AN ABSTRACT OF THE THESIS OF

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Title SPECTROPHOTOMETRY OF MOLYBDENUM, TUNGSTEN AND CHROMIUM CHELATES OF QUERCETIN

Abstract approved [redacted]
(Major professor)

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) reacts with hexavalent molybdenum, tungsten and chromium to form chelates soluble in an ethanol-water medium. This color reaction has been investigated with a view to its possible application in the spectrophotometric determination of trace amounts of these metals.

In the development of the method, several variables affecting the metal-quercetin color system have been studied in order to establish optimum conditions for color development. These include wavelength, pH, ethanol concentration, quercetin concentration and time.

The molybdenum-quercetin chelate obeys Beer's law from 0. 1 to 1. 8 ppm at 420 mμ where maximum absorbance occurs. The molar absorptivity at this wavelength is about 34, 000 $cm^{-1}mole^{-1}l$. The optimum concentration for maximum precision at 420 mμ corresponds to 0. 6 to 1. 2 ppm of molybdenum. By operating at 450 mμ where the molar absorptivity is only about 15, 000 $cm^{-1}mole^{-1}l$

one can extend the workable range to 15 ppm of molybdenum.

The tungsten and chromium chelates with quercetin both show deviations from Beer's law, although a working curve can still be established. The maximum absorbances for the tungsten and chromium complexes occur at 420 mμ and 435 mμ, respectively. The optimum concentration in both cases is 3 to 5 ppm of metal ion. The molar absorptivities are 18,900 $cm^{-1}mole^{-1}l$ for tungsten and 4600 $cm^{-1}mole^{-1}l$ for chromium.

Studies have been made of the composition of the complexes in solution using the slope-ratio method and the "gerade" method of Asmus. Both methods indicated a 1:1 mole ratio of metal to ligand for all three chelates, although there is some evidence that additional species may be present in the case of tungsten and chromium. Approximate values for the instability constants of the chelates have been obtained using a spectrophotometric method. These were found to be about 3.9×10^{-5} for molybdenum, 1.4×10^{-5} for tungsten and approximately 10^{-5} for chromium.

A study of the effect of foreign ions indicates a general lack of specificity of the reagent and points to the probable need for a prior separation of the metal ion before its determination.

An attempt was made to run a potentiometric titration of quercetin, both alone and in the presence of the metal ions, but the results were inconclusive.

SPECTROPHOTOMETRY OF MOLYBDENUM, TUNGSTEN AND CHROMIUM CHELATES OF QUERCETIN

by

LLOYD JAMES MITCHELL

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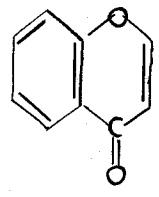
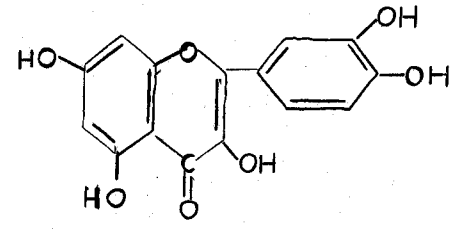
CHAPTER I

INTRODUCTION

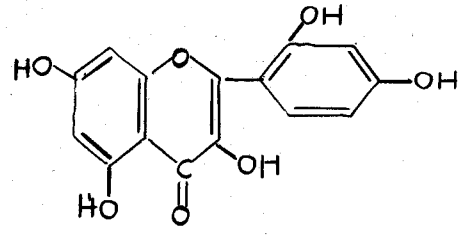
Quercetin (3, 3', 4', 5, 7 - pentahydroxyflavone) is an isomer of morin (2', 3, 4', 5, 7 - pentahydroxyflavone). Both compounds are members of a class of natural coloring matters derived from benzo-γ -pyrone (also called chromone). The structural formulas for these compounds are given below:



Benzo-γ-pyrone
Chromone



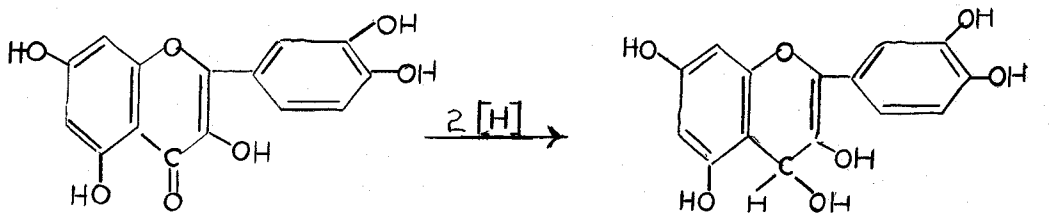
Quercetin



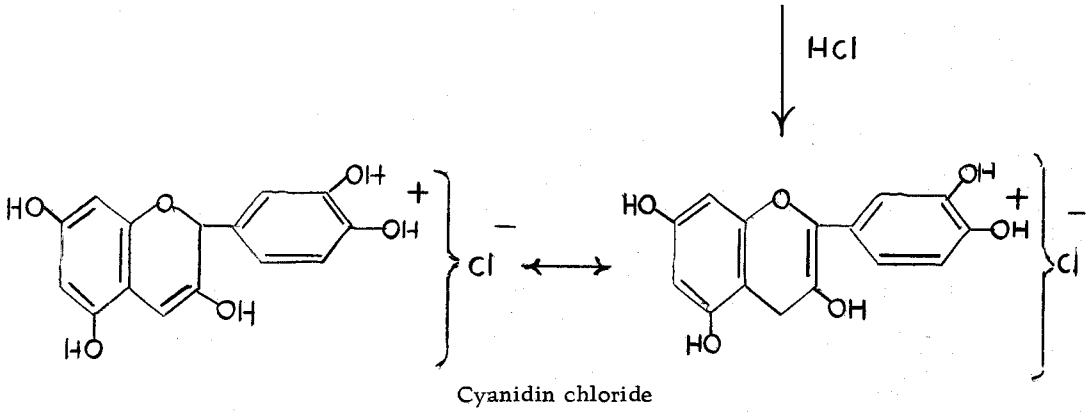
Morin

The flavones and anthocyanidins are closely related as indicated by the conversion of quercetin to cyanidin chloride by reduction with amalgamated magnesium and hydrochloric acid. The secondary

alcohol produced, being an allyl alcohol, is easily converted by hydrochloric acid to the two resonating structures of cyanidin chloride.

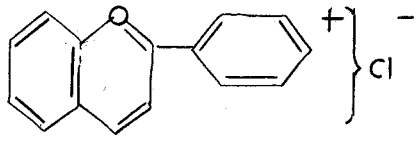


Quercetin



Cyanidin chloride

The anthocyanidins occur both free and combined with sugars as anthocyanins in red, blue and purple flowers, fruits and leaves. Although these compounds are not common constituents of wood, workers in England have reported the presence in certain woody tissues of precursors of anthocyanidins called leucoanthocyanidins. The latter are colorless but turn pink or red upon addition of acid. It is of interest that certain varieties of wood pulp develop a pink color which suggests the possibility of a similar transformation. Flavylium chloride would represent the parent compound of the anthocyanidins.



Flavylium chloride

Quercetin is obtained from dihydroquercetin which in turn is extracted from ground Douglas fir bark by hot water. Douglas fir represents the major lumbering species in the Pacific Northwest and one of the most important species in the production of lumber and plywood in the United States. Since bark represents a large percentage of the waste in the production of pulp and paper, plywood and lumber, increasing attention has been focused in recent years on the possible utilization of this material as a useful source of by-products. Mechanical separation of the material into fractions consisting of cork, fiber, and powder has led to its use chiefly as fillers in the linoleum and plastic industries. Recently there has been some activity in the production of ethanol by saccharification of wood wastes. Another possibility involves the utilization of wood extractives. Although a minor constituent, these substances exert a very considerable influence upon the commercial utilization of the wood. The flavonoids represent just one small group of compounds among the many isolated from these extractives.

Flavonoids, even in very small amount, inhibit sulfite pulping. The flavonoids are fungistatic and hence exert a pronounced influence on the resistance to decay or durability of the lumber. The flavonoids

are prototypes or precursors of phenolic polymers present in large amounts in some species and of importance in leather tanning, oil-well drilling and the preparation of adhesives. Formerly flavone pigments such as fisetin, luteolin, morin and quercetin were used extensively as mordant dyes, especially in the form of crude plant preparations. These have now been largely displaced by synthetic dyestuffs. The color and odor of wood is due to these extractives. Finally, and of special interest in the present instance, a number of these compounds form colored complexes with metal ions, suggesting their possible application as chelating agents for the spectrophotometric determination of trace amounts of various metals.

In this connection, mention should be made of the work of Dr. Harvey Aft and his group at the Forest Products Laboratory of Oregon State University. Dr. Aft's special field of interest lies in products derived from Douglas fir bark, with particular emphasis on dihydroquercetin and quercetin. The former occurs in the bark to the extent of 5 percent. Considerable work has been done by this group on the copper-quercetin complexes with respect to the relative importance of the hydroxyl group positions of quercetin and the metal-complexing sites of the molecule to antioxidant capacity in lard. The possibilities of altering the basic molecule so as to produce such diverse materials as non-toxic food coloring additives and new toxic agents for use as constituents of fungicides and herbicides promises to make Douglas fir bark a veritable chemical warehouse.

CHAPTER II

SURVEY OF PREVIOUS WORK ON QUERCETIN

Earliest references to quercetin isolated from quercetin bark dust (Quercus tinctoria) focused attention on the formation of orange to brown-yellow colorations with mineral acids. Perkins and Pate (30, p. 646-648) prepared and examined the salts of quercetin with sulfuric, hydrobromic, hydrochloric and hydriodic acids. Quercetin was first synthesized by v. Kostanecki, Lampe, and Tambor (24, p. 1402-1405) in 1904 starting with 2'-hydroxy - 3, 4, 4', 6'- tetramethoxychalcone. Ring closure was effected by boiling with dilute hydrochloric acid to yield the tetramethoxyflavanone. This was then treated with nitrous acid to introduce the nitroso group in the 3- position, followed by treatment with mineral acid to yield 3', 4', 5, 7-tetramethoxyflavonol. This was then demethylated by boiling with hydriodic acid to yield quercetin.

The dyeing properties of the hydroxyflavone and hydroxyflavonol coloring matters were studied extensively during the early years of the present century, especially toward aluminum and tin mordants. These studies made it possible to estimate with a reasonable degree of certainty the tinctorial effect of each of the hydroxyl groups present in the compound and thus to predict to some extent the position occupied by the sugar nucleus in their glucosides. Benzo-γ - pyrone

(chromone) by itself is colorless. The chromophoric power of the group -CO - C = C- is enhanced by introduction of a phenyl ring on the pyrone ring, and the simultaneous introduction of hydroxyl groups. The orientation of these hydroxyl groups has a regular effect on the color produced in the sense that hydroxyl groups in the 5- and 7- positions exert only a minor influence, yielding a very pale yellow; hydroxyls in the 3'- and 4'- position are powerful auxochromes and lead to the production of a deep yellow color; a hydroxyl in the 3- position produces only a pale yellow, but it enhances the effect of hydroxyls in the 3'- and 4'- positions to yield a deep orange color. In the hydroxyflavones it is the presence of the group $-\overset{\overset{O}{\|}}{C}-\overset{\overset{OH}{|}}{C}=$ which accounts for the ability to form lakes with mordants.

Extensive application of quercetin as an analytical reagent for the colorimetric determination of trace amounts of various metal ions has developed from about 1950, although a few scattered earlier references can be found. In 1938 E. A. Kocsis (22, p. 13-15) reported a spot test for iron and uranium using quercetin. It was claimed that 0.3γFe and 3.0γU could be detected by this reaction. Ferrous and ferric iron were not distinguished by the test. In 1941 the Russian chemists A. L. Davydov and V. S. Devekki (8) reported the quantitative fluorescent analysis of aluminum using quercetin. These workers had expressed dissatisfaction with the fluorescent intensity of the reaction between aluminum and alizarin under

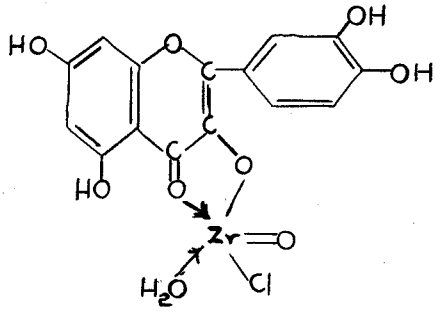
ultraviolet light in weakly acidic medium as insufficient for the determination of aluminum. They likewise were dissatisfied with the method of White and Lowe (36, p. 229-231) which uses the fluorescence of the aluminum salt of morin from the standpoint of the rather narrow pH limits imposed and the rather great variation in fluorescent intensity with the amount of coloring matter added. According to them quercetin was much more satisfactory.

In 1950 Willard and Horton (37, p. 1190-1194) conducted an extensive study of possible colorimetric and fluorimetric indicators for the titration of fluoride with thorium. Their study indicated pure sublimed morin and quercetin to be the best fluorescent indicators. For quercetin the optimum pH was found to be between 3.0 - 4.1, the optimum percent alcohol 50 percent. A high alcohol content with both quercetin and morin led to greater intensity of fluorescence and sensitivity in the titration. At concentrations of alcohol greater than 50 percent interference from foreign ions became appreciable. With excess fluoride the solution was almost colorless; at the end point it was a light green; with a slight excess of thorium a yellow green.

In 1952 Czechoslovakian workers Tomíček and Holeček (35), investigating methods for the determination of niobium and tantalum, reported the application of morin and quercetin for purposes of qualitative testing and rough gravimetric estimation of these elements. Niobium yields a red precipitate and tantalum an orange precipitate

when an alcoholic solution of the flavonoid is added to a strongly acid solution of the element containing 20-25 percent by volume sulfuric acid.

In 1953 Grimaldi and White (13, p. 1886-1889) published a method for the colorimetric determination of zirconium using quercetin as the chelating agent. The method, suitable for trace amounts from 0.1 to 50 γ of ZrO_2, involves separation of zirconium from interfering ions with p-dimethylaminoazophenylarsonic acid prior to its colorimetric estimation with quercetin. The color was developed in a 0.5N- hydrochloric acid solution containing about 30 percent alcohol. Under the conditions applied only the 2:1 complex was formed although under special conditions both a 2:1 and a 1:1 complex can co-exist. Approximate values for the equilibrium constants of the 1:1 and 2:1 complexes were reported to be $K_1 = 0.33 \times 10^{-5}$ and $K_2 = 1.3 \times 10^{-9}$. Chemical analysis of the 1:1 complex (formed in dilute solution of quercetin) indicated the probable formula to be



Likewise in 1953, Komenda (23) reported a method for the colorimetric determination of uranium based on the formation of a rust-brown coloration by reaction of UO_2^{++} ion with quercetin. This reaction was carried out at a pH 7 (ammonium acetate buffer) and a 40 percent alcoholic concentration. In this same article a transient violet colored complex of cerium with quercetin was reported as forming in neutral or weakly acid solution. The possibility of using this as a test for the determination of cerium was suggested. Oka and Shigeki (29) reported the determination of germanium with quercetin using a 40 percent methanol solution, pH 6.4 - 7.1 ($(NH_4)_2HPO_4$ buffer) and measuring the absorbance of the yellow complex at 410 mμ. Beer's law was reported to hold for germanium concentrations of 0.6 to 0.1γ/ml.

The colorimetric determination of tin using the yellow colored complex of Sn(IV) with quercetin in an acidic solution as applied to the determination of tin in brass, bronze, and commercial copper and zinc was reported by Liška (26) in 1955. The determination was carried out in hydrochloric acid solution containing 60 percent alcohol by volume. The Cu and Fe (III) were masked with thiourea.

In 1957 Menis, Manning and Goldstein (27, p. 1426-1430) investigated the color reaction between thorium and quercetin. These workers devised a separation scheme for the removal of interfering ions involving a combination of ion exchange and a

thenoyltrifluoroacetone extraction. Using the yellow complex with a maximum absorbance at 422 mμ and a pH of 2.7 - 3.5, they found Beer's law followed over a range of 10-150 γ thorium in a 25 ml volume. The quercetin-thorium molecular ratio, as determined by the slope-ratio method, was 2:1. The degree of dissociation was calculated to be 0.333; the instability constant was 1.2×10^{-10} which is about the same order of magnitude as the instability constant for the Zr (quercetin)$_2$ complex ($K = 1.3 \times 10^{-9}$) reported by Grimaldi and White.

In 1958 Goldstein, Manning and Menis (10, p. 539-542) applied quercetin to the spectrophotometric determination of molybdenum in an alpha-benzoinoxime-chloroform-ethanol medium. These workers were seeking a rapid and precise method for the determination of molybdenum in aqueous slurries of thorium dioxide containing uranium and contaminated with corrosion products such as iron, nickel and chromium. Separation of the molybdenum from the uranium was necessary since molybdenum interferes seriously in the determination of uranium by the spectrophotometric thiocyanate method as well as by the volumetric oxidation-reduction methods that involve reduction of uranium (VI) and subsequent titration with a standard solution of some oxidizing agent. A combination of a colorimetric method of determination combined with an extraction procedure was indicated, with an obvious advantage to be gained if color development could be

effected directly in an aliquot of the organic phase. In their procedure molybdenum was extracted with a 0.1 percent solution of α-benzoinoxime in chloroform. The complex of molybdenum and quercetin was formed in an aliquot of the organic extract by addition of an alcoholic solution of quercetin. The absorbance of the yellow complex was measured at 420 mμ against a reagent blank. The determination of the empirical formula of the molybdenum-quercetin complex by the slope-ratio method (15, p. 4488-4493) indicated a 1:1 ratio of quercetin to molybdenum. The degree of dissociation of the complex was calculated to be 0.661 for a solution that was 8.32×10^{-6} M in molybdenum, giving an instability constant $K = 1.1 \times 10^{-5}$. Tungsten and vanadium interfere seriously, but since these elements were not present in the material with which they were concerned, no procedure for their separation was developed.

A method for the determination of thorium in monazite sand along with the absorption maxima of the complexes of Th, U, Ti, Al, Be, Ga, In, Cu, and Zr with quercetin was published by Alimarin et al. (1) in 1958. In a similar vein Golovina et al. (11) reported the qualitative color reactions of quercetin and various cations, first in ultraviolet and then in visible light. The ions considered included Cu(II), Hg(I and II), Pb(II), Sn(II and IV), As(III), Zn, Be, Al, Fe(III), Ga, In, Ge, Ti(III and IV), Zr(IV), Th(IV), V(V), Nb(V), Ta(V), U(VI),

Mo(VI), and W(VI). It was noted that at high concentration all of the ions mentioned gave flocculent colored precipitates easily soluble in acetone. A method was described for the determination of Ti(IV). Measurements were made at 440-450 mμ using a 20 percent alcohol solution and a glycolic buffer. Beer's law was followed at concentrations from 0.5 to 1.0 γ/ml. A 1:1 titanium-quercetin complex was indicated. A new colorimetric method for the determination of antimony (III) in the presence of arsenic (III) was reported by Constantinescu and co-workers (7). The stable yellow complex of antimony (III) and quercetin was developed in methanol and allowed the determination of antimony in concentrations of 1 to 11 γ/ml. in the presence of arsenic (III) up to an Sb:As ratio of 1:6.

In 1959 a comparative spectrophotometric study of possible reagents for quadrivalent tin was carried out by Babko and Nazarchuk (5, p. 187-193) at the Institute of General and Inorganic Chemistry at Kiev. It was pointed out that the most fundamental criteria in the selection of a suitable reagent for a given metal was the integral difference between the light absorption of the colored complex MeR and the free dye HR and also the difference in pH range between complete formation of the colored complex and the transition of the dye into its ionic form R^-. On this basis stilbazo was shown to be the most sensitive reagent for the qualitative detection of tin whereas quercetin and hematoxylin were most suitable reagents for the

spectrophotometric determination of tin. In a later issue of this same journal Nazarchuk (28, p. 773-776) reported the determination of tin in nickel metal by means of quercetin. The lemon-yellow Sn-quercetin complex was formed in an aqueous acetate buffered solution of pH 4-5 and then extracted with a 1:1 iso-amyl alcohol-ether mixture. The organic layer was then compared with a series of standard solutions prepared in the same manner. Iron, which interferes, was reduced with thiourea prior to the formation of the complex.

The production of quercetin from Douglas fir bark by Weyerhaeuser Timber Company of Longview, Washington in 1958 prompted the development of a spectrophotometric method for the determination of quercetin by L. E. Dowd (9, p. 1184-1187) for purposes of process control and purity measurements on the final product. The method is based upon the formation of a 1:1 complex of quercetin and aqueous aluminum chloride in a 0.01 M aluminum chloride solution adjusted to pH 4.0 with potassium acetate. Maximum absorbance is at 430 mμ, with Beer's law being followed over the range of 2 to 15 γ quercetin per ml. of solution. It is interesting to note that the complex in aqueous aluminum chloride differs from that in alcoholic aluminum chloride where the ratio is two moles of quercetin per mole of aluminum chloride. Dowd reported a value of 0.773 for the degree of dissociation and a value of 5.2×10^{-5} for the dissociation constant of the 1:1 complex.

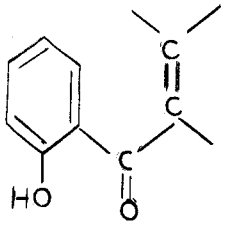
The reaction of quercetin with gallium and indium was reported by Alimarin and co-workers (2) in 1960. These workers observed the formation of colored compounds with gallium and indium in slightly acidic solution, the precipitates being soluble in oxygen-containing organic solvents. Alcoholic solutions were observed to have a bright yellow color and to exhibit a yellowish-green fluorescence when exposed to ultraviolet light. These elements were determined photometrically using a pH of 4 and 20 percent alcohol concentration for gallium and a pH of 5 and 55 percent alcohol concentration for indium. Sensitivities for the color reaction were 0.005 γ/ml. for gallium and 0.01 γ/ml. for indium. Aluminum, fluoride, tartrate, citrate and oxalate were observed to interfere.

A fluorometric method for the determination of zirconium with quercetin in samples containing interfering ions, especially vanadium and titanium, was developed by D. M. Hercules (16, p. 485-491) in 1961. These two elements interfere, not by forming fluorescent compounds with quercetin, but rather by the formation of highly colored complexes which absorb over the same wavelength region as the zirconium-quercetin complex. This difficulty was circumvented by extracting the zirconium with 2-thenoyltrifluoroacetone (TTA). The TTA layer was diluted with xylene and extracted with 12N-hydrochloric acid. The color was developed in an alcohol solution about 2.5 M in hydrochloric acid. An exciting wavelength of 440 mμ was

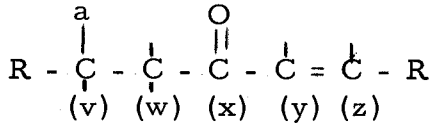
used and the fluorescence measured at 505 mμ. Reproducibility was about four percent with a lower limit of zirconium detection of about 0.05 γ /25 ml. and an upper limit of about 75 γ /25 ml.

A spectrophotometric study of the complex of gallium with quercetin was carried out by Popa and co-workers (31) using a methanol-water medium (≥ 55 percent methanol) for the development of the colored complex. They reported a 1:1 molar ratio of quercetin to metal, with a dissociation constant of 1×10^{-4} for the complex. Measurements were made at 428 mμ in the pH range 4.5 to 5.7. Best results were obtained in the range of 0.08 to 2.28 γ /ml. of solution.

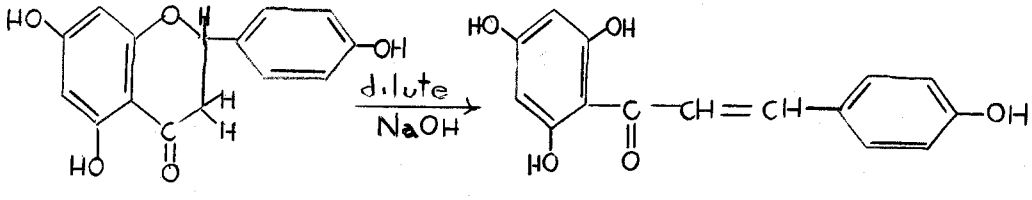
Likewise in 1961, Hiiro (17, p. 1748-1752) applied quercetin in the spectrophotometric determination of boron in graphite samples. A study of the color reaction between boric acid and certain flavones and flavone derivatives in the presence of citric acid had been carried out by C. W. Wilson of the California Fruit Growers Exchange Research Department (38, p. 2303-2306) in 1939, with the result that some very interesting correlations were established between structure and the tendency to give a positive reaction with boric acid. Compounds either having or giving rise to the grouping



on cleavage of the pyran ring with dilute alkali fulfill the boric acid color forming requirement of having an hydroxyl or other auxochromic group on the second carbon from the carbonyl group and a C = C double bond in the α, β - position with respect to the carbonyl. The indicated configuration for reactivity would be

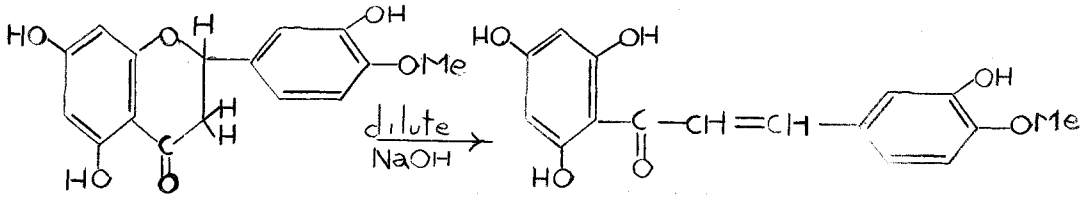


in which a = an auxochromic group such as = O, OH, OCH_3, etc., and in which R, $C_{(v)}$, and $C_{(w)}$ may be a benzene ring and $C_{(x)}$, $C_{(y)}$ and $C_{(z)}$ may be a portion of a pyran ring. Thus the flavanones naringenin and hesperitin gave a negative reaction whereas the polyhydroxychalcones derived from these compounds by cleavage of the pyran ring with dilute alkali gave a positive reaction.



