A STUDY OF COPPER, COBALT, CHROMIUM, NICKEL, AND MANGANESE AS INTERFERENCES WITH THE TPTZ METHOD FOR TRACE IRON

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PREFACE

This dissertation is concerned with the evaluation of the 2, 4, 6-Tripyridyl-s-Triazine (TPTZ) method for trace iron. Each of five known interfering metals (Cu, Co, Cr, Mi, and Mn) was individually paired with Fe. The type and degree of interference was studied as a function of both the concentration of Fe and of each interfering metal.

The aqueous Fe concentration was varied from ten to one hundred parts per billion in all of the systems. Although an attempt was made to keep the TPTZ concentration constant at 3.75×10^{-6} Molar, the standard TPTZ reagent solution decreased with time.

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CHAPTER I

THE PROBLEM

The author is interested in the evaluation of the 2,4,6-Tripyridyl-s-Triazine (TPTZ) method for trace iron in the parts per million range in aqueous solution. The accuracy and reliability of this method will be examined from the viewpoint of its applicability to natural water systems in which the ratio of Fe species to that of all species of interfering metals is greater than unity. Another subject of interest is the degree of applicability to the determination of Fe impurities in other metals or their ores.

The first topic will be developed in terms of the results obtained from five metal-pair systems. Five of the most important metal interferences were individually paired with iron. Interference effects were observed as a function of concentration of both metals within the pair. The Fe was varied from 0.010 to 0.1 parts per million in each of the metal-pair systems. The concentration limits of each of the other metals were not identical with one another.

The second topic will be more briefly discussed and evaluated in terms of the behavior of the metal systems described above. Also, the validity of some attempts to establish the iron impurities contained in Cr and Mn will be treated in relation to the second topic.

CHAPTER II

INTRODUCTION

The number of compounds, which have been synthesized, containing the ferroin group, N=C-C=N, is large. However, the number of these, which is suitable for use as a chromogen for the spectrophotometric determination of iron in a quantitative manner, is less than a dozen. The application of many of the above is restricted by their low solubility in aqueous solution media.

The reactions of 2,2'-bipyridine and 1,10-phenanthroline with ferrous and ferric iron salts and their intense colors have been known and extensively investigated ever since the late nineteenth century (7). The use of 1,10-phenanthroline as a colorimetric method for the quantitative determination of iron was established independently and simultaneously by G. F. Smith, F. C. Hummel, and H. H. Willard, and W. B. Fortune and M. B. Mellon, in 1938 (7).

During the 1940's and the 1950's, G. F. Smith and F. H. Case studied approximately 150 ferroin-type compounds closely related to or derived from bipyridine and 1,10-phenanthroline. As a result, a series of oxidation-reduction indicators was established (7). Also, bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) was deliberately created for use as a colorimetric reagent for determination of iron. Three other ferroin-type iron reagents are presently in use: syn-phenyl-2-pyridyl ketoxime; 2,4,6-tris-(2-pyridyl)-1,2,5-triazine or TPTZ; and bathophenanthrolinedisulfonic acid (7). The four ferroin compounds just mentioned are much more sensitive reagents for iron than 1,10-phenanthroline.

Bathophenanthroline

Bathophenanthroline can be used for the determination of iron in the ferrous state in the parts per billion range. The chelate is best extracted from water by n-hexyl alcohol or nitrobenzene (7). If Fe(II) is to be determined in the presence of Fe(III), then isoamyl acetate must be employed (7). The molar extinction coefficient of the ferrous bathophenanthroline ion in nitrobenzene, of 23,300 at 538 mu, is slightly greater than the value of 22,350 at 533 mu observed in isoamyl alcohol. These are much greater than the value of 11,100 observed for the o-phenanthroline iron complex in isoamyl alcohol at 510 mu (7).

There are several disadvantages associated with the bathophenanthroline method for iron. Bathophenanthroline and the ferrous bathophenanthroline ion both have a low solubility in water, making the addition of ethanol necessary before introduction of chelating reagent into an aqueous solution (7). The cost of bathophenanthroline is high compared with that of some of the other iron reagents and its synthesis is not as direct and simple as one might wish, also, in the neutral solutions needed for maximum color development of ferrous bathophenanthroline ion, if an excess of reagent is used in order to minimize any interference effects, this excess tends to precipitate (7).

Bathophenanthrolinedisulfonic Acid

Bathophenanthrolinedisulfonic acid has a molar absorptivity of 22,140 at 535 mu in water, in the ferrous complex form, and is soluble in aqueous solution media (7). Ferrous tris(bathophenanthrolinedisulfonic acid) is stable over a pH range of three to nine (7).

Two disadvantages are inherent in the nature of the compound. It is more costly and difficult to prepare than bathophenanthroline (7). Iron determinations must be performed directly in aqueous solution, since it cannot be extracted very efficiently into immiscible organic solvents (7). Accordingly, although this compound is as sensitive as bethophenanthroline toward iron, it cannot be used for trace analysis work, unless rather inconvenient and time-consuming sample pre-concentration methods, eg. evaporation, are utilized.

There is one characteristic of the above method that may be considered as a very significant limitation. In the analysis of natural water iron sample, it is common procedure to employ a technique known as "wet-ashing", which involves the addition of concentrated nitric and perchloric acids and heating for several hours (7). The purpose of this step is to liberate any iron species complexed with organic matter. Perchlorate present in the amount required constitutes a considerable interference.

One further unfavorable consideration is that the chelating agent cannot be used to remove iron impurities from the other reagent solutions, since extraction is impossible (7). Therefore, it is necessary to use some other iron reagent to accomplish this.

Phenyl-2-pyridyl Ketoxime

Phenyl-2-pyridyl ketoxime is an unusual ferroin compound inasmuch as it is only effective in strongly alkaline aqueous solution media (7).

In aqueous solution, the molar absorptivity, the maximum limiting concentration for conformance with Beer's Law, the degree of extractability, and the time for full color development of the ferrous complex are all functions of pH (7). Once the complex has been extracted into isoamyl alcohol, the molar absorptivity is no longer a function of pH and has a value of 15,600 at 550 mu (7).

The ferrous complex is unstable in aqueous solution, but stable once extracted into isoamyl alcohol (7). This method is applicable to systems containing oxidized iron, but unfortunately, it does not have sufficient sensitivity for the determination of trace iron (7).

2,4,6-Tripyridyl-s-triazine

2,4,6-Tripyridyl-s-triazine forms a complex with Fe(II), which is cationic in nature and extractable into nitrobenzene in the presence of iodide or perchlorate over a pH range of two to seven (7). The optimum pH is 4.5, and the molar absorptivity of $Fe(TPTZ)_2^{++}$ in nitrobenzene has a value of 24,100 at 595 mu, which is slightly higher than that of 22,600 observed in water at 593 mu (7). The complex has an intense violet color, is stable in aqueous solutions, and is completely formed over the pH range of 3.4 to 5.8 in aqueous media (4), (7).

Physical Constants of TPTZ and the Chelate

Buchanan, Crichton, and Bacon (1) made a study of some of the factors affecting the reaction of Fe(II) with TPTZ. Although the solubility of TPTZ in water is superior to that of most other iron reagents, it is still rather low. Since the solubility of the ligand will determine the potential excess TPTZ that can be in solution, this will ultimately be one of the defining criteria for the type and degree of interference that will be encountered.

TPTZ has a characteristic absorption spectrum in the ultraviolet, and the complex has a peak at 595 mu in the visible spectrum (1). These characteristics were utilized to find the solubility of TPTZ at a pH of 4.5 and the formation constant of the chelate, after the acid dissociation constant had been determined via the difference between titrations of HCl and HCl + TPTZ with NaOH, as a function of pH (1). Since the total TPTZ was known, and the amount of TPTZ that was protonated in the form HTPTZ+ was found as a function of pH, the acid dissociation constant could be determined according to:

$$K_a = \frac{[H^+][TPTZ]}{[HTPTZ+]}$$

The value of K_a was found to be 8.0×10^{-4}. At a pH of 4.5, a Beer's Law plot was employed to find that the solubility of TPTZ was 9.2×10^{-6} molar (1). The equilibrium between chelate and unprotonated ligand can be expressed as

$$Fe^{2+} + 2TPTZ \rightleftharpoons Fe(TPTZ)_2$$

and the formation constant as

$$K_{feq} = [Fe(TPTZ)_2^{2+}] \;/\; [Fe^{2+}][TPTZ]^2$$

In terms of the measured variables and constants A, ε, Fe_t, L_t, K_a, and H^+, where A is the absorbance of the solution at 594 mu, is the molar absorbance of the chelate at the same wavelength, Fe_t is the total iron, L_t is the total TPTZ, H^+ is the hydrogen ion concentration, and K_a is the acid dissociation constant of TPTZ,

$$K_{feq} = \frac{A/\varepsilon}{(Fe^+ - A/\varepsilon)(L_t - 2A/\varepsilon)^2 (K_a/(H^+ - K_a)^{\,2}} .$$

The formation constant was found to be 1.75×10^{10} or $\log K_f = 10.83$ (1).

Dependence of Molar Absorptivity on Refractive Index

The Fe(II) chelate is soluble in both aqueous and organic solution media, which differ greatly in their refractive indicies. Therefore, one would expect to molar absorptivity to be markedly different. Buchanan, Crichton, and Bacon (1) computed tha theoretical molar absorptivities for 80% nitrobenzene-20% ethanol, 48% nitrobenzene-52% ethanol, and 100% nitrobenzene using values of refractive indicies as determined with an Abbe refractometer, which was calibrated at 588 mu, the wavelength of the sodium D line.

The vlue 1.33 was used for the refractive index of the aqueous solution; 1.55 for the 100% nitrobenzene solution; 1.51 for the 80% nitrobenzene-20% ethanol solution; and 1.45 for the 48% nitrobenzene - 52% ethanol solution. The relation between molar absorptivity and refractive index, which was used, is

$$\varepsilon_x = \frac{\varepsilon_{H_2O} \cdot n_{H_2O} (n_x^2+2)^2}{n_x (n_{H_2O}^2+2)^2}$$

where ε is the molar absorptivity, and n is the refractive index of the species indicated by subscript (1).

The observed molar absorptivity values were lower than computed values as shown in Table I. The explanation offered, is that the chelate is known to be insoluble in nitrobenzene unless some water is present (1). The water is principally attached to the chelate and is not evenly dispersed throughout the nitrobenzene medium. Although the bulk refractive index remains unchanged, the molar absorptivity of the chelate is affected (1).

TABLE I

CALCULATED AND OBSERVED MOLAR ASORPTIVITIES

Solvent	Calculated $\times 10^{-4}$	Observed $\times 10^{-4}$	Difference %
Water	-------	2.23	---------
100% Nitrobenzene	2.60	2.52	3.2
80% Nitrobenzene-20% Ethanol	2.52	2.51	0.4
48% Nitrobenzene-52% Ethanol	2.42	2.44	0.8

As alcohol is mixed with the nitrobenzene, the water becomes more soluble in the nitrobenzene, and the bulk refractive index will be affected as well. But, as the proportion of ethanol is increased, the refractive index decreases and approaches that of an aqueous medium, and there will be less apparent change in the molar absorbtivity of the chelate and less deviation from the computed value.

The above workers (1) concluded that, although the application of Beer's Law implies interest in the amount of chelate present, the absorbance should actually be related to the total iron present, since this is more easily accessible or known. In terms of the formation constant, K_f,

$$[FeL_2] = \frac{K_f [L]^2}{1 + K_f [L]^2} \times Fe_t,$$

where $Fe_t = Fe^{2+} + FeL_2{}^{+}$. The Beer's Law expression for absorbance will be

$$A = \left(\frac{K_f [L]^2}{K_f [L^2] + 1} \right) bc_{Fe_t}$$

where is the molar absorptivity of the chelate, b is the cell and c_{Fe_t} is the total iron concentration (1). In view of the above, consistency in regard to the amount of water present in the nitrobenzene and the quantity of ethanol added is desirable and important.

Colline, Diehl, and Smith (4) made a study of the ratio of Fe(II) to TPTZ in the chelate and determined the geometric and bonding structure of the bis [2,4,6-tris(2-pyridyl)-s-triazine] iron(II)$^{++}$ ion. The absorbance readings of solutions containing constant TPTZ and variable Fe(II) became constant when the TPTZ to Fe(II) ratio was 2:1 or greater (4). This ratio was also confirmed by the method of continuous variations. The molar concentration of Fe(II) plus TPTZ was held constant, and the concentration of TPTZ varied from 0 to 2 x 10^{-4} M. Maximum absorbance was achieved when the TPTZ mole fraction was 0.66 (4).

These workers found that in the titration of amino and pyridyl substituted symmetrical triazines, the number of equivalents of acid consumed corresponded to the number of pyridyl groups (4). When tris (1,10-phenanthroline) iron(II) perchlorate was titrated, no endpoint was obtained, indicating that pyridyl nitrogen, which is bonded to Fe(II), cannot be titrated. Two equivalents of acid were used per mole of bis [2,4,6-tris(2-pyridyl)-s-triazine] iron (II) perchlorate. It was concluded that Fe(II) was bonded to two pyridyl nitrogens and triazine ring nitrogen of each TPTZ molecule, as shown in Figure 1 (4).

Interferences

The TPTZ method as established by Collins, Diehl, and Smith (4), (5) involves the use of a pH 4.5 acetate buffer and an hydroxylamine reducer. Also, a source of perchlorate anion is introduced into the sample. According to Collins, Diehl, and Smith (4), the most notable interferences are the transition metals Cu, Co, Cr, and Ni, in addition

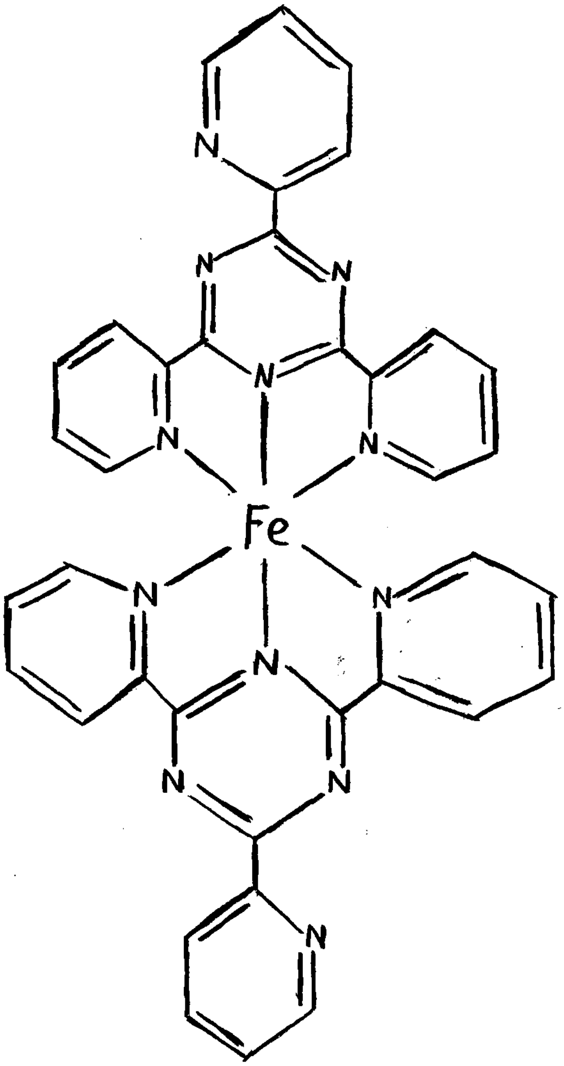

Figure 1. Structure of Bis[2,4,6-tris(2-pyridyl)-s-triazine] Iron(II) Ion

to silver, mercury, bismuth, molybdate, cyanide, oxalate, and nitrite.

Collins, Diehl, and Smith (4) found that Co^{++}, and Ni^{++} form colored compounds with TPTZ, that precipitates form in the presence of Ag^{+}, Hg^{++}, and Bi^{3+}, and that the other ions interfere or retard color development due to their own color. In the study above, a constant Fe concentration of 1.4 p.p.m. was used, and several concentrations of each cation or anion was employed (4). Also, the concentration of TPTZ was varied. These workers concluded that, in aqueous solution, 2.5 p.p.m. of Cu^{++}, or 5.3 p.p.m. of Ni^{++} results in a relative error of less than two percent in the determination of iron under the conditions already mentioned.

The results of (4) are given in Table II. The method used to calculate the relative error is based on the following:

$$C_2 = C_1 \frac{A_2}{A_1} \text{ ; per cent relative error } = \frac{C_2 - C_1}{C_1} \times 100;$$

in which C_1 and A_1 are the concentration of the iron and the absorbance of the solution containing no interfering ion, respectively; and C_2 and A_2 refer to the apparent concentration of the iron and the absorbance of the solution containing the possible interference, respectively.

Collins and Diehl (5) formulated a procedure for analysis of sea water, which will be described in detail later. Fischer and Price (9) modified the Ramsay method for serum iron so that TPTZ could be used instead of bipyridyl. If the $Fe(TPTZ)_2^{++}$ complex is added in known amounts to an unknown containing (ethylenedinitrilo)tetraacetic acid, microgram quantities can be spectrophotometrically determined by difference between blank sample absorbance of the TPTZ complex alone and the absorbance of the unknown (10). C. C. Tsen (20) has devised an improved method for the chemical determination of tocopherols in food

TABLE II

EFFECT OF VARIOUS IONS ON THE FORMATION OF THE VIOLET $Fe(TPTZ)_2^{++}$ ION

Ion	Concentration p.p.m.	Source	Relative Error Per Cent	TPTZ Added Moles x 10^5
Cu^{++}	1.3	$Cu(NO_3)_2$	0.7	0.5
Cu^{++}	2.5	$Cu(NO_3)_2$	1.4	0.7
Cu^{++}	6.3	$Cu(NO_3)_2$	4.8	0.9
Co^{++}	1.2	$CoSO_4$	0.9	0.5
Co^{++}	2.4	$GoSO_4$	1.8	0.7
Co^{++}	4.8	$CoSO_4$	3.6	1.1
Ni^{++}	2.7	$Ni(ClO_4)_2$	0.4	0.8
Ni^{++}	5.3	$Ni(ClO_4)_2$	1.5	1.3
Ni^{++}	10.6	$Ni(ClO_4)_2$	2.7	2.3
Zn^{++}	99.4	$ZnCl_2$	0.2	10.0
Mn^{++}	110.	$Mn(SO_4)$	0.2	2.0
Cr^{++}	10.4	$K_2Cr_2O_7$-SO_2	0.6	0.5
Cr^{++}	20.8	$K_2Cr_2O_7$-SO_2	2.4	0.5
Be^{++}	73	$Be(ClO_4)_2$	0.0	0.5
Al^{+++}	100	$AlCl_3$	0.0	0.5
Mg^{++}	100	$MgSO_4$	0.0	0.5
Ca^{++}	100	$CaCO_3$-HCl	0.0	0.5
Sr^{++}	99	$Sr(ClO_4)_2$	0.2	0.5
Ba^{++}	101	$BaCl_2$	0.2	0.5
Cd^{++}	100	$Cd(NO_3)_2$	-0.7	5.0
Hg^{++}	100	$HgCl_2$	precip.	0.5
Bi^{+++}	100	$Bi(NO_3)_3$	precip.	0.5
Sn^{++}	100	$SnCl_2$	-0.2	0.5
Pb^{++}	101	$Pb(NO_3)_2$	0.2	2.0
Th^{++++}	120	$Th(NO_3)_4$	0.2	0.5

TABLE II (Continued)

UO_2^{++}	115	$UO_2(C_2H_3O_2)_2$	0.4	0.5
Li^+	1020	LiCl	0.0	0.5
K^+	1067	KCl	0.4	0.5
NH_4^+	1033	NH_4Cl	0.0	0.5
Na^+	5600	$NaC_2H_3O_2$	-0.2	0.5
Ag^+	102	$AgNO_3$	precip.	0.5
CN^-	500	NaCN	very large	0.5
$PO_4^{\equiv}$	528	KH_2PO_4	0.2	0.5
F^-	502	NaF	0.2	0.5
$C_2H_3O_2^-$	14,400	$NaC_2H_3O_2$	-0.2	0.5
Br^-	556	NaBr	0.2	0.5
I^-	497	KI	0.7	0.5
NO_3^-	504	KNO_3	0.2	0.5
NO_2^-	500	KNO_2	large	0.5
SO_4^{--}	512	K_2SO_4	0.0	0.5
ClO_4^-	524	$NaClO_4$	0.0	0.5
ClO_3	548	$NaClO_3$	0.2	0.5
$S_2O_5^{--}$	538	$Na_2S_2O_5$	0.0	0.5
SCN^-	507	KSCN	0.2	0.5
$S_2O_3^{--}$	528	$Na_2S_2O_3$	0.4	0.5
BO_3^{--}	545	H_3BO_3	0.0	0.5
BrO_3^{--}	499	$KBrO_3$	0.0	0.5
MoO_4^{--}	34	$(NH_4)_2MoO_4$	very large	2.0

Note: 50 ml. solutions were used to obtain the above.

Source: Diehl, . F. Smith, L. McBride, and R. Cryberg, "The Iron Reagents: Bathophenanthroline, Bathophenanthrolinedisulfonic Acid, 2,4, 6-Tripyridyl-s-triazine, Phenyl-2-pyridyl Ketoxime," 2nd ed., Columbus, Ohio: G. Frederick Smith Chemical Company, 1965.

with either TPTZ or bathophenanthroline. Sensitivity is increased by a factor of 2.5 and maximum color development occurs within fifteen seconds.

Modification of TPTZ Method Using Propylene Carbonate

Stephens and Suddeth (19) discovered that propylene carbonate could be used as an extraction solvent for TPTZ and 1,10-phenanthroline Fe(II) chelates. The principal advantages are associated with the physical and chemical characteristics of the solvent itself. The solvent is colorless, non-hydroscopic, non-corrosive, practically odorless, and is relatively non-toxic compared with nitrobenzene (19). The ferrous TPTZ complex has a molar absorptivity of 22,100 at 593 mu in propylene carbonate (19). Application of the modified TPTZ method to the determination of iron in sea water resulted in an average error of five per cent in the 5-27 p.p.b. range (19).

There are several negative aspects associated with this method, which are related to the properties of the extraction solvent. The nitrobenzene TPTZ method could be used in reverse as a qualitative test for presence of perchlorate and/or iodide. This capability is lost with the modified procedure, since partial extraction can be accomplished in the presence of most common anions (19). However, if one wishes to view the mandatory use of perchlorate or iodide as a disadvantage, then it should be noted that the modified method is no better than the nitrobenzene extraction method in this respect (19).

Although potentially greater concentration of chelate in propylene carbonate than in nitrobenzene via extraction may be possible, much more of the former must be used for a given amount of aqueous solution (19). A minimum of 15 ml. per 100 ml. of aqueous solution is necessary for saturation purposes (19). Stephens and Suddeth (19) determined the solubility of propylene carbonate at 24° C. to be 21.2 ml. per 100 ml. of water and 10.4 ml. per 100 ml. of a 50:50 mixture of water and saturated NaCl solution.

The propylene carbonate-TPTZ method compares favorably with the nitrobenzene-TPTZ method in other ways such as low volatility and high density of solvent. The new method could replace the one now in use, if the cost of propylene carbonate as a solvent decreases in the future.

Other Ferroin-Type Compounds

When Case and Koft (3) first synthesized TPTZ, several other compounds were also reported, which were derivatives of TPTZ. The same trimerization process was applied to the 4-methyl, 4-ethyl, and the 4-phenyl derivatives of 2-cyanopyridine. These TPTZ derivatives have also been found to form deep blue complexes with Fe(II). Tris (4-ethyl-2-pyridyl)-s-triazine and tris(4-phenyl-2-pyridyl)-s-triazine give a more sensitive test for Fe(II), but the yield of the former is very low, and the procedures of synthesis and recovery for both is much more complicated than for TPTZ (3), (6).

Two other compounds that formed violet-colored complexes with Fe(II) were 2-amino-4,6-bis(2-pyridyl)-1,3,5-triazine and 2-amino-4,6-bis(4-ethyl-2-pyridyl)-1,3,5-triazine (3). In general, Case found that, if at least tow pyridyl groups were not connected to the s-triazine ring, or if an electron-withdrawing group such as phenyl was substituted in the 4-position of the pyridyl ring, chelation with Fe(II) would not occur.

