COLORIMETRIC METHODS FOR THE DETERMINATION

OF CARBON MONOXIDE IN AIR AND BLOOD

by

ALBERT T. LIU

B. S., Fu-Jen University, 1971

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KANSAS STATE UNIVERSITY

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Approved by:

Jack L. Lambert

Major Professor

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CHAPTER I

INTRODUCTION

Carbon monoxide is one of the most important of urban
atmospheric pollutants. It is of interest as an air contami-
nant because of its known toxic properties. In spite of
this characteristic, and the known emission of the gas from
internal combustion engines, outdoor measurements of this
ubiquitous chemical have been remarkably scarce until recent
years.

Carbon monoxide, a colorless, odorless gas, is in-
sidious in the onset of its effects; unlike the other major
gaseous pollutants, it has no warning properties such as
odor. The toxic effect of carbon monoxide is associated
with its ability to form carboxyhemoglobin from hemoglobin.
The affinity of carbon monoxide for hemoglobin is about
210 times as strong as that of oxygen. Aspects of carbon
monoxide and human health have been reviewed by Goldsmith
and Landaw.[1]

The determination of carbon monoxide in blood has
been achieved by the gasometric methods,[2,3] optical
methods,[4,5,6] and chromatographic methods.[7,8]

A series of methods for the determination of carbon
monoxide in the atmosphere by chemical methods[9,10] has
been described and is today considered to be classical.
In most of these methods, carbon monoxide is oxidized to
carbon dioxide by means of palladium chloride or iodine
pentoxide or by using an oxidative catalyst, and one of

the reaction products is then determined.

At present, more expensive and sensitive instrumental analytical methods are generally used, in which carbon monoxide is determined electrochemically,[11,12] radioactively[13,14] or chromatographically.[15,16,17,18] Infrared spectroscopy techniques have also been used with success.[19,20] Most of the methods mentioned permit carbon monoxide to be determined down to concentrations of tens or units of parts per million.

Good colorimetric methods for carbon monoxide are limited. The reaction of carbon monoxide with an alkaline solution of silver and sodium para-sulfaminobenzoate is one of the most popular colormetric methods.[21,22] Difficulties with the method involve measurement of a sol rather than a solution and the requirement of a strongly basic medium.

New methods were developed by Hamilin[23] and Weins[24] by using sequential reduction reactions to form a colored compound in aqueous solution at room temperature. The details of these reactions was examined. Different reagents have been studied. The methodology used for each study was similar, and will be described in the next section.
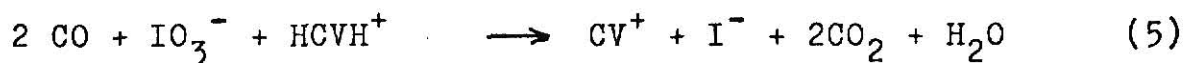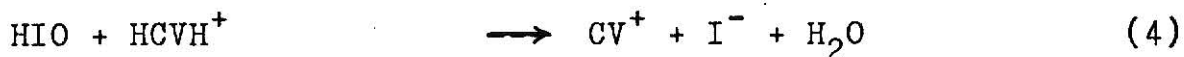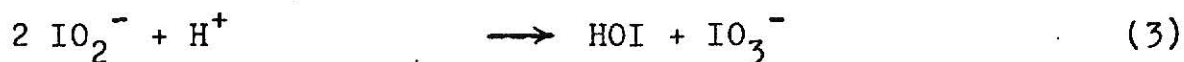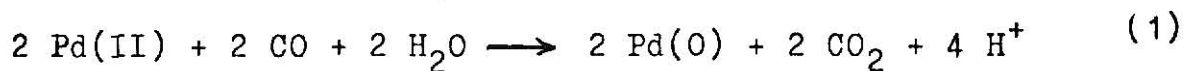
CHAPTER II

A CRYSTAL VIOLET METHOD FOR CARBON MONOXIDE

Previous workers in this laboratory have observed that leuco crystal violet (4,4',4"-methylidynetris(N,N-dimethylaniline) or LCV) and palladium(II) ligand complex, when sequential reactions occurred, exhibit a color change in response to carbon monoxide.[23,24] In this study more detail has been examined and several different sampling methods were utilized.

In the reaction described here, carbon monoxide reduces potassium iodate to hypoiodous acid, HOI, through reduction with a palladium(II) catalyst. Hypoiodous acid then rapidly oxidizes leuco crystal violet to crystal violet[25] which is measured spectrophotometrically to determine the amount of carbon monoxide in the air.

The reaction sequence is presumed to be :

$$2\,Pd(II) + 2\,CO + 2\,H_2O \longrightarrow 2\,Pd(0) + 2\,CO_2 + 4\,H^+ \tag{1}$$

$$2\,Pd(0) + 2\,IO_3^- + 4\,H^+ \longrightarrow 2\,Pd(II) + 2\,IO_2^- + 2\,H_2O \tag{2}$$

$$2\,IO_2^- + H^+ \longrightarrow HOI + IO_3^- \tag{3}$$

$$HIO + HCVH^+ \longrightarrow CV^+ + I^- + H_2O \tag{4}$$

$$\overline{2\,CO + IO_3^- + HCVH^+ \longrightarrow CV^+ + I^- + 2CO_2 + H_2O} \tag{5}$$

where (Pd(II) = $[PdCl_4]^{2-}$), HCVH$^+$ and CV$^+$ are monoprotonated leuco crystal violet and crystal violet, respectively.

Experimental

All solutions were prepared using deionized water with the purest chemicals available from commercial sources.

Apparatus. Absorption spectra and individual absorption measurements were taken on a Coleman 124 spectrophotometer. pH values were measured with a Beckman Zeromatic pH meter using Fisher calomel versus glass electrodes. Hamilton syringes #1705 and # 701 were used to prepare test atmospheres. Round bottom flask (250 ml, S/T 24/40) and glass joint (S/T 24/40, 10/30) were put together as the solution container. Rubber serum stoppers was used to seal the glass joint outlets so that test atmospheres could be easily made and shaken with reagent solution. A Burrell Model BB wrist-action shaker was used to shake the bottles containing reagent solution and gas being tested.

Hydrogen tetrachloropalladate(II) solution (5×10^{-3}M). Suspend 0.8870 gm of palladium(II) chloride in 100 ml of water, then add 1.0 ml of concentrated hydrochloric acid. Agitate the mixture vigorously so as to dissolve the solid, then dilute the solution to 1 liter with water.

Leuco crystal violet solution (2.5×10^{-3}M). Dissolve 0.9340 gm of leuco crystal violet in 200 ml of water and 5 ml of 85% phosphoric acid. Dilute it to exactly 1 liter with water. The solution remains stable for at least six months.

Potassium iodate solution (0.02M). Dissolve 4.28 gm of potassium iodate and dilute to exactly 1 liter with deionized water.

Sodium monohydrogenphosphate solution (0.25M) Dissolve 67.00 gm of sodium monohydrogen phosphate heptahydrate,

$Na_2HPO_4 \cdot 7H_2O$, in water to make 1 liter of solution.

Reagent solution A. Prepare by pipetting 1.0 ml each of leuco crystal violet solution, and hydrogen tetrachloropalladate solution into 25.0 ml of potassium iodate solution. Adjust the pH to 3.60 $\pm$ 0.02 with sodium monohydrogenphosphate solution. Measure absorbance maximum at 591 nm.

Procedures for measuring reagent responses. Pipet 25 ml of reagent solution A into the 250 ml flask and close with rubber serum bottle stopper of appropriate size. The volumes of the flasks had been accurately determined gravimetrically and allowance made for reagent volume. Add a calculated volume of 100% pure carbon monoxide at atmospheric pressure with a gas tight syringe for each of the concentrations studied. The flasks containing the gas mixture and reagent were shaken for an appropriate length of time on the wrist-action shaker.

