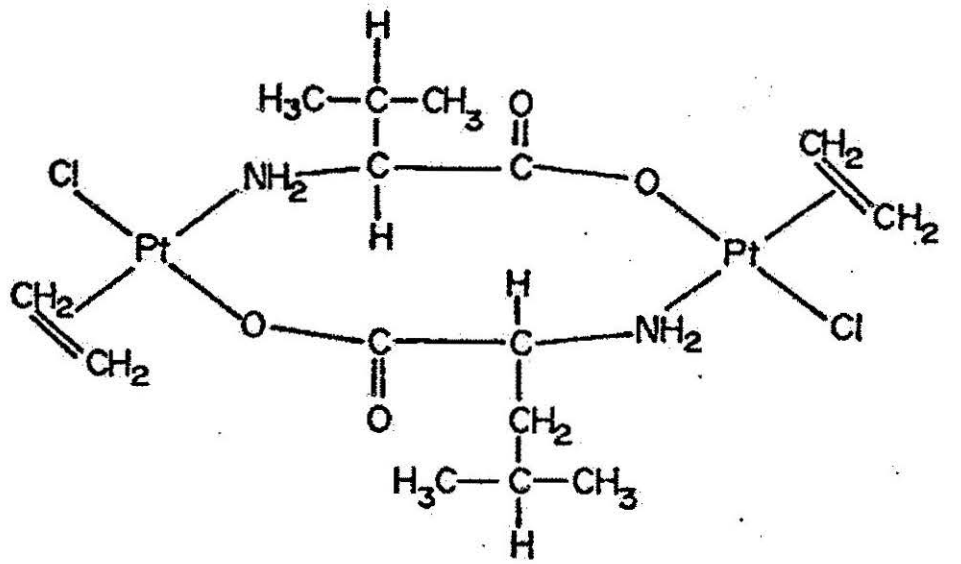
ν (Pt-Cl), ν (Pt-O), and ν (Pt-N). Spectra taken on a Perkin-Elmer 137 instrument show ν (C=O) and δ (NH_2) as sharp peaks that are very similar to those published for compounds I, IV, and V. The compounds all have glycine in a chelated form. An infrared spectrum (Figure 37) of an intermediate of ZS-VV shows doublet structure in the area of δ (NH_2) absorption. This is probably due to both δ (NH_2) and Amide II absorption of the peptide linkage. The bands of the intermediate are at 1540 and 1520 cm^{-1}, whereas δ (NH_2) for ZS-VV is a sharp peak at 1560 cm^{-1}.

The ν (Pt-Cl) bands at 358 and 342 cm^{-1} compare with 360 of the N-trans isomer and 340 of the O-trans isomer of chloro-(glycino)(ethylene)platinum(II), compounds IV and V. The doublet structure of ν (Pt-O) and ν (Pt-N) also agree with this prediction.

ZS-LV, μ-(N-trans-O-trans-leucinato)-μ-(O-cis-N-cis-valinato)chloro(ethene)platinum(II) is assigned the following, as structure VII:



ZS-VL,μ -(N-trans-O-cis-valinato)-μ-(O-cis-N-trans-leucinato)chloro(ethene)platinum(II), is assigned a structure that has both amino acid units N-trans, as follows:



VIII

Structure VII has ν (Pt-Cl) absorption that is similar in position and in doublet structure to that of structure VI, whereas structure VIII has a single peak at 340 cm^{-1}. Since structures VI and VII have doublet ν (Pt-Cl) absorption and IV and V superimpose to show the same, it seems reasonable that structure VIII is either O-trans—O-trans or N-trans—N-trans. Chemical evidence points to the N-trans—N-trans configuration as the correct structure. For example, reaction of glycine with Zeise's salt by Kieft and Nakamoto[36] gave the N-trans isomer of chloro(glycino)(ethylene)platinum (II) by direct treatment of Zeise's salt with glycine. In order to make the O-trans isomer, $K[PtCl_2Gly]$ was dissolved in a minimum

amount of 2 M HCl and ethylene was passed through the solution. After working up, the O-trans isomer was obtained. Consequently, it is seen that the natural order of reactivity is through the trans position.

The formation of cleaved peptide bond compounds does not occur immediately when Zeise's salt is reacted with a dipeptide. It was possible to isolate an intermediate that had a free carboxyl group, as shown in the following procedure:

Zeise's salt (0.0574 g, 0.000149 mole) and ValVal (0.0322 g, 0.000149 mole) were weighed out in a 1:1 ratio. ValVal was added to 30 ml of water and dissolved, then Zeise's salt was stirred in. The reaction mixture was left in an ice bath 5 min, then placed in a water bath at 45°C. for another 5 min. The beaker and contents were allowed to come to room temperature and to sit for 1 hr. The solution was evaporated by means of a "rotovapor" apparatus and placed in a vacuum desiccator. An infrared spectrum was run which showed carboxyl-carbonyl absorption at 1720 cm^{-1} but no appreciable change in the Amide I band.(Figure 37). These results indicate that ValVal was acting as a monodentate ligand, coordinating with Pt(II) at the amino nitrogen. When ethanol was added to the dry products of the above reaction, a yellow solution and a white precipitate of the displaced KCl resulted.

The effect of pH on the formation of crystals from the reaction of Zeise's salt and ValVal gave the following results:

1. The initial pH of a solution made up as directed (p 26) was 4.1 - 4.2. Crystals formed over a period of 24-36 hr. The final pH was 3.3.

2. A solution of Zeise's salt and ValVal had its pH adjusted with KOH from an initial 4.16 to 10.4. No crystals formed.

3. A solution of Zeise's salt and ValVal had its pH adjusted to 7.00. The pH dropped to 4.83 as crystals slowly formed. The crystals that formed at pH 7.00 and at 4.16 had identical infrared spectra (Figure 38).

In view of the work presented here with Zeise's salt and dipeptides, several areas of further research become apparent. One such area would be the X-ray analysis of the crystals obtained from the reaction of Zeise's salt with ValVal. Preliminary investigation under polarized light has shown that this product has suitable properties for X-ray study.

Also of interest would be the investigation of the mechanism for the reaction between Zeise's salt and dipeptides. This should be done with reference to size and relative position of R groups on the dipeptides. pCl and pH versus time studies would be of value here.

A logical extension of the work presented in this research would be a study of the reaction between Zeise's salt and tripeptides as well as between Zeise's salt and sulfur-containing di- and tripeptides.

The investigation of Zeise's salt as a potential anticarcinogenic agent would be of particular interest. Studies have been done showing that cis-diamminedichloroplatinum(II) and cis-diammine-tetrachloroplatinum(IV) have anticarcinogenic properties, while the trans isomers of these compounds do not.[1,2,3] It is possible that

the fissioning of the peptide linkage of ValVal, LeuVal, and ValLeu by Zeise's salt may be related to a Pt(II) protein reaction.

Pd(II)-DIPEPTIDE STUDIES

The first compound that was selected as a source of Pd(II) for reaction with dipeptides was $PdBr_2$. This reacted with ValVal in acetone to form $PdBr_2(ValVal)_2$. (The procedure for this synthesis and the elemental analysis are given on p 23.)

The infrared spectrum of ValVal indicates the absence of the carboxyl group since there is no absorption near 1730 cm^{-1}. However, $PdBr_2(ValVal)_2$ has a strong absorption at 1730 cm^{-1} due to the presence of the carboxyl group. This group arises from the migration of a proton from the "ammonium" nitrogen of the dipeptide to a carboxylate oxygen upon coordination of the amino group to Pd(II).

Comparison of N-H stretching frequencies of the ligand and the complex indicates that the amino nitrogen of ValVal is bonded to Pd(II) in $PdBr_2(ValVal)_2$. There are absorptions at 3230, 3125, and 3270 cm^{-1} in $PdBr_2(ValVal)_2$ and comparable absorptions at 3170 and 3078 cm^{-1} in ValVal. N-H stretching frequencies are expected to be higher in $M—NH_2—$ than in $H—NH_2^+—$ since the N-H bond order is lower in the "ammonium" group.[40] Nakagawa et al.[40] assigned 3246 and 3115 cm^{-1} to NH_2 stretching modes in PdR_2, where R is DL-valine. N-H stretching modes in $NH_3^+—$ of DL-valine were given as 3132 and 2570 cm^{-1}.

The Amide I band of $PdBr_2(ValVal)_2$ was found at 1655 cm^{-1} whereas absorption in ValVal occurred at 1680 cm^{-1}. Even though

there is a lowering of the frequency of the Amide I band by 25 cm^{-1} upon the complexation of ValVal, the peptide linkage is not bonded to Pd(II). The Amide I band of amides is sensitive to hydrogen bonding.[23] The difference in Amide I absorption is probably due to differences in hydrogen bonding in the two compounds.

Primary and secondary amides show a strong absorption in the 1600 - 1500 cm^{-1} region. N-H deformation is likely to be the predominant factor in this band.[25] The infrared spectrum of $PdBr_2(ValVal)_2$ has bands of equal intensity at 1547 and 1532 cm^{-1}, which are assigned as N-H deformation. In the case of ValVal there is a great amount of structure in the 1600 - 1500 cm^{-1} region; consequently, assignment of bands is not a certainty. ValVal has strong absorptions at 1600 and 1515 cm^{-1} that are probably due to antisymmetric carboxylate stretch and to the Amide II band, respectively. Bellamy[46] assigned 1608 and 1400 cm^{-1} to antisymmetric and symmetric carboxylate stretching in GlyGly.

ValVal has bands at 1405 and 1385 cm^{-1} that appear as a doublet. These probably have their origin in the C-H bending vibrations of the gem-dimethyl groups. $PdBr_2(ValVal)_2$ has absorptions at 1392 and 1360 cm^{-1} with the same general band structure as that given for ValVal.

Bair[48] reported infrared frequencies for GlyGly, LeuGly, GlyLeu, and GlyGlyGly. She assigned the bands in comparison with Gly, Leu, their complexes, and their amides. She did not assign absorptions below 1400 cm^{-1} because of the complex nature of the spectra. Bands below 1360 cm^{-1} are not assigned in research presented here. The infrared spectra of ValVal and $PdBr_2(ValVal)_2$

are listed as Figures 2 and 1 in the Appendix.

While attempting to synthesize a crystal for structural studies, $PdBr_2(ValVal)_2$ was found to be in two forms--orange and golden-yellow. $PdBr_2(ValVal)_2$ was dissolved in chloroform and placed in a TLC cell along with a ground glass adaptor. Tape was placed around the lid of the cell so that evaporation could proceed very slowly. After about ten days, golden-yellow platelets and an orange precipitate had formed on the ground glass. Weissenberg studies indicated that the platelets had definite structure but did not possess enough for X-ray determination. The two forms gave identical infrared spectra in the 4000 - 666 cm^{-1} range.

Since lowering of symmetry usually causes d-d electronic transitions to be more probable, the cis form of MX_2Y_2 is ordinarily found to have a more intense color than the trans form. The orange precipitate is probably the cis form since its color is more intense than the golden-yellow platelets. Cis-trans isomerism seems to be a likely explanation for the two compounds since their infrared spectra are the same; but their colors, intensities of color, and crystallization properties are different.

In 1971 Wilson and Martin[4] published a solution circular dichroism study of Pd(II)-dipeptide complexes obtained at a pH near 6. Their work showed that two hydrogen ions were removed from a 1:1 Na_2PdCl_4-GlyGly solution at pH 6 when titrated with a base. They assigned the amino nitrogen, the amide nitrogen, and the carboxylate oxygen as donor atoms to Pd(II) at pH values near 7. No infrared measurements were made and no compounds were isolated.

In this present work, an attempt was made to isolate and characterize chelated compounds obtained from the reaction of K_2PdCl_4 with the dipeptides LeuLeu, LeuVal, ValLeu, and ValVal at pH 10.5 (pp 24-26). Elemental analysis for K[OH(ValVal)Pd], K[OH(LeuVal)Pd], K[OH(ValLeu)Pd], and K[OH(LeuLeu)Pd], did not indicate pure compounds. Circular dichroism spectra showed no isosbestic points in the spectra of Pd(II)-dipeptide complexes at pH values near 3, 7, 11.7, and 13.0.(Figures 10-13), indicating that mixtures of complexes are present in solution. It is doubtful that pure compounds with the above formulas can be isolated.

The N-H stretching frequencies of these complexes are higher than those found for the free dipeptides. The Amide I band decreased at least 50 cm^{-1} with respect to the free dipeptide for each complex. These infrared shifts indicate that both the amino and amide nitrogen atoms are bonded to Pd(II). These values are reported as follows:

	Amide I	ν(N-H)		Amide I	ν(N-H)
K[OH(LeuLeu)Pd]	1617	3290	LeuLeu	1667	3140
K[OH(LeuVal)Pd]	1610	3280 3180	LeuVal	1667	3140
K[OH(ValLeu)Pd]	1611	3290 3200	ValLeu	1690	3170
K[OH(ValVal)Pd]	1611	3180 3310	ValVal	1680	3170 3070

All further studies were done by circular dichroism, nuclear magnetic resonance, and ultraviolet-visible spectroscopies on solutions of Pd(II)-dipeptide complexes.

The results of nuclear magnetic resonance studies of the zwitterion form of GlyVal and Pd(II) complexes of GlyVal (Pd(II)-GV)

at various pD values are presented in conjunction with electronic studies of Pd(II)-VV, Pd(II)-VL, Pd(II)-LV, Pd(II)-LL, Pd(II)-GL, Pd(II)-LG, Pd(II)-VG, and Pd(II)-GV, each at pH values near 1.3, 3.0, 7.15, 11.7, and 13.0. These studies show the different modes of complexation of the dipeptide to Pd(II) at various pH values. The circular dichroism study gives the three-dimensional configuration of the ligand when bound to the amino and amide nitrogen atoms but not to the terminal carboxyl group. All spectra are listed in the Appendix.

The methylene hydrogens of the zwitterion form of GlyVal have a chemical shift of 4.33 ppm. The chemical shift values are 4.01, 4.01, and 3.96 ppm for the Pd(II)-GV complexes at pD values of 2.96, 7.11, and 13.06, respectively. These values are essentially constant and indicate that the amino nitrogen of GlyVal is bound to Pd(II) at all of these pD values. The upfield chemical shift of about 0.3 ppm indicates that Pd(II) is less deshielding on the methylene group than is H^+.

Circular dichroism spectra show a $\Delta\epsilon$ of zero for all Pd(II)-dipeptide combinations at pH 1.3-1.4. Electronic studies (Figure 30) indicate that the d-d transitions observed are those belonging to one or more species of Pd(II)-chloro complexes. Consequently, no complexes were formed at this low pH.

The alpha hydrogen of the valyl residue in GlyVal absorbed as a doublet at 4.53 and 4.58 ppm. This signal is split by the beta hydrogen of the valyl residue (J = 5 cps). At pD 7.11 this doublet was found at 4.37 and 4.40 ppm in the complex. At pD 13.06 the doublet was found at 4.26 and 4.29 ppm. This upfield shift is

commensurate with the carboxyl group, having been displaced from the coordination sphere by OD^- at pD 13.06. The alpha hydrogen is less deshielded in the form $—HC(R)COO^-$ than in the form $—HC(R)COO^-$ Pd(II).

The two methyl groups of the isopropyl unit of GlyVal in the zwitterion form are magnetically nonequivalent and are each split into a doublet by the adjacent proton, giving a quartet structure (J = 7 cps). In Pd(II)-GV these absorptions merged to form a triplet at pD values of 7.11 and 13.06. The triplet at pD 13.06 was upfield from the triplet found at 7.11. This indicates the replacement of the carboxylate group in the coordination sphere of Pd(II) by OD^-.

A triplet and a quartet were found for the complex at pD 2.96. Anomalous structure of the alpha hydrogen doublet was also found at pD 2.96. This indicates a mixture of complexes at this pD. Wilson and Martin[4] reported that Pd(II) induces ionization of the amide hydrogen near pH 3.5. The amide nitrogen falls below the deprotonated amide nitrogen in the spectrochemical series. Therefore, the greater λ (max) at pH 3 compared to pH 7 may be due to this phenomenon.

There was much less circular dichroism near pH 3.0 than at pH 7.0. The molar extinction coefficient for Pd(II)-GV is 685 at pH 3.0 and 870 at pH 7.25 (Table 1). At pH 13.0, ϵ is 528. At pH 7.25 there are two N, one carboxylate O, and probably a mixture of chloro and O from H_2O as donor atoms for Pd(II), whereas there are two N and two O donor atoms at pH 13.0.

If the carboxyl group were bonded to H^+ and to Pd(II), it is believed that the chemical shift values would fall much farther downfield for —COOH than is found experimentally. Therefore, it is probable that the anomalous chemical shift values at pD 2.96 are due to mixtures of complexes utilizing the dipeptide in a tridentate mode but having protonated and deprotonated amide nitrogen atoms as donor atoms.

The center peak for the valyl residue beta hydrogen for the complexes is at 2.68, 2.70, and 2.60 at pD values of 2.96, 7.11, and 13.06, respectively. The upfield chemical shift from 2.70 to 2.60 ppm is still another indication of displacement of the carboxyl group from the coordination sphere.

At a pH near 1.3, the λ (max) of the electronic spectra of the K_2PdCl_4-dipeptide solutions were found to be near 450 nm with ϵ from 168 to 207 (Table 1). These values were essentially those given in the literature for a Pd(II)-chloro complex study.[49] There was no circular dichroism activity at this pH, indicating that the dipeptide had not formed a complex with Pd(II).

In a series of Pd(II)-dipeptide complexes at pH values 3.0, 7.15, 11.7, and 13.0, it was found that λ (max) was at a minimum and ϵ was at a maximum near pH 7.15 (Table 1).

Pitner, Wilson, and Martin[5] showed that $Pd(GlyGly\text{-}L\text{-}Ala)^{-1}$ at pH 7.0 had a λ (max) of 299 nm and an ϵ of 1250. At pH 12.8 λ (max) had shifted to 311 nm and ϵ had a value of 720. However, if the pH of this complex were raised to 10.0 by addition of NH_3, λ (max) became 297 nm and ϵ became 840, commensurate with four nitrogen donor atoms to Pd(II). The complexes had a red shift and

a decrease in ϵ when OH^- replaced COO^- in the Pd(II) coordination sphere. There was a minimum value in λ (max) when all four donor atoms around Pd(II) were N (as found when NH_3 was added to Pd(Gly-Gly-L-Ala)$^{-1}$). These workers showed a chemical shift upfield of 0.12 ppm for the carboxy terminal methylene protons of Pd(GlyGly-Gly)$^{-1}$ when the pD was raised from 7.0 to 12.0.

In Pd(II)-tripeptide complexes there are three nitrogen and one carboxylate oxygen donor atoms at pH values near 7.0.[5] In Pd(II)-dipeptide complexes near pH 7.0, there are two nitrogen, one carboxylate oxygen, and probably chloride or water occupying the fourth position.[5] The fourth donor atom in a tripeptide can be controlled by adjustment of pH. In a Pd(II)-dipeptide complex there are two positions that can have variable donor atoms. These are the position left when the dipeptide is acting as a tridentate ligand and the position vacated by the carboxylate group near pH 13.0. Consequently, a Pd(II)-dipeptide solution contains more than one complex at almost any pH.

In the present work done with Pd(II)-dipeptides, it was found that a red shift of 4-9 nm was encountered when the pH was increased from 7.15 to 13.0. Pitner, Wilson, and Martin[5] found a red shift of 12 nm in the complex Pd(GlyGly-L-Ala)$^{-1}$ and 8 nm in Pd(Gly-L-AlaGly)$^{-1}$ in pH changes of 7.0 to 12.8 and 7.4 to 12.1, respectively. The red shifts were greater for the Pd(II)-tripeptide complexes than for the Pd(II)-dipeptide complexes. These workers showed a chemical shift upfield of 0.12 ppm for the carboxy terminal methylene protons of Pd(GlyGlyGly)$^{-1}$ when the pD was raised from 7.0 to 12.8. An upfield chemical shift of 0.11 ppm was

observed in this present research in the alpha hydrogen doublet of Pd(II)-GV when the pD was increased from 7.11 to 13.06.

The maximum λ(max) was observed in the electronic spectra of Pd(II)-dipeptide complexes at pH 13.0. However, in some cases this wavelength had been reached by pH 11.7. Titration studies by Wilson and Martin[4] of K_2PdCl_4-GlyGly solutions showed that OH^- was evident as a ligand only after pH 8. Therefore, the red shift can start to occur when OH^- begins to replace chloride and water in the coordination sphere and also when OH^- begins to replace the carboxylate group as a ligand. In all cases ϵ was less at pH 13.0 than at pH 11.7.

Pitner, Wilson, and Martin[5] reported CD curves for Pd(Gly-Gly-L-Ala)$^{-1}$ and Pd(Gly-L-AlaGly)$^{-1}$ that resembled very closely the CD curves of Pd(II)-VV, Pd(II)-VL, Pd(II)-LV, Pd(II)-LL, Pd(II)-GL, and Pd(II)-GV when the former and latter complexes are near pH 7.0. The CD curve of Pd(GlyGly-L-Ala)$^{-1}$ inverts in solution where the pH is at 12.77. Electronic absorption studies showed that a red shift of 12 nm occurred when the pH was raised from 7.0 to 12.8, along with a change in the molar extinction coefficient, ϵ , from 1250 to 720. Nuclear magnetic resonance studies showed an upfield chemical shift of 0.12 ppm for the carboxy terminal methylene hydrogens of Pd(GlyGlyGly)$^{-1}$ when the pH was increased from 7.0 to 12.8. These data are indicative of the displacement of the carboxylate group by the hydroxyl ion with consequent rotation of the asymmetric group in Pd(GlyGly-L-Ala)$^{-1}$ from a negative to a positive hexadecant sector.

In corroboration with the former studies, Pitner, Wilson, and Martin found that no such inversion of the CD curve occurred when $Pd(Gly\text{-}L\text{-}AlaGly)^{-1}$ was subjected to a change in pH from 7.0 to 12.1. They also titrated equimolar solutions of $PdCl_4^{-2}$ and glycylglycyl-L-alanineamide that yielded an end point after the addition of four equivalents of base at 8.4. The complex absorbed at 282 nm, which is consistent with four nitrogen donors about the coordination plane. No electronic or CD spectral changes were observed upon addition of base to pH 12.7. After the fourth equivalent of hydrogen ions was added, the CD spectrum became similar to that of $Pd(GlyGly\text{-}L\text{-}Ala)^{-1}$.

Tsangaris and Martin[21] found that the magnitude of CD in Cu(II)-dipeptide complexes, Cu(XX), may be accurately estimated by adding the magnitudes of the corresponding dipeptides, Cu(GX) and Cu(XG). This indicates that the CD is an additive function of independent contributions from amino and carboxyl terminal amino acid residues. Cu(II) complexes of three tripeptides composed of one L-Leucyl and two glycyl residues yielded the value obtained experimentally for the complex of the tripeptide leucylleucylleucine. These results could not be accounted for by any octant or quadrant rule. A hexadecant rule accounted for the sign identity and magnitude additivity of these results for the Cu(II) complexes of dipeptides and tripeptides. Octant or quadrant rules were invalid.

Wilson and Martin[4] found that the CD of Pd(II) complexes of dipeptides and tripeptides near pH 7.0 have additive properties that arise from independent contributions of the amino acid residues.

They assigned D_{4h} microsymmetry to these complexes along with a hexadecant sector rule.