Naringenin
(negative)

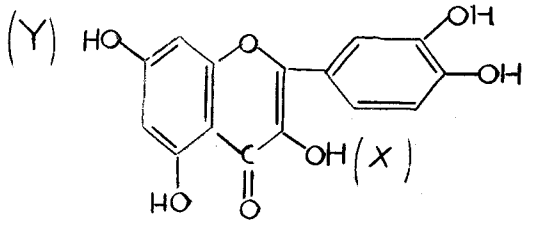
2', 4, 4', 6-tetrahydroxychalcone
(positive)



Hesperitin
(negative)

2', 3, 4', 6'-tetrahydroxy-
4-methoxychalcone
(positive)

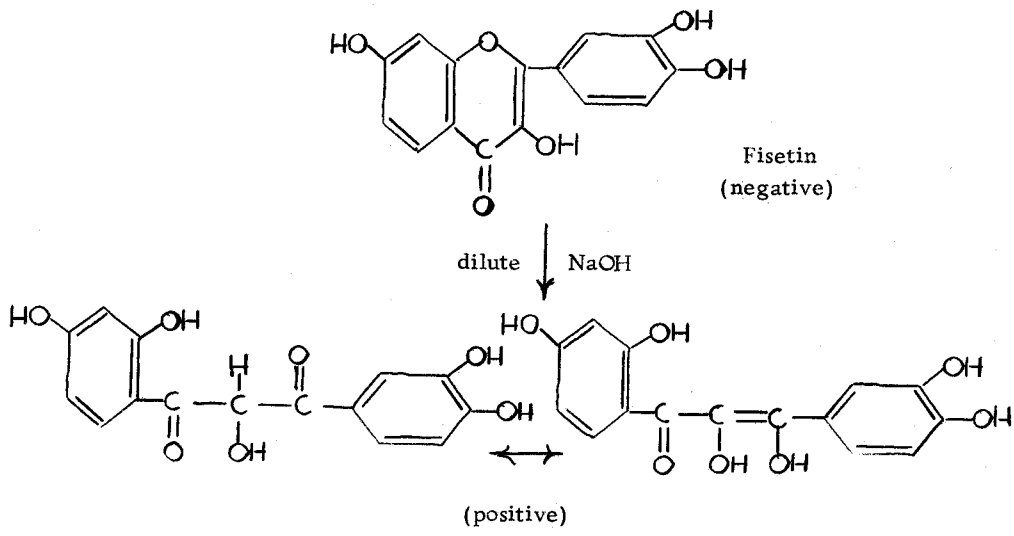
Quercetin, isoquercitrin, quercimeritrin and kaempferol all gave a positive test whereas fisetin (no hydroxyl in the 5- position) gave a negative test. The chalcone obtained on treatment of fisetin with dilute alkali gave a positive test.



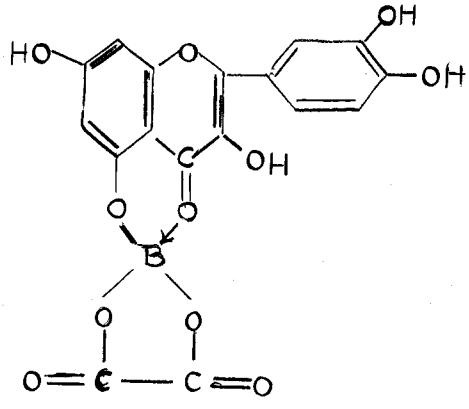
Quercetin
(all three positive)

X = Isoquercitrin

Y = Quercimeritrin



Thirteen years later Hörhammer, Hänsel and Strasser (18, 19, 20) took up the problem of determining the structure of these halochromic boron complexes. The citric acid used by Wilson was replaced by oxalic acid in these investigations. Spectrophotometric measurements indicated a strongly positive boron atom linked through the oxalic acid ligands to the nucleophilic oxygen atom of the flavonoid compound to form a spiro-like boro-oxalic acid complex. Thus the structure of the complex was postulated as



As applied by Hiiro to the determination of trace amounts of boron in graphite, the sample was first ashed in an electric furnace in a stream of oxygen, then the boron in the ash was distilled off as methyl borate into an aqueous solution of sodium hydroxide. The solution, after evaporation, was acidified, treated with oxalic acid and quercetin, evaporated, and the residue kept at 60-90°C for one hour to insure complete color development. The residue was then taken up in acetone and the absorbance measured at 445 mμ against acetone as a reference. It was claimed that this method agreed well with the curcumin method.

Several new elements were added to the growing list of cations determined colorimetrically by quercetin in 1962. Shkrobot (33, p. 185-188) extended the earlier work of Alimarin (2) and Popa (31) on gallium in an article devoted to the quantitative characteristics of the compounds of morin, quercetin, and rutin with gallium and indium. Calculation of the instability constants for these complexes indicated the following order of stability: gallium-morin > gallium-quercetin> indium-quercetin> indium-morin> gallium-rutin. All of the gallium complexes were more stable than those of indium. Only one complex was formed between gallium and indium with each flavone. The mole ratio was 1:1 for compounds of gallium and indium with morin, gallium with rutin, and 1:3 for compounds of indium and gallium with quercetin.

Golovina and Tiptsova (12, p. 521-522) reported the photometric determination of thallium (III) with quercetin. According to these workers a fleeting raspberry coloration develops when Tl (III), Mn (VII), Bi(V), Cr(VI) and V(V) react with quercetin. The qualitative detection of V(V) and Tl(III) with quercetin had been reported earlier by Pribil and Michal (32). With Tl(III) the raspberry color lasted only 20-30 seconds in acetone or alcohol solution, although the stability of the color was increased slightly in a layer of chloroform or carbon tetrachloride and significantly when ether, amyl acetate or iso-amyl alcohol was used as the extractant. In the photometric procedure developed by these workers Tl (I) was oxidized to Tl(III) with ammonium persulfate catalyzed by a trace of silver ion. After removal of excess oxidant, the solution was buffered at pH 5 using a hydrogen phthalate buffer, amyl acetate added, and the color developed by addition of an alcoholic solution of quercetin. The absorbance was read five minutes after mixing at 510 mμ with respect to water. The colored extracts conformed to Beer's law, the molar absorptivity being about 10, 000 at 510 mμ. Acetate, tartrate, chloride, bromide, and iodide interfere. Thallium was determined satisfactorily in the presence of zinc and cadmium at ratios of Tl:Zn = 1:7000 and Tl:Cd = 1:1400.

The spectrophotometric determination of arsenic (V) with quercetin was reported by Tanaka and Hiiro (34). Like boron, color

development with this element seemed to require rather drastic conditions. Alcoholic quercetin solution was added to the arsenic (V) solution followed by evaporation to dryness and heating of the residue at 70°C in an oven for one and a half hours. The residue was taken up in ethanol and the absorbance read at 398 mμ against a reagent blank. An error of 6.2 percent was reported, with the presence of water tending to decrease the absorbance.

Chudinov and Yakovlev (6) reported the photometric determination of the transuranium element neptunium in 1962. Following a separation procedure in which the neptunium was extracted with thenoyltrifluoroacetone (TTA) and then re-extracted from the organic phase with 3 M - hydrochloric acid, the solution was buffered at pH 4.0 with an acetate buffer, alcohol and alcoholic quercetin added to about 48 percent alcohol content and the sample diluted to volume with water. The absorbance was measured after ten minutes at 425 mμ. The molar absorptivity was reported to be 23,000, with the absorbance fairly independent of pH between pH 3.0 to 7.0. The relative error was six percent, with five hours being required for a complete determination. In a variant of the method using KCl-HCl buffer a molar absorptivity of 14,600 was reported at 425 mμ.

A study of the color reactions of the rare earth elements with some polyhydroxyflavones was reported by Nowicka-Jankowska, Szysko and Minczewski (21) at the Institute for Nuclear Research,

Warsaw in 1962. The polyhydroxyflavones with a hydroxyl in the 3-position (morin, quercetin, kaempferol and fisetin) form yellow complexes with lanthanide ions. Attention was called to the slightly different absorption spectra of the quercetin complexes of praseodymium (Z = 59), gadolinium (Z = 64), ytterbium (Z = 70) and holmium (Z = 67) determined at pH 5 to 7 which suggests possible analytical applications. Only one complex, 1:1 for morin and 3:2 for quercetin and kaempferol, was found in each system. It was suggested that the hydroxyl group in the 3-position and the carbonyl group in the 4-position were involved in the chelation process, forming 5 membered rings. Reference was also made to mixed polyhydroxyflavon-pyridine-lanthanide complexes forming in water-pyridine-ethanol systems. Several interesting research problems suggest themselves from these observations.

Scandium is an element possessing few distinctive colorimetric reactions suitable for spectrophotometric application other than the alizarin red S method and the fluorescent reaction with morin, and these are far from being selective. In 1963 Hamaguchi and coworkers (14, p. 61-67) applied the color reaction of scandium with quercetin to the spectrophotometric determination of this element. Like the others, quercetin is sensitive but not selective. A separation procedure using a strongly basic anion exchange resin was used to separate the scandium from interfering ions. Following

separation, color was developed in a 48 percent alcohol solution of pH 4. 4 (acetate buffer) and the absorbance read at 435 mμ against a reagent blank. The solution conforms to Beer's law in the range of 0. 1 to 3 ppm of scandium. The molar absorptivity was reported to be 12, 800. at 435 mμ . A 1:1 complex was indicated, with an instability constant of 2. 7 x 10^{-7} at room temperature.

CHAPTER III

PRELIMINARY EXAMINATION OF QUERCETIN

Preparation of Quercetin

The quercetin used in this investigation was supplied through the courtesy of Dr. E. F. Kurth of the Forest Products Laboratory at Oregon State University. The chemical was obtained from Douglas fir bark by water extraction and oxidation of the resulting solution of dihydroquercetin with sodium metabisulfite (25, p. 2096-2097). The product, after repeated recrystallization from ethanol-water and drying to the dihydrate by heating at 40° C for two hours, was a bright yellow color and melted at 316-317° C with sublimation.

Quercetin is very sparingly soluble in water. Solutions in aqueous alkali are readily prepared but darken gradually on standing as a result of the decomposition of the flavone. The compound dissolves readily in ethanol, and in all but two of the determinations recorded in the literature an alcoholic solution of the reagent was used. Methanol and isopropyl alcohol were tested and found less satisfactory than 95 percent ethanol. In all of the work that follows an alcoholic solution of the quercetin in 95 percent ethanol has been used.

Potentiometric Titration of Quercetin

A preliminary survey of the literature on quercetin failed to reveal any information concerning the ionization constants of the compound itself. Therefore a potentiometric titration of quercetin was carried out.

Reagents and Equipment

0. 1 N-Hydrochloric Acid

Approximately 0. 1 N-HCl was prepared by diluting 8. 3 ml of Baker and Adamson reagent grade concentrated hydrochloric acid to one liter with distilled water.

0. 1 N-Potassium Hydroxide

Approximately 0. 1 N-KOH was prepared in the following manner. Twenty grams of USP potassium hydroxide pellets were dissolved in 15 ml of distilled water and the solution filtered through a Gooch crucible, the filtrate being collected in a clean, dry test tube. The volume of resultant solution was 27 ml. To prepare the 0. 1 N-KOH, 8 ml of the filtered solution (about 13 N in KOH) was diluted to one liter with boiled distilled water.

Quercetin Solution

A 10^{-2}M solution of quercetin was prepared by dissolving 0.3386g of the dry purified material in 95 percent ethanol and diluting to exactly 100.0 ml with ethanol.

pH Meter

A Beckman Model 72 pH meter equipped with a combination silver chloride-glass electrode was used in the potentiometric titrations.

Procedure

Standardization of 0.1 N-HCl and 0.1N-KOH

The hydrochloric acid was standardized against a dried sample of Baker and Adamson reagent grade sodium carbonate using methyl purple as the indicator. An average value of 0.0988 was obtained. The potassium hydroxide was standardized by titrating aliquots of the base against the standard acid using phenolphthalein as the indicator. An average value of 0.1267 was obtained for the normality of the base.

Potentiometric Titration of Quercetin

The potentiometric titration was carried out in duplicate,

although only one curve is shown since the two sets of data are almost identical. The solutions titrated consisted of 20.0 ml of 10^{-2} M-quercetin solution, 20.0 ml of 95 percent ethanol and 60.0 ml of water. Additions of the standard 0.1267 N-KOH were made in 0.1 ml increments, stirring being effected by means of a magnetic stirrer and teflon plug. A one minute interval was allowed after each addition before the pH was read. Readings were taken over a pH range from about 6 to 11. The data is tablulated in Table 1.

Discussion of Results

Both sets of data yielded a curve having a slight but reproducible break at a pH of about 8. The odd shape of this break raises a question as to whether or not it is actually a neutralization point or due to some other factor. If it were the former, it would correspond to a dissociation constant of approximately 2×10^{-8}. A check in the literature failed to disclose any dissociation constants for morin or related hydroxyflavones or hydroxyflavonols.

It was observed that a brown coloration appeared as each drop of base entered the quercetin solution and disappeared on stirring. The solution was considerably darker at the completion of the titration as a result of the decomposition of the quercetin in the presence of alkali. Twelve hours later it was dark brown in color and practically opaque.

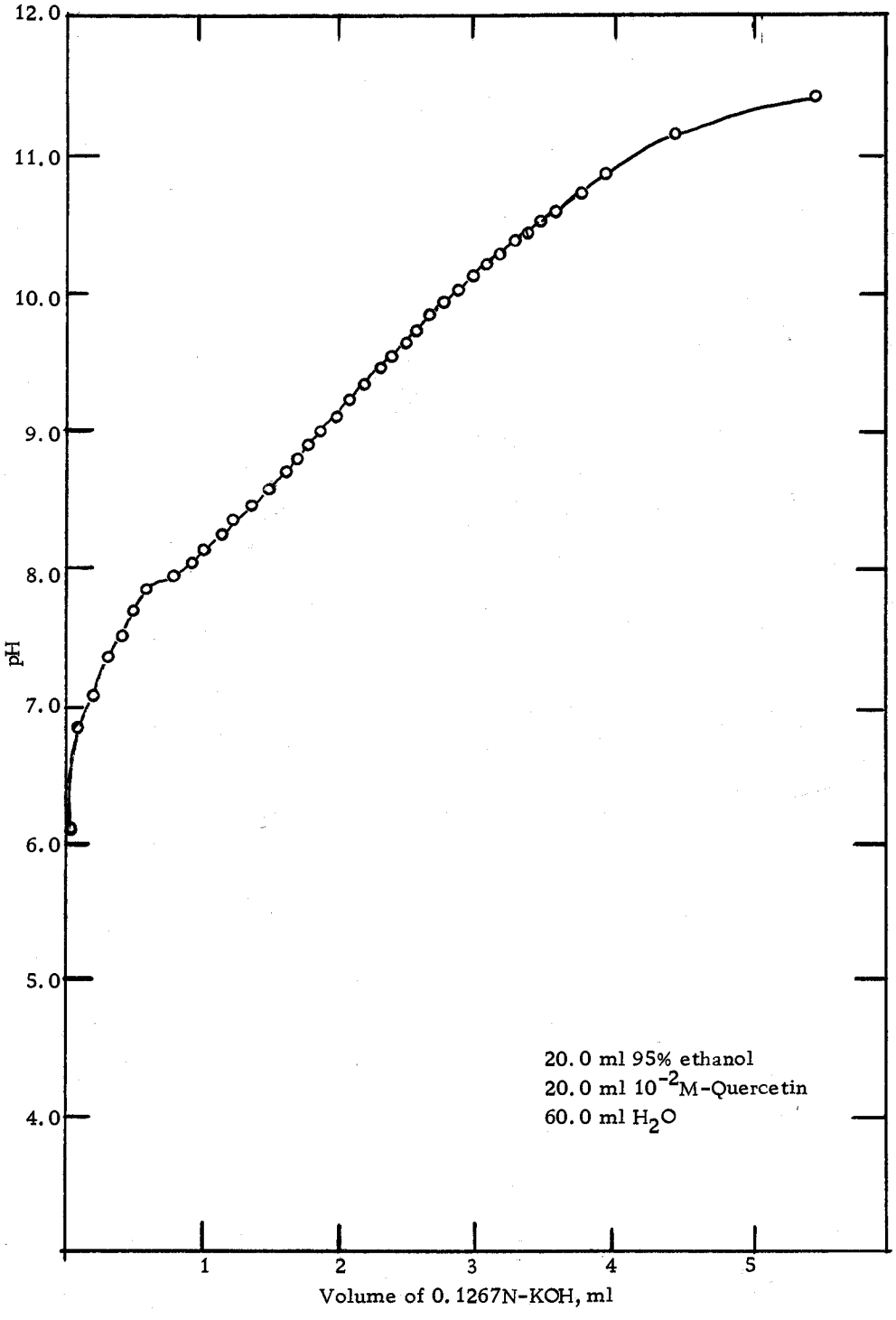


Figure I. Potentiometric Titration of Quercetin

Table 1. Potentiometric Titration of Quercetin

Run 1		Run 2	
Volume of KOH ml	pH	Volume of KOH ml	pH
0.00	6.05	0.00	6.08
0.10	6.85	0.10	6.85
0.20	7.10	0.20	7.08
0.30	7.35	0.30	7.30
0.40	7.50	0.40	7.50
0.50	7.70	0.50	7.75
0.60	7.85	0.60	7.85
0.70	7.90	0.70	7.91
0.80	7.95	0.80	7.97
0.95	8.05	0.90	8.05
1.00	8.15	1.00	8.15
1.10	8.25	1.10	8.25
1.20	8.35	1.20	8.35
1.30	8.40	1.30	8.42
1.40	8.50	1.40	8,50
1.50	8.60	1.50	8.60
1.60	8.70	1.60	8.70
1.70	8.80	1.70	8.80
1.80	8.90	1.80	8.90
1.90	9.00	1.90	9.00
2.00	9.10	2.00	9.14
2.10	9.25	2.10	9.25
2.20	9.35	2.20	9.35
2.30	9.45	2.30	9.45
2.40	9.55	2.40	9.55
2.50	9.65	2.50	9.65
2.60	9.75	2.60	9.75
2.70	9.85	2.70	9.85
2.80	9.95	2.80	9.95
2.90	10.05	2.90	10.05
3.00	10.15	3.00	10.15
3.10	10.20	3.10	10.20
3.20	10.30	3.20	10.30
3.30	10.40	3.30	10.40
3.40	10.45	3.40	10.50
3.50	10.55	3.50	10.55
3.60	10.60	3.60	10.60
3.80	10.75	3.70	10.70
4.00	10.90	3.80	10.80
4.50	11.15	3.90	10.90
5.50	11.45	4.00	10.95
		4.50	11.20
		5.00	11.35
		5.50	11.50

Absorption Curves of the Chelates

In the initial examination of quercetin as a possible chelating agent for the spectrophotometric determination of trace amounts of metals other than those already reported in the literature, a number of absorption curves were run in the Beckman Model DB spectrophotometer. These included the metals barium, beryllium, boron, cadmium, calcium, cesium, chromium (III and VI), cobalt, copper (II), lead (II), lithium, magnesium, mercury (II), molybdenum (VI), nickel (II), selenium (IV), silver (I), strontium, tungsten (VI), vanadium (V) and uranium (VI). Scans were made from 760 mμ to 200 mμ.

Reagents and Equipment

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone)

The purified quercetin supplied by Dr. E. F. Kurth was used in all experiments. Unless noted to the contrary, a 0.01 molar solution of the reagent in 95 percent ethanol was used.

Ethyl Alcohol

The 95 percent U. S. P. ethanol obtained from Commercial Solvents Corporation was used without further purification.

Standard Metal Solutions

Tenth molar solutions of all of the metal ions tested were prepared from Baker's Analyzed Reagents using the nitrates or chlorides whenever possible. In the case of barium and calcium, the carbonates were used and dissolved in the stoichiometric amount of 70 percent perchloric acid.

The various salts used are listed in Table 2 along with the weights per 100 ml of solution. More dilute solutions were prepared from these standard solutions as needed.

Acetate Buffer

An acetate buffer consisting of 27.5g of C.P. anhydrous sodium acetate and 40.0g of glacial acetic acid per 500 ml of solution was used in a few of the later initial scans and in all of the work thereafter.

Spectrophotometer

The Beckman Model DB Recording Spectrophotometer equipped with a Beckman Hydrogen Lamp Power Supply was used with 1.0 cm matched silica cells in all of the initial spectrum scans.

Procedure

In all of the initial spectrum scans a 1:4 alcohol solution was

Table 2. Salts Used in Initial Spectrum Scans

Element	Salt Used	Weight per 100.0 ml solution, g
Ba	$BaCO_3$	1.974
Be	$BeSO_4$	1.051
B	H_3BO_3	0.6182
Cd	$CdCl_2$	1.833
Ca	$CaCO_3$	1.000
Cs	CsCl	1.684
Cr(VI)	K_2CrO_4	1.942
Co	$Co(NO_3)_2 \cdot 6H_2O$	2.911
Cu(II)	$Cu(NO_3)_2 \cdot 3H_2O$	2.416
Pb(II)	$Pb(NO_3)_2$	3.312
Li	$LiNO_3$	0.6895
Mg	$MgCl_2 \cdot 6H_2O$	2.033
Hg(II)	$Hg(NO_3)_2 \cdot H_2O$	3.426
Mo(VI)	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	12.36
Ni(II)	$NiCl_2 \cdot 6H_2O$	2.377
Se(IV)	H_2SeO_3	1.290
Ag(I)	$AgNO_3$	1.699
Sr	$SrCl_2 \cdot 6H_2O$	2.666
W(VI)	$Na_2WO_4 \cdot 2H_2O$	3.299
V(V)	NH_4VO_3	1.170
U(VI)	$UO_2(C_2H_3O_2)_2 \cdot 2H_2O$	4.242
Zn	$Zn(NO_3)_2 \cdot 6H_2O$	2.975

used. Two runs were set up for each metal examined. The first consisted of 2.0 ml of 95 percent ethanol plus 0.1 ml of 10^{-2}M-alcoholic solution of quercetin. The second was identical with the first except for the addition of 0.1 ml of a 10^{-2}M-solution of the metal ion under examination. These solutions were run against a 1:4 alcohol-water reference on the Beckman Model DB spectrophotometer using the medium slit program and a scanning rate of 50 mμ per minute. The scan was extended into the near ultraviolet region by means of a Beckman Hydrogen Lamp Power Supply unit. A pair of matched silica cells was used in all cases.

After selection of molybdenum (VI), tungsten (VI) and chromium (VI) as the most promising, and establishment of a fixed procedure employing optimum conditions for examination, these three metal ions were re-run. These latter runs consisted of 5 ppm of the metal ion under examination, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol, 1.0 ml of 10^{-2}M-alcoholic solution of quercetin and were diluted to a total volume of 10.0 ml with distilled water. The reference solution consisted of 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and 6.5 ml of water. The absorption spectra for these metals, as well as the plots of metal chelate versus quercetin, are reproduced in Figures II through IV.