More recently, Case (2) has prepared hydrazidines and as-triazines related to substituted 2-cyanopyridines. The hydrazidines, of the general formula $RC(=NH)NHNH_2$, were prepared by the action of hydrazine on 2-cyanopyridine and several of its derivatives (2-cyanoquinoline, 2-cyanopyrimidine, and 2-cyano-1,10-phenanthroline). These products, which contain the group = N - C = C - N =, give a deep red color with Fe(II) (2). Benzil and pyridyl condense readily with these hydrazines to form 3,5,6 trisubstituted as-triazines. 1,2,4-Tris(2-pyridyl)-3,5,6-triazine was found to be a very sensitive reagent for Fe(II).

Several 3-substituted as-triazino [5,6-f] [4,7] phenanthrolines were prepared by the action of the hydrazines on 4,7-phenanthroline-5,6-dione (2). Most of these products formed very deep blue chelates with Fe(II). One product, made by Case (2), is of current interest, and the discussion of it immediately follows.

3-(4-Phenyl-2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PPDT)

A. A. Schilt and W. C. Hoyle (15) have recently developed a new method for iron, in which PPDT is used. The ferrous complex has a molar absorptivity of 28,700 at 561 mu in isoamyl alcohol, which is used as the extracting solvent (15). The iron is reduced with ydroxylamine hydrochloride. Then a pH 4-5 sodium acetate-acetic acid buffer, PPDT, and $NaClO_4$ solutions are added before extraction. If copper is believed to be present, pH 8.5-9.2 NH_4Cl-NH_4OH buffer is added until the sample is alkaline (3).

A study of interferences in aqueous solution was made, which revealed that CN^-, Co^{2+}, Cu^+, or Cu^{2+} were the most important interferences (15), in decreasing order. Some of the lesser interferences were Hg^{2+}, Cr^{3+}, NO_2^{2+}, UO_2^{2+}, $C_2O_4^{2-}$, F^-, MoO_4^{2-}, and Mn^{2+}, in decreasing order of importance. Only Cu^+ and Co^{2+} give colored, extractable complexes (15). The Cu(I) complex undergoes color fading in the presence of ammonia buffer, while the $Fe(PPDT)_3^{2+}$ does not fade detectably for half an hour (15).

The molar absorptivity is independent of pH from 3.0 to 8.0, and Beer's Law is followed up to at least 1.5 p.p.m. in aqueous ethanol solution (15). The color stability of the complex is best from pH range of four to six. However, the PPDT reagent and $Fe(PPDT)_3^{2+}$ are both only slightly soluble in aqueous solution, and the rate of formation of the complex is correspondingly quite slow (15).

The presence of either relatively large amounts of ethanol or of soluble perchlorate salt increases the rate of chelation. According to Schilt and Hoyle (15), the ethanol tends to dissolve the PPDT and the perchlorate ions, but it affects the reaction rate by precipitation of the chelate due to the extreme insolubility of perchlorate salt. This salt, however, is readily extractable into and soluble in isoamyl alcohol. Therefore, the requirement for relatively large amounts of ethanol, which would render subsequent extraction into organic solvents inefficient, can be avoided.

Schilt and hoyle have used PPDT specifically for the determination of iron in sea water, beer, and skim milk. The method shows promise for other applications. Quantitative analysis for Fe in some ores, such as those of Co, Ni, Cu, or Cr, would present some difficulties inasmuch as the Fe is only present as a minor impurity.

Terosite

In 1956, Schilt and Smith (16) prepared 2,6-bis(4-phenyl-2-pyridyl)-4-phenylpyridine, which was found to be a very sensitive reagent for iron. Terosite exhibits two sharp peaks in its visible spectrum at 383 mu and 583 mu (16). At 583 mu, the molar absorptivity in ethanol-chloroform mixture is 30,200; at 383 mu, 20,740 (16). The ferrous complex has color stability in the 2-8 pH range (16).

Unfortunately, Terosite is insoluble in water and ethanol, even at fairly low pH values, as well as in dioxane and ethyl acetate, with or without the addition of ethanol (16). The reagent is soluble in chloroform or a 1:1 chloroform-ethanol mixture (by volume) (16). In the procedure, relatively large amounts of chloroform and ethanol must be introduced into aqueous solution. The solution is "extracted" rather inefficiently, and then the extracted portion is diluted to a standard volume with ethanol or chloroform (16).

Terosite also forms a complex with Co(II), which is stable at 466 mu and 528 mu (16). The molar absorptivity at 466 mu is 3,052 and at 3,120 528 mu (16).

CHAPTER III

PREPARATION OF TPTZ AND PURIFICATION OF NITROBENZENE

TPTZ, which was a commercial product of the G. F. Smith Company, was used for preliminary experimentation. However, it became apparent that the cost of the quantity, which would be required to pursue the project, justified the synthesis of the compound. The original procedure of Case and Koft (3) was followed fairly closely.

Three separate syntheses were carried out. The procedure described below is typical. Sodium hydride (50% in oil) and 2-cyanopyridine were mixed in a 20 mm. x 150 mm. Pyrex test tube. The molar ratio of 2-cyanopyridine to NaH was 15:1. The mixture was heated for six hours at 165° C. in a nitrogen atmosphere, as shown in Figure 2.

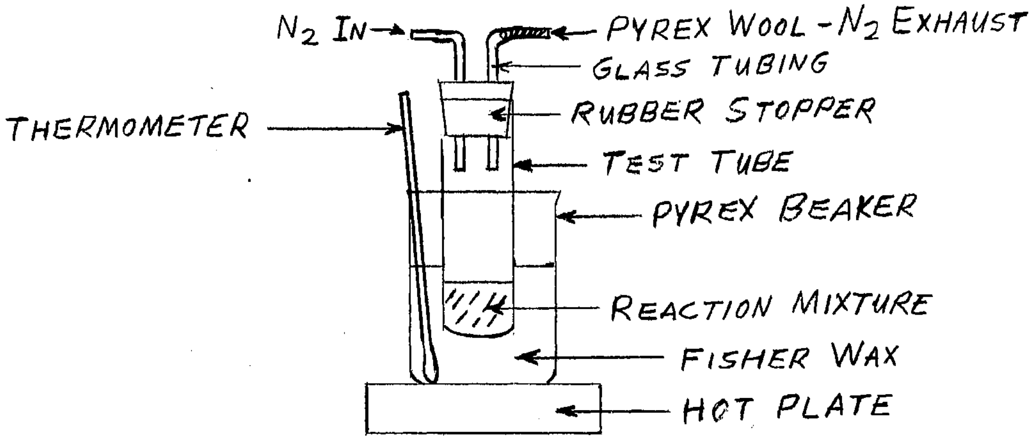

Figure 2. TPTZ Apparatus

The warm residue was extracted with three portions of boiling benzene and was very difficult to remove from the test tube. The residue, which did not dissolve, was separated from the benzene solution by filtration. The benzene solution was then evaporated to dryness, and the remaining solid was dissolved in a minimum amount of boiling ethanol. The ethanol solution was allowed to cool, and a small amount of water was added until precipitation began. An ice bath was used until precipitation was completed. The precipitate was recovered by filtration, and the precipitation of the product from ethanol-water was repeated three times.

The product was dried in the atmosphere and weighed. The final yield was obtained after dehydration, in, in 2.5-3.0 gram increments, of the hydrated TPTZ in a vacuum pistol at a temperature of 183° C. An aniline temperature bath was used, and each increment was in the vacuum pistol for twelve hours. Data concerning the TPTZ produced is in Table III and Table IV.

TABLE III

TPTZ STATISTICS

Batch No.	g. of NaH	g. of 2-Cyanopyridine	g. of TPTZ
1	0.3939	10.98	2.7268
2	0.66	17.20	4.1841
3	2.3793	69.24	14.7708

TABLE IV

TPTZ PRODUCT FORMULAS AND PERCENT YIELDS

Batch No.	% C	% H	% H	% 0 By Difference	Formula	Percent Yield
1	68.96	3.84	26.86	0.34	$C_{18}H_{12}N_6$	24.83
2	67.57	3.78	26.38	2.37	$C_{18}H_{12}N_6 \cdot 0.475\ H_2O$	23.68
3	68.60	4.14	26.46	0.68	$C_{18}H_{12}N_6 \cdot 0.133\ H_2O$	21.17

The analyses were done by Galbraith Laboratories, Inc. The analyses of the second two batches were done in duplicate, and the percentages are the averages. The percent of oxygen was obtained by difference. Apparently the smaller batches had a higher yield. The color of the product varied from a light tan to an orange-tan. The computed molecular weights for each of the products were 312.32, 320.88, and 314.73, respectively.

Purification of Nitrobenzene

Reagent quality nitrobenzene has a sufficient amount of more highly nitrated products present to give an intense yellow color to it. Removal of these impurities may be effected by either distillation or their retention on a chromatographic alumina column. The latter procedure was found to be much more convenient than the former. Even under vacuum conditions, the high boiling point made glass wool insulation of all glass joints and the like, necessary. A four-foot vacuum-jacketed column, packed with glass helices, was used. The rate of distillation was slow, and considerable dry ice had to be used for cooling of the cold trap. In short, the system had to be continuously monitored.

Chromatographic separation was comparatively simple, quicker, and required very little monitoring. A three foot column was packed with ten lbs. of mesh chromatographic grade alumina. The column had an internal diameter of 60 mm., a three liter reservoir at the top, and a #6 Teflon stopcock in a smooth glass bore at the bottom. The solid impurities developed into intense orange bands; and the nitrobenzene was recovered as a pale yellow liquid and was stored in polyethylene bottles. Most of the nitrobenzene was purified via the chromatographic column. Figures 3 and 4 are the basis for comparison of the two methods.

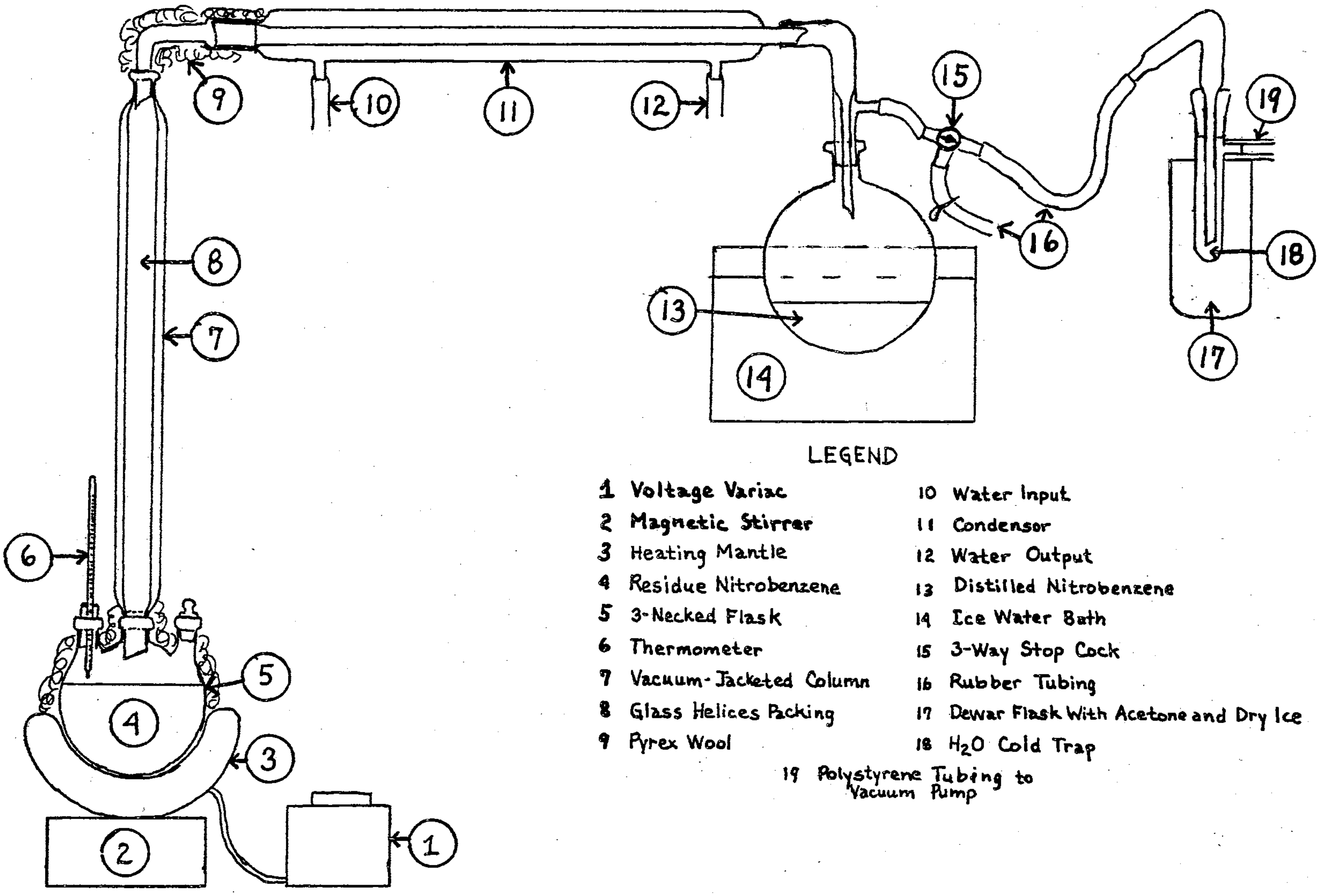

Figure 3. Nitrobenzene Distillation Apparatus

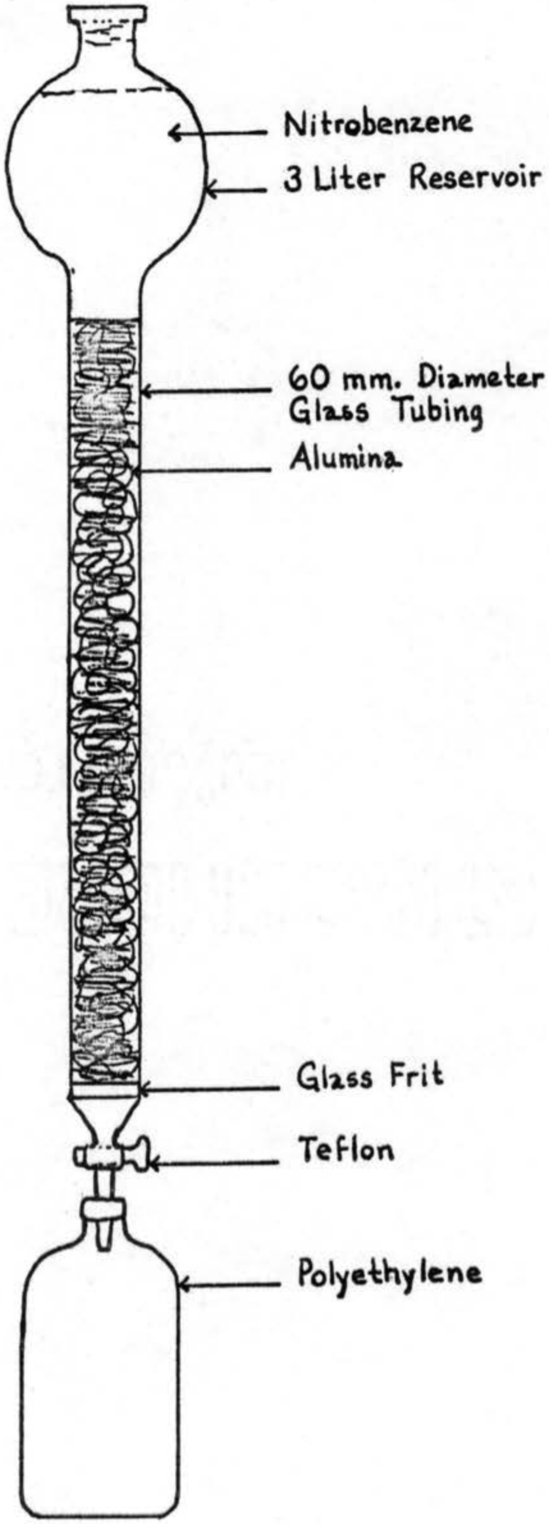

Figure 4. Chromatographic Nitrobenzene Purification Apparatus

CHAPTER IV

SOLUTION STORAGE AND WATER TREATMENT

Evaluation of Containers for Solution Storage

The dangers of contamination resulting from containers are widely recognized, inasmuch as glass tends to absorb Fe from iron solutions or can liberate Fe into solutions, which are initially iron-free; polyethylene can absorb transition metals or organic substances (5), (15), (17), (19). According to neutron activation analytical work done by Robertson (14), glass containers contain a very high level of iron, which is more than twenty-five times that of polyethylene containers. It was also found that glass containers had higher levels of other metals.

Preliminary tests were undertaken to decide the type of container best suited for the storage of reagent buffer-reducer and TPTZ solutions and the purified nitrobenzene. Iron-free water was put into glass and polyethylene bottles. TPTZ was introduced into these bottles. Evaluation was qualitative. Within a period of two weeks, the appearance of a blue color was observed in the glass bottles, but none was detected in the polyethylene bottles.

Deionization of Water

Removal of the metal impurities, fron the distilled water, had to be considered. The use of Fischer REXYN RG 501 (H-OH) cation-anion exchange resin appeared to offer the most convenient method. The 60 mm. diameter column had a flow rate of about 800 ml. per minute.

CHAPTER V

DESCRIPTION OF MODIFIED TPTZ METHOD AS APPLIED TO SYNTHETIC SOLUTION MEDIA

The method of Collins and Diehl (5) can be simplified in its application to synthetic solution media, and there is nothing that prevents the retention of most of the simplifications in the analysis of natural waters of natural waters for their iron content. The standard method involves the use of four standard reagent solutions, excluding standard iron. The 10% $NaClO_4$ solution, as a source of perchlorate, can be eliminated entirely, if perchloric acid is added to the standard iron and interfering metal solutions, and if the buffer and reducer solutions can be reduced to a single one. The buffer solution of Collins and Diehl (5) consists of 2 M $NaC_2H_3O_2$ and 2 M CH_3COOH, and their reducer solution is 10% $NH_2OH \cdot HCl$. The buffer-reducer solution, used in this study, consisted of 2 M $NH_4C_2H_3O_2$, 2 M. CH_3COOH, and 4% $NH_2OH \cdot HCl$. All buffer-reducer solution was made up in increments of three liters and extracted via addition of $HClO_4$ and TPTZ.

Anhydrous reagent grade ammonium acetate (462 g.), 120 g. of reagent grade $NH_2OH \cdot HCl$, and 340 ml of glacial acetic acid (reagent grade) were dissolved in sufficient deionized water to make 3 liters of solution. 30 ml. of 4×10^{-3} M TPTZ and 6 ml of reagent grade 70% $HClO_4$ were added. Then four successive extractions with 20 ml portions of purified reagent grade nitrobenzene were performed.

It was discovered in these studies that aqueous solutions of TPTZ were unstable. Precipitate tends to form after some time has elapsed. TPTZ is usually dissolved in a small volume of a strong acid such as perchloric or hydrochloric and then diluted to volume with water. The presence of 20% ethanol by volume will prevent precipitation. However, if TPTZ with this much alcohol is introduced into an aqueous iron solution, then the efficiency of extraction with nitrobenzene will be reduced due to the increase in the miscibility of the organic and aqueous phases with one another.

The use of acetic acid, instead of hydrochloric or perchloric, renders the use of ethanol unnecessary. TPTZ in 2 M acetic acid (11% glacial acetic acid by volume) does not precipitate. The possibility of chloride interference with iron precluded the use of hydrochloric acid. Sulfuric acid was not used, since some species of ferrous and ferric sulfate are rather insoluble in aqueous solution. Iron was pre-extracted from the deionized water that was used in the preparation of 4.0 or 3.75×10^{-3} M TPTZ solutions. The four solutions of Collins and Diehl have been reduced to two: the chelating agent and the buffer-reducer solution.

CHAPTER VI

DESCRIPTION OF PROCEDURE AS APPLIED TO Cu AND Co SYSTEMS

The study of the Cu system was done in a different manner than that of the Co, Cr, Ni and Mn systems. Therefore, operational detail will be given for the Fe-Cu and Fe-Co metal pairs. The procedure followed for the Fe-Co system is typical of that followed for the Fe-Cr, Fe-Ni, and Fe-Mn metal pairs. The same levels of Fe were used in all systems.

Fe-Cu System

The first step in procedure for any of the systems was the preparation of the standard iron and standard interfering metal solutions. No. 36 iron wire, 99.8% Fe, manufactured by J. T. Baker Chemical Co., was used for the standard iron solutions. 0.1000 gram was weighed with a Mettler single-pan balance and dissolved in 25 ml. of concentrated reagent grade nitric acid in a 100 ml. Pyrex beaker with a 100 mm. watch glass on top of it. A hot plate was used to aid the dissolution process. The solution was allowed to cool before deionized water from a polyethylene wash bottle was slowly added to the solution in the beaker and globules of acid solution washed into the solution, also.

The Cu solution was prepared simultaneously. After the iron wire had been weighed, 4.0000 g. of electrolytic grade 0.008 inch Cu sheet, manufactured by Matheson, Coleman and Bell, was weighed out and dissolved in 100 ml. of 3 N. HNO_3 om a 200 ml. beaker with a 125 ml. watch glass on top. The Cu solution was allowed to cool and diluted in a manner similar to that of the Fe solution. All volumetric flasks were washed with 3 N HCl then deionized water, and finally acetone, and allowed to dry. The Fe solution was carefully transferred in a quantitative manner into a 2 liter volumetric flask. The Cu was similarly transferred into another 2 liter volumetric flask.

Sufficient perchloric acid (70%) was added to these two volumetric flasks and fifteen others so that, in each case, the solution in each one would be 0.25 N in $HClO_4$, upon final dilution. The master Cu solution was 2,000 p.p.m., and the master Fe solution was 50 p.p.m. after final dilution to volume with deionized water.

Aliquots of the master Cu solution were used to prepare 100, 40, 30, and 20 p.p.m. Cu solutions. Aliquots of the 100 p.p.m. Cu solution were employed for the 5, 3, 2, and 1 p.p.m. solutions. 25 ml of 20 p.p.m. Cu made one liter of 0.5 p.p.m. Cu. Similarly, aliquots of the 50 p.p.m. Fe were used for 4, 3, 1, 1 and 0.5 p.p.m. solutions.

Sixteen 2000 ml separatory funnels with teflon stopcocks were used. The funnels were mounted via single-piece O-rings with clamps onto the vertical members of a grill framework in a hood. The Fe-Cu solution combinations were prepared in triplicate. One funnel was reserved for the blank. Aliquots of the standard Fe solutions were placed in the funnels first. Then aliquots of the standard Cu solutions were placed in them.

One level of iron was used until all ten levels of copper had been run. The first three separatory funnels had the lowest Cu level, the second three the next level, etc. A maximum of five triplicate samples with one blank could be prepared at a time. The next step was the addition of deionized water until all solutions were diluted to volume.

2 ml of 70% $HClO_4$ were added to the water in the blank funnel. Then 50 ml of buffer-reducer were added to each funnel. Next, 20 ml of nitrobenzene were added. Finally, 10 ml of 3.75×10^{-3} M TPTZ was added to each of the funnels.

Each funnel was then shaken for ten seconds, starting with the blank. After all funnels had been shaken, they were swirled in the same order. The nitrobenzene phase was subsequently drawn off at the bottom of each funnel into a 100 ml beaker. The beakers were put into four desicators, four occupying each one. Each beaker had an assigned position within its particular desicator. Three additional extractions were performed on each funnel.

The four combined extracts within each beaker were quantitatively transferred to an assigned and numbered 100 ml volumetric flask. Each beaker was washed with absolute ethanol, and the washings transfered to the assigned volumetric flask. All nitrobenzene solutions were diluted to volume with absolute ethanol and mixed with it. The absorbance readings of the solutions were then determined with the Cary 14 Spectrophoto in the same order that they were extracted. The readings were made with reference to the blank.

Sometimes it was desireable to make stability determinations. When this was the case, the solutions were kept and not thrown away. Usually, the solutions were transferred to waste bottles. The volumetric flasks, beakers, and separatory funnels were rinsed with acetone, then with 3N, HCl, and finally washed again with acetone. The volumetrics were inverted on a rack and allowed to dry.

Time and materials available did not permit repetion of all solution combinations in the system. There were five levels of iron and ten levels of copper being considered. In addition, all five Fe levels with no copper were examined for standardization purposes, and some of the Cu levels with no iron were considered as well.