Results

Attempts to vary the reagent solutions described by Weins were unsuccessful. The optimum conditions have been studied very carefully, and the procedure has been modified. Figure 1 shows the spectrum of the product obtained in the experimental conditions specified in the procedure. The absorption maximum is at 591 nm.

A number of carbon monoxide metal complexes have been described.[26] A study was attempted by using other transition metals in place of palladium metal in these sequential reactions. The results are shown in Table I.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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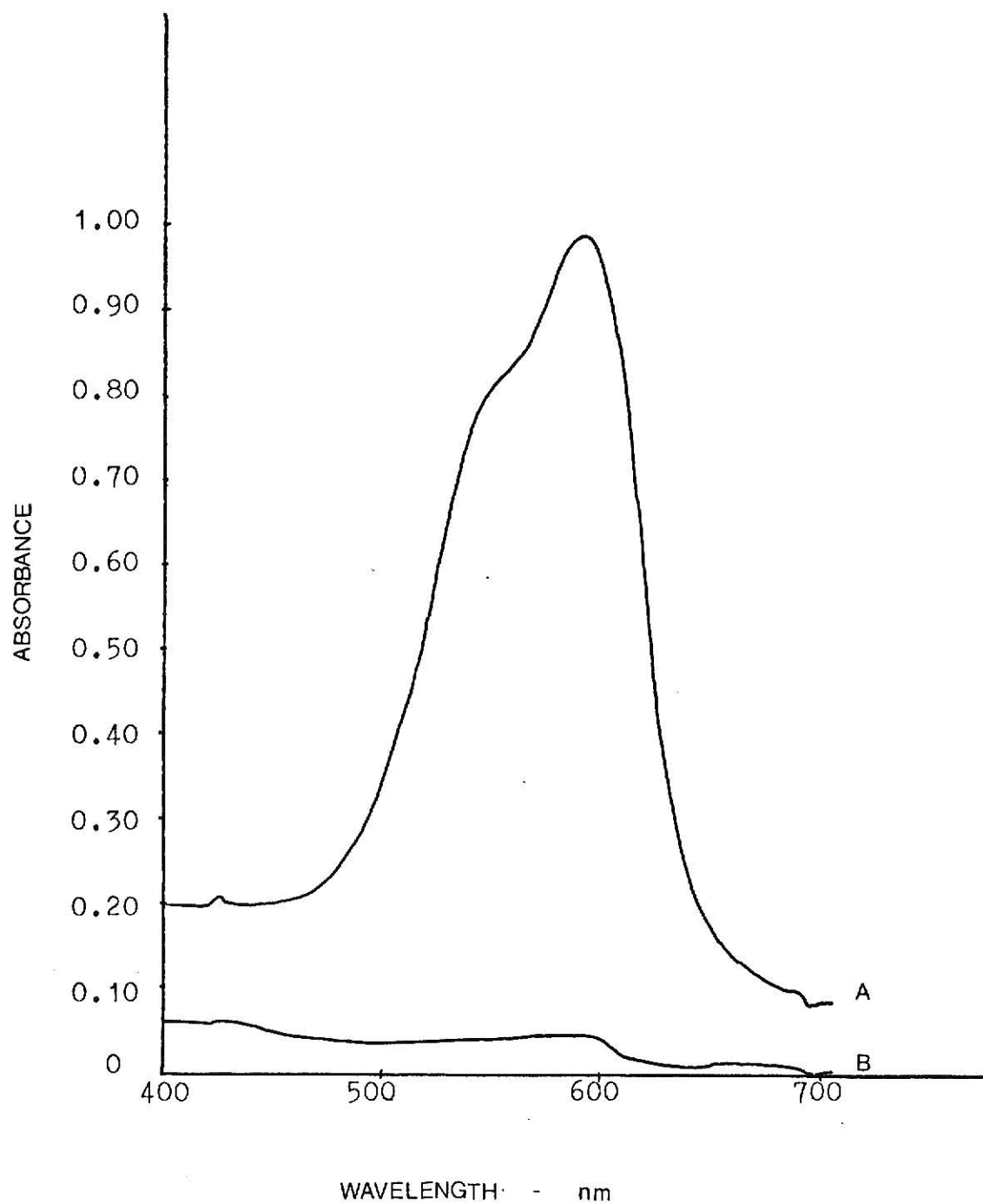
Figure 1. A. Absorption Spectrum of the Product of
Reagent Solution A. B. Spectrum of Blank
Obtained Against Water as Reference.

Table I

Qualitative Tests of Reagents Containing Various

Metal Compounds

Reagent[a]	Absorbance[b]
Leuco crystal violet(2.5×10^{-3}M)	
+ KIO_3(0.02M) with:	
$[PdCl_4]^{2-}$	0.82
$NiCl_2$	0.05
$CoCl_2$	—
$[PtCl_4]^{2-}$	—
$[HgCl_4]^{2-}$	—
$AgNO_3$	L.C.V.(ppt.)

a.   Concentration of each metal solution was 5×10^{-3}M.

Each solution was studied from pH 3.0 to 3.8.

b.   Response by reacting with 73.6 ppm carbon monoxide

— means no response

L.C.V.   Leuco crystal violet precipitated

Reaction time was 30 minutes.

Five dyes were studied in an attempt to get a better response than leuco crystal violet produced. Table II summarizes the qualitative studies of these dyes. Also, a series of palladium compounds have been examined. The qualitative results are reported in Table III.

It is apparent that palladium salts give higher response in this test. Due to consideration of stability, tetrachloropalladate(II) was selected over palladium sulfate, because palladium sulfate solution contained an undetermined precipitate after one month's standing.

Although nickel, palladium, and platinum are in the same family, only palladium showed good response on this method. The absorbance was 0.05 when nickel(II) was used in place of palladium(II) in these sequential reactions. If a small drop of tetrachloropalladate(II) solution was added into nickel(II)-LCV solution, the absorbance increased to 0.29. Platinum(II)-LCV solution gave no response after 30 minutes shaking. Tetrachloropalladate(II) solution did not increase the absorbance of this solution.

Behavior of peroxidase[27] has been observed as a catalyst in the determination of hydrogen peroxide. As carbon monoxide is nearly water-insoluble, an attempt was made to use carbonic anhydrase to increase carbon monoxide solubility in water. This was not successful, as the test solution still had the same absorbance after shaking with carbonic anhydrase enzyme solution as before. Table IV gives the results.

Studies were made of the effects of pH, leuco crystal

Table II

Qualitative Tests of Reagents Containing Various Dyes

Reagent[a]	Absorbance[b]
$PdCl_4^{2-}$ (5×10^{-3} M) + KIO_3 with:	
Leuco Crystal Violet (pH 3.6, 591 nm)	0.70
Leuco Methyl Violet (pH 3.7, 592 nm)	0.60
Leuco Ethyl Violet (pH 3.7, 602 nm)	0.17
Leuco Malachite Green (pH 3.6, 610 nm)	0.35
Leuco Brilliant Green (pH 3.1, 635 nm)	0.07

a. Concentration of each dye was 5×10^{-4} M.

b. Response by reacting with 73.6 ppm carbon monoxide. Reaction time was 30 minutes.

Table III

Qualitative Tests of Reagents Containing Various

Palladium(II) Compounds

Reagent[a]	Absorbance[b]
Leuco Crystal Violet(5×10^{-4}M)	
+ KIO_3 with:	
$[PdCl_4]^{2-}$	0.34
$PdSO_4$	0.33
$Pd(ClO_4)_2$	0.18
$Pd(IO_3)_2$	0.13
$[PdBr_4]^{2-}$	0.15
$Pd(OOCH)_2$	—
$Pd(C_2O_2)$	—
$[Pd(SCN)_4]^{2-}$	—

a. Concentration of each palladium(II) solution was
    5×10^{-3}M.

    pH value of each reagent solution was 3.1.

b. Response by reacting with 73.6 ppm carbon monoxide

—   Means no response

Reaction time was 30 minutes.