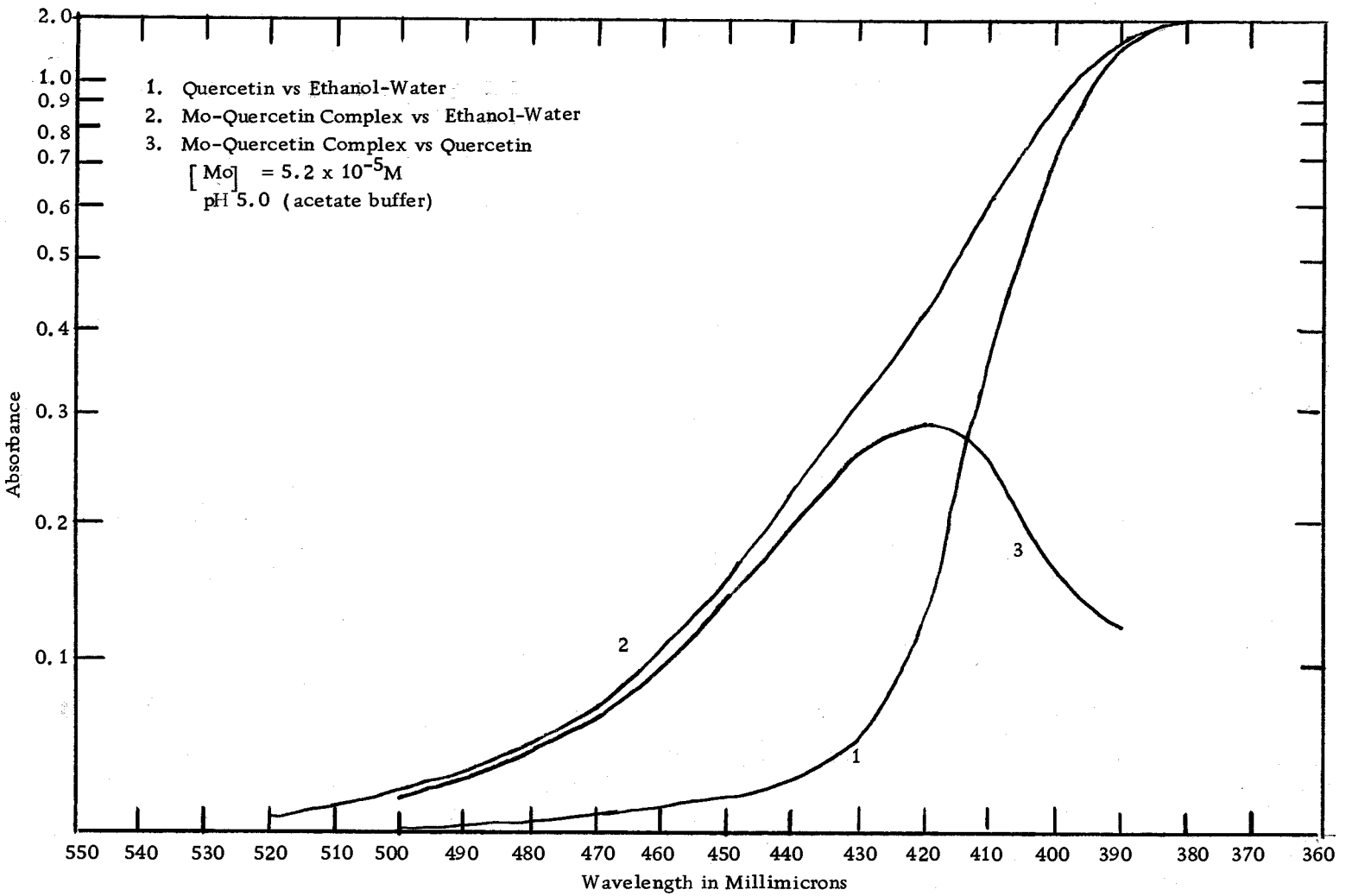


Figure II. Absorption Spectra of Molybdenum-Quercetin Chelate

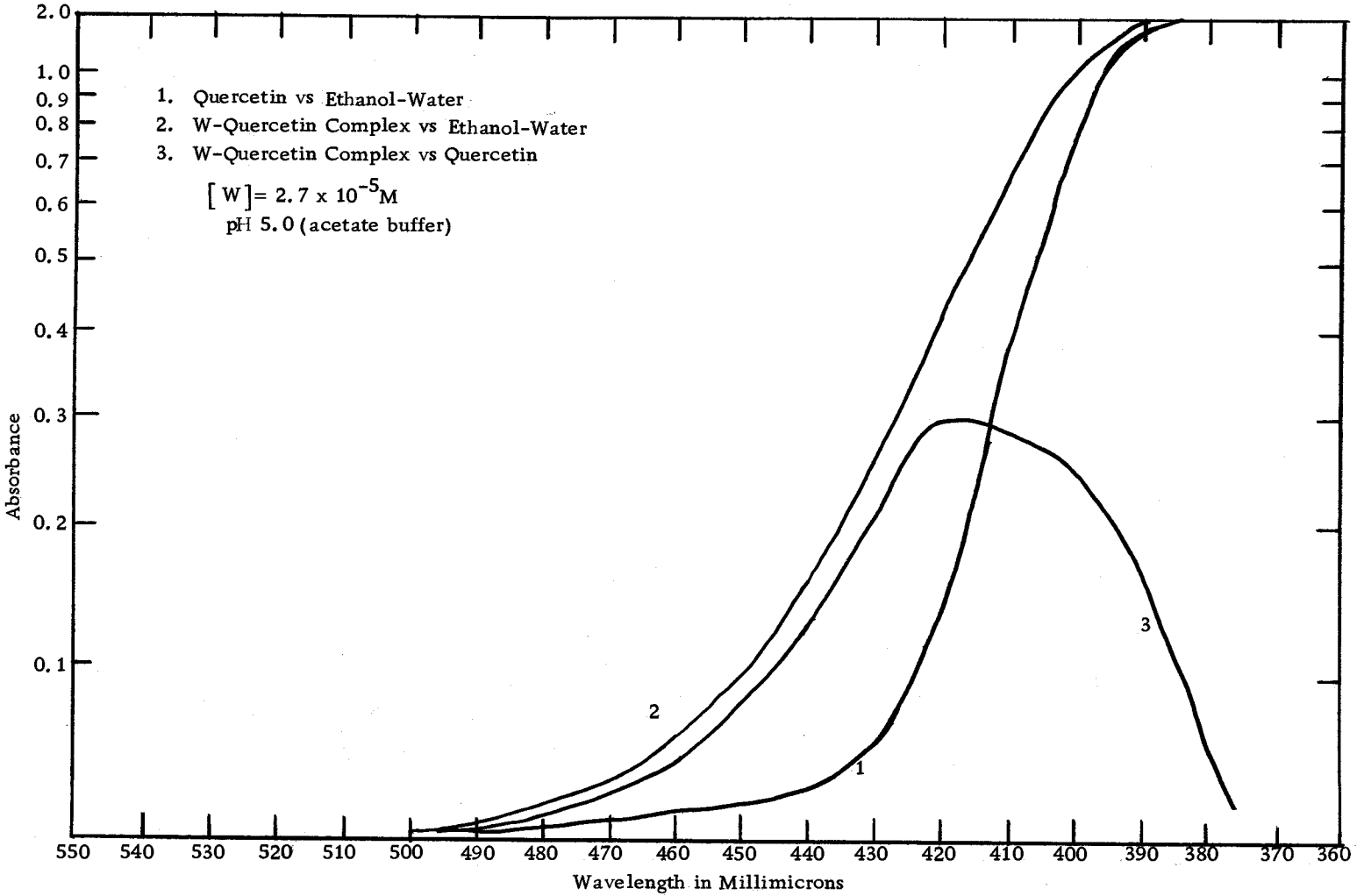


Figure III. Absorption Spectra of Tungsten-Quercetin Chelate

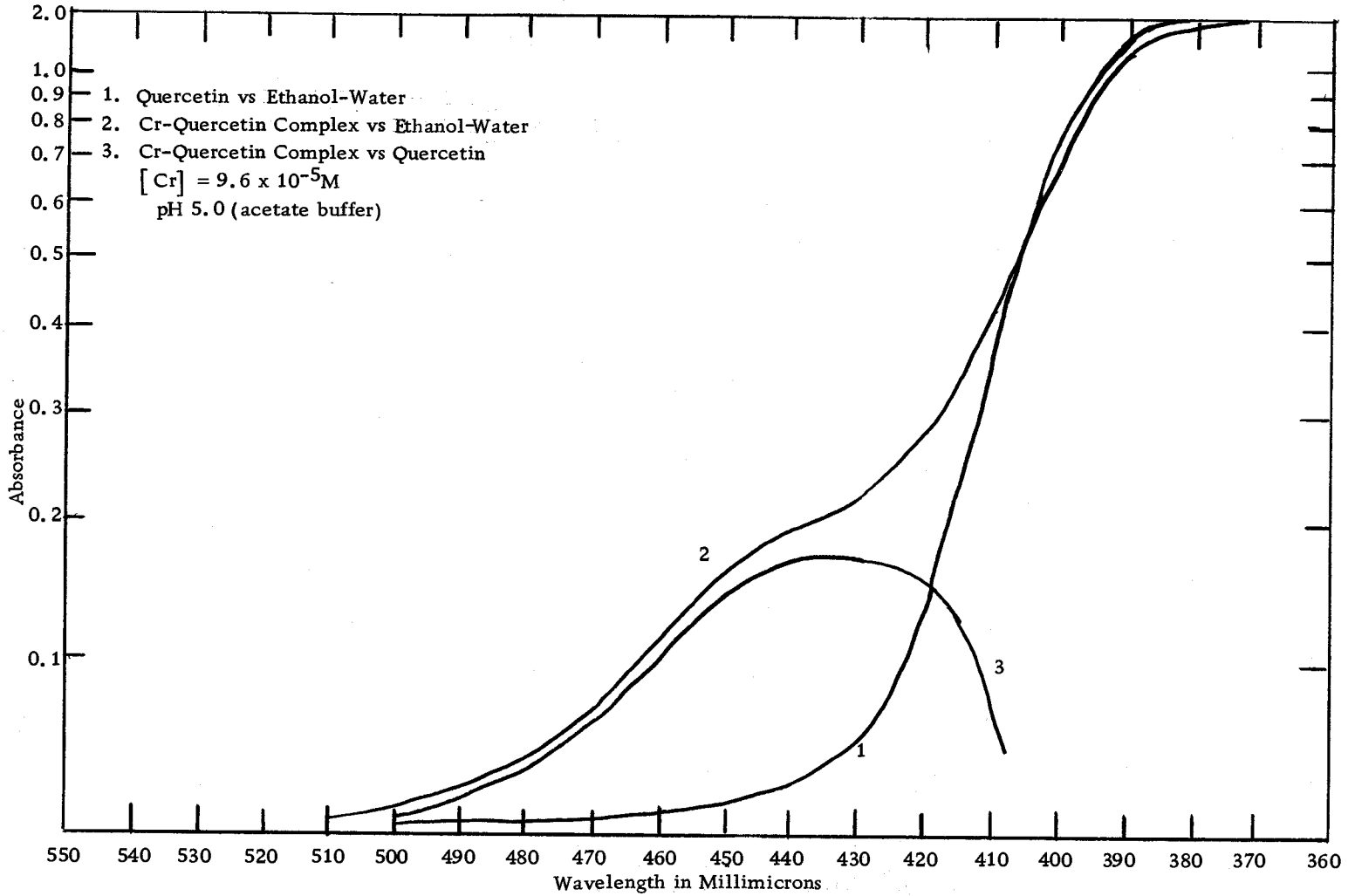


Figure IV. Absorption Spectra of Chromium-Quercetin Chelate

Discussion of Results

On the basis of the initial spectrum scans, molybdenum (VI), tungsten (VI) and chromium (VI) were selected as the most promising and deserving of further investigation. Molybdenum (VI) and chromium (VI) form deep yellow colored complexes with quercetin, while tungsten (VI) gives a pale yellow complex. In the case of the other ions tested, either no color change was apparent and no shift was obtained in the absorption curve, or else, as with uranium, a preliminary literature survey revealed prior investigation.

The optimum wavelengths for measurements as determined by the plots of metal chelate versus quercetin, would seem to be 420 mμ for molybdenum (VI), 420 mμ for tungsten (VI) and 435 mμ for chromium (VI), although higher wavelengths, such as 450 mμ for molybdenum, might be better for analyses since the quercetin absorbance above would be small and not change appreciably with wavelength.

Effect of pH on Absorption Spectra

In order to determine the optimum pH for color development, a series of solutions of each of the three metal ions differing only in pH was prepared. The pH range covered was from about 2 to 7. The absorption spectrum of each solution was then obtained against a 1:4 alcohol-water reference solution on the Beckman

Model DB spectrophotometer.

Reagents and Equipment

The reagents were the same as those described previously. A Beckman Zeromatic pH meter equipped with a standard Beckman Reference Electrode (asbestos fiber type) and a Beckman Glass Electrode of the general purpose type was used in all pH measurements.

Procedure

The solutions used to determine the effect of pH consisted of 20.0 ml of 95 percent ethanol, 1.0 ml of a 10^{-2} molar solution of the metal ion being examined and 1.0 ml of a 10^{-2} molar alcoholic solution of quercetin made up to volume with distilled water. Following addition of the reagents, water was added to within about a milliliter of the 100.0 ml mark. The pH was adjusted to the desired value by addition of the required amount of 1.000 N- or 0.1000 N-perchloric acid and then the volume was adjusted to exactly 100.0 ml with distilled water. The absorption spectrum was then recorded on the Beckman Model DB spectrophotometer against an ethanol-water (1:4) reference. The resulting absorption spectra at various pH values for the molybdenum, tungsten and chromium chelates are shown in Figures V, VI and VII, respectively.

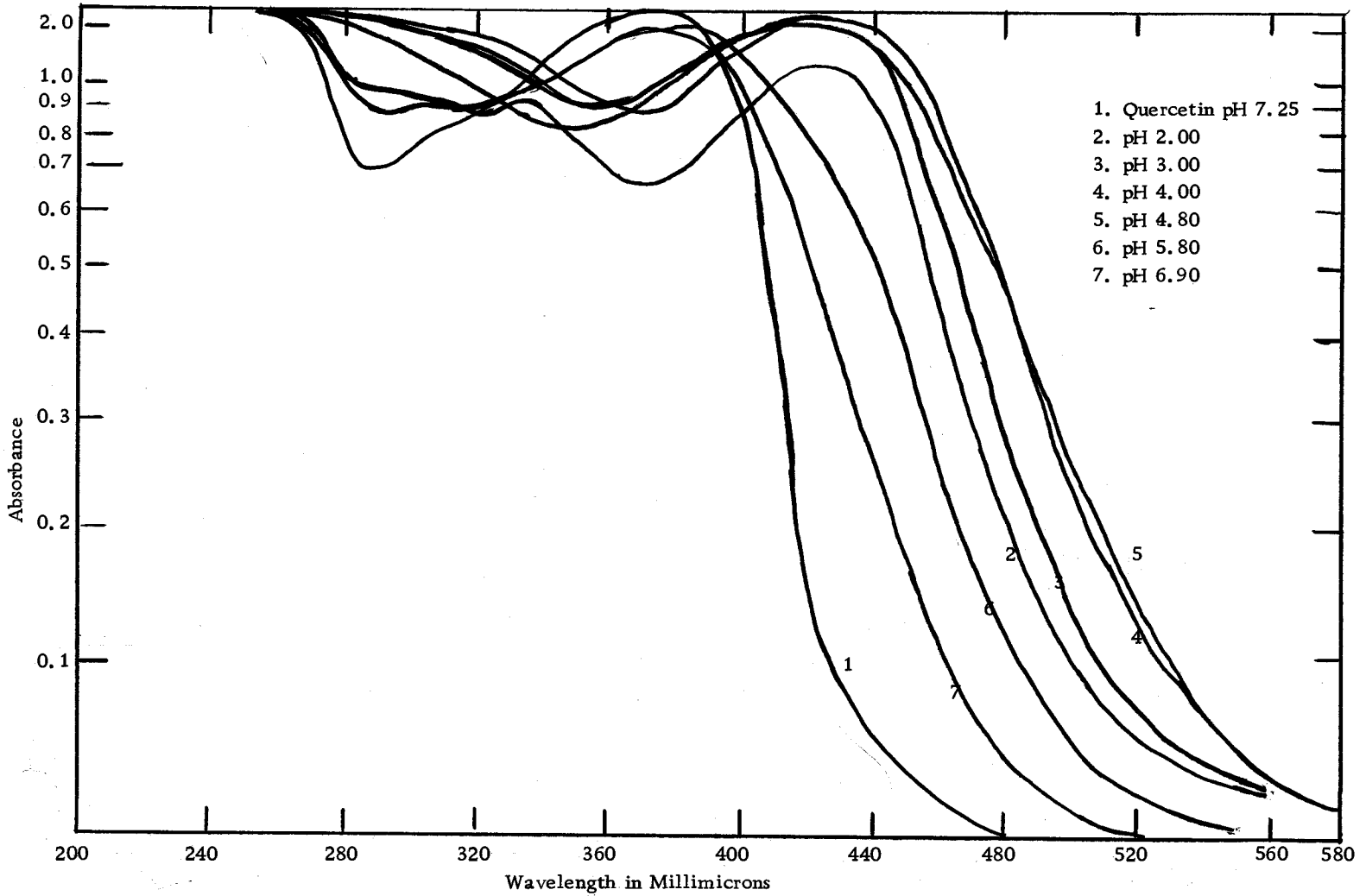


Figure V. Effect of pH on Absorption Spectra of Molybdenum-Quercetin Chelate

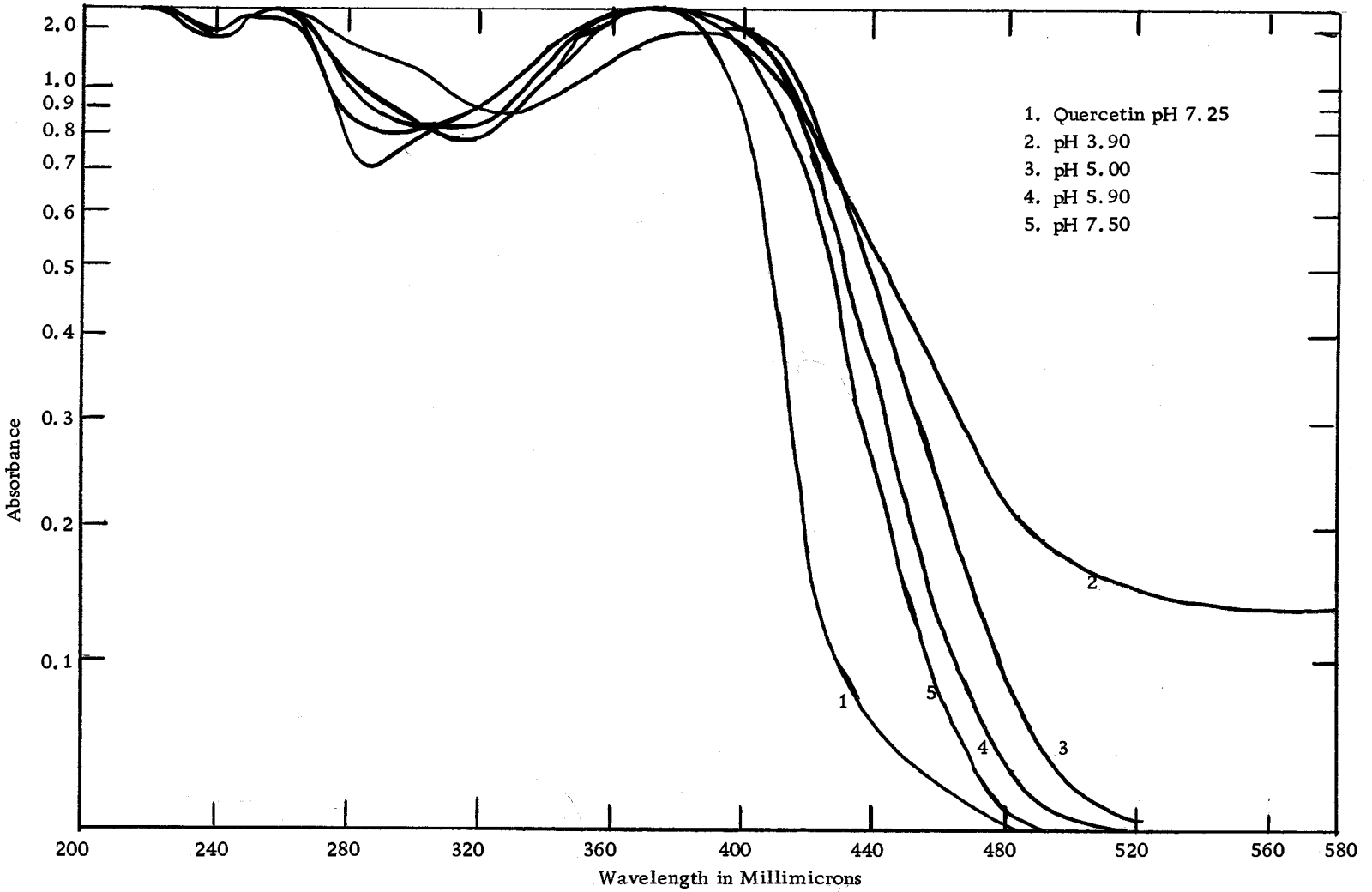


Figure VI. Effect of pH on Absorption Spectra of Tungsten-Quercetin Chelate

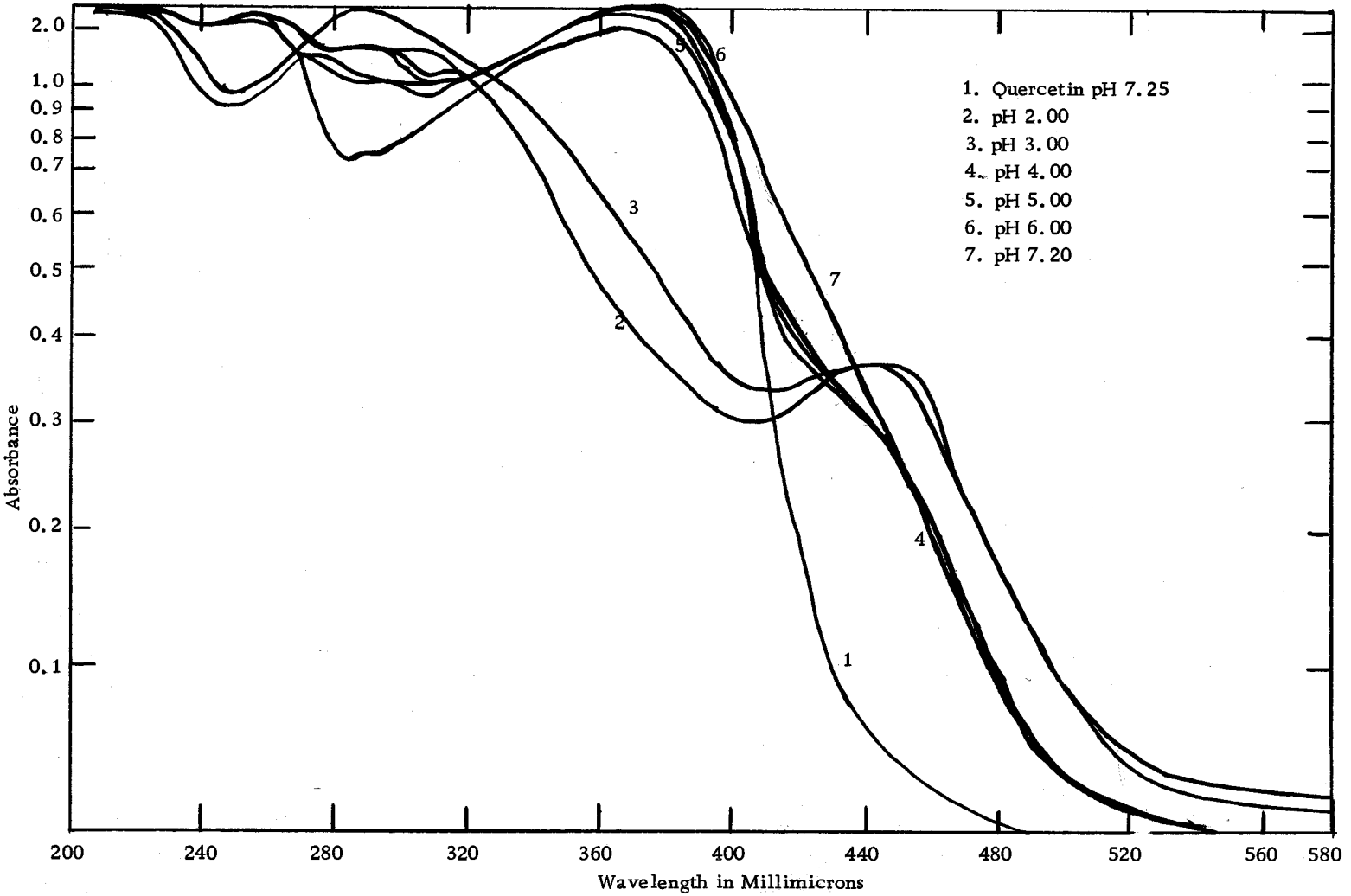


Figure VII. Effect of pH on Absorption Spectra of Chromium-Quercetin Chelate

Discussion of Results

Examination of the curves for the quercetin-molybdenum chelate (Figure V) indicates that those for pH 4.00 and 4.80 (curves 4 and 5) are very similar and are displaced to the greatest degree from the curve for quercetin alone (curve 1 in Figures V, VI and VII). All solutions were deep yellow at the time the spectra were recorded.

In the case of the quercetin-tungsten chelate, the solutions at pH 2.15 and 3.15 were a light greenish-yellow color on initial mixing but a flocculent precipitate separated before these samples could be run in the spectrophotometer. The solution at pH 3.90 remained clear, but gave a definite Tyndall effect, indicating a colloidal dispersion. It was still relatively clear 72 hours later. The solutions of pH 5.0, 5.90 and 7.5 (curves 3, 4 and 5, Figure VI) were pale yellow in color. Again the solution of pH 5.0 seemed to be optimum for color development.

The solution of the quercetin-chromium chelate at low pH (curves 2 and 3, Figure VII) had a slight orange cast on initial mixing which became more pronounced on standing. Curves 4, 5 and 6, corresponding to pH values of 4.0, 5.0 and 6.0, respectively, were very similar to each other indicating little change in the spectra over this range. Solutions of pH 4.0 through 7.2 were all bright yellow on initial mixing although all of them developed a slight

orange cast on standing. In view of the known sensitivity of quercetin to alkali, the evidence again seemed to indicate a pH of 5.0 as optimum for color development.

Effect of Variation of Quercetin Reagent and Alcohol

In order to determine the amount of reagent necessary to produce maximum absorption, a series of solutions of fixed metal ion concentration, but containing variable amounts of quercetin, was prepared. The absorbances were measured against a reagent blank containing the same amount of quercetin.

In conjunction with this series of tests, the effect on the absorbance of variation in the amount of ethyl alcohol present was examined for a sample containing molybdenum.

Reagents and Equipment

Standard Mo(VI) Solution

This solution was prepared by dissolving 1.840 g of dry Baker and Adamson reagent grade $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in distilled water and making up to exactly one liter with water. The solution contained 1 mg Mo(VI)/ml or 1000 ppm of the metal ion.

Standard W(VI) Solution

This solution was prepared by dissolving 1.795g of dry Baker

and Adamson reagent grade $Na_2WO_4 \cdot 2H_2O$ in distilled water and making up to exactly one liter with water. The solution contained 1 mg W(VI)/ml or 1000 ppm of the metal ion.

Standard Cr(VI) Solution

This solution was prepared by dissolving 3.735g of dry Baker and Adamson reagent grade K_2CrO_4 in distilled water and making up to exactly one liter with water. The solution contained 1 mg Cr(VI)/ml or 1000 ppm of the metal ion.

Spectrophotometer

A Beckman Model B spectrophotometer was used with 1.0 cm matched silica cells for the absorbance measurements made in this series of tests.

Procedure

In order to study the effect of reagent concentration on the absorbance, solutions of molybdenum (VI), tungsten (VI) and chromium (VI) containing 100 ppm of the metal ion were prepared. A series of solutions containing 0.05 to 0.1 ml of the 100 ppm solution of the particular metal ion under investigation, 0.5 ml of the acetate buffer, 3.0 ml of 95 percent ethanol and variable amounts of 10^{-3}M- and 10^{-2}M-alcoholic solutions of quercetin was set up, diluted to

10.0 ml total volume with distilled water, and the absorbances read 30 minutes later at the appropriate wavelength on the Beckman Model B spectrophotometer against individual reference solutions containing the appropriate amounts of quercetin, buffer and ethanol. The results of these tests are summarized in Table 3 and Figure VIII.

In conjunction with these tests, the effect of alcohol concentration on the absorbance was examined. A series of solutions was set up containing 0.08 ml of the 100 ppm molybdenum (VI) solution, 0.5 ml of acetate buffer, variable amounts of 95 percent ethanol, and 1.0 ml of 10^{-2}M-alcoholic quercetin solution. These samples were diluted to 10.0 ml total volume with distilled water and the absorbances read 30 minutes later on the Beckman Model B spectrophotometer at 420 mμ against a reference solution containing 0.5 ml of acetate buffer, 3.0 ml of ethyl alcohol and 6.5 ml of distilled water. The results of these tests are summarized in Table 4.

Discussion of Results

An examination of the curves for molybdenum (VI) and tungsten (VI) (Figure VIII) indicates a definite leveling off of the absorbances at about 1.0 ml of 10^{-2}M-quercetin solution. Addition of more reagent has little or no effect on the absorbance. In the case of chromium (VI) no leveling off was observed. Instead, a gradual increase in absorbance was observed up to at least 3.0 ml of 10^{-2}M-quercetin.