Fe-Co System

The same basic procedure was used with regard to solution preparation with several major exceptions. First, only six levels of Co were considered. Five levels of Fe were used, as before. Second, each solution combination was a singlet, not a triplicate. This meant that an entire replicate could be run in two cycles of funnel operation. Third, at the end of the first replicate, new standard Fe and Co solutions were made, and a complete second replicate was done.

Modifications of Other Systems

The Cr, Mn, and Ni systems were exactly the same as the Fe-Co one, with three differences: (a) The Cr metal powder had to be initially dissolved in 5 N H_2SO_4; (b) NiO powder instead of Ni metal powder was used as the source of Ni; (c) and the Mn powder was initially dissolved in 1 N HNO_3.

CHAPTER VII

COMPILATION OF RESULTS AND STATISTICAL ANALYSES OF Cu, Co, Cr, Ni, and Mn INTERFERENCE SYSTEMS

The Fe-Cu system had a different experimental design than the other systems and was consequently statistically treated in another manner. Two analyses of the Fe-Cu system were undertaken. The first was based on a sample size of twenty for an estimate of error, and the second upon a sample size of thirty. The second statistical treatment verified the findings of the first one. This system was considered to be one in which there is unequal replication, and an hierarchial analysis of variance program was used.

The other interference systems had a common experimental design, which entailed singlet solution combination sample size and two complete replicates. A regular factorial analysis of variance was applied in these cases. The Hierarchial AOV and Factorial AOV are part of the Oklahoma State University Computer Center Library. Detailed discussion of statistical treatment of these types of experimental designs is given by Steel and Torrie (18). All parts-per-million designations refer to aqueous concentration before extraction. The asterisks indicate average values of observations for the cells or solution combinations in Tables VI, XI, XVII, XXI, XXIV, XXX, XXXI, XXXIII, and XXXIV. In these Fe-Cu tables, the asterisked value indicates the average of the first two observations within the cell, if there are more than two. The asterisks in the Fe-Cu interaction system tables indicate solution combination positions used for estimating the sample variance. The presence of asterisks in the system AOV tables is the conventional method of indicating degree of significance for F tests used in formulating conclusions in the interpretation of experimental results.

The concentration of the TPTZ solutions used for the evaluation of Cu, Co, and Mn as interferences was never in doubt. Therefore, tables were made showing percentage interference for each cell (solution combination) for each of these systems. The interference was determined with reference to the linear regression value from the standard Fe curve for the particular Fe concentration level. Examination of Table VI shows that for Fe with no interference present, that the maximum standard deviation of the mean, for any of the triplicate measurements, in terms of per cent, is ±2.92%. Hence, absolute interference percentage values, which are less than 2.92, may be disregarded. The various statistical methods, which have been applied, are discussed in Appendix A.

TABLE V

STANDARD Fe CURVE

P.P.M. Fe in H_2O X	Absorbance (1 cm.) Y	1 cm. Regression Absorbance $\underline{Y}$
0.010	0.04485	0.0420967 = 0.0421
0.025	0.1065	0.10887 = 0.109
0.050	0.2195	0.2201665 = 0.220
0.075	0.330	0.336301 = 0.336
0.100	0.4445	0.44275 = 0.443

$$\sum X_i = 0.2600; \quad \sum(X_i)^2 = 0.0676; \quad \sum(X_i)^2/N = 0.01352$$

$$\sum X_i^2 = 0.01885; \quad \sum x_D^2 = \sum X_i^2 - (\sum X_i)^2/N = 0.00533$$

$$\bar{X} = 0.0520; \quad \bar{Y} = 0.22907; \quad \sum Y_i = 1.14535$$

$$\sum X_i Y_i = 0.083286; \quad \sum X_i \sum Y_i = 0.297791$$

$$\sum xy = \sum X_i Y_i - \frac{\sum X_i \sum Y_i}{N} = (0.093286 - 0.0595582)$$

$$\sum xy = 0.0237278$$

$$b = \frac{\sum xy}{\sum x_D^2} = 4.45174484$$

$$(\underline{Y} - \bar{Y}) = b(X - \bar{X}); \quad (\underline{Y} - 0.22907) = 4.45174484X - 0.23149073168$$

$$\underline{Y} = 4.45174484X - 0.0024207317$$

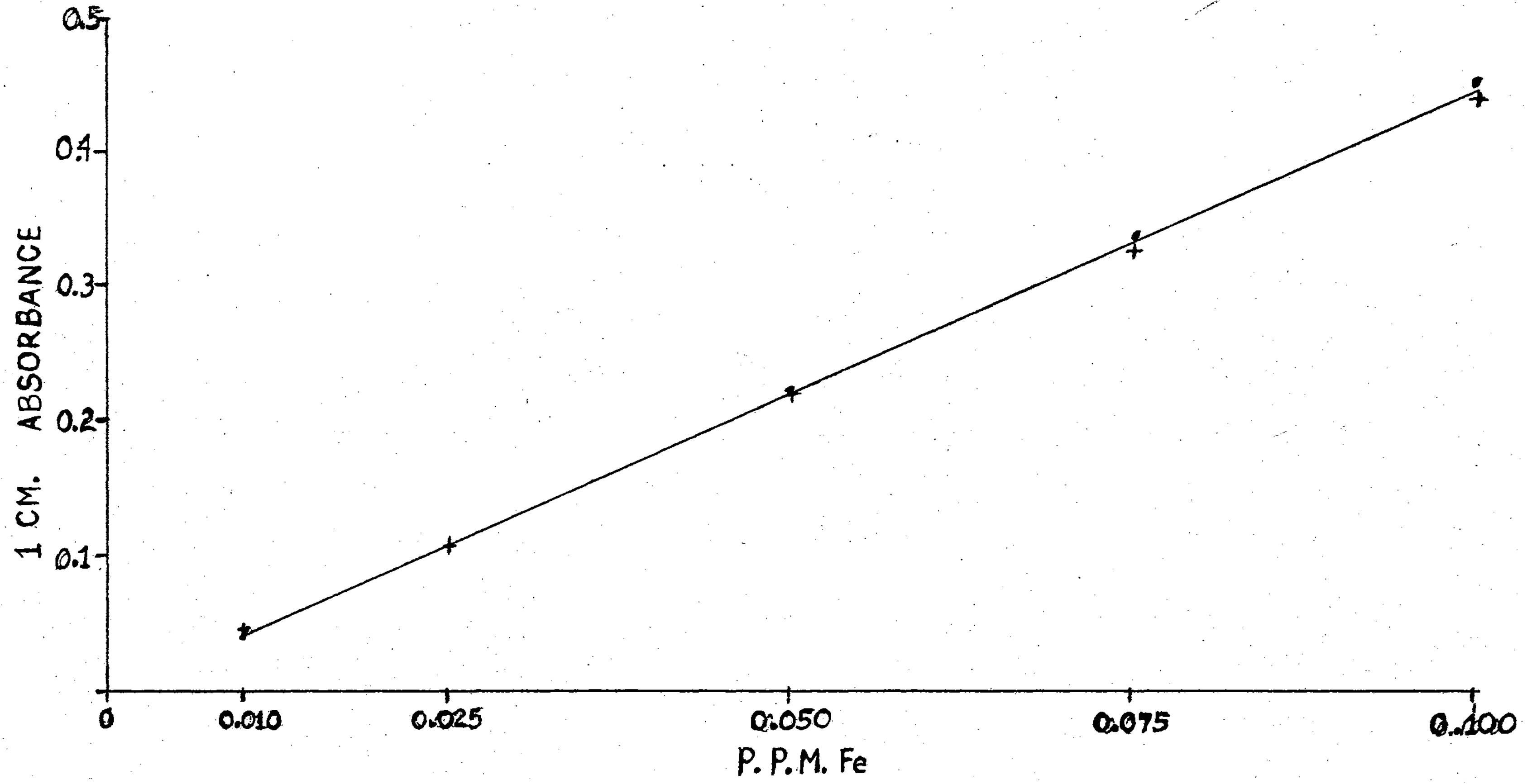

Figure 5. Standard $Fe(TPTZ)_2(ClO_4)_2$ Curve

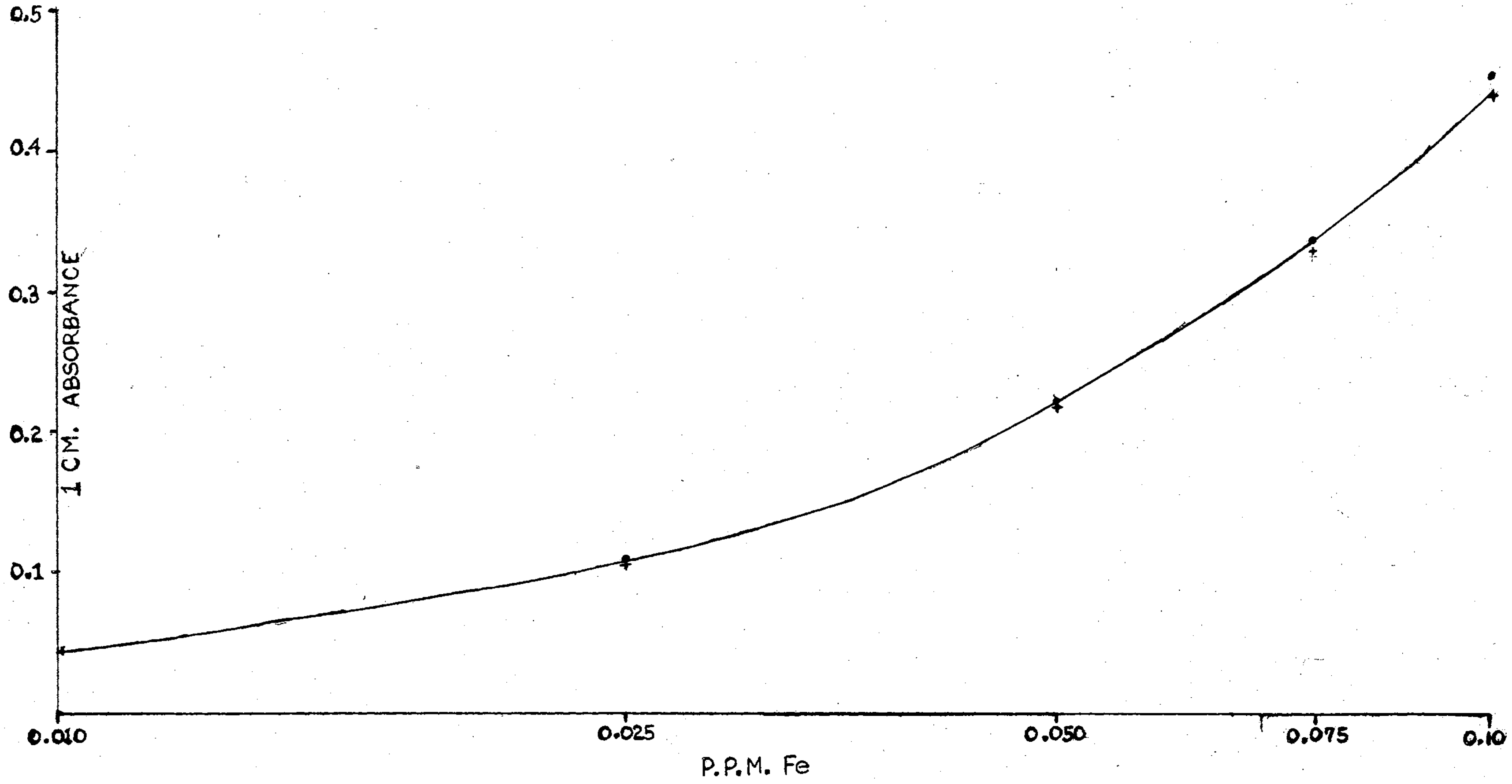

Figure 6. Standard Fe Curve From Linear Regression

TABLE VI

COMPLETE Fe-Cu-TPTZ SYSTEM FOR SAMPLE SIZE = 20

P.P.M. Cu → ↓P.P.M. Fe	0.00	0.010	0.025	0.050	0.075	0.100	0.25	0.50	0.75	1.0	2.5
0.00						0.0144 ±0.0009	0.142 ±0.023	0.187 ±0.039	0.261 ±0.069	0.229 ±0.029	0.116 ±0.015
0.010	0.0446 ±0.0013 0.0451 ±0.0001	0.0470 ±0.0012 0.0475 ±0.0002 *	0.0484 ±0.0012	0.0513 ±0.0025	0.0484 ±0.0025	0.0782 ±0.0172	0.164 ±0.024	0.216 ±0.066 0.202 ±0.026 *	0.310 ±0.072 0.196 ±0.086 *	0.303 ±0.035 0.272 ±0.073 *	0.215 ±0.113 0.082 ±0.040 *
0.025	0.107 ±0.001 0.106 ±0.003	0.112 ±0.001 0.110 ±0.001 *	0.121 ±0.001	0.119 ±0.005	0.133 ±0.012	0.156 ±0.011	0.154 ±0.017	0.209 ±0.081 0.301 ±0.050 *	0.338 ±0.041 0.296 ±0.073 *	0.245 ±0.037 0.183 ±0.040 *	0.094 ±0.068 0.089 ±0.029 *
0.050	0.218 ±0.003 0.221 ±0.0008	0.216 ±0.002	0.227 ±0.002 0.224 ±0.0004	0.217 ±0.002	0.219 ±0.009	0.233 ±0.006	0.200 ±0.002	0.363 ±0.057	0.259 ±0.045	0.544 ±0.044 0.364 ±0.046	0.248 ±0.027
0.075	0.324 ±0.007 0.336 ±0.0009	0.302 ±0.010 0.325 ±0.003 *	0.324 ±0.009	0.307 ±0.024	0.348 ±0.008	0.330 ±0.004	0.434 ±0.016	0.547 ±0.045 0.411 ±0.046 *	0.517 ±0.078 0.482 ±0.059 *	0.319 ±0.016 0.242 ±0.067 *	0.149 ±0.020 0.097 ±0.017 *
0.100	0.451 ±0.002 0.483 ±0.002	0.419 ±0.015 0.452 ±0.002 *	0.418 ±0.006	0.412 ±0.023	0.416 ±0.019	0.444 ±0.014	0.434 ±0.035	0.596 ±0.056 0.432 ±0.090 0.503 ±0.043 *	0.532 ±0.041 0.451 ±0.077 0.567 ±0.016 *	0.554 ±0.022 0.404 ±0.052 *	0.199 ±0.017 0.168 ±0.030 *

TABLE VII

Fe-Cu-TPTZ INTERACTION SYSTEM FOR SAMPLE SIZE = 20

P.P.M. Cu. → ↓ P.P.M. Fe	0.010	0.025	0.050	0.075	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.04635 *	0.0484	0.0513	0.0484	0.0782	0.164	0.209 *	0.253 *	0.288 *	0.149 *
0.025	0.111 *	0.121	0.119	0.133	0.156	0.154	0.255 *	0.317 *	0.214 *	0.0915 *
0.050	0.216	0.227	0.217	0.219	0.233	0.200	0.363	0.259	0.544	0.248
0.075	0.3135 *	0.324	0.307	0.348	0.330	0.434	0.479 *	0.4995 *	0.2805 *	0.123 *
0.100	0.4355 *	0.418	0.412	0.416	0.444	0.434	0.514 *	0.4915 *	0.479 *	0.1835 *

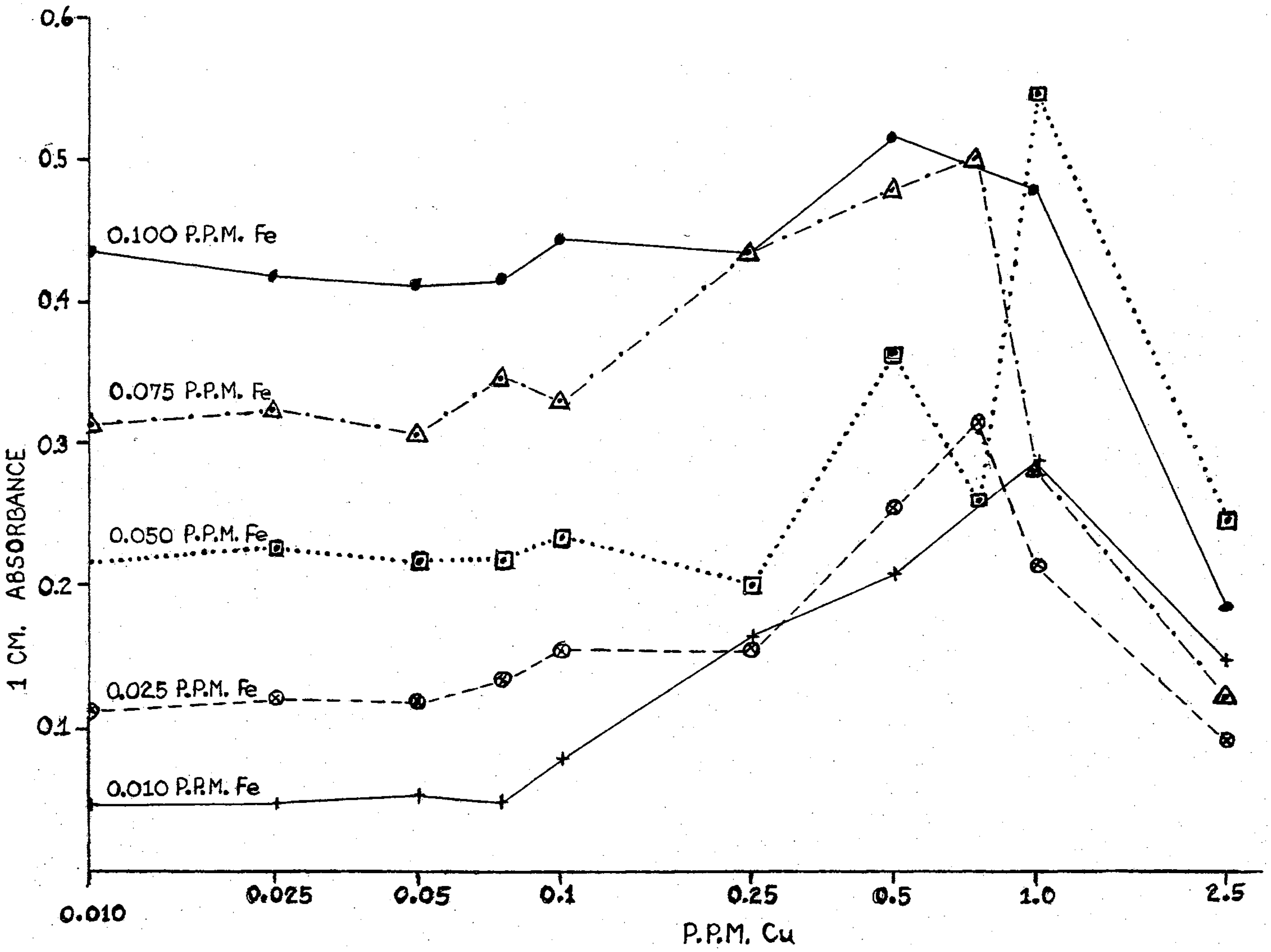

Figure 7. 1 Cm. Absorbance vs. P.P.M. Cu for All Fe Levels

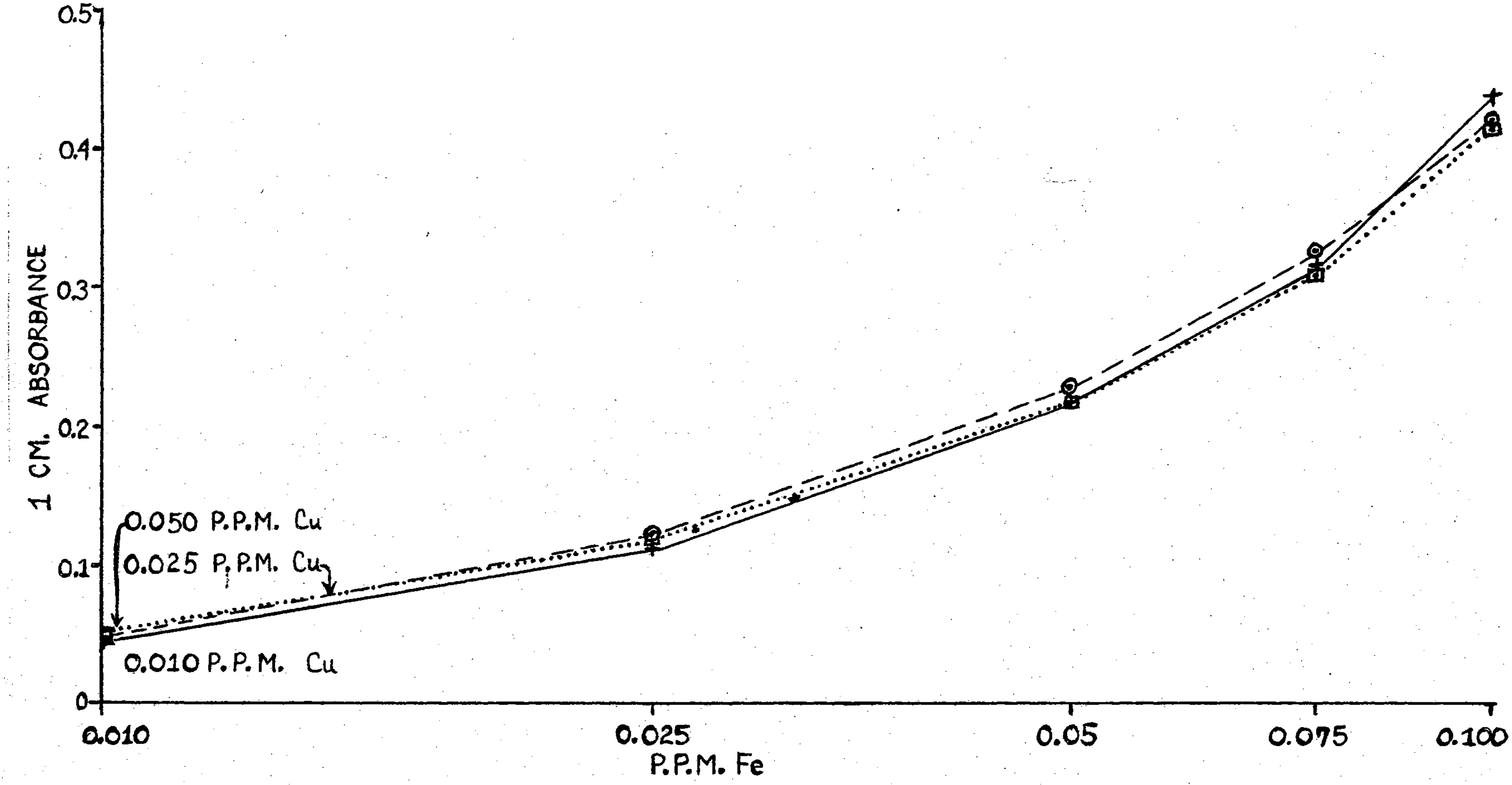

Figure 8. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.010 P.P.M., 0.025 P.P.M., and 0.050 P.P.M. Cu Levels