The CD spectra of Pd(II)-dipeptide complexes did not invert at pH 13.0 as did the Pd(II)-tripeptide complexes of Pitner, Wilson, and Martin. The net CD of the Pd(II)-dipeptide complexes were negative even at pH 13.0. Apparently complete rotation of bulky isopropyl or isobutyl groups out of a negative sector is not possible in a Pd(II)-dipeptide complex where Leu or Val is in the second amino acid residue. In the case of $Pd(GlyGly\text{-}L\text{-}Ala)^{-1}$ there was rotation of the methyl group from a negative to a positive sector with an inversion of the CD spectrum.[5]

Another important difference between the CD of Pd(II)-dipeptide and Pd(II)-tripeptide complexes was found to exist when the carboxy terminal residue was glycine. Pd(II)-LG and Pd(II)-VG had an inversion of the positive peak near 300 nm at pH 13.0. Pitner, Wilson, and Martin found that $Pd(Gly\text{-}L\text{-}AlaGly)^{-1}$ exhibited no inversions and only weakened in magnitude upon addition of base to pH 12.1.[5]

The first residue of a Pd(II)-peptide complex did not transmit its optical activity through the amino nitrogen as readily as did those groups near an amide nitrogen. Apparently the carboxyl group is rotating into a negative sector and overriding the optical activity of the asymmetric group of the first residue. A Pd(II)-tripeptide complex such as $Pd(Gly\text{-}L\text{-}AlaGly)^{-1}$ at pH 13.0 has a CD spectrum similar to that of Pd(II)-GV, Pd(II)-GL, Pd(II)-VV, Pd(II)-VL, Pd(II)-LV, and Pd(II)-LL near pH 7.0. The fact that the CD of Pd(II)-GL and Pd(II)-GV complexes are similar suggests that the

origin of the positive peak is dependent on both the first and second residues of the dipeptide. That the origin of the negative peak is largely dependent on the second residue can be observed in complexes of Pd(II) with GlyLeu and GlyVal when compared to those of LeuGly and ValGly.

It is suggested that the anomalous character of the CD spectra of Pd(II)-dipeptide complexes at pH 13.0 where Leu or Val is in the second residue and Gly, Leu, or Val is in the first residue be explained as follows: The new negative peak near 300 nm is mainly due to the carboxyl group rotated into a negative sector, while the positive peak near 325 nm occurs along with the large negative peak near 375 nm when the asymmetric group of the second residue is in a negative sector. The asymmetric group of the second residue is large and bulky and therefore does not completely rotate out of the negative sector. This partial rotation into a positive sector correlates with the decrease of the absolute value of $\Delta\epsilon$ at the peaks near 325 and 375 at a pH near 13.0. This analysis is based on the assumption that D_{4h} microsymmetry along with a hexadecant sector rule still holds. At pH 13.0 the actual microsymmetry is C_2V. However, the Pd(II)-tripeptide complexes maintained electronic D_{4h} microsymmetry at high pH values.[5]

Pitner, Wilson, and Martin[4] observed tight isosbestic points in the CD curves of $Pd(GlyGly\text{-}L\text{-}Ala)^{-1}$ at pH values of 7.0, 11.13, 11.7, and 12.77, indicating two major species in equilibria. There are no tight isosbestic points in the CD spectra of the dipeptide complexes; consequently, an equilibrium mixture of three or more Pd(II)-dipeptide complexes is present in solution. It is

apparent that isolation of a particular species of Pd(II)-dipeptide complex would be difficult.

This research indicates that further research investigation of the origin of circular dichroism in Pd(II)-dipeptide complexes be pursued. Wilson and Martin[4] suggested that the bands near 320 and 375 nm shown by Pd(II) complexes of di- and tripeptides near pH 7 have their origin in the d_{xz}, $d_{yz} \longrightarrow d_{x^2-y^2}$, and $d_{xy} \longrightarrow d_{x^2-y^2}$ electronic transitions, respectively. A magnetic circular dichroism study of the band near 320 nm would possibly confirm this assignment of bands.

SUMMARY

$PdBr_2(ValVal)_2$ is presented in this work as an example in which a dipeptide bonds only at its amino nitrogen to Pd(II). A detailed study of this compound was not pursued since bonding of Pd(II) and Pt(II) to the peptide linkage was the main problem of interest.

Bidentate and tridentate dipeptide complexes with Pd(II) were obtained when K_2PdCl_4 was reacted with the appropriate dipeptides in water at room temperature. These complexes were studied in solution to determine bonding sites and conformation at pH values near 1.3, 3.0, 7.15, 11.7, and 13.0 by utilizing nuclear magnetic resonance, ultraviolet-visible, and circular dichroism spectroscopies.

Infrared analysis was performed on precipitated products of the K_2PdCl_4-dipeptide reaction. These infrared spectra were valuable in determining that the amino nitrogen and the peptide

nitrogen of the dipeptide were bonded to Pd(II). This determination was made by observing the frequency changes of the (N-H) and the Amide I bands of the free and complexed dipeptides.

$PtCl_2$ was found to react with ValLeu to form $PtCl_2(ValLeu)_2$. In analogy to the reactions of K_2PdCl_4 with dipeptides, K_2PtCl_4 was chosen in an attempt to bond Pt(II) to the peptide linkage. Infrared analysis indicated that there was no bonding of Pt(II) to the peptide oxygen or to the peptide nitrogen when attempts were made to react K_2PtCl_4 with a dipeptide in water solution. Zeise's salt, as a third source of Pt(II), reacted with ValVal, ValLeu, and LeuVal to form compounds with the empirical formulas $(C_2H_4)_2Cl_2(Val)_2Pt_2$, $(C_2H_4)_2Cl_2(Val)(Leu)Pt_2$, and $(C_2H_4)_2Cl_2(Val)(Leu)Pt_2$, respectively. The latter two compounds had the same formula but different crystalline structures and different infrared spectra. Molecular weight determination and infrared analyses as well as elemental analysis indicated that dimer compounds had been formed in which the original dipeptide compound had been fissioned at the peptide linkage. Infrared analyses indicated N-trans—O-trans, N-trans—O-trans, and N-trans—N-trans structures for the products of the reaction of Zeise's salt with ValVal, LeuVal, and ValLeu, respectively.

Table 1

Pd(II)-dipeptide Electronic Spectra Data

Complex	pH	λ max (nm)	ε
Pd(II)-LeuLeu	1.40	450	185
	2.98	329	680
	7.25	327	807
	11.7	332	596
	13.0	332	532
Pd(II)-ValVal	1.40	440	212
	3.0	327	655
	7.25	323	965
	11.7	324	660
	13.0	332	530
Pd(II)-ValLeu	1.35	450	200
	3.0	329	667
	7.15	324	860
	11.7	325	735
	13.0	332	514
Pd(II)-LeuVal	1.30	454	168
	3.0	327	667
	7.80	326	860
	11.7	332	580
	13.0	332	547
Pd(II)-GlyLeu	1.30	450	207
	2.90	331	687
	7.15	329	637
	11.7	333	617
	13.0	333	500
Pd(II)-LeuGly	1.45	445	203
	2.9	330	640
	7.15	328	640
	11.7		665
	13.0	328	340
Pd(II)-GlyVal	1.30	450	205
	3.0	330	685
	7.25	325	870
	11.7	333	565
	13.0	334	528
Pd(II)-ValGly	1.40	445	206
	3.0	329	637
	7.25	327	805
	11.7	332	505
	13.0	332	312

Table 2

Chemical Shift Values

	GlyVal	Pd(II)-GlyVal		
	pD 5.71	pD 2.96	pD 7.11	pD 13.06
Methyl hydrogens	1.32	(1.38)		
	1.35	1.49(1.40)	1.53	1.49
	1.39	1.56(1.45)	1.59	1.56
	1.42	1.63(1.47)	1.67	1.63
Beta hydrogen	2.42			
	2.49			
	2.56			
	2.62			
	2.69			
	2.76			
Beta hydrogen (center)		2.68	2.70	2.60
Methylene hydrogens	4.33	4.01	4.01	3.96
Alpha hydrogen	4.53	4.33	4.37	4.26
	4.58	4.36	4.40	4.29
		4.38		

Table 3

Infrared Frequencies of Metal Complexes of Leucine and Valine

$Pt(Val)_2$	$Pt(Leu)_2$	ZS-VV	ZS-VL	ZS-LV	Mode
3217 s	3217 s	3200	3200	3200	
3137 s	3120 s	3100	3102	3090	NH_2 stretching
			3082		
2962 s	2978 s	3019	3010	3010	
2934 sh	2957 s	2980	2959	2959	
2874 w	2937 s	2922	2930	2930	CH stretch
	2913 s	2871	2910	2908	
	2871 m		2868	2872	
1645 s	1640 s	1660	1660	1660	COO^- asymmetric stretch
1604 s	1600 s	1560	1560	1565	NH_2 scissors
1462 m	1469 m	1460	1460	1462	CH_3 deg def
1360 s	1379 s	1365	1365	1365	COO^- sym str
1387 sh	1389 sh	1386	1382	1387	CH_3 sym def

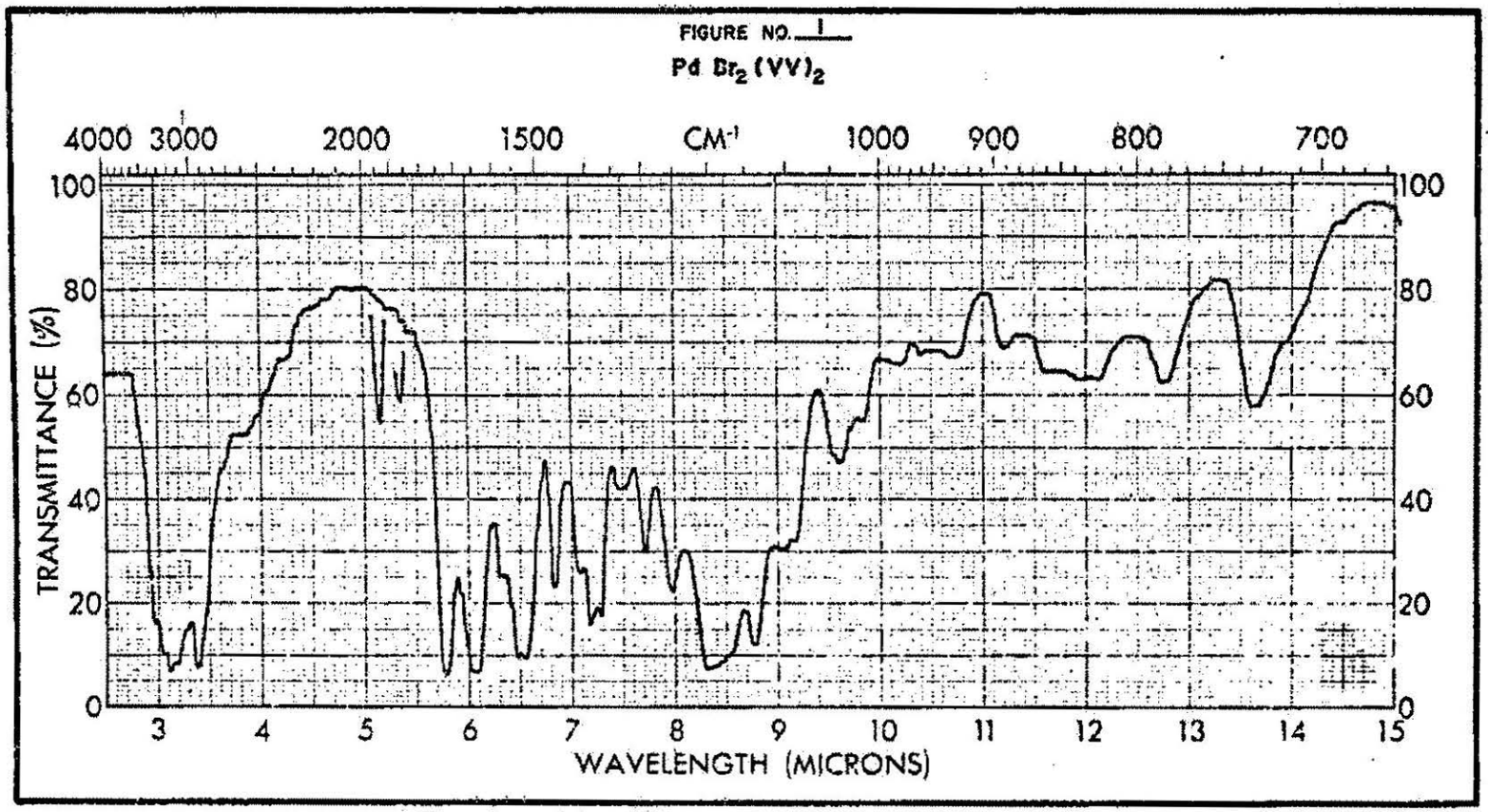


FIGURE NO. 1
Pd Br2 (VV)2
4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

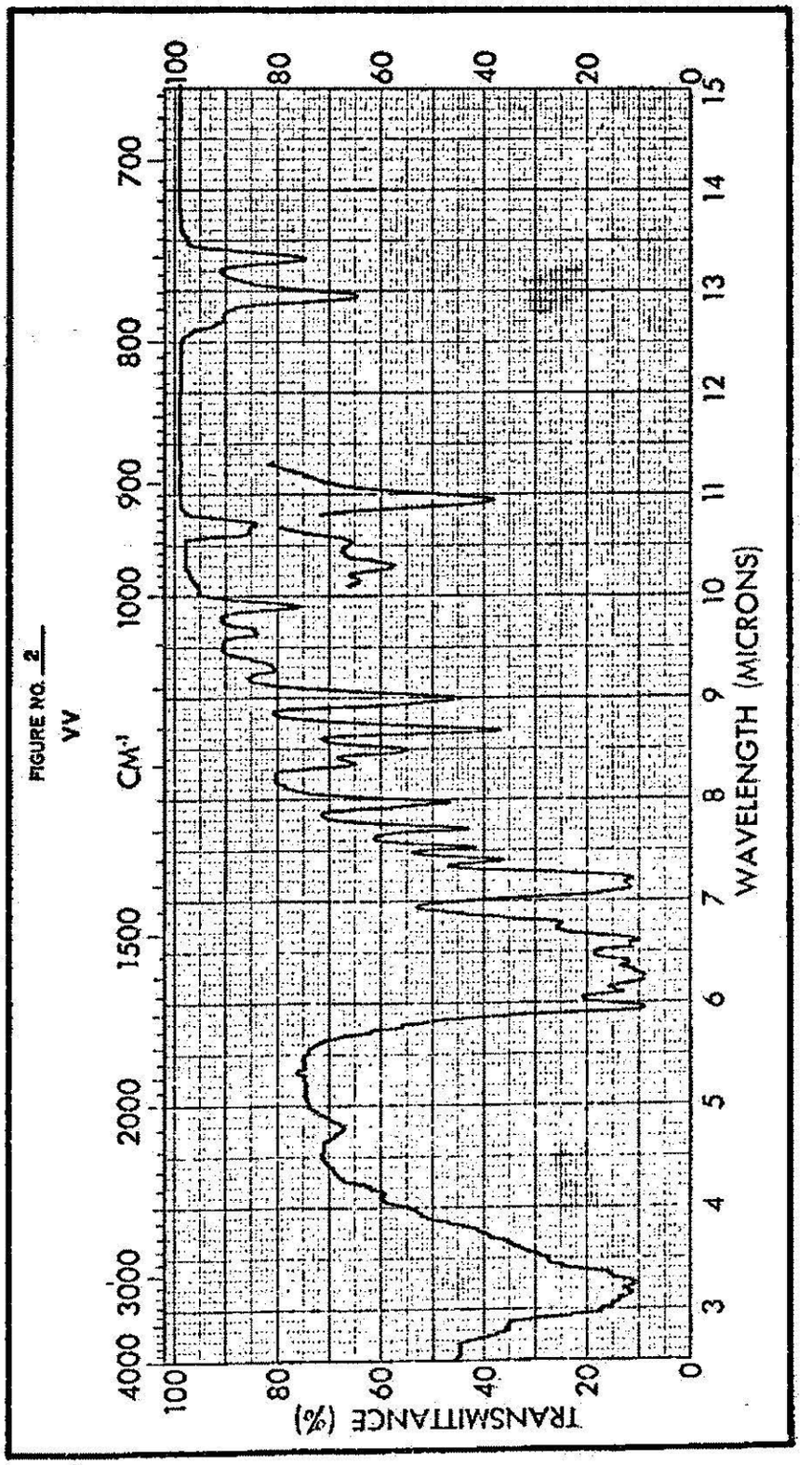


FIGURE NO. 2
VV
CM.-1
4000 3000 2000 1500 1000 900 800 700
100 80 60 40 20 0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

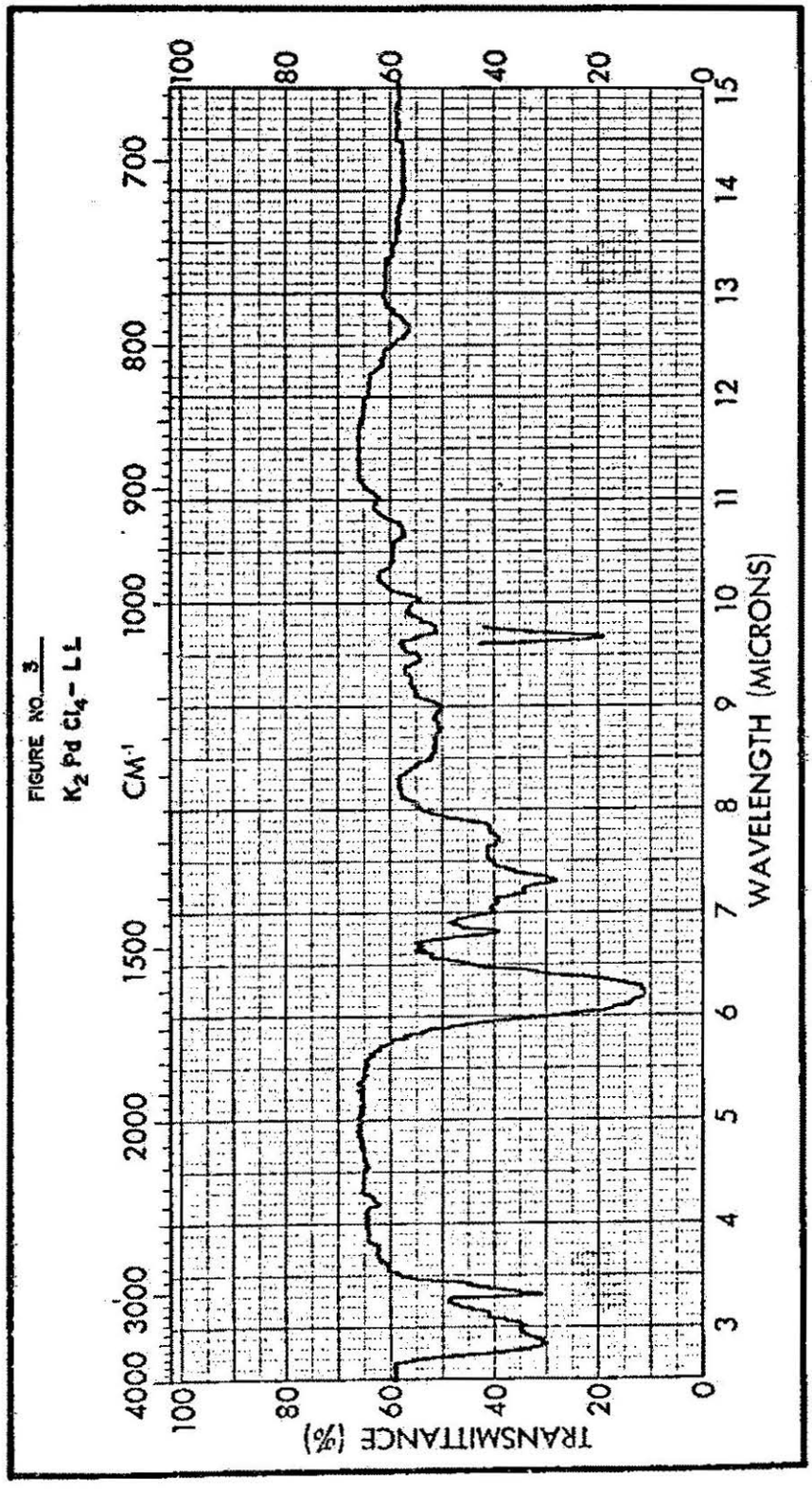


FIGURE NO. 3
$K_2 Pd Cl_4$ - L.L.
CM⁻¹
4000 3000 2000 1500 1000 900 800 700
TRANSMITTANCE (%)
100 80 60 40 20 0
WAVELENGTH (MICRONS)
3 4 5 6 7 8 9 10 11 12 13 14 15

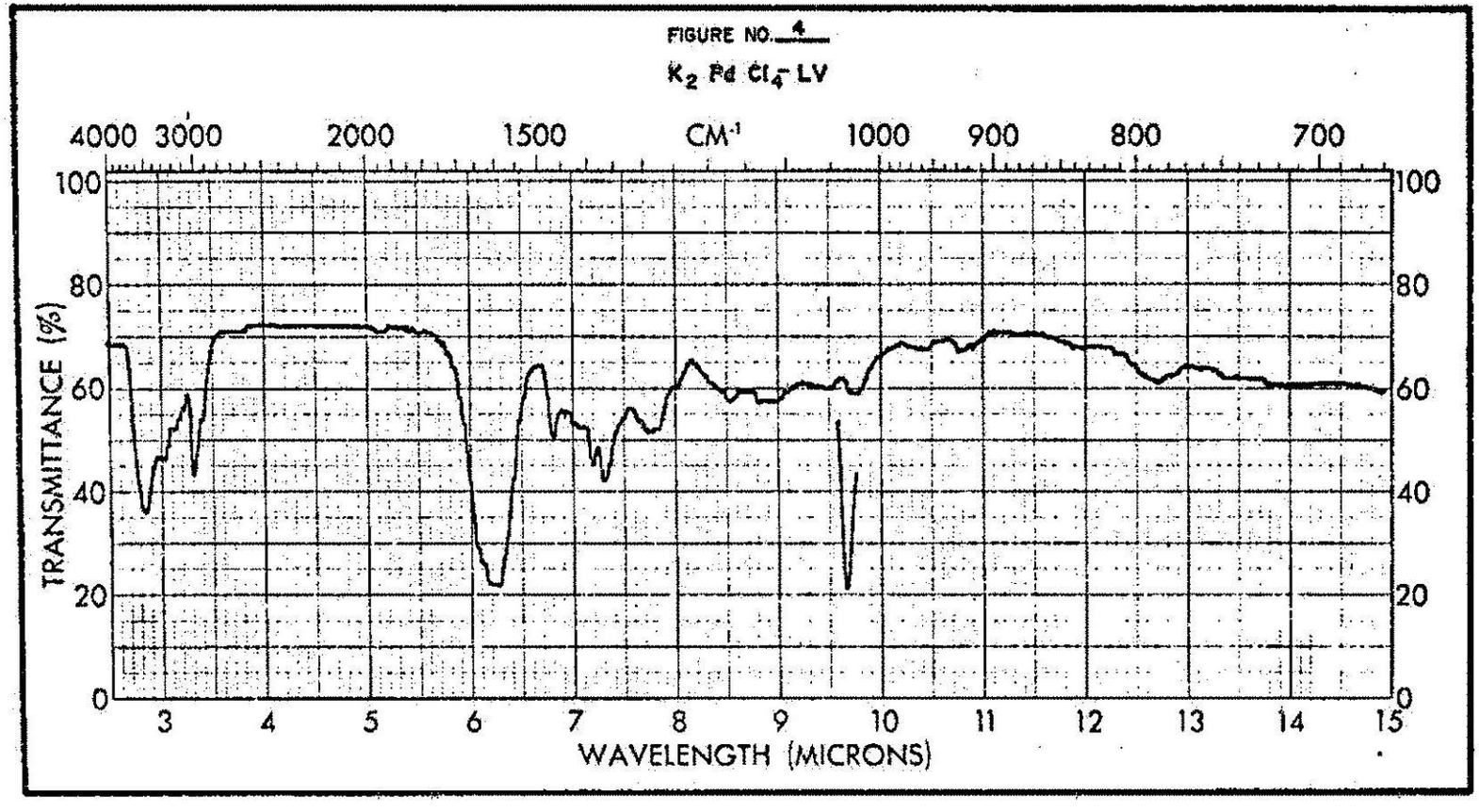
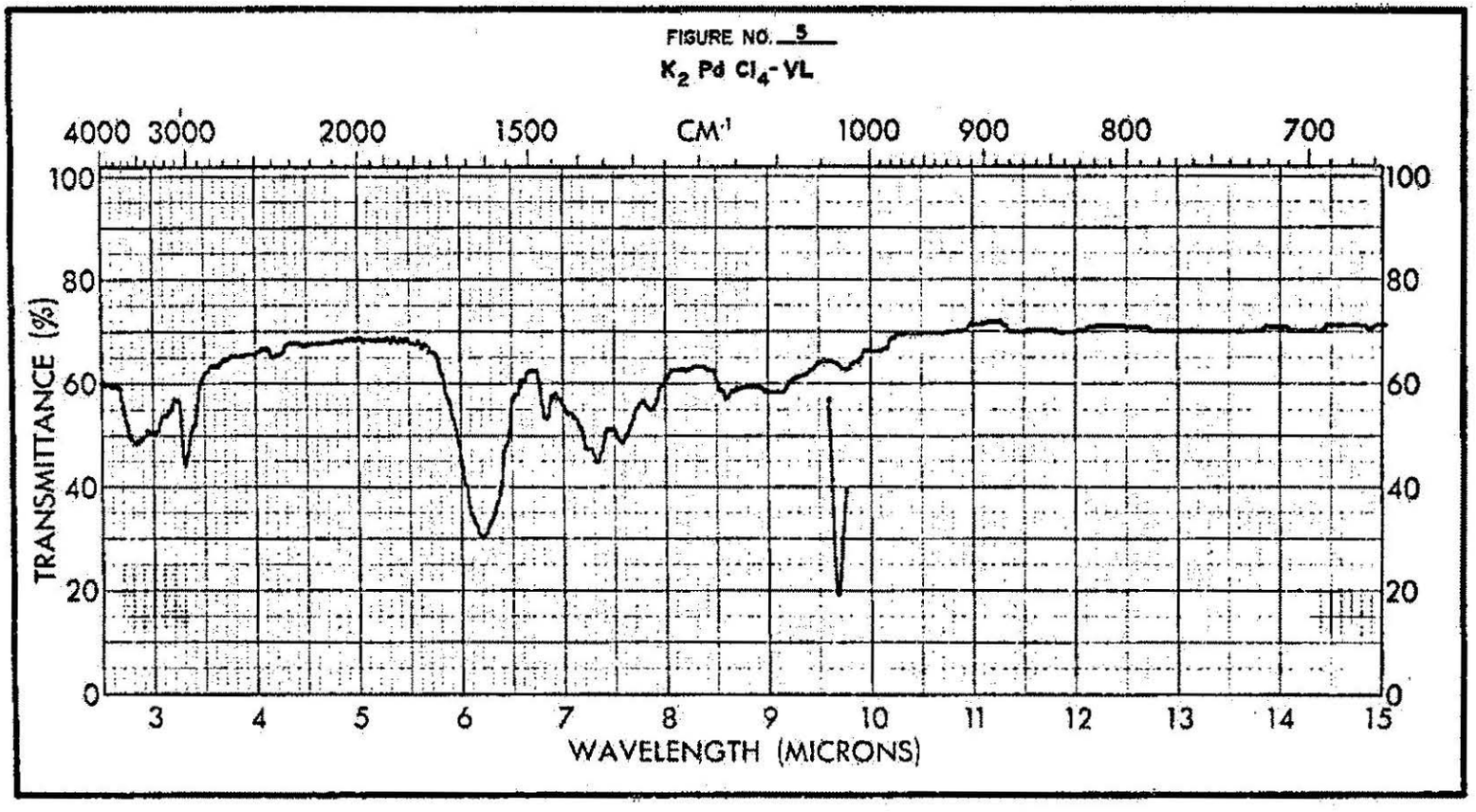