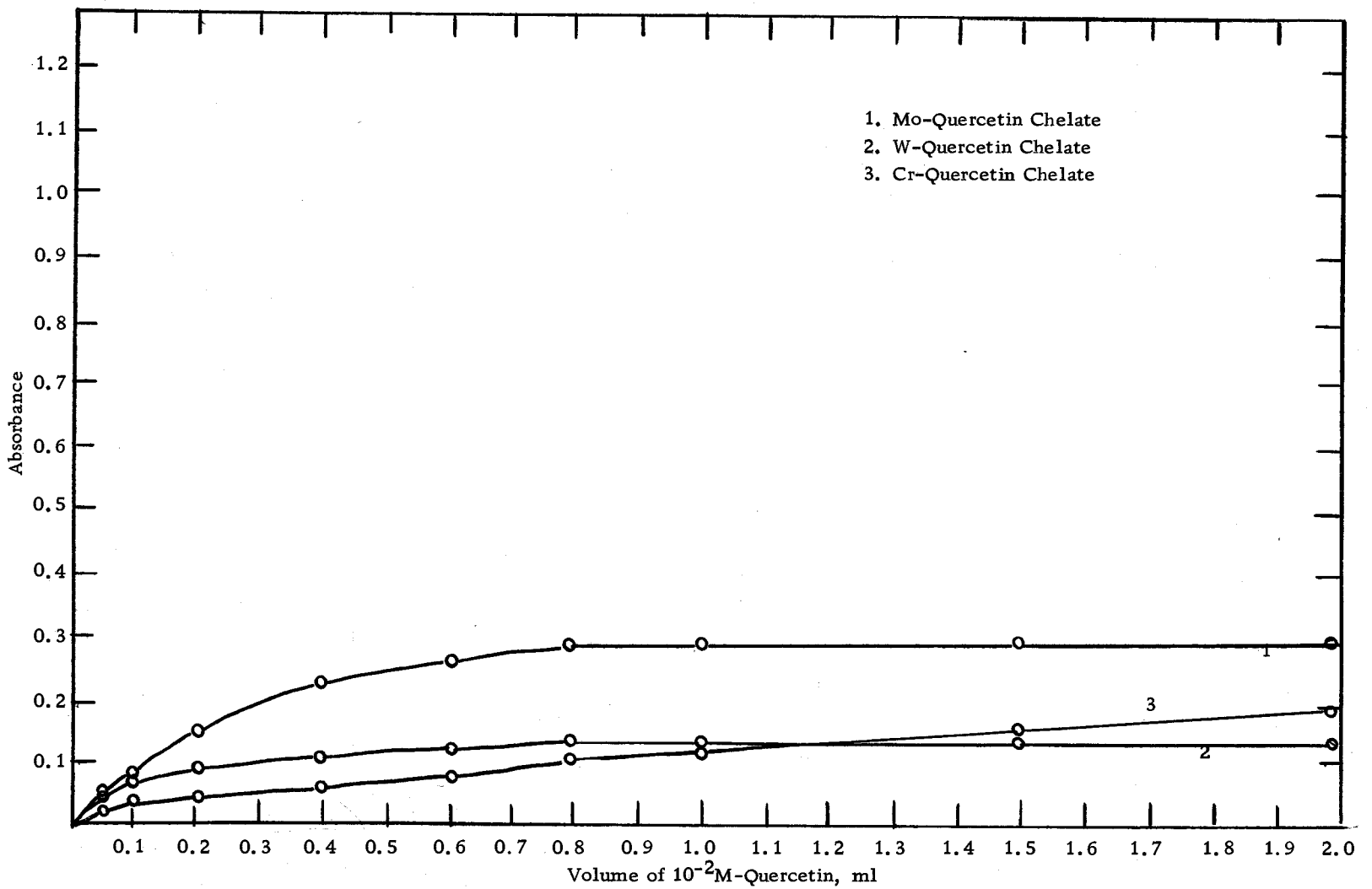


Figure VIII. Effect of Quercetin Concentration on Absorbance at Fixed Metal Ion Concentration

Table 3. Effect of Quercetin Concentration on Absorbance at Fixed Metal Ion Concentration

Solution No.	Volume of 10^{-2}M-Quercetin Solution, ml	Absorbances		
		Mo (VI) 420 mμ	W (VI) 420 mμ	Cr (VI) 435 mμ
1	0.05*		0.020	0.016
2	0.08*	0.013		
3	0.10*	0.018	0.027	0.020
4	0.50*	0.043	0.048	0.029
5	1.00*	0.076	0.063	0.036
6	0.20	0.149	0.073	0.039
7	0.40	0.222	0.100	0.056
8	0.60	0.256	0.112	0.071
9	0.80	0.280	0.125	0.102
10	1.00	0.280	0.125	0.111
11	1.50	0.284	0.127	0.149
12	2.00	0.264	0.103	0.174
13	3.00	0.233	0.105	0.233

* 10^{-3}M-Quercetin solution pH 5.0 (acetate buffer)

[Mo] = 8.33×10^{-6}M

[W] = 5.4×10^{-6}M

[Cr] = 9.6×10^{-6}M

Table 4. Effect of Alcohol Concentration on Absorbance

Solution No.	Total Volume of Ethanol ml	Volume Percent Ethanol	Absorbance
1	2.0	20.0	pptn.
2	3.0	30.0	0.310
3	4.0	40.0	0.272
4	5.0	50.0	0.280
5	5.0	60.0	0.292
6	7.0	70.0	0.292
7	8.0	80.0	0.284
8	9.0	90.0	0.291

All samples contain 0.8 ppm Mo

pH 5.0 (acetate buffer)

Each sample contains 1.0 ml 10^{-2}M-Quercetin

Total volume 10.0 ml

This, along with the change in color observed on standing, may be indicative of the presence of more than one complex in the solutions of chromium (VI) and quercetin.

Since quercetin and the quercetin-metal chelates have only a very limited solubility in water, ethyl alcohol must be added to increase the solubility of the complex. At concentrations less than 30 percent by volume immediate precipitation of reagent occurs. At 30 percent by volume alcohol concentration the solution was slightly turbid when read 30 minutes after dilution to volume, and within one hour extensive precipitation had occurred. No significant change in absorbance was observed for alcohol concentrations ranging from 40 to 90 percent by volume. Since it is desirable to maintain the alcohol concentration as low as possible because of the limited solubility of many inorganic salts in ethanol and still have enough alcohol present to stabilize the solution of the chelate, an optimum working concentration of 40 percent by volume was selected.

Rate of Color Development

Before arriving at a uniform procedure to be followed in the investigation of the three metal chelates, it was necessary to examine the absorbance as a function of time and to determine the time required for maximum color development.

Reagents and Equipment

All reagents and equipment used have been previously described.

Procedure

Samples containing 4 ppm of the metal ion, 0.5 ml of the acetate buffer, 3.0 ml of 95 percent ethanol and 1.0 ml of 10^{-2} molar quercetin solution were diluted to a total volume of 10.0 ml with distilled water and the absorbances read at regular intervals through the day. The results are tabulated in Table 5.

Discussion of Results

In the case of molybdenum and tungsten the absorbances seemed to have leveled off after one half hour, indicating maximum color development. This was not the case with chromium where the color seemed to be 80 percent developed at the end of 30 minutes, but continued to increase in absorbance over the next three to five hours. For convenience and reproducibility of results the time was arbitrarily taken as 30 minutes following dilution to volume, and this time factor has been observed in all of the readings taken in the work that follows.

At this stage a uniform procedure for all three metals was evolved. Because of the limited solubility of both the reagents and

Table 5. Rate of Color Development

Metal Ion	Time hr.	Absorbance	Percent Transmittance
Mo	0.25	0.469	34.0
	0.50	0.600	25.1
	1.0	0.595	25.4
	2.0	0.616	24.2
	3.0	0.611	24.5
	5.0	0.606	24.8
	7.0	0.622	23.9
	8.0	0.629	23.5
W	0.5	0.248	56.5
	1.0	0.250	56.2
	2.0	0.250	56.2
	3.0	0.243	57.2
	5.0	0.229	59.0
	6.0	0.231	58.8
	7.0	0.226	59.5
	8.0	0.216	60.8
Cr	0.5	0.740	18.2
	1.0	1.20	6.26
	2.0	2.00	1.00
	3.0	2.08	0.84
	5.0	2.22	0.60
	23.0	2.52	0.30

All samples contain 4 ppm of metal ion

1.0 ml of 10^{-2}M-quercetin added

the metal chelates the order of addition of the various reactants was important. The aqueous solution of the metal ion was added first, followed by 0.5 ml of the acetate buffer. Then 3.0 ml of 95 percent ethanol was added, followed by 1.0 ml of a 10^{-2} molar solution of quercetin in 95 percent ethanol. The sample was made up to 10.0 ml with distilled water. The absorbance was read one half hour later against a reference solution containing the same amounts of buffer, ethanol and quercetin reagent.

CHAPTER IV

PROPERTIES OF THE Mo(VI)-QUERCETIN CHELATE

When an alcoholic solution of quercetin is added to a solution buffered at pH5 and containing hexavalent molybdenum, a deep yellow coloration develops due to the formation of the molybdenum-quercetin chelate. It is the purpose of this investigation to determine whether or not the chelate formed under these conditions conforms to Beer's law, the combining ratio of quercetin to metal ion in the chelate, and the effect of various metal ions on the absorbance using the method of color development previously described.

Adherence to Beer's Law

The linearity between the absorbance of the molybdenum-quercetin complex and the molybdenum concentration was tested by setting up a series of solutions containing varying amounts of the metal ion and measuring the absorbances. Following the standard procedure, appropriate aliquots of a 100 ppm solution of molybdenum (VI) were added to each flask, followed by 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol, 1.0 ml of 10^{-2}M-alcoholic quercetin solution, and dilution to a total volume of 10.0 ml with distilled water. The absorbances were read 30 minutes later at 420mμ on the Beckman Model B spectrophotometer. The results are

summarized in Table 6 and Figure IX.

The concentration range covered by the solutions above varied from 0. 1 ppm to 2. 0 ppm. A similar series of measurements was made at 450 mμ using concentrations of molybdenum (VI) ranging from 1 to 15 ppm. These results are summarized in Table 7 and Figure X.

Reagents and Equipment

Standard Mo(VI) Solution

This solution was prepared by dissolving 1. 8400g of Baker and Adamson reagent grade $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in distilled water and making up to exactly one liter with water. The solution contained 1 mg Mo per milliliter or 1000 ppm of metal ion. Less concentrated solutions were prepared by diluting this standard solution to the desired volume.

Buffer Solution

The solution is the same as that described previously. It was prepared by dissolving 27. 5 g of C. P. anhydrous sodium acetate and 40. 0 g of glacial acetic acid in water and diluting to 500. 0 ml with distilled water. The pH was checked on a Beckman Model 72 pH meter and found to be 4. 45

Table 6. Beer's Law Plot for Molybdenum-Quercetin Chelate at 420 mμ

Solution No.	Concentration of Mo(VI) ppm	Absorbance	Percent Transmittance	Absorptancy (100 minus Transmittancy)
1	0.1	0.025	94.5	5.5
2	0.2	0.071	85.0	15.0
3	0.4	0.144	71.8	28.2
4	0.6	0.220	60.2	39.8
5	0.8	0.286	51.8	48.2
6	1.0	0.354	44.3	55.7
7	1.2	0.407	39.2	60.8
8	1.5	0.516	30.5	69.5
9	1.8	0.602	25.0	75.0
10	2.0	0.642	22.8	77.2

Wavelength 420 mμ
pH 5.0 (acetate buffer)
1.0 cm cells

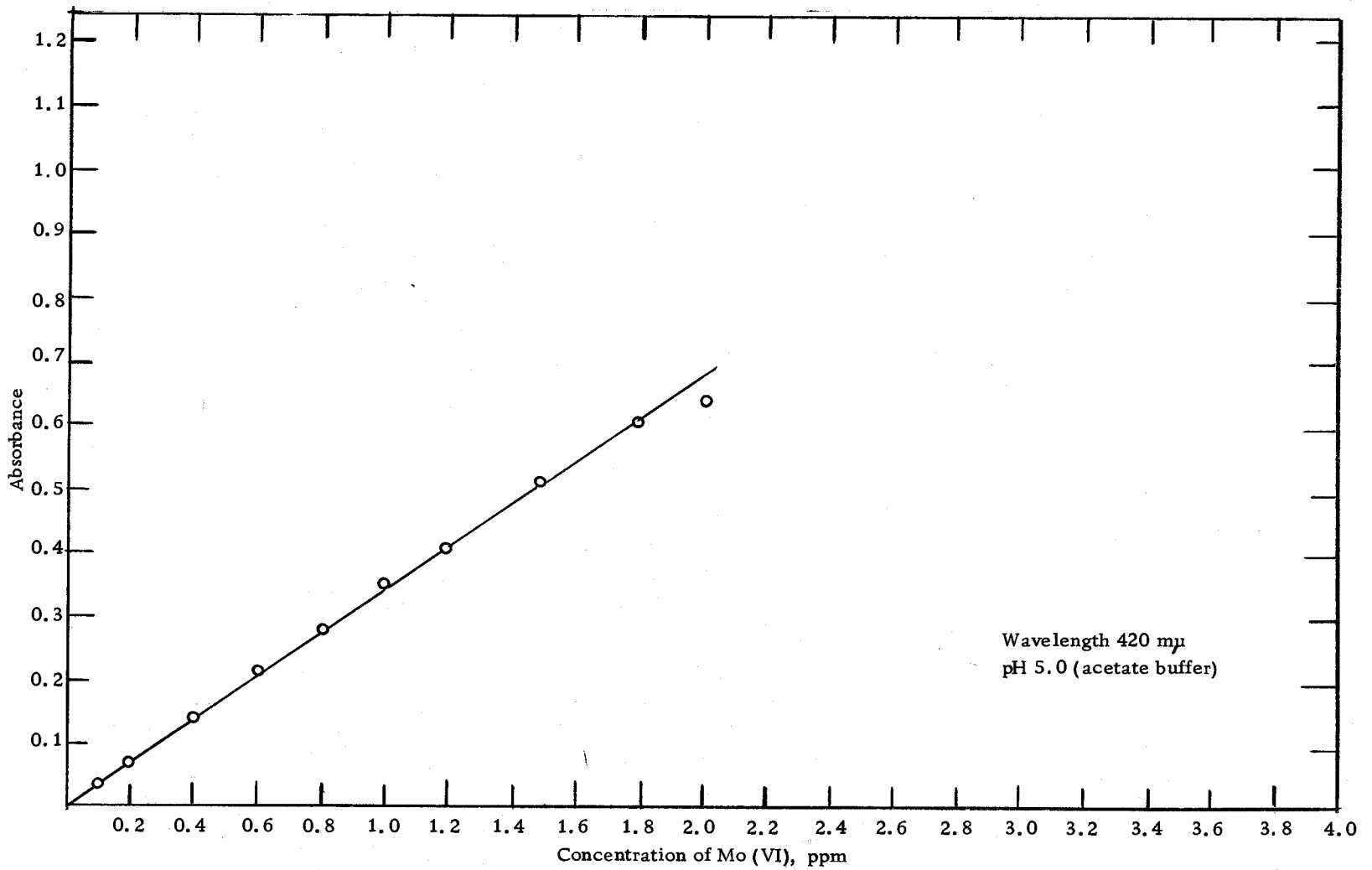


Figure IX. Beer's Law Plot for Molybdenum-Quercetin Chelate at 420 mμ

Table 7. Beer's Law Plot for Molybdenum-Quercetin Chelate at 450 mμ

Solution No.	Concentration of Mo(VI) ppm	Absorbance	Percent Transmittance	Absorptancy (100 minus transmittancy)
1	1	0.187	65.0	35.0
2	3	0.486	32.7	67.3
3	5	0.777	16.7	83.3
4	7	1.09	8.22	91.8
5	9	1.34	4.58	95.4
6	11	1.65	2.25	97.8
7	13	1.88	1.32	98.7
8	15	2.19	0.65	99.4

Wavelength 450 mμ
pH 5.0 (acetate buffer)
1.0 cm cells

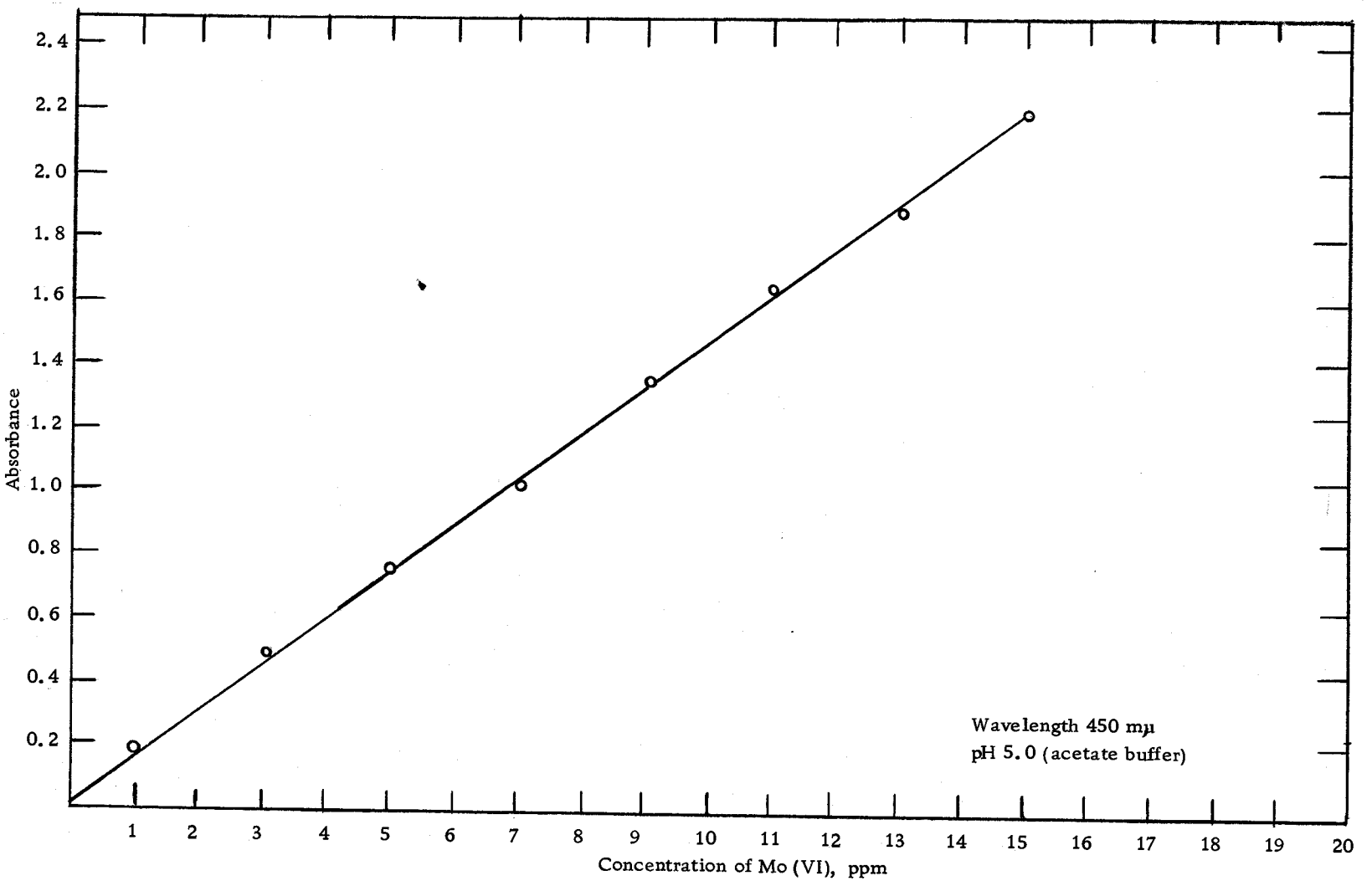


Figure X. Beer's Law Plot for Molybdenum-Quercetin Chelate at 450 mμ

Spectrophotometer

Absorbance measurements were made on a Beckman Model B spectrophotometer and on a Beckman Model DU spectrophotometer using matched 1.0 cm pyrex cells.

Discussion of Results

The straight line plots through the origin obtained at both 420 mμ in the concentration range of 0.1 to 1.8 ppm of molybdenum and at 450 mμ in the concentration range of 1 to 15 ppm of molybdenum indicates conformity to Beer's law. The molar absorptivity for the chelate at 420 mμ is 34,000 cm^{-1} $mole^{-1}$ l. At 450 mμ the molar absorptivity is 15,000 cm^{-1} $mole^{-1}$ l.

As pointed out by Ayres (1, p. 652-657) a straight line plot merely indicates adherence to Beer's law and does not show directly the concentration range for maximum precision. The optimum range for highest precision was determined by plotting the percent absorptancy (100 minus the percent transmittance) against the log of the molybdenum concentration. An "S" shaped curve results, with the optimum range of concentration being established from the linear portion of the curve having maximum slope. At 420 mμ this would be in the range of 0.1 to 1.2 ppm molybdenum (Figure XI). The calculated value of the coefficient of variation derived from the maximum

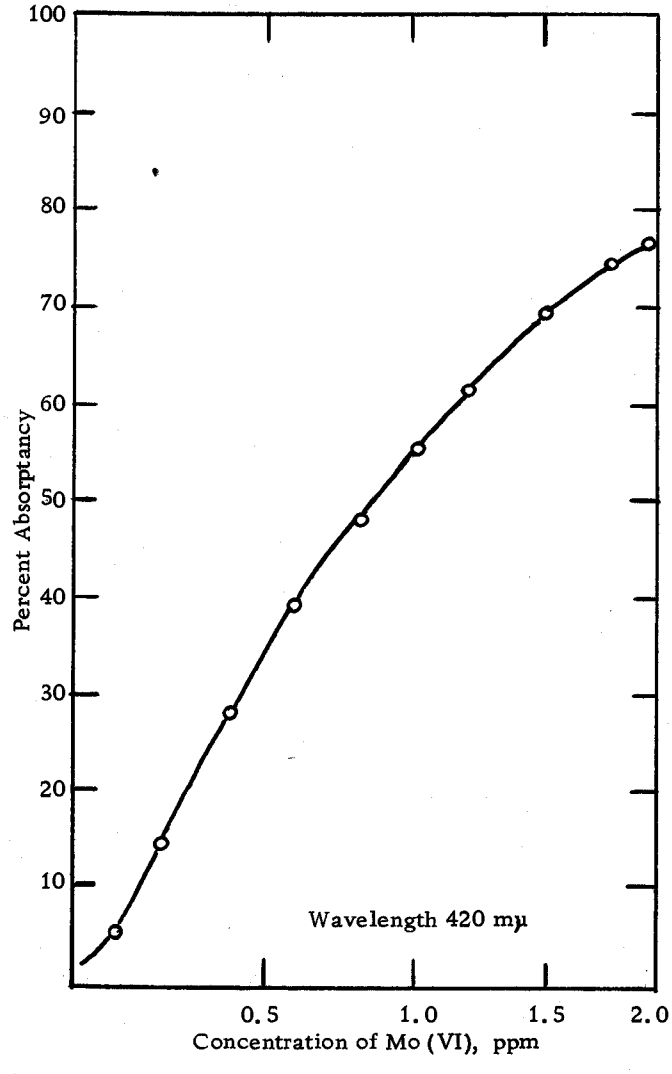


Figure XI. Ringbom Plot for Molybdenum-Quercetin Chelate at 420 mμ

slope, for a variation of 1 percent in the photometric error, is about 3 percent over this range. At 450 mμ, the optimum concentration range would be in the neighborhood of 1 to 3 ppm of molybdenum (Figure XII).

Stability of Mo(VI) Chelate

A check on the rate of color formation and the stability of the Mo(VI)-quercetin chelate was obtained for solutions number 1 and 3 of Table 7. The absorbances read 15 minutes after dilution to volume were 0.108 and 0.580 respectively; after 30 minutes the absorbances were 0.187 and 0.777. Some further slight increase in color occurred on longer standing, but for convenience and reproducibility the standard time was arbitrarily set at 30 minutes. The color was stable 24 hours later and the absorbance only slightly greater than that reached at the end of 30 minutes.

Spectrophotometric Determination of the Empirical Formula Slope-Ratio Method

According to Harvey and Manning (15, p. 4488-4493) the empirical formula of a soluble chelate may be determined in the following manner. Two series of solutions are prepared. In the first, variable amounts of the chelating agent are added to a large excess of the metal ion; in the second, different quantities of the metal ion

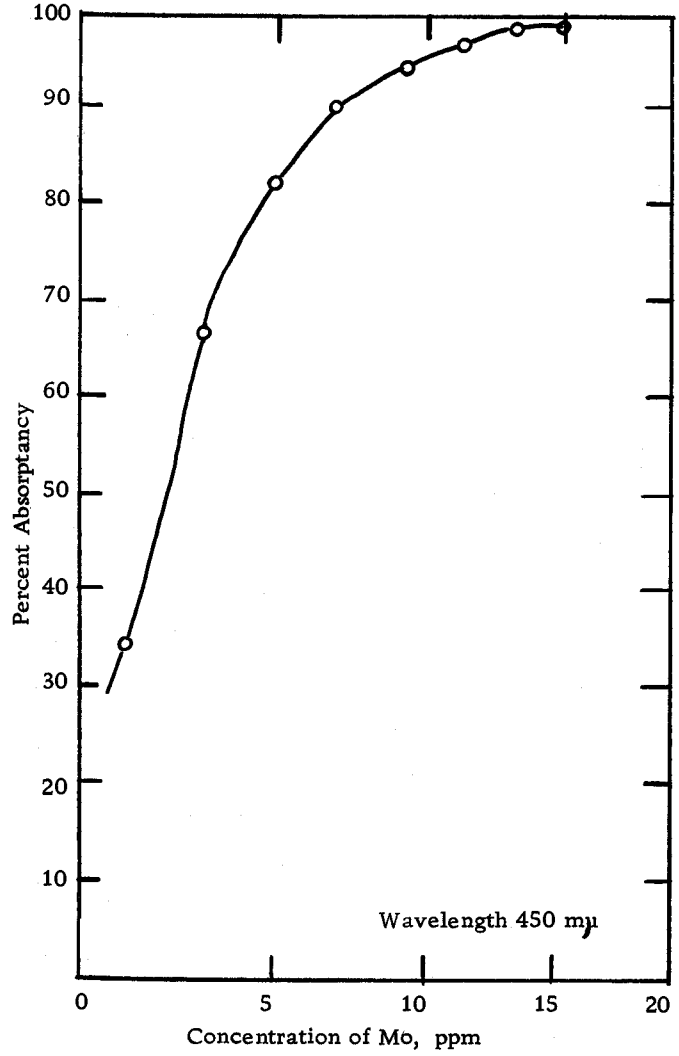


Figure XII. Ringbom Plot for Molybdenum-Quercetin Chelate at 450 mμ

are added to a large excess of the chelating agent. In each case the absorbance is plotted against the concentration of the variable component. The combining ratio of the components in the complex is equal to the ratio of the slopes of the two straight lines obtained.

Procedure

In the first series of solutions the standard Mo(VI) solution was diluted with an equal volume of water to give a molybdenum concentration of 5.2×10^{-3}M. The quercetin solution was diluted to a concentration of 1×10^{-3}M. The general composition of the solutions in this series was as follows: 1.0 ml of the 5.2×10^{-3} M-molybdenum solution, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and variable amounts of the 10^{-3}M-quercetin solution. The samples were diluted to 10.0 ml total volume and the absorbances read one half hour later in the Beckman Model B spectrophotometer against individual reference solutions containing the same amount of acetate buffer, ethanol and quercetin reagent.