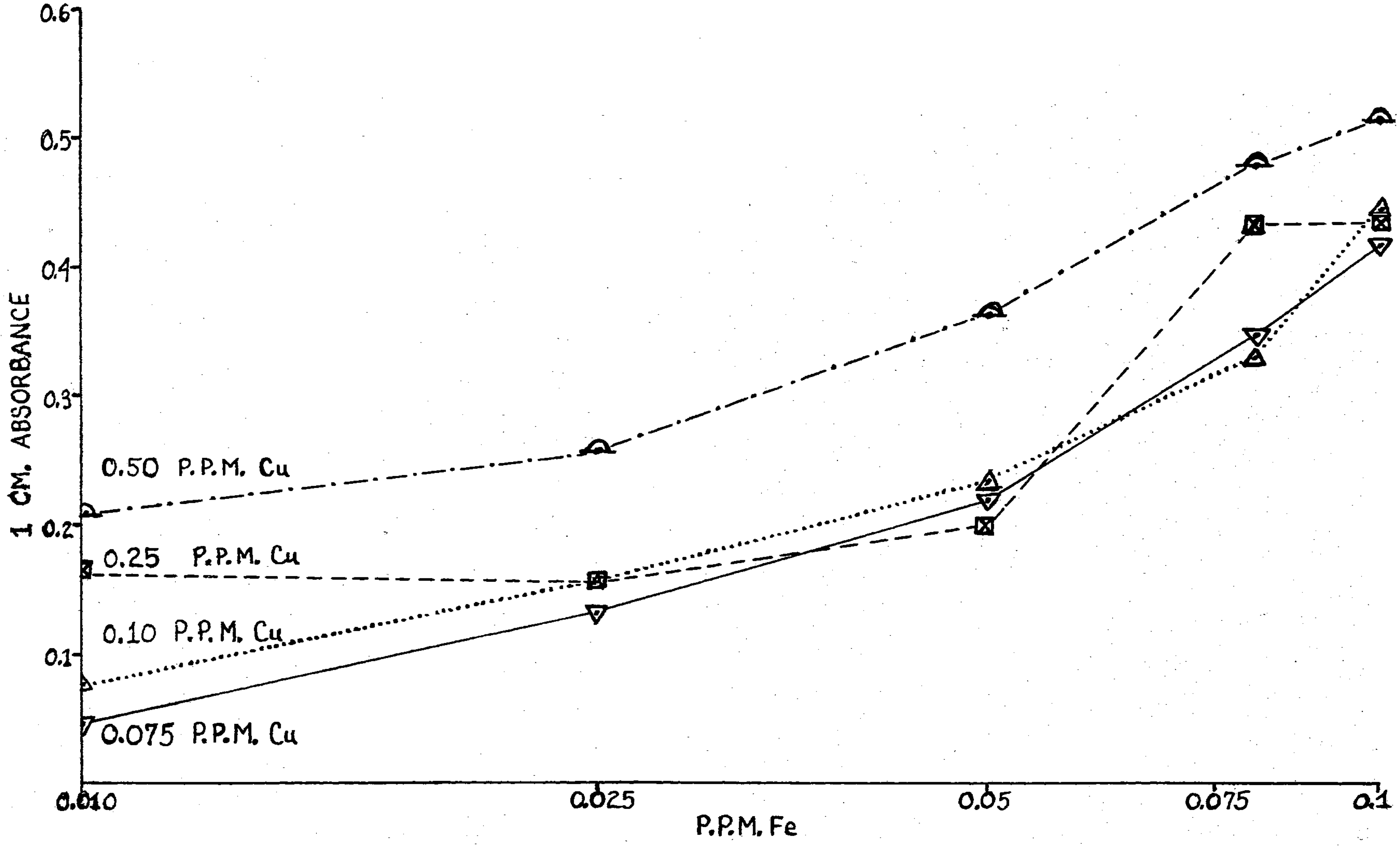

Figure 9. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.075 P.P.M., 0.10 P.P.M., 0.25 P.P.M., and 0.50 P.P.M. Cu Levels

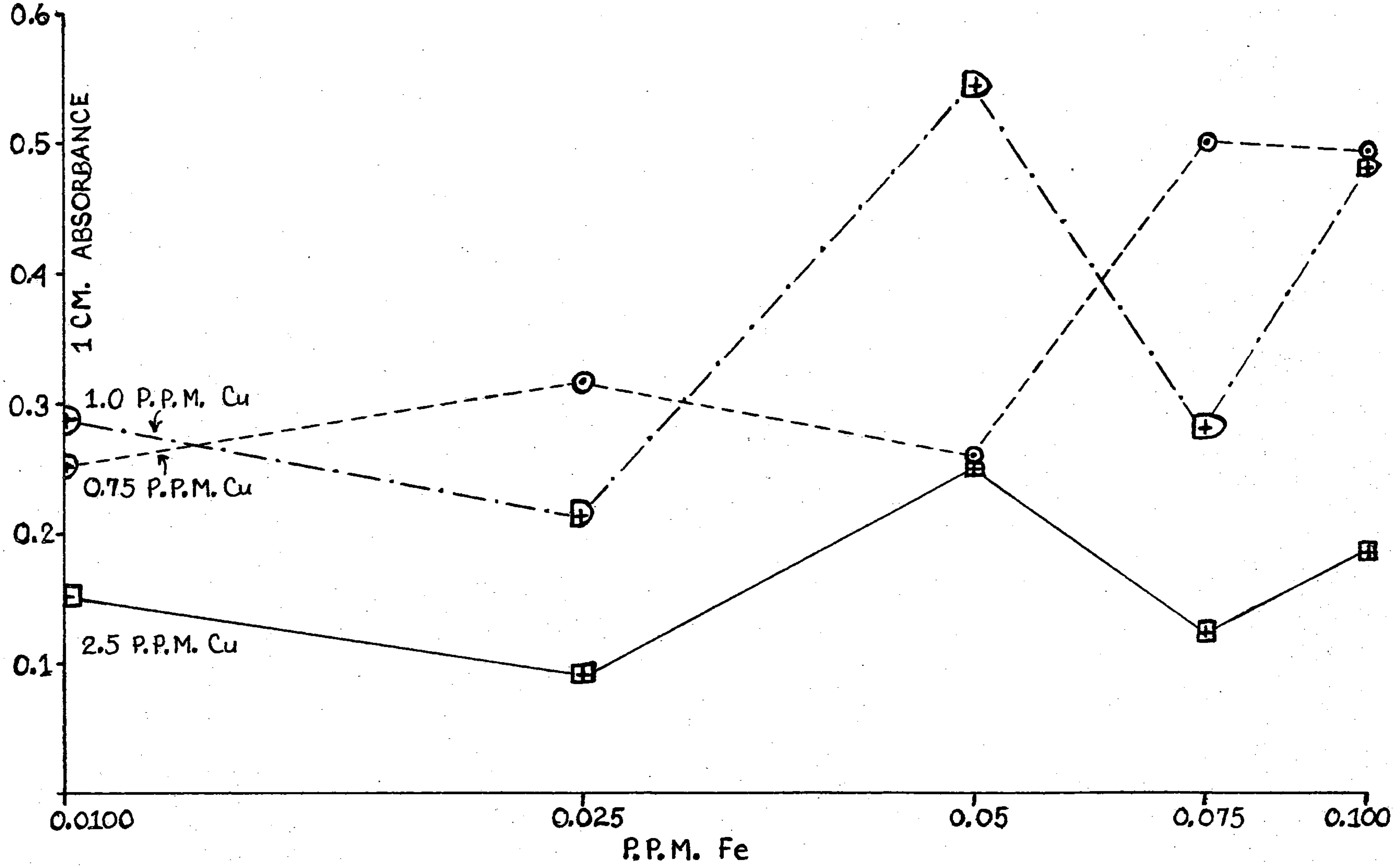

Figure 10. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.75 P.P.M., 1.0 P.P.M., and 2.5 P.P.M. Cu Levels

TABLE VIII

Cu ANALYSIS OF VARIANCE TABLE FOR SAMPLE SIZE = 20

Source	df	Sum of Squares	Mean Square	F
Total	(70-1) = 69	1.489997	0.00000000	
Cu	(10-1) = 9	0.404401	0.044933444	13.53 ** F 9,20(0.005) = 3.96
Fe in Cu	4 x 10 = 40	1.019178625	0.025479465	7.673 ** F 40,20(0.005) = 3.02
Sample	4 x 5 = 20	0.066417375	0.0033208687	

TABLE IX

Fe ANALYSIS OF VARIANCE TABLE FOR SAMPLE SIZE = 20

Source	df	Sum of Squares	Mean Square	F
Total	(70-1) = 69	1.489997	0.00000000	
Fe	(5 - 1) = 4	0.762415875	0.19060396875	57.40 ** F 4,20(0.005) = 5.17
Cu in Fe	9 x 5 = 45	0.6611638125	0.01469252905273	4.424 ** F 45,20(0.005) = 3.05
Sample	4 x 5 = 20	0.0664173125	0.0033208656	

TABLE X

ANALYSIS OF VARIANCE FOR Fe-Cu SYSTEM SAMPLE SIZE = 20

Source	df	Sum of Squares	Mean Square	F
Total	(70-1) = 69	1.489997	0.00000000	
Cu	(10-1) = 9	0.404401	0.044933444	13.53 ** F 9,20(0.005) = 3.96
Fe	(5-1) = 4	0.762415875	0.19060396875	57.40 ** F 4,20(0.005) = 5.17
Cu x Fe	9 x 4 = 36	0.2567628	0.00713231	2.148 ** F 36,20(0.05) = 2.01
Sample	4 x 5 = 20	0.0664173125	0.00332087	

Interaction Sum of Squares

Fe x Cu(ss) = Cu in Fe(ss) - Cu(ss) = 0.2567628

Cu x Fe(ss) = Fe in Cu(ss) - Fe(ss) = 0.2567628

TABLE XI

COMPLETE Fe–Cu–TPTZ SYSTEM FOR SAMPLE SIZE = 30

P.P.M. $\overrightarrow{Cu}$	• 0.00	• 0.010	• 0.025	• 0.050	• 0.075	• 0.100	• 0.25	• 0.50	• 0.75	• 1.0	• 2.5
P.P.M. Fe 0.00 ↓						0.0144 ±0.0099	0.142 ±0.023	0.187 ±0.039	0.261 ±0.069	0.229 ±0.029	0.116 ±0.015
0.010	0.0446 ±0.0013 0.0451 ±0.0001 *	0.0470 ±0.0012 0.0457 ±0.0002 *	0.0484 ±0.0012 0.0443 ±0.002	0.0513 ±0.0023	0.0484 ±0.0025	0.0782 ±0.0172	0.164 ±0.024	0.216 ±0.066 0.202 ±0.026 *	0.310 ±0.072 0.196 ±0.086 *	0.303 ±0.035 0.272 ±0.073 *	0.215 ±0.113 0.082 ±0.040 *
0.025	0.107 ±0.001 0.106 ±0.003 *	0.112 ±0.001 0.110 ±0.001 *	0.121 ±0.001 0.108 ±0.0004	0.119 ±0.005	0.133 ±0.012	0.156 ±0.011	0.154 ±0.017	0.209 ±0.081 0.301 ±0.050 *	0.338 ±0.041 0.296 ±0.073 *	0.245 ±0.037 0.183 ±0.040 *	0.094 ±0.068 0.089 ±0.029 *
0.050	0.218 ±0.003 0.221 ±0.0008 *	0.216 ±0.002 0.222 ±0.001 *	0.227 ±0.002 0.224 ±0.0004	0.217 ±0.002	0.219 ±0.009	0.233 ±0.006	0.200 ±0.002	0.363 ±0.057 0.229 ±0.004 *	0.259 ±0.045 0.295 ±0.070 *	0.544 ±0.044 0.364 ±0.046 0.260 ±0.032 *	0.248 ±0.027 0.032 ±0.012 *
0.075	0.324 ±0.007 0.336 ±0.0009 *	0.302 ±0.010 0.325 ±0.013 *	0.324 ±0.009 0.333 ±0.002	0.307 ±0.024	0.348 ±0.018	0.330 ±0.004	0.434 ±0.016	0.547 ±0.045 0.411 ±0.046 *	0.517 ±0.078 0.482 ±0.059 *	0.319 ±0.016 0.242 ±0.067 *	0.149 ±0.020 0.097 ±0.017 *
0.100	0.451 ±0.002 0.438 ±0.002 *	0.419 ±0.015 0.452 ±0.002 *	0.418 ±0.006 0.436 ±0.004	0.412 ±0.023	0.416 ±0.019	0.444 ±0.014	0.434 ±0.035	0.596 ±0.056 0.432 ±0.090 0.503 ±0.043 *	0.532 ±0.041 0.451 ±0.077 0.567 ±0.016 *	0.554 ±0.022 0.404 ±0.052 *	0.199 ±0.017 0.168 ±0.030 *

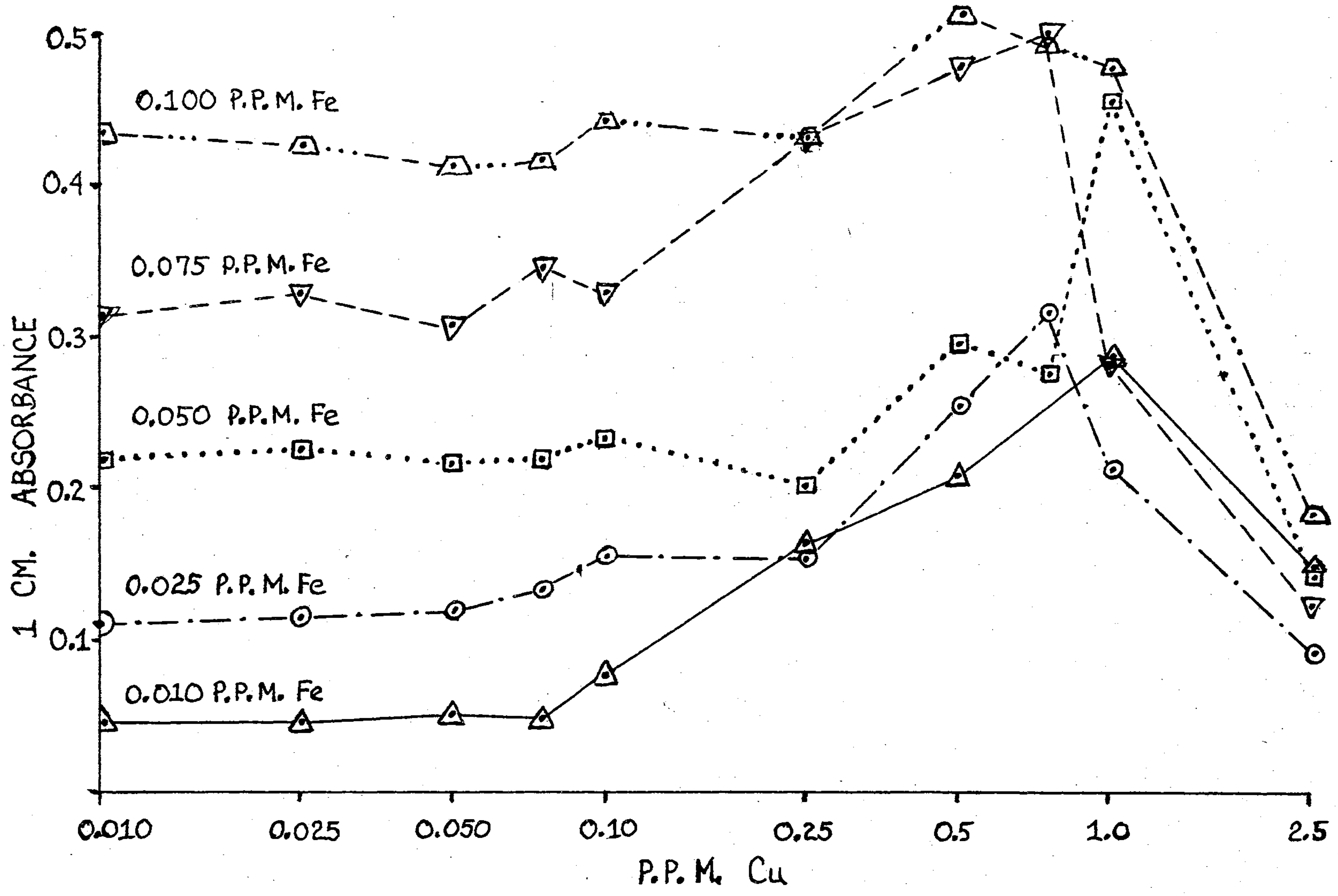

Figure 11. 1 Cm. Absorbance vs. P.P.M. Cu for All Fe Levels With Sample Size = 30

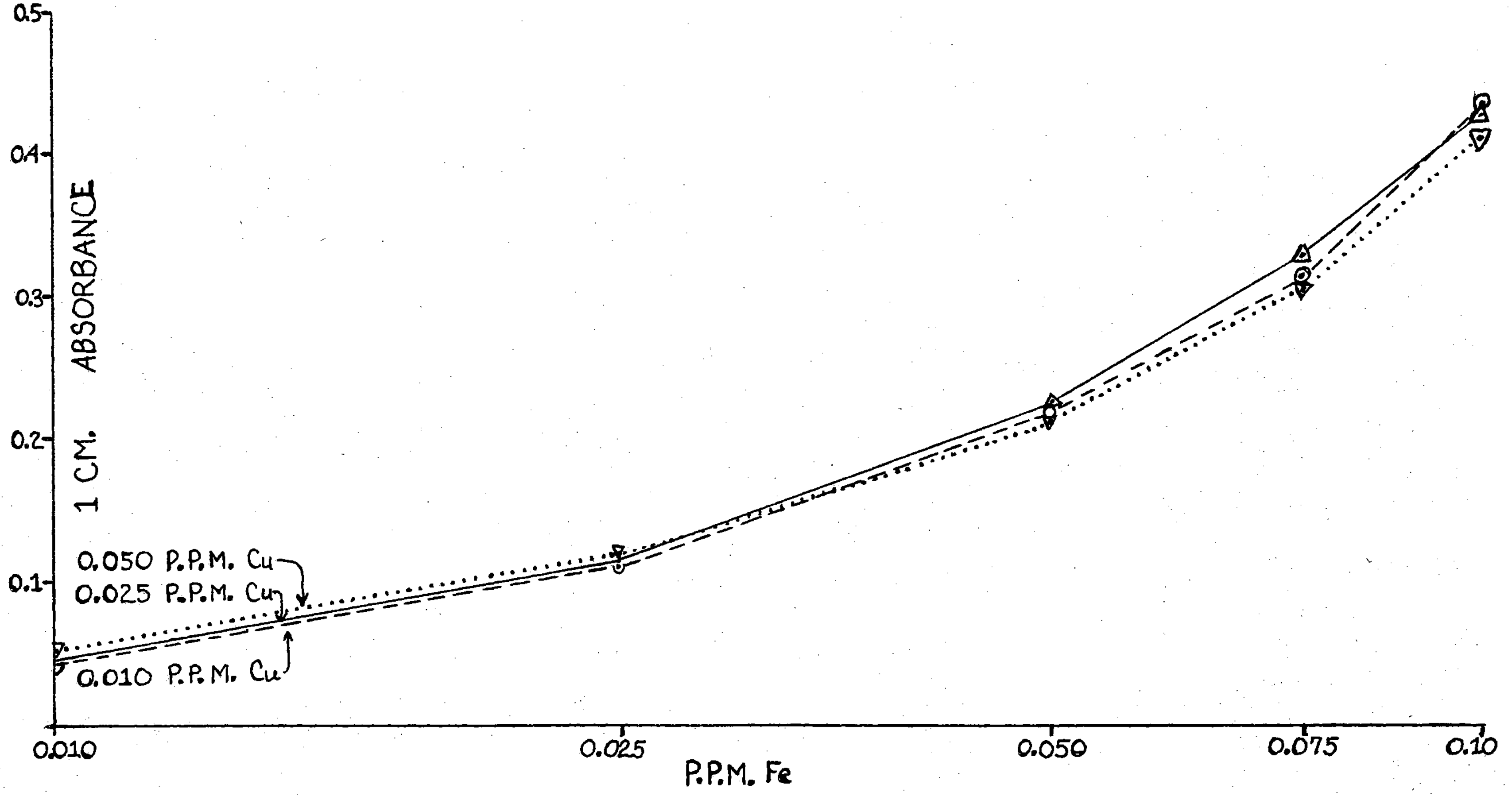

Figure 12. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.010 P.P.M., 0.025 P.P.M., and 0.050 P.P.M. Cu Levels With Sample Size = 30

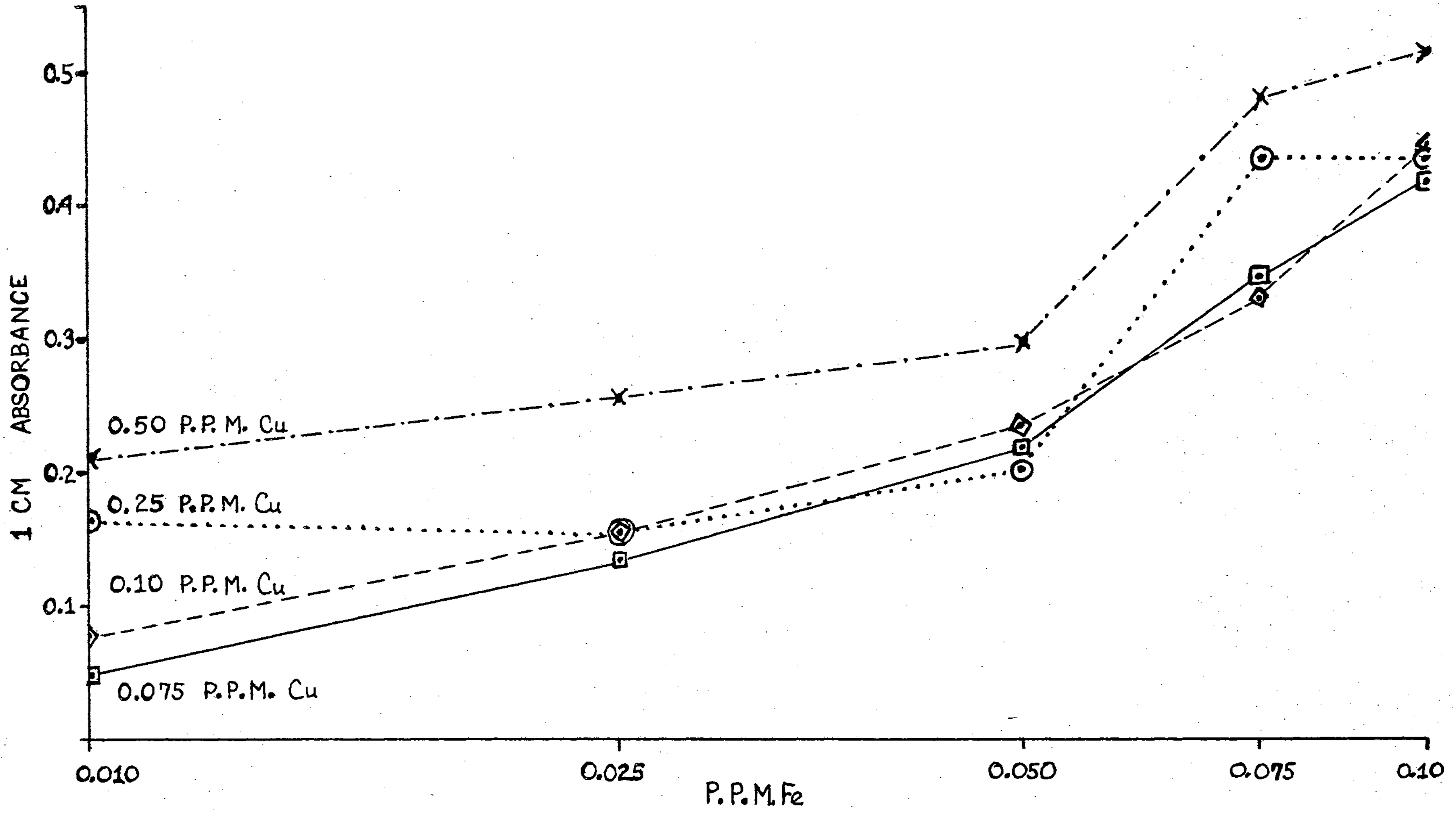

Figure 13. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.075 P.P.M., 0.10 P.P.M., 0.25 P.P.M., and 0.50 P.P.M. Cu Levels With Sample Size = 30