FIGURE NO. 4
K_2 Pd Cl_4 - LV
4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

FIGURE NO. 5

K_2 Pd Cl_4 - VL



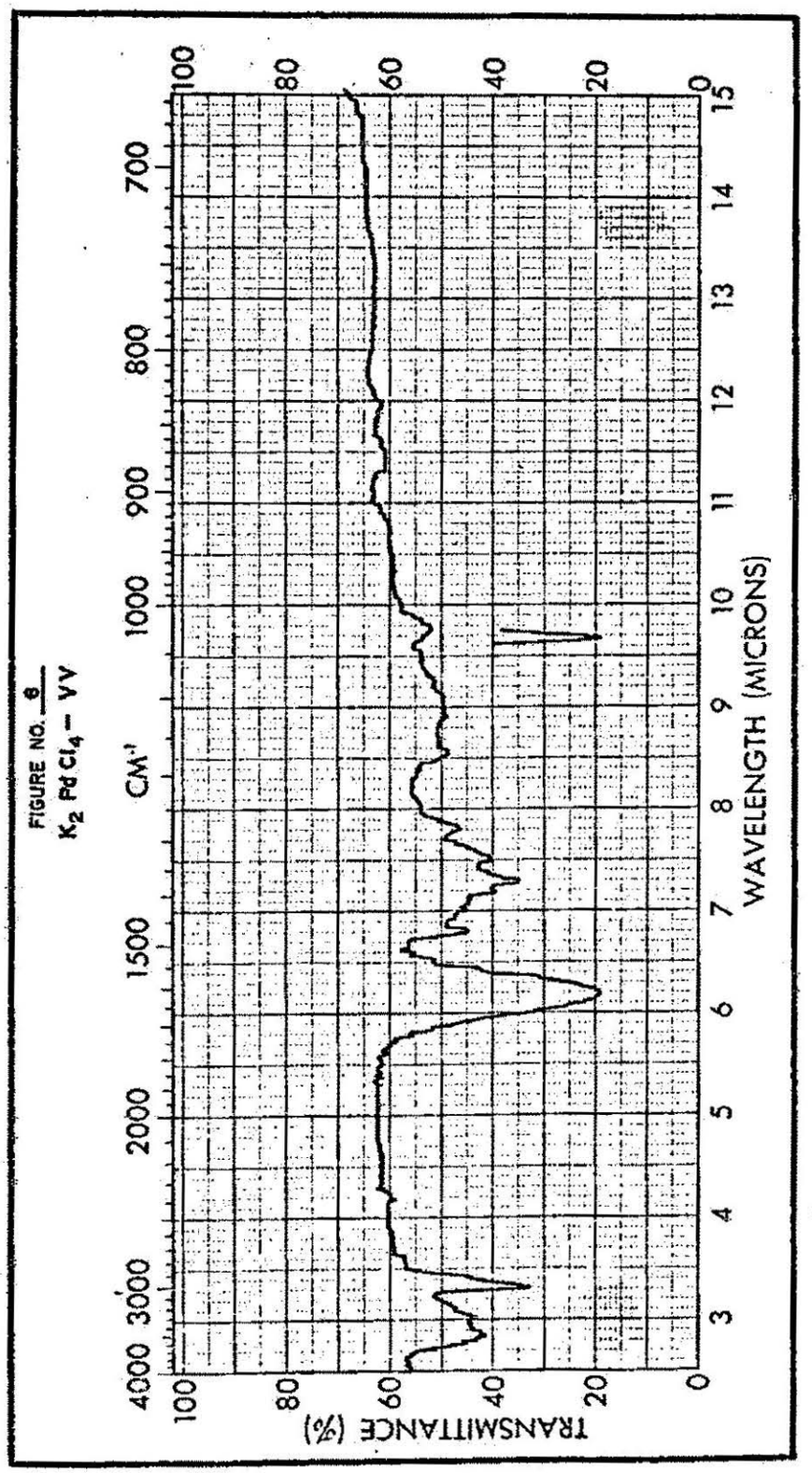


FIGURE NO. 6
$K_2 Pd Cl_4$ – VV
CM⁻¹
4000 3000 2000 1500 1000 900 800 700
WAVELENGTH (MICRONS)
3 4 5 6 7 8 9 10 11 12 13 14 15
TRANSMITTANCE (%)
100 80 60 40 20 0

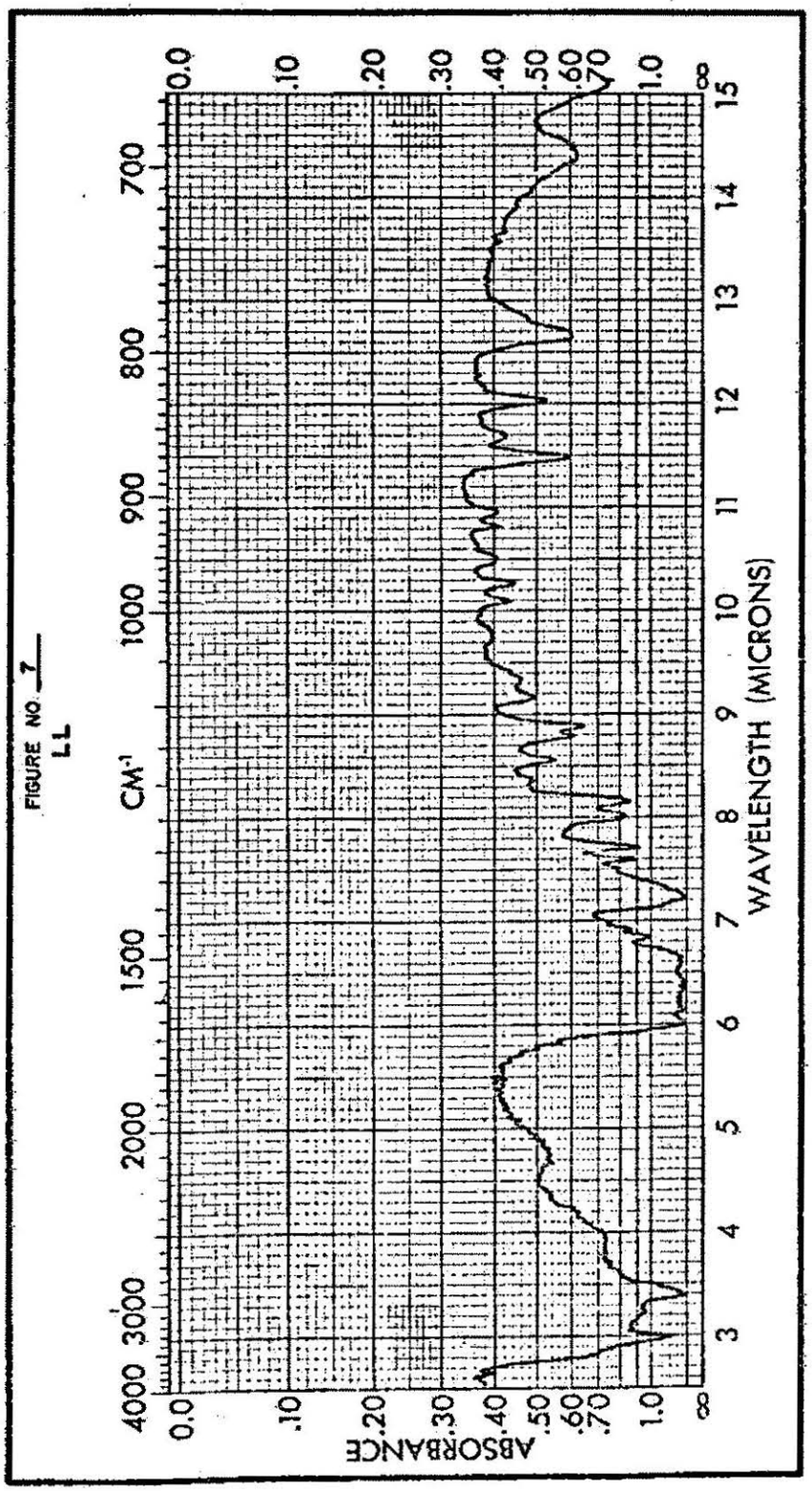


FIGURE NO 7
LL
CM-1
4000
3000
2000
1500
1000
900
800
700
ABSORBANCE
0.0
.10
.20
.30
.40
.50
.60
.70
1.0
∞
3
4
5
6
7
8
9
10
11
12
13
14
15
WAVELENGTH (MICRONS)

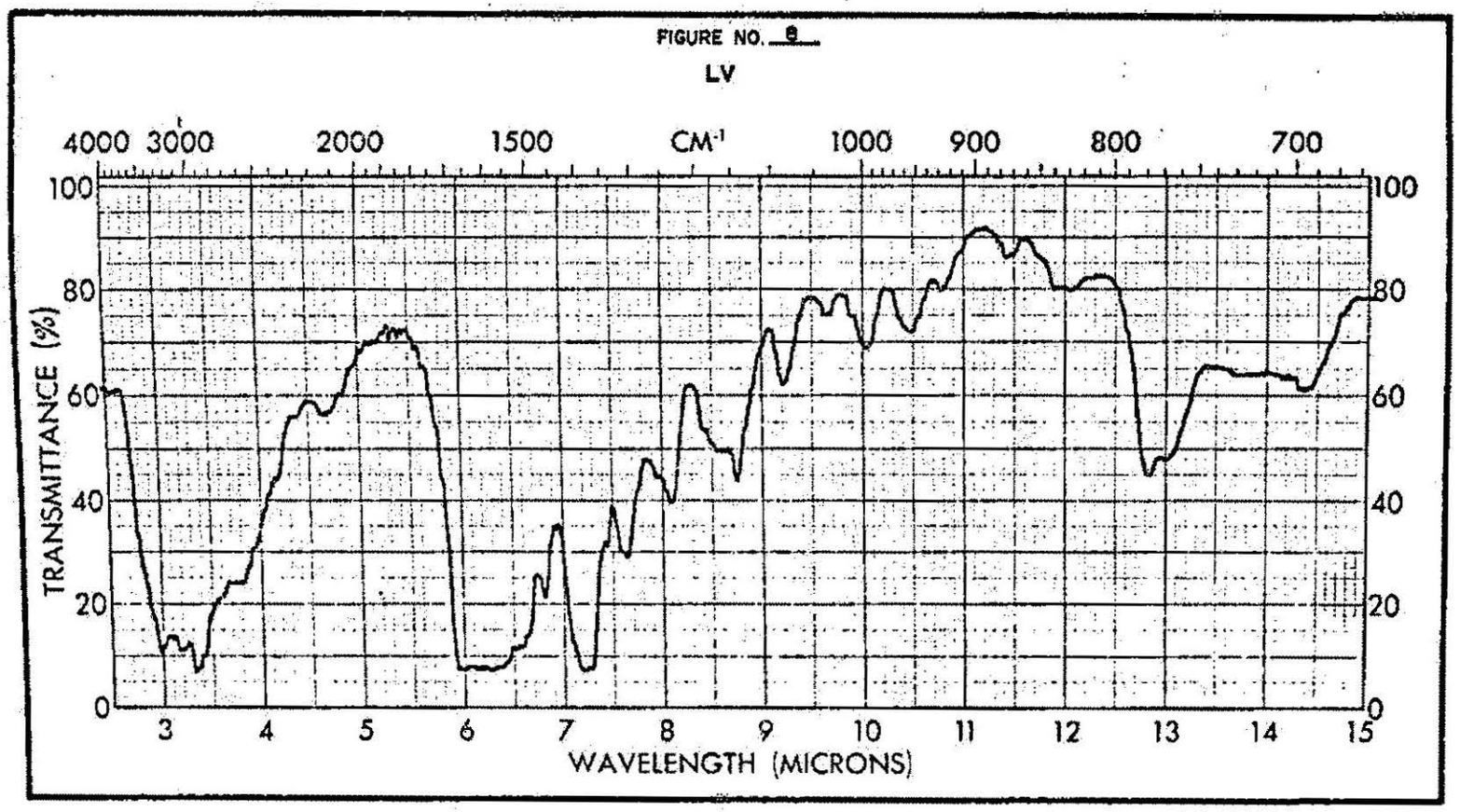


FIGURE NO. 8
LV
4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

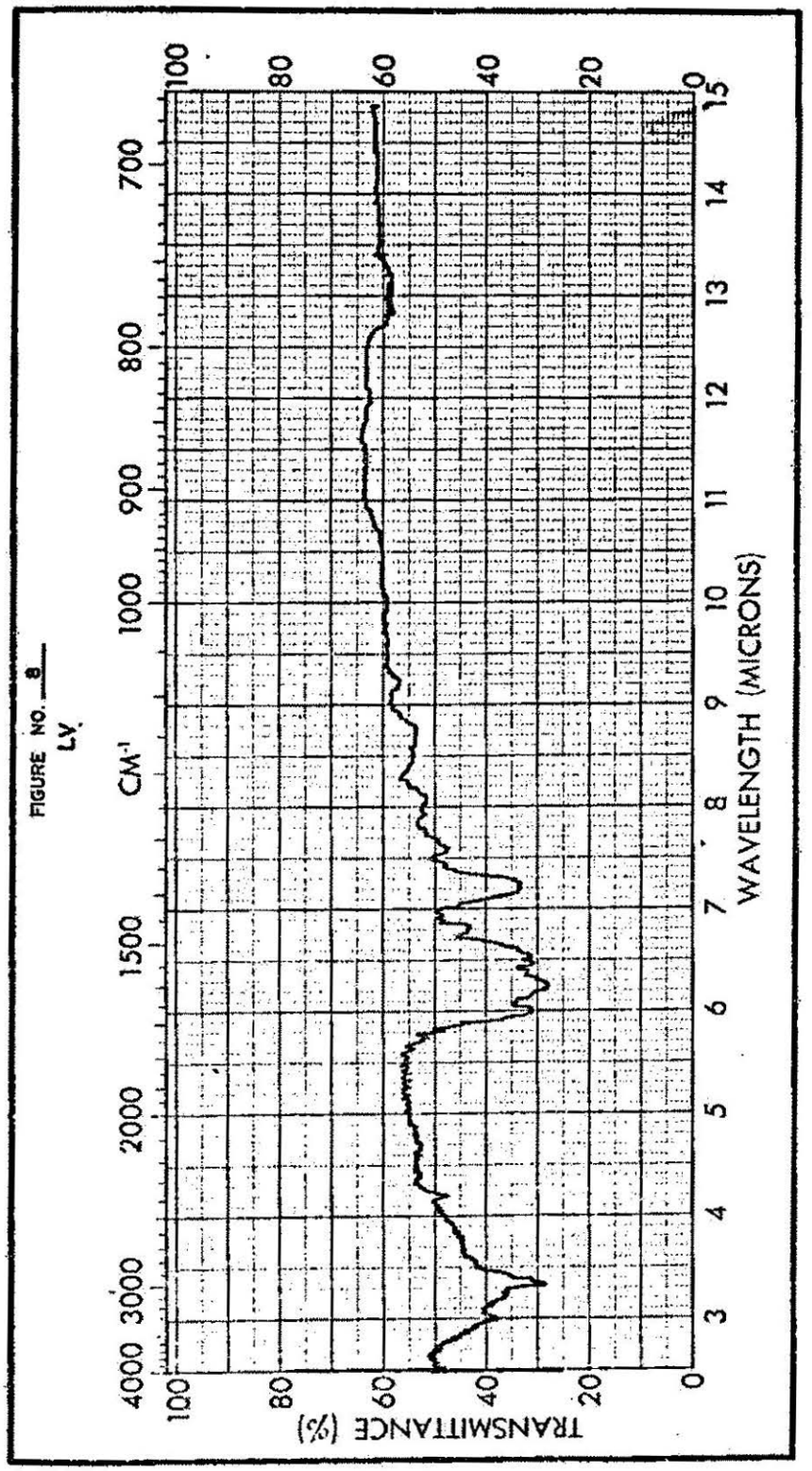


FIGURE NO. 8
LV
CM-1
4000 3000 2000 1500 1000 900 800 700
100 80 60 40 20 0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

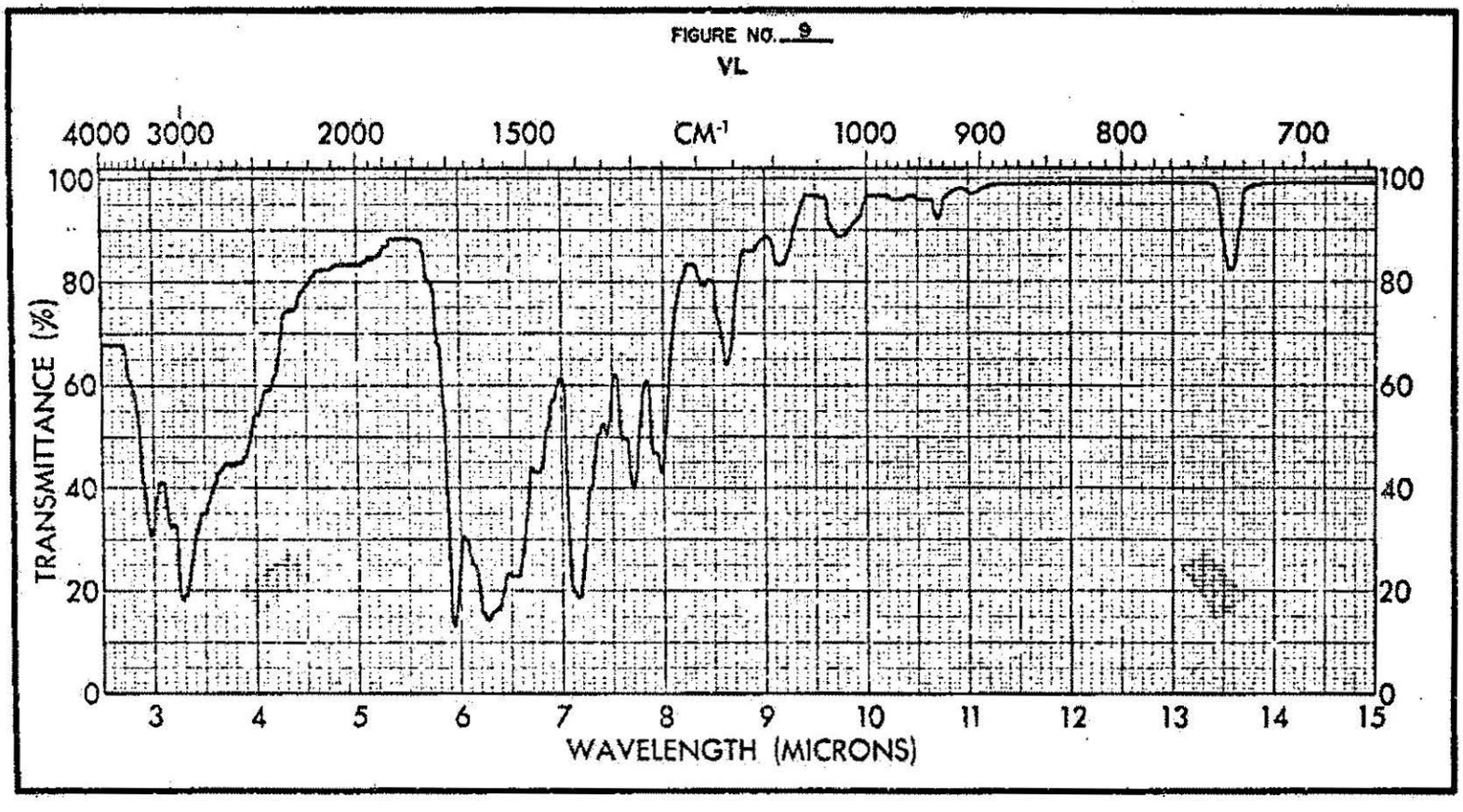


FIGURE NO. 9
VL
4000 3000 2000 1500 CM⁻¹ 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15
WAVELENGTH (MICRONS)