A second series of measurements was made holding the quercetin concentration constant and varying the molybdenum concentration. In this case the standard quercetin solution was diluted with ethanol to give a 5.2×10^{-3}M solution. The standard molybdenum solution was diluted with water so as to obtain a 1.04×10^{-3}M solution. The general composition of the solutions in this series

was as follows: variable amounts of the 1.04×10^{-3}M-molybdenum solution, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and 1.0 ml of the 5.2×10^{-3}M-quercetin solution. The samples were diluted to 10.0 ml total volume and the absorbances read one half hour later at 450 mμ in the Beckman Model B spectrophotometer against a reagent blank.

The data for these measurements are tabulated in Tables 8A and 8B and plotted in Figure XIII.

Spectrophotometric Determination of the Empirical Formula "Gerade" Method of Asmus

The determination of the empirical formula by the slope-ratio method was checked by the "gerade" method of Asmus (3, p. 104-116). This method is particularly useful for complexes of intermediate strength which are only moderately dissociated. For a reaction of the general type $A + n\,B \rightleftarrows AB_n$, where A is the coordinating metal ion and B the ligand, the method involves the addition of increasing volumes of the chelating agent to a constant volume of the metal ion in a volumetric flask. The absorbances are measured for all solutions prepared and the extinction modulus, m , calculated. This quantity is defined as the quotient of the absorbance divided by the path length. The curves obtained by plotting $\frac{1}{m}$ versus $\frac{1}{v^n}$ where v is the volume of the ligand solution and $n = 1, 2, 3, \ldots$

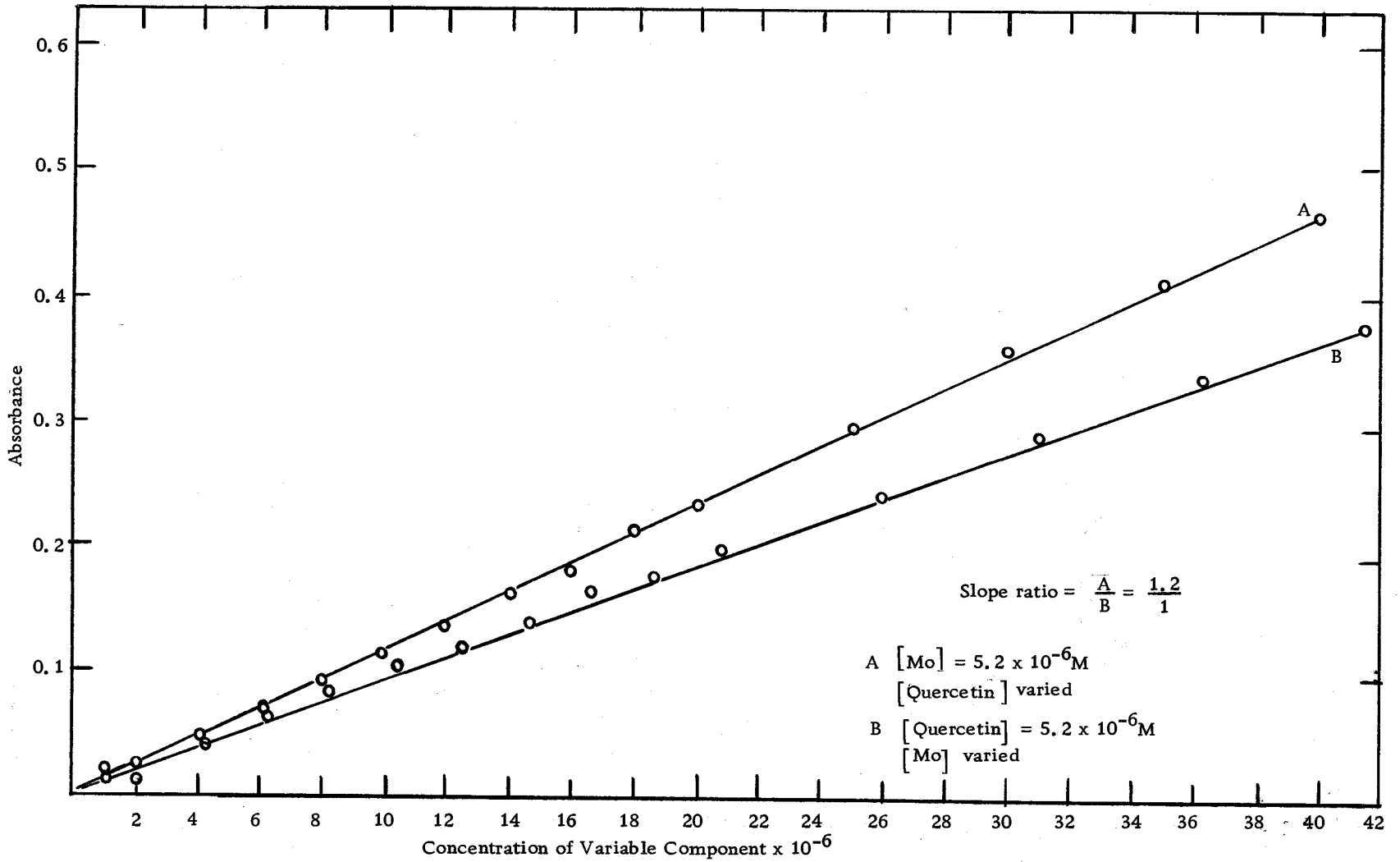


Figure XIII. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Molybdenum-Quercetin Chelate

Table 8A. Spectrophotometric Determination of Empirical Formula. Slope-Ratio Method - Variation of Quercetin Concentration

Sample No.	Volume of Quercetin Soln. ml	[Quercetin] $x10^{-6}$	A	Percent T
1	0.01	1.0	0.007	98.5
2	0.02	2.0	0.013	97.0
3	0.04	4.0	0.037	92.0
4	0.06	6.0	0.056	88.0
5	0.08	8.0	0.081	83.0
6	0.10	10.0	0.097	80.0
7	0.12	12.0	0.122	75.5
8	0.14	14.0	0.155	70.0
9	0.16	16.0	0.174	67.0
10	0.18	18.0	0.208	62.0
11	0.20	20.0	0.226	59.5
12	0.25	25.0	0.292	51.0
13	0.30	30.0	0.362	43.5
14	0.35	35.0	0.409	39.0
15	0.40	40.0	0.456	35.0
16	0.45	45.0	0.523	30.0
17	0.50	50.0	0.585	26.0

[Mo] = 5.2×10^{-4}M after dilution

[Quercetin] varied

Absorbances at 450 mμ

Table 8B. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Variation of Molybdenum Concentration

Sample No.	Volume of Mo Solution ml	[Mo] $x10^{-6}$	A	Percent T
1	0.01	1.04	0.023	94.8
2	0.02	2.08	0.025	94.5
3	0.04	4.16	0.039	91.5
4	0.06	6.24	0.062	86.8
5	0.08	8.32	0.081	83.0
6	0.10	10.4	0.102	79.0
7	0.12	12.5	0.119	76.0
8	0.14	14.6	0.136	73.2
9	0.16	16.6	0.161	69.0
10	0.18	18.7	0.174	67.0
11	0.20	20.8	0.194	64.0
12	0.25	26.0	0.233	58.5
13	0.30	31.2	0.284	52.0
14	0.35	36.4	0.328	47.0
15	0.40	41.6	0.367	43.0
16	0.45	46.8	0.409	39.0
17	0.50	52.0	0.456	35.0

[Quercetin] = 5.2×10^{-4}M after dilution

[Mo] varied

Absorbances at 450 mμ

will include a straight line for that value of n equal to the coordination number of the complex.

Reagents and Equipment

Standard Mo(VI) Solution

A standard solution of Mo(VI) was prepared by dissolving 2.7603 g of dry Baker and Adamson reagent grade $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in distilled water and making up to exactly 100.0 ml with water. This solution contains 15 mg of Mo per ml.

Standard Quercetin Solution

A 10^{-4} M-quercetin solution was prepared by diluting 1.0 ml of the standard 10^{-2} molar solution of quercetin with 95 percent ethanol to a final volume of 100.0 ml.

All other reagents and equipment have been described previously.

Procedure

A series of ten 50.0 ml volumetric flasks was set up, and to each was added 1.0 ml of the Mo(VI) solution (15 mg Mo(VI) per ml), 2.5 ml of the acetate buffer, and 15.0 ml of 95 percent ethanol. Variable amounts of the 10^{-4}M solution of quercetin, ranging from

3.0 ml to 30.0 ml in increments of three ml, were then added and the solutions made up to volume with distilled water. The absorbances were read 30 minutes after dilution to volume. All measurements were made at 450 mμ using the Beckman Model B spectrophotometer, against a reagent blank.

The data for these measurements are tabulated in Table 9 and plotted in Figure XIV.

Discussion of Results

In the slope-ratio method, the combining ratio in the complex is given by the ratio of the slopes of the two straight lines formed when the absorbance is plotted against the concentration of the variable component. The ratio of the slopes of lines A and B in Figure XIII is 1.2:1. In all probability this corresponds to a 1:1 complex.

Since the "gerade" method of Asmus is particularly useful for complexes of intermediate strength which are only moderately dissociated, it was felt that this might be a better method to employ as a check on the results given by the slope-ratio method than the classical method of continuous variations of Job. The latter is much more satisfactory when one is dealing with strong complexes which are only very slightly dissociated. All elements of doubt were removed when this was done. The straight line obtained for n = 1 in the family of curves in Figure XIV clearly indicates a 1:1 complex.

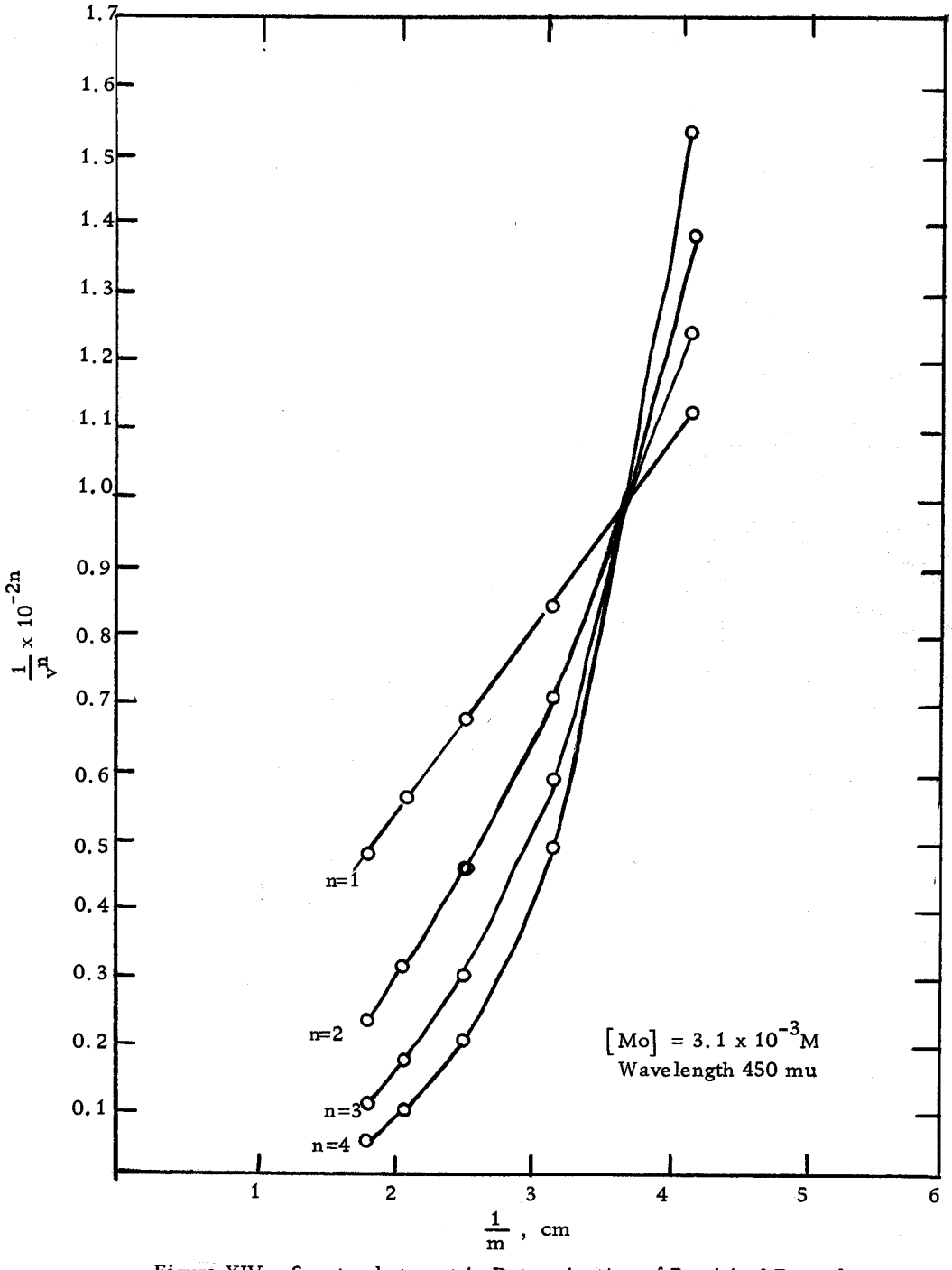


Figure XIV. Spectrophotometric Determination of Empirical Formula
"Gerade" Method of Asmus
Molybdenum-Quercetin Chelate

Table 9. Spectrophotometric Determination of Empirical Formula "Gerade" Method of Asmus Molybdenum-Quercetin Chelate

Solution No.	Volume of Quercetin ml	m cm^{-1}	$\frac{1}{m}$ cm	$\frac{1}{v} \times 10^{-2}$ l^{-1}	$\frac{1}{v^2} \times 10^{-4}$ l^{-2}	$\frac{1}{v^3} \times 10^{-6}$ l^{-3}	$\frac{1}{v^4} \times 10^{-8}$ l^{-4}
1	3.0	0.081	12.35				
2	6.0	0.158	6.33				
3	9.0	0.240	4.17	1.111	1.235	1.373	1.524
4	12.0	0.314	3.18	0.833	0.695	0.579	0.484
5	15.0	0.398	2.51	0.667	0.445	0.297	0.198
6	18.0	0.479	2.07	0.556	0.309	0.171	0.095
7	21.0	0.553	1.81	0.476	0.227	0.108	0.051
8	24.0			0.417	0.174	0.072	0.030
9	27.0			0.370	0.137	0.051	0.019
10	30.0			0.333	0.111	0.037	0.012

[Mo] = 3.12×10^{-3} M constant in all runs

[Quercetin] varied

Wavelength 450 mμ 1.0 cm cells

Spectrophotometric Determination of the Instability Constant

As the combining ratio of molybdenum to quercetin in the chelate has been shown to be 1:1, the dissociation of the complex can be expressed as

$$Mo\,Q \rightleftarrows Mo + Q$$

If c represents the total concentration of the complex and α its degree of dissociation, the instability constant can be expressed as

$$K = \frac{(\alpha c)(\alpha c)}{c(1-\alpha)} = \frac{\alpha^2 c}{(1-\alpha)}$$

Thus the overall instability constant can be calculated from the measurement of the degree of dissociation. The value of α can be determined spectrophotometrically from the relationship

$$\alpha = \frac{E_m - E_s}{E_m}$$

where E_m is the maximum absorbance of the chelate when it does not dissociate into metal ion and ligand and E_s is the actual absorbance of the complex.

The method of continuous variations of Job offers a method of determining the values E_m and E_s. E_s is the actual maximum on the continuous variations plot while E_m is obtained graphically from the same plot and is given by the intersection of the two slopes which are tangent to the continuous variations plot at the points where the

mole fraction of quercetin is zero and one.

Procedure

For this experiment 10^{-3} molar solutions of both molybdenum (VI) and quercetin were prepared. To each of a series of eleven flasks was added 0.5 ml of acetate buffer and varying volumes of 1.0×10^{-3}M-molybdenum (VI), 1.0×10^{-3}M-alcoholic quercetin solution and 95 percent ethanol so that for each sample the total number of mmoles of metal ion and ligand together was maintained constant at 1×10^{-3} mmole per 10.0 ml total volume, while the total volume of alcohol per 10.0 ml total volume was maintained constant at 4.0 ml. The flasks were then diluted to the mark with distilled water and the absorbances read 30 minutes later in the Beckman Model B spectrophotometer at 420 mμ. These results are summarized in Table 10 and Figure XV.

Discussion of Results

The method of continuous variations, while ideal when applied to strong complexes, suffers severe limitations when applied to weak (i.e. appreciably dissociated) complexes. Instead of obtaining two straight lines intersecting in a point the abscissa of which indicates the mole ratio of metal ion to ligand in the complex, one obtains a curve flattened at the top, the degree of flattening becoming

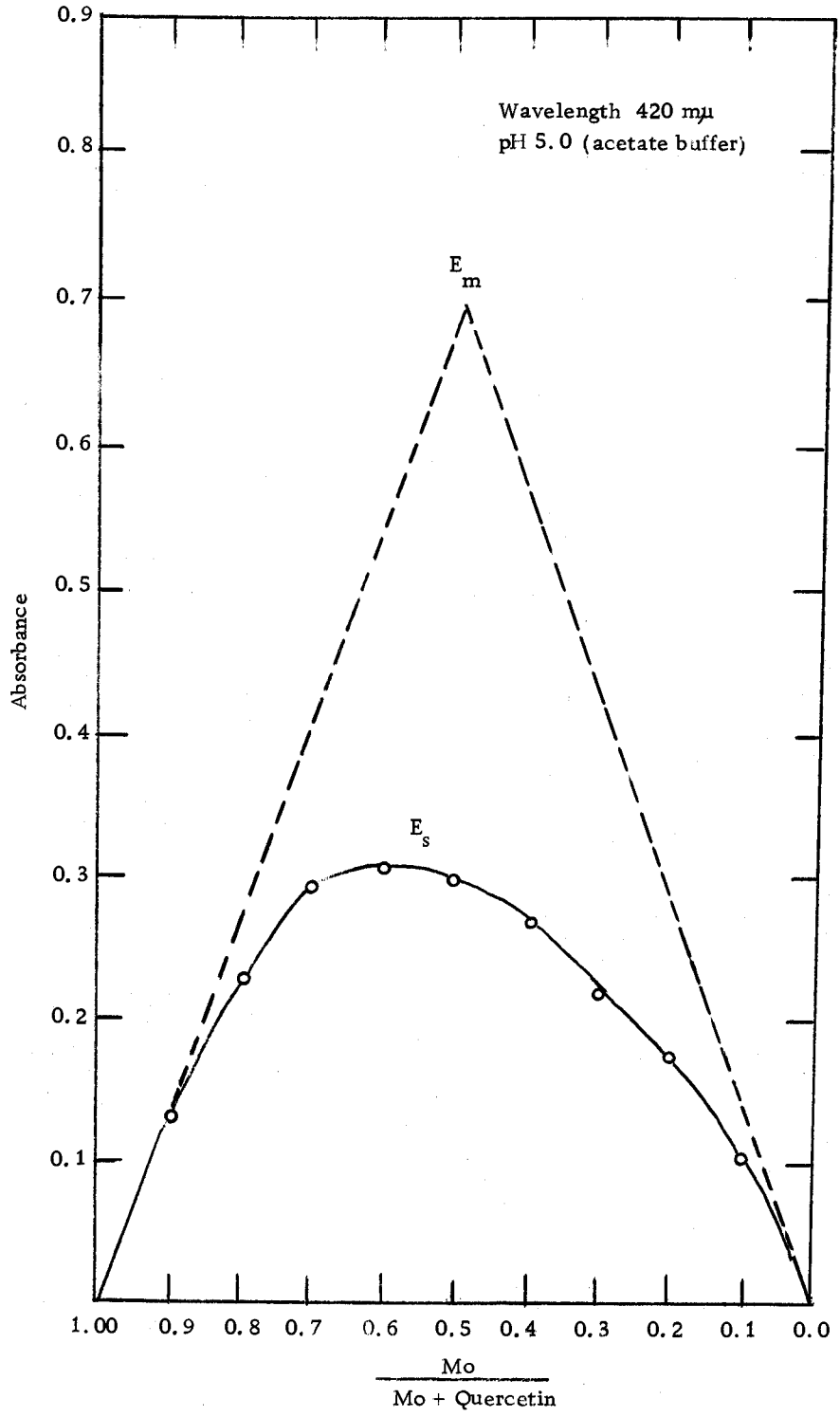


Figure XV. Job's Plot for Molybdenum-Quercetin Chelate

Table 10. Job's Plot for Molybdenum-Quercetin Chelate

Solution No.	Volume of Mo(VI) Solution ml	Volume of Quercetin Solution ml	Volume of Ethanol ml	Absorbance	$\frac{\text{Mo}}{\text{Mo + Quercetin}}$
1	1.00	0.00	4.00	0.000	1.00
2	0.90	0.10	3.90	0.131	0.90
3	0.80	0.20	3.80	0.229	0.80
4	0.70	0.30	3.70	0.292	0.70
5	0.60	0.40	3.60	0.305	0.60
6	0.50	0.50	3.50	0.297	0.50
7	0.40	0.60	3.40	0.268	0.40
8	0.30	0.70	3.30	0.222	0.30
9	0.20	0.80	3.20	0.174	0.20
10	0.10	0.90	3.10	0.104	0.10
11	0.00	1.00	3.00	0.000	0.00

[Mo] = 1.0×10^{-3}M before dilution

[Quercetin] = 1.0×10^{-3}M before dilution

pH 5.0 (acetate buffer), 1.0 cm cells

Total volume 10.0 ml

greater the weaker the complex. Thus, while one can usually recognize a 1:1 complex where the maximum is in the neighborhood of 50 percent, it becomes considerably more difficult to recognize a complex of the general form MX_2 and for a complex of the type MX_n when $n \geq 3$, it becomes almost impossible to recognize it from the curve unless it happens to be the special case of a very stable complex. In addition, with weak complexes there is great uncertainty in drawing the tangents to the curve at the points where the mole fraction of ligand is zero and one, thus producing a high degree of uncertainty in the value of E_m, the intersection of the two tangents.

The shape of the Job's plot obtained with molybdenum indicates a weak complex. In view of the above limitations, it is recognized that the value of the instability constant determined by this procedure is only approximate. For molybdenum, the values of E_m and E_s from Figure XV are 0.700 and 0.305 respectively, yielding a value of $a = 0.564$ and an approximate value of 3.9×10^{-5} for the instability constant.

Interfering Ions

The effect of a number of other ions on the absorbance of the molybdenum-quercetin chelate at concentration levels of 1 and 5 ppm of molybdenum (VI) has been determined. The concentration level of the interfering ion was varied from 1 to 10 ppm. The ions

tested included aluminum, bismuth, boron, cadmium, cerium (III and IV), cobalt (II), copper (II), iron (III), lead, mercury (I and II), nickel, silver, thallium (I), thorium (IV), uranium (VI), vanadium (V), zinc and zirconium (IV). The alkali metal ions and the alkaline earths were checked qualitatively, but since no difference in the intensity of color could be detected visually when compared with the solutions containing molybdenum alone, the actual absorbances were not measured.

Reagents and Equipment

Standard Metal Ion Solutions

Baker and Adamson reagent grade chemicals were used in the preparation of all of the standard metal ion solutions unless noted to the contrary. All solutions contain 100 ppm of the metal ion. The amounts of the various salts used, along with the solvent solution used, are recorded in Table 11.

The sample of thallous nitrate was supplied by Amend Drug and Chemical Company, Inc. The cerous chloride was purchased from Fisher Scientific Company and the ceric ammonium sulfate from G. Frederick Smith Company.

Procedure

The following procedure was used to determine the effect of

Table 11. Standard Metal Ion Solutions for Interference Tests

Ion	Salt Used	Amount per liter, g	Solvent
Al	$Al(NO_3)_3 \cdot 9H_2O$	1.3910	0.024M HNO_3
Bi	$Bi(NO_3)_2 \cdot 5H_2O$	0.2350	3.0M HNO_3
B	$Na_2B_4O_7 \cdot 10H_2O$	0.8800	Water
Cd	$Cd(NO_3)_2 \cdot 4H_2O$	0.2750	Water
Ce(III)	$CeCl_3 \cdot 7H_2O$	0.2660	0.6M HNO_3
Ce(IV)	$(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$	0.3090	0.6M HNO_3
Co	$Co(NO_3)_2 \cdot 6H_2O$	0.4950	Water
Cu	$Cu(NO_3)_2 \cdot 3H_2O$	0.3800	Water
Fe(III)	$Fe(NO_3)_3 \cdot 9H_2O$	0.7230	0.024M HNO_3
Pb	$Pb(NO_3)_2$	0.1600	Water
Hg(II)	$Hg(NO_3)_2$	0.1680	0.16M HNO_3
Hg(I)	$Hg_2(NO_3)_2 \cdot 2H_2O$	0.1430	0.6M HNO_3
Ni	$Ni(NO_3)_2 \cdot 6H_2O$	0.4970	Water
Ag	$AgNO_3$	0.1580	Water
Tl(I)	$TlNO_3$	0.1300	Water
Th(IV)	$Th(NO_3)_4 \cdot 4H_2O$	0.2400	Water
U(VI)	$UO_2(C_2H_3O_2)_2 \cdot 2H_2O$	0.1780	Water
V(V)	NH_4VO_3	0.2300	0.6M HNO_3
Zn	$Zn(C_2H_3O_2)_2 \cdot 2H_2O$	0.3360	Water
Zr(IV)	$Zr(NO_3)_4 \cdot 5H_2O$	0.4710	0.6M HNO_3

these various metals on the absorbances of the molybdenum-quercetin chelate. Six samples were set up in each case, one containing 1 ppm Mo (VI) and the other five containing 5 ppm Mo (VI). Various volumes of the standard metal ion solution were added to each flask, sufficient to supply 1, 1, 3, 5, 7, and 10 ppm of interfering ion, respectively. Then to each solution was added 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol, and 1.0 ml of 10^{-2} molar solution of quercetin in 95 percent ethanol. The solutions were made up to 10.0 ml total volume with water and the absorbances read 30 minutes later on the Beckman Model DU spectrophotometer at 450 mμ against a reagent blank.

The results are tabulated in Table 13.

Discussion of Results

Analysis of the results of the interference tests indicates that Al, Cu, Fe (III), V (V), and Zr (IV) interfere seriously with the molybdenum determination. Bismuth (III) and Pb(II) interfere moderately. Under the conditions employed B, Cd, Ce(III and IV), Co, Hg(I and II), Ni, Ag, Tl(I), Th(IV), U(VI) and Zn did not interfere.