Table IV

Qualitative Tests of Reagent Solution A Containing
Carbonic Anhydrase

Time	Absorbance I	Absorbance II
10 minutes	0.470	0.450
20 minutes	0.690	0.695
30 minutes	0.870	0.890
40 minutes	0.970	1.040

Absorbance I	Reagent Solution A with 0.5 mg enzyme
Absorbance II	Reagent Solution A without enzyme.

violet concentration, potassium iodate concentration and tetrachloropalladate concentration. The results of pH dependence studies of the leuco crystal violet-palladate solution are plotted in Figure 2. From this figure the optimum pH appears to be near 3.6. Bubbles appeared in solution after shaking when the pH was greater than 3.8. The pH of the reagent solution was adjusted with sodium mono-hydrogenphosphate solution.

The optimum concentrations of leuco crystal violet, iodate and tetrachloropalladate(II) were determined by varying the concentration of each in the presence of fixed concentrations of the other two. Different concentrations of potassium iodate versus absorbance were plotted in Figure 3. Within this sequential reaction, potassium iodate exhibited a plateau when the concentration is above 0.015M. Excess amount of potassium iodate beyond that concentration did not increase the absorbance. Leuco crystal violet also exhibited a concentration plateau. If the concentration of LCV was higher than 2.5×10^{-3}M, the absorbance did not increase. The results of the absorbance dependence studies of the concentration of leuco crystal violet are plotted in Figure 4. Theoretically, the higher the concentration of tetrachloropalladate used, the higher the response should be. Too much tetrachloropalladate, however, causes the reagent solution to become turbid after shaking. 5×10^{-3}M Tetrachloropalladate(II) appears to be the optimum for this reaction.[24] The optimum conditions which were required for the best response are shown in Table V.
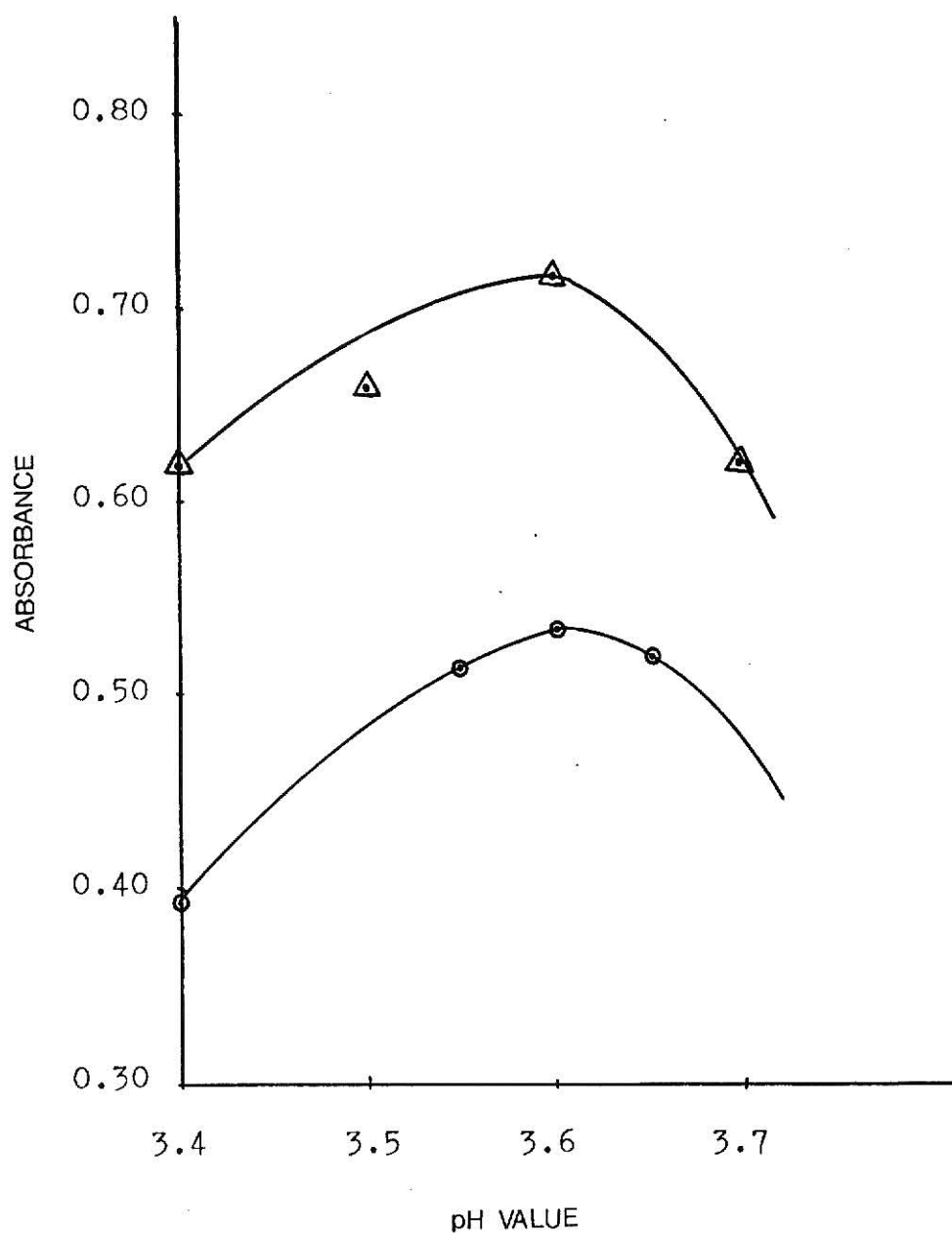
Figure 2. Effect of pH on LCV-Palladate Solution