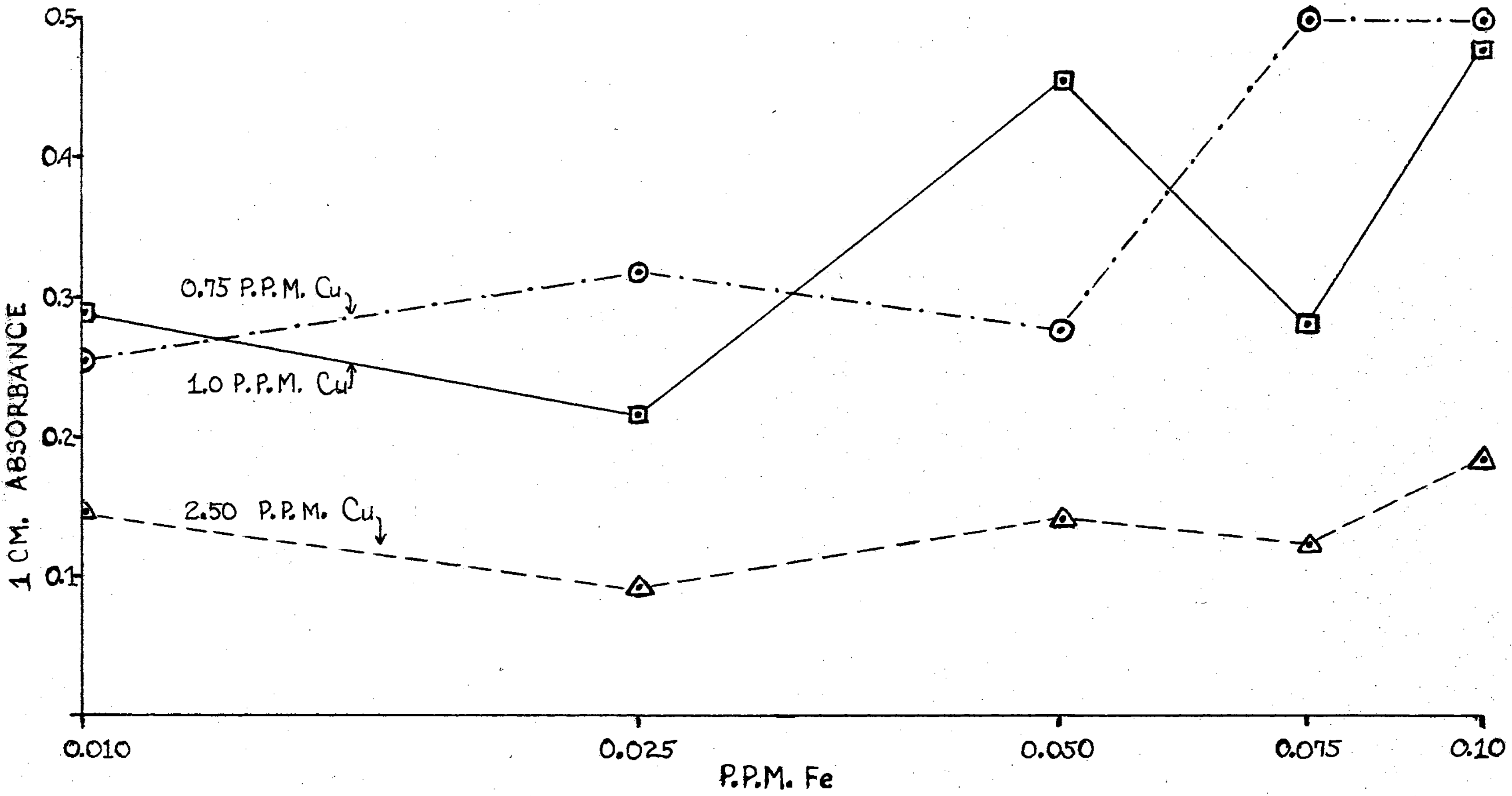

Figure 14. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.75 P.P.M., 1.0 P.P.M., and 2.5 P.P.M. Cu Levels With Sample Size = 30

TABLE XII

Fe-Cu-TPTZ INTERACTION SYSTEM FOR SAMPLE SIZE = 30

P.P.M. Cu → P.P.M. Fe ↓	0.010	0.025	0.050	0.075	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.04635 *	0.04635 *	0.0513	0.0484	0.0782	0.164	0.209 *	0.253 *	0.2875 *	0.1485 *
0.025	0.111 *	0.1145 *	0.119	0.133	0.156	0.154	0.255 *	0.317 *	0.214 *	0.0915 *
0.050	0.219 *	0.2255 *	0.217	0.219	0.233	0.200	0.296 *	0.277 *	0.454 *	0.1415 *
0.075	0.3135 *	0.3285 *	0.307	0.348	0.330	0.434	0.479 *	0.4995 *	0.2805 *	0.123 *
0.100	0.4355 *	0.427 *	0.412	0.416	0.444	0.434	0.514 *	0.4915 *	0.479 *	0.1835 *

TABLE XIII

INTERFERENCE PERCENTAGES FOR Fe-Cu-TPTZ SYSTEM

P.P.M. Cu P.P.M. Fe	0.010	0.025	0.050	0.075	0.10	0.25	0.50	0.75	1.0	2.5
0.010	10.1	10.1	21.9	15.0	85.7	299	396	502	583	252
0.025	1.83	5.05	9.17	20.2	43.1	41.3	134	200	96.3	-16.1
0.050	-0.454	2.50	-1.36	-0.454	5.91	-9.09	34.5	25.9	106	-35.7
0.075	-6.70	-2.23	-8.63	3.57	-1.79	29.2	42.6	49.3	-16.5	-63.4
0.100	-1.47	-3.49	-6.78	-5.88	0.452	-1.81	16.3	11.2	8.37	-58.5

$$\% \text{ Interference} = \frac{\text{Observed Absorbance} - \text{Standard Fe Absorbance}}{\text{Standard Absorbance}} \times 100$$

TABLE XIV

Cu ANALYSIS OF VARIANCE TABLE FOR SAMPLE SIZE = 30

Source	df	Sum of Squares	Mean Square	F
Total	(80-1) = 79	1.6698454375	0.00000000	
Cu	(10-1) = 9	0.447316875	0.049701875	12.94 ** $F_{9,30}(0.005) = 3.45$
Fe in Cu	4 x 10 = 40	1.1072828125	0.0276820703125	7,206 ** $F_{40,30}(0.005) = 2.52$
Sample	5 x 6 = 30	0.11524575	0.0038415249939	

TABLE XV

Fe ANALYSIS OF VARIANCE TABLE FOR SAMPLE SIZE = 30

Source	df	Sum of Squares	Mean Square	F
Total	(80-1) = 79	1.6698455	0.00000000	
Fe	(5-1) = 4	0.8589438125	0.214735953125	55.90 ** $F_{4,30}(0.005) = 4.62$
Cu in Fe	9 x 5 = 45	0.6956559375	0.01545902075195	4.024 ** $F_{45,30}(0.005) = 2.52$
Sample	5 x 6 = 30	0.11524575	0.0038415249939	

TABLE XVI

ANALYSIS OF VARIANCE FOR Fe-Cu SYSTEM SAMPLE SIZE = 30

Source	df	Sum of Squares	Mean Square	F
Total	(80-1) = 79	1.6698454	0.00000000	
Cu	(10-1) = 9	0.447316	0.049701875	12.94 ** F 9,30(0.005) = 3.45
Fe	(5-1) = 4	0.8589438125	0.21473595	55.90 ** F 4,30(0.005) = 4.62
Cu x Fe	9 x 4 = 36	0.248339	0.00689831	1.796 F 36,30(0.05) = 1.81
Sample	5 x 6 = 30	0.11524575	0.00384152	

Interaction Sum of Squares

Fe x Cu(ss) = Cu in Fe(ss) – Cu(ss) = 0.24833906

Cu x Fe(ss) = Fe in Cu(ss) – Fe(ss) = 0.24833900

TABLE XVII

COMPLETE Fe-Co-TPTZ FACTORIAL SYSTEM

P.P.M. Co → P.P.M. Fe ↓	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.0436 0.0464 0.0450*	0.0551 0.0510 0.05305*	0.0605 0.0576 0.05905*	0.0661 0.0635 0.0648*	0.0726 0.0701 0.07135*	0.0553 0.0546 0.05495*
0.025	0.1153 0.1176 0.11645*	0.1240 0.1235 0.12375*	0.1318 0.1337 0.13275*	0.1347 0.1347 0.1347*	0.1366 0.1450 0.1408*	0.1032 0.1022 0.1027*
0.050	0.228 0.218 0.223*	0.234 0.223 0.2285*	0.241 0.234 0.2375*	0.243 0.244 0.2435*	0.245 0.241 0.243*	0.185 0.177 0.181*
0.075	0.343 0.332 0.3375*	0.330 0.336 0.333*	0.359 0.342 0.3505*	0.369 0.348 0.3585*	0.343 0.337 0.340*	0.249 0.223 0.236*
0.100	0.456 0.446 0.451*	0.469 0.440 0.4545*	0.456 0.450 0.453*	0.457 0.456 0.4565*	0.449 0.440 0.4445*	0.319 0.303 0.311*

TABLE XVIII

Fe-Co-TPTZ FACTORIAL INTERACTION SYSTEM

P.P.M. Co → P.P.M. Fe ↓	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.0450	0.05305	0.05905	0.0648	0.07135	0.05495
0.025	0.11645	0.12375	0.13275	0.1347	0.1408	0.1027
0.050	0.223	0.2285	0.2375	0.2435	0.243	0.181
0.075	0.3375	0.333	0.3505	0.3585	0.340	0.236
0.100	0.451	0.4545	0.453	0.4565	0.4445	0.311

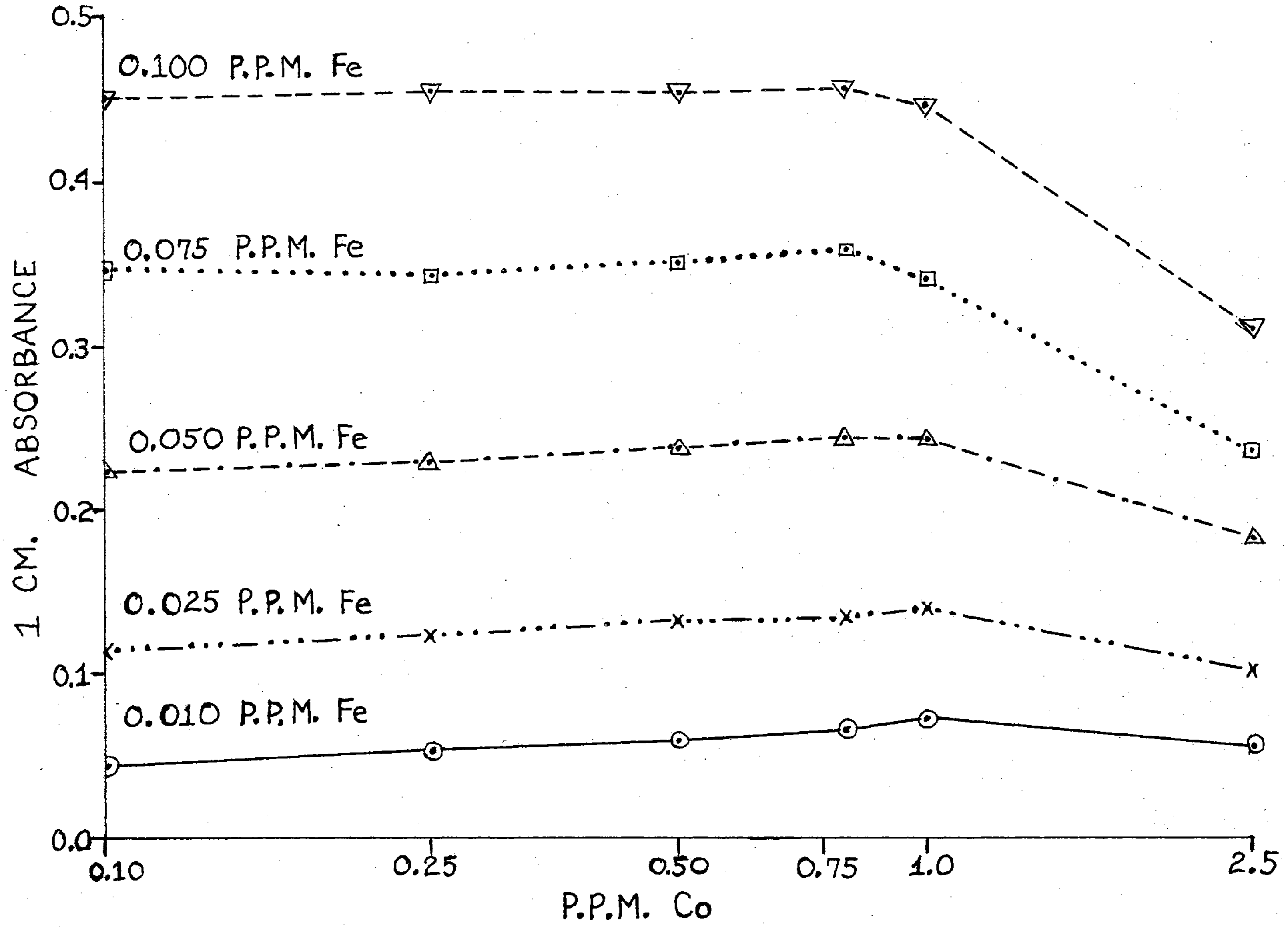

Figure 15. 1 Cm. Absorbance vs. P.P.M. Co for All Fe Levels

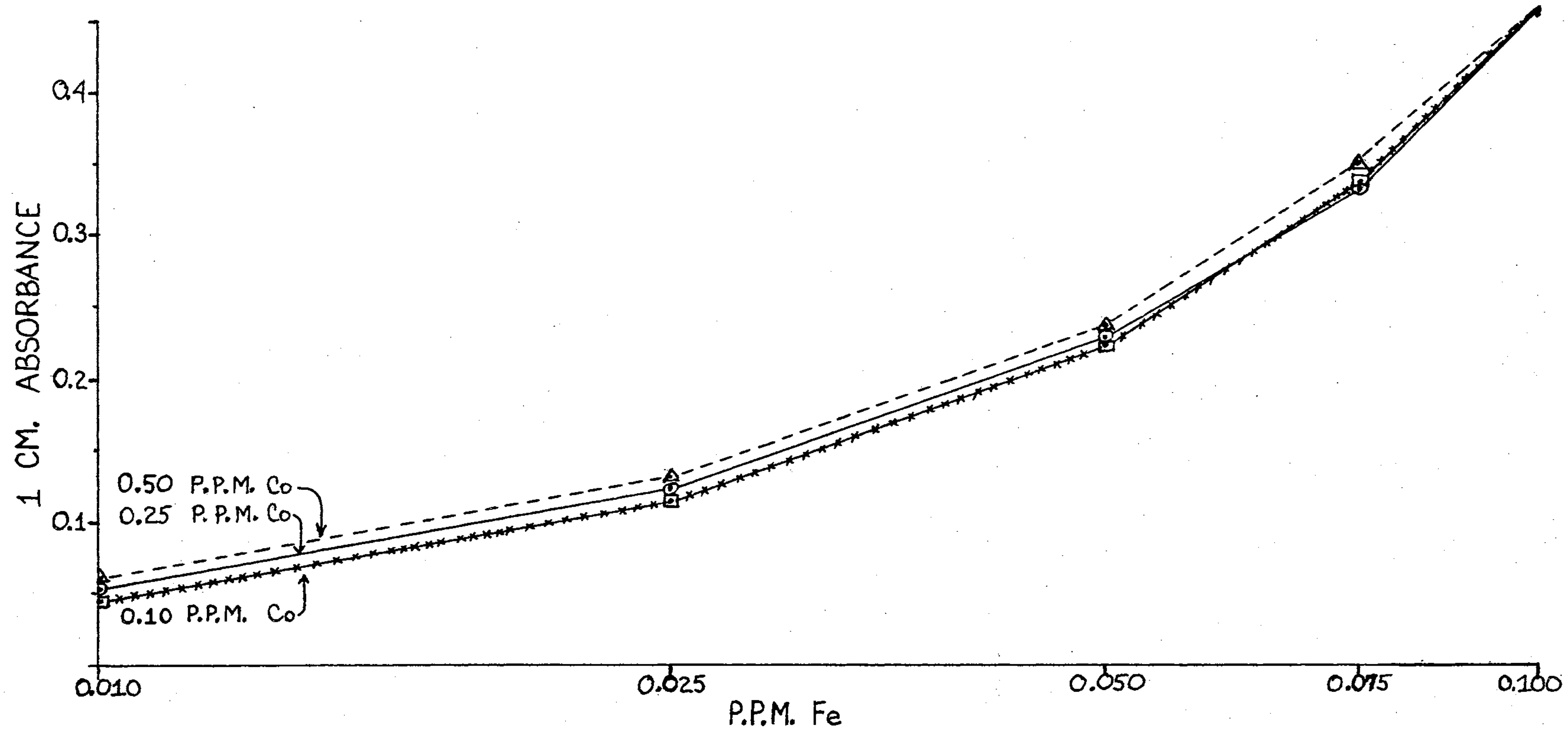

Figure 16. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.10 P.P.M., 0.25 P.P.M., and 0.50 P.P.M. Co Levels

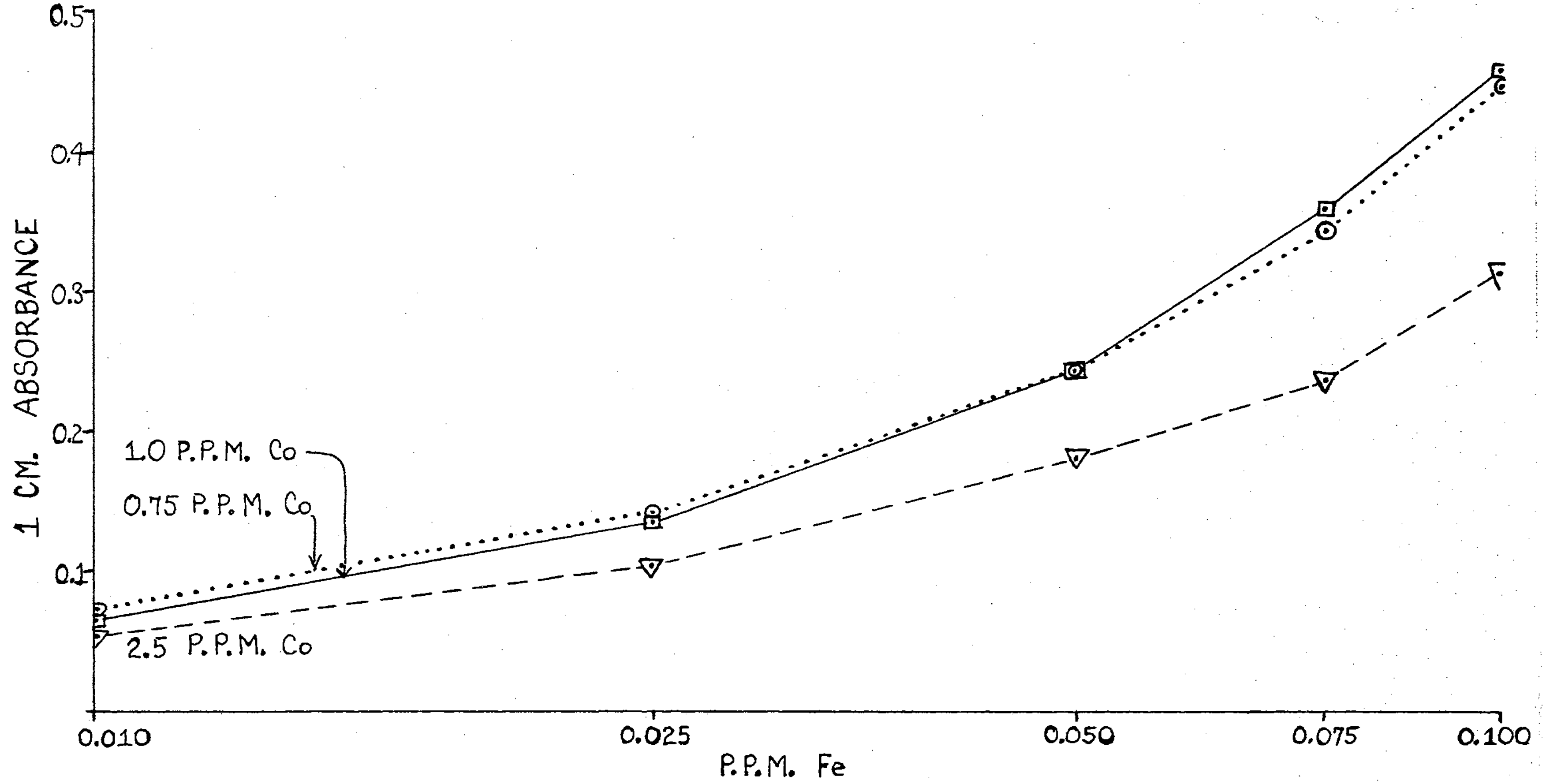

Figure 17. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.75 P.P.M., 1.0 P.P.M., and 2.5 P.P.M. Co Levels

TABLE XIX

COMPLETE ANALYSIS OF VARIANCE OF Fe-Co FACTORIAL SYSTEM

Source	df	Sum of Squares	Mean Square	F
Total	59	1.13281964063		
Replicates	1	0.00056360689	0.00056360689	14.4319496823 F 1,29(0.005) = 9.23
Fe	4	1.06925546875	0.26731386719	6844.94875619 F 4,29(0.005) = 4.66
Co	5	0.03907188086	0.00781437616	200.098127864 F 5,29(0.005) = 4.26
Fe x Co	20	0.02279615503	0.00113980774	29.1863854809 F 20,29(0.005) = 2.86
Rep. x Fe	4	0.00047057919	0.00011764480	3.01246110386 F 4,29(0.025) = 3.27 F 4,29(0.050) = 2.70
Rep. x Co	5	0.00008873815	0.00001774763	0.454453108515 F 5,29(0.10) = 2.06
Rep. x Fe x Co	20	0.00057321178	0.00002866059	0.733894847785 F 20,29(0.10) = 1.68
Error	29	0.00113252912	0.00003905272	

Error Sum of Squares = (Rep. x Fe) + (Rep. x Co) + (Rep. x Fe x Co).

TABLE XX

INTERFERENCE PERCENTAGES FOR Fe-Co-TPTZ SYSTEM

P.P.M. Co → P.P.M. Fe ↓	0.10	0.25	0.50	0.75	1.0	2.5
0.010	6.88	26.0	40.3	53.8	69.4	30.5
0.025	6.84	13.5	21.8	23.6	29.2	-5.78
0.050	1.36	3.86	7.95	10.7	10.5	-17.7
0.075	0.446	-0.892	4.32	6.69	1.19	-29.8
0.100	2.04	2.83	2.49	3.48	0.566	-29.6

TABLE XXI

COMPLETE Fe-Cr-TPTZ FACTORIAL SYSTEM UNCORRECTED FOR Cr IRON CONTENT

P.P.M. Cr → P.P.M. Fe ↓	0.25	0.50	0.75	1.0	2.5	5.0
0.010	0.0366 0.0625 0.04955*	0.0392 0.0666 0.0529*	0.0330 0.0733 0.05315*	0.0486 0.0637 0.05615*	0.0672 0.0970 0.0821*	0.0796 0.1570 0.1183*
0.025	0.108 0.107 0.1075*	0.114 0.102 0.108*	0.114 0.117 0.1155*	0.119 0.127 0.123*	0.139 0.161 0.150*	0.157 0.220 0.1885*
0.050	0.204 0.215 0.2095*	0.218 0.224 0.221*	0.217 0.220 0.2185*	0.223 0.231 0.227*	0.244 0.264 0.254*	0.259 0.327 0.293*
0.075	0.316 0.318 0.317*	0.320 0.328 0.324*	0.330 0.331 0.3305*	0.328 0.328 0.328*	0.347 0.374 0.3605*	0.373 0.430 0.4015*
0.100	0.415 0.408 0.4115	0.409 0.402 0.4055*	0.419 0.414 0.4165*	0.425 0.422 0.4235*	0.436 0.445 0.4405*	0.457 0.509 0.483*

TABLE XXII

Fe-Cr-TPTZ FACTORIAL SYSTEM UNCORRECTED FOR Cr IRON CONTENT

P.P.M. Cr → P.P.M. Fe ↓	0.25	0.50	0.75	1.0	2.5	5.0
0.010	0.04955	0.0529	0.05315	0.05615	0.0821	0.1183
0.025	0.1075	0.108	0.1155	0.123	0.150	0.1885
0.050	0.2095	0.221	0.2185	0.227	0.254	0.293
0.075	0.317	0.324	0.3305	0.328	0.3605	0.4015
0.100	0.4115	0.4055	0.4165	0.4235	0.4405	0.483

TABLE XXIII

DETERMINATION OF IRON IMPURITIES IN CHROMIUM

P.P.M. Fe Put In	P.P.M. Fe Observed	595 mu 1 Cm. Cell Absorbance	P.P.M. Cr	% Fe
0	0.004497277	0.0196	1.0	0.4497
0	0.012718772	0.0542	2.5	0.5088
0	0.026151708	0.114	5.0	0.5230
0	0.052658168	0.232	10.0	0.5266

Note: $\underline{Y} = 4.45174484X - 0.0024207317$ solved for X where $\underline{Y}$ = 1 cm. Absorbance, and X = the concentration of Fe in P.P.M..

$X = 0.224631023Y + 0.000543771439$.