It is interesting to note that neither Th(IV) nor U(VI) interfered at the concentrations used, although both of the ions have been reported to form complexes with quercetin. Tungsten (VI) and

Table 12. Calibration Data for Molybdenum Determination

ppm Mo(VI)	Absorbance	Absorptivity
1.0	0.187	187
3.0	0.486	162
5.0	0.777	155
7.0	1.090	156
9.0	1.340	149
11.0	1.650	150
13.0	1.880	145
15.0	2.190	146

Average absorptivity a = 156

Wavelength 450 mμ 1.0 cm cells

Table 13. Interfering Ions - Molybdenum Determination

Ion Tested	Interfering Ion ppm	Mo(VI) ppm	Absorbance	Absorptivity	Ion Tested	Interfering Ion ppm	Mo(VI) ppm	Absorbance	Absorptivity
Al	1	1	0.801	801	Hg(II)	1	1	0.182	182
	1	5	1.06	212		1	5	0.796	159
	3	5	2.40	480		3	5	0.793	159
	5	5	3.00	600		5	5	0.824	165
	7	5	ppt	--		7	5	0.883	177
	10	5	ppt	--		10	5	1.00	200
Bi	1	1	0.218	218	Hg(I)	1	1	0.188	188
	1	5	0.866	173		1	5	0.767	153
	3	5	0.939	188		3	5	0.726	145
	5	5	0.991	198		5	5	0.839	168
	7	5	1.12	224		7	5	0.955	191
	10	5	1.28	256		10	5	0.987	197
B	1	1	0.174	174	Ni	1	1	0.171	171
	1	5	0.755	151		1	5	0.796	159
	3	5	0.767	153		3	5	0.775	155
	5	5	0.780	156		5	5	0.818	164
	7	5	0.752	150		7	5	0.799	160
	10	5	0.745	149		10	5	0.818	164
Cd	1	1	0.202	202	Ag	1	1	0.189	189
	1	5	0.757	151		1	5	0.712	142
	3	5	0.678	136		3	5	0.721	144
	5	5	0.775	155		5	5	0.818	164
	7	5	0.791	158		7	5	0.721	144
	10	5	0.717	143		10	5	0.720	145
Ce(III)	1	1	0.136	136	Tl(I)	1	1	0.124	124
	1	5	0.710	142		1	5	0.697	139
	3	5	0.688	138		3	5	0.724	145
	5	5	0.712	142		5	5	0.679	136
	7	5	0.724	145		7	5	0.703	141
	10	5	0.717	143		10	5	0.717	143
Ce(IV)	1	1	0.168	168	Th(IV)	1	1	0.260	260
	1	5	0.728	146		1	5	0.815	163
	3	5	0.726	145		3	5	0.886	177
	5	5	0.731	146		5	5	0.765	153
	7	5	0.740	148		7	5	0.733	147
	10	5	0.733	147		10	5	0.928	186
Co	1	1	0.192	192	U(VI)	1	1	0.202	202
	1	5	0.791	158		1	5	0.777	155
	3	5	0.757	151		3	5	0.750	150
	5	5	0.783	157		5	5	0.818	164
	7	5	0.770	154		7	5	0.821	164
	10	5	0.775	155		10	5	0.863	173
Cu(II)	1	1	0.392	392	V(V)	1	1	0.426	426
	1	5	0.971	194		1	5	0.925	185
	3	5	1.39	278		3	5	1.23	246
	5	5	1.72	344		5	5	1.40	280
	7	5	1.97	394		7	5	1.76	352
	10	5	ppt	--		10	5	1.97	392
Fe III)	1	1	1.04	1040	Zn	1	1	0.161	161
	1	5	1.57	314		1	5	0.770	154
	3	5	2.74	548		3	5	0.740	148
	5	5	ppt	--		5	5	0.777	155
	7	5	ppt	--		7	5	0.796	159
	10	5	ppt	--		10	5	0.810	162
Pb	1	1	0.122	122	Zr(IV)	1	1	0.393	393
	1	5	0.745	149		1	5	0.796	159
	3	5	0.662	132		3	5	0.956	191
	5	5	0.498	100		5	5	1.00	200
	7	5	0.444	89		7	5	1.11	222
	10	5	0.233	47		10	5	1.54	308

Cr(VI) interfere seriously, of course, although data for these two metals are not included here.

The alkali metals and the alkaline earths do not interfere. These were examined qualitatively to determine whether any increase or decrease in color occurred when they were present with 1 and 5 ppm Mo(VI).

CHAPTER V

PROPERTIES OF THE W(VI)-QUERCETIN CHELATE

When an alcoholic solution of quercetin is added to a solution containing tungstate ion buffered at pH 5.0, a pale yellow color develops due to the formation of the tungsten-quercetin chelate. It is the purpose of this investigation to ascertain whether or not this chelate conforms to Beer's law, and to determine the empirical formula of the complex and the nature and extent of the interferences offered by the presence of various ions on the spectrophotometric determination of tungsten utilizing the tungsten-quercetin chelate.

Adherence to Beer's Law

Conformity to Beer's law was checked by setting up a series of solutions using appropriate aliquots of the standard tungsten (VI) solution (1 mg W(VI) per ml) so that, after dilution to volume, they would contain 1, 3, 5, 7, 9, 11, 13, and 15 ppm W(VI), respectively. Then, following the same standard procedure as with molybdenum, 0.5 ml of acetate buffer was added to each sample, followed by 3.0 ml of 95 percent ethanol. One milliliter of 10^{-2}M-quercetin solution was added next, and the samples diluted to exactly 10.0 ml with distilled water. The absorbances were read 30 minutes after dilution

to volume, the readings being taken at 420 mμ on a Beckman Model B spectrophotometer using a pair of matched 1.0 cm pyrex cells and a reagent blank as reference. The results are tabulated in Table 14 and plotted in Figure XVI.

Reagents and Equipment

Standard W(VI) Solution

This solution was prepared by dissolving 1.7950 g of dry Baker and Adamson reagent grade $Na_2WO_4 \cdot 2H_2O$ in distilled water and diluting to exactly one liter with water. The solution contained 1 mg W(VI) per ml or 1000 ppm. Less concentrated solutions were prepared from this by appropriate dilution.

All other reagents have been described previously.

Instruments

The absorbance measurements were made with either a Beckman Model B spectrophotometer or a Beckman Model DU quartz spectrophotometer using a set of matched pyrex cells. The pH measurements were made with a Beckman Zeromatic pH meter equipped with a standard glass (fiber type) electrode and a standard calomel reference electrode.

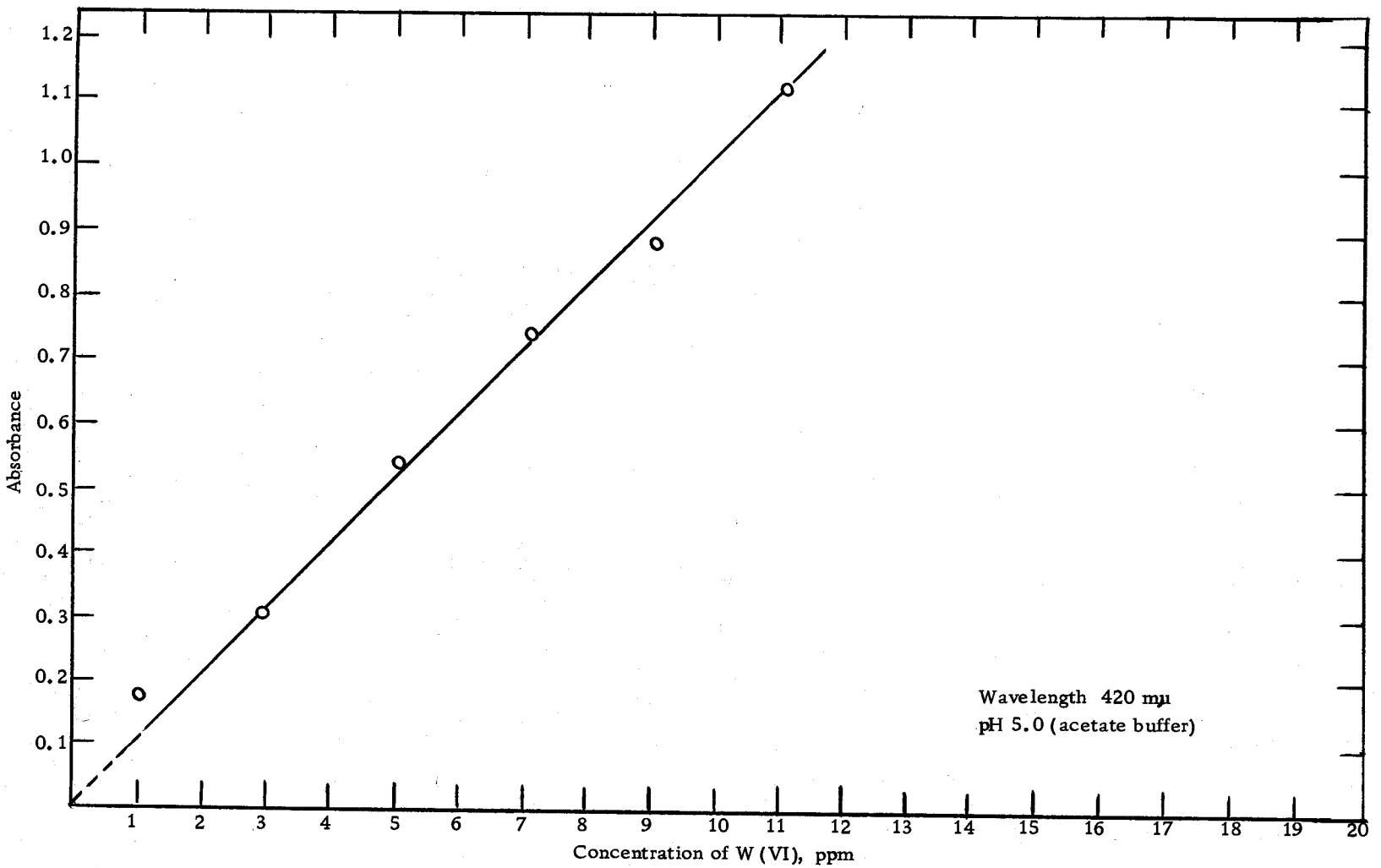


Figure XVI. Beer's Law Plot for Tungsten-Quercetin Chelate at 420 mμ

Table 14. Beer's Law Plot for Tungsten-Quercetin Chelate at 420 mμ

Solution No.	Concentration of W(VI) ppm	Absorbance	Percent Transmittance	Absorptancy (100 minus transmittancy)
1	1	0.178	66.3	33.7
2	3	0.294	50.8	49.2
3	5	0.536	29.1	70.9
4	7	0.745	18.0	82.0
5	9	0.883	13.1	86.9
6	11	1.13	7.36	92.6
7	13	1.34	4.57	95.4
8	15	1.56	2.75	97.3

Wavelength 420 mμ

1.0 cm cells

Stability of W(VI) Chelate

Solutions of the pale yellow tungsten-quercetin chelate seemed to be stable for several hours but a slight yellow precipitate was observed on standing overnight. As with the molybdenum, there was a slight increase in color intensity after 30 minutes, but for convenience and reproducibility, the time for color development was arbitrarily set at one half hour.

Discussion of Results

The tungsten(VI)-quercetin chelate shows deviations from Beer's law, although a working curve can be established over the range 1 to 15 ppm W(VI). The molar absorptivity for the chelate at 420 mμ is 18,900 cm^{-1} $mole^{-1}$ l.

The optimum concentration range for maximum precision was determined by plotting the percent absorptancy (100 minus the percent transmittance) versus the log of the tungsten concentration. From the data in Table 14 the optimum concentration range is 3 to 5 ppm W(VI) (Figure XVII). The calculated value of the coefficient of variation derived from the maximum slope, for a variation of one percent in the photometric error, is about 3 percent over this range.

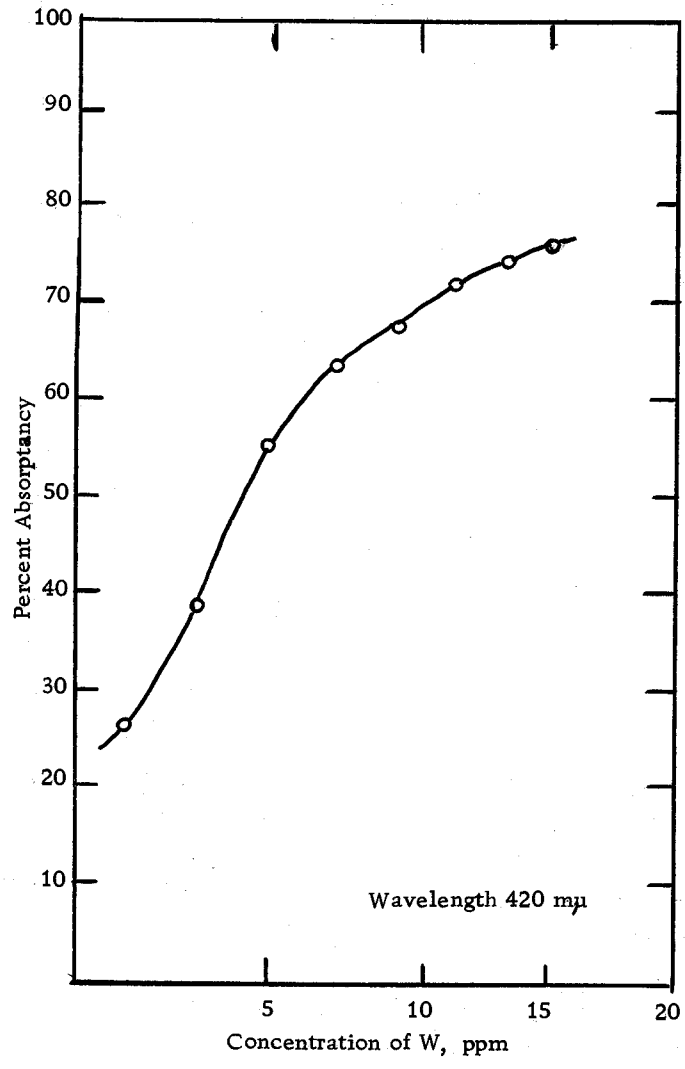


Figure XVII. Ringbom Plot for Tungsten-Quercetin Chelate at 420 mμ

Spectrophotometric Determination of Empirical Formula Slope-Ratio Method

In the slope-ratio method two series of solutions were prepared. In one, various amounts of quercetin were added to a constant amount of tungstate ion present in large excess. In the other, variable amounts of tungstate ion were added to a constant amount of quercetin present in large excess. The absorbances of the resulting solutions, plotted against the concentration of the variable component, yield two straight lines. The ratio of the slopes of these two lines indicates the combining ratio of the two components in the complex.

Procedure

In the first series of solutions the standard W(VI) solution was used directly, giving a 5.2×10^{-4}M-tungstate concentration after dilution. The quercetin solution was diluted to an initial concentration of 1×10^{-3}M. The general composition of the solutions in this series was as follows: 1.0 ml of the 5.2×10^{-3}M-tungsten solution, 0.5 ml of the acetate buffer, 3.0 ml of 95 percent ethanol and variable amounts of the 10^{-3}M-quercetin solution. The samples were diluted to a total volume of 10.0 ml with distilled water and the absorbances read 30 minutes later at 430 mμ in the Beckman Model B spectrophotometer against solutions containing the same amounts

of buffer, alcohol, and quercetin reagent.

In the second series of solutions the quercetin was diluted with ethanol to yield a 5.2×10^{-3}M solution. Similarly, the standard tungstate solution was diluted with water so as to obtain a 1.0×10^{-3}M-tungstate solution. The general composition of the solutions in this series was as follows: variable amounts of the 1.0×10^{-3}M-tungstate solution, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and 1.0 ml of the 5.2×10^{-3}M-quercetin solution. The samples were diluted to 10.0 ml total volume with distilled water and the absorbances read 30 minutes later at 430 mμ in the Beckman Model B spectrophotometer against a reagent blank.

The data for these measurements are tabulated in Tables 15A and 15B and plotted in Figure XVIII.

Spectrophotometric Determination of Empirical Formula "Gerade" Method of Asmus

The 1:1 combining ratio indicated by the slope-ratio method was checked by the "gerade" method of Asmus. Due to the limited solubility of the tungsten-quercetin chelate, it was not possible to use as large a range of volumes of the complexing reagent as in the case of the molybdenum chelate. However, by reducing the size increment of the variable component added it was possible to obtain a sufficient number of points so that the method could be applied.

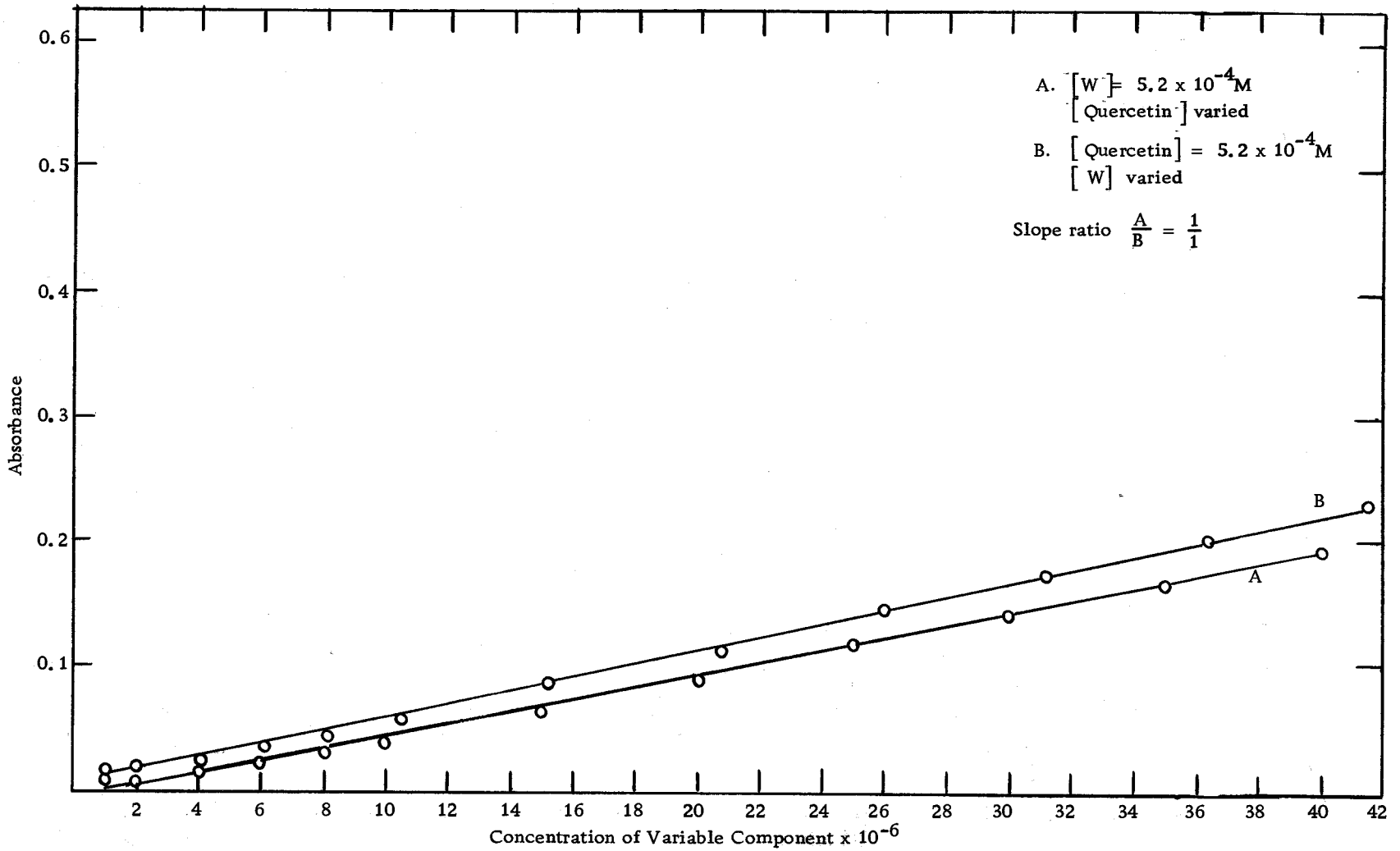


Figure XVIII. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Tungsten-Quercetin Chelate

Table 15A. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Variation of Quercetin Concentration

Solution No.	Volume of Quercetin Soln. ml	[Quercetin] $\times 10^{-6}$	A	Percent T
1	0.01	1.0	0.004	99.0
2	0.02	2.0	0.005	98.8
3	0.04	4.0	0.013	97.0
4	0.06	6.0	0.020	95.5
5	0.08	8.0	0.028	93.8
6	0.10	10.0	0.037	91.8
7	0.15	15.0	0.063	86.5
8	0.20	20.0	0.086	82.0
9	0.25	25.0	0.116	76.5
10	0.30	30.0	0.140	72.5
11	0.35	35.0	0.167	68.0
12	0.40	40.0	0.194	64.0
13	0.45	45.0	0.215	61.0
14	0.50	50.0	0.237	58.0
15	0.75	75.0	0.414	38.5
16	1.00	100.0	0.569	27.0
17	1.50	150.0	0.801	15.8
18	2.00	200.0	1.09	8.2

[W] = 5.2×10^{-4}M after dilution

[Quercetin] varied

Absorbances at 430 mμ

Table 15B. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Variation of Tungsten Concentration

Solution No.	Volume of W Solution ml	$[W] \times 10^{-6}$	A	Percent T
1	0.01	1.04	0.016	96.5
2	0.02	2.08	0.018	96.0
3	0.04	4.16	0.023	94.8
4	0.06	6.24	0.033	92.8
5	0.08	8.32	0.041	91.0
6	0.10	10.40	0.057	87.8
7	0.15	15.6	0.086	82.1
8	0.20	20.8	0.110	77.6
9	0.25	26.0	0.145	71.6
10	0.30	31.2	0.174	67.0
11	0.35	36.4	0.200	63.1
12	0.40	41.6	0.230	58.9
13	0.45	46.8	0.260	54.9
14	0.50	52.0	0.298	50.3

[Quercetin] = 5.2×10^{-4} M after dilution

[W] varied

Absorbances at 430 mμ

Reagents and Equipment

Standard W(VI) Solution

A standard solution of W(VI) was prepared by dissolving 2.6910g of dry Baker and Adamson reagent grade $Na_2WO_4 \cdot 2H_2O$ in distilled water and making up to exactly 100.0 ml with water. The solution contained 15 mg W(VI) per ml.

Standard Quercetin Solution

A 10^{-4}M-quercetin solution was prepared by diluting 1.0 ml of the standard 10^{-2}M solution with 95 percent ethanol to a final volume of 100.0 ml.

All other reagents and equipment have been described previously.

Procedure

A series of eight 50.0 ml volumetric flasks was set up, and to each was added 1.0 ml of the W(VI) solution (15 mg W(VI) per ml), 2.5 ml of acetate buffer, and 15.0 ml of 95 percent ethanol. Variable amounts of the 10^{-4}M-quercetin solution, ranging in amount from 3.0 to 15.5 ml in increments of 1.5 ml, were then added and the solutions made up to volume with distilled water. The absorbances were read

30 minutes after dilution to volume. Measurements were made at 430 mμ on the Beckman Model B spectrophotometer against a reagent blank containing identical amounts of acetate buffer, alcohol and quercetin.

The data are tabulated in Table 16 and plotted in Figure XIX.

Discussion of Results

In the slope-ratio method, the combining ratio of metal ion and ligand in the complex is given by the ratio of the slopes of the two straight lines formed when the absorbance is plotted against the concentration of the variable component. From Figure XVIII this is indicated as 1:1. The "gerade" method is less reliable in this case as a result of the limited concentration range over which clear solutions were obtained. Nevertheless, the curve obtained from n = 1 is closer to a straight line than any of the others, and indicates the likelihood of a 1:1 complex (Figure XIX).

Spectrophotometric Determination of the Instability Constant

An approximate value for the instability constant of the tungsten-quercetin chelate was obtained by the same method as applied previously in the case of molybdenum.