△ =73.6 ppm CO, 30 minutes contact time

⊙ =73.6 ppm CO, 15 minutes contact time

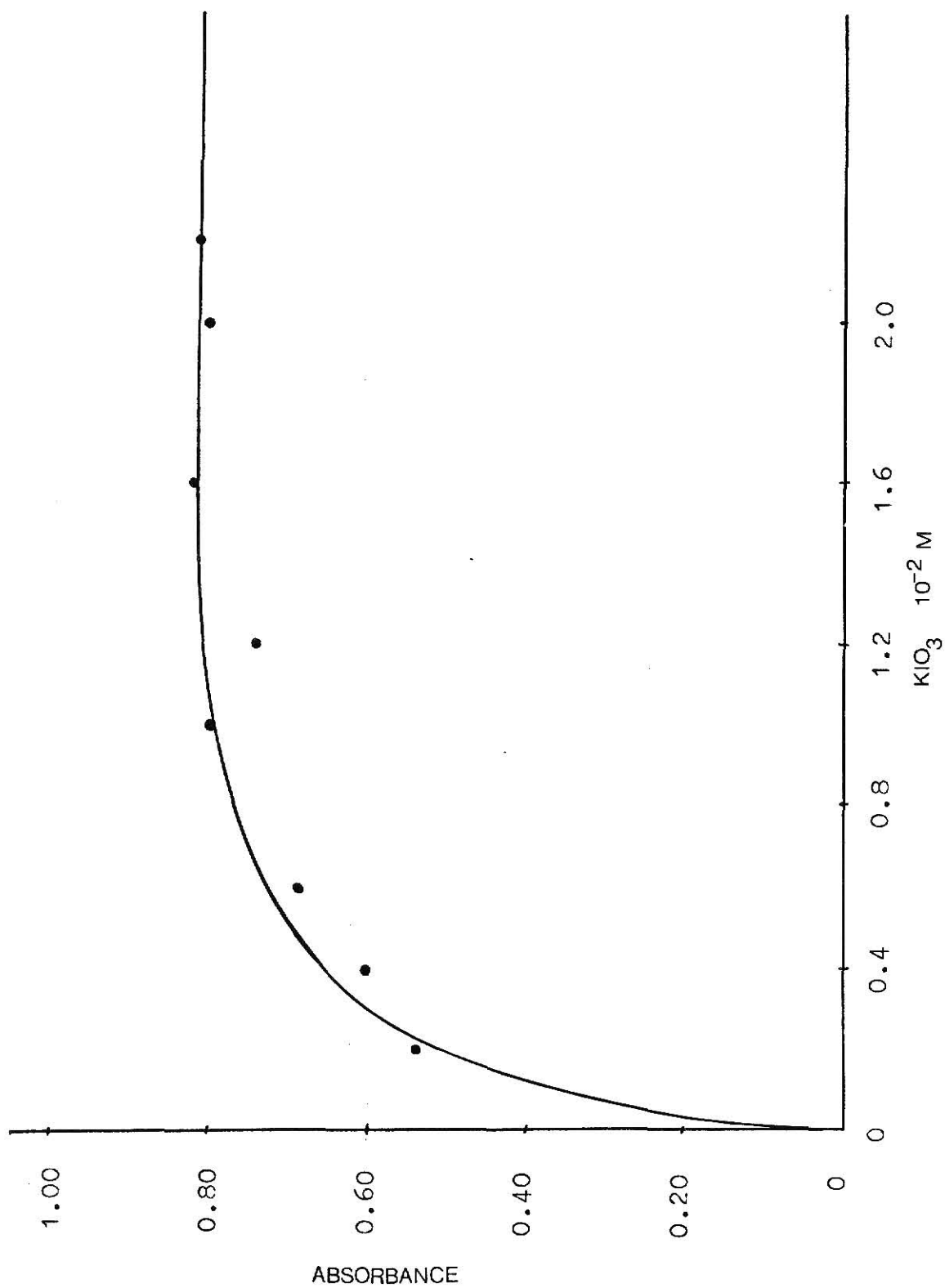Absorbance measured at 591 nm

Figure 3. Effect of Potassium Iodate on LCV-Palladate
Solution.   73.6 ppm CO, 30 minutes contact
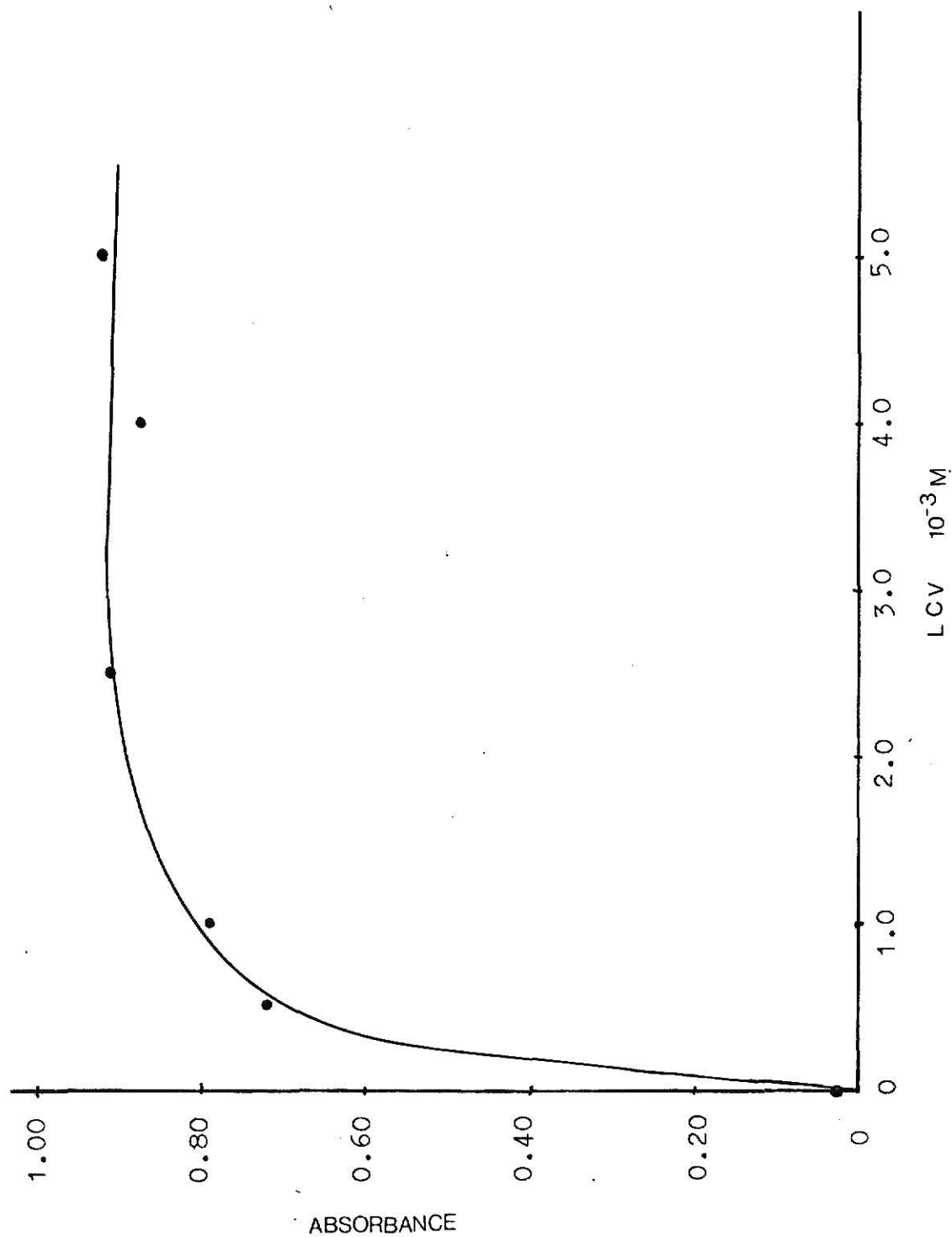time.

Figure 4. Effect of LCV on Solution Reagent A.
76.0 ppm CO, 30 minutes contact time.

Table V
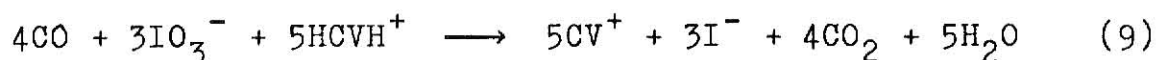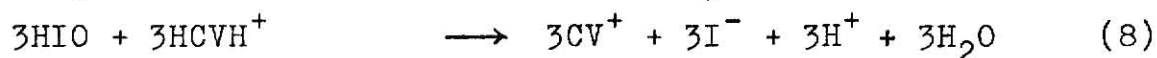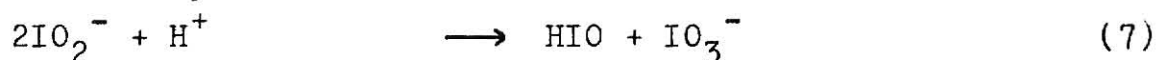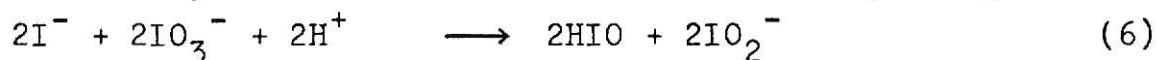
The Optimum Conditions of Reagent Solution A

pH Value	3.6
Concentration of LCV	2.5×10^{-3} M
Concentration of H_2PdCl_4	5.0×10^{-3} M
Concentration of KIO_3	2.0×10^{-2} M