The Average Fe % in Cr = 0.5020 %

TABLE XXIV

COMPLETE Fe-Cr-TPTZ FACTORIAL SYSTEM CORRECTED FOR Cr IRON CONTENT

P.P.M. Cr → P.P.M. Fe ↓	0.25	0.50	0.75	1.0	2.5	5.0
0.010	0.0316 0.0575 0.04455*	0.0292 0.0566 0.0429*	0.0181 0.0584 0.03825*	0.0287 0.0438 0.03625*	0.0174 0.0472 0.0323*	0.0100 0.0574 0.0337*
0.025	0.1030 0.1020 0.1025*	0.1040 0.0920 0.0980*	0.0991 0.1021 0.1006*	0.0991 0.1071 0.1031*	0.0892 0.1112 0.1002*	0.0574 0.1204 0.0889*
0.050	0.1990 0.2100 0.2045*	0.2080 0.2140 0.2110*	0.2021 0.2051 0.2036*	0.2031 0.2111 0.2071*	0.1942 0.2142 0.2042*	0.1594 0.2274 0.1934*
0.075	0.3110 0.3130 0.3120*	0.3100 0.3180 0.314*	0.3151 0.3161 0.3156*	0.3081 0.3081 0.3081*	0.2972 0.3242 0.3107*	0.2734 0.3304 0.3019*
0.100	0.4100 0.4030 0.4065*	0.3990 0.3920 0.3955*	0.4041 0.3991 0.4016	0.4051 0.4021 0.4036*	0.3862 0.3952 0.3907*	0.3574 0.4094 0.3834*

TABLE XXV

Fe-Cr-TPTZ FACTORIAL SYSTEM CORRECTED FOR Cr IRON CONTENT

P.P.M. Cr → P.P.M. Fe ↓	0.25	0.50	0.75	1.0	2.5	5.0
0.010	0.04455	0.0429	0.03825	0.03625	0.0323	0.0337
0.025	0.1025	0.0980	0.1006	0.1031	0.1002	0.0889
0.050	0.2045	0.2110	0.2036	0.2071	0.2042	0.1934
0.075	0.3120	0.314	0.3156	0.3081	0.3107	0.3019
0.100	0.4065	0.3955	0.4016	0.4036	0.3907	0.3834

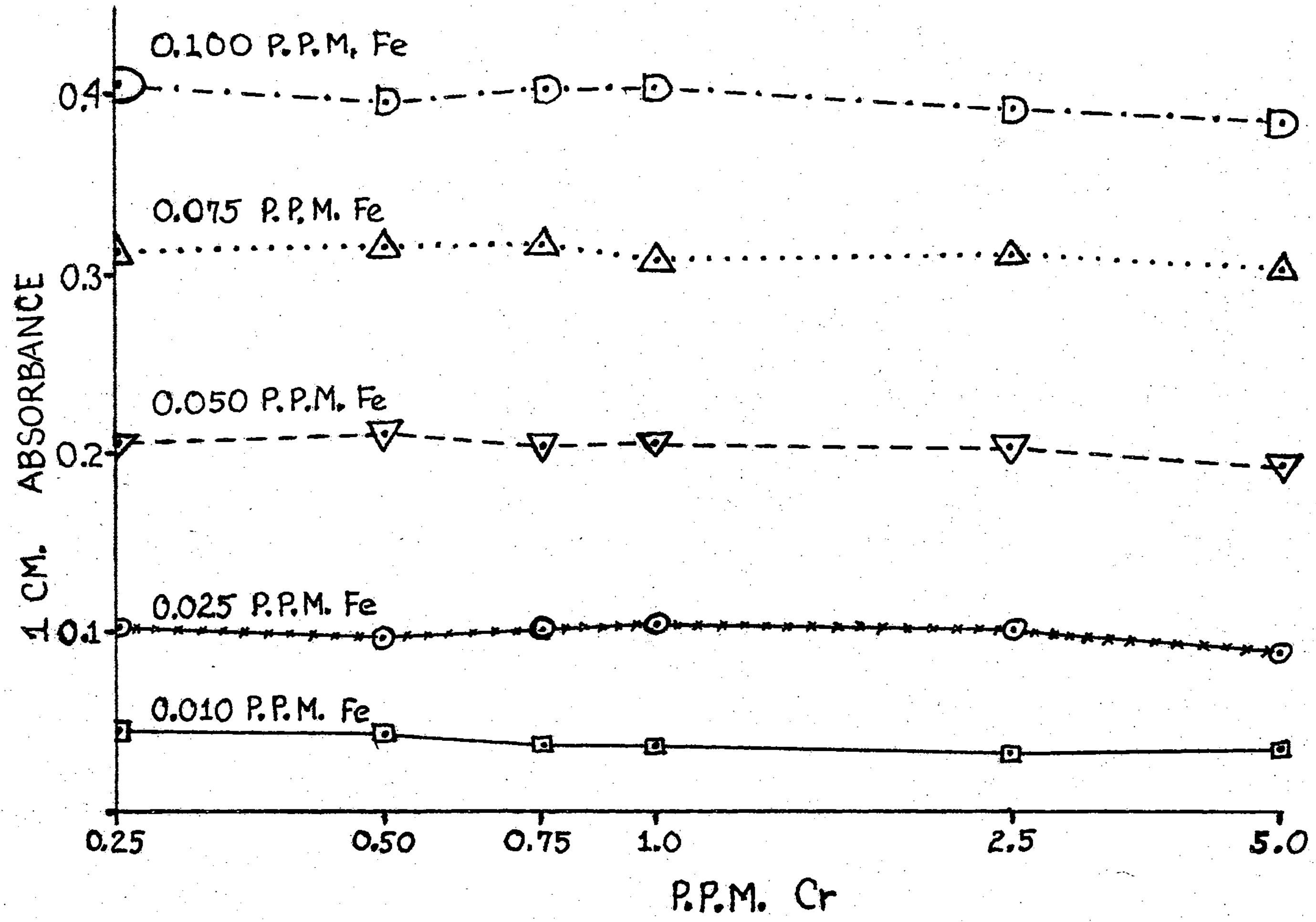

Figure 18. 1 Cm. Absorbance vs. P.P.M. Cr for All Fe Levels

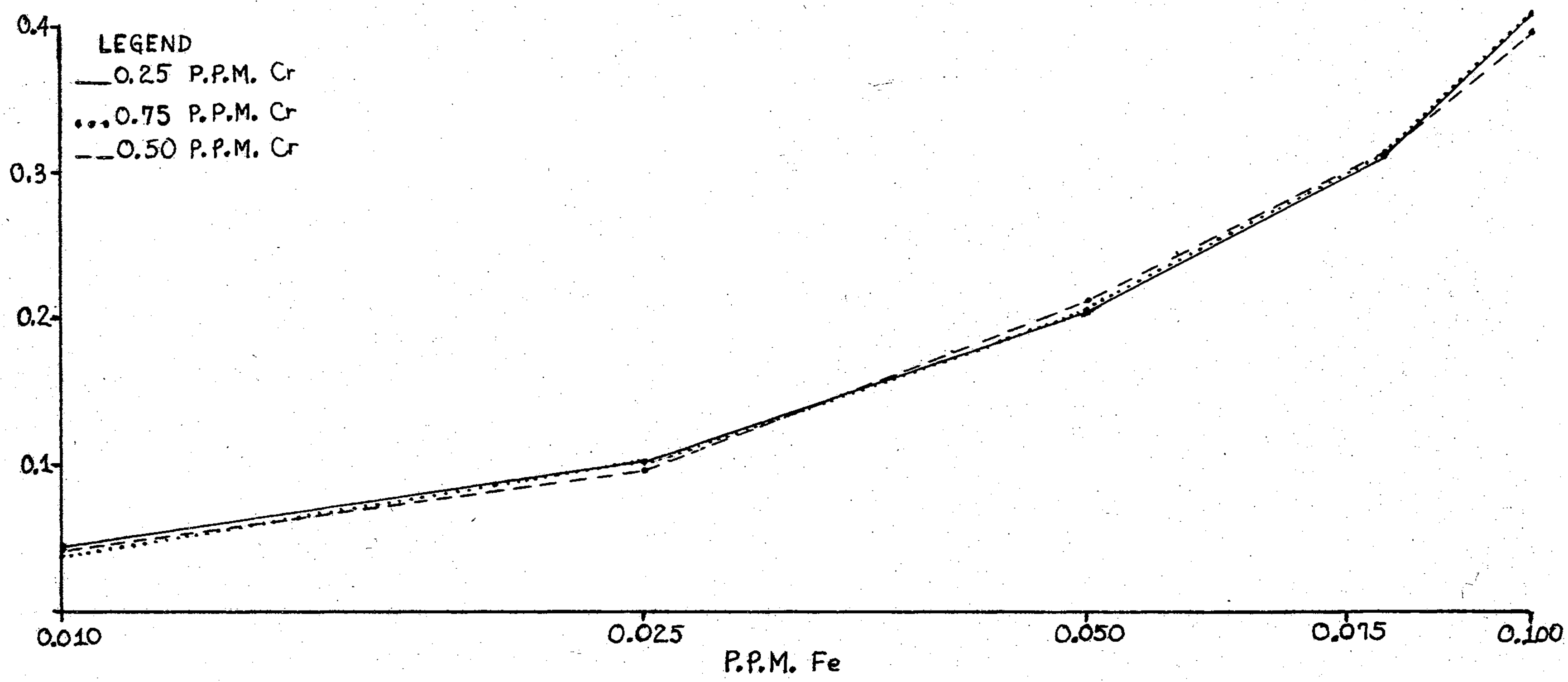

Figure 19. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.25 P.P.M., 0.50 P.P.M., and 0.75 P.P.M. Cr Levels

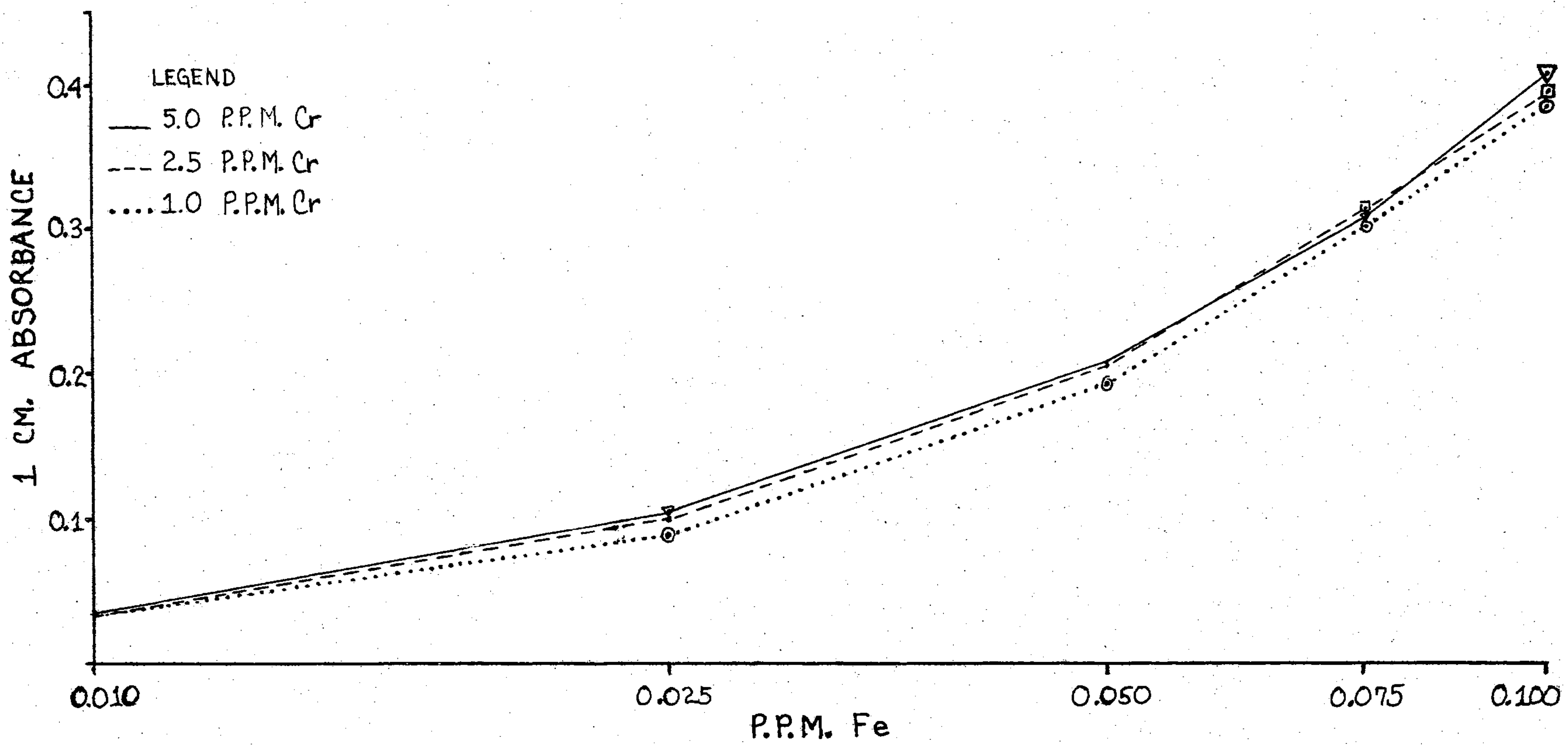

Figure 20. 1 Cm. Absorbance vs. P.P.M. Fe for the 1.0 P.P.M., 2.5 P.P.M., and 5.0 P.P.M. Cr Levels

TABLE XXVI

COMPLETE ANALYSIS OF VARIANCE OF Fe-Cr FACTORIAL SYSTEM

Source	df	Sum of Squares	Mean Square	F
Total	59	1.05716942188		
Replicates	1	0.00448740533	0.00448740533	18.1718705657 F 1,29(0.005) = 9.23
Fe	4	1.04365973438	0.26091493359	1056.36039767 F 4,29(0.005) = 4.66
Cr	5	0.00127324120	0.00025464824	1.03120500975 F 5,29(0.10) = 2.06
Fe x Cr	20	0.00058771864	0.00002938593	0.118999126921 F 20,20(0.10) = 1.68
Rep. x Fe	4	0.00096684425	0.00024171106	0.978815545649 F 4,29(0.10) = 2.15
Rep. x Cr	5	0.00533816205	0.00106763242	4.32340667297 F 5,29(0.005) = 4.26
Rep. x Fe x Cr	20	0.00085632341	0.00004281617	0.173385250973 F 20,29(0.10) = 1.68
Error	29	0.00716132971	0.00024694240	

TABLE XXVII

COMPLETE Fe-Ni-TPTZ FACTORIAL SYSTEM

P.P.M. Ni → P.P.M. Fe ↓	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.0380	0.0421	0.0337	0.0317	0.0214	0.0
	0.0396	0.0395	0.0400	0.0343	0.0153	0.0
	0.0388*	0.0408*	0.03685*	0.0330*	0.01835*	0.0*
0.025	0.101	0.0961	0.0981	0.0863	0.0413	0.0
	0.0866	0.0950	0.0900	0.0823	0.0066	0.0
	0.0938*	0.09555*	0.09405*	0.0843*	0.02395*	0.0*
0.050	0.190	0.213	0.195	0.172	0.0542	0.0
	0.204	0.197	0.182	0.132	0.0836	0.0
	0.197*	0.205*	0.1885*	0.152*	0.0689*	0.0*
0.075	0.276	0.315	0.284	0.248	0.162	0.0
	0.308	0.307	0.287	0.188	0.0741	0.0
	0.292*	0.311*	0.2855*	0.218*	0.11805*	0.0*
0.100	0.353	0.412	0.322	0.325	0.135	0.0
	0.409	0.348	0.394	0.307	0.113	0.0
	0.381*	0.380*	0.358*	0.316*	0.124*	0.0*

TABLE XXVIII

Fe-Ni-TPTZ FACTORIAL INTERACTION SYSTEM

P.P.M. Ni → P.P.M. Fe ↓	0.10	0.25	0.50	0.75	1.0	2.5
0.010	0.0388	0.0408	0.03685	0.0330	0.01835	0
0.025	0.0938	0.09555	0.09405	0.0843	0.02395	0
0.050	0.197	0.205	0.1885	0.152	0.0689	0
0.075	0.292	0.311	0.2855	0.218	0.11805	0
0.100	0.381	0.380	0.358	0.316	0.124	0

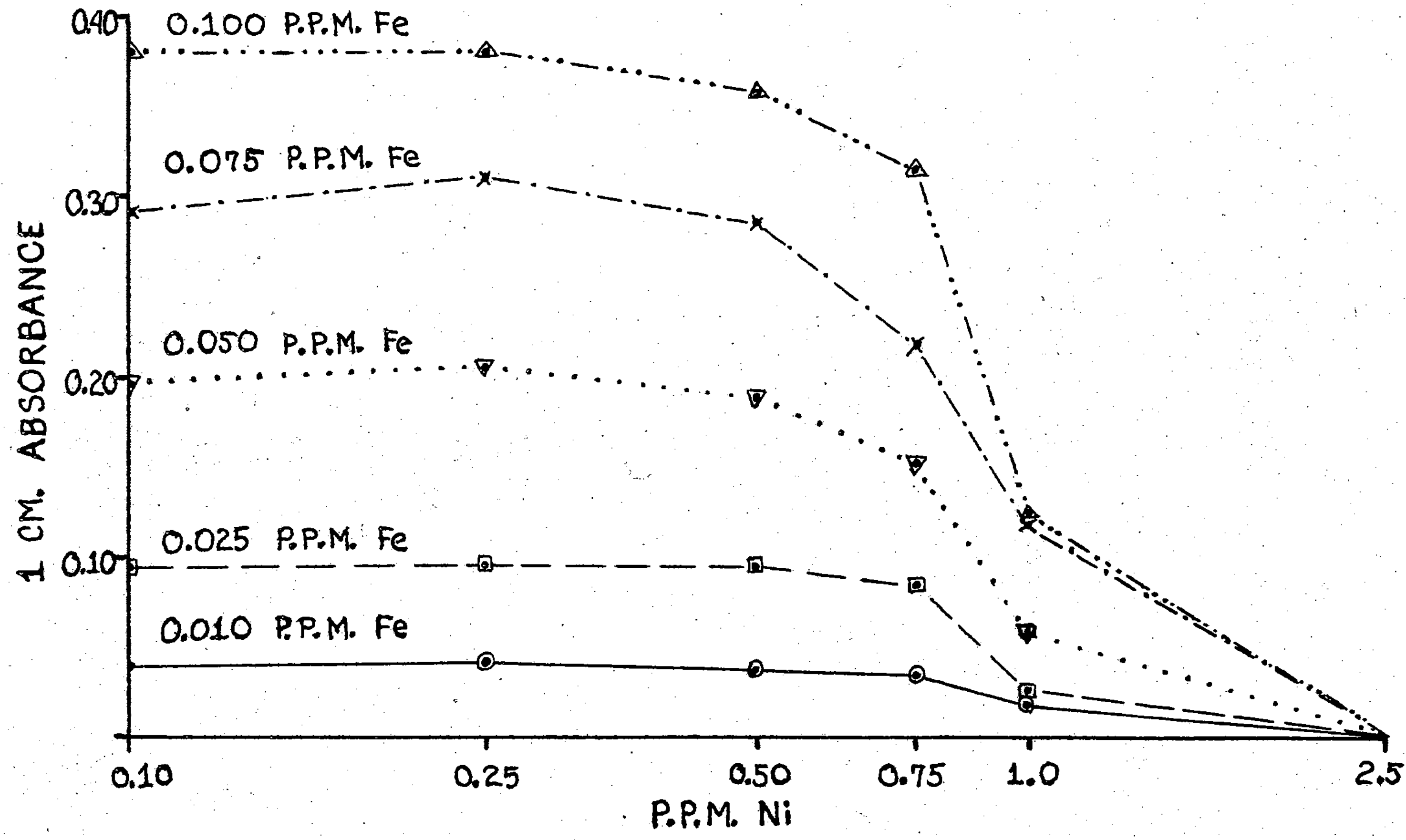

Figure 21. 1 Cm. Absorbance vs. P.P.M. Ni for All Fe Levels

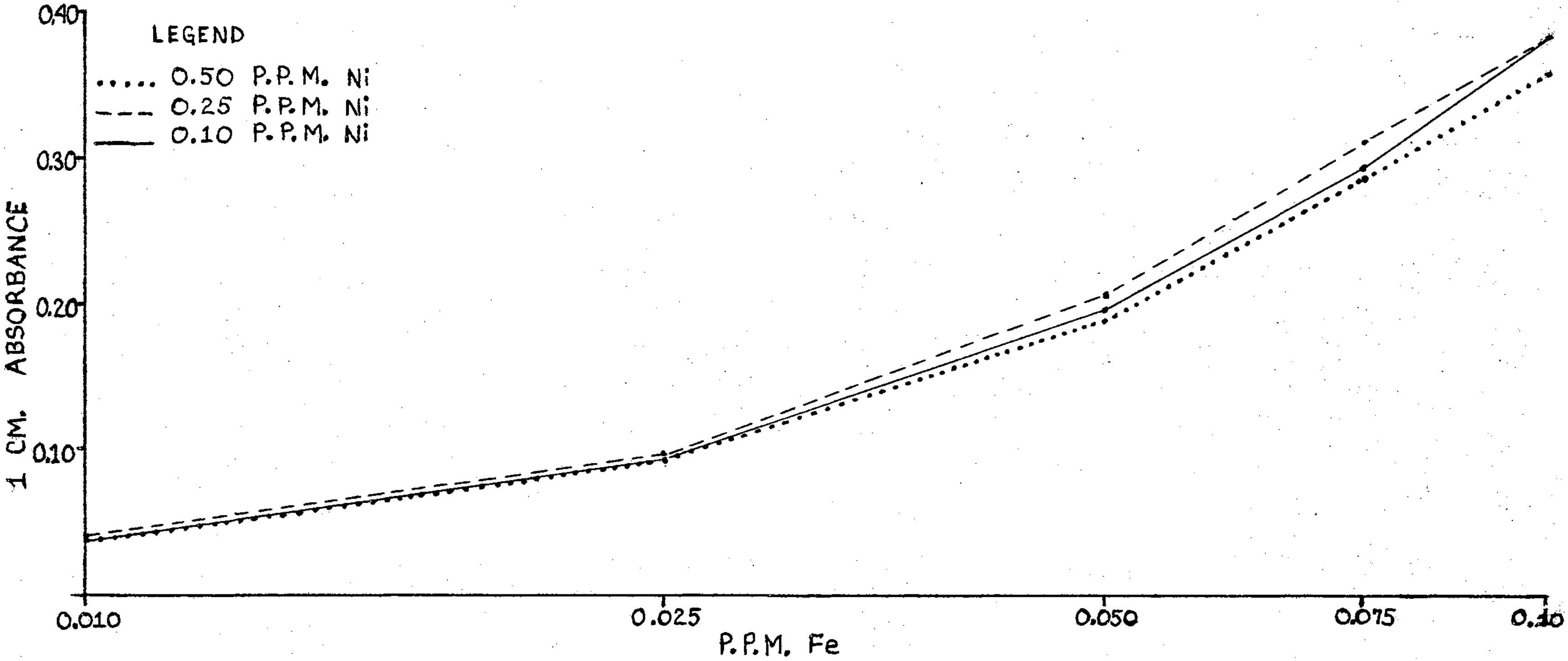

Figure 22. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.10 P.P.M., 0.25 P.P.M., and 0.50 P.P.M. Ni Levels