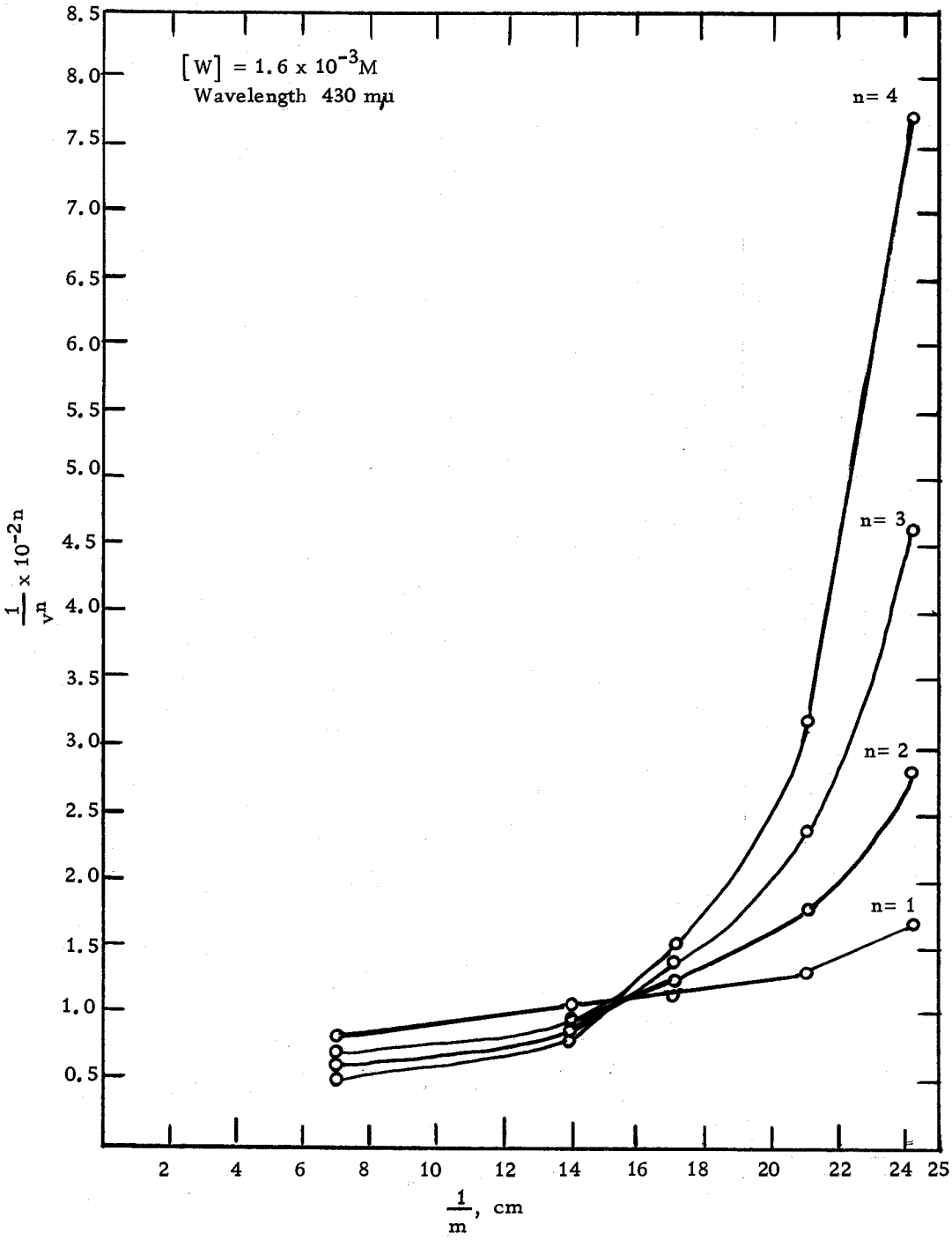


Figure XIX. Spectrophotometric Determination of Empirical Formula
"Gerade" Method of Asmus
Tungsten-Quercetin Chelate

Table 16. Spectrophotometric Determination of Empirical Formula "Gerade" Method of Asmus Tungsten-Quercetin Chelate

Solution No.	Volume of Quercetin ml	m cm^{-1}	$\frac{1}{m}$ cm	$\frac{1}{v} \times 10^{-2}$ l^{-1}	$\frac{1}{v^2} \times 10^{-4}$ l^{-2}	$\frac{1}{v^3} \times 10^{-6}$ l^{-3}	$\frac{1}{v^4} \times 10^{-8}$ l^{-4}
1	3.0	0.013	77.0				
2	4.5	0.027	37.0				
3	6.0	0.041	24.4	1.667	2.770	4.630	7.710
4	7.5	0.047	21.2	1.333	1.780	2.370	3.160
5	9.0	0.058	17.2	1.111	1.235	1.373	1.524
6	10.5	0.071	14.1	0.952	0.907	0.863	0.823
7	12.0	0.143	7.0	0.833	0.695	0.579	0.484
8	15.0	---	--				

[W] = 1.6×10^{-3} M

[Quercetin] varied

Wavelength 430 mμ

100 cm cells

Procedure

For this experiment 1 x 10^{-3}M solutions of both tungsten (VI) and quercetin were prepared. To each of a series of eleven flasks was added 0. 5 ml of acetate buffer and varying volumes of 1.0 x 10^{-3}M-tungsten(VI), 1. 0 x 10^{-3}M-alcoholic quercetin solution and 95 percent ethanol so that for each sample the total number of mmoles of metal ion and ligand together was maintained constant at 1 x 10^{-3} mmole per 10. 0 ml total volume, while the total volume of alcohol was maintained constant at 4. 0 ml. The flasks were diluted to the mark with distilled water and the absorbances read 30 minutes later at 420 mμ on the Beckman Model B spectrophotometer, against individual reagent blanks containing the same amount of quercetin, acetate buffer and ethanol. The results are summarized in Table 17 and Figure XX.

Discussion of Results

As pointed out previously when evaluating the Job's plot for molybdenum, the method of continuous variations suffers severe limitations when applied to weak complexes and permits only an approximate estimate of the magnitude of the instability constant. From Figure XX the value of E_m is estimated to be about 0. 34. E_s has a value of 0. 200, yielding a value of $\alpha = 0.41$ and an instability

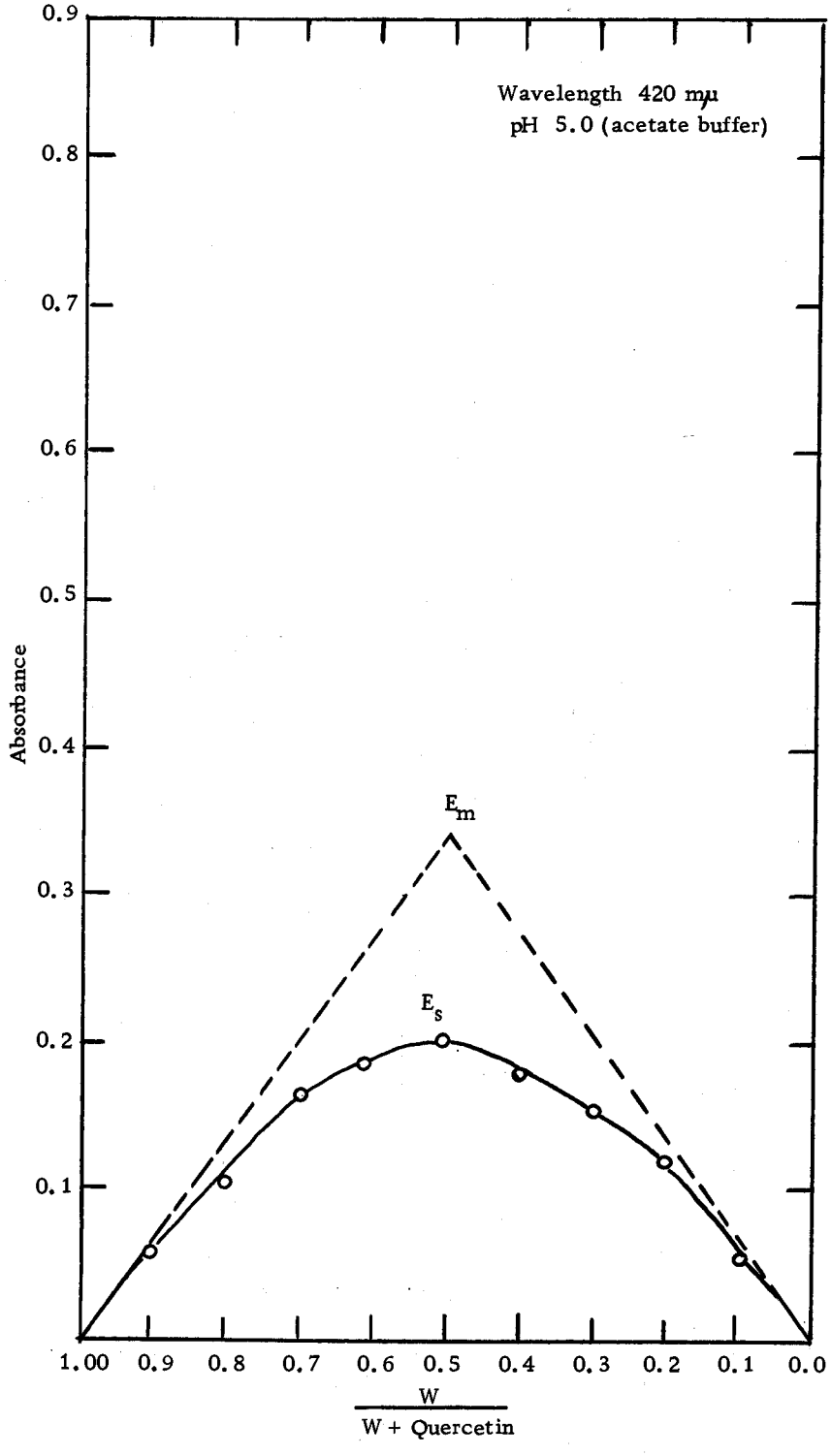


Figure XX. Job's Plot for Tungsten-Quercetin Chelate

Table 17. Job's Plot for Tungsten-Quercetin Chelate

Solution No.	Volume of W(VI) Solution ml	Volume of Quercetin Solution ml	Volume of Ethanol ml	Absorbance	$\frac{W}{W + Quercetin}$
1	1.00	0.00	4.00	0.000	1.00
2	0.90	0.10	3.90	0.056	0.90
3	0.80	0.20	3.80	0.104	0.80
4	0.70	0.30	3.70	0.140	0.70
5	0.60	0.40	3.60	0.178	0.60
6	0.50	0.50	3.50	0.200	0.50
7	0.40	0.60	3.40	0.180	0.40
8	0.30	0.70	3.30	0.152	0.30
9	0.20	0.80	3.20	0.115	0.20
10	0.10	0.90	3.10	0.049	0.10
11	0.00	1.00	3.00	0.000	0.00

[W] = 1.0×10^{-3}M before dilution

[Quercetin] = 1.0×10^{-3}M before dilution

pH 5.0 (acetate buffer), 1.0 cm cells

Total volume 10.0 ml

constant of about 1.4×10^{-5}.

Interfering Ions

The effect of a number of foreign ions on the absorbances of the tungsten-quercetin chelate at concentration levels of 1 and 5 ppm W(VI) has been determined. The concentration level of the interfering ion was varied from 1 to 10 ppm. The metals tested were the same as in the case of Mo(VI).

Reagents and Equipment

The preparation of the test solutions of the various metal ions has been described in Table 11.

Procedure

Six samples were set up for each metal ion tested, one containing 1 ppm W(VI) and the other five containing 5 ppm W(VI). Various volumes of the test solutions were added to each flask, sufficient to supply 1, 1, 3, 5, 7, and 10 ppm of interfering ion, respectively. Then to each solution was added 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and 1.0 ml of 10^{-2} molar quercetin solution. The samples were made up to 10.0 ml total volume with distilled water. The absorbances were read 30 minutes later at 430 mμ against a reagent blank. All readings were made on a Beckman Model DU

Table 18. Calibration Data for Tungsten Determination

ppm W(VI)	Absorbance	Absorptivity
1.0	0.178	178
3.0	0.294	98
5.0	0.536	107
7.0	0.745	106
9.0	0.883	98
11.0	1.13	103
13.0	1.34	103
15.0	1.56	104

Average absorptivity a=103

Wavelength 430 mμ 1.0 cm cells

spectrophotometer using a pair of matched 1.0 cm pyrex cells.

The results are tabulated in Table 19.

Discussion of Results

In examining the results in Table 19 it should be kept in mind that the average absorptivity of 103 was calculated from the amount of W(VI) known to be present. This is an ideal value, and if the presence of a foreign metal does not cause appreciable deviation from this it is assumed that the ion will not interfere at that concentration.

Most of the ions examined interfered to some extent, although some were much more serious than others. As with Mo(VI), aluminum, copper, ferric iron, zirconium (IV) and vanadium (V) interfered seriously. Only Ce(III) gave no interference. All of the other ions gave low values for the absorbance and for the absorptivity, tending to increase as the concentration of diverse ion increased. Both thorium (IV) and uranium (VI) interfered slightly, thorium more so than uranium. It seems likely that some type of separation of W(VI) from other ions would have to be employed before this procedure could be used to determine W(VI). Masking with an appropriate sequestering agent might be effective with some diverse ions, but probably actual separation by means of ion exchange or an extraction procedure would be more feasible.

Table 19. Interfering Ions - Tungsten Determination

Ion Tested	Interfering Ion ppm	W(VI) ppm	Absorbance	Absorptivity	Ion Tested	Interfering Ion ppm	W(VI) ppm	Absorbance	Absorptivity
Al	1	1	1.50	1500	Hg(II)	1	1	0.052	52
	1	5	1.58	316		1	5	0.228	46
	3	5	2.70	540		3	5	0.237	47
	5	5	2.72	544		5	5	0.285	57
	7	5	ppt	--		7	5	0.350	70
	10	5	ppt	--		10	5	0.406	81
Bi	1	1	0.200	200	Hg(I)	1	1	0.069	69
	1	5	0.409	82		1	5	0.288	58
	3	5	0.472	94		3	5	0.253	51
	5	5	0.510	102		5	5	0.154	31
	7	5	0.609	122		7	5	0.132	26
	10	5	0.745	149		10	5	0.098	20
B	1	1	0.114	114	Ni	1	1	0.082	82
	1	5	0.471	94		1	5	0.467	93
	3	5	0.435	87		3	5	0.401	80
	5	5	0.389	78		5	5	0.379	76
	7	5	0.466	93		7	5	0.455	91
	10	5	0.467	93		10	5	0.457	91
Cd	1	1	0.215	215	Ag	1	1	0.115	115
	1	5	0.347	69		1	5	0.499	100
	3	5	0.292	58		3	5	0.375	75
	5	5	0.348	70		5	5	0.378	76
	7	5	0.371	74		7	5	0.470	94
	10	5	0.403	81		10	5	0.527	105
Ce(III)	1	1	0.069	69	Tl(I)	1	1	0.086	86
	1	5	0.460	92		1	5	0.471	94
	3	5	0.521	104		3	5	0.375	75
	5	5	0.528	106		5	5	0.408	81
	7	5	0.535	107		7	5	0.367	73
	10	5	0.506	101		10	5	0.438	88
Ce(IV)	1	1	0.100	100	Th(IV)	1	1	0.106	106
	1	5	0.365	73		1	5	0.067	13
	3	5	0.386	77		3	5	0.229	46
	5	5	0.400	80		5	5	0.449	90
	7	5	0.445	89		7	5	0.623	125
	10	5	0.423	85		10	5	0.975	195
Co	1	1	0.077	77	U(VI)	1	1	0.110	110
	1	5	0.419	84		1	5	0.454	91
	3	5	0.351	70		3	5	0.507	101
	5	5	0.359	72		5	5	0.567	113
	7	5	0.420	84		7	5	0.614	123
	10	5	0.521	104		10	5	0.654	131
Cu(II)	1	1	0.411	411	V(V)	1	1	0.284	284
	1	5	0.550	110		1	5	0.328	66
	3	5	1.00	200		3	5	0.585	117
	5	5	1.47	294		5	5	0.793	159
	7	5	1.81	362		7	5	0.967	193
	10	5	2.30	460		10	5	1.14	228
Fe(III)	1	1	1.12	1118	Zn	1	1	0.075	75
	1	5	1.41	282		1	5	0.347	69
	3	5	2.39	478		3	5	0.350	70
	5	5	ppt	--		5	5	0.379	76
	7	5	ppt	--		7	5	0.458	92
	10	5	ppt	--		10	5	0.469	94
Pb	1	1	0.134	134	Zr(IV)	1	1	0.249	249
	1	5	0.491	98		1	5	0.238	48
	3	5	0.458	92		3	5	0.466	93
	5	5	0.359	72		5	5	0.821	164
	7	5	0.350	70		7	5	1.15	230
	10	5	0.222	44		10	5	1.65	330

CHAPTER VI

PROPERTIES OF THE Cr (VI) -QUERCETIN CHELATE

When an alcoholic solution of quercetin is added to a solution containing hexavalent chromium buffered at pH 5. 0, a yellow color develops, apparently as a result of the formation of a Cr (VI) -quercetin chelate. On standing for several hours these solutions developed an orange coloration. It is the purpose of this investigation to determine whether or not this chelate conforms to Beer's law,, and also to determine the empirical formula of the complex and the nature and extent of the interference offered by the presence of diverse ions on the spectrophotometric determination of Cr (VI) utilizing the Cr (VI) -quercetin chelate.

Adherence to Beer's Law

Conformity to Beer's law was checked by setting up a series of solutions using appropriate aliquots of the standard Cr (VI) solution (1 mg Cr (VI) per ml) so that, after dilution to volume, they would contain 1, 3, 5, 7, 9, 11, 13, and 15 ppm Cr (VI), respectively. Then, following the same standard procedure as with molybdenum and tungsten, to each sample was added 0. 5 ml of the acetate buffer, 3. 0 ml of the 95 percent ethanol and 1. 0 ml of 10^{-2}M-quercetin

solution. The samples were diluted to 10.0 ml total volume with distilled water. The absorbances were read 30 minutes after dilution to volume, the readings being made at 435 mμ on a Beckman Model DU spectrophotometer using a pair of matched 1.0 cm pyrex cells and a reagent blank as reference. The results are tabulated in Table 20 and plotted in Figure XXI.

Reagents and Equipment

Standard Cr(VI) Solution

This solution was prepared by dissolving 3.7350 g of dry Baker and Adamson reagent grade K_2CrO_4 in distilled water and diluting to exactly one liter. The solution contained 1 mg Cr(VI) per ml or 1000 ppm. Less concentrated solutions were prepared from this by appropriate dilutions.

Instruments

All absorbance measurements were made with a Beckman Model DU quartz spectrophotometer using a set of matched 1.0 cm pyrex cells. All pH measurements were made with a Beckman Model 72 pH meter equipped with a combination silver chloride and glass electrode.

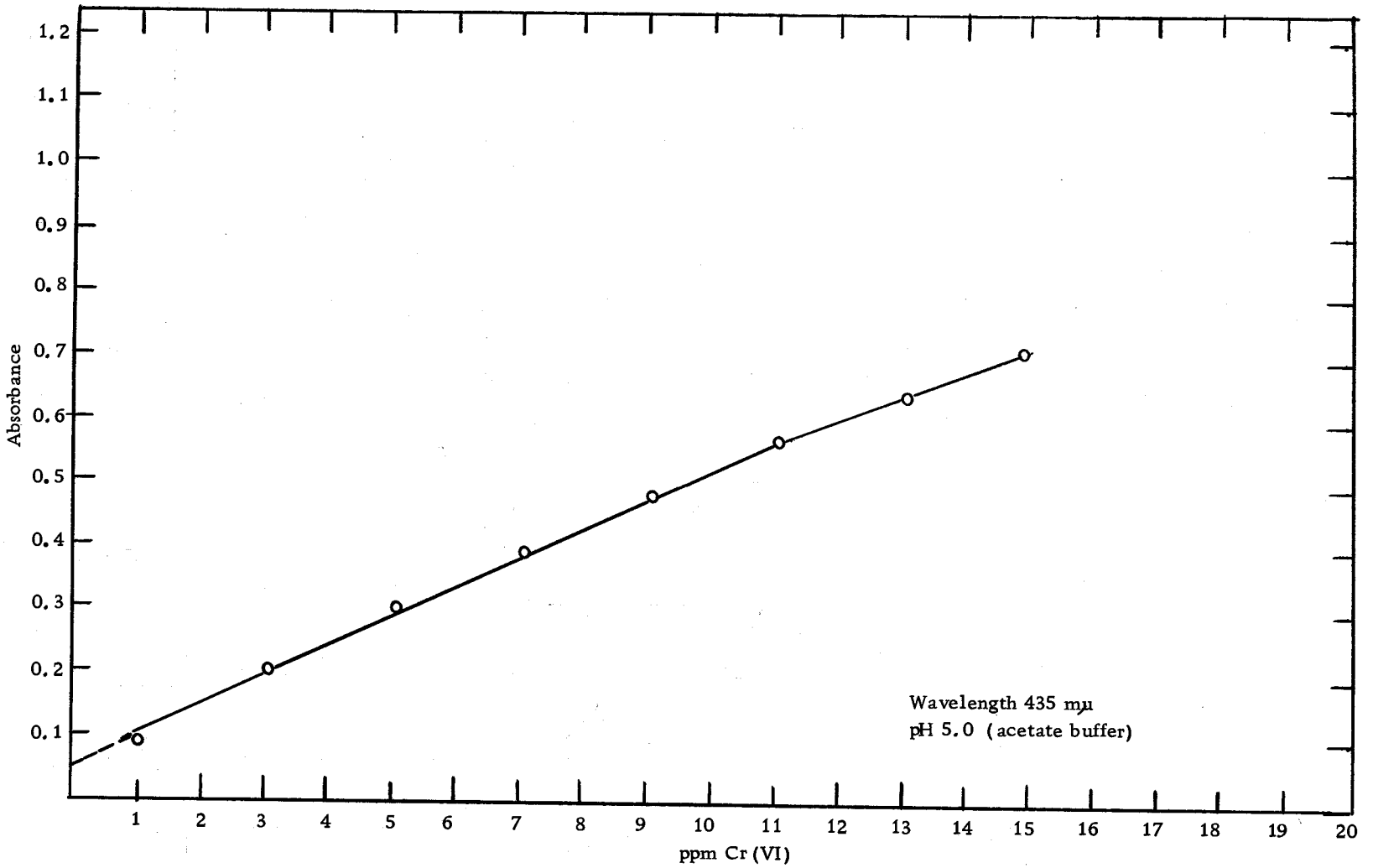


Figure XXI. Beer's Law Plot for Chromium-Quercetin Chelate at 435 mμ

Table 20. Beer's Law Plot for Chromium-Quercetin Chelate at 435mμ

Solution No.	Concentration of Cr(VI) ppm	Absorbance	Percent Transmittance	Absorptancy (100 minus transmittancy)
1	1	0.082	82.8	17.2
2	3	0.202	62.8	37.2
3	5	0.304	49.7	50.3
4	7	0.390	40.7	59.3
5	9	0.482	33.0	67.0
6	11	0.569	27.0	73.0
7	13	0.642	22.8	77.2
8	15	0.710	19.5	80.5

Wavelength 435 mμ

1.0 cm cells

Stability of the Cr(VI) Chelate

As indicated previously, solutions containing chromium (VI) and quercetin were initially a bright yellow in color but developed an orange tint on standing over a 24 hour period, especially those of higher chromium content. Also, chromium was the only one of the three metals being examined that did not exhibit a leveling off of absorbance when the quercetin concentration was varied at fixed metal ion concentration. Both pieces of evidence may indicate the presence of more than one complex in the solution.

Discussion of Results

The chromium(VI)-quercetin chelate does not adhere to Beer's law, although there seems to be a linear relationship between absorbance and concentration between 3 and 11 ppm of chromium, with derivations at either end of this range. The molar absorptivity for the chelate at 435 mμ is only 4600 cm^{-1} $mole^{-1}$ l.

The optimum concentration range for maximum precision was determined by plotting the percent absorptancy (100 minus the percent transmittance) versus the log of the chromium (VI) concentration. From the data in Table 20 the optimum concentration range is 3 to 5 ppm of Cr(VI) (Figure XXII). The calculated value of the coefficient of variation derived from the maximum slope, for a

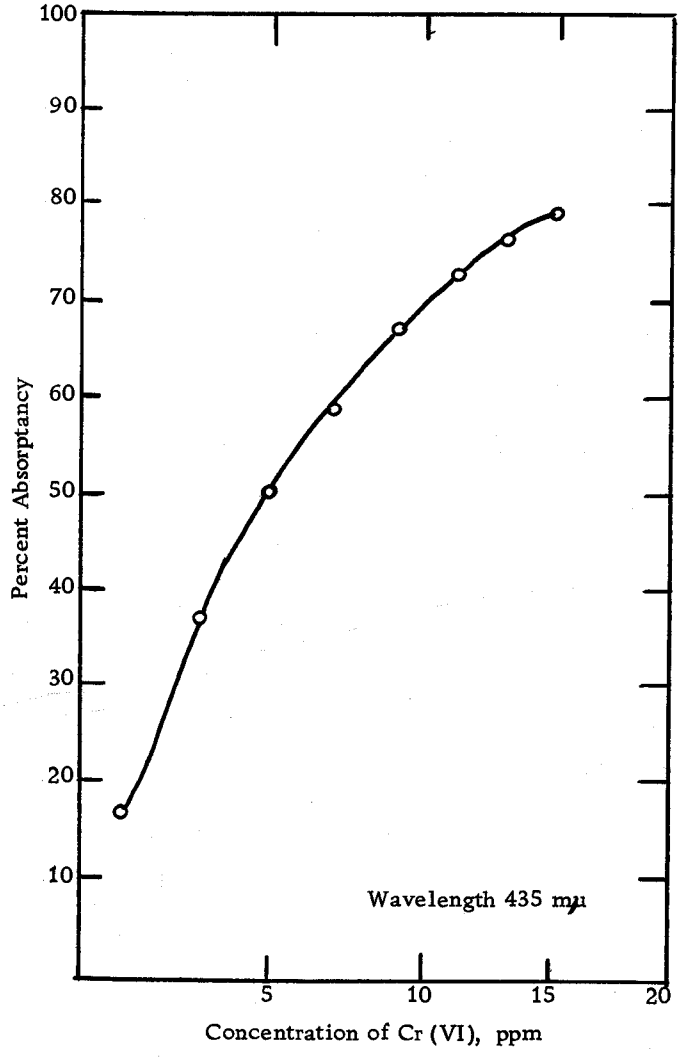


Figure XXII. Ringbom Plot for Chromium-Quercetin Chelate at 435 mμ

variation of one percent in the photometric error, is about 3 percent over this range.

Spectrophotometric Determination of Empirical Formula Slope-Ratio Method

The composition of the complex was determined by the slope-ratio method as in the case of molybdenum and tungsten.

Procedure

In the first series of solutions the standard Cr (VI) solution was diluted so as to yield a 5.2×10^{-3}M-chromium (VI) concentration. The standard quercetin solution was diluted to a concentration of 1×10^{-3}M. The general composition of the solutions in this series was as follows: 1.0 ml of the 5.2×10^{-3}M-chromium solution, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and variable amounts of the 10^{-3}M-quercetin solution. The samples were diluted to a total volume of 10.0 ml with distilled water and the absorbances read 30 minutes later at 435 mμ in the Beckman Model DU spectrophotometer against solutions containing the same amounts of buffer, alcohol, and quercetin reagent.

In the second series of solutions the quercetin was diluted with ethanol so as to yield a 5.2×10^{-3}M solution. Similarly, the standard chromium (VI) solution was diluted with water so as to obtain a

1×10^{-3} M-chromium concentration. The general composition of the solutions in this series was as follows: variable amounts of the 1×10^{-3}M-chromium(VI) solution, 0.5 ml of acetate buffer, 3.0 ml of 95 percent ethanol and 1.0 ml of the 5.2×10^{-3}M-quercetin solution. The samples were diluted to 10.0 ml total volume with distilled water and the absorbances read 30 minutes later at 435 mμ in the Beckman Model DU spectrophotometer against a reagent blank.

The data for these measurements are tabulated in Tables 21A and 21B and plotted in Figure XXIII.

Spectrophotometric Determination of Empirical Formula "Gerade" Method of Asmus

The empirical formula of the chromium-quercetin chelate was checked by the "gerade" method of Asmus as in the case of tungsten and chromium.