FIGURE NO. 10

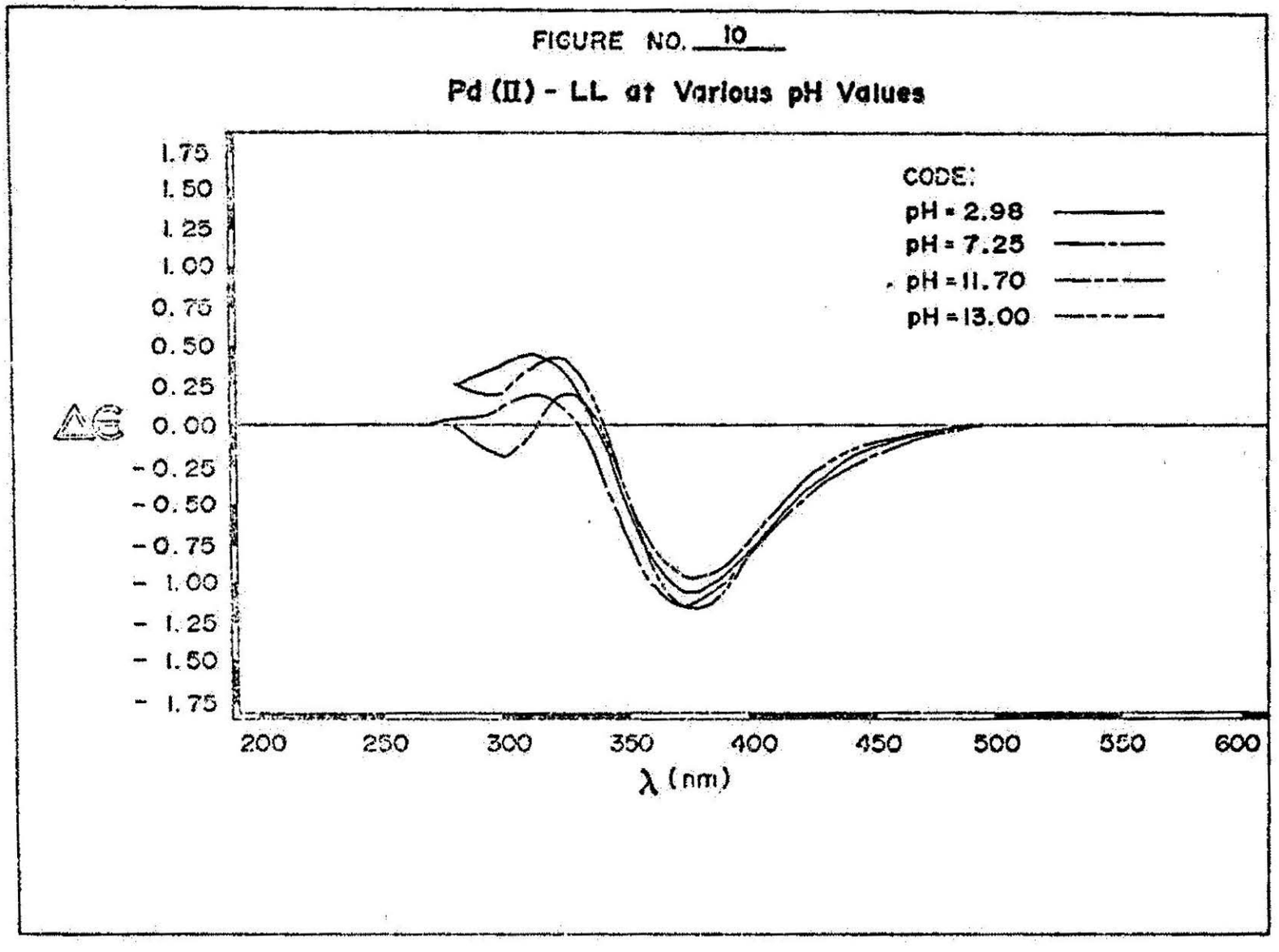

Pd (II) - LL at Various pH Values
CODE:
pH = 2.98
pH = 7.25
pH = 11.70
pH = 13.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 11

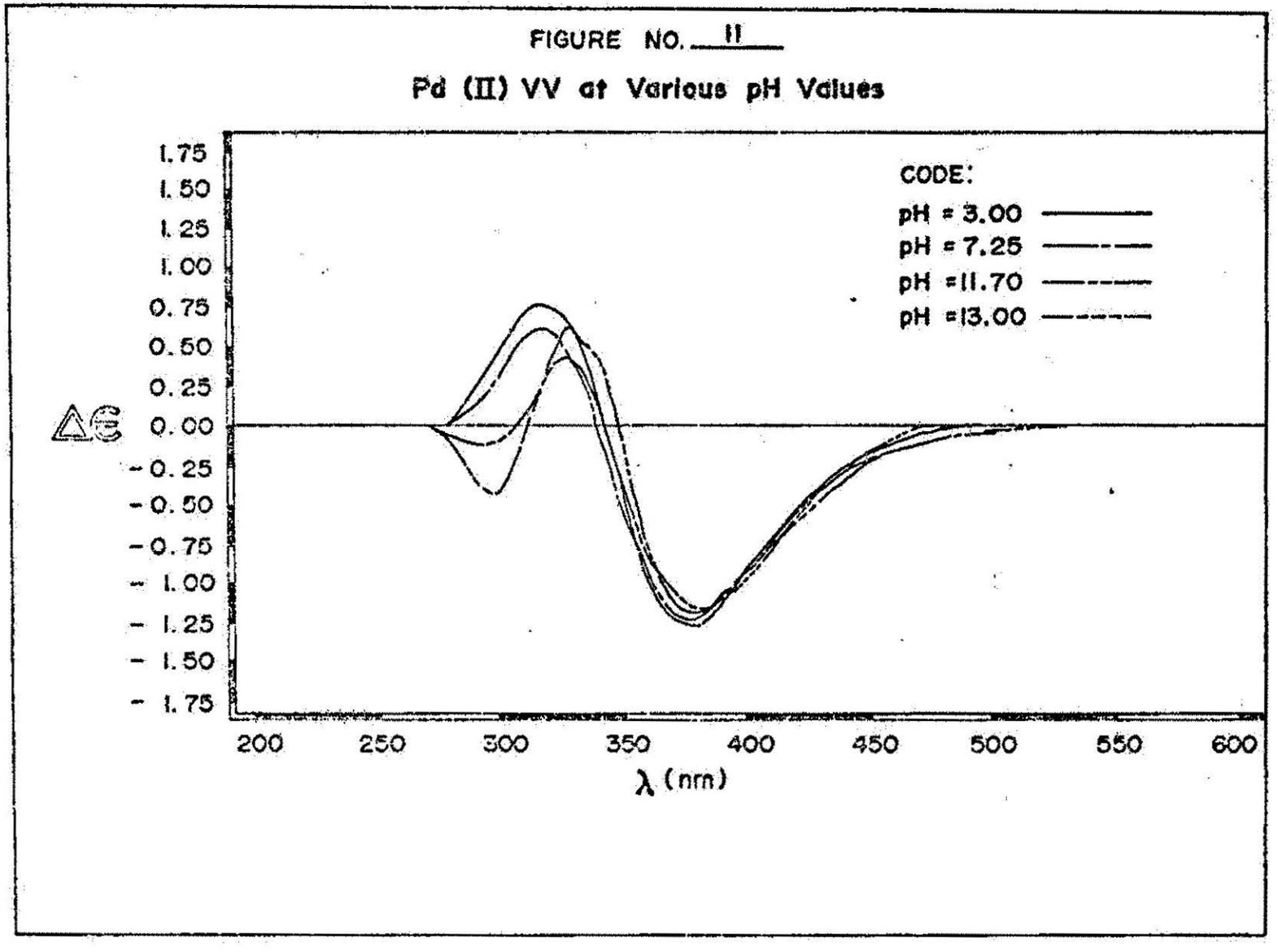

Pd (II) VV at Various pH Values
CODE:
pH = 3.00
pH = 7.25
pH = 11.70
pH = 13.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 12

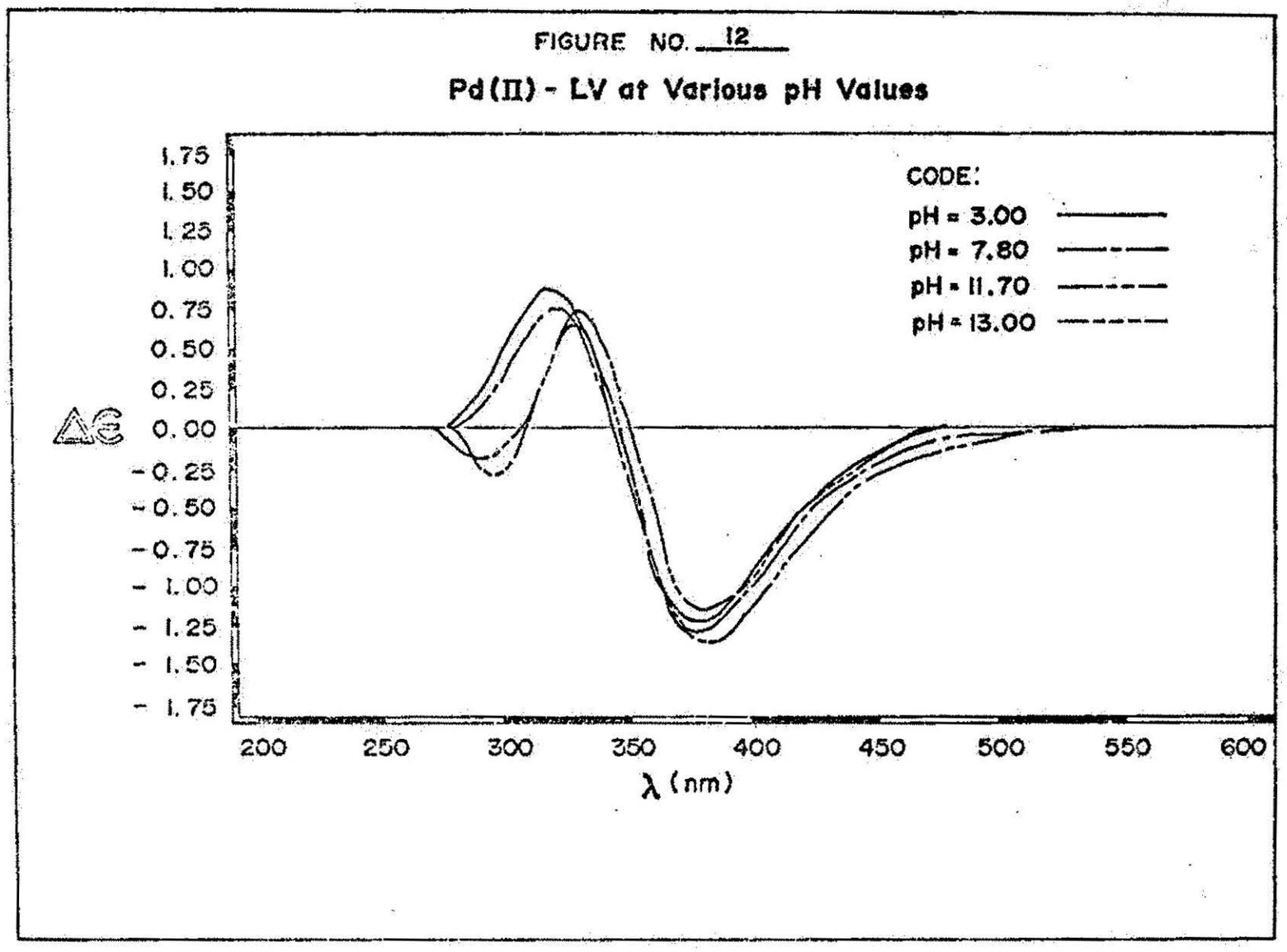

Pd(II) - LV at Various pH Values
CODE:
pH = 3.00
pH = 7.80
pH = 11.70
pH = 13.00
Δε
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 13

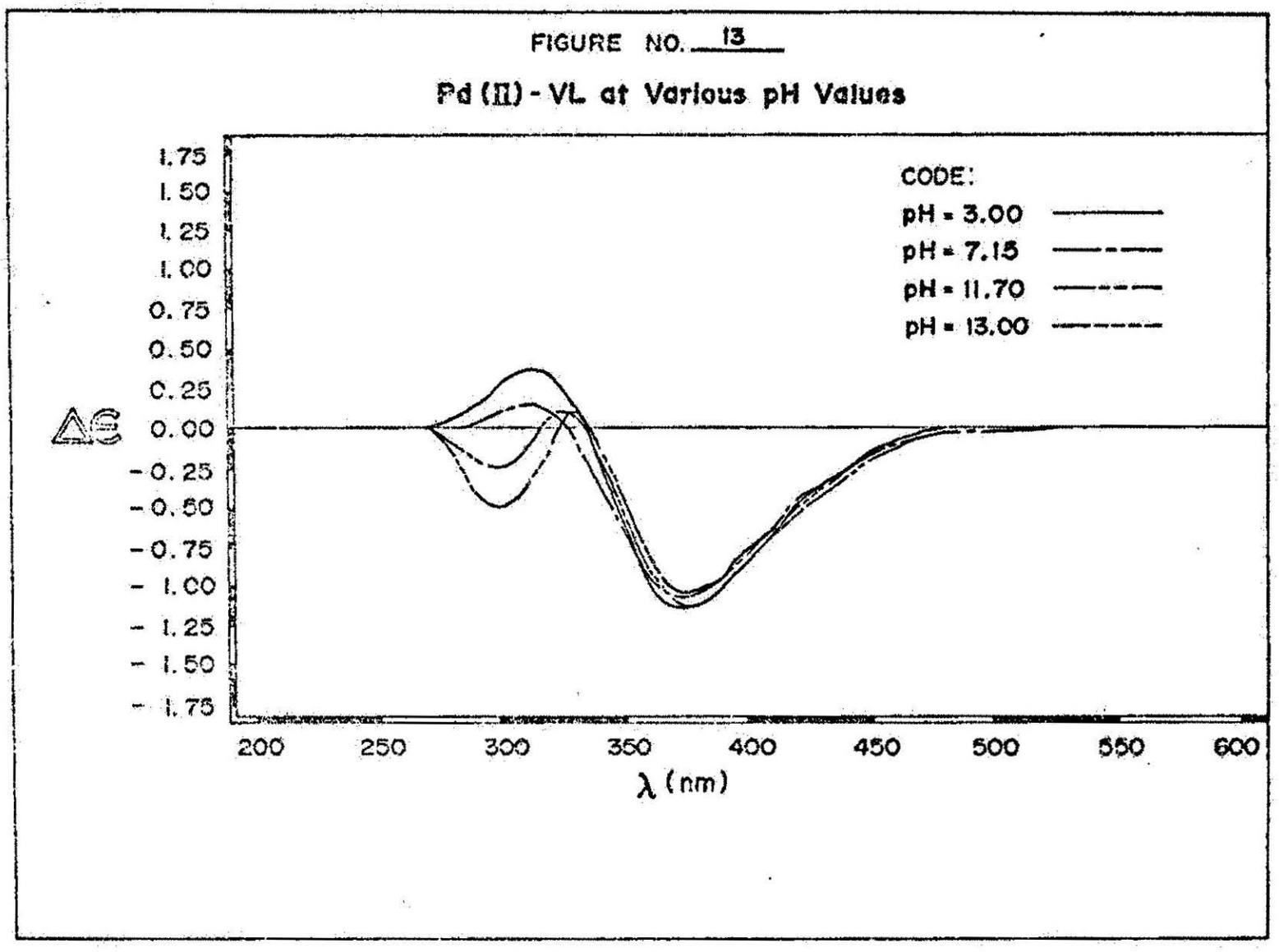

Pd (II) - VL at Various pH Values
CODE:
pH = 3.00
pH = 7.15
pH = 11.70
pH = 13.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 14

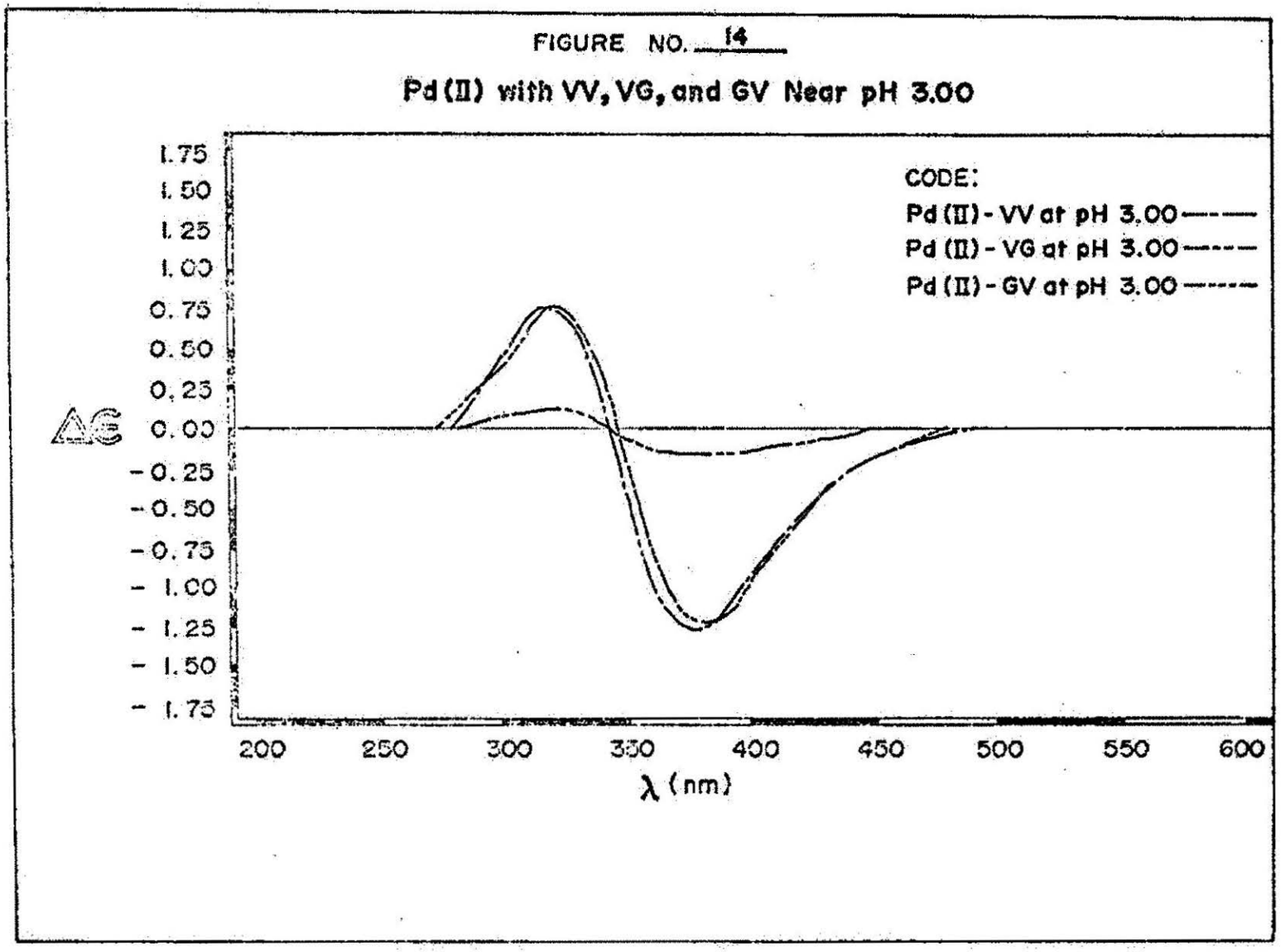

Pd (II) with VV, VG, and GV Near pH 3.00
CODE:
Pd (II) - VV at pH 3.00
Pd (II) - VG at pH 3.00
Pd (II) - GV at pH 3.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 15

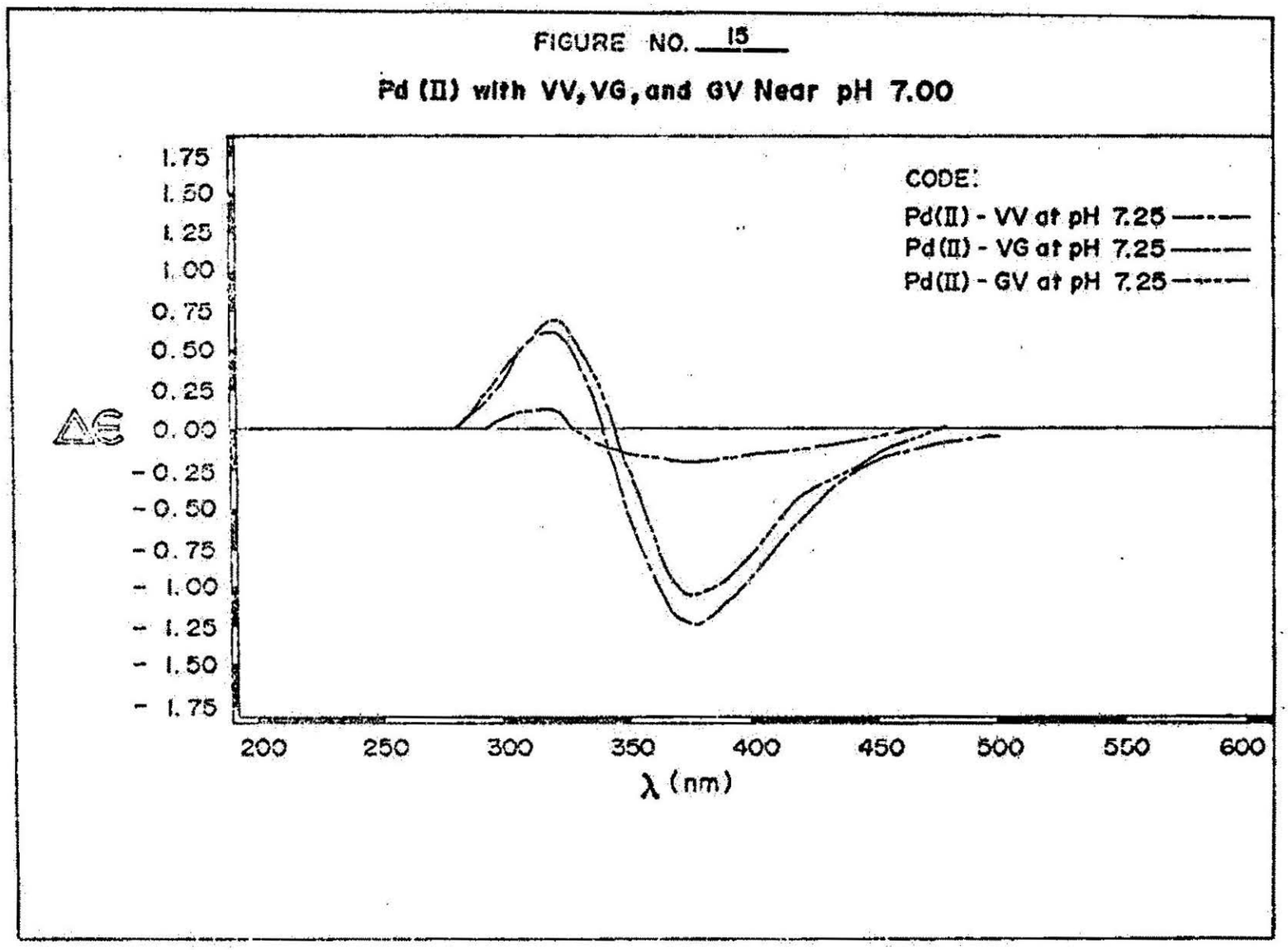

Pd (II) with VV, VG, and GV Near pH 7.00
CODE:
Pd(II) - VV at pH 7.25
Pd(II) - VG at pH 7.25
Pd(II) - GV at pH 7.25
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 16