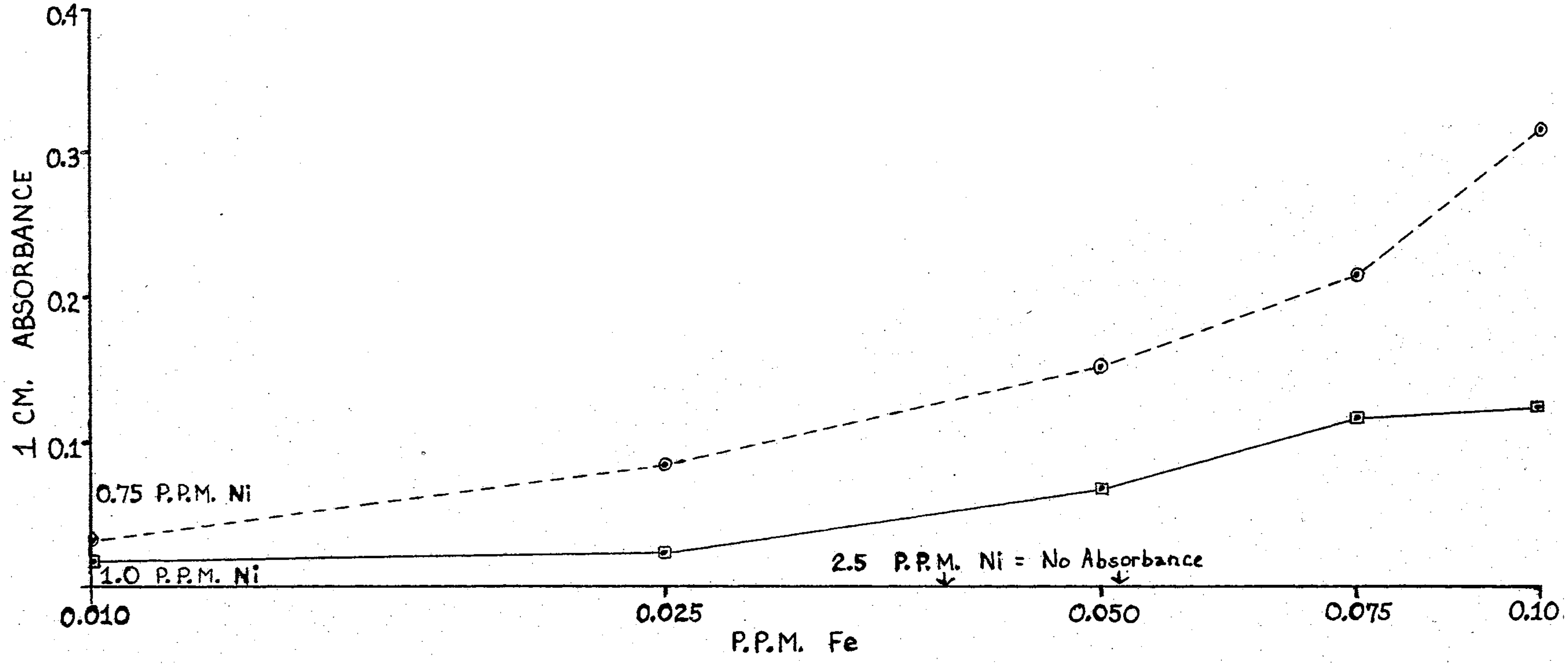

Figure 23. 1 Cm. Absorbance vs. P.P.M. Fe for the 0.75 P.P.M., 1.0 P.P.M., and 2.5 P.P.M. Ni Levels

TABLE XXIX

COMPLETE ANALYSIS OF VARIANCE OF Fe-Ni FACTORIAL SYSTEM

Source	df	Sum of Squares	Mean Square	F
Total	59	0.94963133594		
Replicates	1	0.00055812309	0.00055812309	1.10845727243 F 1,29(0.10) = 2.89
Fe	4	0.43937076953	0.10984269238	218.152470976 F 4,29(0.005) = 4.66
Ni	5	0.35667981250	0.07133596191	141.676392147 F 5,29(0.005) = 4.26
Fe x Ni	20	0.13842074023	0.00692103705	13.7454873097 F 20,29(0.005) = 2.86
Rep. x Fe	4	0.00108627753	0.00027156938	0.539348863406 F 4,29(0.10) = 2.15 F 4,29(0.050) = 2.70
Rep. x Ni	5	0.00433785333	0.0086757067	1.72303392521 F 5,29(0.050) = 2.55 F 5,29(0.10) = 2.06
Rep. x Fe x Ni	20	0.00917775684	0.00045888784	0.911371653664 F 20,29(0.05) = 1.94 F 20,29(0.10) = 1.68
Error	29	0.01460188770	0.0005035134	

TABLE XXX

COMPLETE Fe-Mn-TPTZ FACTORIAL SYSTEM UNCORRECTED FOR Mn IRON CONTENT

P.P.M. Mn → P.P.M. Fe ↓	1.0	2.5	5.0	10	25	50
0.010	0.0424 0.0423 0.04235*	0.0398 0.0567 0.04825*	0.0431 0.0557 0.0494*	0.0458 0.0513 0.04855*	0.0514 0.0549 0.05315*	0.614 0.0672 0.0643*
0.025	0.103 0.104 0.1035*	0.104 0.103 0.1035*	0.108 0.104 0.106*	0.118 0.113 0.1155*	0.123 0.121 0.122*	0.138 0.134 0.136*
0.050	0.221 0.217 0.219*	0.217 0.215 0.216*	0.217 0.218 0.2175*	0.220 0.224 0.222*	0.227 0.234 0.2305*	0.245 0.243 0.244*
0.075	0.316 0.325 0.3205*	0.316 0.331 0.3235*	0.318 0.327 0.3225*	0.316 0.326 0.321*	0.334 0.335 0.3345*	0.346 0.357 0.3515*
0.100	0.442 0.437 0.4395*	0.417 0.439 0.428*	0.439 0.439 0.439*	0.426 0.443 0.4345*	0.436 0.436 0.436*	0.448 0.456 0.452*

TABLE XXXI

Fe-Mn-TPTZ FACTORIAL SYSTEM UNCORRECTED FOR Mn IRON CONTENT

P.P.M. Mn → P.P.M. Fe ↓	1.0	2.5	5.0	10	25	50
0.010	0.04235	0.04825	0.0494	0.04855	0.05315	0.0643
0.025	0.1035	0.1035	0.106	0.1155	0.122	0.136
0.050	0.219	0.216	0.2175	0.222	0.2305	0.244
0.075	0.3205	0.3235	0.3225	0.321	0.3345	0.3515
0.100	0.4395	0.428	0.439	0.4345	0.436	0.452

TABLE XXXII

DETERMINATION OF IRON IMPURITIES PRESENT IN MANGANESE

P.P.M. Mn	595 mu 1 Cm. Absorbance	P.P.M. Fe Calculated from Regression Equation	% Fe
800	0.370	0.083657249949	0.0104571
600	0.271	0.0614187786720	0.0102365
400	0.183	0.0416512486480	0.0104128

Note: The Regression Equation is:
$X = 0.224631023\underline{Y} + 0.000543771439$

Average % Fe = 0.0104

TABLE XXXIII

COMPLETE Fe-Mn-TPTZ FACTORIAL SYSTEM CORRECTED FOR Mn IRON CONTENT

P.P.M. Mn → P.P.M. Fe ↓	1.0	2.5	5.0	10	25	50
0.010	0.0420 0.0419 0.04195*	0.0387 0.0556 0.04715*	0.0409 0.0535 0.04720*	0.0414 0.0469 0.04415*	0.0404 0.0439 0.04215*	0.0395 0.0453 0.0424*
0.025	0.1026 0.1036 0.1031*	0.1029 0.1019 0.1024*	0.1058 0.1018 0.1038*	0.1136 0.1086 0.1111*	0.1120 0.1100 0.1110*	0.1161 0.1121 0.1141*
0.050	0.2206 0.2166 0.2186*	0.2159 0.2139 0.2149*	0.2148 0.2158 0.2153*	0.2156 0.2196 0.2176*	0.2160 0.2230 0.2195*	0.2231 0.2211 0.2221*
0.075	0.3156 0.3246 0.3201*	0.3149 0.3239 0.3194*	0.3158 0.3248 0.3203*	0.3116 0.3266 0.3191*	0.3230 0.3240 0.3235*	0.3241 0.3351 0.3296*
0.100	0.4416 0.4366 0.4391*	0.4159 0.4379 0.4269*	0.4368 0.4368 0.4368*	0.4216 0.4386 0.4301*	0.4250 0.4250 0.4250*	0.4261 0.4341 0.4301*

TABLE XXXIV

Fe-Mn-TPTZ FACTORIAL SYSTEM CORRECTED FOR Mn IRON CONTENT

P.P.M. Mn → P.P.M. Fe ↓	1.0	2.5	5.0	10	25	50
0.010	0.04195	0.04715	0.04720	0.04415	0.04215	0.0424
0.025	0.1031	0.1024	0.1038	0.1111	0.1110	0.1141
0.050	0.2186	0.2149	0.2153	0.2176	0.2195	0.2221
0.075	0.3201	0.3194	0.3203	0.3191	0.3235	0.3296
0.100	0.4391	0.4269	0.4368	0.4301	0.4250	0.4301

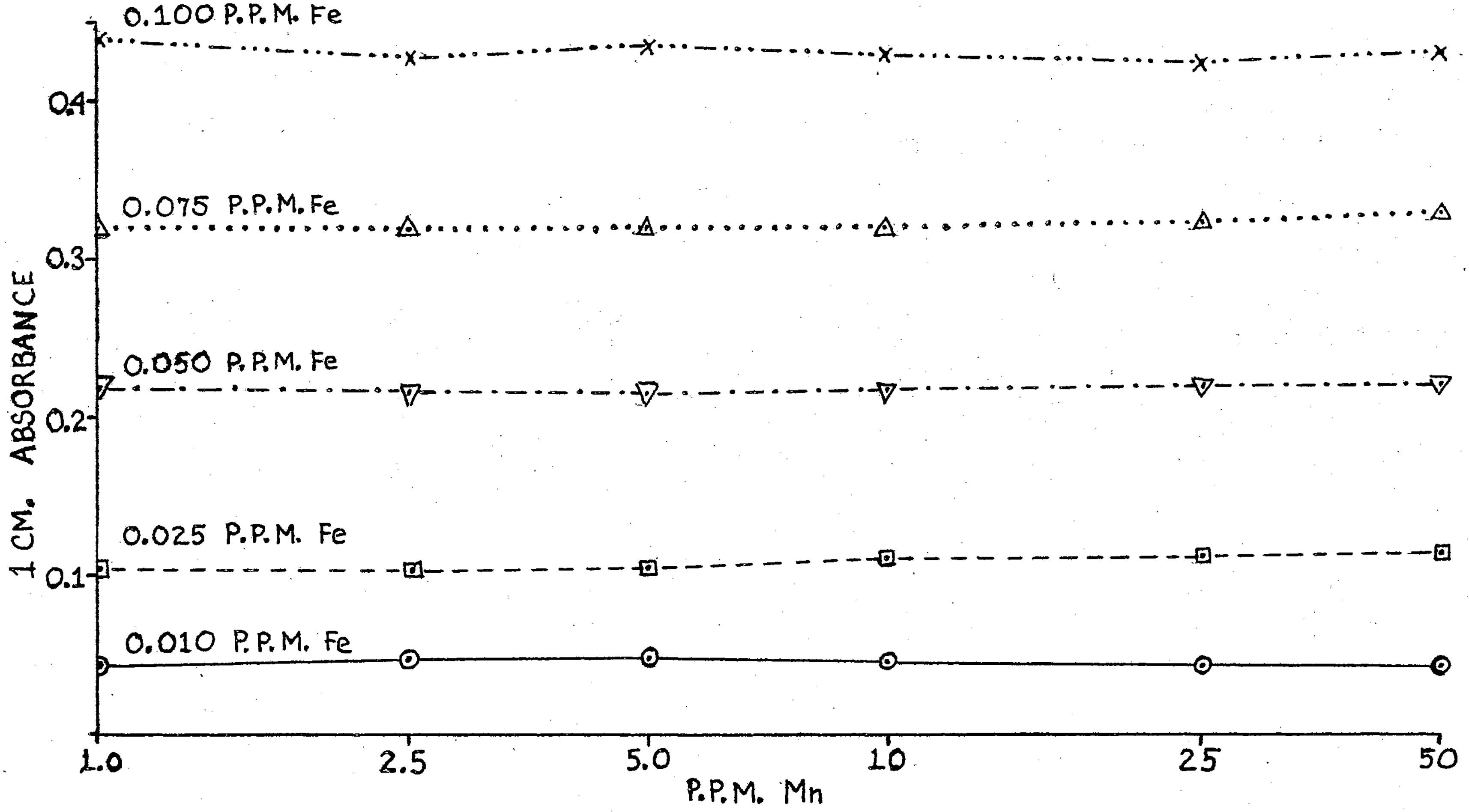

Figure 24. 1 Cm. Absorbance vs. P.P.M. Mn for All Fe Levels

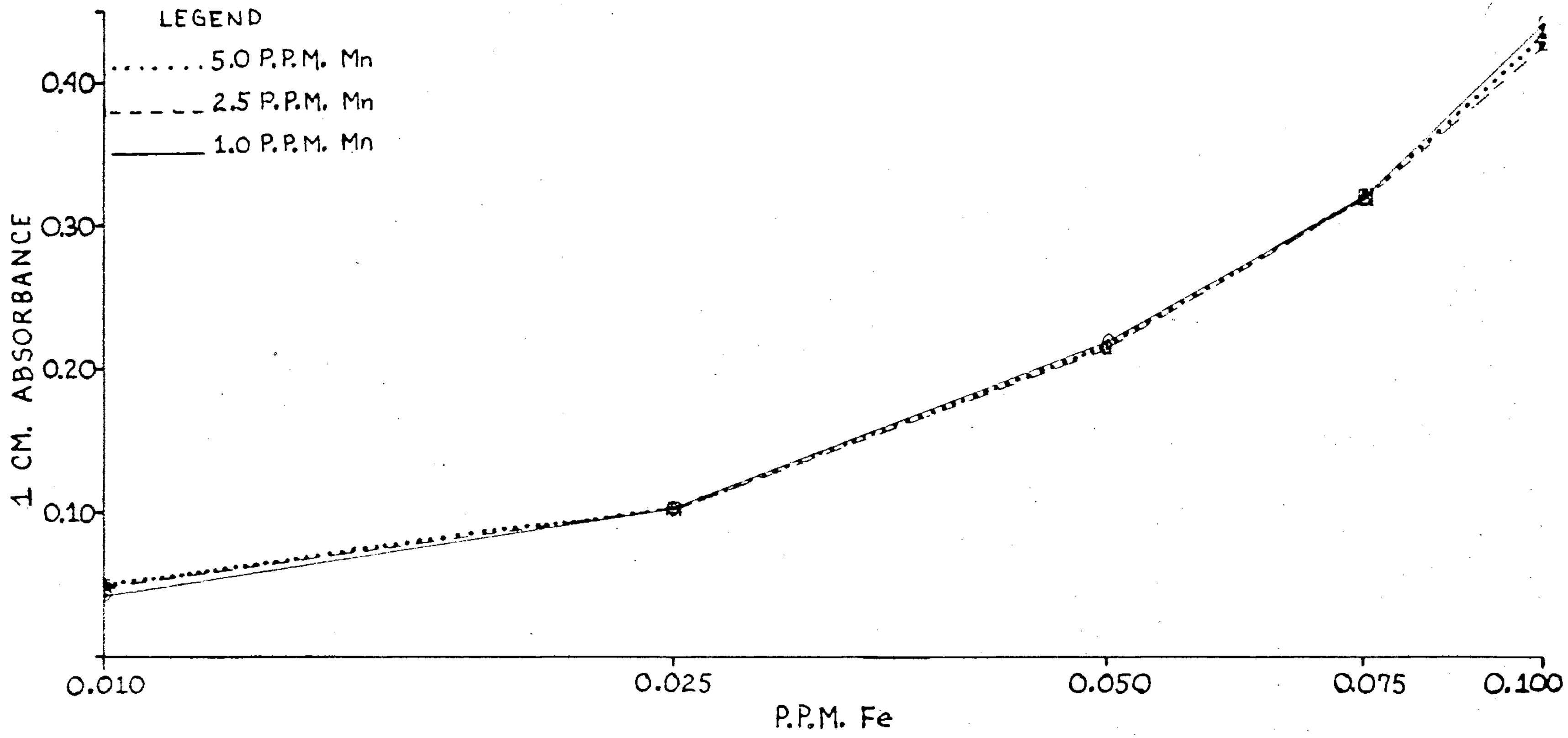

Figure 25. 1 Cm. Absorbance vs. P.P.M. Fe for the 1.0 P.P.M., 2.5 P.P.M., and 5.0 P.P.M. Mn Levels

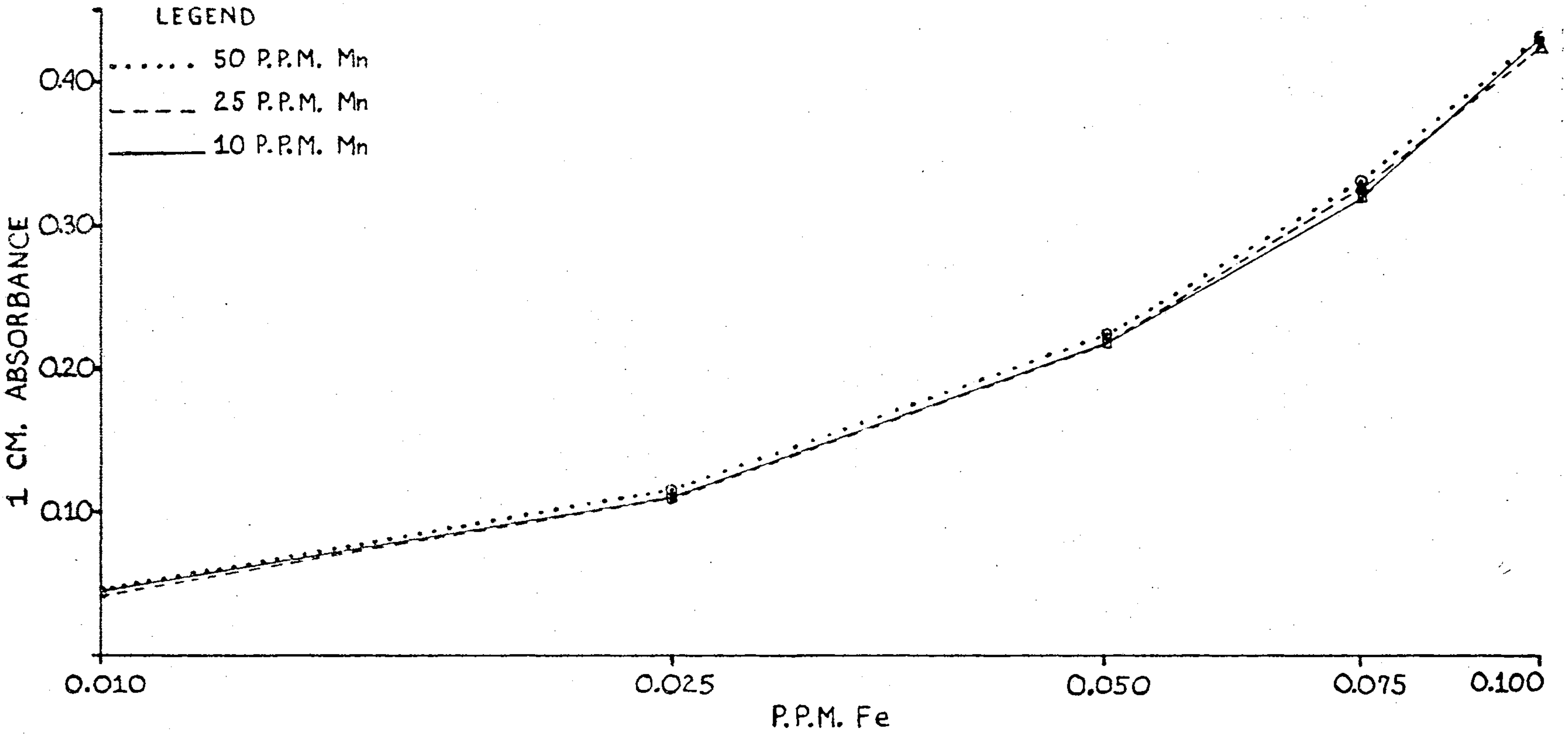

Figure 26. 1 Cm. Absorbance vs. P.P.M. Fe for the 10 P.P.M., 25 P.P.M., and 50 P.P.M. Mn Levels

TABLE XXXV

INTERFERENCE PERCENTAGES FOR Fe-Mn-TPTZ SYSTEM

P.P.M. Mn → P.P.M. Fe ↓	1.0	2.5	5.0	10	25	50
0.010	-0.357	12.0	12.1	4.86	0.119	0.712
0.025	-5.42	-6.06	-4.78	1.93	1.83	4.68
0.050	-0.636	-2.32	-2.14	-1.09	-0.227	-0.955
0.075	-4.73	-4.94	-4.67	-5.03	-3.72	-1.91
0.100	-0.657	-3.42	-1.18	-2.69	-3.84	-2.69

TABLE XXXVI

COMPLETE ANALYSIS OF VARIANCE OF Fe-Mn FACTORIAL SYSTEM

Source	df	Sum of Squares	Mean Square	F
Total	59	1.18413801563		
Replicates	1	0.00027798665	0.00027798665	10.2725364590 F 1,29(0.005) = 9.23
Fe	4	1.18221665625	0.29555416406	10,921.7148591 F 4,29(0.005) = 4.66
Mn	5	0.00015533294	0.0003106659	1.14801440441 F 5,29(0.05) = 2.55 F 5,29(0.10) = 2.06
Fe x Mn	20	0.00070325191	0.00003516260	1.29937567324 F 20,29(0.05) = 1.94 F 20.29(0.10) = 1.68
Rep. x Fe	4	0.00029502251	0.00007375563	2.72551720824 F 4,29(0.05) = 2.70
Rep. x Mn	5	0.00013596064	0.00002719213	1.00484014906 F 5,29(0.05) = 2.55
Rep. x Fe x Mn	20	0.00035379028	0.00001768951	0.653686558036 F 20,29(0.05) = 1.94
Error	29	0.00078477343	0.0002706115	

TABLE XXXVII

STANDARDIZATION OF 4×10^{-3} MOLAR TPTZ SOLUTION

Concentration of TPTZ in Moles/Liter	310 mu UV. Absorbance Referred to 330 mu (1 Cm. Cell)	Avg. 1 Cm. Absorbance	Regression Absorbance Value
4×10^{-5}	0.342	0.325	0.32654166 = 0.327
4×10^{-5}	0.350		
4×10^{-5}	0.324		
4×10^{-5}	0.286		
3×10^{-5}	0.222	0.24725	0.244916666 = 0.245
3×10^{-5}	0.250		
3×10^{-5}	0.260		
3×10^{-5}	0.257		
2×10^{-5}	0.165	0.1625	0.163291666 = 0.163
2×10^{-5}	0.163		
2×10^{-5}	0.170		
2×10^{-5}	0.152		

$\underline{Y} = 8162.5X - 0.0000416666$ describes the TPTZ system where $\underline{Y}$ = 1 cm. absorbance, and X = the concentration of TPTZ in moles/liter.

In terms Of X:

$X = 0.000122511485451\underline{Y} - 0.000000005104645$

TABLE XXXVIII

DETERMINATION OF CONCENTRATION OF UNKNOWN TPTZ SOLUTION

Size of Aliquot in ml.	310 mu UV. Absorbance Referred to 330 mu (1 Cm. Cell)	Avg. 1 Cm. Absorbance	Computed Moles/Liter Concentration By Comparison of Avg. Absorbance with Standard TPTZ Curve
20 ml.	0.207	0.204	$2.49872384 \times 10^{-5}$
20 ml.	0.221		
20 ml.	0.207		
20 ml.	0.181		
15 ml.	0.135	0.154	$1.88616641 \times 10^{-5}$
15 ml.	0.166		
15 ml.	0.157		
15 ml.	0.158		
10 ml.	0.106	0.10375	$1.27054620 \times 10^{-5}$
10 ml.	0.116		
10 ml.	0.075		
10 ml.	0.118		

Computed concentration of unknown TPTZ solution = 2.50×10^{-3} M. as determined by the linear regression equation:
$X = 0.000122511485451\underline{Y} - 0.000000005104645.$

CHAPTER VIII

DISCUSSION AND INTERPRETATION OF RESULTS

The experimental results have been presented in a concise, tabular form in the preceding Chapter. In this section, explanation of the use of these tables and the significance of all results will be given. Tables XXIII and XXXII indicate the iron found to be in the Cr and Mn, respectively. Tables XXI, XXII, XXX, and XXI give the uncorrected absorbances of these systems, and Tables XXIV, XXV, XXXIII, and XXIV show the absorbances obtained by correction for the Fecontent in the interfering metal reagents, themselves. Tables XXXVII and XXXVIII give information concerning the determination of the TPTZ solution after it was apparent that the strength or concentration had decreased.

Fe-Cu System

The F values for both the Fe and Cu main effects were much larger than that for the Fe x Cu interaction, and highly significant in both of the statistical analyses. In the analysis in which the sample variance estimate was based upon twenty out of a population of fifty, the F value for the Fe x Cu interaction was very slightly significant; the F value obtained in the second analysis, in which the sample estimate of variance was based upon thirty solution combinations, was not significant.