Reagents and Equipment

Standard Cr(VI) Solution

A solution of Cr(VI) was prepared by dissolving 3.7350 g of dry Baker and Adamson reagent grade K_2CrO_4 in distilled water and diluting to exactly one liter. The solution contained 1 mg Cr(VI) per ml or 1000 ppm. For the "gerade" method 27.08 ml of this standard solution were diluted to exactly 100.0 ml with water to yield a

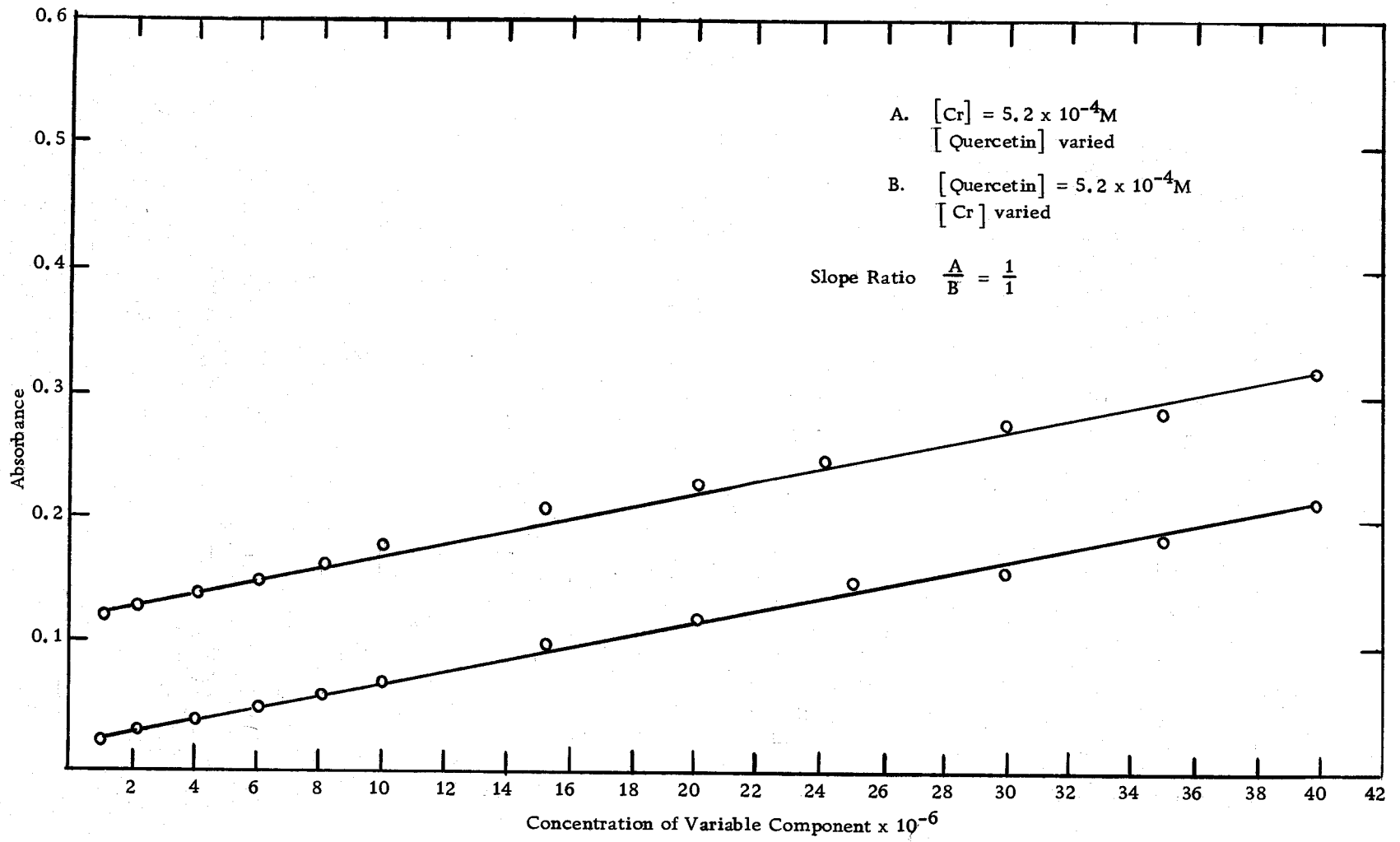


Figure XXIII. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Chromium-Quercetin Chelate

Table 21A. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Variation of Quercetin Concentration

Solution No.	Volume of Quercetin Soln. ml	[Quercetin] $\times 10^{-6}$	A	Percent T
1	0.01	1	0.124	75.2
2	0.02	2	0.131	74.0
3	0.04	4	0.142	72.1
4	0.06	6	0.150	70.8
5	0.08	8	0.162	68.8
6	0.10	10	0.181	66.0
7	0.15	15	0.215	61.0
8	0.20	20	0.232	58.6
9	0.25	25	0.267	54.1
10	0.30	30	0.280	52.5
11	0.35	35	0.287	51.6
12	0.40	40	0.310	49.0
13	0.45	45	0.318	48.1
14	0.50	50	0.328	47.0
15	0.75	75	0.372	42.5
16	1.00	100	0.444	36.0
17	1.50	150	0.587	25.9
18	2.00	200	0.710	19.5

[Cr] = 5.2×10^{-4} M constant in all runs

[Quercetin] varied

Absorbances at 435 mμ

Table 21B. Spectrophotometric Determination of Empirical Formula Slope-Ratio Method - Variation of Chromium Concentration

Solution No.	Volume of Cr(VI) Solution ml	$[Cr]_6 \times 10^{-6}$	A	Percent T
1	0.01	1	0.016	96.4
2	0.02	2	0.025	94.4
3	0.04	4	0.036	92.0
4	0.06	6	0.050	89.1
5	0.08	8	0.059	87.2
6	0.10	10	0.070	85.1
7	0.15	15	0.102	79.1
8	0.20	20	0.123	75.3
9	0.25	25	0.150	70.8
10	0.30	30	0.155	70.0
11	0.35	35	0.184	65.5
12	0.40	40	0.206	62.2
13	0.45	45	0.214	61.0
14	0.50	50	0.246	56.8
15	0.75	75	0.333	46.5
16	1.00	100	0.408	39.1
17	1.50	150	0.530	29.5
18	2.00	200	0.650	22.4

[Quercetin] = 5.2×10^{-4} M constant in all runs

[Cr] varied

Absorbances at 435 mμ

5.2×10^{-3}M-chromium solution.

All other reagents and equipment have been described previously.

Procedure

A series of ten 50.0 ml volumetric flasks was set up, and to each was added 1.0 ml of the 5.2×10^{-3}M-Cr(VI) solution, 2.5 ml of the acetate buffer, and 15.0 ml of 95 percent ethanol. Various amounts of a 10^{-3}M-quercetin solution ranging from 3.0 ml to 30.0 ml in increments of three ml, were then added and the solutions were made up to volume with distilled water. The absorbances were read 30 minutes after dilution to volume. All measurements were made at 435 mμ on a Beckman Model DU spectrophotometer against a reagent blank using a pair of 1.0 cm matched pyrex cells.

The data for these measurements are tabulated in Table 22 and plotted in Figure XXIV.

Discussion of Results

In the slope-ratio method, the combining ratio of metal ion to ligand in the complex is given by the ratio of the slopes of the two straight lines formed when the absorbance is plotted against the concentration of the variable component. From Figure XXIII this is indicated as 1:1.

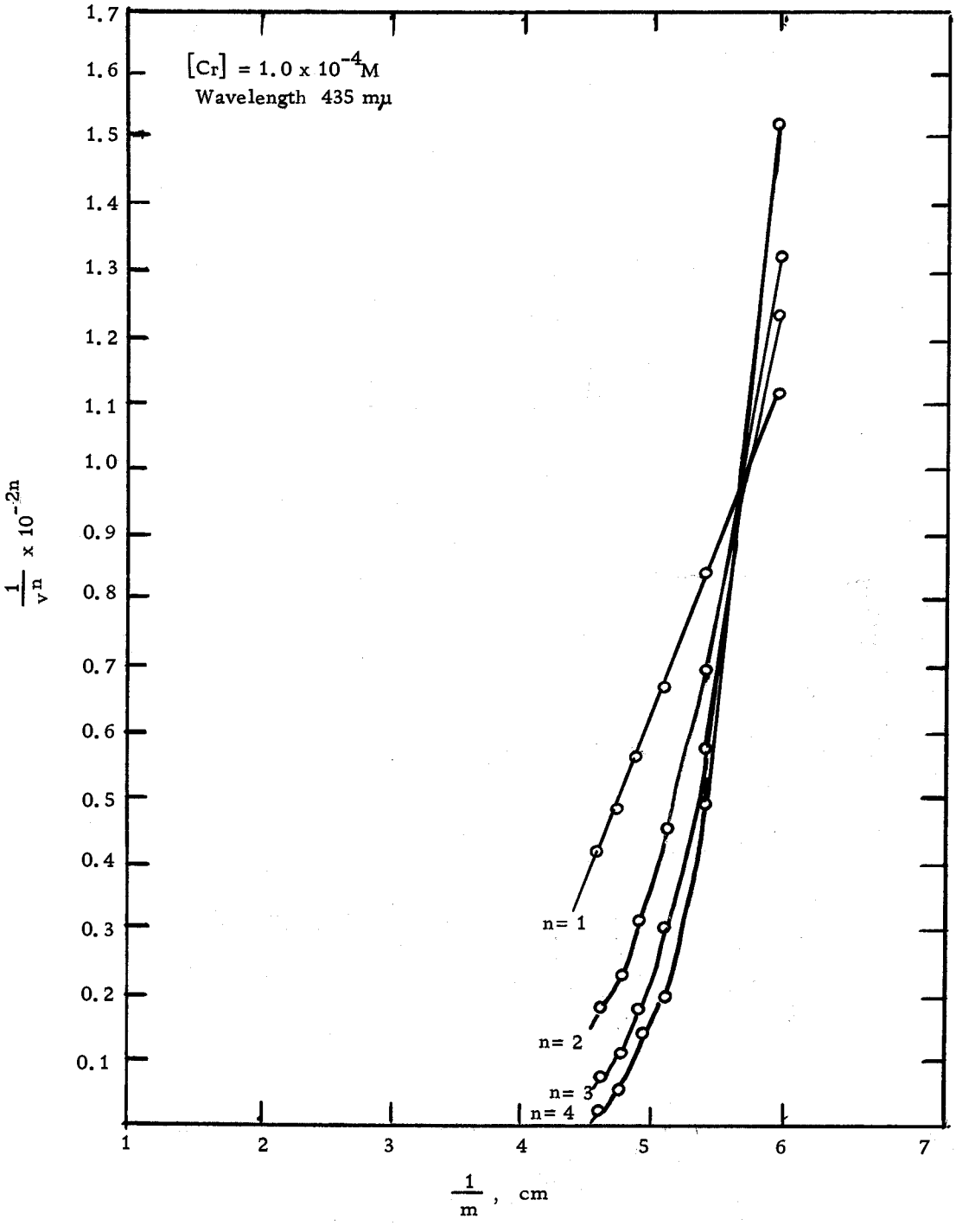


Figure XXIV. Spectrophotometric Determination of Empirical Formula
"Gerade" Method of Asmus
Chromium-Quercetin Chelate

Table 22. Spectrophotometric Determination of Empirical Formula "Gerade" Method of Asmus Chromium-Quercetin Chelate

Solution No.	Volume of Quercetin ml	m cm^{-1}	$\frac{1}{m}$ cm	$\frac{1}{v} \times 10^{-2}$ l^{-1}	$\frac{1}{v^2} \times 10^{-4}$ l^{-2}	$\frac{1}{v^3} \times 10^{-6}$ l^{-3}	$\frac{1}{v^4} \times 10^{-8}$ l^{-4}
1	3.0	0.135	7.40				
2	6.0	0.151	6.62				
3	9.0	0.167	6.00	1.111	1.235	1.373	1.524
4	12.0	0.184	5.43	0.833	0.695	0.579	0.484
5	15.0	0.195	5.12	0.667	0.445	0.297	0.198
6	18.0	0.203	4.93	0.556	0.309	0.171	0.095
7	21.0	0.209	4.78	0.476	0.227	0.108	0.051
8	24.0	0.216	4.63	0.417	0.174	0.072	0.030
9	27.0	0.218	4.59				
10	30.0	0.222	4.50				

$[Cr] = 1.0 \times 10^{-4} M$ Wavelength 435 mμ
[Quercetin] varied 1.0 cm cells

In the "gerade" method of Asmus a plot of the extinction modulus versus the reciprical of the n^{th} power of v, the volume of chelating agent added, yields a straight line for n = 1 (Figure XXIV). Thus, a combining ratio of 1 mole of quercetin per mole of Cr (VI) is indicated.

Spectrophotometric Determination of the Instability Constant

An attempt was made to determine the instability constant of the quercetin-chromium complex by the same method as applied previously in the case of molybdenum and tungsten.

Procedure

To each of a series of eleven flasks was added 0.5 ml of the acetate buffer and varying volumes of 1.0×10^{-2} M-chromium (VI), 1.0×10^{-2} M-alcoholic quercetin solution and 95 percent ethanol so that for each sample the total number of mmoles of metal ion and ligand together was maintained constant at 1×10^{-2} mmole per 10.0 ml total volume, while the total volume of alcohol was maintained constant at 4.0 ml. The flasks were diluted to the mark with distilled water and the absorbances read 30 minutes later at 435 mμ on the Beckman Model B spectrophotometer against individual reagent blanks containing the same amount of quercetin, acetate buffer and ethanol. The results are summarized in Table 23 and Figure XXV.

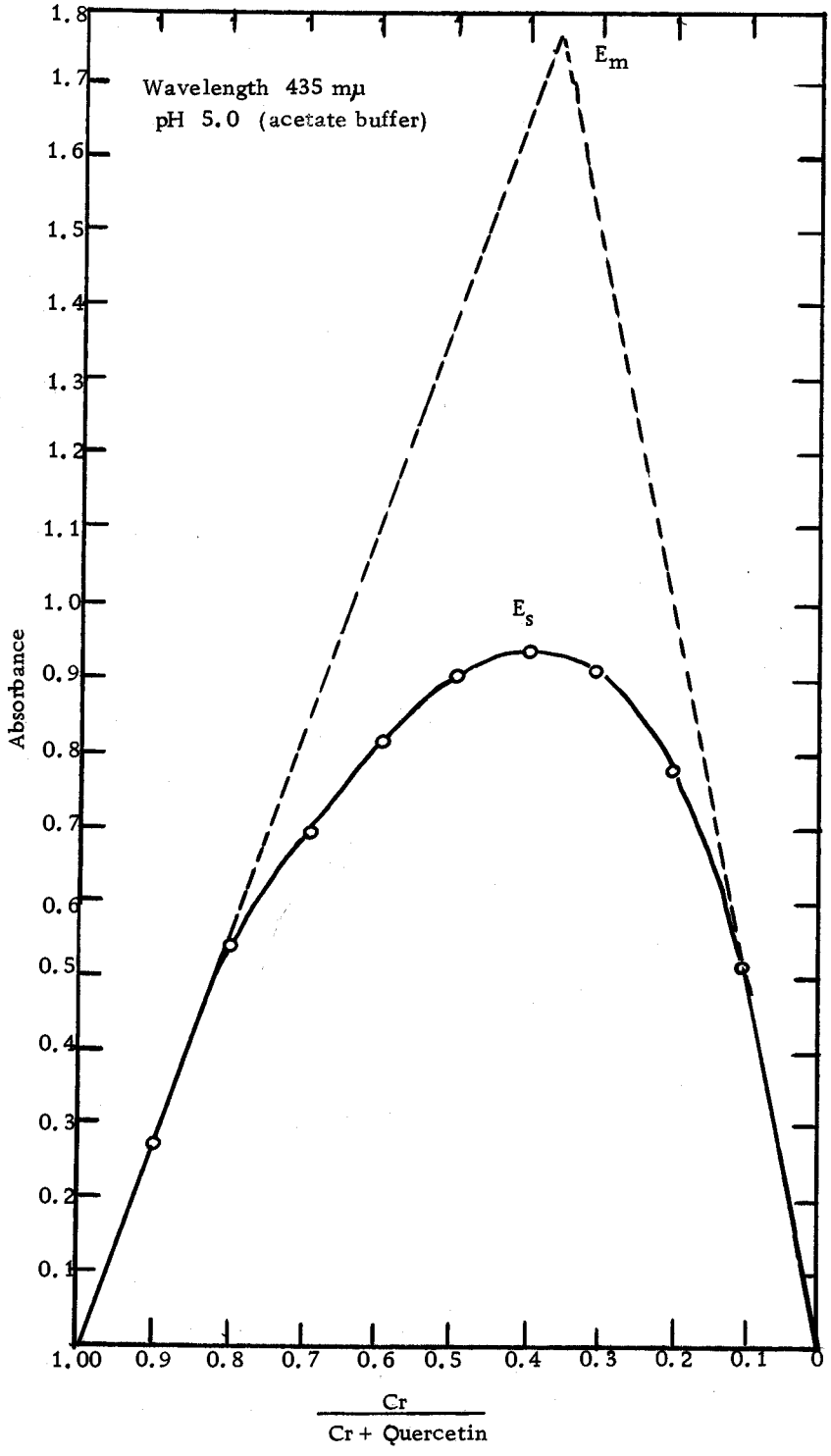


Figure XXV. Job's Plot for Chromium-Quercetin Chelate

Table 23. Job's Plot for Chromium-Quercetin Chelate

Solution No.	Volume of Cr(VI) Solution ml	Volume of Quercetin Solution ml	Volume of Ethanol ml	Absorbance (corr) *	$\frac{Cr}{Cr + Quercetin}$
1	1.00	0.00	4.00	0.000	1.00
2	0.90	0.10	3.90	0.276	0.90
3	0.80	0.20	3.80	0.544	0.80
4	0.70	0.30	3.70	0.698	0.70
5	0.60	0.40	3.60	0.836	0.60
6	0.50	0.50	3.50	0.909	0.50
7	0.40	0.60	3.40	0.942	0.40
8	0.30	0.70	3.30	0.914	0.30
9	0.20	0.80	3.20	0.763	0.20
10	0.10	0.90	3.10	0.505	0.10
11	0.00	1.00	3.00	0.000	0.00

[Cr] = 1.0×10^{-2}M before dilution

[Quercetin] = 1.0×10^{-2}M before dilution

pH 5.0 (acetate buffer), 1.0 cm cells

Total volume 10.0 ml

* Absorbances corrected for color produced by same amount of Cr(VI) in 10.0 ml total volume.

Discussion of Results

As with molybdenum and tungsten, the uncertainty involved in constructing the tangents so as to determine the value of E_m is evident. Using an estimated value of 1.8 for E_m and 0.94 for E_s, the value of α is calculated to be 0.46 giving a value of about 2×10^{-4} for the instability constant.

Interfering Ions

The effect of a number of foreign ions on the absorbances of the chromium(VI)-quercetin chelate at concentration levels of 1 to 5 ppm of Cr(VI) has been determined. The concentration level of the interfering ion was varied from 1 to 10 ppm. The metals tested were the same as in the case of molybdenum and tungsten.

Reagents and Equipment

The preparation of the test solutions of the various metal ions has already been described in Table 11.

Procedure

Six samples were set up for each metal ion tested, one containing 1 ppm Cr(VI) and the other five containing 5 ppm Cr(VI). Various volumes of the test solutions were added to each flask,

sufficient to supply 1, 1, 3, 5, 7, and 10 ppm of interfering ion respectively. Then to each solution was added 0. 5 ml of acetate buffer, 3. 0 ml of 95 percent ethanol and 1. 0 ml of 10^{-2} molar quercetin solution. The samples were made up to 10. 0 ml total volume with distilled water and the absorbances read 30 minutes later at 435 mμ against a reagent blank. All readings were made on a Beckman Model DU spectrophotometer using a pair of matched 1. 0 cm pyrex cells.

The results are tabulated in Table 25.

Discussion of Results

In examining the results of Table 25 it should be kept in mind that the average absorptivity of 58.5 was calculated from the amount of chromium(VI) known to be present. This is an ideal value, and if the presence of a foreign ion does not cause appreciable deviation from this value it is assumed that the ion will not interfere at that concentration.

Aluminum, copper (II), iron (III), vanadium (V), and zirconium (IV) interfere seriously. The interference offered by bismuth (III), thorium (IV) and uranium (VI) was moderately severe. The divalent metals cadmium, cobalt, lead, mercury, nickel and zinc, as well as monovalent silver and mercury, offered slight interference. The effect of boron, cerium (III and IV) and thallium (I) was insignificant.

Table 24. Calibration Data for Chromium Determination

ppm Cr (VI)	Absorbancy	Absorptivity
1	0.082	82.0
3	0.202	67.3
5	0.304	60.8
7	0.390	55.7
9	0.482	53.5
11	0.569	51.7
13	0.642	49.4
15	0.710	47.3

Average absorptivity a = 58.5

Wavelength = 430 mμ 1.0 cm cells

Table 25. Interfering Ions - Chromium Determination

Ion Tested	Interfering Ion ppm	Cr(VI) ppm	Absorbance	Absorptivity	Ion Tested	Interfering Ion ppm	Cr(VI) ppm	Absorbance	Absorptivity
Al	1	1	1.51	151	Hg(II)	1	1	0.148	148
	1	5	1.76	352		1	5	0.441	88
	3	5	2.72	544		3	5	0.438	88
	5	5	3.05	610		5	5	0.482	80
	7	5	ppt	--		7	5	0.572	114
	10	5	ppt	--		10	5	0.656	131
Bi	1	1	0.266	266	Hg(I)	1	1	0.080	80
	1	5	0.674	135		1	5	0.349	70
	3	5	0.928	186		3	5	0.344	69
	5	5	1.120	224		5	5	0.369	74
	7	5	1.27	254		7	5	0.652	130
	10	5	1.46	292		10	5	0.703	141
B	1	1	0.069	69	Ni	1	1	0.140	140
	1	5	0.373	75		1	5	0.569	114
	3	5	0.404	81		3	5	0.602	120
	5	5	0.407	81		5	5	0.590	118
	7	5	0.389	78		7	5	0.620	124
	10	5	0.407	81		10	5	0.635	127
Cd	1	1	0.148	148	Ag	1	1	0.148	148
	1	5	0.495	99		1	5	0.488	98
	3	5	0.517	103		3	5	0.503	101
	5	5	0.523	105		5	5	0.520	104
	7	5	0.553	111		7	5	0.538	108
	10	5	0.562	113		10	5	0.538	108
Ce(III)	1	1	0.136	136	Tl(I)	1	1	0.107	107
	1	5	0.388	78		1	5	0.355	71
	3	5	0.397	79		3	5	0.341	68
	5	5	0.416	83		5	5	0.352	70
	7	5	0.440	88		7	5	0.344	69
	10	5	0.472	94		10	5	0.339	68
Ce(IV)	1	1	0.093	93	Th(IV)	1	1	0.310	310
	1	5	0.276	55		1	5	0.719	144
	3	5	0.277	55		3	5	0.951	190
	5	5	0.282	56		5	5	1.14	229
	7	5	0.297	59		7	5	1.46	292
	10	5	0.311	62		10	5	1.82	364
Co	1	1	0.123	123	U(VI)	1	1	0.207	207
	1	5	0.413	83		1	5	0.585	117
	3	5	0.434	87		3	5	0.638	128
	5	5	0.434	87		5	5	0.654	131
	7	5	0.494	99		7	5	0.654	131
	10	5	0.498	100		10	5	0.750	150
Cu(II)	1	1	0.466	466	V(V)	1	1	0.523	523
	1	5	0.767	153		1	5	1.36	271
	3	5	1.15	229		3	5	1.59	317
	5	5	1.55	310		5	5	1.61	321
	7	5	1.92	384		7	5	1.75	350
	10	5	ppt	--		10	5	1.92	384
Fe(III)	1	1	1.20	1200	Zn	1	1	0.108	108
	1	5	1.41	282		1	5	0.428	86
	3	5	ppt	--		3	5	0.466	93
	5	5	ppt	--		5	5	0.507	101
	7	5	ppt	--		7	5	0.523	105
	10	5	ppt	--		10	5	0.572	114
Pb	1	1	0.156	156	Zr(IV)	1	1	0.369	369
	1	5	0.505	101		1	5	0.604	121
	3	5	0.527	105		3	5	0.921	184
	5	5	0.514	103		5	5	1.20	241
	7	5	0.553	111		7	5	1.57	313
	10	5	0.580	116		10	5	1.98	396

CHAPTER VII

SUMMARY

Molybdenum(VI) and its congeners, tungsten(VI) and chromium (VI), have been observed to form soluble yellow colored chelates with quercetin (3, 3', 4', 5, 7-pentahydroxyflavone). The properties of the chelate formed in each case and the possible application to the colorimetric determination of the metal have been studied.

The factors affecting the metal-quercetin color system were studied first in order to establish optimum conditions for color development. It was apparent from initial experiments that a certain amount of ethanol would be required in order to hold the reagent and the chelate in solution, and this was included in the variables studied, along with pH, wavelength, and quercetin concentration. The optimum pH was found to lie between 4.0 and 5.0 for all three metals, and was achieved by means of an acetate buffer. Optimum wavelengths for absorbance measurements were selected at 420 mμ for the molybdenum chelate, 420 mμ for the tungsten chelate and 435mμ for the chromium chelate. A reagent concentration of 1.0 ml of 10^{-2}M-quercetin for 10.0 ml total volume was found to be satisfactory. The final alcohol concentration was established at 40 percent by volume which includes that contributed by the reagent solution

added. Absorbance readings were arbitrarily taken 30 minutes after dilution to volume for convenience and reproducibility. Finally, the same order of addition of reagents was consistently followed. The metal ion was added first, followed by acetate buffer, 95 percent ethanol and quercetin. The sample was diluted to volume with water.

The molybdenum-quercetin chelate is an intense yellow and exhibits maximum absorbancy at 420 mμ. The chelate conforms to Beer's law, and has a molar absorptivity of about 34,000 $cm^{-1}mole^{-1}l$ at 420 mμ. The ratio of metal to ligand in the chelate is 1:1 as indicated by the slope-ratio method and the "gerade" method of Asmus. The instability constant has been estimated to be approximately 3.9×10^{-5} by a spectrophotometric method. Aluminum, chromium (VI), copper (II), iron (III), tungsten (VI), vanadium (V) and zirconium (IV) interfere seriously.

The tungsten-quercetin chelate is a pale yellow color and also exhibits maximum absorbance at 420 mμ. Deviations from Beer's law occur, although a working curve was set up between 1 to 11 ppm of W. The molar absorptivity of the chelate at 420 mμ is 18,900 $cm^{-1}mole^{-1}l$. Both the slope-ratio method and the "gerade" method indicate a 1:1 complex. The instability constant is estimated to be about 1.4×10^{-5}. The same ions interfered as in the case of molybdenum.

The yellow chromium-quercetin chelate shows maximum

absorbance at 435 mμ. This chelate does not conform to Beer's law although a working curve has been set up over the range of 1 to 15 ppm of chromium. The chelate has a molar absorptivity of 4600 cm^{-1} $mole^{-1}$ l at 435 mμ. The slope-ratio method and "gerade" method both indicated a 1:1 complex, although there is some evidence that other chelates may be present. An estimated magnitude for the instability constant of about 2×10^{-4} was obtained. The same ions offered serious interference here as with molybdenum and tungsten.

An attempt was made to run a potentiometric titration of quercetin, both alone and in the presence of the metal ions, but the results were inconclusive.

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