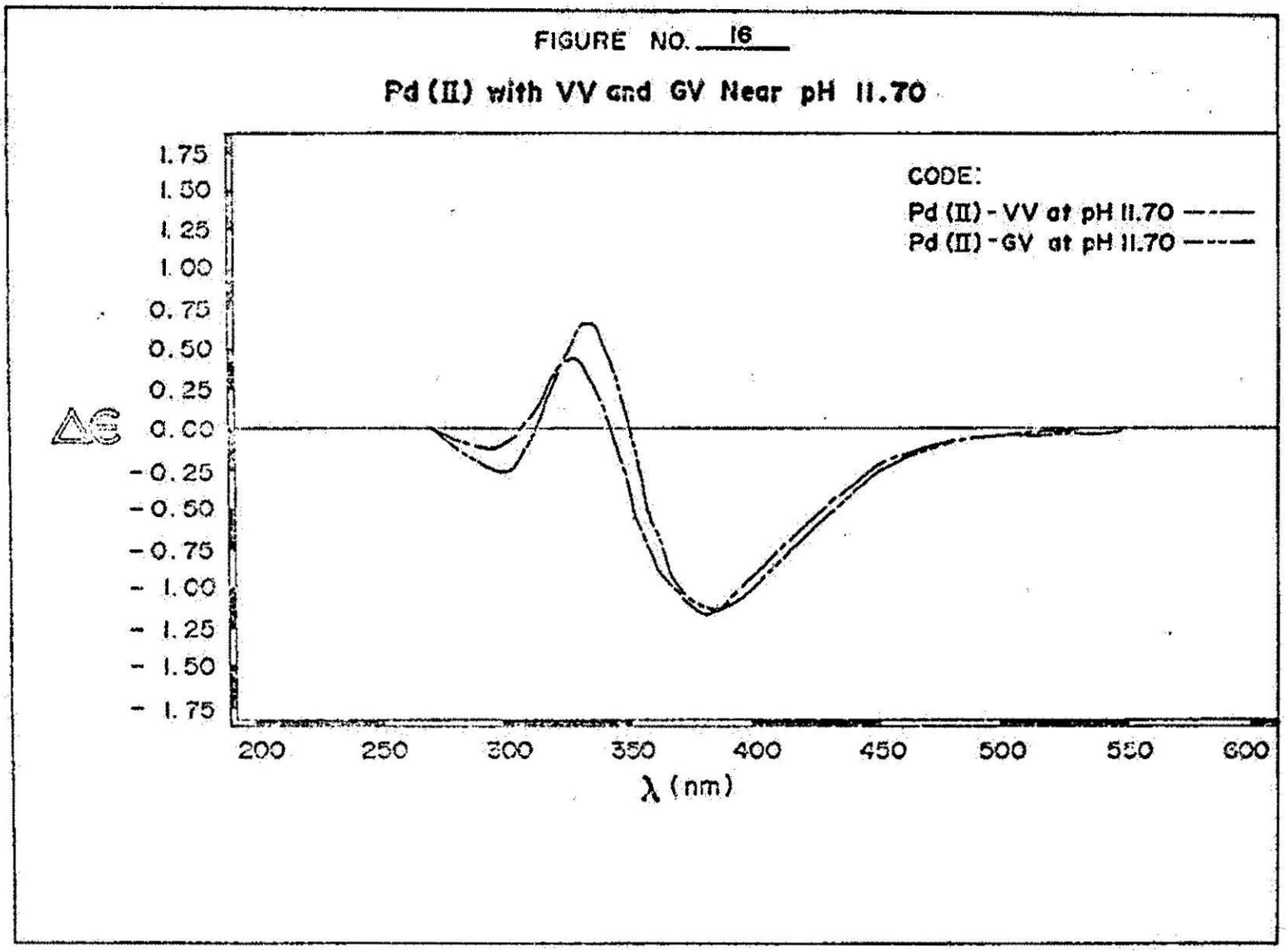

Pd (II) with VV and GV Near pH 11.70
CODE:
Pd (II) - VV at pH 11.70
Pd (II) - GV at pH 11.70
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
Δε
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 17

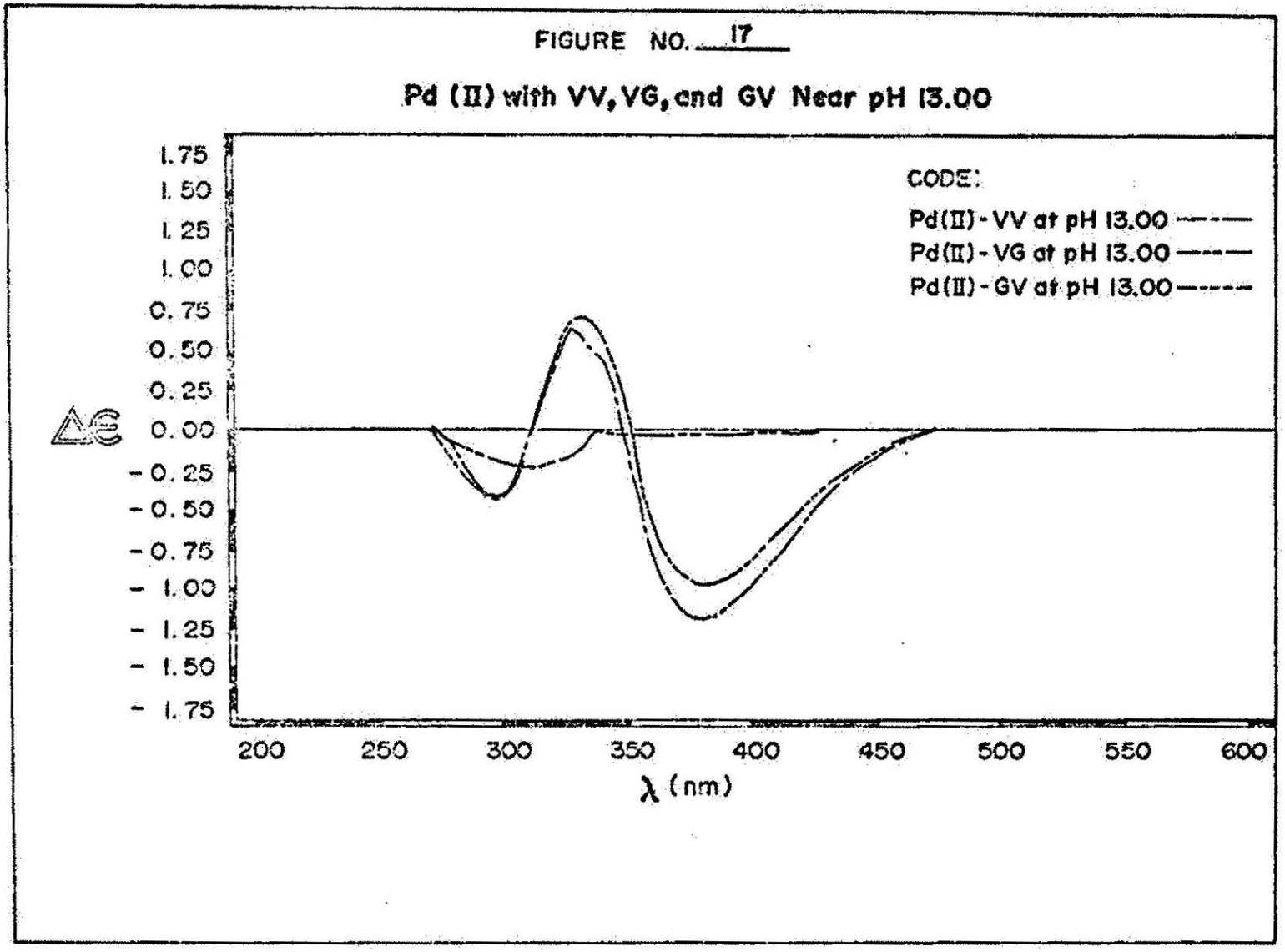

Pd (II) with VV, VG, and GV Near pH 13.00
CODE:
Pd(II) - VV at pH 13.00
Pd(II) - VG at pH 13.00
Pd(II) - GV at pH 13.00
Δε
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 18

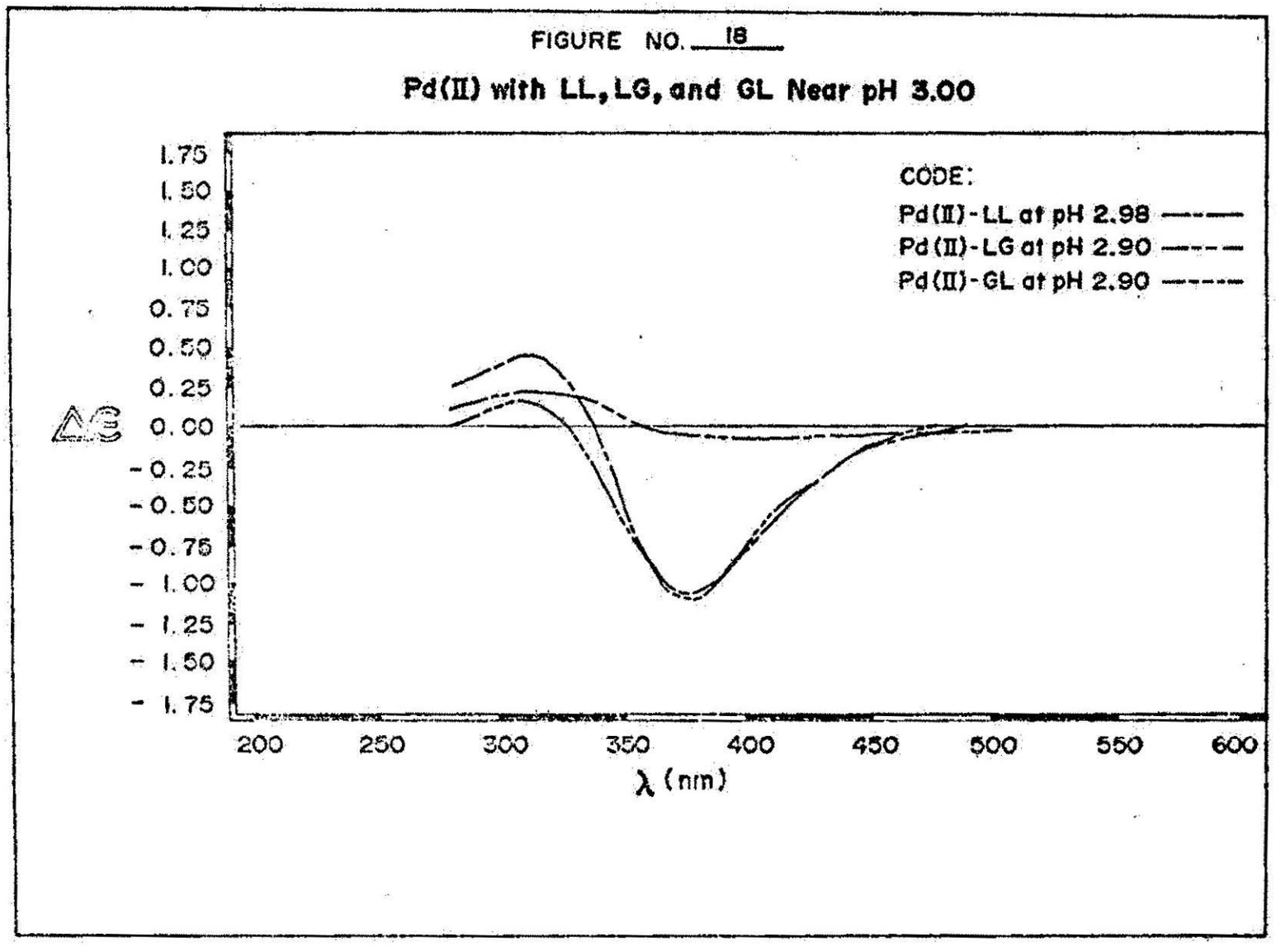

Pd(II) with LL, LG, and GL Near pH 3.00
CODE:
Pd(II)-LL at pH 2.98
Pd(II)-LG at pH 2.90
Pd(II)-GL at pH 2.90
Δε
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 19

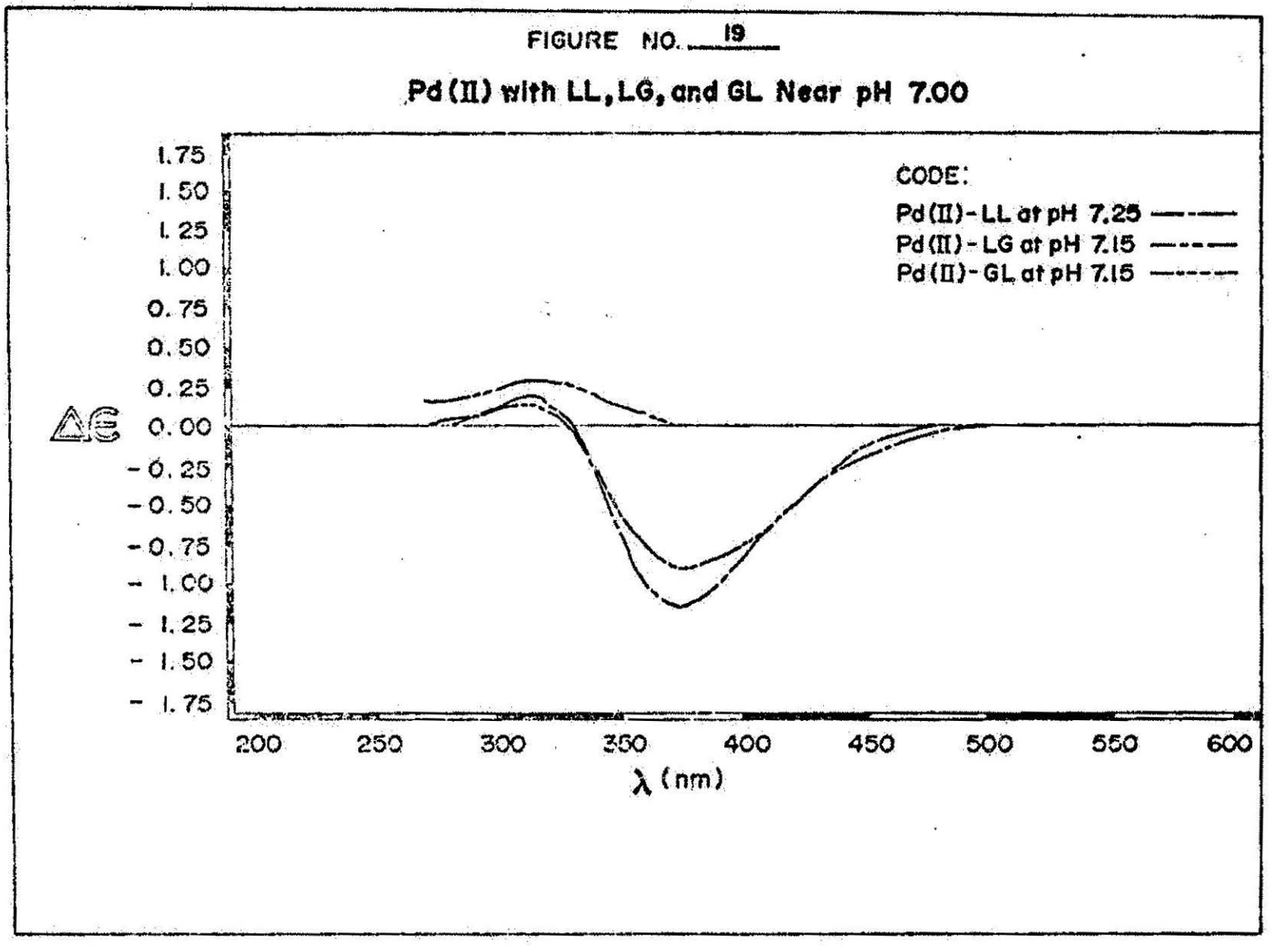

Pd(II) with LL, LG, and GL Near pH 7.00
CODE:
Pd(II) - LL at pH 7.25
Pd(II) - LG at pH 7.15
Pd(II) - GL at pH 7.15
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 20

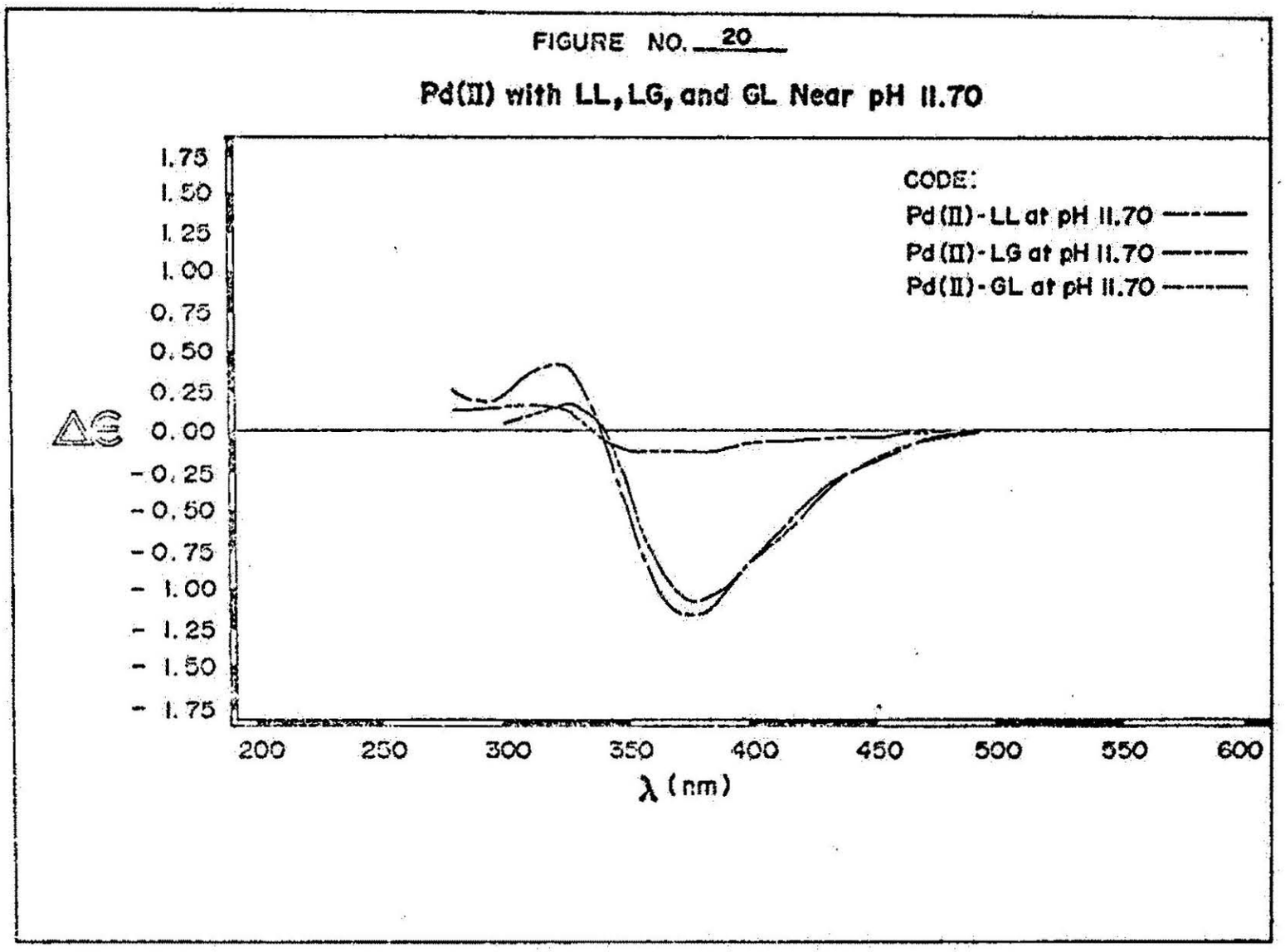

Pd(II) with LL, LG, and GL Near pH 11.70
CODE:
Pd(II)-LL at pH 11.70
Pd(II)-LG at pH 11.70
Pd(II)-GL at pH 11.70
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 21

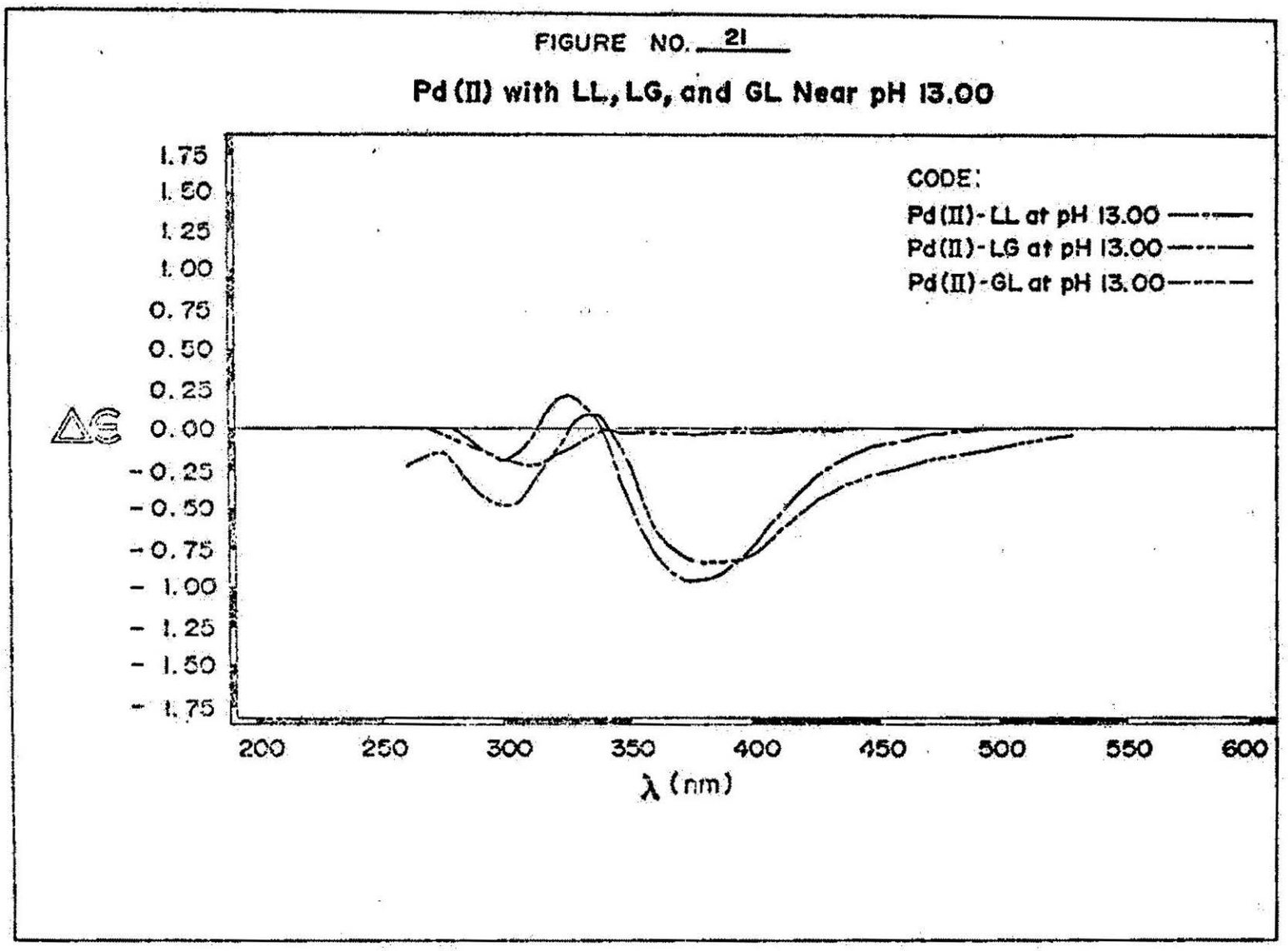

Pd(II) with LL, LG, and GL Near pH 13.00
CODE:
Pd(II)-LL at pH 13.00
Pd(II)-LG at pH 13.00
Pd(II)-GL at pH 13.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 22