Blanks of reagent solution A were studied at different contact times. Table VI summarizes the results. Several methods were examined in order to reduce those blanks. The first method tried was to purify the potassium iodate.[28] The further reaction of iodide with iodate would be

$$4CO + 2IO_3^- + 2HCVH^+ \longrightarrow 2CV^+ + 2I^- + 4CO_2 + 2H_2O \qquad (5)$$

$$2I^- + 2IO_3^- + 2H^+ \longrightarrow 2HIO + 2IO_2^- \qquad (6)$$

$$2IO_2^- + H^+ \longrightarrow HIO + IO_3^- \qquad (7)$$

$$3HIO + 3HCVH^+ \longrightarrow 3CV^+ + 3I^- + 3H^+ + 3H_2O \qquad (8)$$

___

$$4CO + 3IO_3^- + 5HCVH^+ \longrightarrow 5CV^+ + 3I^- + 4CO_2 + 5H_2O \qquad (9)$$
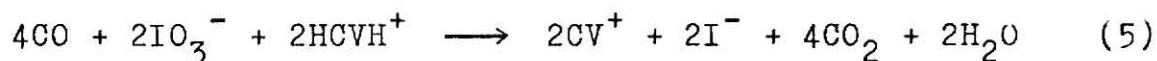
where $HCVH^+$ and CV^+ are leuco crystal violet and crystal violet, respectively. A trace amount of iodide was thought to be present in potassium iodate. A flask which was coated with a silver mirror formed by reaction of Tollen's reagent with d-glucose was used to trap iodide ions. Cupric(II) chloride and mercury(II) chloride were also added to reagent solution A in an attempt to reduce blanks, because qualitative tests showed that mercury(II) inhibits the reaction.[29] Another idea was to prepare reagent solution A in a dry box which was filled with pure nitrogen gas in order to preclude any trace amount of carbon monoxide. All of these efforts failed to improve the blanks appreciably. As the method did not require much contact time and the blank absorbance was not very high, the blanks were not considered to be a serious defect in the method.

The data from the calibration response studies of reagent solution A are given in Table VII. Four determina-

Table VI

Blanks of Reagent Solution A at Different Contact Time

Time(Minute)	Absorbance
0	0.030
5	0.050
10	0.055
20	0.065
30	0.070

The pH value of each solution was 3.6.

Table VII

Absorbance and Standard Deviations for Calibration
Curve of Reagent Solution A

Time(min.)	Concentration of CO(ppm)			
	7.4	18.4	36.8	73.6
5	0.0220±0.0006	0.055±0.005	0.108±0.008	0.220±0.010
10	0.044 ± 0.008	0.110±0.010	0.213±0.012	0.395±0.015
20	0.076 ± 0.006	0.178±0.010	0.337±0.016	0.665±0.022
30	0.092 ± 0.015	0.227±0.016	0.411±0.020	0.810±0.032

The pH value was 3.6.

tions were made for four concentrations of carbon monoxide
The results are plotted in Figure 5. The original blank
absorbance has already been subtracted from the values in
Figure 5.

There is a constant K which was calculated from

$$K = Ct/A \qquad (10)$$

where C is the concentration of carbon monoxide, t is the
time of exposure, and A the absorbance at 591 nm. The cal-
culations of constant K are summarized in Table VIII. From
those data, short exposure times gave linear responses;
longer exposure time and higher carbon monoxide concen-
tration gave non-linear responses which were, however,
highly reproducible.

Interference effects produced by nitrogen dioxide,
hydrogen sulfide, sulfur dioxide and chlorine were in-
vestigated. Nitrogen dioxide and hydrogen sulfide were
found not to interfere, but sulfur dioxide and chlorine
produced higher response at 36 ppm carbon monoxide. Table
IX gives the results.
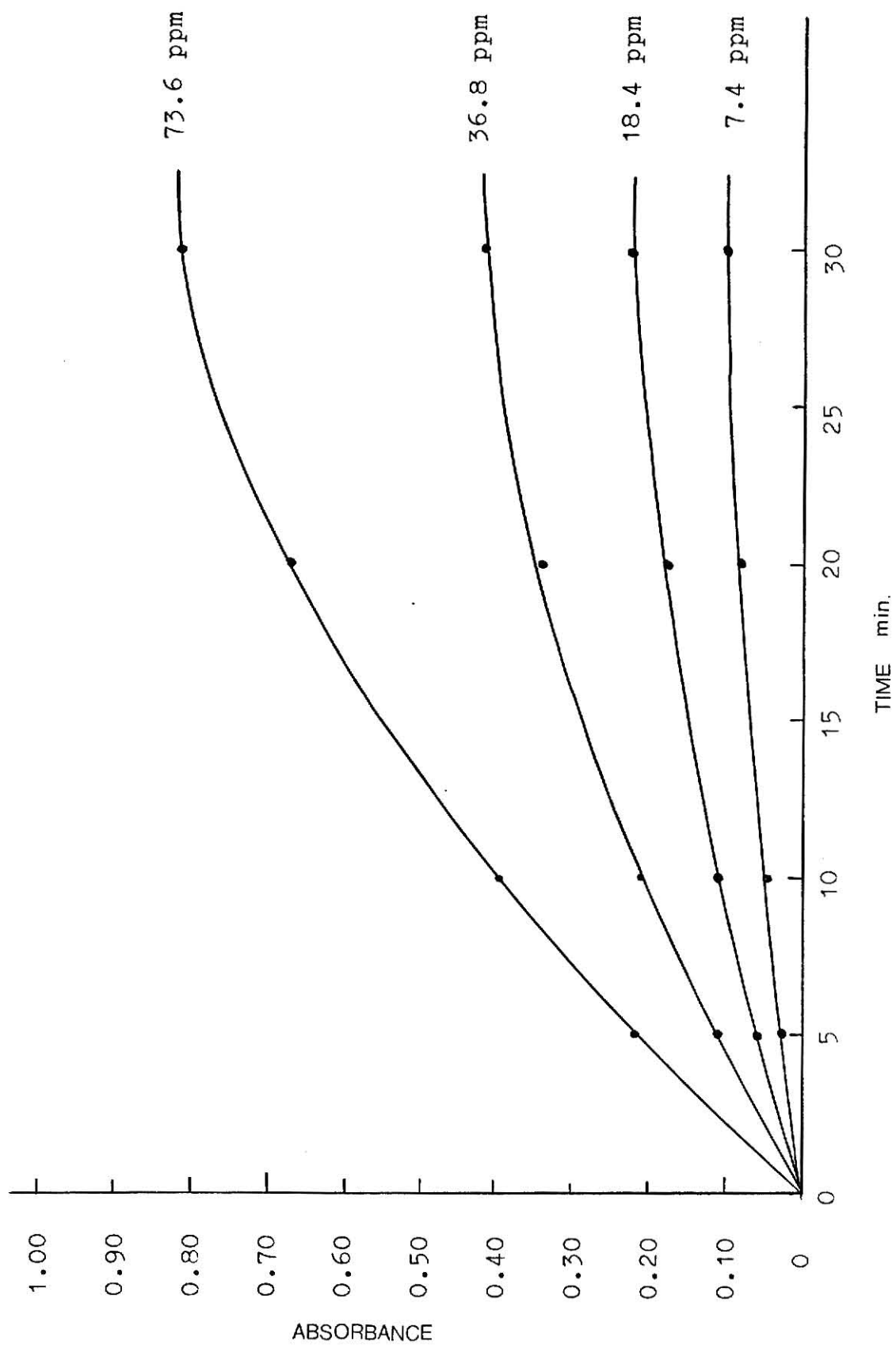
Figure 5. Absorbance Versus Contact Time of Different
Carbon Monoxide Concentrations.

Table VIII.

The Calculations of Constant $K(X\ 10^3)$

Time(min)	Concentration of CO			
	7.4 ppm	18.4 ppm	36.8 ppm	73.6 ppm
5	1.68	1.67	1.70	1.67
10	1.68	1.67	1.73	1.86
20	1.95	2.07	2.18	2.21
30	2.41	2.43	2.69	2.73

Table IX

Interference Studies

Species	Concentration	Net Abs.	Change
CO	36 ppm	0.41	
NO_2	40 ppm	0.43	+ 5
H_2S	38 ppm	0.42	+ 2
SO_2	40 ppm	0.53	+30
Cl_2	46 ppm	0.55	+34

Value is for 36 ppm CO plus interferent at indicated concentration.