The results indicate that, in general, the main effects due to copper and iron are predominant, and that there is no metal-metal interaction. From a graphical standpoint, the lines in Figures 7 and 11, which illustrate the main effects due to iron, are parallel to one another. Similarly, the lines in Figures 8, 9, 10, 12, 13, and 14, which describe the main effect due to copper, are also parallel. The graphs in Figures 11, 12, 13, and 14, which are a summary of the analysis based upon a sample size of thirty, are visually satisfying, i.e., the lines have a greater appearance of parallelism. A statistical analysis based on a sample size of fifty, would have rendered a stronger verdict in favor of the hypothesis that the behavior of the system can be attributed to the main effects of Fe and Cu, alone.

Examination of Tables VI and XI show, that at high levels of Cu, the difference between successive determinations of a given solution combination tends to be large, and that the standard deviation of the mean for a given triplicate also tends to be large, regardless of the Fe level.

The highest level of Cu, 2.5 p.p.m., completely overpowered the Fe, regardless of its level; and the observed 595 mu absorbance was attributable to the Cu(I) complex. These large deviations occurred in spite of all efforts to maintain consistency in daily solution extraction operations. The Cu(I) complex of TPTZ had a black appearance in nitrobenzene-ethanol solution and was very unstable with respect to time. The complex appeared to dissociate, and no color due to it would be apparent twelve hours after preparation of a solution.

Fe-Co System

Agreement between the two replicates seems to be fairly good. Differences between the two singlet determinations of a given solution combination are much less than in the case of the Fe-Cu system. The Co(II) complex with TPTZ is orange in nitrobenzene-ethanol or water solutions and is stable over a period of several days. The absorbance at 595 mu was small and did not show any proportionality to concentration over the range covered. The maximum absorbance of this complex occurred at 531 mu, but showed no proportionality to concentration above 1.0 p.p.m. in aqueous solution.

At 2.5 p.p.m., the Co(II) complex overwhelmed the Fe, regardless of its level. The Fe-Co system exhibited a spectacular variety of colors. Purple, violet, red, maroon, blue, green, yellow, gray, orange, and a mixture of these colors were observed. If 595 mu is used as a reference for zero absorbance, then the 1 cm. 531 mu absorbance for 10 p.p.m. of the complex in nitrobenzene is 0.0674; for 7.5 p.p.m. it is 0.0516. The proportionality is evident. The molar absorptivity of the complex is about 300.

Although the F values for main effects due to Fe, main effects due to Co, and to Fe x Co interaction are all significant at the 0.005 confidence level, the values for the main effects due to Co and Fe are so much more significant than that for Fe x Co interaction, that it can definitely be concluded that the behavior of the Fe-Co interference system can be attributed principally to main effects of Fe and secondarily to main effects of Co. Figures 15, 16, and 17 lend strong support to this interpretation.

Fe-Cr System

The agreement of the two replicates was close, except for the lowest Fe level. Cr(III) does not form a complex with TPTZ. The Cr metal powder, which was used had Fe impurities to the extent of 0.502% as determined via the TPTZ method. The initial absorbance readings were corrected for iron content of the chromium used, before statistical analysis. The character of this system is attributable to the main effects of Fe alone. Since the hypotheses for main effects of Cr and Fe x Cr interaction affecting the system are negative, according to the F values, it is rather unlikely that even main effects of Cr influence the system. Although the concentration of the TPTZ solution was of a lower level, than originally, Figures 18, 19, and 20 support the contention that Cr did not cinstitute an interference within the Cr/Fe ratios studied.

Fe-Ni System

The concentration of the TPTZ solution used for this system was lower than the original value of 3.75×10^{-3} M. The higher Fe levels saturated the system, as evidenced by the manner in which the absorbance readings increased with the higher level of Fe used. The percentage difference between the observed absorbance values and the values expected for iron without any interference, increased with the iron level, regardless of the Ni level, but the experimental values agreed with the periodic checks made with the standard iron solutions.

The F values obtained for main effects due to iron, main effects due to Ni, and Fe-Ni interaction are all significant at the 0.005 level of confidence, as shown by Table XXIX. The first two are of so much greater significance, that it is evident that this system is characterized by the main effects due to nickel and iron under the particular experimental conditions. Examination of Figure 21 indicates that Ni was a negligible source of interference below 0.50 p.p.m.

Aqueous solutions developed a yellowish color when 2.5 p.p.m. of Ni was present, regardless of the Fe level. Comparison of the visible spectrum of these solutions with those of aqueous solutions containing only TPTZ indicated that the yellowish color might be caused by the free and uncomplexed TPTZ. Both types of spectra showed only a small peak at 436 mu. If Ni did not form a complex with TPTZ as reported by Collins, Diehl, and Smith (4), but retarded development of the Fe(II) complex, instead, then a high level of Ni could completely inhibit its formation, and either the protonated or unprotonated form would be yellow in aqueous solution. Unfortunately, an insufficient amount of investigation of this point was completed. This topic will be developed later.

Fe-Mn System

Tables XXXIII, XXXIV, and XXXVI, and Figures 24, 25, and 26 illustrate the character of the Fe-Mn system. There is no Fe-Mn interaction, and the main effects attributable to Mn are insignificant. The main effects due to Fe are the only consideration of importance. In brief, Mn had no influence upon this system over the range of Mn to Fe ratios evaluated. As in the case of the Fe-Cr system, corrections were applied for the Fe found to be present in the reagent grade Mn, which was used in the synthetic solutions. A fresh solution of 4.00 x 10^{-3} M. TPTZ was employed.

Standardization of TPTZ Solutions

The possibility of the concentration of the TPTZ solutions changing, was not investigated until after Cu, Co, Cr, and Ni had been investigated as interferences. The first standard TPTZ solution was exhausted in four months and applied exclusively to the Fe-Cu system. There were no apparent difficulties indicated by the standard checks, which were made periodically, with Fe solutions.

The second TPTZ solution was used to complete the Fe-Cu study as well as the examination of the Fe-Co, Fe-Cr, and Fe-Ni interference systems. This solution was used for a period of about three and one-half months, and the absorbance readings obtained for the Fe-Cu and Fe-Co systems were satisfactory. After the work for the Fe-Cr and Fe-Ni systems had been completed, it was evident that the concentration of the second TPTZ solution had diminished.

An examination of the ultraviolet spectrum of a third TPTZ solution of 4.00×10^{-3} $\underline{M}$ concentration, which had been freshly prepared, showed that there was a peak at 305 mu. Unfortunately, the servo slit on the Cary 14 Spectrophotometer was at a maximum, so that a proportionality relationship to concentration was not observed at the peak maximum.

This relationship was evident, when 310 mu absorbance readings were referred to 330 mu. Since, the aperature of the servo slit was rapidly changing in this range of the spectrum, the peak was obtained in the form of an increasing stair-step function, and a smooth curve had to be drawn in order to obtain an interpretable function. Linear regression procedure was applied to the data, and a close-fitting linear relation was found between concentration and absorbance.

The TPTZ solution in which the concentration had decreased, was discovered to have an identical ultraviolet spectrum. Accordingly, the inverse of the linear regression equation obtained for the 4.00 x 10^{-3} M standard TPTZ solution was applied to the unknown. The unknown was determined to be 2.50 x 10^{-3} M in TPTZ. Thus its concentration had diminished by 33.3% from the original value of 3.75 x 10^{-3} M.

CHAPTER IX

SUMMARY OF RESULTS AND INTERPRETATIONS

Copper(I) forms a very unstable complex with TPTZ, which absorbs in the same region of the visible spectrum that the ferrous TPTZ chelate absorbs. Copper presents an interference of more than two per cent, in general, if the Cu to Fe ratio is greater than four to one. When the Cu is present in a very large excess, the observed absorbance is attributable to the Cu complex with TPTZ, alone. The amount of interference seen at higher Fe levels would have been smaller, if a higher TPTZ concentration had been used. The Fe-Cu system exhibited no evidence of metal-metal interaction. Its behavior was characterized by main effects due to Cu and Fe.

Cobalt(II) forms a stable xomplex with TPTZ, which has an intense orange color. This complex has some absorbance at 595 mu, but no proportionality to concentration was evident. Cobalt does not appear to be as strong as interference as Cu. A Fe to Co ratio, which is greater than five to one, will result in an interference of at least two per cent. Only main effects due to Co and Fe influence this system.

Since, there was uncertainty as to the concentration of the TPTZ reagent solution when Cr was being studied as an interference it is more difficult to evaluate the Fe-Cr system. Within the range of Cr to Fe ratios which were studied, Cr does not offer much interference. The system is influenced solely by the main effects of Fe.

The concentration of the TPTZ reagent solution used for the Fe-Ni system was found to be 2.50 x 10^{-3} M., after the work had been completed. Since the maximum absorbances were much lower than they would have been if 3.75 x 10^{-3} M. TPTZ solution had been used, interpretation of this system is not as direct as one might wish. The graphical and statistical analyses indicate that the main effects due to Fe and Ni, and possibly a small metal-metal interaction, determine the behavior characteristics of the Fe-Ni system. Nickel appears to have approximately the same degree of influence upon the formation of the ferrous TPTZ complex as Cr.

The question as to the nature of the Ni(II) complex with TPTZ can be resolved by doing two things, which time did not permit in this study. A comparison of the ultraviolet spectra of TPTZ and Ni aqueous solutions should be made. Also, the ultraviolet spectra of aqueous iron-free Cr and Mn solutions should be completed in order to determine if the appearance and behavior of TPTZ in solutions having a large molar excess ratio of Cr or Mn to TPTZ is similar to that observed in the Ni solutions.

The graphical and statistical analyses, as well as the interference percentages table indicate that Mn(II) was not a source of interference within the range of Mn to Fe ratios employed. The maximum Mn to Fe ratio of the system was 5,000.

CHAPTER X

APPLICATION OF THE TPTZ METHOD TO ANALYSES FOR IRON

The principal interest of this study was to determine the feasibility of this TPTZ method for the determination of iron in natural waters. None of the most abundant cationic or anionic species, which are in sea water offer direct interference which is very troublesome.

It is true that some of these species such as phosphate tend to form rather insoluble complexes with iron. The influence of bicarbonate, carbonate, and phosphate can be minimized by the wet-ashing procedure, which introduces a relatively large amount of anionic species such as NO_3^- and ClO_4^-, which form soluble iron species. Fluoride tends to complex iron, also. Again, the wet-ashing procedure remedies this, by volatilizing the fluoride.

None of the metal interferences studied here would be likely to interfere, since Fe usually occurs in large excess to them (8). Mn would never interfere, simply because it is never present in sufficiently large excess. According to Morgan and Stumm (12), the average Fe:Mn ratio is fifty to one. In lake water this ratio tends to be lower, but the amount of Mn present is still much lower than that of Fe.

The results of the Fe-Cr and Fe-Mn interference systems suggest that the TPTZ method should be readily applicable to the determination of iron impurities in Cr and Mn ores. There is no evident barrier to adaptation of this method for the determination of iron in plutonium

(13), hydrocarbon materials (17), or the like.

CHAPTER XI

SUGGESTIONS FOR FUTURE WORK

One result of this study has been the acquisition of experience in experimental design. Optimum experimental design will yield a maximum amount of information and the best estimate of error, which is possible for a given amount of work. Several complete replications with singlet sample size fulfills these requirements. Studies of this type, should have a minimum of four complete replications, in order to obtain reliable averages for the various solution combinations.

The synthesis of TPTZ involves the trimerization of 2-cyanopyridine, which is catalyzed by NaH. Case and Koft (3) found that the synthesis of 2,4,6-tris(4-phenyl-2-pyridyl)-1,3,5-triazine required less drastic conditions than that of TPTZ. The synthesis was accomplished via trimerization of 4-phenyl-2-cyanopyridine catalyzed by NaH. Trimerization of 4-nitro-2-cyanopyridine should not be difficult either. 2,4,6-Tris(4-nitro-2-pyridyl)-1,3,5-triazine would be expected to be fairly soluble in basic aqueous solution media. Reduction of the nitro groups would result in 2,4,6-tris(4-amino-2-pyridyl)-1,3,5-triazine (TAPTZ).

TAPTZ would be expected to have a better solubility in acidic solution media than TPTZ. Extraction characteristics can not be predicted with certainty, but the compound would surely form some type of chelate with Fe(II), in view of all the known compounds which are closely related to TPTZ, that do. The acid-base reactions of the ligand would undoubtedly affect the extraction characteristics of the chelate. If the optimum pH for chelate formation is acidic, then TPTZ could be very useful as a reagent for iron.

BIBLIOGRAPHY

1. Buchanan, E. B. Jr., Crichton, D., and Bacon, J. R. "Study of the Factors Which Affect the Reaction of the Ferrous Ion with Tripyridyltriazine." Talanta, XIII (Dec. 1966), 903-909.

2. Case, Francis H. "The Preparation of Hydrazidines and as-Triazines Related to Substituted 2-Cyanopyridines." Journal of Organic Chemistry, XXX (March, 1965), 931-933.

3. Case, Francis H., and Koft, Emil. "The Synthesis of Certain Substituted 1,3,5-Triazines Containing the Ferroin Group." Journal of the American Chemical Society, LXXXI (February, 1959), 905-906.

4. Collins, Peter F., Diehl, Harvey, and Smith, G. Frederick. "2,4,6-Tripyridyl-s-triazine as a Reagent for Iron." Analytical Chemistry, XXXI (November, 1959), 1862-1867.

5. Collins, Peter F., and Diehl, Harvey. "Tripyridyltriazine, a Reagent for the Determination of Iron in Sea Water." Journal of Marine Research, XVIII (1960), 152-156.

6. Diehl, Harvey, Buchanan, Edward B., and Smith, G. Frederick. "Iron (II) Derivatives of Pyridyl-substituted s-Triazines." Analytical Chemistry, XXXII (August 1960), 1117-1119.

7. Diehl, Harvey, Smith, G. Frederick, McBride, Loren, and Cryberg, Richard. The Iron Reagents: Bathophenanthroline, Bathophenanthrolinedisulfonic Acid, 2,4,6-Tripyridyl-s-triazine, Phenyl-2-pyridyl Ketoxime, 2nd ed. Columbus: G. Frederick Smith Chemical Company, 1965.

8. Fairbridge, Rhodes W., ed. The Encyclopedia of Oceanography, New York: Reinhold Publishing Corporation, 1966.

9. Fischer, David S., and Price, David C. "A Simple Serum Iron Method Using the New Sensitive Chromogen Tripyridyl-s-triazine." Clinical Chemistry, X (1964), 21-30.

10. Kratochvil, Byron, and White, Martha Clasby. "Spectrophotometric Determination of Microgram Quantities of (Ethylenedinitrilo) tetraacetic Acid with Bis(2,4,6-tripyridyl-s-triazine)iron (II)." Analytical Chemistry, XXXVII (January, 1965), 111-113.

11. Lewis, George J. Jr., and Goldberg, Edward D. "Iron in Marine Waters." Journal of Marine Research, XIII (1954), 183-197.

BIBLIOGRAPHY (Continued)

12. Morgan, J. J., and Stumm, Werner. "The Role of Multivalent Metal Oxides in Limnological Transformations, as Exemplified by Iron and Manganese." The Proceedings of the Second International Water Pollution Research Conference, Tokyo, 1964, New Your: Pergamon Press, 1965.

13. Plock, C. E., and Caldwell, C. E. "Colorimetric Determination of Iron in Plutonium Metal Using Nitrobenzene Extraction Technique." Analytical Chemistry, XXXIX (October, 1967), 1472-1473.

14. Robertson, David E. "Role Contamination in Trace Element Analysis of Sea Water." Analytical Chemistry, XL (June, 1968), 1067-1072.

15. Schilt, Alfred A., and Hoyle, William C. "Improved Sensitivity and Selectivity in the Spectrophotometric Determination of Iron by Use of a New Ferroin-Type Reagent." Analytical Chemistry, XXXIX (January, 1967), 114-117.

16. Schilt, A. A., and Smith, Frederick, G. "2,6-Bis(4-phenyl-2-pyridyl)-4-phenyl-, and 2,6-Bis(6-phenyl-2-pyridyl)-4-phenylpyridine. Their Spectrophotometric Constants in Reactions of Chelation with Fe(II), Cu(I), and Co(II). Analytical Chimica Acta, XV (1956), 567-572.

17. Short, Frank R., Eyster, H. Clyde, and Schribner, William G. "Spectrophotometric Determination of Parts-per-Billion Iron in High-Temperature Hydrocarbon Jet Fuels." Analytical Chemistry, XXXIX (February, 1967), 251-253.

18. Steel, Robert G. D., and Torrie, James H. Principles and Procedures of Statistics. New York: McGraw-Hill, 1960.

19. Stephens, B. G., and Suddeth, H. A. "10-Phenanthroline, and 2,4,6-Tripyridyl-sym-triazine Complexes of Iron(II) into Propylene Carbonate." Analytical Chemistry, XXXIX (October, 1967), 1478-1480.

20. Tsen, C. C. "An Improved Spectrophotometric Method for the Determination of Tocopherols Using 4,7-Diphenyl -1,10-phenanthroline." Analytical Chemistry, XXXIII (June, 1961), 849-851.

APPENDIX A

AN EXPLANATION OF SOME OF THE STATISTICAL METHODS AND TERMINOLOGY USED IN THIS PAPER

An attempt will be made to define some of the terms, which have been most frequently used throughout the body of this paper. First, several designations requisite to these definitions will be explained. A deviate is the difference between a mean value of a population of results or observations and a given observation of this population. The variance or mean square of a finite population is the sum of the squres of the deviates divided by the degrees of freedom (one less than the number of deviates, i.e.,

$$s^2 = \sum_i (X_i - \bar{x})^2/(n - 1) = \left[\sum X_i^2 - (\sum X_i)^2/n\right]/(n - 1),$$

where X_i is the ith observation, $\bar{\bar{x}}$ is the mean value of the population, and n is the total number of observations within the population.

The variance of an infinite population would be the actual variance for a given experiment and is denoted by σ^2. Thus, s^2 is an estimate of σ^2. The square root of the variance is known as the standard deviation of a single measurement or value from the given population. The standard deviation of the mean, itself, is obtained by dividing the variance by the square root of the total number of observations. This is often called the standard error of a mean. The designation F refers to an estimate of σ^2 from the means divided by an estimate of σ^2 from the individuals. More generally, F is defined as the ratio of two independent estimates of the same variance.

The term replicate refers to the number of times a specific operation or treatment is repeated. If a sample, which is 2.5 p.p.m. in Co, 0.10 p.p.m. in Fe, and 3.75×10^{-5} M. in TPTZ, is prepared and the 595 mu. absorbance is measured, and then the entire operation is repeated, then two replicates of this solution combination have been completed.

The term treatment sum of squares refers to the sum of squares attributable to a given operation, eg Fe sum of squares. Treatment SS $= \sum X_i^2/r - (X_{ij})^2/rt$, where X_i is the ith treatment total over all replicates, X_{ij} is the ith treatment observation for the jth replicate, r is the number of replicates, and t is the number of treatments. The second term is called the correction factor, C. The total sum of squares is the sum of the squares of all the individual observations, i.e., Total SS $= \sum X_{ij}^2 - C$.

The error sum of squares (residual sum of squares) is obtained by subtracting all the different treatment sums of squares from the corrected total sum of squares and is often referred to as a generalized error term, since it is an average of the components contributed by the several populations or treatments. Thus, it is an estimate of a common , the variation among observations being treated alike. Only if the assumption of a common σ^2 is true, is s^2 a valid estimate of σ^2.

A description of the F test and its applications is of importance. This is a method for testing hypotheses of differences existing between treatments, eg. Fe levels in an experiment. The term factor refers to a group of treatments. For example, if concentration of Fe is a factor in an experiment, then ten different Fe concentration levels may be used. Thus, the F test may be used to detect differences between levels within a factor. The ratio of the mean square of Fe to the error mean square expresses the Fe mean square as a multiple of the error mean square.

As an example in the application of the F test, Table XVI will be considered. The F value for testing the hypothesis of differences existing among Fe levels is 55.90. This is expressed as $F_{4,30} = 55.90$ since there are four degrees of freedom associated with Fe and the error mean square has thirty degrees of freedom. The experimental F value is compared with the tabulated value at a given confidence level. The 0.005 confidence level was chosen. This means that there is only one chance in two hundred of the experimental value being larger than the tabulated one. Tabulated $F_{4,30(0.005)} = 4.62$, from Table A.6 in Steel and Torrie (8).

The experimental F value is said to be very highly significant and the hypothesis that differences exist between the Fe levels is accepted. Therefore, there are main effects due to iron, that is iron concentration affects the absorbances of the Fe-Cu system. In the same table, experimental $F_{36,30} = 1.80$ for the hypothesis that there is Fe x Cu interaction. Tabulated $F_{36,30(0.05)} = 1.81$ and experimental F is not significant at the 0.05 confidence level. Thus, it is improbable that any metal-metal interaction is occurring.

Available time and materials did not permit equal replication of all solution combinations within the Fe-Cu system. The Oklahoma State University Computer Center Library did not have a program for factorial systems with unequal replication. The Fe-Cu system was statistically treated as a hierarchial (nested) classification. This means that the system could be considered on the basis of the variation Fe within Cu and on the inverse basis of the variation of Cu within Fe. The cell (solution combination) positions, where two replicates were completed were chosen in a symmetrical manner, as can be verified by examination of Tables VI and Xi. The sample (error) variance was estimated by these cells. <u>Linear regression</u> is a method of fitting a straight line to data consisting of more than two pairs of values. It is also referred to as the least squares method. The sum of the squares of the deviations from the straight-line moving average. The procedure is illustrated in Table V.

Information for the complete analysis of variance was obtained in two separate computer runs. The first run obtained the sums of squares and mean squares for Fe, Cu in Fe, and samples. The second run of the hierarcial AOV program obtained the sums of squares and mean squares for Cu, Fe in Cu, and samples. The sum of squares for Fe x Cu interaction was computed by combining these results: Fe x Cu (SS) = Cu in Fe (SS) - Cu (SS) or Fe x Cu (SS) = Fe in Cu (SS) - Fe (SS).

All of the other metal interference systems were of a factorial design with equal replication and a factorial analysis of variance computer program was applied to their statistical treatment. A factorial experiment is designed to give independent comparisons of a type dependent upon the choice of treatments,

APPENDIX B

CHEMICALS AND APPARATUS

Aldrich Chemical Company, Inc.

2-Cyanopyridine	Catalog No. 09460

Alfa Inorganics, Inc.

Sodium Hydride (50% in Oil)	Catalog No. H-106

Allied Chemical Company

70% Perchloric Acid	Reagent Grade	Code 1144
No. 36 Iron Wire	99.8% Fe	

J. T. Baker Chemical Company

Anhydrous Ammonium Acetate	Reagent Grade	0.0002% Fe	Cat. No. 0596
Acetone	Reagent Grade	0.000005% Fe	Catalog No. 9006
37.6% Hydrochloric Acid	Reagent Grade	0.000004%	Cat. No. 9535

E. I. Du Pont De Nemours & Co.

Nitrobenzene	Reagent Grade

Fisher Scientific Company

Glacial Acetic Acid	Reagent Grade	0.00002% Fe	Cat. No. A-38
Benzene	Reagent Grade		Cat. No. B-245
Chromium Powder	99^{+} % Cr		Cat. No. C-318
Hydroxylamine Hydrochloride	Reagent Grade	0.0003 % Fe	Cat. No. H-330

Mickel(ous) Oxide Reagent Grade 0.002 % Fe Cat. No. N-69

71.0 % Nitric Acid Reagent Grade 0.00002 % Fe Cat. No. A-200

REXYN RG 501 (H-OH) Resin 0.005 % Fe

Matheson Coleman & Bell

0.008" Electrolytic Grade Copper Sheet Cat. No. CB324

Merck & Company, Inc.

Aluminum Oxide Chromatographic Grade 100 Mesh Cat. No. 71695

G. Frederick Smith Chemical Company

2,4,6-Tripyridyl-s-Triazine Cat. No. 291

U.S. Industrial Chemical Company

Absolute Pure Ethyl Alcohol

Applied Physics Corporation

Cary 14 Recording Spectrophotometer Serial No. 375

Beckman Instruments, Inc.

Beckman Zeromatic pH Meter Model 96 Cat. No. 9600 Ser. No. 236061

Mettler Instrument Corporation

Single-pan Balance Type H-5 Capacity 160 g. Ser. No. 5631 1

VITA

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Master of Science

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Biographical:

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Professional Experience: Technician for Electrochemical Labs, Tulsa, Oklahoma, the Summers of 1959, 1960, and 1961; graduate teaching assistant, Chemistry Department, Oklahoma State University, in 1966, 1967, and 1968.