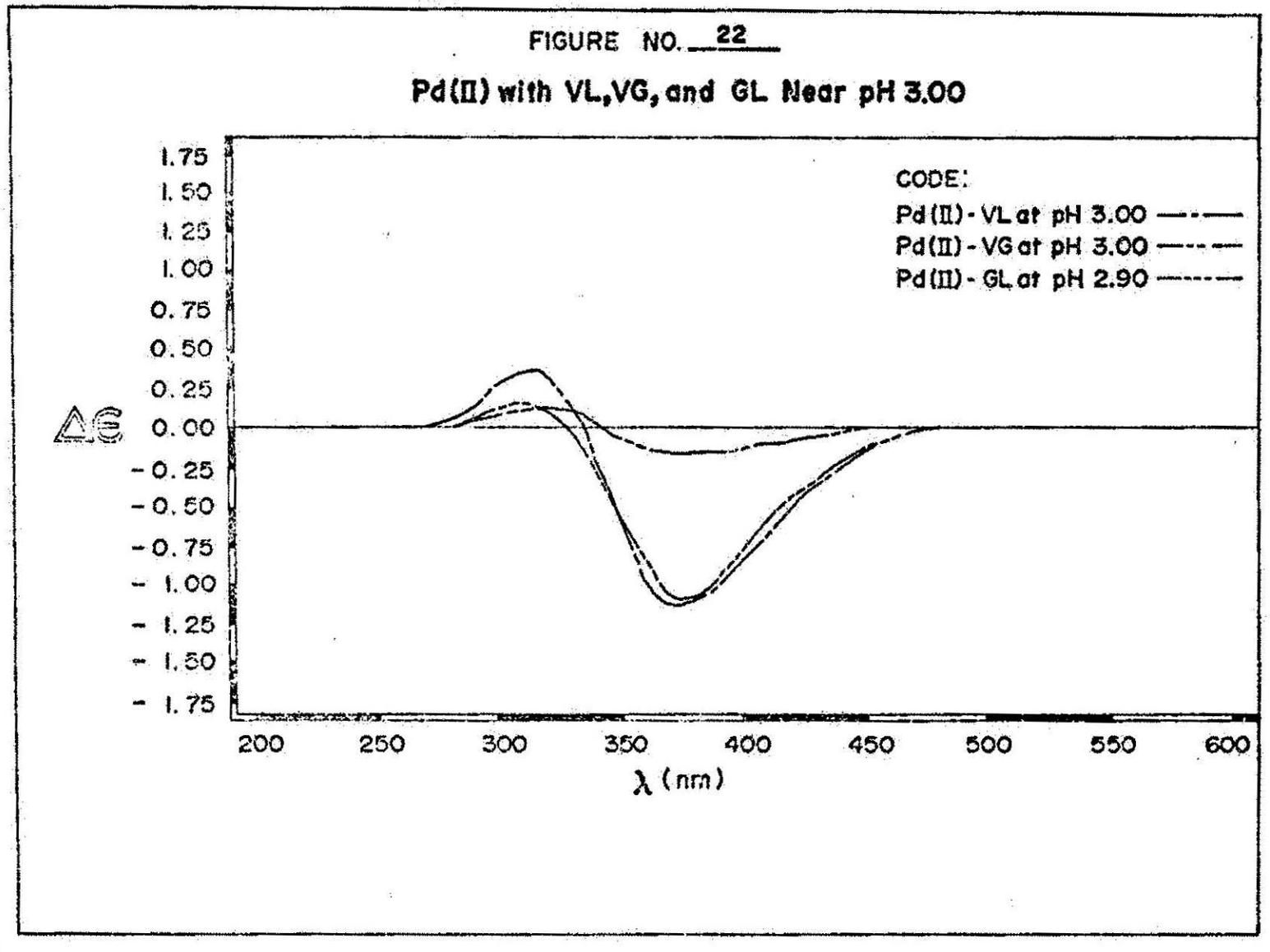

Pd(II) with VL, VG, and GL Near pH 3.00
CODE:
Pd(II) - VL at pH 3.00
Pd(II) - VG at pH 3.00
Pd(II) - GL at pH 2.90
Δε
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 23

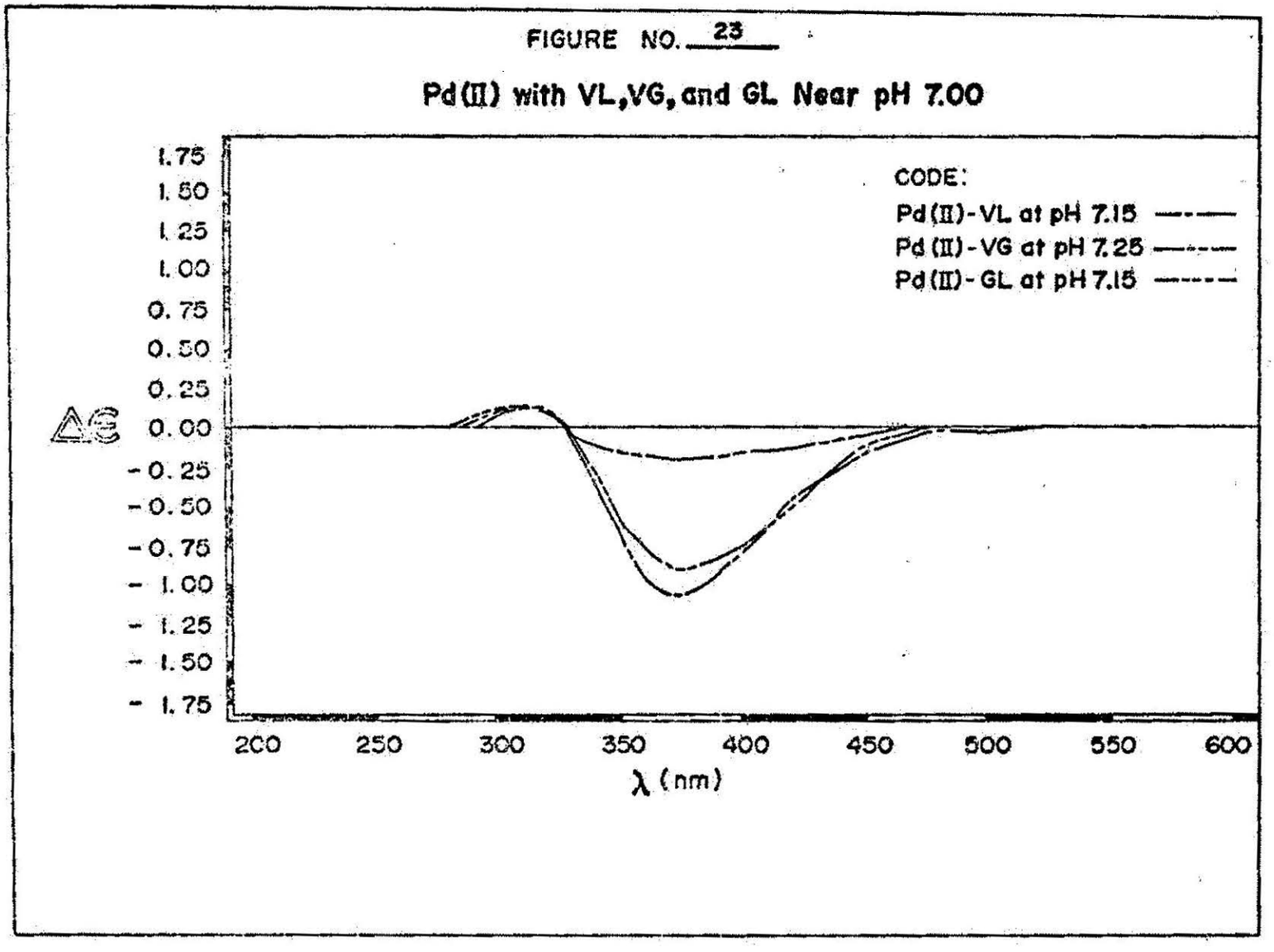

Pd(II) with VL, VG, and GL Near pH 7.00
CODE:
Pd(II)-VL at pH 7.15
Pd(II)-VG at pH 7.25
Pd(II)-GL at pH 7.15
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
Δε
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 24

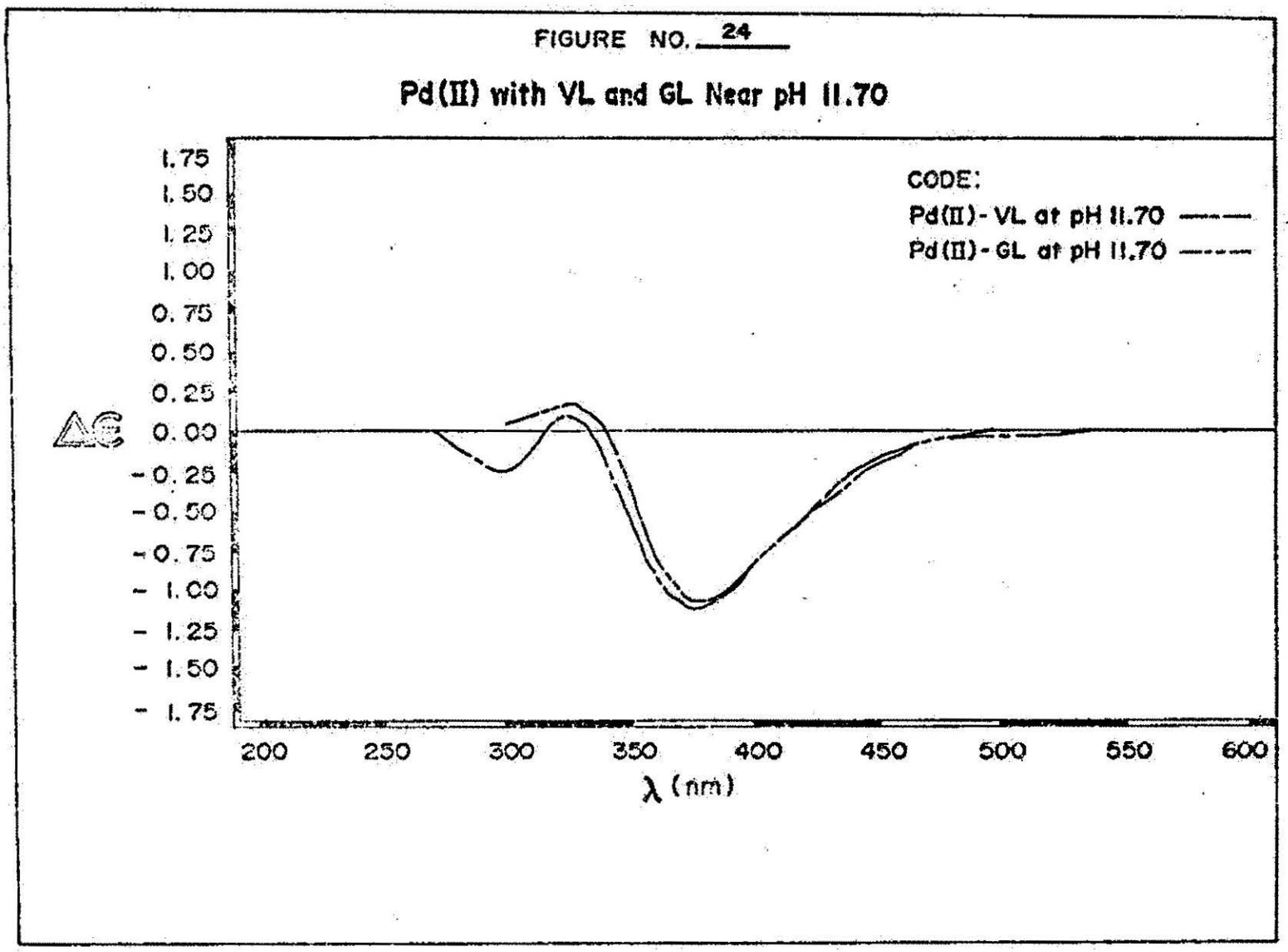

Pd(II) with VL and GL Near pH 11.70
CODE:
Pd(II) - VL at pH 11.70
Pd(II) - GL at pH 11.70
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 25

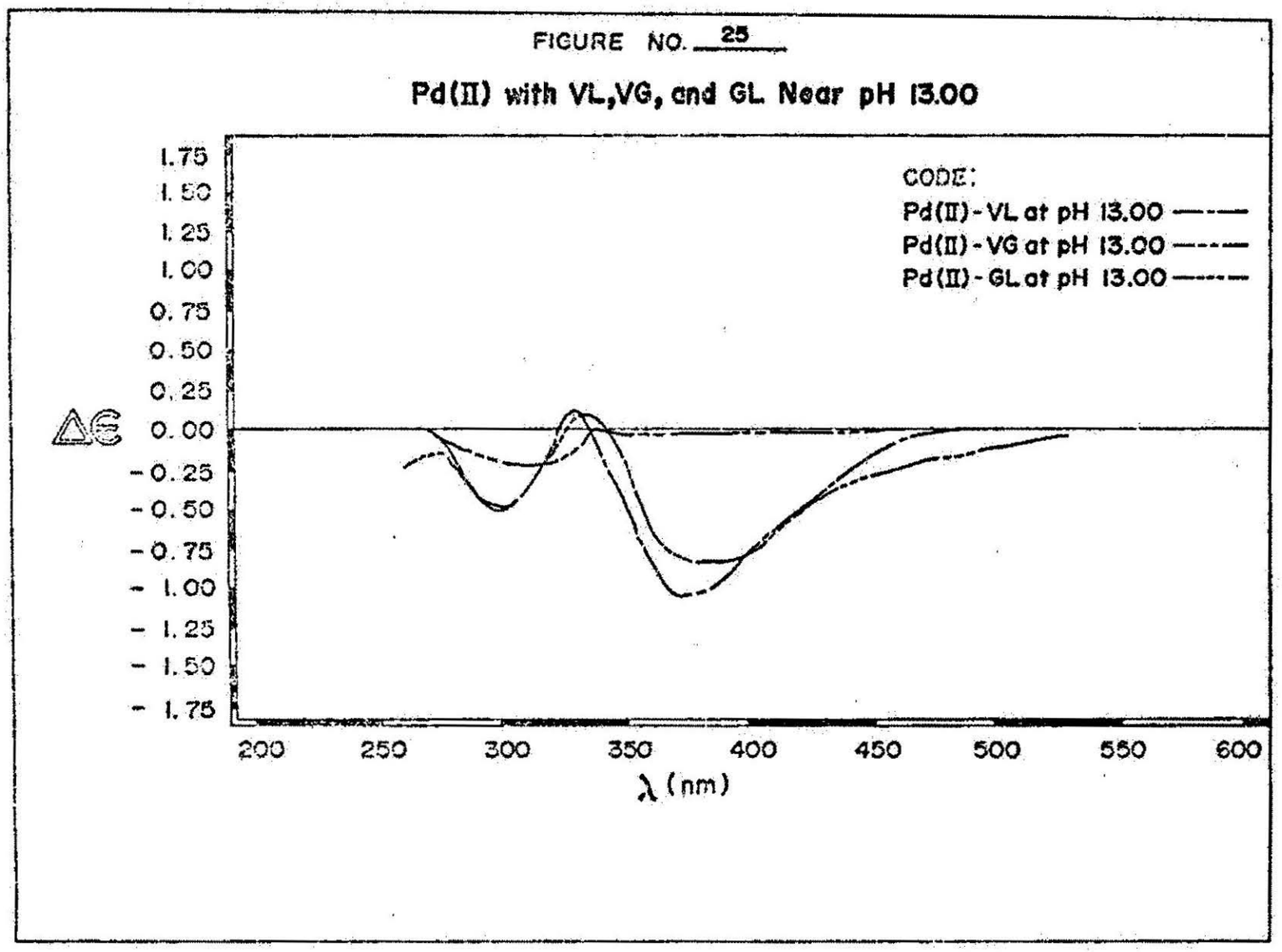

Pd(II) with VL,VG, and GL Near pH 13.00
CODE:
Pd(II) - VL at pH 13.00
Pd(II) - VG at pH 13.00
Pd(II) - GL at pH 13.00
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 26

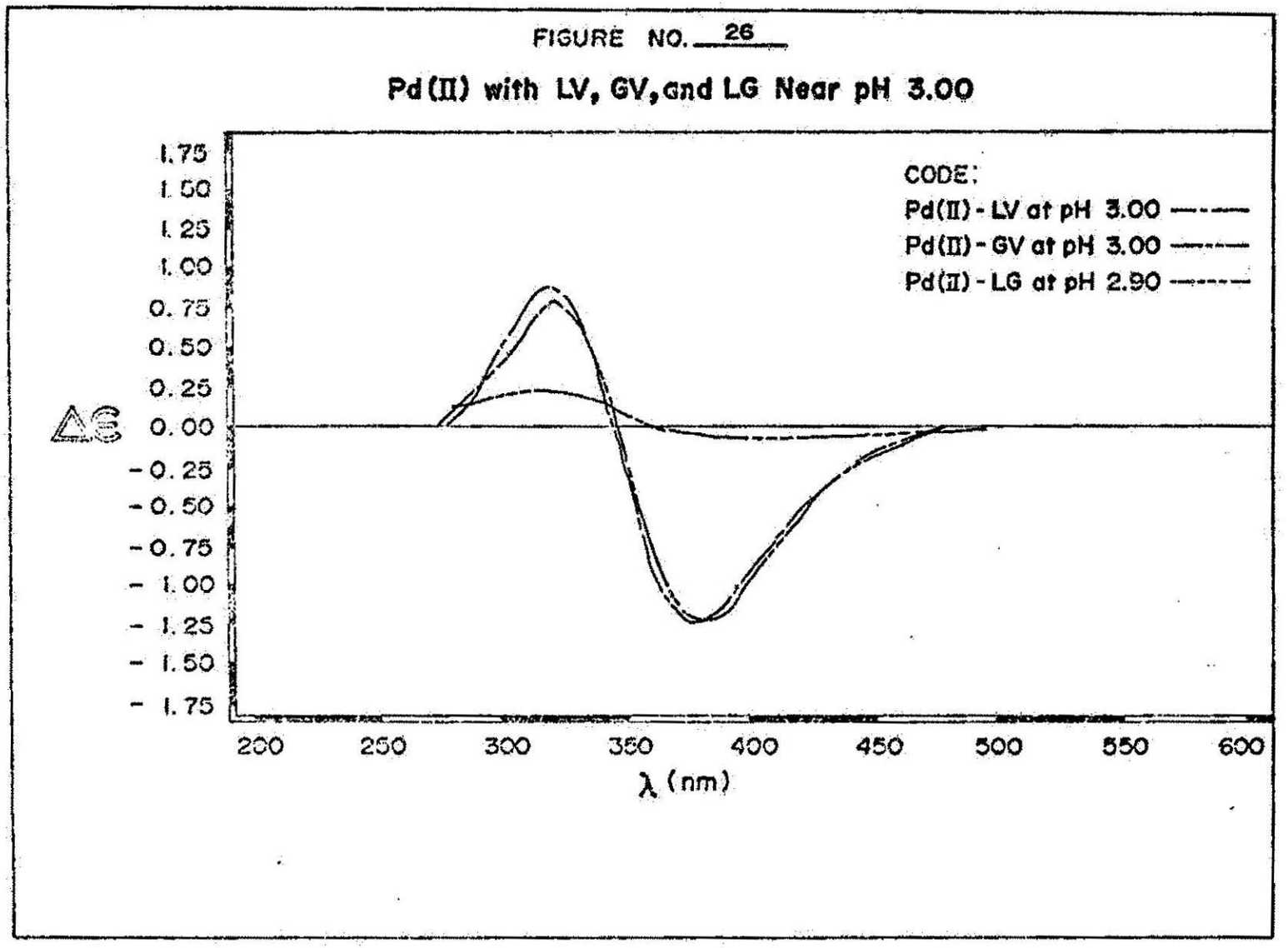

Pd(II) with LV, GV, and LG Near pH 3.00
CODE:
Pd(II) - LV at pH 3.00
Pd(II) - GV at pH 3.00
Pd(II) - LG at pH 2.90
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 27

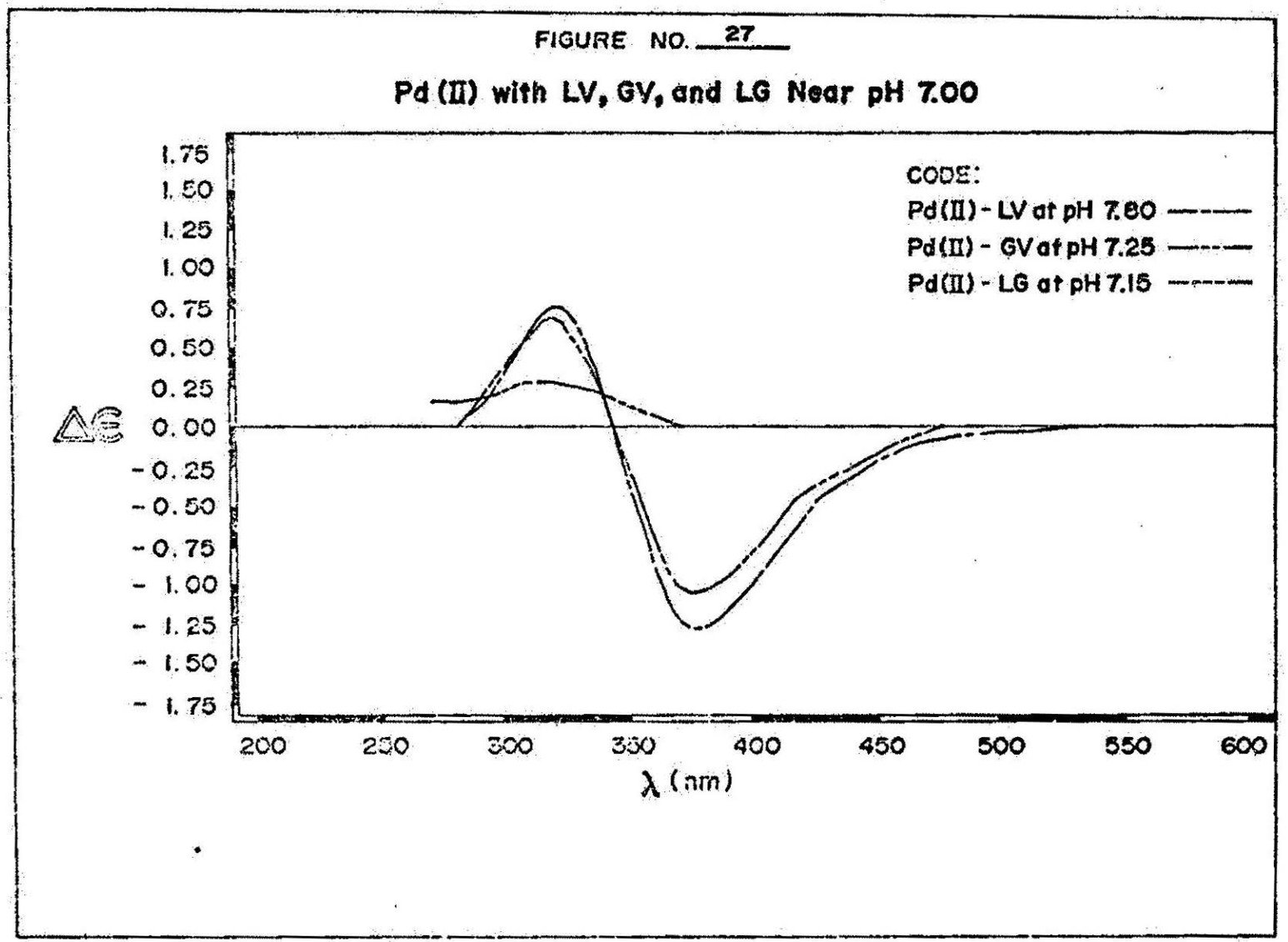

Pd (II) with LV, GV, and LG Near pH 7.00
CODE:
Pd(II) - LV at pH 7.80
Pd(II) - GV at pH 7.25
Pd(II) - LG at pH 7.15
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 28