Contact time was 30 minutes.

The pH value was 3.6.

Discussion

Leuco crystal violet is selectively oxidized by hypo-iodous acid, HOI. The color development depends both on the contact time and the concentration of carbon monoxide. The sensitivity and speed of reaction will allow this method to be used at the parts per million range for carbon monoxide determination.

From Figure 5 and Table VIII, the response of the reagent is linear for a carbon monoxide concentration up to 20 ppm or 10 minutes contact time. Higher concentration or longer contact time gives non-linear but highly reproducible responses.

Reduction of the blanks was attempted, based on several assumptions. Chloride ion was considered to be one of the factors which caused the blanks. Adding a large amount of sodium chloride to leuco crystal violet-iodate reagent solution reduced the response almost to zero. Several other attempts were made to get rid of iodide or trace amount of carbon monoxide from the reagent solution but these efforts were not successful. The causes of the blank are still unknown.

Replacement of palladium(II) by nickel(II) in reagent solution A gave very poor response to carbon monoxide. Nickel(II) could be catalyzed by even a trace amount of palladium(II) which was added to the nickel reagent solution or already adsorbed on the glass wall of the reaction flask. The minimum requirement of palladium(II) on this experiment was not identified. The reason for the reactivity of nickel(II) with carbon monoxide in the sequential

reactions is still unknown. The phenomenon that nickel(II) can be catalyzed by even a trace amount of palladium(II) to yield a higher response also is not understood. More work needs to be done on this apparent catalyzation.

pH sensitivity of reagent solution A and optimum concentartion of each component were problems that were studied. As the series of sequential reactions is very complex, the optimum conditions are difficult to elucidate. Optimum pH values may shift under different conditions. More phosphoric acid can produce a more stable leuco crystal violet solution, but more dibasic sodium phosphate buffer solution must then be used to adjust the pH value. Sodium phosphate appears to be the most effective buffer solution for this method, but more study should be made of the buffer.

Sulfur dioxide and chlorine were found to interfere at high concentrations. These substances could be removed from a sample of air by selective adsorption on diphenylamine oxalate(NO_2) and primary alkylamines(SO_2) prior to analysis by this method.[30] These might also be effectively removed by passing the air sample through a silica gel filter containing mercury(II) sulfate[21] or ascarite.[29]

In summary, the method developed here is a simple, rapid, inexpensive, sensitive and reliable method for the determination of carbon monoxide in the ambient atmosphere. The exact reaction mechanism is not completely known, although the oxidation of leuco crystal violet by hypo-

iodous acid is evidently the final step in the production
of crystal violet by the oxidation of carbon monoxide.[29]

CHAPTER III

AN ALIZARIN COMPLEXAN METHOD FOR CARBON MONOXIDE

Alizarin complexan (3-(di-(carboxymethyl) amino-methyl)-1,2-dihydroxyanthraquinone), whose structure is shown in Figure 6, has been used for the determination of fluoride ion since 1960.[31-33]

Alizarin complexan, H_4I, has four ionizable protons, the removal of which produces changes in the resonance system of the molecule. The yellow form of alizarin complexan exists at pH values less than 5.0. At the range 6-10, the solution developes a red color. Beyond pH 13, a blue color will form.

Leonard and West[31] concluded that the (1:1) cerium(III)-alizarin complexan chelate reacted with one fluoride ion, the latter ion replacing one of the coordinated water molecules remaining on the cerium atom.

This study was made to determine whether CO forms complexes with the alizarin complexan chelate of palladium:
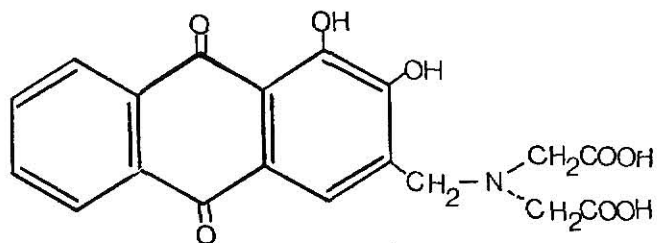

(11)
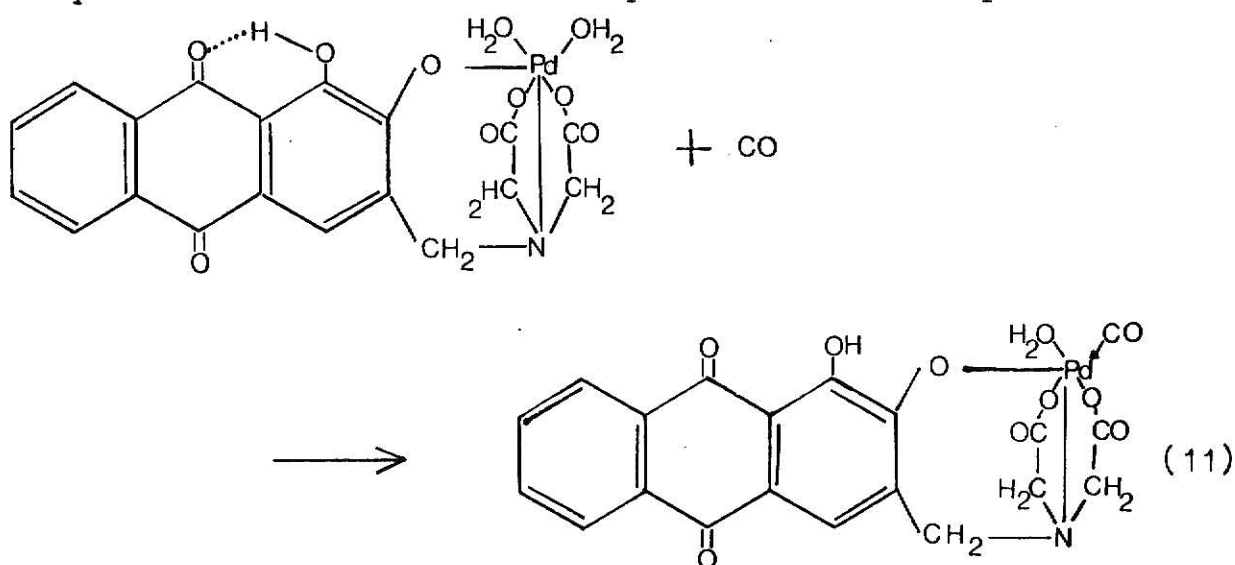
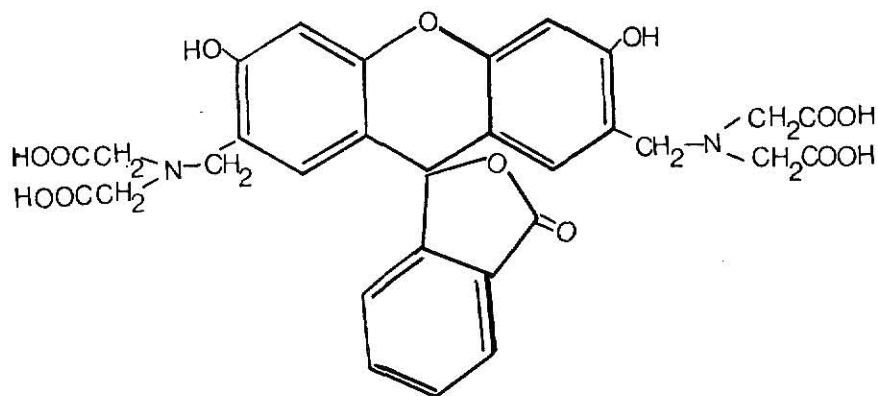Figure 6.   Structure of Alizarin Complexan

Figure 7.   Structure of Calcein

Experimental

Procedures used were similar to those described in Chapter II. In this study, only qualitative work was done.