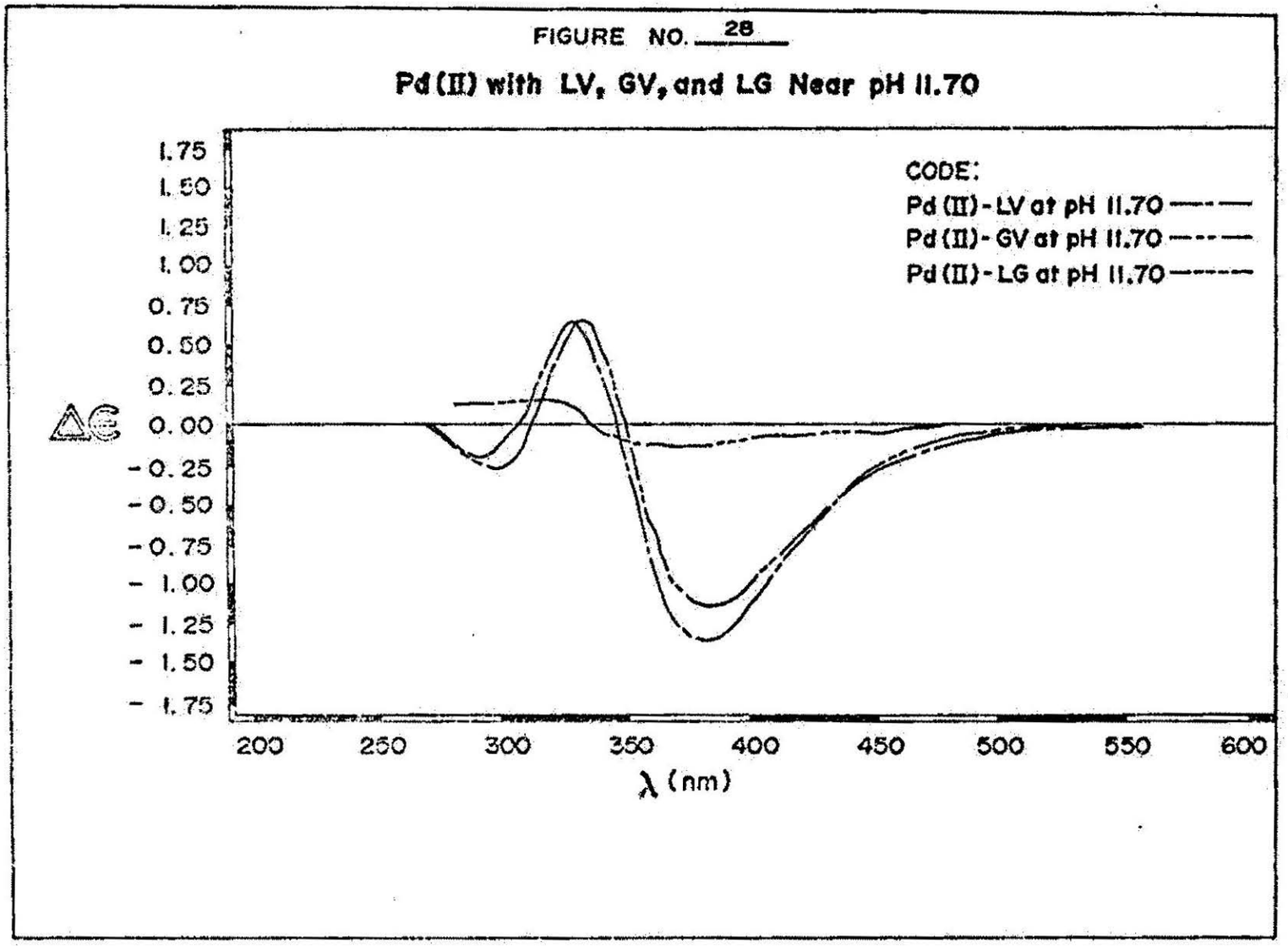

Pd(II) with LV, GV, and LG Near pH 11.70
CODE:
Pd(II) - LV at pH 11.70
Pd(II) - GV at pH 11.70
Pd(II) - LG at pH 11.70
ΔE
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
200
250
300
350
400
450
500
550
600
λ (nm)

FIGURE NO. 29

Pd(II) with LV, GV, and LG Near pH 13.00

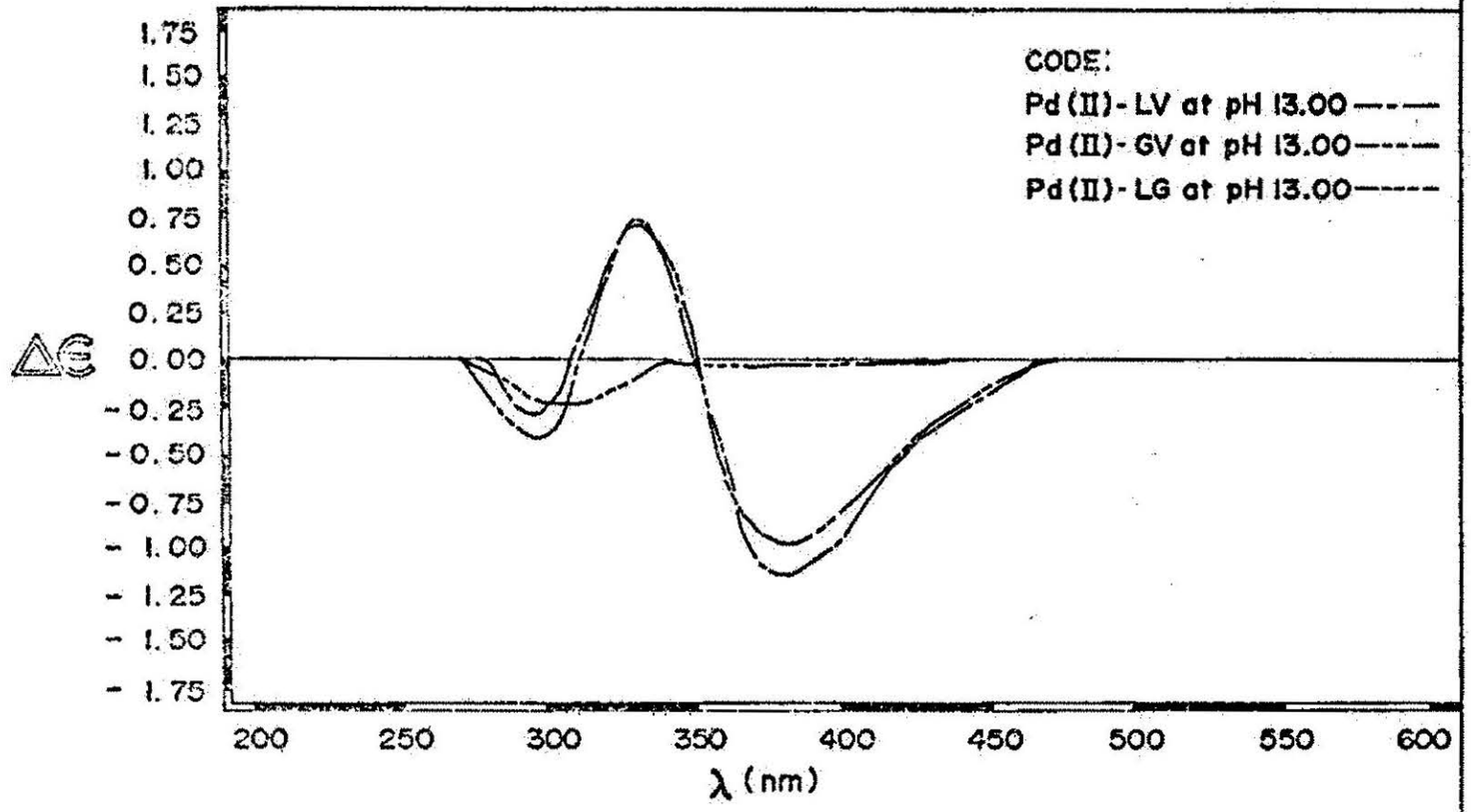
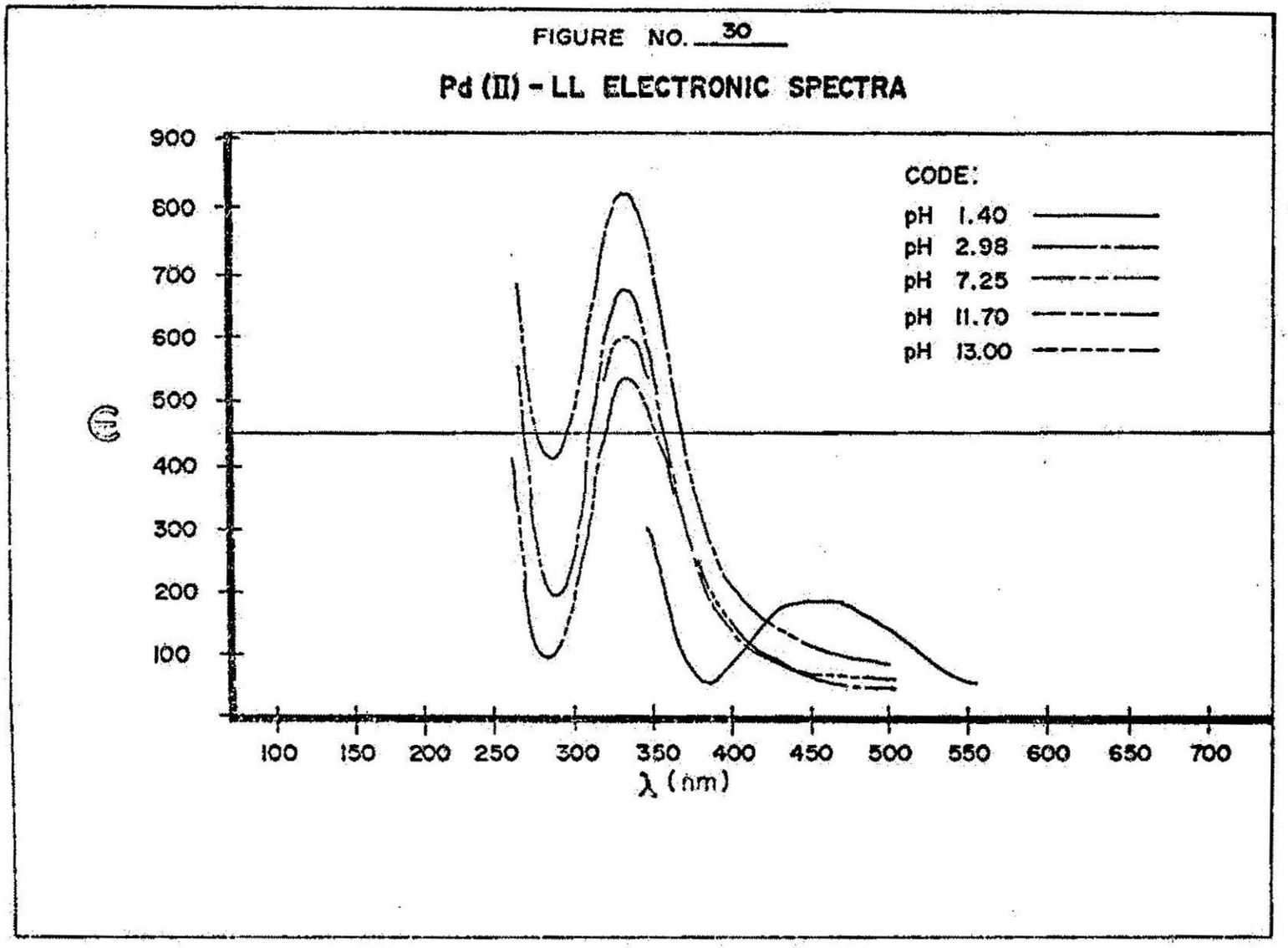


FIGURE NO. 30


Pd (II) - LL ELECTRONIC SPECTRA
900
800
700
600
500
400
300
200
100
ε
CODE:
pH 1.40
pH 2.98
pH 7.25
pH 11.70
pH 13.00
100 150 200 250 300 350 400 450 500 550 600 650 700
λ (nm)

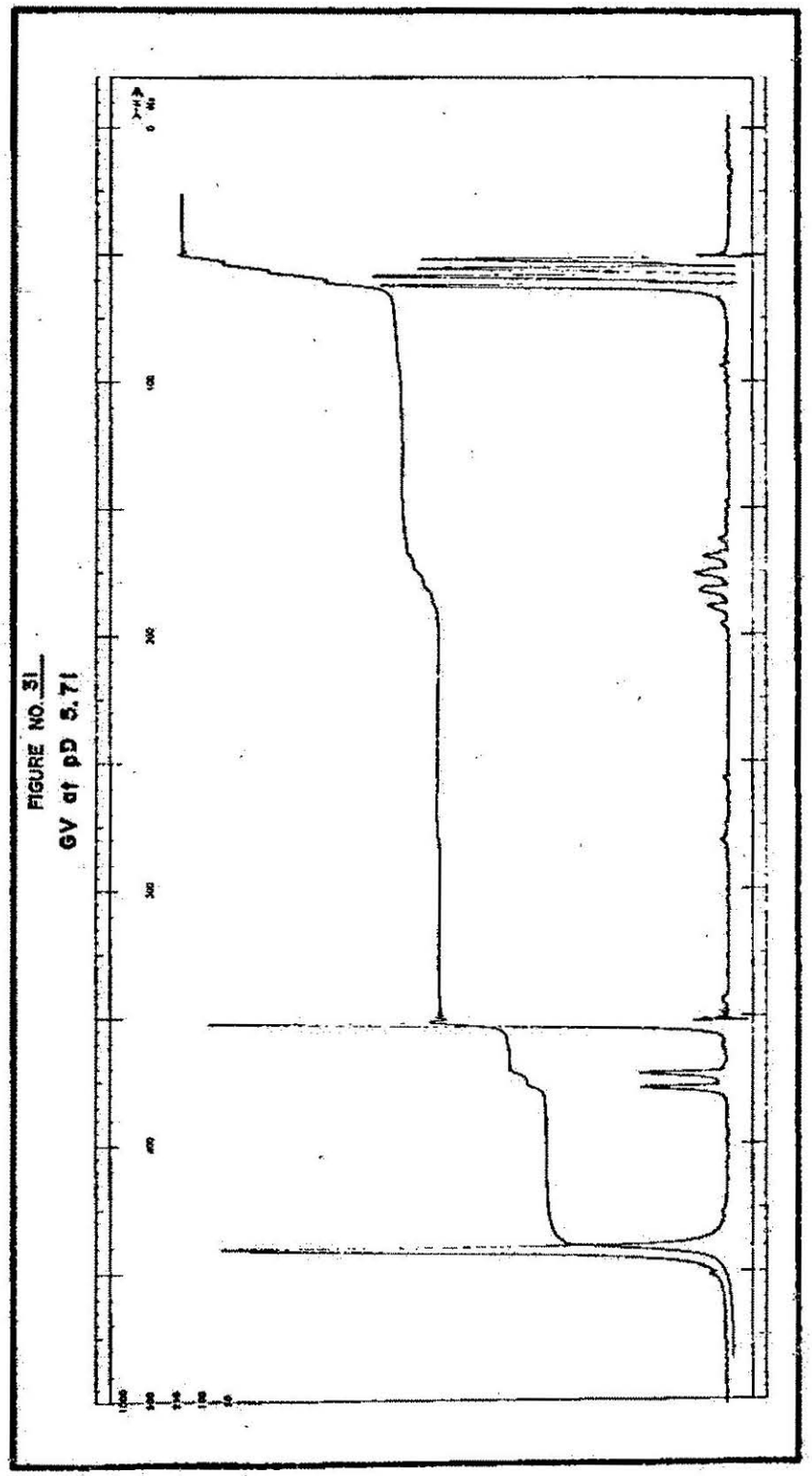


FIGURE NO. 31
GV at pD 5.71

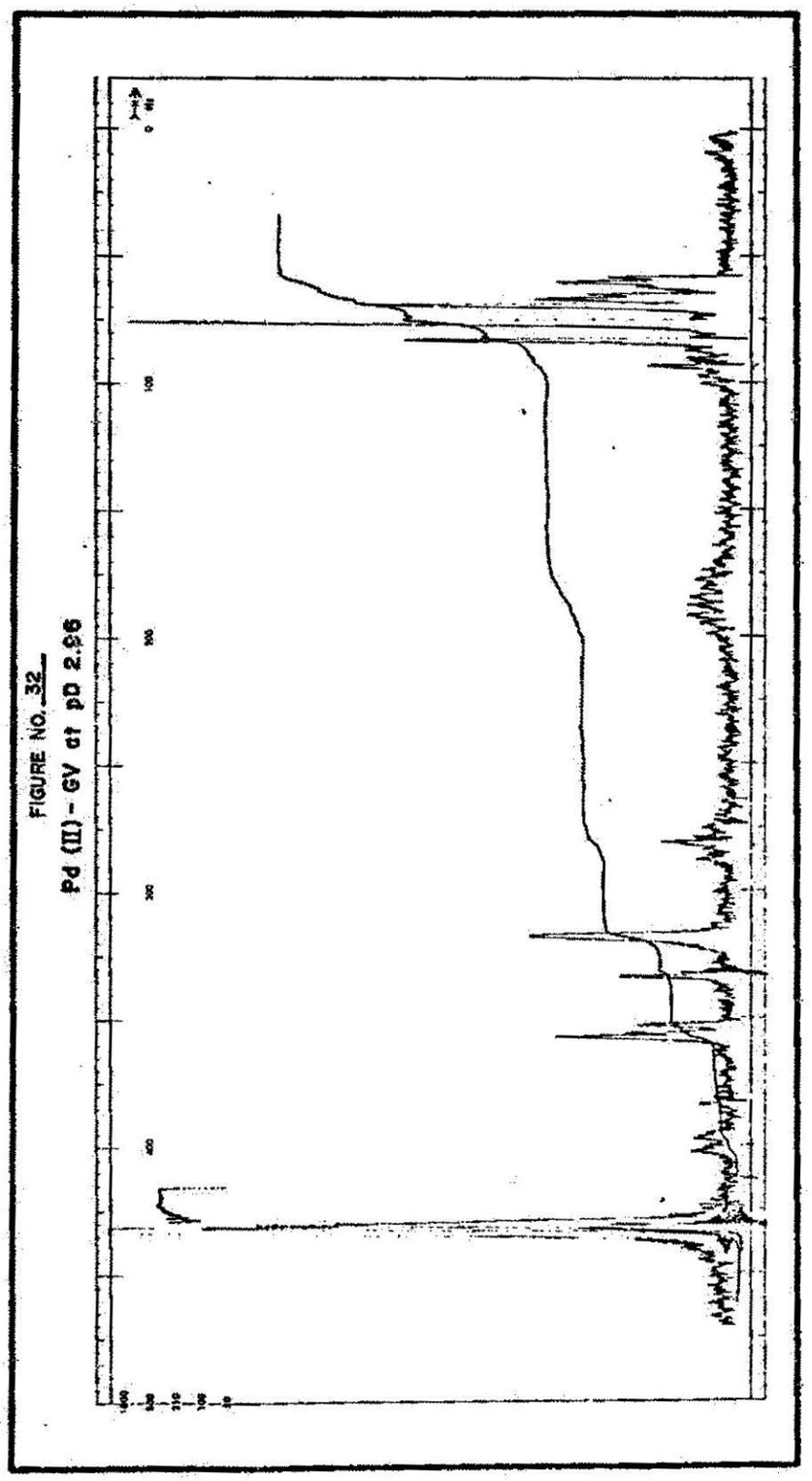


FIGURE NO. 32

Pd (II) - GV at pD 2.96

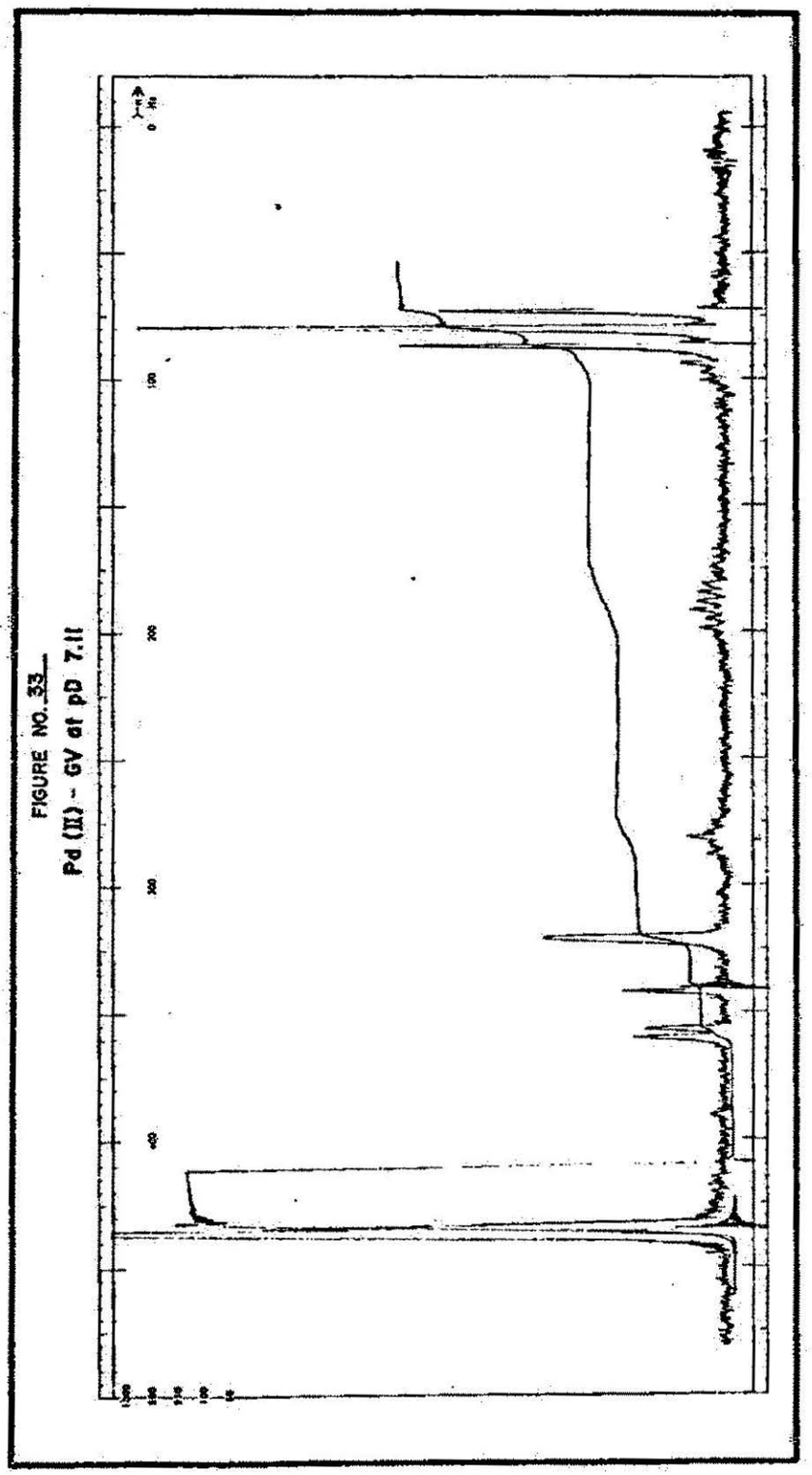
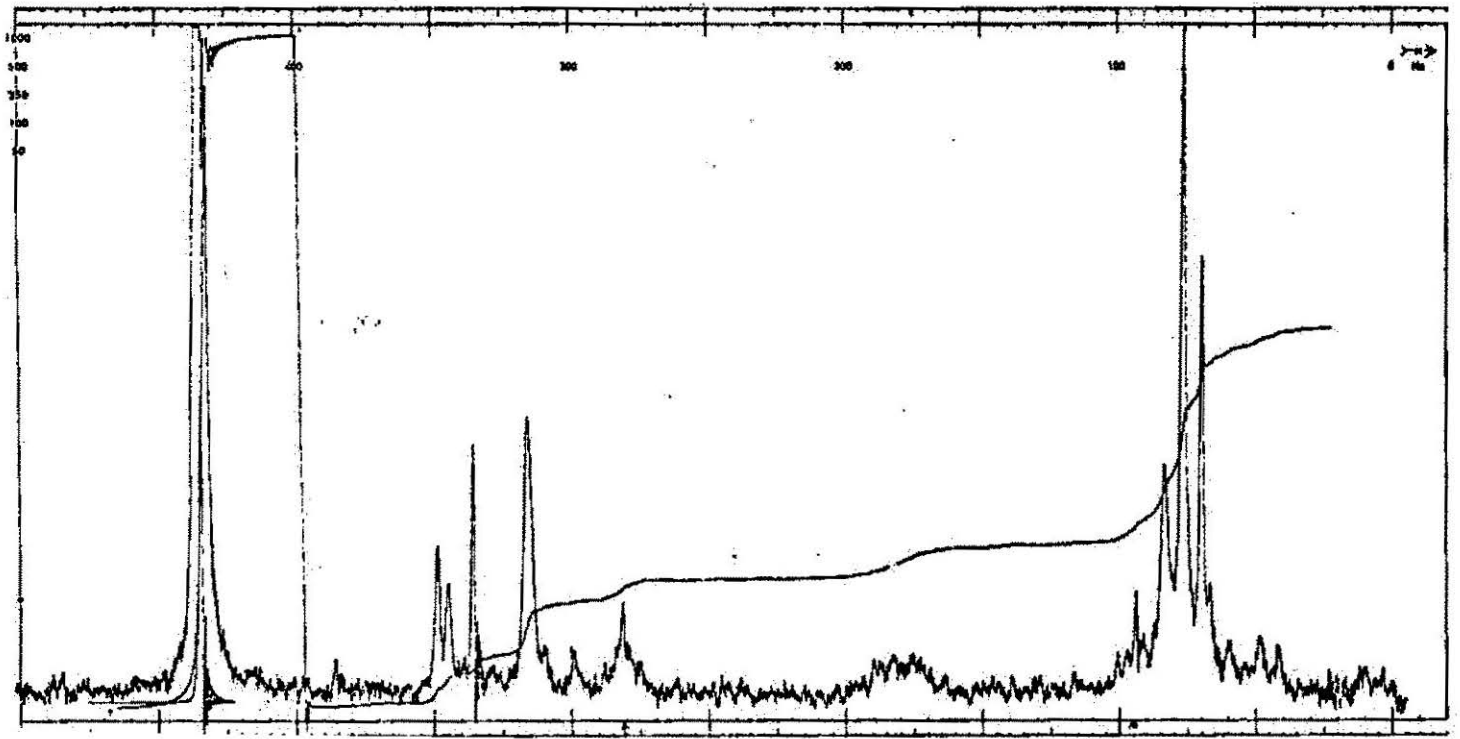