Alizarin Complexan Solution (5×10^{-4}M). Alizarin complexan(192.5 mg) was suspended in water and dissolved by addition of 2 ml (4 equivalents) of 1 N sodium hydroxide solution. Sodium acetate trihydrate(250 mg) was added and the pH of the solution adjusted to 5.0 by dilute hydrochloric acid. The solution was then diluted to 1 liter, and was stored away from direct sunlight.

pH 4.3 Buffer. This contained sodium acetate trihydrate(105 gm) in glacial acetic acid(100 ml), diluted to 1 liter with deionized water.

Sodium Tetrachloropalladate(II) Solution (5×10^{-4}M). Suspend 8.9 mg of palladium(II) chloride in 50 ml of water, add 5.9 mg of sodium chloride, agitate the mixture vigorously, then dilute the solution to 100 ml.

Reagent Solution B. Pipet 1.0 ml of 5×10^{-4}M alizarin complexan and 1.0 ml of pH 4.3 buffer into the flask. Add 1.0 ml of 5×10^{-4}M sodium tetrachloropalladate(II).Bring the volume up to 25 ml with deionized water and set aside for 1 hour in diffuse daylight to allow the color system to reach equilibrium.

Results and Discussion

At pH values less than 4.5, the color of reagent solution B changed from orange to dark brown when the solution was ex-

posed to 100% pure carbon monoxide. The absorbance gained 0.052 at 490 nm. Higher pH (6.0, 10.0. 12.0) did not give any color change. Compared to the leuco crystal violet method, it is not sensitive enough. The studies were not pursued further.

A similar study was done by using Calcein (bis-(N,N-bis(carboxymethyl)-aminomethyl)-fluorescein), whose structure is shown in Figure 7, in place of alizarin complexan. No change was observed when the solution was exposed to carbon monoxide.

CHAPTER IV

DETERMINATION OF CARBON MONOXIDE IN BLOOD

Previous workers[5] in this laboratory have modified the
Conway microdiffusion cell for determination of carbon mon-
oxide in blood.

In this study, another modification was made on this
cell. The modified cell pictured in Figure 8 was designed
for Bausch and Lomb Spectronic 20 spectrophotometer. Ports
equipped with rubber septums were designed to permit in-
jection by syringe of the reagent into the inner tube of
the cell, and injection of the blood sample and 10% sul-
furic acid solution into the outer portion of the cell. A
solid glass rod was fused to the bottom of the tube in
order to minimize the amount of the reagent solution. A
Teflon rod was machined with air-tight O-rings to fit the
upper tube, so that all reactions can be carried out without
contact with the atmosphere.

An aqueous solution of tetrachloropalladate(II),
leuco crystal violet and potassium iodate at pH 3.1 ± 0.1
was prepared as described by Weins.[24] The reagent was
freshly prepared before each test.

Procedure

Heparized cow blood with 10.0 ± 0.1 grams of hemo-
globin per 100 ml was equilibrated with 100% pure carbon
monoxide in a 125 ml flask which was shook in a mechanical
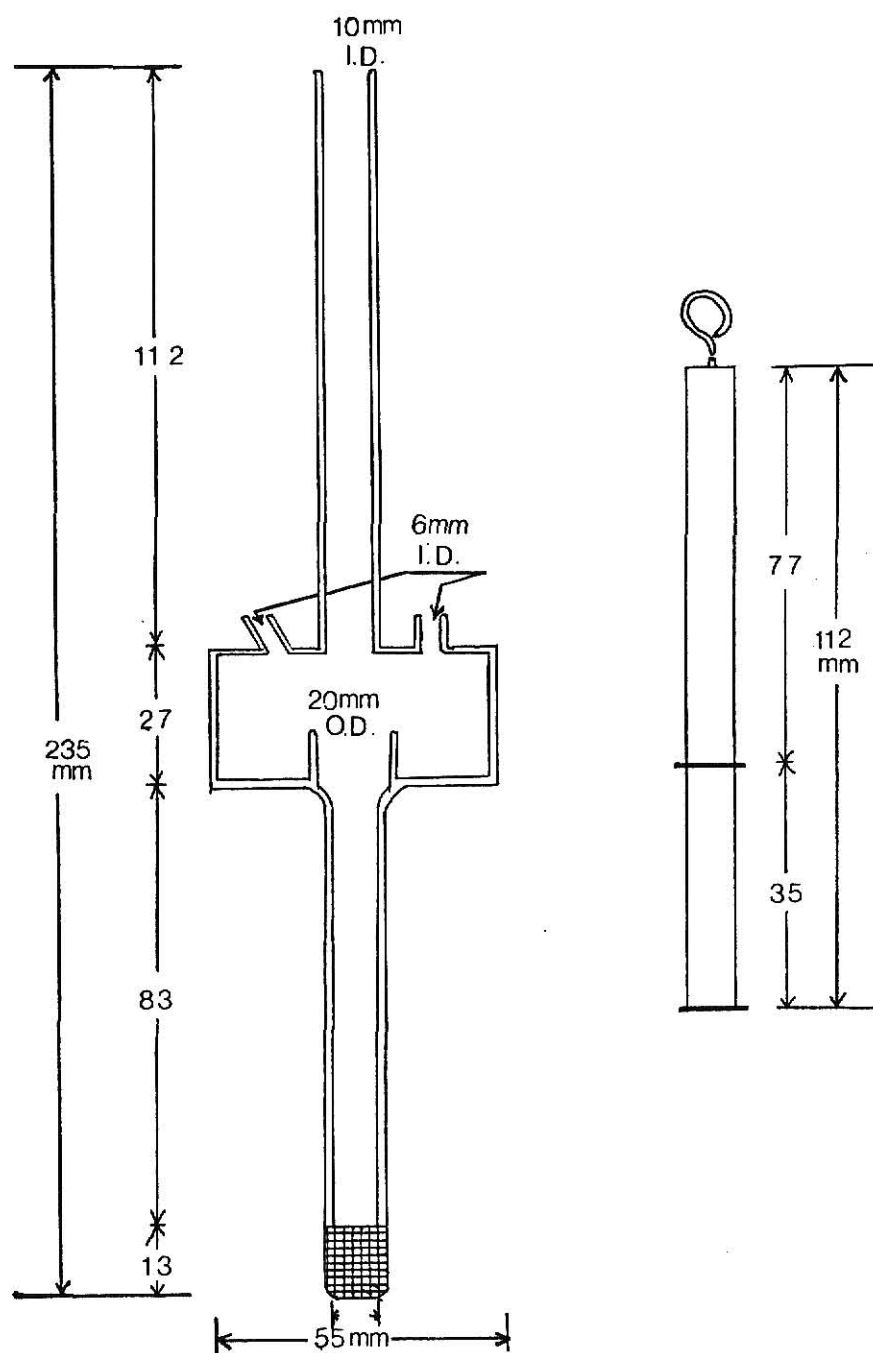shaker at 37°C for 30 minutes. Blood dilutions of 25, 50

Figure 8.   Modified Conway Microdiffusion Cell with

Plunger.

and 75% carbon monoxide were made with carbon monoxide-free blood. The spectrophotometer was set at zero absorbance at 587 nm. A Teflon rod was inserted into the cell until it reached the bottom of upper cell. One milliliter of reagent was injected into the inner tube. By using microsyringes, 50 μl of blood sample and 50 μl of 10% sulfuric acid were injected into the outer compartment of the cell. The cell was shaken on the wrist-action shaker. Absorbance measurements were made at 5-minute intervals for a period of one hour.

Results and Discussion

Table X shows the results obtained at five concentrations after one hour contact time. The relation between reaction time and absorbance produced in the reagent is shown in Figure 9.

Attempts to use the same cell described by Hamlin[5] were unsuccessful. Purple crystal violet was absorbed by the Teflon rod that made the absorbances decrease unpredictable. The same shape of stainless steel rod also has been utilized, but it did not produce good results either.

For this method, several determinations were made at each concentration, because the diffusion rate of carbon monoxide in this cell is variable. For this reason, the standard deviations in this experiment are higher. The primary advantage of this method is the production of a soluble colored compound rather than the colloidal metal dispersions produced in other methods. A very small amount

Table X

Data for Calibration Curve

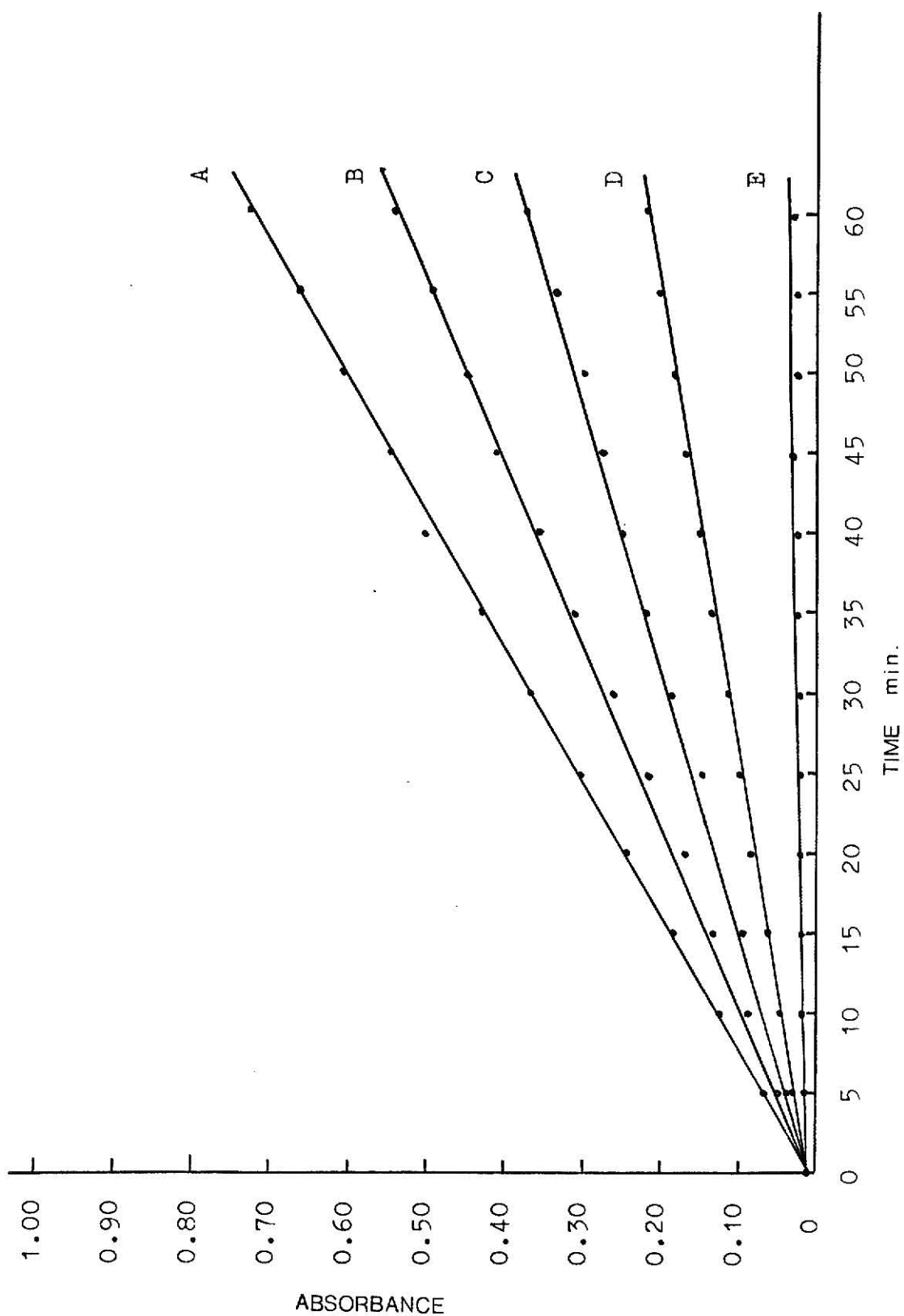| Saturation, %	Mean(Abs.)	Std. Deviation
0	0.031	0.003
25	0.217	0.013
50	0.373	0.010
75	0.545	0.034
100	0.731	0.017

Figure 9. Rate of Color Development in Reagent.

per cent saturation of carbon monoxide in blood:

A=100%, B=75%, C=50%, D=25%, E=0%

of blood sample is required; in each test, only 50 μl of blood sample was injected. It is an inexpensive and re-liable method for quantitative determination of carbon mon-oxide in blood.

CHAPTER V

EXTENSIONS AND CONTINUATIONS

Although a considerable amount of work has been done, there is a great deal left to do. More work needs to be done to find the best palladium(II) complex and the optimum conditions. The buffer solution is another item which should be studied further. An arsenate buffer system was found to enhance the absorbance, but its effect was not reproducible. Studies on concentration and pH values of this reagent solution A should be continued. Studies should also be continued with the aim of reducing the blanks. Nickel(II) could be an important reagent for further study.

More study of the alizarin complexan compounds may still have some value. Optimum concentration and pH might provide a stable reagent for the carbon monoxide test.

Regardless of the fact that the Teflon rod may absorb crystal violet and drop the absorbance, it should be possible to find some other material to replace Teflon, and make a new plunger for the original cell. Once this deficiency of the plunger can be overcome, the opportunity to develop a better method for determination of carbon monoxide in blood would be good.

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VITA

Albert T. Liu was born on August 31, 1949 in Taipei, Taiwan. He is the second son and youngest of six children in his family. He received the B. S. degree in chemistry from Fu-Jen Catholic University in May 1971, spent the next two years in the army as a second lieutenant, being honorably separated in July 1973. In September of 1973 he began his studies as a full time graduate student in chemistry at Kansas State University. He married Theresa T. Huang on October 26, 1974.

COLORIMETRIC METHODS FOR THE DETERMINATION

OF CARBON MONOXIDE IN AIR AND BLOOD

by

ALBERT T. LIU

B. S., Fu-Jen University, 1971

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Colorimetric methods for the determination of carbon monoxide concentrations in air are described. The process of a previously developed reaction sequence involved oxidation of carbon monoxide by a palladium(II) complex, followed by reduction of iodate by palladium(0) to hypoiodous acid(HOI). HOI selectively oxidizes leuco crystal violet(4,4', 4"-methylidynetris(N,N-dimethylaniline)) to crystal violet, which is measured spectrophotometrically. Optimum concentrations of each reagent were studied. At pH 3.6, the method has maximum sensitivity. Attempts to use other palladium(II) complexes and compounds to replace tetrachloropalladate were unsuccessful. Nickel(II) showed some reactivity but platinum(II) compounds are non-reactive. Attempts to use other dyes to replace leuco crystal violet were unsuccessful, also. High concentrations of sulfur dioxide and chlorine were found to interfere in this method. Further investigations showed that short exposure times gave linear plots for absorbance, longer contact times and higher carbon monoxide concentrations gave non-linear but highly reproducible responses. Reduction of the reagent blank was one of the major aims; several attempts were made but they were unsuccessful.

Chelates formed by "alizarin complexan" with palladium(II) gave a color reaction at about pH 4 after carbon monoxide was bubbled through the solution. A similar reagent was made with Calcein, but no response was observed.

Determinations of carbon monoxide in bovine blood is described by using a modified Conway microdiffusion cell

and the leuco crystal violet-palladate(II) reagent. A quantitative analysis was done. Although this method has higher standard deviation, it is an effective method for the determination of carbon monoxide in blood.