FIGURE NO. 33
Pd (II) - GV at pD 7.11

FIGURE NO. 34

Pd (II) - GV at pD 13.06



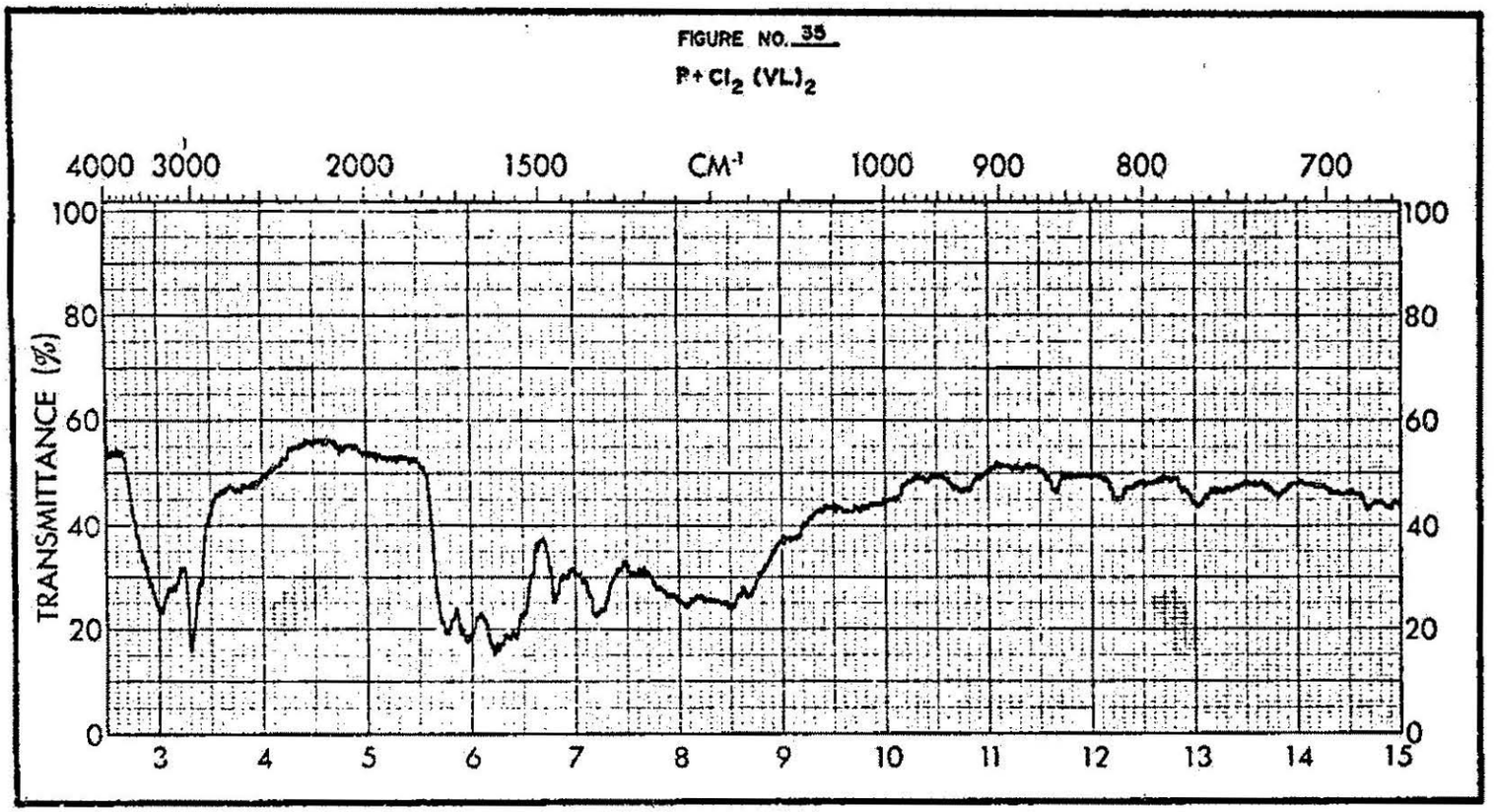


FIGURE NO. 35
P + Cl2 (VL)2
4000 3000 2000 1500 CM-1 1000 900 800 700
100
80
60
40
20
0
TRANSMITTANCE (%)
3 4 5 6 7 8 9 10 11 12 13 14 15

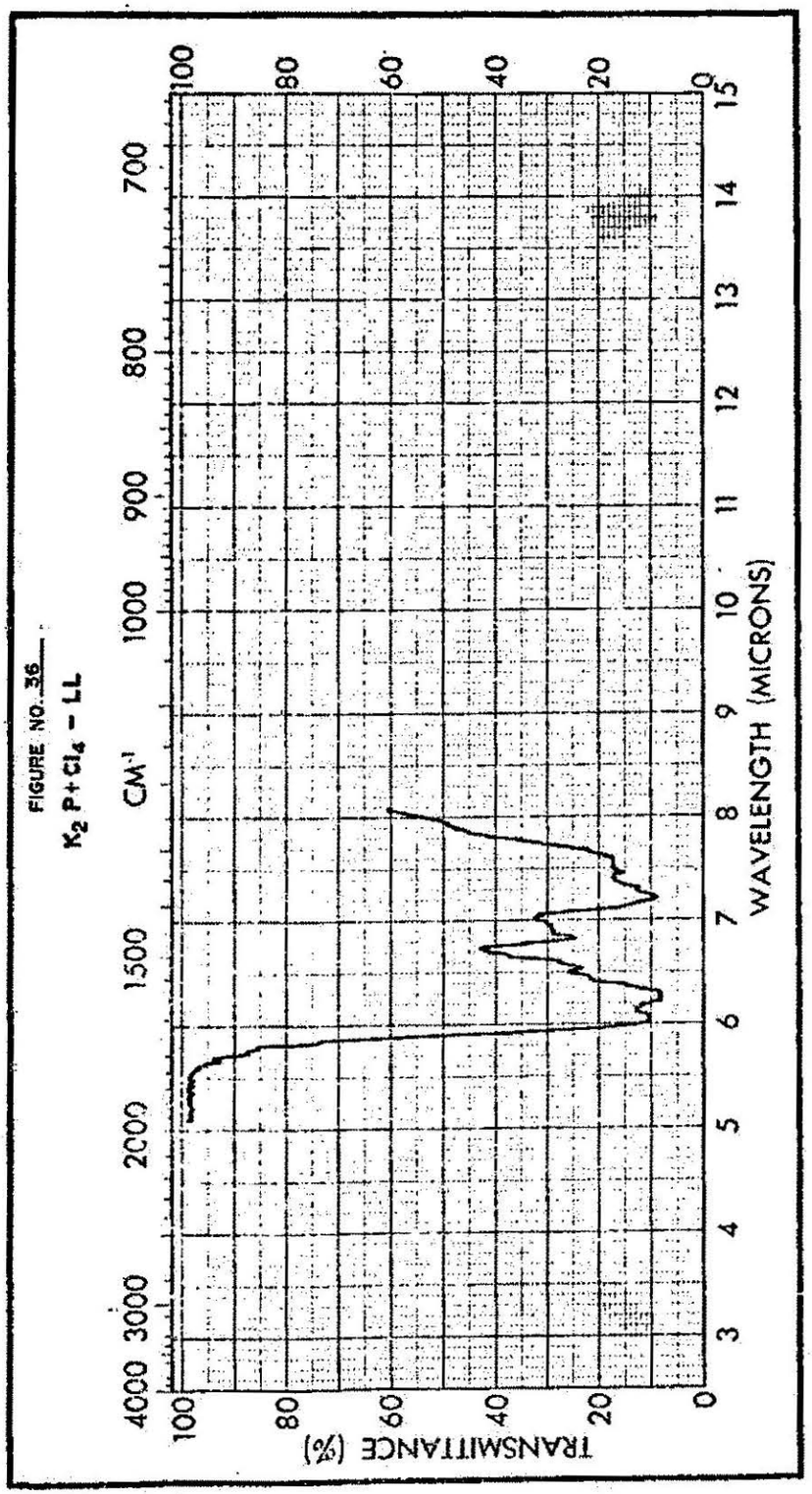


FIGURE NO. 36
K_2P+Cl_4 – LL
TRANSMITTANCE (%)
CM⁻¹
WAVELENGTH (MICRONS)
4000
3000
2000
1500
1000
900
800
700
100
80
60
40
20
0
3
4
5
6
7
8
9
10
11
12
13
14
15

FIGURE NO. 37

ZS-VV Intermediate

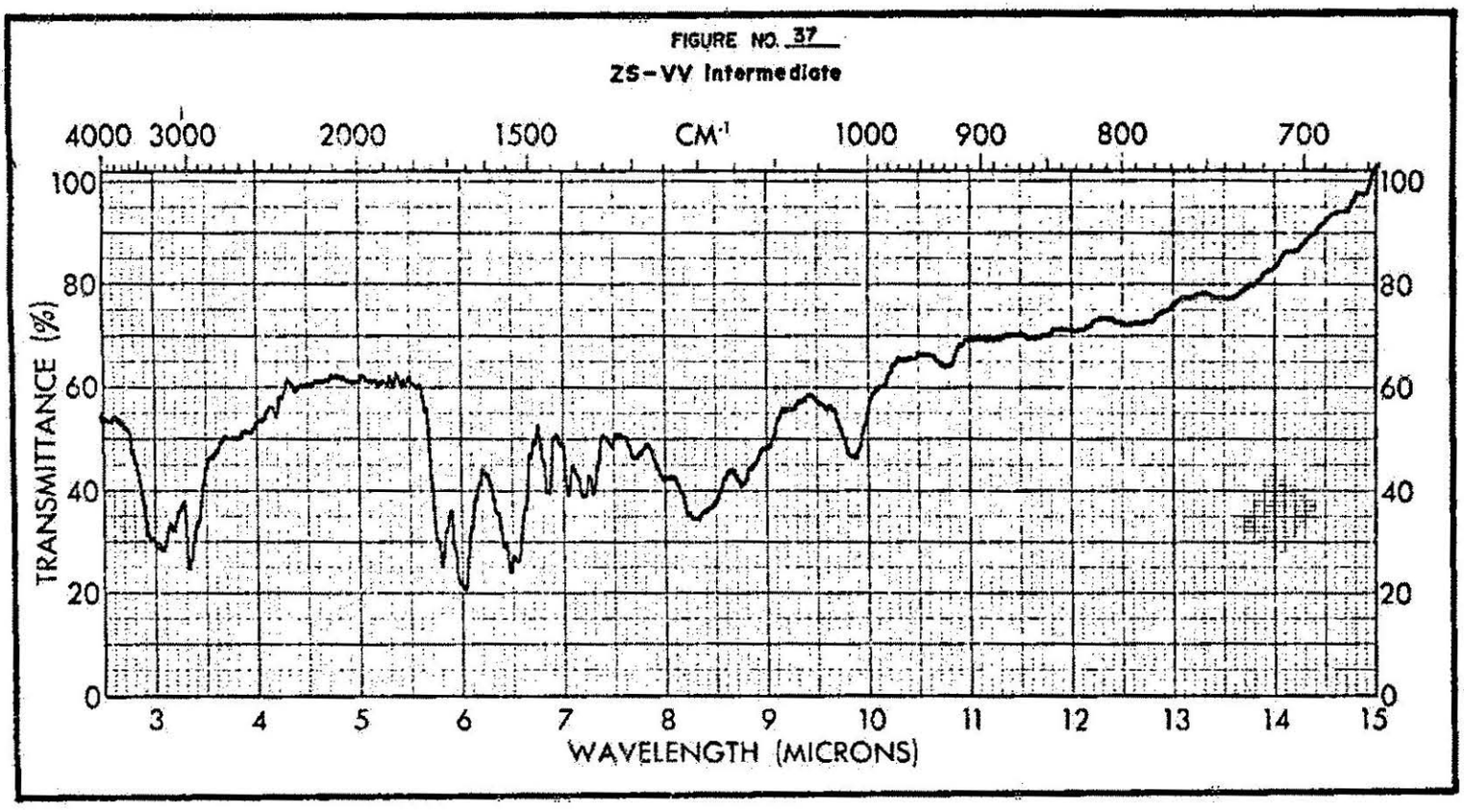
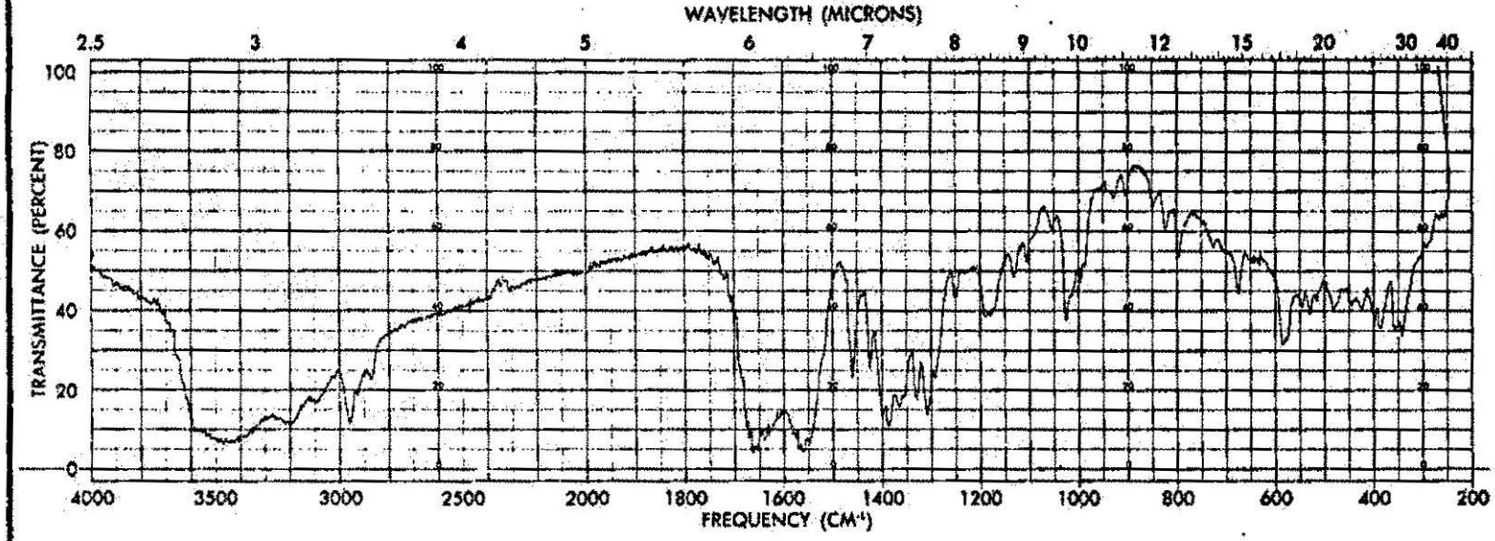


FIGURE NO. 38

ZS-VV



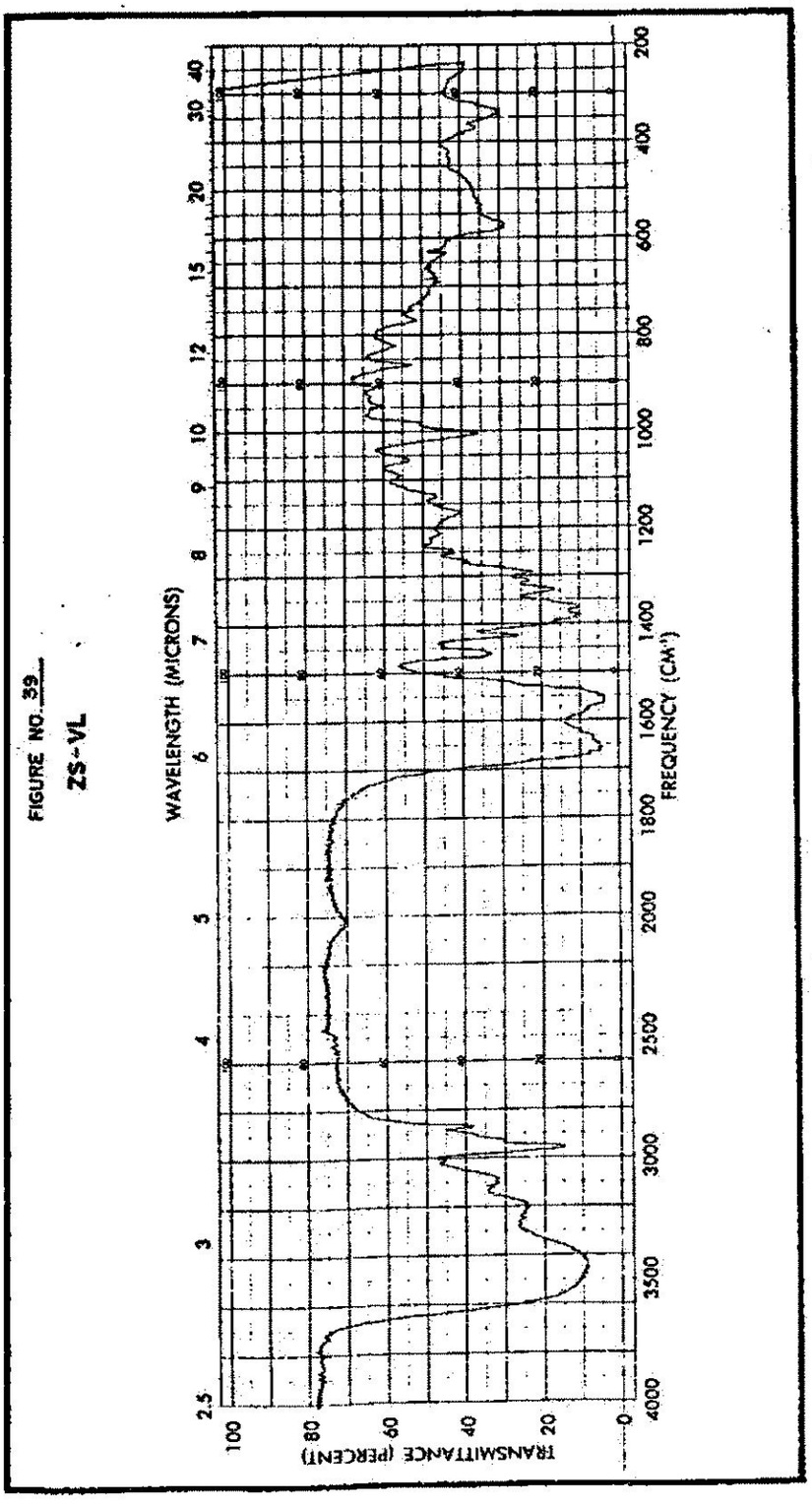
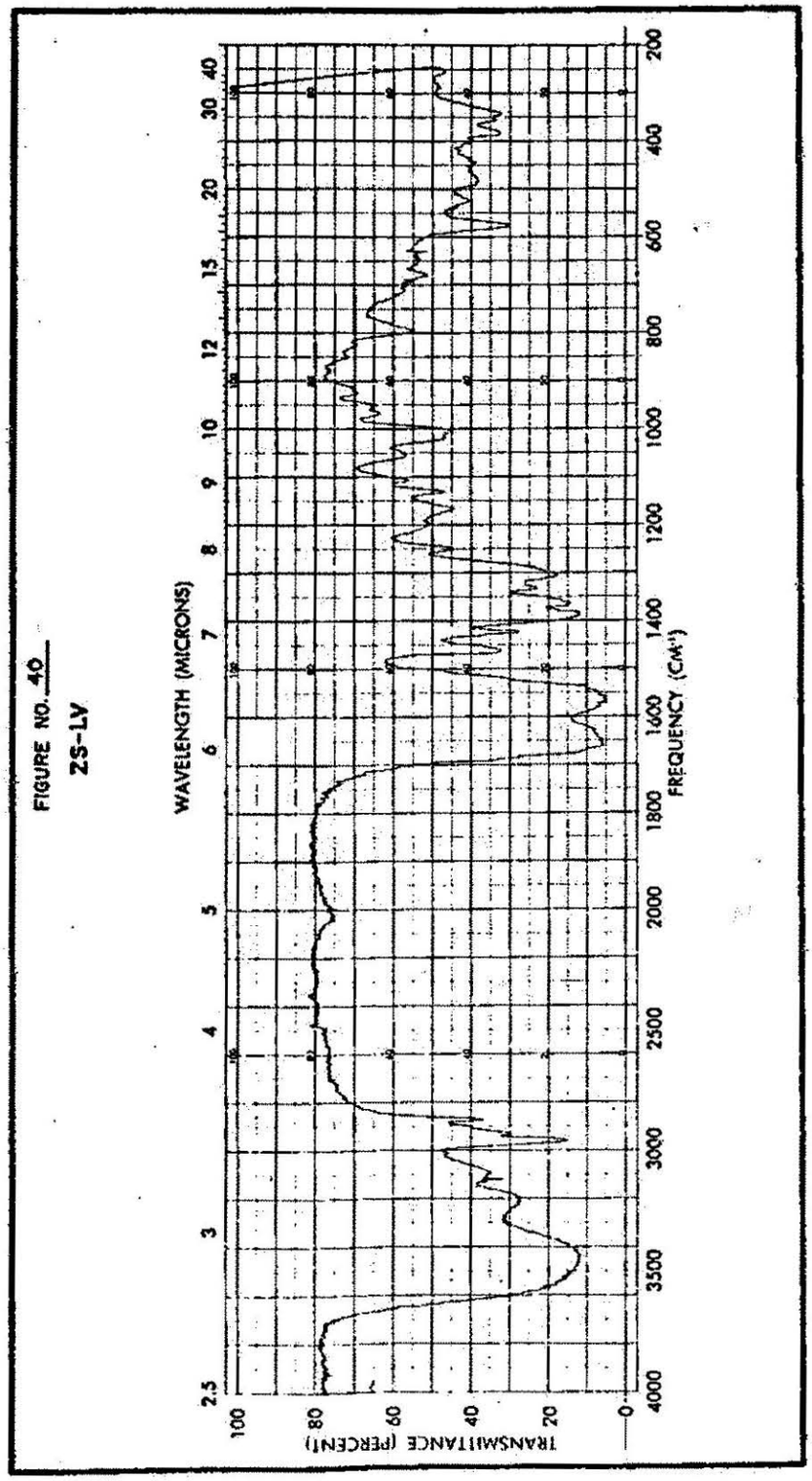


FIGURE NO. 39

ZS-VL

FIGURE NO. 40

2S-LV



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